THE RYUMACHI

English Edition

THE 25TH ANNIVERSARY

COMMENORATIVE ISSUE

VOL.21 SUPPLEMENT 1981

OFFICIAL JOURNAL OF THE JAPAN RHEUMATISM ASSOCIATION

ISSN 0300-9157
Contents

Preface ................................................................. 1
Editorial
Yoshio Oshima ...................................................... 3

Reviews & Epidemiology
Balneotherapy of chronic rheumatism currently being performed in Japan
H. Morinaga ............................................................. 5
A clinical evaluation of arthroscopic synovectomy of the knee joint
with the electric resectoscope
H. Ariomi & M. Yamamoto .......................................... 11
The Kodama-Yamamoto knee prosthesis — Its design and clinical
result —
S. Yamamoto, M. Hachinota, O. Yuzuki & T. Miyake ............ 21
A retrospective study on the cause of death, in Japan, of patients
with gout
K. Nishioka & K. Mikanagi ........................................ 29
A longitudinal population survey of rheumatoid arthritis in a rural
district in Wakayama
K. Shichikawa, Y. Takenaka, A. Maeda, K. Yostuno,
M. Tsujimoto, H. Oka, T. Kashiwade & I. Hongo .............. 35
Ankylosing spondylitis in Japan
H. Mitsui & H. Sonozaki ............................................ 43

Clinical Investigations
Immunological studies on the nature of anemia in rheumatoid
arthritis and leukopenia in systemic lupus erythematosus
M. Sugimoto, Y. Wakabayashi, Y. Shiohawa & F. Takaku ........ 51
Basic and clinical studies on continuous cryofiltration — A new
treatment for rheumatoid arthritis —
Hans Carossa wrote in his article, “Das Jahr der Schönen Täuschungen (The Year of Beautiful Delusion)” “If all the apple trees in the world withered leaving only one seed, what would people do with it? Would they sow it and let it survive at the risk of its extirpation? Or would they cut it into pieces to analyse it and to describe its detailed character for future generations? Fortunately, in the field of literature, both can be accomplished; that is by describing one’s history in detail, one can expect to plant and let it grow in the readers’ hearts.” With this beginning, he started to tell his own story of youth using elegant sentences.

The same is true for a boy, tentatively called Jr. A. He was born in 1957, in Japan, which had not yet recovered from its serious defeat in World War II. He was hungry, had few clothes to wear and no house to live in. However, in spite of these obstacles, he grew up with the kind help and support of many—both Japanese and foreign—people. How he graduates from medical school, and reaches his 25th birthday is our story.

Now Mr. Jr. A has become a man, and we would like to acknowledge those who helped him with open hands and to revise his name, replacing the small letter with a large one. In this way Mr. JRA is now ready to progress and to fly into the wide world of unknown. His real name is the Japan Rheumatism Association.

We, who have taken parts in bringing him up and encouraging him, want to record a scene of his youth following Carossa’s precedent.

The motivation originated in Wiesbaden, where the 2nd International Conference of Editors in Rheumatology was held in 1979. Among many discussions, “how to overcome language barriers” and the problem of duplicate publications were the most stimulating.

Rheumatology is developing more and more as is now as international as any other branch of science, English is the most commonly used language communicated with in many international meetings as well as in the literature. His official journal is published primarily in Japanese, including some papers in English.

Unfortunately, the Japanese language is so unique that little was read and called attention to not only in English-speaking countries but throughout the world, unless they were published in European languages.
In view of these facts, it was decided to issue a commemorative supplement of his official journal in English on his 25th anniversary.

In this issue, we have collected 6 reviews and epidemiological surveys, 16 original articles and 4 case-reports as well as an Editorial from Professor Oshima, the General Secretary of the Japan Rheumatism Foundation. In addition, a list of Winners of the JRA Scientific Prize awarded each year along with their references is included. The titles and authors of the reviews were selected by the Editorial Board from the standpoint of characteristics of our country. (marked with *) Original papers and case reports were collected in three ways, namely 1) public subscription, 2) translation of excellent papers which had been published in Japanese and 3) recommendations from Chairmen at the 25th annual Meeting at Kobe held with Prof. K. Hirohata as President. Papers in the first and the second category are marked (and) respectively, the third, into which the majority of papers belong, are unmarked.

Another point that we paid attention was to exclude duplicate publications. There must be many excellent papers from our country besides the contents of this issue, which have appeared or will appear in overseas journals. But to save extra labor and time in reviewing the literature, they were omitted.

For these reasons, this issue does not carry all the representative achievements which have been accomplished in Japan, but only a part of them.

Although they may be less beautiful but delusive, we are quietly confident that this issue, full of excellent articles from Japan, will act as the seed of an apple—not only by stopping and revealing a frame of a film in 1981 to the spectators of the world including ourselves, but by stimulating and encouraging Japanese authors to present further progress in international languages.

We sincerely pray and expect that it will grow into a large tree, with a bounty of beautiful and delicious fruit.

The Editorial Board
Editor-in-Chief: Tatsuo Suzuta, Tokyo Medical College
Editors: Masahiko Okuni, Nihon University
Masakazu Yamamoto, Kaitak University
Hisayoshi Yoshizawa, National Sagamihara Hospital
Shunichi Hirose, University of Tokyo
Yasuo Yamauchi, Juntendo University
Yasuhiro Hosoda, Keio University
Takeshi Tojo, Keio University
Yutaka Nagai, Tokyo Medical and Dental University
Shigeru Arimori, Tokai University
Advisers: Yoshih Oshima, Saitama Medical School
Yuichi Ota, Tokyo Medical College
Takehumi Morotomi, Nippon Medical School
Satoshi Sasaki, University of Tokyo
Mitsuo Homma, Keio University

The Japan Rheumatism Association was practically founded in April 1957 holding scientific lectures at the general meeting of the Japan Rheumatism Foundation. In April 1958, the Association published an official journal, “The Ryumachi” (vol. 1, no. 1), in which the late Professor Isaharu Miki reported that the Association had joined “La Ligue Internationale contre le Rhumatisme” at the 9th International Congress of Rheumatology held in Toronto, Canada, in June 1957 (the year the Association was founded). The name “Japan Rheumatism Association” has been officially used since the 6th general meeting in 1963 (the President was Professor Ryoichi Yano). Both the Foundation and the Association have been growing satisfactorily since they were established and held their 25th general meeting this year. The number of members of the Foundation is over 2,700, including organization members belonging to 54 companies, and the number of presentations increased to 377 although only 3 commemorative lectures were held in the year the Association was founded.

The Association not only promotes research on rheumatism and exchange of the results by opening scientific meetings and by publishing an official journal but also functions in cooperation with the Foundation and is endeavoring to level up the research on rheumatism and rheumatology praxis in Japan by sending its members abroad and by training young rheumatologists. Furthermore, the Foundation makes the results public in “Rheumatism Digest” and provides continuing education for general practitioners.

Also, the Foundation has given encouragement to its members by presenting the Japan Rheumatism Foundation Prize for long-term antirheumatic activities and the Japan Rheumatism Association offers the Japan Rheumatism Association Prize for excellent research on rheumatism. It is regrettable that an ideal eradicative medicine has not been found yet, but we are proud that the Foundation itself, in cooperation with the Association, has conducted scientific evaluation of anti-rheumatic drugs which must be administered for a long period. There are only a few precedents in this field among other associations.

Dixon reported in the Journal of Rheumatology published recently that rheumatology includes systemic disorders of connective tissue, inflammatory arthritis, osteoarthritides, back troubles and soft tissue or non-articular rheumatism, as defined by W.H.O. As a matter of fact, he deals mainly with the latter 4 dis-
Balneotherapy of Chronic Rheumatism Currently Being Performed in Japan

Hiroshi MORINAGA
Misasa Branch Hospital of Okayama University Hospital, Misasa Spa, Misasa-cho Tohaku-gun, Tottori-ken 682-02

Chronic rheumatism is known to be the main indication for balneotherapy in Japan. Balneotherapy is one of the natural treatments whose efficacy has been recognized empirically. The Book on Hot Springs in Japan for 1941 lists about 150 hot spring resorts for the treatment of chronic rheumatism. With the development of new drugs for rheumatism, the range of indications for balneotherapy has been extended and its practice has become easy. As a result, the concomitant use of hot springs and drugs is now able to produce an effect in about 80% of the cases of chronic articular rheumatism. Furthermore, this concomitant therapy is also capable of returning more than 90% of patients with lumbago, sciatica or cervico-brachial syndrome to their daily jobs. Balneotherapy for chronic rheumatism is superior to other therapeutic methods in that it is capable of being used concomitantly with basic therapy.

Gout has been an indication for balneotherapy from olden times. Since the pathophysiology of gout has been gradually clarified today, it is reasonable to consider that the efficacy of balneotherapy for this disease is based on (1) an increase in diuresis by the absorption of hot spring water, (2) alkalization of the urine and (3) alkalosis of the blood through hot spring bathing. Therefore, I recommend the use of alkaline simple thermalas as "Gichtbader." The Book on Mineral Springs in Japan for 1954 lists 47 resorts having alkaline simple thermalas whose pH is 8.0 or more.

INTRODUCTION

Japan is known worldwide for her hot springs, which number some 1,800. Although hot spring cures probably date back to an early age, it was only in 1931 that a systematic investigation of hot springs was started by the Institute of Balneotherapeutics established by Kyushu University at the Beppu Hot Spring. However, research laboratories were successively established by Hokkaido University (Noboribetsu Hot Spring, 1936), Kagoshima University (Kurisima Hot Spring, 1937), Okayama University (Misasa Hot Spring, 1939), Tohoku University (Naruto Hot Spring, 1944), Keio University (Izu-Tsukigase Hot Spring, 1945) and Gunma University (Kusatsu Hot Spring, 1952) which individually reported the results of their investigations on the therapeutic effects of hot springs.

Until the Japan Rheumatism Association was established in 1957, patients with chronic rheumatism were treated at branch university hospitals located at the above hot springs or at national hot spring hospitals at Ito, Narugo, Shirahama and Ureshino hot spring resorts. Hot spring cure is one of those natural therapies whose effects have been proven by time and experience. The hot spring cure has long been popular among its users,
but its image as folk medicine has not been wiped out to date because the essential constituent element composition of hot springs is extremely complicated and the therapeutic effects depend largely upon factors such as the climate of the watering places and environmental changes. Hydrotherapy as normally prescribed is unaffected by such factors.

Furthermore, because of the complexity of the mechanism of balneotherapy, and as criteria for evaluation of therapeutic effects on symptoms such as chronic rheumatoid arthritis are not established in Japan as yet, it is still difficult for physicians in general to understand the mode of action of hot springs. Nonetheless, many patients with chronic rheumatism visit hospitals at hot spring resorts, and in fact we have found in our daily clinical practices that in addition to treatment with newly developed antirheumatic agents including steroids, balneotherapy is indicated for more patients with chronic rheumatism.

**CHRONIC RHEUMATISM AND BALNEOTHERAPY**

Other than various valuable data obtained by the above medical research institutes, the therapeutic effects of hot springs on human physiology have been reported by Honorary Professor Yoshio Ohshima, Tokyo University, and his colleagues. Honorary Professor Takashi Sugiyama, Tohoku University, referred to the balneotherapeutic effects in the treatment of rheumatism by classifying them under five different merits, namely (1) thermal effects, (2) effects on the peritoneal cavity and abdominal system, (3) adjustment of autonomic nervous system functions, (4) improvement in various metabolic abnormalities and (5) improvement in vascular permeability (1977)[1], while Honorary Professor Ryoichi Yano, Kyushu University, gave a detailed description of balneotherapeutics in rheumatoid arthritis in his paper entitled "Guidance of medical care at hot springs"[2](1979).

Since a hot spring medical system has been established in Japan for only a few years, previously patients with rheumatism were not fully advised as to practical hot spring medical care. Thus, upon arriving at hot spring resorts, patients with chronic rheumatism were apt to take baths many times on the same day in the hope of finding earlier relief from their symptoms. Because the Japanese people prefer a bathing temperature as high as 42-45°C, higher than normal in Europe and America, and in their haste to achieve the therapeutic effect in a shorter time, they often became exhausted and developed the "bathing reaction" (Badekurreaktion).

In view of such past experiences, we have been advising those with chronic symptoms not to take baths more than once or twice a day during the 1st week after admission to a hot spring hospital and not more than three days thereafter. Since patients with chronic rheumatism often develop anemia, we have been advising them to take a lukewarm bath (38-40°C) for a longer period of time (15-20 minutes) so that they won't eventually suffer from cerebral anemia. It is naturally important in winter to install heat in a bath room, particularly in the dressing room, and to advise the patients to thoroughly wipe the skin, particularly the hands and feet, with a dry towel after bathing, and to take a rest for about 30 minutes. After bathing had been repeated approximately 10 times, i.e. around the 3rd day for patients who took baths twice a day and the 3rd day for patients who took baths three times a day, either local or general symptoms due to the "bathing reaction" such as transient nausea, general malaise, anorexia, dull headache, insomnia and recurrence of pain or swelling of the affected region were observed; a fever, as in a cold, as high as 37.5-38°C was also noted in some patients.

According to our experience at Misasa Hot Spring, approximately 50% of patients with chronic rheumatism (about 70% of rheumatoid arthritis and 30% of other degenerative arthropathy) showed some kinds of symptoms due to the "bathing reaction," but they always disappeared in 1-2 days when bathing was discontinued with ample rest. However, there could possibly be complications when the symptoms do not disappear in 1-2 days. Therefore, it is reasonable for physicians at hot spring resorts to combine mud stupes, other hydrotherapy and physiotherapy with bathing after 1-2 weeks have passed.

**Balneotherapy of Rheumatoid Arthritis**

**Case Report** (Fig. 1)

A 64-year-old female with a 10-year medical history was admitted to this hospital since she developed a "moon face" because of steroid treatment, and was mostly confined to bed ever since she suffered from disturbed gait with increasing pain in both knees; this was stage II-III and class 3. Balneotherapy was combined with aspirin given by mouth. Hubbard tank baths were prescribed during the 1st week and hot spring baths once a day from the 2nd week. The patient was allowed to walk in a pool three times a week so that she could regain confidence in walking. After the patient was locally treated with usual baths and mud stupes, she walked more easily as stiffness and pain of joints subsided gradually.

Around the 4th week after treatment, the patient could take care of herself. Chryotherapy was then performed, and the patient was discharged approximately 3 months after admission. Although long-lasting effects from balneotherapy alone could hardly be expected in patients with rheumatoid arthritis visiting hot spring resort hospitals because they are considered inadequately cured by other therapies, there were some patients in whom swelling of finger joints was cured by baths and local mud stupes alone.

Results with 70 patients with rheumatoid arthritis, who were admitted once every 3 months on the average and given antirheumatic agents orally or intra-articular injection of steroid preparations in addition to local mud baths and mud stupes centering on Hubbard tank pool and bath, showed functional improvement rates of 75% in stage I, 48% in stage II and 13% in stage III, but an improvement as high as 48% was observed in stage IV as well. Fig. 2 shows the severity (class) of functional disturbances which these 70 patients suffered from at the times they were admitted and discharged, compared with data on the functional conditions of 36 patients obtained by communication 1-4 years after their discharge.

The patients' seemingly favorable condition at the time of discharge is not promising as continued long-term improvement, and thus not appreciably different from spontaneous remission of rheumatoid arthritis. It is therefore considered necessary to repeat balneotherapy in these patients once or twice a year, although favorable results were observed in patients with a medical history of less than 5 years.

Balneotherapy is not contraindicated even in patients with increased ESR and higher CRP levels when they are properly cared for under the guidance of qualified physicians.
controlling their lifestyle and adjusting the balance of the metabolic disorder. It is advisable for these patients to take the waters of sulphated bitter springs, real bitter springs, sulphur springs and bicarbonate springs as well as "alkaline simple thermal" with diuretic effects, known as "gout springs" (Giebhublieder). It may be worth noting that Badgastein (Austria), Plomhütre (France) and Fiuggi (Italy) which are well known as "gout springs" have diuretic effects due to their properties of being alkaline simple thermal in addition to being "radioactive springs.""**

**CONCLUSION**

There are about 150 hot springs which have long been known to be effective in the treatment of rheumatism, including 36 common salt springs, 36 simple thermal, 28 sulphur springs, 22 bitter springs, 9 alkaline springs and 8 radioactive springs (Fig. 4). Less stimulative salt springs, simple thermal and bitter springs are suitable for rheumatoid arthritis shortly after disappearance of the acute symptoms, but highly stimulative springs such as sulphur springs, radioactive springs and acid springs are also suitable for the symptoms of established disease remaining in a stage of degeneration and contracture. However, it is a more common trend of late not to specify a particular type of spring but to recommend balneotherapy at a hot spring where physicians well qualified in the treatment of chronic rheumatism are stationed. Effective results in the balneotherapy of chronic rheumatism are being achieved in Japan as the

---

**Fig. 3** Changes in serum concentrations after the internal use of Indomethacin with spring water

Balneotherapy in rheumatoid arthritis is presumably more advantageous and acceptable than other therapies in that a routine basic treatment can be combined with it.

**Balneotherapy of Degenerative Rheumatism**

According to investigations made by questionnaire of 100 patients given balneotherapy because of lumbago, sciatica and cervicothoracic syndrome, 98% of patients with lumbago and sciatica and 77% with cervicothoracic syndrome were found to have returned to normal life 6 months after discharge.

**Balneotherapy of Gout**

Gout is still indicated for gout, as this condition has long been considered rheumatism since its main symptoms originate in the joints despite the fact that it is a metabolic disease. Although the pathophysiology of gout is only gradually being unveiled, it may be reasonable to consider that (1) an increase of diuretics by drinking of hot spring water, (2) alkalinisation of the urine and (3) the alkaliotic trend of blood after taking lukewarm baths are the main therapeutic effects that can be expected in the treatment of gout. Thus, it may be necessary for patients with gout requiring lengthy drug therapy to be treated at hot spring resort hospitals for 2–3 weeks once or twice a year and to see attending physicians well experienced in the treatment of gout to receive a routine check of their physical condition, thereby

---

**Fig. 4** Distribution map of "Rheuma-Bad" in Japan

---

**Fig. 2** Evaluation of functional abilities before and after balneotherapy

Although some kinds of symptoms due to the "bathing reaction" were seen in 71% of patients with rheumatoid arthritis during the course of balneotherapy, no similar symptoms were noted in patients requiring discontinuance of bathing. Steroid treatment was no longer required in 54% of the patients and the dose of steroid was reduced in 23%.

We have recently used "gold salts" and D-penicillamine or immunosuppressive agents, depending on the patient, in combination with balneotherapy and observed a therapeutic effect in 89%, including a marked effect in 49% (ineffective in 11%). Side effects due to gold salts were seen in 14% which was, however, lower than the percentage reported by non-balneotherapeutic institutions. When Indomethacin was given with hot spring water, over-rapid absorption of the drug was inhibited more effectively and an effective serum concentration of Indomethacin was preserved for a longer period of time than when control city supply water was used (Fig. 3). This may prove to be a source of a much lower incidence of side effects due to drug therapy of rheumatoid arthritis at hot spring resort hospitals.
A Clinical Evaluation of Arthroscopic Synovectomy of the Knee Joint with the Electric Resectoscope

Hiroshi ARITOMI1 and Makoto YAMAMOTO

Department of Orthopedic Surgery, Kitaato University School of Medicine, Kitaato, Sagamihara 228

The technique, design and function of the electric resectoscope, consisting of arthroscopy with an electric surgical knife, is described along with the attached equipment for arthroscopic surgery of the knee joint.

Arthroscopic surgery with the electric resectoscope can additionally be used for synovectomy, synovial biopsy and removal of loose bodies as well as for cartilage shaving. Clinical follow-up suggests that synovectomy performed with the electric resectoscope produces almost comparable results with those of conventional synovectomy.

The advantages of arthroscopic surgery with the electric resectoscope are as follows. It can be used to perform a synovectomy as well as for the removal of loose bodies after observation with the resectoscope following a small skin incision. It can be used to effect intraarticular hemostasis by electrocoagulation. Postoperative pain is far less than with conventional synovectomy. It offers a much more rapid recovery of range of motion of the joint without any postoperative manipulation. Consequently, it shortens the period of hospitalization.

A few complication were, however, noted with this method. None were serious, except for two episodes of infection with Pseudomonas during early utilization of this technique.

With the development of the arthroscope in daily practice, further progress of the electric resectoscope is expected to be beneficial not only physical diagnosis, but in arthroscopic surgery for the treatment of some joint diseases.

Arthroscopy has become widely accepted for pathologic study of the knee joint. Employment of the urological electric resectoscope, equipped with an electric surgical knife, in the knee joint suggested that it might be applicable to synovectomy. Since 1972 we have used this instrument, clinically, to overcome the problems of conventional synovectomy, such as disturbance in function during rehabilitation. In this article, our experience and clinical results are reported along with some observations.

Keywords: rheumatoid arthritis, arthroscopy, arthroscopic synovectomy, knee joint, surgical treatment

The Design and Function of the Electric Resectoscope

There are two types of resectoscopes, a punching type and an electric type. We employ the Iglesias electric resectoscope, which has been improved and modified for the knee joint. This instrument can be easily operated with one hand (Fig. 1).

The Femorobique Telescope

This instrument is an endoscope with a diameter of 4.9 mm with an oblique or square distal tip. The length varies according to its use. The recent

REFERENCES

1) T. Sugiyma: A comment about the effect of balneological treatment on rheumatic disease. The Ryumachi 17: 147-180, 1977
cutting and coagulating currents are necessary to perform surgery. The high frequency current produced by this instrument varies from 1.5 to 5 megacycles. The tube oscillating circuit generates the cutting currents, and the spark gap oscillating circuit generates the coagulating current, while the blended current produced by mixing the two currents coagulates as well as resects. Switching from one of these currents to the other is controlled by a foot pedal. The metal plates, usually placed on the buttocks, needed to complete the electrical circuit should be as close as possible to the area of surgery. Although many electrical current sources are available, the Bovie (U.S.A.) and Mizuho GT-S4 (Japanese) devices were used by the authors.

Illumination Source
Although older instruments required tungsten illumination, newer models are equipped with a safer and more efficient fiberglass light source.

Irrigation Apparatus
The irrigating solution is usually placed in a covered container with a capacity of 5.0 liters. This is suspended from the ceiling or from a stand in the theatre. The container for irrigating fluid is placed 50 to 60 cm above the operating table. The fluid pressure varies between approximately 40 and 80 cm of water, depending on the height of the irrigating fluid above the operating table.

The Cleaning Device
A cleaning device is used to remove resected tissue or blood clots from the joint cavity and to clean within the joint following synovectomy with the electric resectoscope.

STERILIZATION OF THE RESECTOSCOPE AND PREPARATION OF THE IRRIGATING SOLUTION

The electric resectoscope is separated into its various elements. The device may then be steri-
When resection is satisfactorily accomplished, the inside of the joint cavity is washed out fully with physiologic saline solution or irrigating fluid to remove resected tissue and blood clots. The wound is then closed.

POSTOPERATIVE TREATMENT

After operation, a compression bandage is applied to the knee joint with mild pressure and the knee is maintained in slight flexion. No plaster fixation is needed. On the day of the operation the extremity is supported in an elevated position. On the first postoperative day the patient is encouraged to flex and extend the knee within the limits of pain. Weight bearing, as tolerated, is encouraged on the third day. Most patients can ambulate with minimal pain. A normal range of motion is usually rapidly recovered. It is advisable to aspirate the occasional joint effusion that persists for more than one week. Usually, however, the patient can be discharged from the hospital after the third or fourth postoperative day.

CLINICAL RESULTS

Synovectomy with the electric resectoscope was performed in 68 joints of 60 patients with knee joint pain or arthritis who visited our clinic or who were hospitalized in our hospital. Their ages ranged from 16 to 84 years. Eight were males and 52 females (Table 1). The patients were divided into three groups according to the surgical procedure. In 14 cases the aim of the operation was to view the surface of the synovial membrane and obtain a biopsy. In six other cases resection of the anterior synovial membrane and debridement were carried out following establishment of the diagnosis by biopsy. In 29 cases the operation was performed because of rheumatoid arthritis which had been progressing. The indications for surgery in these patients were persistent and marked symptoms of inflammation, such as pain, swelling of the knee joint, or effusion, which resulted from chronic disease present for at least six months. The procedure was also used when clinical assessment indicated a high degree of inflammatory activity. One specific merit of the electric resectoscope is that it can be used in conjunction with conventional arthroscopic examination and to perform biopsies at the same time. In this manner, various diagnoses were established after careful observation of the synovial membrane surface and histopathologic examination of the biopsy tissue. Debridement of the knee joint was carried out when indicated.

In three patients with chronic rheumatoid arthritis, conventional resection of the anterior synovial membrane was performed after use of the resectoscope. Manipulation, under general anesthesia, was necessary two to three weeks after the operation in all patients so treated. In the other 36 joints in 29 patients with chronic rheumatoid arthritis, synovectomy was performed with the electric resectoscope. By observing the synovial membrane with the resectoscope in the joint cavity, two types of change were noted—hypotrophic and hypertrophic. In the former case, the synovial membrane shows congestion and swelling. However, the prolif-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical cases for synovectomy with the electric resectoscope (SER) and arthroscopic surgery (1972–79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. SER</td>
<td>Rheumatoid arthritis ................................................................. 29 cases (36 joints)</td>
</tr>
<tr>
<td></td>
<td>Chronic synovitis (hydrarthrosis) ............................................................................. 3 cases (6 joints)</td>
</tr>
<tr>
<td></td>
<td>Pigmented villonodular synovitis ...................................................................... 5 cases (2 joints)</td>
</tr>
<tr>
<td>2. SER</td>
<td>For the purpose of biopsy only .................................................................. 14 cases (14 joints)</td>
</tr>
<tr>
<td>3. Anterior conventional synovectomy or debridement following SER</td>
<td>Rheumatoid arthritis ........................................................................... 3 cases (3 joints)</td>
</tr>
<tr>
<td></td>
<td>Pigmented villonodular synovitis ................................................................ 1 case (1 joint)</td>
</tr>
<tr>
<td></td>
<td>Tuberculosis .......................................................................................... 1 case (1 joint)</td>
</tr>
<tr>
<td></td>
<td>Chronic synovitis ................................................................................... 1 case (1 joint)</td>
</tr>
<tr>
<td>4. Removal of intraarticular loose bodies .................................................. 4 cases (4 joints)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>......................................................................................................... 60 cases (68 joints)</td>
</tr>
</tbody>
</table>
In order to determine the amount of synovial membrane resected and its effect, scanning of the joint with 99m-technetium pertechnetate was performed. In some cases the uptake of 99m-technetium pertechnetate was markedly reduced as compared to the preoperative uptake. These findings almost coincide with the subsidence of inflammatory activity.

After the operation the range of joint motion returned to normal in most cases. Rapid recovery was noted in four to five days and in the delayed case, in about 10 days. In some cases the range increased, whereas in other cases a slight loss of approximately 10 to 20 degrees was noted. Significant atrophy or decrease in power of the quadriceps femoris was not observed.

As for complications, a postoperative bloody joint effusion was observed in one case. Infection with pseudomonas aeruginosa occurred in two other cases. The infection was controlled during rehospitalization by continuous irrigation of the joint with large amounts of physiologic saline solution containing antibiotics. When the patient was discharged, some arthritic destruction was noted. The irrigating fluid can cause a systemic reaction resulting from an abrupt increase in extracellular fluid provoked by absorption of the irrigating fluid from the surgical site. Such cases are reported in urologic patients, but with the resectoscope only two patients showed pyrexia, possibly resulting from the use of Urigal (Cytal). The condition was transitory in each case, and with adjustments in the electrolyte levels in the intravenous solution there were no additional problems.

### Table 2
Clinical results of synovectomy with the electric resectoscope (SER) for rheumatoid arthritis

<table>
<thead>
<tr>
<th>X-ray stage at the time of SER</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No pain, no effusion</td>
<td>2(1)</td>
<td>3(2)</td>
<td>5(4)</td>
<td>10(7)</td>
</tr>
<tr>
<td>Slight pain on motion</td>
<td>4*(0)</td>
<td>9(6)</td>
<td>13*(6)</td>
<td></td>
</tr>
<tr>
<td>Occasional effusion</td>
<td>1(0)</td>
<td>2(0)</td>
<td>5(4)</td>
<td>8(4)</td>
</tr>
<tr>
<td>Occasional pain</td>
<td>1(0)</td>
<td>1*(0)</td>
<td></td>
<td>2*(0)</td>
</tr>
<tr>
<td>Profuse effusion</td>
<td>1(0)</td>
<td>2(0)</td>
<td></td>
<td>3(1)</td>
</tr>
<tr>
<td>Total</td>
<td>4(1)</td>
<td>10*(2)</td>
<td>19(14)</td>
<td>33*(17)</td>
</tr>
</tbody>
</table>

(*: case complicated by infection)

(15 cases, 17 joints: asomic acid injected intra-articularly prior to operation)

### PATHOLOGIC EXAMINATION OF TISSUES

Most specimens of the synovial membrane, obtained by resectoscope, were tissue sections with an approximate size of 0.5 by 0.7 by 1 cm, as shown in Figure 4. Macroscopic examination of the resected synovial membrane showed that the resected surface was cut sharply by the loop, with tissue coagulation, almost uniformly linear in nature. The width of tissue coagulation in the resected tissue was approximately 500 to 1000 μ where coagulation and necrosis had occurred. In two cases joint inflammation recurred; the resectoscope was used again in the same manner.

Histologic examination in one case showed a synovial membrane with changes of rheumatoid arthritis (Fig. 5B). There was evidence of marked inflammation, such as multiple large vili and lymphocytic follicles. Eleven months after the first operation with the resectoscope the inflammation recurred and marked retention of synovial fluid in the joint was observed. Therefore, the operation was performed again. The surface layer of the synovial membrane showed slight congestion and hyperplasia and a ridgelike protrusion throughout the tissues. No proliferated villi or other specific changes were noted. Histologic study revealed that most portions of the synovial membrane consisted of blood vessels and granulation tissue with predominant fibrinosis with which the inflammatory lesion and the accompanying exudate of fibrin-like substance are associated (shown by arrow in Fig. 5A). The electric resectoscope caused no obviously harmful effects.

### DISCUSSION

Many surgeons have noted subsidence or relief of pain after conventional synovectomy. However, there often is either loss of joint motion or a prolonged recovery period. When changes were observed on x-ray examination, the incidence of

![Fig. 4 Synovial tissue resected with the electric resectoscope (21.5 g., 56 year old female)]
Fig. 5 Histologic findings in the synovial membrane. A: The regenerated membrane with recurrence of the inflammation (shown by arrow), 11 months after the first operation. B: The findings, by synovectomy with the resectoscope, at the first operation.

Recurrence was high, possibly because of a mechanical factor. X-ray changes often develop in spite of long lasting symptomatic improvement. Thus, many authors believe that synovectomy should be performed before roentgenographic changes are seen. However, since chronic rheumatoid arthritis is a systemic, although self-limiting, disease, very few authors believe that synovectomy can arrest the rheumatic process in all cases. Patients with progressive multiple joint disease that have shown a high level of activity usually show only a brief period of improvement following surgery. This is true even though many reports have indicated improvement in laboratory findings following synovectomy. This is the main reason for the differences seen in the excellent results following synovectomy for cases with chronic synovitis.

Histologic findings in the regenerated synovial membrane vary. An increase in cicatrical and connective tissue is reported by some investigators, whereas others state that the synovial membrane appears normal clinically. Resection of the inflamed tissue interrupts the abnormal foci and the progress of the disease is thereby impeded.

Since he had observed no systemic exacerbation and marked symptomatic relief, Paradie emphasizes the importance of early synovectomy. Resection of the inflamed tissues seems indicated especially in patients who early flexion contractures with a hyperplastic synovium and excessive joint fluid with a poulpe-like cyst. Symptomatic and functional improvement appears to persist until the regenerated tissues are invaded by the inflammatory process. This occurs in less than one third of the operated cases.

Recently electron microscopic scans of the minute synovial blood vessel patterns have been reported. The specimens were produced by the injection of methyl methacrylate into the femoral artery. Four layers of blood vessels have been noted in the synovium. The vascular connections between the layers appeared to be minimal. The progress of pathologic changes in the area may thus be estimated from the distribution of the vessels in each layer. These findings have suggested that resection of a 1 mm layer of synovium may suffice in the early rheumatoid joint.

Synovial resection, even by conventional methods, is often difficult and, at times, incomplete. The extensive incision that is required often results in a prolonged convalescence. In spite of the beneficial effects of synovectomy, the patient may postpone or refuse the procedure. The use of the electric resectoscope was first proposed because of the foregoing problems. The arthroscopic features of the instrument offer a simple method of examination and synovial biopsy. The advantages of this new modality over conventional synovectomy are indicated as follows. Because of the small incision and electrocoagulation, postoperative bleeding is minimized. Postoperative pain is also greatly decreased, recovery of function is greatly accelerated, and hospitalization is shortened. An added benefit resides in the fact that only one surgeon is needed to perform the operation.

Except for patients with postoperative complications, the average period of hospitalization was 10.3 days. Although some patients with severe radiologic changes again developed joint effusion shortly after operation, their pain was comparatively relieved. When the operation is performed with the electric resectoscope, it provides the benefit of fewer side effects than the conventional procedure with comparable clinical results.

Although use of the electric resectoscope has the aforementioned benefits, the operation is not without its problems (Table 3). Some of the difficulties reside in the structure of the resectoscope per se and its accessories. Furthermore, it is essential that the surgeon acquire adequate skill in the use of the instrument.

At present, a urologic resectoscope is employed. It is believed that with an improved instrument the area of resectable tissue will be about the same as that now removed with the open procedure. Currently, the available area of resection is increased by use of both the interstitial and the inferior interstellar approaches.

In order to identify the tissue to be resected more clearly, 1 percent procaine with Xylocaine is injected into the joint about two hours prior to surgery. The synovial surface assumes a grayish hue. Estimation of the area of the tissue to be resected is thereby facilitated.

Postoperative bleeding and infection are not unusual when a conventional synovectomy is performed. The ability to perform electrocoagulation with the resectoscope greatly reduces hemorrhage both during and after the procedure. With the use of a sterile hood and administration of antibiotics, postoperative infection has been minimized. Although reactions to the irritating fluid have been reported by urologists, we have encountered no severe reactions except for two instances of hypotonotematia.

Although other problems such as postoperative quadriiceps weakness may occur, they are minimal in duration when the resectoscope is used.

CONCLUSIONS

The electric resectoscope, used for transurethral resection in urology, was modified to perform synovectomy and arthroscopic surgery in the knee joint. Our clinical results have also been reported.

The advantages and problems of synovectomy and arthroscopic surgery with the electric resectoscope, as used in the knee joint, are discussed.

Table 3 Complications of SER

<table>
<thead>
<tr>
<th>Complication</th>
<th>Case Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Infection with pseudomones aeruginosa</td>
<td>three cases</td>
</tr>
<tr>
<td>2) Oliguria and general weakness due to hypotonotematia</td>
<td>two cases</td>
</tr>
<tr>
<td>3) Excessive retention of intrarticular bloody exudate</td>
<td>one case</td>
</tr>
<tr>
<td>4) Muscle weakness in the operated extremity</td>
<td>one case</td>
</tr>
</tbody>
</table>
especially in comparison with conventional resection of the anterior two-thirds of the synovial membrane. Clinical follow-up observation indicated that synovectomy performed with the electric resectoscope produced results almost comparable with those of conventional synovectomy.

The procedure is performed through a small skin incision with fewer untoward effects. The operation is relatively painless and offers more rapid recovery following surgery.

A few complications, however, were noted using this method. None were serious. There were two episodes of infection during the early use of this technique.

With the development of the arthroscope, further development of the electric resectoscope is expected to be beneficial not only in making diagnoses, and in biopsies, but also in arthroscopic surgery for treatment of joint diseases.

ACKNOWLEDGMENT

We thank Professor Takeki Koshiba of the Urological Department at Kiasato University for his kind advice and help with the electric resectoscope.

REFERENCES


☆

The Kodama-Yamamoto Knee Prosthesis

Its Design and Clinical Result

Sumiko YAMAMOTO†, Minoru HACHINOTA,
Osamu YUZUKI* and Takahiro MIYAKE**

The Centre for Rheumatic Diseases, Matsuyama Red Cross Hospital, Bunkyo-cho, Matsuyama 790
*Department of Orthopaedic Surgery, Kawasaki Hospital
**Department of Orthopaedic Surgery, Kojima City Hospital

In 1970, the first operation was performed using the Kodama-Yamamoto knee prosthesis. The fundamental aspects of this prosthesis are that it is semiconstrained and that bone cement is not used. The postoperative results of one hundred and thirty two knees replaced with the Kodama-Yamamoto Mark II prosthesis revealed that in patients with osteoarthritis operated upon unilaterally with a favourable preoperative range of motion, a highly stable reconstruction of joint function can be expected through this total joint replacement.

DESIGN OF THE KODAMA-YAMAMOTO KNEE PROSTHESIS

In 1968, we started the studies on knee prosthesis with the late Professor Kodama. The operation on the first case was performed for the first time in 1970. As a fundamental aspects of the design, neither hinge nor bone cement was used.

In the initial stage, we modified the femoral mold of Sharples for use as the femoral component. This is based on an expectation of physiological joint movement with a combination of femoral and tibial joint surfaces of physiological shape.

By 1973, 20 operations had been performed using Mark I, type D shown in Fig. 1. The results were not always satisfactory. An analysis of the postoperative results revealed greater improvement of pain than expected. The method of fixation, however, was not successful because the prosthesis did not fit the Japanese knee joint.

In 1973, a revision was carried out to design Mark II, type D. The emphasis was placed on improving the following point. The shape of the joint surface was simplified, since replacement with a joint surface of physiological shape did not frequently induce effective mobility.

Between 1973 and 1975, 23 operations were performed with Mark I, type D. Since this was designed to fit the Japanese knee joint, stability was achieved in the early postoperative period. The monoscalene shape of the joint surface, adopted to increase stability, was effective for this purpose but limited rotatory and sliding movement, so that it was not possible to provide a sufficient degree of flexion postoperatively.

Based on these experiences, and the results of a study by the author (Yamamoto) for 1 year in The London Hospital with Mr. M.A.R. Freeman, the Mark II was designed as shown in Fig. 2.

The femoral component of the Mark II has a radius of 20 mm at the center, 23 mm at the pos-
terior condyle and 38 mm in the anterior portion in the lateral view. The radius of the concave joint surface of the tibial component facing this is 38 mm. Through the combination of these two radii, the stability is increased at the extended position, and a rotating movement became possible at the flexed position.

Since the femoral component had an anterior flange as a succession from the Mark I, hardly any trouble was encountered with reference to the patello-femoral joint.

Since the operation consists of an osteotomy with the use of instruments, the femoral component is driven on to the femur with an excellent fit on the resected surface, without any need for the use of bone cement.

The tibial component, on the other hand, consists of high density polyethylene with an inferior indented surface facing the surface of the resected tibia. The tibial component is fixed with two staples driven in at an angle of 45° from the anterior direction. These 2 staples prevent anterior floating of the tibial component and also accomplish temporary fixation until permanent fixation is ready. No bone cement is at all required for the fixation. While bone cement is generally thought to be necessary for fixation of artificial joints, a prosthesis without the requirements of bone cement appears to be ideal, in view of its potential toxicity and its injurious effects. Based on our operative experience (Fig. 3), bone cement is not at all necessary in knee prostheses as long as a careful design is used.

**OPERATIVE RESULTS WITH THE KODAMA-YAMAMOTO MARK II KNEE PROSTHESIS**

Postoperative results were followed up in 132 knee joints operated with the use of the Kodama-Yamamoto Mark II knee prosthesis between 1975 and 1979.

Table 1 summarizes 132 joints of 90 patients, 23 joints of 18 males and 109 joints of 72 females. The diagnosis was RA in 100 joints of 63 patients, OA in 31 joints of 26 patients and TB in 1 joint of 1 patient. A unilateral operation was performed in 44 patients and a bilateral operation in 46 patients. As to the stage of this survey, more than 2 years have elapsed since the operation in 66 joints of 45 patients, and less than 2 years in

<table>
<thead>
<tr>
<th>Sex of Patient</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>23 knees</td>
</tr>
<tr>
<td>Female</td>
<td>109 knees</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>100 knees</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>31 knees</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1 knee</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within 2 years</td>
<td>64 patients</td>
</tr>
<tr>
<td>More than 2 years</td>
<td>66 knees</td>
</tr>
<tr>
<td>Died</td>
<td>4 knees</td>
</tr>
</tbody>
</table>
64 joints of 42 patients. Three patients, in whom 4 joints had been operated, are now dead.

Table 2 summarizes the history of previous operations in the operated knee joints. Synovectomy had been performed in 9 joints, application of a tibial plateau prosthesis in 5 joints, meniscectomy in 1 joint, and a high tibial osteotomy in 1 joint.

Since many patients in this series had RA, operations had been performed on other joints in a large number of patients. Total hip replacement had been performed in 14, fusion of the cervical spine in 2 and arthrodesis of the contralateral knee in 2.

The operative results were evaluated according to the knee score shown in Table 3. This knee score is the method of evaluation of knee joint function most widely used in Japan.

**Pain**

As shown in Table 4, knee joint pain markedly improved postoperatively. Moderate pain was experienced postoperatively in 6 joints. Popliteal pain was experienced in 2 patients and pain on standing in 4 patients, but the cause remains unknown.

**Range of Motion**

As shown in Fig. 4, the range of motion of the joint increased from 82° preoperatively to 87° postoperatively. Patients with a favorable pre-

---

### Table 2 Previous surgery

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Number of Joints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synovectomy</td>
<td>9</td>
</tr>
<tr>
<td>Tibial plateau prosthesis</td>
<td>5</td>
</tr>
<tr>
<td>Meniscectomy</td>
<td>1</td>
</tr>
<tr>
<td>High tibial osteotomy</td>
<td>1</td>
</tr>
</tbody>
</table>

Patient data on those who had had other surgery

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hip replacement</td>
<td>14</td>
</tr>
<tr>
<td>Anterior fusion of the cervical spine</td>
<td>2</td>
</tr>
<tr>
<td>Arthrodesis of the opposite knee</td>
<td>2</td>
</tr>
<tr>
<td>Osteotomy of the opposite knee</td>
<td>1</td>
</tr>
</tbody>
</table>

---

### Table 3 Knee score

(Niwa, Terayama, Yamamoto)

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>30</td>
</tr>
<tr>
<td>Occasional</td>
<td>25</td>
</tr>
<tr>
<td>Moderate</td>
<td>15</td>
</tr>
<tr>
<td>Severe</td>
<td>5</td>
</tr>
<tr>
<td>Severe pain at rest</td>
<td>0</td>
</tr>
<tr>
<td>Range of motion</td>
<td></td>
</tr>
<tr>
<td>&gt;120°</td>
<td>20</td>
</tr>
<tr>
<td>90°–110°</td>
<td>15</td>
</tr>
<tr>
<td>60°–89°</td>
<td>10</td>
</tr>
<tr>
<td>30°–59°</td>
<td>5</td>
</tr>
<tr>
<td>0°–29°</td>
<td>0</td>
</tr>
<tr>
<td>Extension lag or flexion deformity</td>
<td></td>
</tr>
<tr>
<td>&gt;10°</td>
<td>10</td>
</tr>
<tr>
<td>11°–30°</td>
<td>5</td>
</tr>
<tr>
<td>&gt;31°</td>
<td>0</td>
</tr>
<tr>
<td>Varus or valgus deformity, or lateral instability</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>10</td>
</tr>
<tr>
<td>&lt;15°</td>
<td>5</td>
</tr>
<tr>
<td>&gt;15°</td>
<td>0</td>
</tr>
<tr>
<td>Walking ability</td>
<td></td>
</tr>
<tr>
<td>Unlimited</td>
<td>20</td>
</tr>
<tr>
<td>More than 500 meters</td>
<td>15</td>
</tr>
<tr>
<td>Less than 500 meters</td>
<td>10</td>
</tr>
<tr>
<td>Indoors only</td>
<td>5</td>
</tr>
<tr>
<td>Bedridden</td>
<td>0</td>
</tr>
<tr>
<td>Activity of daily living</td>
<td></td>
</tr>
<tr>
<td>Get out of chair</td>
<td>210</td>
</tr>
<tr>
<td>Go upstairs</td>
<td>210</td>
</tr>
<tr>
<td>Go downstairs</td>
<td>210</td>
</tr>
<tr>
<td>Stand on one leg</td>
<td>210</td>
</tr>
<tr>
<td>Run</td>
<td>210</td>
</tr>
</tbody>
</table>

---

### Table 4 Pain (One hundred and twenty-four knees)

<table>
<thead>
<tr>
<th></th>
<th>Before operation</th>
<th>After operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Occasional</td>
<td>0</td>
<td>58</td>
</tr>
<tr>
<td>Moderate</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Severe</td>
<td>117</td>
<td>0</td>
</tr>
<tr>
<td>Severe pain at rest</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

---

**Fig. 5A** In cases with varus deformities, the deformity is mainly a consequence of the bony defect on the medial tibial plateau, the stepped tibial component is used to obtain a correct alignment and to tense the medial collateral ligament.

**Fig. 5B** In cases with valgus deformities, in order to obtain good alignment, more of the medial femoral condyle is removed than the lateral femoral condyle with a consequent slackening of the medial collateral ligament. The operation is performed by advancing the medial collateral ligament distally, it is tightened and fixed so that ligamentous stability and good alignment are the result.
operative range of motion generally preserved this favorable range postoperatively, but those with a restricted preoperative range also showed some limited range postoperatively according to our impression.

In patients with a preoperative flexion contracture of less than 30°, complete extension was achieved with relative ease, postoperatively. It thus appears possible to perform a beneficial and effective reconstruction of the range of movement of the joint with this procedure.

**Valgus and Varus Deformities and Walking Ability**

Lateral instability and valgus and varus deformities are correctable with surgery. Among operative techniques used, the establishment of a correct alignment and tension of the ligaments are most important as shown in Fig. 5. With a correct operative technique, reconstruction of a stable knee joint is feasible.

According to the evaluation of the walking ability shown in Table 5, the predicted improvement apparently did take place. The results of the evaluation of walking ability are markedly influenced not only by the state of the operated joints, but also by disturbances of other joints frequently seen in generalized diseases, such as RA.

Consequently, in patients bedridden preoperatively, even successful reconstruction of painless stable knee joints with bilateral operations frequently achieves only limited indoor activity because of the restriction of the hip and ankle joints. Even when the postoperative score is low, the operation itself is not necessarily worthless. Individual symptoms should be given an overall analysis in order to set a specific goal for each patient before the operation is carried out. Replacement with an artificial joint may thus prove to be useful even in patients with multiple serious impairments.

### Table 6 Total knee score

<table>
<thead>
<tr>
<th>Before operation</th>
<th>After operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>One hundred and twenty-one knees</td>
<td>35.2 ± 10</td>
</tr>
</tbody>
</table>

### Table 7 Knee score RA vs. OA

<table>
<thead>
<tr>
<th>Before operation</th>
<th>After operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA 34.8 ± 11</td>
<td>75.3 ± 10</td>
</tr>
<tr>
<td>OA 37.1 ± 9</td>
<td>80.4 ± 9</td>
</tr>
</tbody>
</table>

*not significant

### Table 8 Knee score (Total cases vs. severe varus deformity)

<table>
<thead>
<tr>
<th>Before operation</th>
<th>After operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greater than 15° varus</td>
<td>54.2 ± 10</td>
</tr>
<tr>
<td>Total cases</td>
<td>55.3 ± 10</td>
</tr>
</tbody>
</table>

*not significant

### Table 9 Knee score (Total cases vs. severe valgus deformity)

<table>
<thead>
<tr>
<th>Before operation</th>
<th>After operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greater than 20° valgus</td>
<td>29.5 ± 6</td>
</tr>
<tr>
<td>Total cases</td>
<td>35.3 ± 10</td>
</tr>
</tbody>
</table>

*not significant

### Table 10 Knee score (Total cases vs. severe flexion deformity)

<table>
<thead>
<tr>
<th>Before operation</th>
<th>After operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>More than 45° flexion</td>
<td>22.6 ± 5</td>
</tr>
<tr>
<td>Total cases</td>
<td>35.3 ± 10</td>
</tr>
</tbody>
</table>

*P<0.05

### Table 11 Knee score (Unilateral vs. bilateral)

<table>
<thead>
<tr>
<th>Before operation</th>
<th>After operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral 37.3 ± 10</td>
<td>80.9 ± 9</td>
</tr>
<tr>
<td>Bilateral 34.9 ± 11</td>
<td>74.9 ± 10</td>
</tr>
</tbody>
</table>

*P<0.01

### Table 12 Knee score (Period of follow-up)

<table>
<thead>
<tr>
<th>Before operation</th>
<th>After operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 2 years</td>
<td>55.3 ± 10</td>
</tr>
<tr>
<td>More than 2 years</td>
<td>55.0 ± 12</td>
</tr>
</tbody>
</table>

*not significant

### Table 13 Complications (One hundred and thirty-two knees)

- Early
  - 1 knee
  - Avulsion of patellar ligament 1 knee
  - Subdislocation of patella 1 knee
- Infection
  - 1 knee
- Late
  - Infection 1 knee
  - Pin hole corrosion of the femoral component 1 knee
  - Tilt of the tibial component 1 knee
and local administration of antibiotics constituted the only treatment. One month later, complete healing was achieved without any restriction of the range of joint motion.

Reoperation was performed in 2 patients. In the first patient, joint hydrops appeared in the second year after surgery. Since the X-ray revealed mild bone absorption on the femoral side, a reoperation was performed. The removed femoral component had a pinhole, from which metal corrosion had taken place. This was probably responsible for the joint hydrops. After replacing the femoral component the postoperative course was uneventful.

As a complication following the total knee replacement, loosening of the tibial component presented a problem. Among our patients, none required reoperation for this reason. In order to avoid complications, including loosening of the prosthesis, the following conditions should be provided: 1. Excellent design of the prosthesis. 2. Proper processing of the metal. 3. Avoiding the use of bone cement in the fixation of the prosthesis. 4. Accurate performance of the operation. 5. Correct guidance of the patient.

As to postoperative infection, 1 patient suffered from infection 6 months after the operation. Healing occurred after systemic administration of antibiotics alone, without leaving any joint dysfunction. This case may be regarded as a lucky one, but the avoidance of bone cement probably contributed a great deal to the successful outcome.

REFERENCES


A Retrospective Study on the Cause of Death, in Japan, of Patients with Gout

Kusuki NISHIOKA and Kiyounobu MIKANAGI*

Rheumatology Division, Department of Medicine, School of Medicine, Mie University, Edobashi, Tsu 514

*Rheumatology Division, Department of Medicine, School of Medicine, Jichi Medical School

To clearly determine whether hyperuricemia participates directly in atherosclerotic disease or not, the cause of death and associated factors were studied, based on data from 104 patients whose serum uric acid had been completely maintained at normal levels with prolonged medication.

104 patients had been treated with either allopurinol (54.3%), or probenecid (35.3%), for a mean 7.8 ± 6.6 years. The mean age at death was 65.8 ± 10.5 years. The causes of death were as follows: cardiovascular disease (26.9%), cerebral disease (26.2%), malignant neoplasms (26.0%), uremia (7.6%) and miscellaneous disease (18.3%). Serum uric acid, urinary uric acid excretion, renal function, serum lipids, blood pressure, body weight, FBS, drugs (allopurinol or probenecid) were compared respectively, in each group. Allopurinol or probenecid had no correlation with the cause of death. Most common complications were in the cardiovascular disease group; hypertension and hyperlipidemia.

These data suggested that the apparent increased incidence of cardiovascular disease in gout rather than renal failure bore a relationship to such complications as hypertension or hypertriglyceridemia. Hyperuricemia alone may not be an atherosclerotic risk factor. There was no correlation between treatment with allopurinol and probenecid and cardiovascular complications.

INTRODUCTION

It has been well known that the incidence of gout has been increasing in Japan. Since the war it has been increasing noted amongst patient of middle age.

Since approximately 1965, patients with gout have become more prevalent and nowadays, it has become one of the most frequent diseases observed in rheumatology or metabolic units.

Keywords: allopurinol, gout, hyperuricemia, probenecid, purine metabolism

1 To whom reprint requests should be addressed.

Epidemiological studies have been carried out since 1970 to clarify the incidence of these patients. Based on these studies, the incidence of hyperuricemia was 0.4% in the normal population and that of hyperuricemia 4-7%.

The clinical and pathological features of these patients have changed in these past ten years. For example, extensive tophaceous gout and nephropathy directly associated with gout has been markedly diminished in recent years.

On the other hand, there has been a suggested close linkage between hyperuricemia and ischemic heart disease.

In this study, to determine whether hyperuricemia is directly associated with atherosclerotic dis-
case or not, the cause of death and associated factors were studied based on patients who died from various diseases from 1965 to 1980.

**MATERIALS AND METHODS**

104 cases of primary gout diagnosed by the ACR criteria or the ARA criteria who died between 1965 and 1980 were analysed. Hyperuricemia in these patients had been successfully controlled either with allopurinol or probenecid.

These 104 patients were divided into the following four groups depending upon the cause of their death: cerebrovascular disease, ischemic heart disease, malignant tumors and others. The following items were compared in each group.

1. The cause and age at death.
2. The age of onset of the initial gout attack.
3. The following complications: hyperlipidemia, hypertension, renal failure, and diabetes. Diagnosis of diabetes was assessed on the basis of an oral, 100 g, glucose tolerance test.
5. Serum uric acid level, urinary uric acid excretion, proteinuria and uric acid to creatinine ratio were analysed before treatment of hyperuricemia was instituted.

**RESULTS**

**The Cause of Death**

28 patients (26.5%) expired from cardiovascular disease, 21 patients (21.2%) from malignant tumors, 22 patients (21.2%) from cerebrovascular disease, 8 patients (7.6%) from uremia and 19 patients (18.3%) died from miscellaneous diseases. The mean age at the time of death was 65.3 years and at the onset was 53.1 years. (Fig 1)

The mean age of those dying within the vascular disease group was 65.2 years and in the others it was 67.6 years.

**Hyperuricemia and Its Treatment**

Before treatment the mean serum uric acid level of patients who died from cerebrovascular disease was 10.9 ± 3.6 mg/dl, those of ischemic heart disease 9.9 ± 1.7 mg/dl and those of malignant tumors 9.8 ± 1.0 mg/dl. Urinary uric acid excretion was 685.3 ± 267.3, 747.2 ± 236.6 and 721.5 ± 289.4 mg/day, respectively. The ratio of urinary uric acid excretion to creatinine excretion was 55.0 ± 24.2%, 54.1 ± 21.0 and 68.0 ± 15.0 respectively. (Table 1)

Hyperuricemia in 56 patients had been treated allopurinol and 38 patients had been treated with probenecid. (Table 2)

In 95.7% of these patients, the hyperuricemia was controlled almost to normal levels during the treatment period. 58.7% of patients who died with ischemic heart disease had been treated with allopurinol and 41.7% with probenecid. In subjects who died with cerebrovascular disease, the method used to treat the hyperuricemia was similar to the previous group. 66.7% of patients who died with malignant tumors had been treated with allopurinol, and probenecid had been used in 33.2% of the patients.

In most patients in these three groups, hyperuricemia had been well controlled, however, in the group with cerebrovascular disease, 15.4% of patients had not been successfully controlled.

**Complications**

Among the 104 subjects, 63.8% were complicated with hypertension, 53.3% with hyperlipidemia, 25.5% with kidney diseases, and 17.0% with diabetes mellitus.

**Table 1** The effects of uric acid levels on prognosis

<table>
<thead>
<tr>
<th>Disease</th>
<th>Mean serum uric acid level before treatment (mg/dl)</th>
<th>Urinary uric acid excretion (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic heart disease</td>
<td>9.9 ± 1.7</td>
<td>747.2 ± 236.6</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>10.9 ± 3.6</td>
<td>685.5 ± 267.3</td>
</tr>
<tr>
<td>Malignant tumors</td>
<td>9.8 ± 1.0</td>
<td>721.5 ± 289.4</td>
</tr>
<tr>
<td>Others</td>
<td>9.4 ± 2.8</td>
<td>800.3 ± 272.3</td>
</tr>
</tbody>
</table>

**Table 2** The effects of drug on prognosis

<table>
<thead>
<tr>
<th>Follow up term (years)</th>
<th>Primary drugs for hyperuricemia</th>
<th>Evaluation</th>
<th>Age at time of death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allopurinol</td>
<td>Probenecid</td>
<td>Good</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>8.0 ± 6.5</td>
<td>58.3%</td>
<td>41.7%</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>7.0 ± 4.3</td>
<td>53.8%</td>
<td>46.2%</td>
</tr>
<tr>
<td>Malignant tumors</td>
<td>7.1 ± 4.5</td>
<td>66.7%</td>
<td>33.3%</td>
</tr>
<tr>
<td>Others</td>
<td>7.8 ± 4.2</td>
<td>80.0%</td>
<td>20.2%</td>
</tr>
</tbody>
</table>

**Table 3** Frequency of complications in each cause of death group

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Hypertension</th>
<th>Diabetes mellitus</th>
<th>Hyperlipidemia</th>
<th>Kidney diseases</th>
<th>No complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrovascular disease</td>
<td>76.9%</td>
<td>38.3%</td>
<td>61.5%</td>
<td>20.0%</td>
<td>19.4%</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>75.0%</td>
<td>8.3%</td>
<td>66.5%</td>
<td>8.3%</td>
<td>16.0%</td>
</tr>
<tr>
<td>Malignant tumors</td>
<td>41.7%</td>
<td>—</td>
<td>40.7%</td>
<td>25.0%</td>
<td>25.0%</td>
</tr>
<tr>
<td>Others</td>
<td>60.5%</td>
<td>20.2%</td>
<td>40.0%</td>
<td>40.0%</td>
<td>20.0%</td>
</tr>
<tr>
<td>Total</td>
<td>63.8%</td>
<td>17.0%</td>
<td>53.5%</td>
<td>25.5%</td>
<td>25.5%</td>
</tr>
</tbody>
</table>

**Fig. 1** Causes of death in 104 patients with gout

**Table 3** Frequency of complications in each cause of death group

In the group of subjects who died from cerebrovascular diseases, the frequency of hypertension was 76.9%, followed by 61.5% with hyperlipidemia, primarily hypertriglyceridemia, 38.3% with diabetes mellitus and 30.0% with kidney diseases. In the malignant tumors group, the frequency of hypertension and hyperlipidemia were 41.7% and 40.7%, respectively. (Table 3)

**DISCUSSION**

The classical prognosis in a patient with gout...
had been thought of as chronic renal failure or uremia caused by gouty nephropathy. However, it has been shown that the most important diseases influencing the prognosis are vascular disorders such as ischemic heart disease or cerebral disease.

On the other hand, recent studies have revealed evidence of a close linkage between purine metabolism disorders and other metabolism disorders such as carbohydrates, lipids and hypertension. To study the prognosis of patients with gout whose hyperuricemia had been controlled with probenecid or allopurinol, we evaluated the associations between multiple factors except hyperuricemia and vascular disease. The studies were done from that point of view. The findings in the main three groups; ischemic heart disease, cerebral vascular disease and malignant tumors might be summarized as follows.

1. Patients who died from cerebral vascular disease had an increased serum uric acid level and decreased urinary uric acid excretion. Complications of hypertension and diabetes mellitus were most common in this group.

2. In the group with ischemic heart disease, hyperlipidemia was the most frequent complication, complications of hypertension and hyperlipidemia were relatively low in patients who died from malignant tumors as compared to the others.

3. In the vascular disease group there were no differences noted between the drugs used to control hyperuricemia, allopurinol and probenecid.

The study shows that patients with gout frequently have vascular disease complications. Atherosclerotic disorders associated with hypertension, hyperlipidemia and diabetes mellitus may play an important role in the pathogenesis of these vascular disorders.

Fessel showed that gouty and hyperuricemic people tend to be overweight and to have an increased risk of hypertension and atherosclerosis. However, in his study series, no uric acid was detected from any specimens obtained from the atherosclerotic aorta at autopsy in seven gouty subjects.

At present as a general concept, hyperuricemia alone may not be associated with the formation of atherosclerosis.

Our data suggest this opinion because hyperuricemia had been maintained at normal levels for an extensive period in our cases. However, our studies confirmed that, in gouty patients, these vascular or atherosclerotic changes could be formed through the mediation of such complications as hypertension, hyperlipidemia and diabetes mellitus.

On the other hand, it is of considerable importance to determine whether the drugs, allopurinol or probenecid are related to these complications. There is no difference between allopurinol and probenecid from the view point of their effect on prognosis. This is the first report which accredits this fact. In conclusion, we can reduce the risk of renal failure in patients with gout by administering allopurinol or uricosuric agents. However, regardless of the success in treating hyperuricemia, the high risk of vascular diseases mediated by hypertension, lipid or carbohydrate metabolic disorders can not at this time be reduced in patients with gout.

REFERENCES

A Longitudinal Population Survey of Rheumatoid Arthritis in a Rural District in Wakayama

Kanji SHICHIKAWA*, Yoshitaka TAKENAKA, Akira MAEDA*, Ryohei YOSHINO*, Masaki TSUJIMOTO**, Hiroshi OTA*, Tozo KASHIWADA*# and Ichiro HONGO*#

Department of Orthopaedic Surgery, Shiga University of Medical Science, Seta, Otsu 520-21
* Department of Orthopaedic Surgery, National Osaka Minami Hospital
** Department of Orthopaedic Surgery, Osaka Rosai Hospital
# Department of Orthopaedic Surgery, Osaka National Railway Hospital
# Department of Orthopaedic Surgery, National Toneyama Hospital
# Department of Orthopaedic Surgery, Sakai Municipal Hospital

A longitudinal population survey of rheumatoid arthritis was made in Kamitonda districts, a rural area in Wakayama prefecture, located 120km south of Osaka. From 1965 to 1980 five surveys were performed at intervals of three or five years. Three methods were used concomitantly or selectively. 1. The use of questionnaires concerning the rheumatic complaints 2. Household interview surveys 3. Medical examinations of inhabitants aged thirty years and over. The district is composed of about 3000 persons, seventy percent of whom engage in agriculture. The mobility of population in the districts has been relatively low throughout the period of the survey. The result of the fifth survey, in comparison with those of the preceding four surveys is presented here.

The prevalence of rheumatoid arthritis ranged from 0.3 to 0.5 per cent (0.4 per cent on average) and the estimated annual incidence from 0.02 to 0.04 per cent (0.03 per cent on average), This slight variation in the prevalence of rheumatoid arthritis, confirmed by a longitudinal population survey in Kamitonda districts, suggests that a predisposing factor dominates the production of this disease.

It was also clearly demonstrated that the prevalence of rheumatoid arthritis, in Japanese, is definitely low as compared to populations of other countries, especially Europe and the United States. Etiological implications, in this respect, are discussed.

Regarding the rheumatoid factor, it appears that, contrary to general view, it is as reliable an indicator of involvement of rheumatoid arthritis in population surveys as in clinical practice. Seronegativity is considered a sign of good prognosis in polyarthritis involvement and also of ambiguity in diagnosis.

A population survey of rheumatic disease has been carried out in Kamitonda, a rural area in Wakayama prefecture, since 1965. In 1980, the fifth survey was performed.

The aim of these population surveys mostly concerns the prevalence of rheumatoid arthritis in Japan, which appears considerably low, as compared with that of the United States and European countries. It is also concerned about possible variations in the prevalence as well as the annual

Keywords: epidemiology, incidence of rheumatoid arthritis, prevalence of rheumatoid arthritis, rheumatoid arthritis, rural district

* To whom reprint requests should be addressed.
incidence which may occur in long term observations, related to some causative factors of the disease. The results of the fifth survey performed in this scope, compared with those of the preceding four surveys will be presented.

SAMPLE AND METHODS

Surveyed Area

Kamitonda in Wakayama is located 150km south of Osaka (Fig. 1). The surveyed areas are the Oka and Iwata districts of Kamitonda, which include about 3000 inhabitants. These districts belong to a rural area, which includes small residential sections. Seventy per cent of the population are engaged in agriculture. Recently, several small factories with residences for worker's families have been established and the population is always increasing, but the mobility of the population in the districts has been relatively low throughout the period of the surveys. The distribution by sex and age of the population has the same general trend as seen in rural and residential areas of Japan (Fig. 2).

Survey Method

For the preceding four surveys the following three methods were used concomitantly or selectively.
1. Questionnaires concerning the constitution of the family members and their professions and rheumatic complaints were distributed through the town office to all households in the districts.
2. Inhabitants aged thirty years and over were called to attend the center of examination established in the prefectural hospital for disabled in the districts. The histories and the results of the physical examination, which was made with accent on the joints and spine were recorded on special recording cards, coded for computer use. In addition, an inquiry was made on absence from work, medical care expenses from the last year and annual income which is useful for evaluating social class. Blood was taken for uric acid determination and serologic tests. Both hands and cervical spine were X-rayed during two surveys, the former in anteroposterior view and the latter in lateral view. Both hips, including the pelvis and lower lumbar spine were X-rayed during one survey for males aged thirty years and over and females aged fifty years and over.
3. All households in the districts were visited by a team, composed of a doctor, who is competent in the field of rheumatology, a public health nurse and an officer in charge of public health. If there was a family member who had rheumatic complaints and he or she did not attend the center for examination, he or she was examined in the household and if necessary blood was taken and the joints X-rayed.

During the first and third surveys all three methods were used and at the second and fourth surveys, method 3 was omitted.
For diagnosis, the ARA criteria (Rome criteria) and New York criteria were used.
Response rates of the household interview survey and that of the examination at the center were 85–90 per cent and 65–75 per cent respectively.
In 1980 (the fifth survey) only questionnaires were used to find persons with rheumatoid arthritis. In addition, national health insurance bills submitted by family doctors in the districts were used to find patients that possibly may not have responded. Moreover some information was obtained from the check list on rheumatoid arthritis patients prepared by a public health nurse in Kamitonda who has been a collaborator in this population survey for more than ten years and makes regularly community surveys on other diseases asked for by the Ministry of Public Welfare.
The answers to questionnaires were classified in four groups. People belonging to group A are those who should be examined, by all means. They were called to attend the center for examinations established in the districts and if it was impossible to come the center, they were examined at home by visiting doctors. People in group B are those who were called to attend the center, but if they did not respond, examination at home was omitted. People in group C were called to attend at the center, if possible. People in group D are those who have no rheumatic complaints.

RESULTS

Answers to questionnaires were obtained in 2276 of 3021 persons to be surveyed, that is in 75 per cent. The number of persons classified into the four classes were 118 in A class, 38 in B, 114 in C and 2006 in D.
According to our classification, seven of nine previously detected patients with RA, belonged to A class, and one to C. The remaining person had died. One patient classified in C had no activity or joint inflammation and was diagnosed as osteoarthritis. Four newly detected patients belonged to A class.
Judging from this result, we had the impression that the survey method with questionnaires and examinations of selected cases was efficient for a population survey of rheumatoid arthritis, providing that the survey was made not only once, but had been previously made, repeatedly in the same population.
The survey, as mentioned above, found eight definite and four probable rheumatoid arthritis patients, according to the ARA criteria (Table 1, 2). Eight patients had already been detected by previous surveys, five of whom are classical or definite and three probable rheumatoid arthritis patients. Since the last survey in 1975, at intervals of five years, one patient died and four patients were newly detected, of whom three were classical or definite and one probable (Table 3). One patient moved from the district and two moved into the district. In one patient the diagnosis was changed from probable RA to osteoarthritis. The prevalence of definite plus probable RA per people examined is 0.5 per cent. If the people who were not surveyed are assumed to be without rheumatoid arthritis, the prevalence of definite plus probable RA is 0.4 per cent and that of only
Table 1: Prevalence of RA in Kamitondai population in five surveys (1965–1980)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>2,594</td>
<td>2,754</td>
<td>2,998</td>
<td>3,045</td>
<td>3,021</td>
</tr>
<tr>
<td>Number of people examined</td>
<td>m</td>
<td>f</td>
<td>m</td>
<td>f</td>
<td>m</td>
</tr>
<tr>
<td>1,186</td>
<td>1,139</td>
<td>1,113</td>
<td>1,118</td>
<td>1,107</td>
<td>1,104</td>
</tr>
<tr>
<td>994</td>
<td>1,139</td>
<td>1,113</td>
<td>1,118</td>
<td>1,107</td>
<td>1,104</td>
</tr>
<tr>
<td>2,345</td>
<td>2,135</td>
<td>2,264</td>
<td>2,302</td>
<td>2,276</td>
<td>2,267</td>
</tr>
<tr>
<td>Number of people with RA</td>
<td>m</td>
<td>f</td>
<td>m</td>
<td>f</td>
<td>m</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>11</td>
<td>9</td>
<td>10</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Prevalence of RA (%)</td>
<td>(m + f) *</td>
<td>0.3</td>
<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Prevalence of def. RA (%) **</td>
<td>0.19</td>
<td>0.36</td>
<td>0.24</td>
<td>0.23</td>
<td>0.26</td>
</tr>
</tbody>
</table>

* Prevalence per person examined
** Prevalence per total population

definite 0.3 per cent. The authors are of the opinion that this assumption is reasonable, because the surveys were repeated several times on the same relatively small population, they believe that they have not neglected to include any person who suffers from inflammatory polyarthritis.

Table 1 shows the data obtained from the five surveys made between 1965 to 1980. It is remarkable that the prevalence of rheumatoid arthritis, probable cases being included or not, is of minor variability and always low, as compared to those of the European and American population, which were found in numerous publications. Table 2 shows the age and sex specific distribution of rheumatoid arthritis patients. Rheumatoid arthritis patients were seen from 15 years of age and distributed, almost within a range, from 45 to 74 years of age.

The Table 3 shows the estimated incidence of rheumatoid arthritis in the districts which I obtained from a calculation as follows: the number of newly detected patients was divided by the number of years of interval, and the result was again divided by the number of population at the time of the survey. This estimated annual incidence is, if probable RA is included, within a range of 0.03 to 0.05 per cent of persons examined (0.04 per cent on average). If people who were not examined are assumed to be without

Table 2: Age and sex specific distribution of patients with def. + prob. RA in Kamitondai, Wakayama (1965–1980)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4 M</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-14 M</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-24 M</td>
<td>F</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-34 M</td>
<td>F</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35-44 M</td>
<td>F</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-54 M</td>
<td>F</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>55-64 M</td>
<td>F</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>65-74 M</td>
<td>F</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>75 M</td>
<td>F</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

Number without circle: definite rheumatoid arthritis
Number with circle: probable rheumatoid arthritis

Table 3: Estimated annual incidence of def. + prob. RA in Kamitondai population

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>2,594</td>
<td>2,754</td>
<td>2,998</td>
<td>3,045</td>
<td>3,021</td>
</tr>
<tr>
<td>Number of persons with RA</td>
<td>7</td>
<td>11</td>
<td>9</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Prevalence of RA (%)</td>
<td>0.3</td>
<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Newly detected cases</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>4**</td>
<td></td>
</tr>
<tr>
<td>Death cases</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Excluded cases</td>
<td>1**</td>
<td>1**</td>
<td>1**</td>
<td>1***</td>
<td>1***</td>
</tr>
<tr>
<td>Estimated annual incidence of RA (%)</td>
<td>0.04</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* Two patients moved into the district.
** Because of change of diagnosis.
*** One patient moved from the district.

Table 4: Age and sex specific distribution of patients with rheumatoid arthritis newly detected by four surveys made at intervals of three to five years in Kamitondai, Wakayama (1965–1980)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4 M</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-14 M</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-24 M</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-34 M</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35-44 M</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-54 M</td>
<td>F</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>55-64 M</td>
<td>F</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>65-74 M</td>
<td>F</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>75 M</td>
<td>F</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

Number without circle: definite rheumatoid arthritis
Number with circle: probable rheumatoid arthritis

Rheumatoid arthritis, as mentioned above, falls to a range of 0.02-0.04 and an average 0.03. This also gives the impression that rheumatoid arthritis occurred almost regularly in this small population.

Table 4 shows the age and sex specific distribution of rheumatoid arthritis patients newly detected at the time of the surveys made at intervals of three to five years in Kamitondai districts. The ages of onset are 45 years and over. It is interesting that the age of onset of RA in the population survey is higher than that found in the clinics.

**Newly Detected Cases**

Four patients were newly detected. Patient Y.T., a 58 year old woman, a master of Ikebana, had been diagnosed as normal eight years ago. Since 1979 she has been suffering from polyarthritis and was diagnosed as definite rheumatoid arthritis at the 1980 survey X-ray films of the joints showed erosive arthritis of grade 3. Her serological test had been positive in 1972 and was also positive in 1980.

Patient S.I. is a 56 year old farmer. He complained only of low back pain in 1972 and was seronegative. In 1979 he had an episode of arthralgia. At the 1980 survey he was diagnosed as definite rheumatoid arthritis with seropositivity.

**Two Patients Moved into the District**

Patient M.N., a 70 year old woman, had been suffered from rheumatoid arthritis for 13 years. It was definite seropositive rheumatoid arthritis. Patient S.N. is a nurse of 45 years of age who has had arthralgia in her fingers and toes for one year. At the 1980 survey, she had a slight symmetrical swelling in her toes. She was diagnosed as probable RA with seronegativity.

These results appear to prove that seropositivity
is intimately related to the severity and prognosis of the disease.

**Probable RA Cases**

In the course of six surveys, nine persons were diagnosed one or more times or always to have probable RA. Among them, two patients (S. Tan—; K. Yas—) developed seropositive definite RA. One patient (T. Oga—) has always suffered from probable RA with seronegativity, throughout the surveys. Two patients (M. Mit—; S. Nag—) were diagnosed as probable RA only during the last one or two surveys. One probable RA patient (N. Yam—) with seropositivity at the 1980 survey had definite RA at the 1972 and 1975 surveys. This change of diagnosis is due to a decrease in his rheumatoid activity. One patient (Y. Shi—) diagnosed as probable RA with seropositivity in 1972 and 1975 was considered in 1980 to be with enthesopathy. In the remaining two cases, the diagnosis was changed twice due to the uncertainty of the diagnostic criteria. In one, (T. Ito—) the diagnosis of probable RA was doubtful and polyarthritis was strongly suggested at the 1965 survey. At the next survey, in 1969, the patient was diagnosed as definite RA, but with seronegativity. Through the following surveys the patient has always been diagnosed as polyarthritis with seronegativity. In another (N. Yam—), the diagnosis was benign polyarthritis in 1965, then changed to flexion contracture of the PIP joint of the right little finger. At the 1969 survey, the diagnosis was changed again into definite RA with seronegativity. In 1972 the diagnosis was probable RA and in 1980, again, flexion contracture of the little finger. The serology was always negative. Table 5 shows a list of these patients.

In these probable cases, also, seropositivity seems to have a prognostic value. Seronegative probable RA was always benign and the diagnosis was uncertain due to the lack of criteria. In one case, however, the diagnosis was probable RA in 1972 and 1975 and always seropositive, but in 1980 it was changed to enthesopathy, because in the course of eight years, the main clinical symptoms were invariably polyarthritis.

**DISCUSSION**

The prevalence rate of rheumatoid arthritis in Kumamoto districts obtained by a longitudinal population survey showed minimal variability. During the 15 years of the survey it was almost consistently 0.4 per cent of those people examined and 0.3 per cent of the total population, which is very low as compared to those of other countries.

Very recently, in the ninth scientific meeting of the International Epidemiological Association, in 1981, it was reported to be 3 per cent in England and 1.5 per cent in Poland. On the other hand, previously reported prevalence rates of rheumatoid arthritis, in Japan, are regardless of attitude, uniformly low centering around 0.3 per cent of the total population as seen in Table 6. One of the present authors (Shichikawa) had already published results of population surveys of rheumatic diseases made in eight areas of Kinki region (in the Central part of Japan), including 10,272 inhabitants, of whom 7,364 was surveyed, of all ages and 2,908 in the 45–64 age group.

In the survey of the population of all ages, the prevalence of rheumatoid arthritis was invariably 0.3 per cent. In the population of the 45–64 age group the prevalence of rheumatoid arthritis was 0.6 per cent, ranging between 0 and 1 per cent. From these results it seems justified to say that the previously observed prevalence rate of rheumatoid arthritis was definitely corroborated by our longitudinal population survey in Kumamoto districts.

The estimated annual incidence also showed minimal variability, showing 0.03 per cent, on the average.

What does this uniform occurrence of rheumatoid arthritis observed over a long term mean? Also, how is the relatively low prevalence in the Japanese population explained? We think that there must be, very probably, a predisposing factor or factors in the occurrence of rheumatoid arthritis. It is suggested that HLA DR4 is one of these factors. It does not seem, however, as Table 7 shows, that an analogy of spondylitis, the difference of frequency of HLA DR4 in differing populations can explain the difference of prevalence of the disease in differing populations; because positivity of HLA DR4 in the population is rather higher in Japanese than in Europeans or Americans.

Some recent studies have shown that HLA DR4 concerns the seropositivity and progression of disease, although there are some reports which do not confirm these facts. On the other hand, it has been mentioned that HLA DR2 is related to good prognosis and a better response to disease modifying drugs.

---

**Table 5 Clinical courses in nine cases with probable RA detected in Kumamoto population (1965–1980)**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Tan— (F)</td>
<td>past arthritis of elbow, seronegative</td>
<td>prob RA seropositive</td>
<td>def RA seronegative</td>
<td>prob RA seropositive (55y.o.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K. Yas— (M)</td>
<td>prob RA seronegative</td>
<td>class RA seronegative</td>
<td>class RA seropositive</td>
<td>class RA seropositive (54y.o.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. Oga— (F)</td>
<td>prob RA seronegative</td>
<td>prob RA seronegative</td>
<td>prob RA seronegative</td>
<td>prob RA seronegative (74y.o.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. Mit— (M)</td>
<td>polyarthritis</td>
<td>prob RA</td>
<td>(moved in 1977)</td>
<td>prob RA seronegative (51y.o.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. Nag— (F)</td>
<td>limitation of extension of the elbow joint</td>
<td>low back pain, cervical spondylosis, seronegative</td>
<td>def RA seronegative</td>
<td>def RA seronegative (67y.o.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y. Shi— (M)</td>
<td>prob RA seropositive</td>
<td>polyarthritis</td>
<td>def RA seronegative</td>
<td>enthesopathy seronegative (50y.o.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. Ito— (F)</td>
<td>prob RA polyarthritis (62y.o.)</td>
<td>def RA seronegative</td>
<td>polyarthritis</td>
<td>polyarthritis seronegative (77y.o.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. Yam— (F)</td>
<td>benign polyarthritis (55y.o.)</td>
<td>flexion contracture of the left little finger, seronegative</td>
<td>def RA seronegative</td>
<td>prob RA seropositive</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Table 6 Prevalence of rheumatoid arthritis in Japan**

<table>
<thead>
<tr>
<th>Population</th>
<th>Authors</th>
<th>Age</th>
<th>Prevalence (%)</th>
<th>Females: Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hokkaido</td>
<td>Moriwaka (1967)</td>
<td>20*</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Tohoku</td>
<td>Kosaka et al. (1967)</td>
<td>all</td>
<td>0.3</td>
<td>3.9</td>
</tr>
<tr>
<td>Kanto</td>
<td>Osima et al. (1960)</td>
<td>all</td>
<td>0.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Shizuoka</td>
<td>Osima et al. (1960)</td>
<td>6*</td>
<td>0.9</td>
<td>3.6</td>
</tr>
<tr>
<td>Meie</td>
<td>Sonozaki (1968)</td>
<td>all</td>
<td>0.3</td>
<td>3.6</td>
</tr>
<tr>
<td>Chihokawa</td>
<td>Shichikawa (1967)</td>
<td>all</td>
<td>0.3</td>
<td>10.0</td>
</tr>
<tr>
<td>Shonan</td>
<td>Morita (1967)</td>
<td>30*</td>
<td>1.1</td>
<td>17.0</td>
</tr>
<tr>
<td>Shonan</td>
<td>Kodama (1967)</td>
<td>all</td>
<td>0.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Kyushu</td>
<td>Yano (1967)</td>
<td>all</td>
<td>0.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Hiroshima</td>
<td>Wood et al. (1967)</td>
<td>15*</td>
<td>0.35</td>
<td>4.4</td>
</tr>
</tbody>
</table>
Table 7 Prevalence of HLA antigens in rheumatoid arthritis

<table>
<thead>
<tr>
<th>Antigen</th>
<th>RA No. exam</th>
<th>%pos</th>
<th>Control No. exam</th>
<th>%pos</th>
<th>F²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>St Denis (1980) U.S.A.</td>
<td>DW4 45</td>
<td>54</td>
<td>11</td>
<td>16</td>
<td>22.92</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>DR4 54</td>
<td>70</td>
<td>68</td>
<td>28</td>
<td>21.77</td>
<td>&lt;0.00001</td>
<td></td>
</tr>
<tr>
<td>Batchelor et al. (1979) U.K.</td>
<td>DRW4 55</td>
<td>55.8</td>
<td>67</td>
<td>33.5</td>
<td>12.35</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Gabon (1981) France</td>
<td>DRW4 58</td>
<td>50.00</td>
<td>116</td>
<td>12.93</td>
<td>8.123</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Maeda et al. (1981) Japan</td>
<td>DRW4 88</td>
<td>70.5</td>
<td>104</td>
<td>46.1</td>
<td>4.84</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

thesis has been revived that seropositive polyarthritis is quite different in its etiopathology than seronegative polyarthritis. In the present survey, a follow up of seronegative probable RA regularly showed a good prognosis and also diagnostic ambiguity between inflammatory and degenerative disease. It appears, that contrary to the general view, the rheumatoid factor is as reliable an indicator of the involvement of rheumatoid arthritis in population surveys as in clinical practice.

It has long been discussed that diagnostic fluctuations among authors owing to a lack of precise diagnostic criteria, strongly influenced the prevalence of rheumatoid arthritis. In this population survey, probable rheumatoid arthritis cases are considerably fewer, as compared to definite cases, while in European and American surveys, probable rheumatoid arthritis cases are quite numerous. The foreign authors claim that the reduced number of probable cases are proof that Japanese investigators have not faithfully followed the diagnostic criteria in population surveys. In the authors opinion, however, it seems that foreign investigators did sufficiently not exclude polyarthrosis cases, which are not described in terms of exclusion in the ARA diagnostic criteria. This view is supported by the fact that in the course of our population surveys diagnoses of polyarthrosis and rheumatoid arthritis were occasionally interchanged. In Europe and the United States are polyarthrosis cases, apparently indistinguishable from rheumatoid arthritis, more numerous? It is not conceivable, however, because the frequency of osteoarthritic changes in the X-ray films of the fingers and hands and cervical spine of Japanese people are quite similar to those of English people, according to the epidemiological study of the population in Kanisawa. If some seronegative polyarthrosis cases are to be included with the rheumatoid arthritis, this surely represents another problem concerning the classification of rheumatoid arthritis.

It is also possible that the difference in foods eaten by differing populations influences the prevalence of rheumatoid arthritis, as in case of coronary heart disease. The prevalence of coronary heart disease is consistently very low in the Japanese population, as compared to European and American populations and with less lipid content food taken by the latter populations in recent years the death rate due to coronary heart disease has clearly decreased. If foods are related to the causes or morbidity of rheumatoid arthritis, the prevalence of this disease will not decrease, but rather it will increase in Japan, in the future, because the Japanese have a tendency to eat more European meals than before. In this respect, further epidemiological studies along this line will be requested.

REFERENCES

Ankylosing Spondylitis in Japan

Hiroshi MITSUI and Hidekichi SONOZAKI
Department of Orthopedic Surgery, Mitsubishi Memorial Hospital, Kanda, Chiyoda-ku, Tokyo 101

One hundred Japanese patients with ankylosing spondylitis were studied. The prevalence of ankylosing spondylitis among Japanese was expected to be approximately 0.02% to 0.04%, The disease is more frequent in men than in women, in an almost 16:1 ratio: actually 15.7 (M:2[F]. As compared to Caucasians, the disease is less common in Japanese.

Genetic factors are important in the etiology of this disease. Familial aggregations were confirmed, not only in Caucasians, but also in Japanese. HLA-B27 antigen was frequently found (and significantly one might add) in Japanese patients as well as in Caucasians. HLA-B27 antigen was thought to be the single most important genetic factor after race.

Detailed clinical features, complications and treatment are not discussed in this paper. However, differential diagnosis is thought to be of considerable significance as diagnostic difficulties still exist in the criteria used.

Ankylosing spondylitis (AS) has a long history. The first description of the disease was probably made in 1695 by B. Connor. Among the end of the 19th century many descriptions were made—such as, Wilks (1858), von Thaden (1863), Bressinger (1864) and Strümpell (1883). In 1899 Vladimir von Bechterew named it “ankylosing inflammation of the spine and large limb joints.” The term “ankylosing spondylitis” is thought to have originated from his description. Strümpell and Marie reported several cases of ankylosing spondylitis in 1897 and 1898, respectively. Ankylosing spondylitis is still referred to as “Bechterew’s syndrome” or “Marie-Strümpell disease.” Since the Second World War, many clinical, pathological, immunological and genetic studies have been conducted. Among these studies, particularly those in immunology and genetics, ankylosing spondylitis has been firmly established as a different disease than rheumatoid arthritis. In 1973 Brewerton and Schlostein discovered the strong association between ankylosing spondylitis and HLA-B27. Since this discovery, numerous intensive studies of this association have been done, in many races. Most of these have been done by Europeans and Americans. It was found that the prevalence of ankylosing spondylitis varies considerably according to race. Ankylosing spondylitis is thought to be more frequent in Caucasians than in Asian people. According to recent studies of HLA antigens, HLA-B27 is also more frequently found in Caucasians. Consequently, it is likely that the discrepancy in incidence of HLA-B27 indicates that the incidence of this disease varies with race. However, there have only been a few studies on the incidence of ankylosing spondylitis or HLA antigens in Asian people, including Japanese. We examined one hundred Japanese patients with ankylosing spondylitis and at the same time noted the frequency of their HLA antigens.

Keywords: ankylosing spondylitis, epidemic study, rheumatic disease, spondylitis
† To whom reprint requests should be addressed.

PREVALENCE

The prevalence of ankylosing spondylitis varies considerably according to sex, age and race. The
most satisfactory study regarding the prevalence of ankylosing spondylitis in Caucasians was performed in 1949 by West6 in Bristol, England, who found that the incidence of the disease was 0.05% within a population of 420,000. Kellgren (1964),4 reviewing epidemiological studies prior to 1963, suggested a mean incidence of 0.1%, among Caucasians. However, to date, almost no epidemiological studies on the prevalence of ankylosing spondylitis have been performed in Japan. We have, however, from 1970 to 1977, kept track of the incidence of ankylosing spondylitis in patients who have visited our clinic in the department of orthopedic surgery, Tokyo University Hospital.10 Out of the 54,000 individuals who visited the clinic during the above mentioned time, we found that the mean incidence of the disease was approximately 0.02%. This frequency indicates that ankylosing spondylitis is much less frequent in Japanese than in Caucasians. However, another study on the prevalence of this disease failed to support the rarity of the disease in Japanese; Shichikawa,11) Tsuchimoto et al, in 1973, examined 7364 normal people in Osaka and Wakayama, Japan, and the mean incidence of ankylotic spondylitis was reported as 0.04%. This figure seems to be closer to that of Caucasians, however, the number of people examined was inadequate for a complete incidence study. Consequently, the prevalence of ankylosing spondylitis in Japanese should be considered to be 0.02% to 0.04%; a little less than half the incidence found in Caucasians.

GENETICS AND TISSUE TYPING

Familial aggregations in ankylotic spondylitis have been studied by West, Stecher and Hersht, De Blecourt and Lawerence.12) According to their results, taking an approximate prevalence for ankylotic spondylitis of 0.1% for controls and 4.0% for relatives, the degree of familial aggregation is 40%. In Japan, Tsuchimoto, Shichikawa and Shirakura13) noted the incidence of patients with ankylotic spondylitis in 311 relatives. The prevalence in relatives was 2.3%, while the degree of familial aggregation was 115%, making the prevalence for ankylotic spondylitis in Japan 0.02%. The results of our family studies indicate a prevalence of 3%, in a total of 400 relatives with a 150 degree of aggregation. This strong tendency of familial aggregation indicates that genetic factors are of considerable importance in the etiology of the disease. HLA-B27 antigen is thought to be the most important genetic factor. In most reports dealing with Caucasian populations, the HLA-B27 antigen has been present in 85-95% of patients with ankylotic spondylitis. Our examinations of HLA antigens found that HLA-B27 was present in 76.3% of our patients with ankylotic spondylitis. While the frequency is a little lower than that found in Caucasian patients, the frequency of HLA-B27 in the Japanese population is also much lower than in the Caucasian population. The frequency of HLA-B27 in the Japanese population is almost 0%, while it is reported to be about 8% in Caucasians. This low incidence of HLA-B27 in the normal Japanese population is of great interest. The discrepancy in HLA-B27 frequency between Japanese and Caucasians may explain the reason why the frequency of HLA-B27 in Japanese patients with ankylotic spondylitis is much lower, and furthermore, why ankylotic spondylitis is rare in Japanese (Table 1).

CLINICAL FEATURES

The clinical features of ankylotic spondylitis are documented in detail in most modern textbooks of rheumatology published in Europe and the U.S.A. Almost all the clinical features of our Japanese patients with ankylotic spondylitis are thought to approximate those descriptions, however, there has been a dearth of literature on this disease in Japan, as compared to many original studies in Europe and the U.S.A. The clinical features of 100 Japanese patients with ankylotic spondylitis are shown in the tables. However, details of the clinical features of these 100 ankylotic spondylitis patients are not documented here. The primary purpose of this report is to contrast and compare the disease in Japanese patients with that in Caucasian patients, not to describe its clinical features. The diagnosis of ankylotic spondylitis diagnosis was made using the New York clinical criteria14) (Bennet and Wood, 1968). All 100 individuals were definite ankylotic spondylitis patients by these criteria—probable patients with ankylotic spondylitis were excluded from this study. The sex incidence, with a predilection for men, was 15:7 (Table 2). Age, at onset, was from 10 to 49 years of age, with none below 9 years nor above 50. (Table 3). The fourteen patients with onset before 14 years of age should be grouped as juvenile ankylotic spondylitis. Their clinical features were atypical of ankylotic spondylitis, and much more similar to those of peripheral arthropathy, such as juvenile rheumatoid arthritis. The chief complaints of the 100 patients were almost identical to the descriptions seen in most text-books. (Table 4). Chest and spinal rigidity were measured (Table 4) by the tape measure method or by Schober’s method. (Table 5). Cervical mobility was relatively normal, particularly, in the early phase of the disease. Radiological features of our patients were also typical for ankylotic spondylitis; with bilateral sacro-ilium observed in all 100 patients. Several cases of ankylotic spondylitis with normal sacro-iliac joints were excluded from our series. Laboratory findings indicated non-specific in-

---

**Table 1** HLA antigen incidence in patients with AS and controls

<table>
<thead>
<tr>
<th>A-locus</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=80</td>
<td></td>
<td></td>
<td>N=181</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2.5</td>
<td>3</td>
<td>1.7</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>37.5</td>
<td>61</td>
<td>33.7</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1.3</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>9</td>
<td>42</td>
<td>52.5</td>
<td>71</td>
<td>42.5</td>
</tr>
<tr>
<td>10</td>
<td>11</td>
<td>15.8</td>
<td>31</td>
<td>17.1</td>
</tr>
<tr>
<td>11</td>
<td>28</td>
<td>35.0</td>
<td>22</td>
<td>12.2</td>
</tr>
<tr>
<td>22</td>
<td>2</td>
<td>2.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ref</td>
<td>2</td>
<td>2.4</td>
<td>2</td>
<td>1.1</td>
</tr>
<tr>
<td>W33</td>
<td>1</td>
<td>1.3**</td>
<td>24**</td>
<td>14.8</td>
</tr>
</tbody>
</table>

**Table 2** Patients with AS

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Male</th>
<th>Female</th>
<th>Male-female ratio</th>
<th>Age (yrs.)</th>
<th>Mean age (yrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>98</td>
<td>6</td>
<td>15.7 : 1</td>
<td>14-72</td>
<td>34.2</td>
</tr>
</tbody>
</table>

**Table 3** Age at onset

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9 yrs.</td>
<td>0</td>
</tr>
<tr>
<td>10-19</td>
<td>14</td>
</tr>
<tr>
<td>20-29</td>
<td>21</td>
</tr>
<tr>
<td>30-39</td>
<td>26</td>
</tr>
<tr>
<td>40-49</td>
<td>2</td>
</tr>
<tr>
<td>50-59</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 4** Chief complaints in patients with AS

<table>
<thead>
<tr>
<th>Chief Complaint</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Back—Back Pain</td>
<td>56</td>
</tr>
<tr>
<td>Gonalgia</td>
<td>20</td>
</tr>
<tr>
<td>Neck—Shoulder girdle pain</td>
<td>16</td>
</tr>
<tr>
<td>Gonalgia</td>
<td>14</td>
</tr>
<tr>
<td>Stiffness of the spine</td>
<td>15</td>
</tr>
<tr>
<td>Periarthritis</td>
<td>4</td>
</tr>
<tr>
<td>Sciatic pain</td>
<td>3</td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
</tr>
</tbody>
</table>

**Table 5** Mobility of the spine and the chest

<table>
<thead>
<tr>
<th>Dorsal and lumbar spine</th>
<th>Complete restriction</th>
<th>Moderate restriction</th>
<th>Mild or none</th>
</tr>
</thead>
<tbody>
<tr>
<td>81%</td>
<td>15%</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Cervical spine</td>
<td>Complete restriction</td>
<td>Moderate restriction</td>
<td>Mild or none</td>
</tr>
<tr>
<td>20%</td>
<td>10%</td>
<td>54%</td>
<td></td>
</tr>
<tr>
<td>Chest expansion</td>
<td>Remarkable restriction*</td>
<td>Moderate restriction</td>
<td>Normal</td>
</tr>
<tr>
<td>54%</td>
<td>24%</td>
<td>22%</td>
<td></td>
</tr>
</tbody>
</table>
Table 6 CRP in patients with AS

<table>
<thead>
<tr>
<th>CRP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1+~2+</td>
<td>45%</td>
</tr>
<tr>
<td>3+</td>
<td>38%</td>
</tr>
</tbody>
</table>

Inflammatory patterns. The erythrocyte sedimentation rate was definitely high in approximately 56% of the patients. C-reactive protein was positive in 83% of the patients, while 38% of those were strongly positive. (Table 6). Rheumatoid factor was not detected in any of the patients.

COMPLICATIONS

Iritis is said to occur in approximately 20% of the patients with ankylosing spondylitis, while the incidence of iritis was 19% in our 100 patients—100% had unilateral iritis. Aortic incompetence and cauda equina syndrome are rare complications of ankylosing spondylitis. We have not encountered any patients with these complications. Atlanto-axial subluxation was encountered in 15% of the 90 patients however, neurological disturbances (13) were not noted in any of them.

DIFFERENTIAL DIAGNOSIS

The diagnosis is usually not difficult when the disease has progressed to the degree where there are characteristic physical and radiological findings. In the early phases of the disease, however, there are various difficulties in the diagnosis. The clinical and radiological features of early ankylosing spondylitis may be indistinguishable from those of other seronegative disorders such as juvenile rheumatoid arthritis, psoriatic arthritis, Reiter’s disease and the intestinal arthropathies. Another important disease in the differential diagnosis of ankylosing spondylitis, is ankylosing spinal hyperostosis. Reference has already been made to descriptions differentiating these two disorders (Forester and Lagier, 1971). According to this reference, sacro-iliac joints and apophyseal joints are never affected in ankylosing spinal hyperostosis. However, from our experience there have been a few cases of hyperostosis which revealed partial or complete ankylosis of the sacro-iliac joints or apophyseal joints. When differential diagnosis is difficult, both radiological findings and clinical features are useful and important in helping to distinguish between the two conditions.

MANAGEMENT AND TREATMENT

The management and treatment of ankylosing spondylitis consists of physical measures and appliances, medication, irradiation and surgery. We have never used radiation, and have only rarely performed surgery. All of our surgical procedures were total hip replacements. However, physical appliances and medication are our principal treatment for this disorder. All patients should be encouraged to exercise in order to prevent postural abnormalities and joint contractions while receiving proper medication to reduce pain and inflammation.

Indomethacin is most commonly used to accomplish this. Phenybutazone has the remarkable effect of helping to relieve the pain of ankylosing spondylitis, however, unfortunately, this drug has many toxic side effects. Therefore, phenybutazone is carefully used when pain and inflammation have not been controlled by other anti-rheumatic agents, such as indomethacin.

REFERENCES

1) Connor B : An extract of letter from B Connor to Sir Charles Walgrave, The bones of a skeleton united without joints or cartilage, Phil Trans 19 : 21, 1695
4) Strumpel A : Bemerkungen uber die chronische ankylosierende Entziendung der Wirbelsaule und Hufgelenke, Dusch Z. Neuronesh 11 : 338-342, 1897
5) Marie P : Sur la spondylose rhumatoide, Rev Med 18 : 293-345, 1898
8) West HF : The aetiology of ankylosing spondylitis, Ann rheum Dis 8 : 143-152, 1949
12) Wright V : Ankylosing spondylitis, Seronegative polyarthritis (Wright V, Mall JMH ed), Amsterdam, North-Holland publishing Company, 1976
Immunological Studies on the Nature of Anemia in Rheumatoid Arthritis and Leukopenia in Systemic Lupus Erythematosus

Masakuni SUGIMOTO, Yoshihisa WAKABAYASHI, Yuichi SHIOKAWA and Fumimaro TAKAKU*

Department of Internal Medicine, Juntendo University, Hongo, Bunkyo-ku Tokyo 113
* Department of Medicine, Jichi Medical School

The pathogenesis of the anemia associated with rheumatoid arthritis (RA) and the leukopenia accompanying systemic lupus erythematosus (SLE) remains obscure. Considering the possibility that immunological mechanisms are involved in these changes, the effects of lymphocytes on the heme synthesis in human bone-marrow erythroblasts and on the colony forming units in cultures (CFU-C) were compared, with the following results:

1) Heme synthesis in the erythroblasts in the bone-marrow of RA patients is lower than that of normal healthy subjects.

2) Serum and articular fluid exerted practically no effect on the heme synthesis of erythroblasts in healthy individuals.

3) Lymphocytes in the peripheral blood and articular fluid of RA patients markedly inhibited the heme synthesis of erythroblasts of healthy individuals.

4) The colony formation of the bone-marrow CFU-C of SLE patients was decreased as compared to that of the healthy subjects.

5) The serum of some of the SLE patients had an inhibitory effect on the colony formation by bone-marrow CFU-C.

6) Lymphocytes of SLE patients markedly inhibited colony formation by bone-marrow CFU-C.

The results shown above suggest the possibility that immunocompetent cells of the patients are involved in the pathogenesis of the anemia of RA and the leukopenia of SLE.

INTRODUCTION

Anemia is frequently observed as an extra-articular symptom of rheumatoid arthritis (RA) and leukopenia associated with SLE correlates well with the severity of these diseases and it is well known that the hemoglobin level improves and the leukocyte count increases with improvement in the clinical picture of these diseases with the administration of steroidal agents, etc. The exact etiology of the anemia and the leukocytopenia in these diseases remains obscure. However, we have

Keywords: anemia, leukopenia, lymphocytes, rheumatoid arthritis, systemic lupus erythematosus

† To whom reprint requests should be addressed.
already reported on the decreased ability of the bone-marrow erythroblasts of RA patients to synthesize hemoglobin on the decrease of granulocyte-macrophage colony forming units in cultures (CFU-C) by the bone-marrow cultures of SLE patients. Recently, the involvement of immunocompetent cells, especially that of lymphocytes, in the abnormal differentiation and proliferation of blood cells has been pointed out. Considering the possibility that peripheral lymphocytes are involved in the pathogenesis of the anaemia and the leukocytopenia associated with diffuse collagen diseases arising from immunological abnormalities, studies of the effects of the lymphocytes in the RA patients' peripheral blood and articular fluid on heme synthetic activities of the bone-marrow erythroblasts of normal healthy individuals, and of the effects of the lymphocytes in the peripheral blood of SLE patients on the granulocyte-macrophage colony formation by human-bone marrow were made. It was found that lymphocytes of these patients significantly inhibit heme synthesis in bone-marrow erythroblasts as well as colony formation in vitro.

**SUBJECTS AND METHODS**

**Subjects**

Thirteen RA patients, falling under “definite” or higher categories as established by the American Rheumatism Association, ages between 22 and 73, an averaging 49.5 years, 2 males and 1 female, in Class 2-4 of Stage II in accordance with the classification of Steinblocker et al. were used. Peripheral blood from these patients showed an RBC count of 300 ± 43.3 × 10^6/mm^3, hemoglobin 8.4 ± 1.2 g/dl, hematocrit 25.9 ± 3.8%, and serum iron 46.8 ± 16.5 μg/dl.

The SLE patients consisted of 8 females, between 16 and 50 years of age, averaging 29.8 years, all meeting the preliminary diagnostic criteria of the American Rheumatism Association and in the active phase of SLE, showing leukopenia prior to the administration of large doses of steroidal agents. These patients showed a peripheral WBC count of 1000-3000/mm^3 (average 2050 ± 756/mm^3), seven of whom had positive LE cells or LE tests, all of whom showed positive antinuclear antibodies/anti-DNA antibodies, with a complement level (CH50) of 20.2 in one patient and 20.0 or less in all the others. During the period of this study, these patients received steroidal agents equivalent to 0 to 30 mg/day of prednisolone, or 9.7 ± 9.7 mg/day on the average (Table 1).

**Methods**

As previously reported, the heme synthesis of erythroblasts was determined by the following procedures: 2 to 4 ml of bone-marrow aspirate was collected into a heparin-wetted syringe and was then filtered in 5–10 ml of phosphate-buffered saline (PBS) of pH 7.4, after which it was centrifuged at 2000 rpm for 5 minutes to remove the layer of nucleated cells and then washed twice to isolate the bone-marrow cells. Lymphocytes were isolated from the peripheral blood by means of specific gravity centrifugation using a Lymphoprep solution, while lymphocytes in the articular fluid were isolated by the method described by Tukida et al. with some modifications, that is, the articular fluid was collected into a heparin-added syringe and after the addition of 10 units of hyaluronidase per 1.0 ml of the articular fluid, the mixture was allowed to stand at room temperature for 20 minutes, and the isolation of the lymphocytes was done in a manner similar to that followed in the isolation of lymphocytes from the peripheral blood. By morphologic classification, by means of Wright’s/Giemsa staining after isolation, the articular fluid of the RA patients showed 68.7 ± 4.1% lymphocytes, 21.2 ± 5.0% mononuclear cells of series of T and 6.8 ± 2.1%, others, while the articular fluid taken from the patients with osteoarthritis (OA) showed 81.3 ± 8.3% lymphocytes, 12.4 ± 7.3% monocytic series of cells and 5.8 ± 3.1% others. The isolated human bone-marrow cells, in the amount of 1 × 10^6, were placed in small test tubes containing 100 units of penicillin G, 100 μg of streptomycin, and 1.0% of 20% inactivated human-type AB serum added NCTC 109, to which 1 unit of erythropoietin (EPO, Step 1, Connaught Laboratories, Ontario, Canada), or depending on the cases, 20% of the plasma or the articular fluid and 1 × 10^7 of lymphocytes from the peripheral blood or articular fluid of the patients were added, together with 1 μCi of 59FeCl3 (16 m Ci/mg Fe, Japan Isotope Assm.) as an index of the heme synthesis, and the mixture was incubated at 37°C for 72 hours in an incubator containing 5% CO2.

**Table 1**

<table>
<thead>
<tr>
<th><strong>Methods</strong></th>
<th><strong>Bone-marrow finding</strong></th>
<th><strong>Lymphocytes (%, mm^3)</strong></th>
<th><strong>Bone-marrow finding</strong></th>
<th><strong>Lymphocytes (%, mm^3)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fr. Age</strong></td>
<td><strong>WBC</strong></td>
<td><strong>LE cell</strong></td>
<td><strong>Hb</strong></td>
<td><strong>FBC</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After the incubation, the cells were washed three times with physiological saline at 4°C and after adding 1.0 ml of distilled water, freezing and thawing were repeated four times in an acetone dry-ice bath to lyse the cultured cells. The extraction of heme from the cells was accomplished following the method of Teale. That is, after the addition of 1.0 ml of 5N Drabkin solution, the mixture was allowed to stand for 2 hours at 4°C to allow the hemoglobin into stannetemoglobin. Following this, 0.5 ml of 0.4N hydrochloric acid and 3.0 ml of methylythylketone were added and the mixture was shaken vigorously. After allowing the mixture to stand for 20 minutes, it was centrifuged at 2000 rpm for 20 minutes to extract the heme which had moved into the upper layer. The radioactivity of 3HFe incorporated into heme was measured with an automatic well-type y-scintillation counter and shown in units of cpm. As a rule, each sample was tested with 3 test tubes, the mean value of which was considered the value of the heme synthesis. In the experiments using the patients’ plasma or articular fluid, the cpm level was adjusted by determining the amount of iron contained in the plasma/articular fluid.

The bone-marrow cell culture for CFU-C assay was performed as previously reported. Approximately 1.0 ml of bone-marrow aspirate was taken at the time of puncture of the bone-marrow, using a syringe whose internal cylinder was wets with heparin. The aspirate was centrifuged at 100 g for 5 minutes to collect the buffy coat and the bone-marrow nucleated cells were isolated by washing twice with McCoy’s 5 A solution. The measurement of CFU-C by bone-marrow cells was done by the slow-agar mono-layer method of Robinson et al. in which a supernatant of human placental culture was used as the colony stimulating factor (CSF). 2 × 10^6 each of bone-marrow nucleated cells were embedded in 1.0 ml of McCoy’s solution mixed with 0.3% agar gel (Bacto agar, Difco) containing 20% bovine fetal serum (FCS) and 10% CSF and placed in the incubation plate (Falcon # 3001). In one part of the experiment, lymphocytes obtained from the patients’ venous blood by means of specific centrifugation using Lymphoprep solution and 2 × 10^5 of the lymphocytes or 10% serum were added to the incubation medium.

Bone marrow cells in soft agar were cultured at 37°C for 7 days in an incubator containing 5% CO2, after which the culture was examined under a microscope in order to count the colonies originating from the bone-marrow CFU-C, with a cluster of 40 or more cells.
qualifying as a colony. As a rule, each sample was incubated in 4 separate Petri dishes, the mean value of which was used for computation of the number of colonies.

RESULTS

A Study of Heme Synthesis of Bone-Marrow Erythroblasts of Normal Healthy Subjects and of RA Patients

1 × 10⁶ bone-marrow erythroblasts of normal healthy subjects or RA patients, after the addition of 1 unit of EPO, were incubated for 72 hours to compare their heme synthesis. While the mean value of the heme synthesis by the normal bone-marrow erythroblasts was 4312 ± 902 cpm (n = 21), that of the RA patients was 3153 ± 746 cpm (n = 10), showing a statistically significant lowering of heme synthesis in RA patients as compared to the normal, healthy subjects (t = 4.1395, p < 0.01). In patients with iron deficiency anemia, who showed nearly equal levels of serum iron (SIBC = 48 ± 13.1 μg/dl), the mean heme synthesis per 1 × 10⁶ erythroblasts of the bone-marrow was 8261 ± 1338 cpm (n = 10), considerably higher than the others (Fig. 1).

The Effects of Plasma and Articular Fluid of RA Patients on Heme Synthesis of Erythroblasts from the Bone-Marrow of Normal Subjects

To 1 × 10⁶ each of erythroblasts from the bone-marrow of normal human subjects, plasma or articular fluid of RA patients was added in a ratio of 20% to evaluate the effect on heme synthesis. As shown in Fig. 2, heme synthesis was somewhat inhibited when 20% of the articular fluid was added, but it was not statistically significant (t = 1.399, 0.05 < p < 0.1).

The Effects of RA Patients’ Peripheral Blood Lymphocytes on Heme Synthesis of Bone-Marrow Erythroblasts of Normal Healthy Subjects

To 1 × 10⁶ each of bone marrow erythroblasts of normal human subjects, allogeneic peripheral lymphocytes or RA patients’ peripheral lymphocytes were added at a ratio of 1 × 10⁶ to determine the effect on heme synthesis. As shown in Fig. 3, peripheral lymphocytes of the normal human subjects had little effect on heme synthesis. Heme synthesis following the addition of the lymphocytes of normal human subjects was 94.6 ± 14.8% (n = 10) of the heme synthesis without lymphocytes. However, when the lymphocytes of the peripheral blood of the RA patients were added, heme synthesis was suppressed in proportion to the number of lymphocytes added, and following the addition of 1 × 10⁶ of RA patients’ lymphocytes, heme synthesis had decreased to 63.9 ± 15.9% (n = 15).

The Effects of Lymphocytes in Articular Fluid on Heme Synthesis of Normal Human Bone-Marrow Erythroblasts

0.5–1.0 × 10⁶ each of lymphocytes collected from the articular fluid of the OA or RA patients were added to 1 × 10⁶ each of normal human bone-marrow erythroblasts to determine any effects on their heme synthesis. Heme synthesis was suppressed in proportion to the number of lymphocytes added. That is, heme synthesis, following the addition of 1 × 10⁶ each of lymphocytes from RA patients, decreased to 52.8 ± 18.2% (n = 6) and this was remarkable as compared to 96.5 ± 9.4% (n = 5) following the addition of lymphocytes from the articular fluid of OA patients (Fig. 4). The effects of the lymphocytes from the articular fluid and peripheral blood of the same patient on heme synthesis were compared; as shown in Fig. 5. The lymphocytes from the patient's articular fluid sup-

![Fig. 1](image1)

![Fig. 2](image2)

![Fig. 3](image3)

![Fig. 4](image4)

![Fig. 5](image5)
pressed heme synthesis of the human bone-marrow erythroblasts much more than the lymphocytes from the peripheral blood of the same patient.

An Evaluation of the Number of CFU-C of Normal Subjects and SLE Patients

2 x 10^5 each of bone-marrow cells of normal subjects and SLE patients were cultured for 7 days to compare the number of colonies formed. Using normal human bone-marrow cells, the number of colonies was 81.7 ± 23.4 (n = 19), but in the case of active SLE patients showing leukopenia, the number of colonies decreased markedly to 23.8 ± 14.3 (n = 6) (Fig. 6).

The Effects of Serum of SLE Patients on the Formation of Colonies Attributable to Normal Human Bone-Marrow CFU-C

To 2 x 10^5 each of human bone-marrow cells, either normal human plasma, or that from SLE patients, was added at a rate of 10% and their influence was studied after 7 days of incubation. It was found that normal human serum had very little influence on the formation of colonies as the number of colonies following the addition of normal human serum at 10% was 93.3 ± 9.9% (n = 7) of those without the addition. On the other hand, the serum from two SLE patients prior to receiving steroid agents showed a marked inhibition of colony formation. However, in 6 cases receiving 13 ± 9 mg/day (n = 6) prednisolone equivalent of steroid hormones, the number of colonies following the addition of the patients’ plasma was 87.0 ± 18.9%, and this did not show any significant deviation from those following the addition of normal human plasma at 10% (Fig. 7).

The Influence of Peripheral Blood Lymphocytes of SLE Patients in Active Phase Shown by Leukopenia on CFU-C

2 x 10^5 each of lymphocytes derived from the peripheral blood of normal healthy subjects or SLE patients were added to 2 x 10^5 each of bone-marrow cells, which were then incubated for 7 days in order to compare the influence of the lymphocytes on colony formation. Assuming that the number of colonies without the addition of lymphocytes was 100%, the colonies in the culture with lymphocytes from normal human peripheral blood was 88.1 ± 6.0% (n = 6), while those in the culture with 2 x 10^5 lymphocytes from the peripheral blood of SLE patients in the active phase of the disease accompanied by leukopenia were markedly lower, or 39.4 ± 16.3% (n = 7) (Fig. 8). On the other hand, lymphocytes from the peripheral blood of SLE patients in the post-therapy, inactive phase tended to inhibit colony formation in some of the cases, however, the number of colonies following the addition of lymphocytes from the peripheral blood of the SLE patients was 83.4 ± 19.3% (n = 6) and, therefore, about the same as those following addition of lymphocytes from the peripheral blood of normal human subjects (Fig. 9).

Fig. 6 Colony formation of the bone-marrow CFU-C of SLE patients, especially in active phase, is decreased as compared with that of healthy subjects.

Fig. 7 Serum from some SLE patients had an inhibitory action on colony formation by bone-marrow CFU-C. ● indicates serum of SLE patients without steroid therapy.

Fig. 8 Lymphocytes of active SLE patients markedly inhibited colony formation by bone-marrow CFU-C. ● indicates lymphocytes of SLE patients without steroid therapy.

Fig. 9 Lymphocytes of inactive SLE patients did not inhibit colony formation by bone-marrow CFU-C.

DISCUSSION

In view of the possibility that immunocompetent cells were involved in the etiology of the anemia associated with RA and the leukopenia related to SLE, the effects of RA patients’ plasma, articular fluid, lymphocytes from peripheral blood, and lymphocytes from articular fluid on the heme synthesis of normal human bone-marrow erythroblasts were studied, together with the influence of serum and peripheral lymphocytes of SLE patients in the active phase of the disease on the formation of the colonies of human bone-marrow CFU-C. It was found that lymphocytes derived from the peripheral blood and articular fluid of the RA patients inhibited the heme synthesis of human bone-marrow erythroblasts and that lymphocytes from the peripheral blood of SLE pa-
tients in the active phase, as well as some of their plasma, suppressed the formation of colonies originating from human bone-marrow CFU-C.

In the articular fluid of RA patients, lymphokine-like substances and an immune complex are known to be present and the relationship between the lymphokine-like substances and the onset of arthritis has been pointed out. However, their effect on heme synthesis seems to be negligible as there was no significant difference observed in the synthetic activities even when 20% articular fluid was added. It is known that so-called antileukocyte antibodies exist in the serum of SLE patients. However, on the effects of the serum of SLE patients on colony formation, Fitchen et al. proposed that colony formation is inhibited only when complement is added to the serum of SLE patients, while Duckman et al. reported that the serum of 43% of SLE patients suppressed, without the addition of any complement, the formation of colonies originating from mice bone-marrow CFU-C. We found colony formation in the sera of patients with RA in some sera of RA patients to receiving steroid agents, but not in the sera of patients receiving steroid agents. In view of this, it seems necessary to use sera of patients prior to treatment to investigate colony formation suppressing factors in serum.

During recent years, investigations have been made of the hematopoietic stem cells and the factors involved in the differentiation and proliferation of blood cells. Metcalf et al. and Nathan et al. reported that peripheral T-lymphocytes and their supernatants have something to do with the differentiation and proliferation of hematopoietic stem cells. In some patients with aplastic anemia, their own lymphocytes from peripheral blood are known to act suppressively on the hematopoietic stem cells and it is a well-known fact that immunocompetent cells are involved in the differentiation and proliferation of hematopoietic stem cells.

With regards to the effects on the hematopoietic stem cells exerted by the lymphocytes of patients with collagen diseases which exhibit immunological abnormalities, it is known that peripheral lymphocytes of patients with Felty's syndrome suppress the formation of colonies originating from CFU-C, that such action of these lymphocytes is attenuated after treatment with steroids and that the lymphocytes from the spleen of New Zealand black mice inhibit formation of the colonies originating from the colony forming units in erythroid cells. Wakabayashi, who is one of the authors of this report, has shown in previous studies that peripheral lymphocytes of SLE patients inhibit DNA synthesis of human bone-marrow cells, and this action is decreased by the addition of prednisolone.

On the other hand, lymphocytes from the peripheral blood of RA patients can damage cultured human renal cells, fibroblasts and the synovial layer of the joints of RA patients, while peripheral lymphocytes of SLE patients likewise will cause damage to cultured human renal cells and fibroblasts. In addition, it is a well-known fact that when autologous or homologous white blood cells are injected subcutaneously in SLE patients, an Arthus phenomenon and delayed allergic reaction will occur. Considering these, in conjunction with the results of the present experiment, it is possible that peripheral lymphocytes of patients with collagen diseases have some damaging effects on heme synthesis of bone-marrow erythroblasts and on the process of differentiation/proliferation of bone-marrow CFU-C and that these may be the cause of anemia and leukopenia in SLE. It has been suggested that articular fluid contains more mononuclear phagocytes than peripheral blood. In the articular fluid we collected from RA patients during the present study, we found that 25.2 ± 5.9% of the cells were large mononuclear cells which could be considered monocytes morphologically, and they were clearly greater in number than those (12.4 ± 7.3%) found in the articular fluid of OA patients. As the involvement of reticulohistiocytic cells in the etiology of anemia in RA patients has been pointed out, the effects of the large mononuclear cells can not be ignored in the investigation on the etiology of anemia associated with RA.

REFERENCES

12) Teale FWH: Cleavage of the heme-peptide link by acid methylsulfonylketone. Biochim Biophys Acta 35: 543, 1959
Basic and Clinical Studies on Continuous Cryofiltration
A New Treatment for Rheumatoid Arthritis

Kuniaki SHIMO, Tatsuo SUZUTA,† Mitsuji SUZUKI* and Yukihiko NOSE**
Department of Immunology and Serology, * Department of Surgery, Tokyo Medical College, Shinjuku, Shinjuku-ku, Tokyo 160
** Departments of Artificial Organs and Rheumatology, Cleveland Clinic Foundation, Cleveland, Ohio, USA

In order to provide a fundamental support for continuous cryofiltration (CCF), a newly developed treatment for rheumatoid arthritis (RA), in vitro experiments on cryoprecipitation, as well as preliminary clinical trial treatments were performed.

1. Incubation of fresh plasma, not inactivated sera, from RA patients revealed a clear precipitate after several hours of incubation at 0°C, and it increased in the passage of time up to 24 hours. Rheumatoid factors (RF) as well as immune complexes (IC) gradually diminished from the supernatant, while those in the precipitate increased.

2. A possible role of complement was suggested, because the addition of fresh normal human sera partially restored cryoprecipitation.

3. CCF on 3 RA patients was followed by considerable clinical improvement, such as grip strength, pain index, morning stiffness and ADL. Their laboratory data also revealed considerable improvement in ESR, RF titer, Ig levels, and serum viscosity, but there was no reduction in the blood cell counts, nor in the hemoglobin determination. It was determined that about 3/4 of the IC were removed.

4. Successful elution of the cryoprecipitates from the filters was accomplished. Immunocytological analysis revealed that it consisted of IgG, IgA, IgM and fibrinogen, containing many IC of each Ig class and it had a high RF titer. These observations clearly establish the usefulness of CCF that is removed during the CCF of RA patients is nothing other than RF complexed with its reactant IgG.

INTRODUCTION
A possible role of immune complexes (IC) in the pathogenesis of rheumatoid arthritis (RA) has been suspected. In fact, studies with analytical ultracentrifugation and detection of IC in RA sera have provided evidence that rheumatoid factor(s) (RF) bind to form complexes with auto-

Keywords: cryoglobulin, hemolysis, immune complexes, rheumatoid arthritis (RA), rheumatoid factor

† To whom reprint requests should be addressed.

logous IgG as the reactant and activate complement which play a major role in the process of inflammation. It is therefore likely to be of therapeutic value to remove such immune complexes from the circulating blood. The plasma exchange treatment developed for this particular purpose has been used in some institutions with good results. This procedure, however, has disadvantages in that the plasma preparations are not readily available in large quantities, and also, it involves some risks, such as allergic reactions, infections and adverse effects on cardiopulmonary hemodynamics. These considerations led Nose,
one of our co-workers, to develop the continuous cryofiltration (CCF) technique based on the principle of hemodialysis combined with cryoprecipitation. The usefulness of the new technique was reported. However, there seem to be some questions to be answered before the procedure will be applied for wide clinical use. Does cryoprecipitation take place in every RA patient? Does the procedure remove IC as cryoprecipitate? To answer these questions, the authors attempted some fundamental in vitro experiments as well as preliminary clinical trials to evaluate this method.

MATERIALS AND METHODS

CCF Procedure. Each subject had a blood access prepared to introduce two different kinds of hollow fiber filters into a closed circuit of extracorporeal circulation. The primary filter, which was composed of hollow fibers, 0.5 μ in pore size, enables separation of the blood plasma from blood corpuscles, the former being led to the secondary filter while it was ice-cooled to facilitate cryoprecipitation. The secondary filter consisted of hollow fibers with 0.2 μ pores to trap the cryoprecipitate. The filtrated plasma was warmed to 37°C and reconstituted with the blood corpuscles prior to being returned to the subject’s circulation. Each procedure usually took several hours.

Rheumatoid Patients. Three patients with definite RA (according to the ARA diagnostic criteria) were investigated.

Determination of RF Titer. The RAHA test, a passive hemagglutination test using tanned sheep red blood cells coated with rabbit IgG was used. The initial dilution was 1 to 20, and the RF titer was expressed with the concentration of the end point of serial twofold dilutions.

Detection and Titration of IC. The JM cell method, developed in our laboratory, was employed. It is based on the principle of the indirect fluorescent antibody test. Briefly, a pellet of 10⁶ JM cells, an established human lymphoblastoid cell line, which bear no surface IgG but have Fc receptors, per sample was incubated with 50 μl of a test sample at 37°C for 1 hr, washed three times with phosphate-buffered saline (PBS), pH 7.2, and then 50 μl of FITC-labeled anti-human Ig antibody was added to react at 37°C for 30 minutes. After washing twice with PBS, the cells were observed with a fluorescent microscope. A sample was considered reactive when clear capping or patch-formation was noted. The IC titer was expressed with the end point of serial twofold dilutions.

Purification of the Cryoprecipitate. The precipitate was retained in the secondary filter after the CCF was thoroughly washed by passing heparinized cold saline through the filter and then being eluted with warm saline at 37°C. The eluate was concentrated and then cooled overnight at 4°C. The resultant precipitate was collected by centrifugation in cold, dissolved in warm saline and purified by repeating these processes.

Immunoelectrophoresis (IEP) and Quantitation of the Plasma Protein. A modification of Scheidegger’s microtechnique, is routinely used in our laboratory, and the radial immunodiffusion in agar plates by Mancini were employed respectively.

Clinical Evaluation of CCF. Clinical evaluation of CCF was made in terms of semiquantitative criteria of the ARA Committee for estimating degrees of disease activity.

RESULTS

Fundamental Studies

Cryoprecipitation of RA Plasma. Fresh patients’ plasma were incubated at 0°C, samples were removed and centrifuged at intervals of time to estimate the amount of the precipitate. As shown in Fig. 1, the quantity of the precipitate increased with the time passage. At the same time, the RF and IC titers in the precipitate increased and those in the supernatant diminished, until the supernatant lost all of its original RF activity.

Cryoprecipitation of RA Sera and the Effect of Fresh Normal Plasma. As shown in Fig. 2, similar studies with patients’ sera showed little precipitate produced by cooling nor any substantial changes in RF and IC titers in the supernatant. However, the addition of fresh normal human

**Table 1**  An analysis of the immune complexes (IC) and rheumatoid factor (RF) in the cryoprecipitate

<table>
<thead>
<tr>
<th>Patient</th>
<th>Protein (mg/ml)</th>
<th>IgG</th>
<th>IC IgM</th>
<th>IgA</th>
<th>RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>TK</td>
<td>1</td>
<td>14.5</td>
<td>40</td>
<td>40</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>19.5</td>
<td>40</td>
<td>40</td>
<td>160</td>
</tr>
<tr>
<td>EM</td>
<td>1</td>
<td>14.0</td>
<td>520</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13.0</td>
<td>40</td>
<td>40</td>
<td>160</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>32</td>
<td>16</td>
<td>32</td>
<td>10240</td>
</tr>
<tr>
<td></td>
<td>IC IgM</td>
<td>32</td>
<td>16</td>
<td>32</td>
<td>2360</td>
</tr>
<tr>
<td></td>
<td>IgA</td>
<td>64</td>
<td>128</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>RF</td>
<td>4</td>
<td>4</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>
**Clinical Studies**

The clinical effects of CCF observed are summarized in Table 2. Patients received 14 to 31 CCF treatments, about 3 to 5 liters of plasma were filtered at each occasion. Marked alleviation of pain and morning stiffness was noted, along with some beneficial objective changes such as increased grip strength and reduced gait disturbances. The mean disease activity before and after CCF was 20 and 14, respectively, which means a 30% improvement.

Above all, their motor functions improved remarkably. Case 1 and 3 could not take care of themselves at first but remained lying in bed. After a series of CCF treatments, they could walk with a little help or with a stick.

**Laboratory Findings.** Following CCF a marked decrease of immunoglobulins in the patients'; plasma was observed (Table 3). IgG by 45.4%, IgM by 42.2%, and IgA by 40.2%. Of the complement components, C3 was decreased by 51.9%, and C4 by 53.8%. CH50 also decreased by as much as 47.3%. The RF titer was sharply diminished to an undetectable level. There was also a fall in the levels of some other plasma proteins and components: α1-protein by 50%, cholesterol by 30%, GOT by 33%, and GPT by 38%. The albumin level remained unchanged. While the RBC, platelet counts and hemoglobin remained unchanged following CCF, which, on the other hand, resulted in a slight elevation of the WBC counts.

**DISCUSSION**

The etiology of RA remains obscure, although viruses(10) or some other infectious agents(14) and genetic predisposition(15) have been implicated.

It has been strongly suggested that autoimmune mechanisms were involved in the pathogenesis of the disease(16). There are a number of observations(17-21) suggestive of a type III allergic mechanism: an inflammatory process induced by immune complexes (IC) made up of RF bound to autologous IgG.

These considerations led to the therapeutic use of some techniques for removing the IC from the circulation: plasma exchange (PE) and plasmapheresis (PP). These procedures, although of some clinical value(22,22) in RA, and in Goodpasture's syndrome, hyperc viscosity syndrome, immune thrombocytopenia and autoimmune hemolytic anemia, turned out to have some disadvantages as they came to be in wider clinical use: e.g. low efficiency of removal, a requirement of a large quantity of fresh human plasma or plasma products, and the risk of infection by hepatitis virus, cardiopulmonary hemodynamic insufficiency and even shock. In order to overcome these problems, the continuous cryofiltration (CCF) technique has been developed(23).

The principle of this method consists of precipitating the IC by cooling the plasma and removing the precipitate by filtration during an extracorporeal circulation. Nise reported good clinical results with this procedure.

The CCF method has advantages in that it is highly efficient, can minimize the use of plasma preparation because the plasma from which the ICs are removed is returned to the patient, and also, it is free from the risk of immune reactions against allo genic plasma. In our study to evaluate the clinical usefulness of this procedure in terms of Lombay's parameters, including grip power and ADL, marked improvement was noted in these parameters, as in cases reported by Malchesky et al.(9).

Laboratory studies also revealed a marked decrease in the ESR along with a fall in the immunoglobulin levels, complement titer, serum viscosity and RF titer in concert with the clinical improvement, the latter diminished to an undetectable level.

In contrast, only an approximately 30% decrease in enzymes and plasma proteins other than those of the immune system were noted after the treatment. The levels of low molecular weight substances as well as albumin remained virtually unchanged. The fact that there was a marked decrease in those plasma proteins which have a high molecular weight or are involved in IC formation, such as immunoglobulins or complement components irrespective of their molecular weight may be suggestive that the sieving was taking place by molecular size and the precipitate trapped by the secondary filter represented IC. There is no fundamental evidence available, however, which can provide support for the clinical benefits described above. This is one of the reasons why the present study was attempted. It seems significant to decide whether the CCF is actually removing the IC and also to confirm experimentally the effect of cooling on the solubility of the IC in vivo.

There are several methods available for the detection of IC, each of which has its own advantages and disadvantages(22). The method used in our study utilizes JM cells, cultured T lymphoblastoid cells which themselves have no surface Ig or C3 receptors but only have receptors for FC. This method provides not only highly reliable and reproducible results, if enough care is taken in the culturing of the cells and in the fluorescent antibody technique, but also enables us to detect.
identify and quantify the IC in each immunoglobulin class if class-specific anti-IgG labeled fluorescent antibody is used.90 The evidence that the ICs were actually removed from the patient's blood was obtained not only in an indirect way, by comparing RF and IC titers of the patient's serum before and after CCF, but also in more direct way, by detecting and quantitating the IC in the cryoprecipitate trapped on the secondary filter. The high RF activity of the IC thus eluted together with the immunoneutrophoric finding that the IC contained complement components in addition to IgG, IgM and IgA, but no other proteins except fibrinogen, is strongly suggestive that RF combined with reactant autologous IgG represents the main constituent of the cryoprecipitate.

Cryoglobulins are frequently detectable in sera from patients with monoclonal gammapathies,90 chronic infectious diseases90 or autoimmune diseases, especially in SLE,90 but less common in RA,91 where it is presumed that the IC consisting of RF with reactant IgG exists as cryoglobulin. It is therefore appropriate to determine whether the IC is really precipitated by chilling in vitro experiments. In fact, our initial study showed that even prolonged cooling of sera from RA patients resulted in no appreciable cryoprecipitation nor little substantial changes in RF and IC titers of the supernatant. The situation changed drastically, however, when the RA plasma was substituted in place of the RA sera: a few hours of cooling resulted in a visible precipitate, which increased in amount in the passage of time. In concert with the increase, both the IC and the RF in the supernatant decreased until they reached almost zero at 24 hrs, whereas the IC and the RF in the precipitate increased. This indicates that the IC consisted of RF and reactant IgG in the plasma and can be removed as a precipitate. The fact that cryoprecipitate takes place, not in inactivated sera, but in plasma strongly suggests the possible role of complement in this reaction. This is supported by the experiment and also by the fact that the IC can bind complement.92 Even in the presence of enough complement, however, cryoprecipitate appeared only to a lesser extent than in plasma, a finding which is suggestive of the possibility that some proteins other than complement, such as those of the clotting system, may also be involved. It is not unlikely that part of the RF-IgG complexes were removed from the plasma into the clot during the separation of the serum, as suggested by the immunoneutrophoric finding that the cryoprecipitate contained fibrinogen. Further investigation is necessary to make this clear.

In vitro experiments showed that it took from several hours to about 20 hrs for the cryoprecipitation to be finished. While in CCF, it requires only 40 minutes for the plasma to undergo cooling by passing through the cooling unit and secondary filter. Although there seems to be a big gulf between them, the conditions are quite different in the former study one observed visual precipitate and in the latter one trapped even an invisible precipitate with the aid of a micro pore filter, which must have served to shorten the cooling time in the extracorporeal system, because from 50% to 100% of the circulating ICs were actually removed after a 4 hours run as shown in Fig. 4. At any rate, it is advisable to establish the optimal cooling time, necessary and sufficient to remove the IC to the filter—and to operate the instrument in the condition as near as possible to it.

Thus, we have succeeded in providing an experimental basis for CCF—evidence that ICs have actually been removed. We have also confirmed the usefulness of the procedure by trying it clinically, for the first time in Japan. However, the number of patients treated by the procedure, so far, are so small in number, that further well controlled studies, on a larger scale, are necessary for proper evaluation and for indications of this method. No serious side-effects have thus far been encountered with the procedure. Clinical experience must be accumulated to confirm its safety.

Although CCF may basically be considered a somewhat casualistic approach to remove circulating IC more directly, after all, it is nothing more than symptomatic in nature, since, like other treatments in current use, it can only bring about symptomatic relief but never a specific cure of the disease. IC, which may be harmful to the patient himself, also play some role in regulating immune reactions as a monitor.93 Care should therefore be taken in the possibility that removal of IC from the circulation might prove an adverse immune response in increased RF production.93 The fact that low molecular substances are not removed from the blood by CCF but are returned intact to the circulation also might make it necessary to find some means to eliminate any such substance if they are found to be involved in the pathogenesis of the disease.

CCF is a unique method for the treatment of RA based on a new principle and can be expected to be a useful therapeutic tool if these problems are solved in further investigations.

The value of the procedure consists not only in its therapeutic effect but in its probable contribution in fundamental studies: it provides specimens, in quantities large enough for the analysis of the circulating IC, whose antigenic component is of particularly critical importance in the elucidation of the etiology of the disease.

Immunological studies, with this in view, are now under way.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr. Jun Minowada, Roswell Park Memorial Institute, Buffalo, NY, USA for his kind supply of the J774 cells. A part of the study was supported by grants for Systemic Vascular Diseases (Chairman: Prof. Y. Fukuda) from the Government of Japan, Ministry of Health and Welfare.

REFERENCES

1) Winchester RJ, Aguello V, Junkel HG: Gam
globulin complexes in synovial fluids of pa
citiers with rheumatoid arthritis. Partial charac
2) Junkel HG, Mueller-Eberhard HJ, Fadenbog
toid arthritis and certain other conditions. J Clin
Invest 40 : 117-129, 1961
3) Suzuki T, Shimoh K: Quantization of com
pound and free rheumucid factor in each immunoglobulin class by absorption of Fc-receptor bearing cell. New Horizons in Rheumatoid Arthritis, (Shokawa Y, Abe T, Yamauchi Y, ed) 22-28, Amsterdam, Excerpta Medica, 1981
4) Jain HI: Mechanism of trapping to immune
complexes in joint collagenous tissues. Clin Exp

ase. Transfusion 15 : 570-576, 1975
munosuppression and plasma exchange in the treat-
ment of Goodpasture's syndrome. Lancet 1 : 711-
715, 1976
9) Malchesky PS, Ansanna Y, Zawicki I, et al : On line separation of macromolecules by mem-
brane filtration with cryogelation. Arif Organ
s 3 : 205-207, 1980
12) Scheidegger JJ : Une micro méthode de l'im
munoelectrophoretic. Int Arch Allergy 7 : 103-110, 1953
13) Mancini G, Carbonaro AO, Heerema JF : Immunochemical quantitation of single radial immu
nodiffusion. Immunochimistry 2 : 235-254, 1965
14) Oakhons E, American Rheumatism Association Committee on Diagnostic and Therapeutic Crite
ria. A Progress Report submitted to Executive Committee and Membership at the Annual Meet-
ing, Colorado Springs, Colorado, May 16-20, 1977
15) Neumann T, Hollos I, Farkas K : Virus-like par
2 : 21-28, 1973
16) Bennett JC : The infectious etiology of rheuma
17) Staats P, Fink CW : Different HLA-D asso-
20) Kinsella TD, Baum J, Ziff M : Studies of iso-
Depressed Natural Killer Activity in Rheumatoid Arthritis and Its in vitro Augmentation with Interferon and N-(2-Carboxyphenyl)-4-Chloroantranilic Acid Disodium Salt (CCA), an Anti-Arthritis Agent

Kyogo ITOH; Fumio SAITO, KatsuO KUMAGAI and Shiro KOSAKA

Department of Microbiology, Tottori University School of Dentistry, Seiryyo-cho, Tottori 680
*Department of Internal Medicine, Asahi Prefectural Central Hospital

Natural killer (NK) activity against a tumor cell line, K-562, was studied using peripheral blood mononuclear cells of patients with rheumatoid arthritis and age- and sex-matched normal subjects. NK activity of patient's cells were significantly lower than that of the control group. However, NK activity of RA cells was increased following incubation with 500 units/ml of human interferon and restored almost to normal levels. The NK activity of mononuclear cells, either from RA patients or normal subjects, was significantly increased following incubation with 10 ng/ml of N-(2-carboxyphenyl)-4-chloroantranilic acid disodium salt (CCA), which is therapeutically effective in experimental arthritis in rats and under investigation as a therapeutic agent for RA. The expression of the receptors for IgG-Fc (FcγR) on human lymphocytes were also augmented by CCA as well as by interferon. At present, the significance of this depressed NK activity in RA disease, which can be restored to normal levels by interferon and an anti-arthritis agent, remains unknown. However, application of the NK assay in RA patients may add valuable information to investigations of immunologic abnorality observed in these patients.

INTRODUCTION

Although the etiology of rheumatoid arthritis (RA) remains unknown, there is evidence suggesting that abnormal humoral and cellular immune responses may be involved in the pathogenesis of the disease.1-3 Several investigators, using a number of immunologic in vitro techniques, have reported deficiencies of the T-cell system and normal B-cell function,4-7 although results often have been conflicting and difficult to interpret because of differences in patient selection, clinical analysis and techniques used. Natural killer (NK) cells involved in spontaneous target cell killing and K cells in antibody-dependent cell-mediated cytotoxicity (ADCC) have been characterized as nonimmune lymphoid cells which are found in the population of non-T and non-B cell fractions.8 Studies in mice suggest that NK (K) cells may be of importance in tumor surveillance, early host response to bacterial and viral infection (due to interferon activation),9-10 and in bone marrow graft rejection.11 Patients

Keywords: IgG-Fc receptor, interferon, natural killer (NK) cell, N-(2-carboxyphenyl)-4-chloroantranilic acid disodium salt (CCA), rheumatoid arthritis (RA);* To whom reprint requests should be addressed.
with active systemic lupus erythematosus show significantly reduced NK and K activity, as well as a number of other abnormal humoral and cellular immune responses. In this study, we demonstrate that patients with RA, who are clinically active, also show significantly reduced NK. In addition, we demonstrate that the mononuclear cells of RA patients respond in vitro with a significant increase in NK activity to human interferon, or a new drug, N-(2-carboxyphenyl)-4-chloroanthranilic acid disodium salt (CCA) which is therapeutically effective in experimental arthritis in rats and is now being investigated as a possible chemotherapeutic agent for RA in human beings.

MATERIALS AND METHODS

Patients. Twenty patients with classical or definite rheumatoid arthritis (RA) were studied. There were 19 female and 1 male patients between the ages of 43 and 70. They were controlled without the administration of steroid or any other immunosuppressive drugs and free from infection when studied. Age- and sex-matched controls consisted of 10 healthy donors studied in parallel.

Preparation of Lymphocytes. Peripheral blood mononuclear cells from RA patients and healthy donors were prepared by the technique of Ficoll-Isooaque gradient centrifugation as described previously. The mononuclear cells were counted using a hemocytometer. For assay of the Fe-receptors and culture experiments, lymphocyte preparations, depleted of monocytes by adherent techniques, were usually used as described previously, and suspension of lymphocytes were adjusted to a concentration of 1 x 10^6 cells/ml in plastic tubes with varying concentrations of CCA or interferon and incubated in 5% CO2/95% air at 37°C. RPMI1640 medium supplemented with 10% fetal calf serum (FCS) (RPMI/10%; FCS) was used in all the experiments.

Assay for Natural Killer Activity. A human erythroleukemic cell line, K-562, was used as target cells. Approximately 1 x 10^3 K-562 cells were incubated with [3H] thymidine (du - 2.5 mCi) for 1 hr at 37°C. The labeled cells were then washed three times, resuspended in RPMI/10%; FCS and incubated further for 2 hrs at 37°C. After incubation, the cells were washed twice and adjusted to 5 x 10^6 cells/ml in the medium. For the assay of cytotoxicity, 0.2 ml lymphocyte suspensions at varying concentrations were mixed with 2 x 10^6 41Cr-labeled K-562 cells in Nunclo round-bottomed microtiter plates in triplicate. After rocking, the plates were cultured in 5% CO2/95% air at 37°C for 4 hrs. At the end of the incubation period, 0.1 ml of the supernatant was removed from the microtiter wells and counted in an autogamma counter. The percentage of natural cytotoxicity was calculated from the means of the triplicate assay by the following formula:

Experimental release—spontaneous release

Maximum release—spontaneous release

where maximum release is cpm released from target cells in 1N NaOH, and spontaneous release is from target cells in the medium. Spontaneous release rarely exceeded 10%.

Detection of IgG-Fe Receptors. Ox red blood cells (ORBC) coated with IgG (EAg) was prepared essentially as described previously. Rosette formation of test lymphocytes with EAg was performed as described previously. Briefly, one-tenth milliliter of lymphocyte suspension (1 x 10^6/ml) and an equal volume of 1 x 10^6/ml EAg were mixed, spun down at 1000 rpm for 10 min at 4°C, and then kept at 4°C for 2 hrs. After incubation, mixtures were resuspended very gently by pipetting. A drop of resuspended cells was placed on a hemocytometer and at least 250 cells were counted. The adherence of three or more erythrocytes was considered positive. All data were determined in duplicate.

Treatment of Cells with Reagents. Human β-interferon was kindly provided by Toray, Industries, Inc., Japan. The specific activity of this preparation was 1.0 x 10^9 international units/mg protein. N-(2-carboxyphenyl)-4-chloroanthracetic acid disodium salt (CCA) was supplied by Chugai Pharmaceutical Co., Ltd., Japan. In order to examine their effect on NK activity, effector lymphocytes were incubated with 41Cr-labeled K-562 cells in the presence or absence of these reagents at 37°C for 4 hrs, and assayed for their cytotoxicity. To examine their effect on the expression of IgG-Fe receptors, lymphocytes were incubated with varying concentrations of these reagents for 3 to 24 hrs at 37°C in 5% CO2/95% air, washed and then examined for values of EAg-rosette forming cells.

RESULTS

NK Activity in Patients with Rheumatoid Arthritis

The mean number of mononuclear cells in the peripheral blood from RA patients was 1.59 x 10^9/ml which was significantly lower than that found in healthy donors (2.41 x 10^9/ml). Fig. 1 shows the NK activity against K-562 cells of peripheral blood mononuclear cells from RA and healthy individuals at an E/R ratio of 20. NK activity in 17 of 20 RA patients was lower than the mean activity of the healthy group. The mean activity of the diseased group was also significantly lower than that of the control group (p<0.01). The mean ± SD of NK activity at effector:target (E/T) ratios of 5, 10 and 20 of both groups also showed depressed NK activity in the diseased group in all E/T ratios tested (Fig. 2).

Fig. 1. The percentage of natural killer (NK) activity in healthy donors and patients with RA. The NK activity of peripheral blood mononuclear cells from 10 healthy donors and 20 RA patients was examined at an effector:target ratio of 20:1 in triplicate assay. The arithmetic mean of the observation for each group is also shown.

Fig. 2. The percentage of NK activity in healthy (—) and RA patients (—) at different effector:target ratios.

The values represent the mean ± SD of the percentage of NK activity in 10 healthy and 20 RA patients.

In vitro Effects of Interferon and CCA on NK Activity

Mononuclear cells from both groups were divided into three specimens. Each was incubated with target cells in the medium containing 10 ng/ml CCA, 500 u/ml of interferon or in the medium alone for 4 hrs at 37°C, and then assayed for its cytotoxicity. As clearly shown in Fig. 3, CCA augmented the cytotoxicity of mononuclear cells from 9 of 10 healthy donors and from 18 of 20 RA patients. Table 1 shows that NK activity similar to, but somewhat stronger than that of CCA was obtained with interferon-treated cultures. The mononuclear cells of one healthy subject and 2 patients whose NK activity was not augmented by CCA also did not respond to interferon.

Enhancement of IgG-Fe Receptor Expression with CCA and Interferon

Interferon can augment not only NK activity but also the expression of the receptors for IgG-Fe (Fcy-R) on the effector cells. Therefore, CCA, which was found to augment NK activity of human peripheral blood, was also examined for its effect on the expression of Fcy-R on human...
Table 2 The effect of CCA and interferon on the expression of IgG-Fc receptors

<table>
<thead>
<tr>
<th>Cultivation time (hr)</th>
<th>Control (medium)</th>
<th>CCA, μg/ml</th>
<th>IFN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EA γ-RFC (×100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>30.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>27.5</td>
<td>37.2</td>
<td>57.1</td>
</tr>
<tr>
<td>6</td>
<td>26.0</td>
<td>35.8</td>
<td>55.8</td>
</tr>
<tr>
<td>24</td>
<td>27.1</td>
<td>27.3</td>
<td>29.0</td>
</tr>
</tbody>
</table>

*: Monocyte-depleted lymphocytes were cultured at 37°C for 24 hrs in the presence of 1 to 100 μg/ml of CCA or 200 units/ml of interferon β, α in the medium alone.

Similar results were obtained with lymphocytes from RA patients (data not shown).

DISCUSSION

The present studies demonstrate depressed NK activity in peripheral blood mononuclear cells from RA patients as compared to age- and sex-matched healthy donors. The finding of depressed NK activity provides further evidence of abnormal cell-mediated immune responses in RA as previously reported. A significant decrease of mononuclear cells in the peripheral blood of the RA patients tested so far was noted. Therefore, the total NK activity in the circulation of RA patients should be considerably more depressed than that of healthy controls.

The significance and mechanisms involved in this immunologic abnormality remain unknown. However, regarding the mechanisms that lead to the observed depression of NK activity in RA, anti-lymphocyte antibodies or immune complexes, which have been demonstrated in RA patients, have to be considered. To test if these factors were responsible for the suppression of NK activity, effector lymphocytes were incubated with target cells in the presence of aggregated IgG or the Fc fragment of IgG, and then assayed for their cytotoxicity. NK activity was severely suppressed by 5 μg/ml of aggregated IgG but not by 10 μg/ml of the Fc fragment of IgG (data not shown). These results suggest that immune complexes present in the serum of RA patients might be a factor responsible for the depression of NK activity. However, previous studies have failed to confirm that Fcγ-R is involved directly in NK cytotoxicity. In spite of this, we do not exclude the fact that Fcγ-R on NK cells are closely linked to the active site of NK cytotoxicity. Therefore, it is possible that the structural alteration of the cell membrane induced by the binding of immune complexes to the Fcγ-R leads indirectly to impaired function of the active site of cytotoxic activity of NK cells.

An alternative explanation could be a metabolic effect, on NK cells, of humoral or chemical mediators such as prostaglandin, which might act as release of the serum of patients with chronic inflammatory diseases such as RA.

This would then result in a reduction of the intrinsic function necessary for differentiation or activation of NK cells.

In the present studies, it has also been shown that the depressed NK activity of blood mononuclear cells of RA patients, when incubated with interferon, a known NK-activating molecule, was restored to near normal levels. CCA, which has been shown to be therapeutically effective, at least on experimental arthritis in rats, also induced in vitro augmentation of the depressed NK activity of mononuclear cells of RA patients. Our unpublished observation has shown that, at least in mice, CCA is an interferon inducer. Indeed, mononuclear cells from normal and diseased individuals, whose NK activity was augmented by interferon, also responded to CCA. CCA, as well as interferon, was also shown to augment the expression of Fcγ-R on peripheral blood lymphocytes.

Restoration of the depressed NK activity in RA patients, by these reagents, suggests that the depressed NK in RA might be derived from an impaired function of the NK cells not simply from a reduction in the number of effector cells.

The significance of the depressed NK activity in RA patients remains unknown. Further investigation of a possible relationship between disease activity and depressed NK activity in RA patients will be needed. At present, no information is available as to whether interferon or interferon inducers have a beneficial effect on the course or prognosis of RA disease. However, the application of an NK assay in RA patients or in animals with experimental arthritis who received anti-arthritis drugs or interferon may add valuable information to the investigation of the immunologic abnormalities in RA or experimental arthritis.
REFERENCES


Enhanced Glucose Consumption by Activated Monocytes in Rheumatoid Arthritis

Kazuhide YAMANE1, Ichiro KONO, Teizo KABASHIMA, Tetsushi SAKURAI and Hiehachi KASHIWAGI

Department of Medicine, Institute of Clinical Medicine, The University of Tsukuba, Saitama

Enhanced glucose consumption by activated monocytes in patients with rheumatoid arthritis (RA) was activated by lipopolysaccharide (LPS) and their glucose consumption was measured and compared with that of the normal control. The following results were obtained:

1) Human peripheral blood monocytes could be activated by LPS to consume glucose.

2) Lymphocytes alone consumed a negligible amount of glucose.

3) The rate of glucose consumption was higher in RA patients with a Lansbury activity index greater than 50 (25.4 ± 6.1%) than that in patients with less clinical activity (7.2 ± 4.5%).

4) The rate of glucose consumption was higher in patients without chryotherapy (19.6 ± 8.9%) than that in patients with chryotherapy (44 ± 5.6%).

5) Pretreatment of monocytes with gold sodium thiomolate, in vitro, did not affect glucose consumption in RA patients without previous chryotherapy.

6) There was a good correlation between the rate of glucose consumption by activated monocytes and the level of circulating immune complexes in RA patients.

INTRODUCTION

When peripheral blood monocytes or macrophages are stimulated, in vivo or in vitro, they become activated to show various biological properties, for example, enhanced phagocytosis, the acquisition of the capacity to inhibit the multiplication of tumor cells and intracellular pathogens and an increased rate of glucose oxidation. Lipopolysaccharide (LPS) has been shown to activate macrophages by morphologic and functional criteria. Activation of macrophages in vitro by LPS leads to the secretion of lymphoo enzyme, lymphocyte activating factor and prostaglandin. Macrophages activated, in vitro, by LPS are also cytotoxic for lymphoma cells. In 1979, Ryan et al10 described a method by which glucose consumption by activated macrophages could be assessed in vitro. We have utilized this technique to measure the degree of activation of human monocytes obtained from peripheral blood.

We found that monocytes from patients with rheumatoid arthritis (RA) demonstrated enhanced glucose consumption when activated by LPS. Data are presented which suggest a relationship between glucose consumption by activated monocytes and soluble immune complexes in patients with RA.

STUDY SUBJECTS

Peripheral blood was obtained from 12 patients with classical or definite RA according to the criteria of the American Rheumatism Association Subcommittee on the Classification of Rheumatic Diseases (ARA) who fulfilled the recent criteria for the classification of RA. Monocytes were isolated from whole blood by Ficoll-Hypaque density gradient centrifugation. The purity of monocytes was more than 95% as confirmed by the presence of mono- nuclear cell marker in monocytes. Monocytes were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and then cultured for 24 hours. Monocytes were cultured for 24 hours in RPMI 1640 medium supplemented with 10% FBS and glycerol (1 M/L) and D-glucose (5.5 M/L). Monocytes cultured in RPMI 1640 medium supplemented with 10% FBS only served as control. Monocytes cultured in RPMI 1640 medium supplemented with 10% FBS and LPS (100 ng/ml) served as activated monocytes.

Keywords: glucose consumption, gold, immune complex, rheumatoid arthritis

To whom reprint requests should be addressed.
teria of the American Rheumatism Association.\textsuperscript{11} There were 10 women and 2 men, ranging in age from 30 to 44 (mean, 35 years). As a control group, 5 healthy subjects matched in age and sex were studied. The clinical activity of the RA was judged according to the activity index of Lumsden.\textsuperscript{18} Four patients showed an activity index of more than 50 and all 4 patients had no previous chrysotherapy. The activity index of the remaining 8 patients was lower than 50. Five of these patients had received chrysotherapy (total doses>10 mg). All patients who had received chrysotherapy showed an activity index of less than 50. All patients had been taking nonsteroidal anti-inflammatory drugs. Patients were excluded from the study who were taking prednisolone or immunosuppressants.

**Cell Preparation.** Peripheral blood mononuclear cells were obtained by Ficoll-Hypaque density gradient centrifugation.\textsuperscript{15} Interphase cells were utilized as unfraccionated mononuclear cell population. Cells were adjusted to $5 \times 10^8$/ml with RPMI 1640 medium (Gibco, New York) containing 15% heat inactivated fetal calf serum (FCS) (Gibco, New York). Aliquots of 5 ml each were placed into 90 x 15 mm plastic culture dishes (Terumo, Tokyo) followed by incubation for 2 hours at $37^\circ$C in a CO$_2$ incubator. Nonadherent cells were collected by aspirating the suspension and were used as monocyte-depleted lymphocytes. Cells adherent to the culture dishes were isolated by a modification of the method of Rinehart et al.\textsuperscript{14-16} After nonadherent cells were removed by washing the culture dishes three times, 4 ml of 3% lidocaine hydrochloride aqueous solution (Fujiwawa Pharmaceuticals, Osaka) mixed with 2 ml RPMI 1640 medium containing 15% FCS (pH 7.2) were added to each culture dish, followed by incubation for 15 minutes at $37^\circ$C in a CO$_2$ incubator. Adherent cells detached by this treatment were recovered by gently streaming RPMI 1640 medium containing 15% FCS onto the culture dish. Cells that had been detached and suspended in the medium were then washed to remove the lidocaine and were used as a monocyte-rich population.

The unfraccionated mononuclear cell population contained 76-92%, lymphocytes and 6-20% monocytes. Monocyte-depleted lymphocytes contained 92-96% lymphocytes and 3-7% monocytes. The monocyte-rich population was found to contain 96% or more monocytes in repeated samples. The viability of these cells was greater than 90%, as assessed by trypan blue exclusion. The monocytes were identified by the nonspecific esterase staining method according to Yan et al.\textsuperscript{16} The lymphocytes were identified on Giemsa stained smears.

**Assay for Glucose Consumption.** The glucose consumption assay was performed by the modified method of Ryan et al.\textsuperscript{40} The cells were adjusted to $1 \times 10^6$/ml in RPMI 1640 medium containing 15% FCS. They were distributed in 0.2 ml aliquots in flat bottom microwells of microtest plates (Nunc, Denmark) and cultured with 0.5 $\mu$g/ml of E ceill 0111:B4 LPS (Difco Laboratories, Mich) for 96 hours at $37^\circ$C in a CO$_2$ incubator. After the 96 hour incubation, an aliquot of the culture medium was removed, and the glucose content was measured. The glucose assay was performed according to the glucose oxidase method using a "Glucose-B-test-Wako" kit (Wako Chemicals, Osaka).\textsuperscript{17} For each glucose assay, 20 $\mu$l of medium was added to solutions consisting of glucose oxidase, phenol, and 4-aminoantipyrine. The solution was mixed with a Pasteur pipette and after the reaction was completed (37°C, 20 minutes) the OD$_{490}$ was read to determine the quantity of red dye which is produced from glucose.

The rate of glucose consumption was expressed as follows.

Rate of glucose consumption (mg/dl) in glucose (mg/dl) in medium with LPS = glucose (mg/dl) in medium without LPS

In some experiments, monocytes (1 x 10$^6$/ml) from RA patients were pretreated with gold sodium thiomalate (GST) (Shionogi Pharmaceutical, Osaka) (25 $\mu$g/ml) at $37^\circ$C for 96 hours followed by three washes with Hank's balanced salt solution (Gibco, New York). They were then assayed for glucose consumption.

**Circulating Immune Complexes (CIC).** Levels of CIC were assayed by the Clq-deviation test.\textsuperscript{18-19} The basic principle of this method is the following. Serum samples are incubated for 30 minutes at

56°C to inactivate intrinsic Clq, followed by the addition of $191-I$-Clq to the reaction mixture and they are allowed to react for 30 minutes at room temperature. Under these conditions, a part of the Clq remains free while the rest of the Clq binds to the IC in the serum. To this reaction mixture sensitive erythrocytes (EA) are added to bind the free Clq on to the EA. After incubation, the reaction mixture is then overlaid on a 40% sucrose solution in a microtube. By centrifugation Clq with IC is separated from those with EA. The results of the IC assay are expressed as a percent of inhibited by CIC in the test serum.

The Student's t-test was used to evaluate possible statistical significance.

**RESULTS**

**The Effects of LPS Concentration on Glucose Consumption by Monocytes**

In order to evaluate the ability of LPS to enhance the metabolic activity of monocytes, monocytes from controls as well as RA patients were activated with various concentrations of LPS and the glucose consumption by the monocytes were measured. The stimulation of increased glucose consumption was highest in both controls and RA patients when monocytes were activated with 0.5 or 2.5 $\mu$g/ml of LPS (Fig. 1). In subsequent experiments, LPS was used at a final concentration of 0.5 $\mu$g/ml.

![Fig. 1](image) The effects of LPS concentration on glucose consumption by monocytes. Monocytes ($2 \times 10^6$) from 2 controls and 2 RA patients were cultured with various concentrations of LPS in 15% FCS-RPMI 1640 for 96 hours. The rate of glucose consumption was measured as described in Materials and Methods.

The Effects of Lymphocytes on Glucose Consumption

In order to evaluate the role of lymphocytes in glucose consumption, glucose consumption by lymphocytes alone, mixtures of lymphocytes and monocytes, and monocytes alone, in the presence of LPS were compared. In 2 RA patients with an activity index of more than 50, lymphocytes alone consumed a negligible amount of glucose, whereas there was more consumption of glucose when a mixture of lymphocytes and monocytes in a ratio of 1:1 was cultured (Fig. 2). As long as the same number of monocytes were cultured, the addition of lymphocytes in varying ratios did not significantly affect glucose consumption. Glucose consumption by monocytes alone approximately equaled that of the mixtures of lymphocytes and monocytes. When cells from 2 normal subjects were studied, similar consumption of glucose, although to a lesser degree, was demonstrated.

Glycose Consumption in RA Patients and Controls

The glucose consumption, by activated monocytes, from RA patients and controls was examined. As may be seen in Fig. 3, the mean rate of glucose consumption in RA patients (14.5 ± 9.5%) was not statistically different from that of the controls (7.2 ± 2.7%). The patients were then divided into two groups according to clinical activity and the glucose consumption was compared between them. The mean rate of glucose consumption in patients with active RA was significantly lower ($p < 0.05$) than in those with no activity.
there is a close correlation between the degree of glucose consumption and the level of circulating immune complexes (r=-0.90, p<0.01).

**DISCUSSION**

Although the important role of monocytes in immune reactions has been documented, little is known about their functions in RA patients. In the present study we measured glucose consumption as an indicator of monocyte function. Activated macrophages have been shown to manifest the ability to metabolize glucose. 29-31 Recently Ryan et al. 32 reported a simple, reproducible and sensitive method of glucose consumption for measuring the activation of mouse macrophages in culture, but little work had been performed on its application in human monocytes.

Our observations applying this method provide additional evidence that peripheral blood monocytes from RA patients demonstrate enhanced glucose consumption when activated by LPS. There was negligible consumption of glucose by lymphocytes, and when monocytes were added to the lymphocytes the consumption was similar to that of monocytes alone. Thus, glucose consumption seems to be primarily due to monocytes, although a possible effect of monocyte-lymphocyte interaction cannot be excluded. RA patients as a whole demonstrated a slight increase in glucose consumption over the controls. Clinically active patients showed, however, significantly enhanced glucose consumption as compared to those with less active disease.

It has been suggested that chryotherapy can halt the progress of RA. 34-35 It has also been proposed that the therapeutic efficacy of gold compounds may involve their ability to interfere with the functional capability of macrophages. 36-37 To determine whether gold compounds exert any direct effect on glucose consumption by activated monocytes in vitro, glucose consumption was measured after monocytes were pretreated with 25 μg/ml GST for 96 hours. This gold concentration was within the range found in synovial tissues of patients with chryotherapy. 38 From our data the possibility of a direct effect of gold on glucose consumption by activated monocytes was found unlikely. Thus, it may be that low glucose consumption by activated monocytes seen in clinically inactive patients is not induced by the gold compounds per se, but is due to intrinsic changes in the monocytes of clinically inactive patients.

Immune complexes have been implicated in the pathogenesis of RA. 39 It is known that such complexes may alter monocyte functions. 40-41 In our work, a linear correlation was established between the glucose consumption by activated monocytes and the level of immune complexes in RA patients. Our data raise the possibility that circulating immune complexes may be responsible for enhanced glucose consumption by monocytes in RA. Further studies are needed to confirm these observations.

**ACKNOWLEDGEMENTS**

The authors wish to thank Dr. K. Onozaki of the Institute of Basic Medical Sciences, University of Tsukuba, for valuable discussions and Miss Masayo Yamaguchi for excellent technical assistance.

Supported in part by a grant from the Ministry of Health and Welfare, Research Committee for the Treatment of Connective Tissue Diseases, Japan.

**REFERENCES**


A Study of Essential Fatty Acid Metabolism as a Precursor of Prostaglandin in Collagen Disease

Tosihio TOHJIMA* and Yuichi SHIOKAWA

Department of Internal Medicine, Juntendo University School of Medicine, Hongo, Bunkyo-ku Tokyo 113

In the present study, we investigated the prostaglandin (PG) metabolism by measuring essential fatty acids (EFA) in total lipids of plasma and platelets, and both PG and EFA assays were undertaken in synovial fluid in order to clarify the correlation between the levels of these two substances.

EFA compositions were analyzed by gas chromatography in 45 collagen disease patients (RA 20; SLE 9; PSS 2; MCTD 2; Behcet's disease 3), 50 controls (18 normal subjects and 32 diabetics) and four osteo-arthritis patients as non-rheumatic controls for synovitis. PGS were assayed by radioimmunoassay in 10 patients with synovitis (six with rheumatoid arthritis, four with osteo-arthritis).

Results

1) A. Plasma linoleic acid levels were lower and plasma palmitic acid levels were higher in collagen disease patients than on the controls (p<0.005).

B. Sex and age differences in plasma linoleic acid levels were observed in the controls; they were higher in females than in males (p<0.05) and had a tendency to decrease with age in subjects more than 30 years old.

C. Decreased linoleic acid levels in plasma were observed in three groups of patients, i.e., rheumatoid arthritis patients with positive CRP test results (p<0.005), lupus erythematous patients with negative CRP test results (not significant) and collagen disease patients with anemia (low hemoglobin group, p<0.05; low hematocrit group, p<0.005).

2) Platelet arachidonic acid levels were lower in rheumatoid arthritis than in normal subjects (p<0.005).

3) A correlation was found between the levels of PGs and both linoleic and arachidonic acid in the synovial fluid (r=-0.6425, r=-0.5973).

Conclusion

The decreased levels of linoleic acid in collagen disease patients might be caused by an increased PG requirement resulting from inflammation.

INTRODUCTION

Prostaglandins (PG) are synthesized from polyunsaturated fatty acids produced on the y-linolenic pathway.† The essential fatty acids (EFA) are produced from linoleic acid by desaturation or chain elongation to dihomo-γ-linolenic acid (DHLA). These fatty acids are released from cell membrane phospholipids through the action of membrane-bound enzymes, phospholipase A2 or phospholipase C-diglyceride lipase. DHLA, AA and EPA are metabolized via cyclooxygenase to yield cyclic endoperoxides and PGs.

† To whom reprint requests should be addressed.

References


(DHAL 1 series): PGG1, HB, E, F2a, etc.; AA (2 series): PGG2, H3, thromboxane (TXA2), PGE3, E3, D3, etc.; PGE3 (3 series): PGG3, HB, TXA3, PGD3, E3, D3, etc.; 4. Furthermore, these fatty acids, i.e., PG precursors, can be metabolized via lipoxigenase to yield hydroxy-eicosatetraenoic acid (HETE) and leukotrienes (LT A, B, C, D and E). Exogenous administration of PGIs induces erythema, edema, pain, fever, increased cell migration (1) and collagen synthesis (2) and enhances granuloma formation (3) as well. In addition, leukoty lipoxigenase products, 5 (8)- hydroxyeicosatetraenoic acid (HETE) and leukotriene B4, elicit human neutrophil and eosinophil chemotactic responses. (4, 5)

These PGs and their related substances have been detected in several types of inflammation. (6-9) In addition, anti-inflammatory drugs of an aspirin-like nature (10) and steroids (11, 12) prevent PG generation. These studies indicate that PGs may be mediators of inflammation and, thus, may contribute to the pathogenesis of inflammatory rheumatic diseases. However, there have been relatively few studies which investigate the possible role of endogenous PG formation in rheumatic diseases despite the fact that the levels of PGB in the synovial fluids are elevated in these diseases. (13, 14) Monoocytes of patients with systemic lupus erythematosus (SLE) prevent immune-complex-induced production of normal amounts of PGE(15). This may be explained by the fact that 1) precise PG detection in organisms is limited due to the quick removal of PGs from the circulation and 2) the test tube generation of PGs from the cellular elements present is occurred when their membranes are injured in vitro.

Thus, an attempt has been made to investigate the disturbed metabolism of PG precursors rather than directly measure endogenous PGs. Hagenfeldt et al. (16) reported that a decrease of free AA was observed in the plasma pool of male rheumatoid arthritis (RA) patients. However, it would be difficult to evaluate its metabolism because the measurement of free AA due to the fact that fatty acid used in PG synthesis is derived not only from the free fatty acid pool, but also from other pools of the PG metabolism. Furthermore, the turnover rate of free AA in plasma is too rapid for it to be used as an indicator of the PG metabolism. Thus, we have examined the PG metabolism in collagen disease by measuring the EFA, primarily linoleic acid of total lipids, which is the substrate of all PG precursors.

MATERIALS AND METHODS

Subjects

For the fatty acid measurement in plasma, two men and 43 women with collagen disease (20 with RA, 20 with SLE, 2 with PSS, 2 with MCTD and 1 with Behcet's disease) were the subjects of the study. The ages of the patients ranged from 21 to 56 years, averaging 37.9 ± 12.6 years. Twelve RA patients, all of the SLE patients and three other patients were receiving steroids, while all of the RA and SLE patients and three others were using non-steroidal anti-inflammatory medication. Eight men and nine women aged 26-47 (average 30.7 ± 9.5 years) served as the control group, while 19 men and 14 women with diabetes mellitus aged 18-72 (average 52.1 ± 14.6 years) served as the controls for chronic diseases.

For measurement of fatty acid in the synovial fluid, six RA patients were the subjects of the study, and four osteoarthritis (OA) patients served as the controls for synovitis. All were using anti-inflammatory medication.

Seven RA and two SLE patients took part in the study for the fatty acid measurement of platelets; five normal subjects served as the controls.

Materials

Plasma. Blood samples obtained from an antecubital vein in the post-absorptive state after 12 hours of fasting were drawn into tubes containing anti-coagulants (one part 1% EDTA-2Na, 1 mg/ml AS and nine parts blood), which were immediately placed in an ice bath. The plasma was separated by centrifugation at 4°C and 3000 rpm for 30 minutes before being snap-frozen and freeze-dried.

Synovial fluid. After adding a drop of glacial acetic acid, the fluid was mixed, and the supernatant was separated by centrifugation at 4°C and 3000 rpm for 30 minutes before freeze-drying.

Platelets. Blood samples were obtained by the same method as for the plasma. Platelet-rich plasma was separated by centrifugation at 20°C and 700 rpm. Washed platelets were obtained by Mustard's method (20) and freeze-dried.

METHODS

Gas Chromatographic Analysis of Total Lipids. Freeze-dried materials were extracted and transesterified according to the hydrochloric acid methanol method. Then they were analyzed by gas chromatography.

Prostaglandin Assay. The materials were dissolved in a Tris-maleate buffer solution, and the PGs were then extracted according to Jaffe's method. (21) The PGE assay was performed by measuring the amount of PGE obtained after the conversion of PGE or PGA to PGE with alkali treatment. The PGE radioimmunoassay, using 1H PGB2 and an anti-PGB2 rabbit serum, was carried out according to the method of Levien et al. (22).

Other Examination Methods. Red cells, Hb and Hct were measured by the Coulter counter method. Plasma cholesterol and triglycerides were measured by a Technicon 500 enzyme assay and plasma NEFA by a modification of the Un-Itaya method. Plasma protein fractions were separated by electrophoresis.

Statistical Methods. The data in the figures and tables are the mean ± SD. The statistical significance was evaluated by the student's t-test and the chi square test.

RESULTS

Total Fatty Acid Composition in Plasma

A. Fatty Acid Composition without Essential Fatty Acids (Table 1)

Plasma palmitic acid levels were higher among the collagen disease patients and diabetics than among the normal subjects (collagen disease, p < 0.01; RA, p < 0.01; SLE, p < 0.01; others, p < 0.05; diabetes, p < 0.05). The same was true for plasma oleic acid levels (collagen disease, p < 0.005; RA, p < 0.005; SLE, p < 0.005; others, p < 0.005).

B. Essential Fatty Acid Composition (Table 1)

Plasma linoleic acid levels were significantly lower among collagen disease patients than either the normal subjects or the diabetics used as controls for chronic disease (collagen disease, p < 0.005; RA, p < 0.005; SLE, p < 0.001; others, p < 0.005).

Table 1 Plasma fatty acids compositions in total lipids

<table>
<thead>
<tr>
<th>Diseases</th>
<th>n</th>
<th>Myristic acid</th>
<th>Palmitic acid</th>
<th>Palmitoleic acid</th>
<th>Stearic acid</th>
<th>Oleic acid</th>
<th>Linoleic acid</th>
<th>Arachidonic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>10</td>
<td>0.79 ± 0.02</td>
<td>24.38 ± 1.19</td>
<td>3.22 ± 0.55</td>
<td>7.76 ± 0.50</td>
<td>21.78 ± 2.07</td>
<td>35.83 ± 2.97</td>
<td>6.25 ± 1.22</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>1.00 ± 0.08</td>
<td>26.81 ± 2.61</td>
<td>4.28 ± 1.00</td>
<td>7.37 ± 0.95</td>
<td>24.73 ± 3.08</td>
<td>30.88 ± 3.55</td>
<td>5.31 ± 1.86</td>
</tr>
<tr>
<td>Collagen RA</td>
<td>20</td>
<td>0.99 ± 0.04</td>
<td>26.23 ± 0.23</td>
<td>4.64 ± 0.97</td>
<td>8.02 ± 0.60</td>
<td>25.11 ± 2.84</td>
<td>29.01 ± 3.33</td>
<td>5.18 ± 2.08</td>
</tr>
<tr>
<td>Diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLE</td>
<td>20</td>
<td>1.06 ± 0.05</td>
<td>27.02 ± 2.79</td>
<td>5.92 ± 0.95</td>
<td>6.90 ± 0.76</td>
<td>23.78 ± 3.01</td>
<td>32.59 ± 5.09</td>
<td>5.02 ± 1.36</td>
</tr>
<tr>
<td>Others</td>
<td>5</td>
<td>0.98 ± 0.53</td>
<td>28.06 ± 3.03</td>
<td>4.46 ± 0.61</td>
<td>6.85 ± 1.21</td>
<td>27.29 ± 2.18</td>
<td>27.35 ± 3.26</td>
<td>5.26 ± 0.06</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>33</td>
<td>0.86 ± 0.53</td>
<td>25.04 ± 1.94</td>
<td>3.34 ± 0.91</td>
<td>7.22 ± 1.10</td>
<td>23.64 ± 3.45</td>
<td>34.59 ± 1.52</td>
<td>3.92 ± 1.55</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.005

Mean values significantly different from the corresponding value for the normal subjects (student t). * Differences significantly from the corresponding value for the normal subjects and diabetics used as control of chronic diseases (student t).
Arachidonic acid levels, on the other hand, were lower among diabetics than normal subjects (p<0.05), whereas collagen disease patients did not show a significant decrease in these values.

Table 2 Sexual difference of plasma linoleic acid

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Linoleic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>27</td>
<td>34.1±3.9*</td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>36.4±3.2*</td>
</tr>
</tbody>
</table>

*p<0.05

*Differs significantly from the corresponding value for both sexes.

Table 3 Age distribution of plasma linoleic acid composition of total lipids control subjects

<table>
<thead>
<tr>
<th>Age</th>
<th>20-29</th>
<th>30-39</th>
<th>40-49</th>
<th>50-59</th>
<th>60-69</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>34.9±2.3</td>
<td>36.5±3.4</td>
<td>35.5±4.6</td>
<td>34.4±2.6</td>
<td>32.9±3.7</td>
<td>35.0±2.9</td>
</tr>
</tbody>
</table>

*p<0.01, **p<0.001, ***p<0.0001

C. Factors Affecting Plasma Linoleic Acid Composition

Demographics

Plasma linoleic acid levels were significantly higher among female than male control subjects (p<0.05) (Table 2). This emphasizes the fact that the decrease in plasma linoleic acid levels in collagen disease was of considerable value and significance, since a large majority of the patients studied were female.

Plasma linoleic acid levels were the highest in the 30-39 age group (Table 3, Fig. 1) and tended to decline thereafter with age. There were statistically significant differences in linoleic acid levels between those in this age group and those in the 20-29 (p<0.01), 40-49 (p<0.01) and greater-than-59 (p<0.01) age groups, respectively.

Comparing collagen disease patients with controls by decades in the 30-39 age range, plasma

Fig. 1 Collagen disease patients

Fig. 2 Changes of plasma linoleic acid composition of total lipids in collagen disease

Table 4 Changes of plasma linoleic acid composition of total lipids in collagen disease

A. Negative and positive groups of GRP test

<table>
<thead>
<tr>
<th>GRP test</th>
<th>N</th>
<th>Linoleic acid levels</th>
<th>Decrease</th>
<th>Decrease/Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>18</td>
<td>8</td>
<td>10</td>
<td>55.6%</td>
</tr>
<tr>
<td>Positive</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

RA***

B. Anemic and non-anemic groups

<table>
<thead>
<tr>
<th>GRP test</th>
<th>N</th>
<th>Linoleic acid levels</th>
<th>Decrease</th>
<th>Decrease/Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>25.0%</td>
</tr>
<tr>
<td>Positive</td>
<td>15</td>
<td>0</td>
<td>15</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Differs significantly from the corresponding value between 20-29 and 30-39 yrs of age*, 30-39 and 40-49 yrs of age**, 30-39 and 50-59 yrs of age*** (Student's t).

Differs significantly from the corresponding value for the control subjects divided into a decade (30-39***, 40-49*** and 50-59 yrs of age* (Student's t).
linoleic acid levels were significantly lower in the former than the latter (30.0 vs. 30.0 ng/ml, p < 0.05; 40.0 vs. 40.0 ng/ml, p < 0.05). Duration of Morbidity. There was no relationship between the plasma linoleic acid levels and the duration of morbidity of this disease (Fig. 2).

Clinical Differences. Inflammatory Reaction (Table 4A): A significant decrease in plasma linoleic acid levels was observed among patients with a positive CRP test (p < 0.005) and those with RA, but not among those with SLE.

Anemia (Table 4B): Significant decreases in plasma linoleic acid levels were observed among collagen disease patients with anemia as indicated by their Hb and Hct (p < 0.05 and p < 0.025, respectively).

Others: No significant differences between plasma linoleic acid levels and changes of plasma lipids (cholesterol, triglycerides, phospholipids and MEFA) or protein levels (albumin, lipoprotein and γ-globulin) were observed in collagen diseases.

Table 5 Platelet linoleic and arachidonic acid composition of total lipids in collagen disease patients

<table>
<thead>
<tr>
<th></th>
<th>C18:2</th>
<th>C18:4</th>
<th>AL-index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subject</td>
<td>5</td>
<td>14.0±1.2</td>
<td>19.8±0.8</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>7</td>
<td>13.8±1.7</td>
<td>17.1±1.7*</td>
</tr>
<tr>
<td>Lupus erythematosus</td>
<td>2</td>
<td>13.1±2.2</td>
<td>19.3±0.1</td>
</tr>
</tbody>
</table>

* **Diffs significantly from the corresponding value for the normal subjects (student t).

Table 6 Comparison of fatty acids composition and prostaglandins levels between rheumatoid arthritis and osteo-arthritis in synovial fluid

A. Fatty acids composition of total lipids (%)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>0.75</td>
<td>±0.37</td>
<td>±1.93</td>
<td>±1.19</td>
<td>±0.50</td>
<td>±2.17</td>
<td>±4.66</td>
<td>±0.30</td>
<td>±1.25</td>
<td>±0.18</td>
<td>±1.78</td>
</tr>
<tr>
<td>OA</td>
<td>0.40</td>
<td>±0.36</td>
<td>±1.97</td>
<td>±1.71</td>
<td>±0.82</td>
<td>±2.59</td>
<td>±3.20</td>
<td>±0.66</td>
<td>±1.80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B. Prostaglandins levels (mg/ml)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>IPGE*</th>
<th>IPGF1**</th>
<th>IPGF2*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>6</td>
<td>2.19±0.48</td>
<td>0.52±0.16</td>
<td>0.61±1.36</td>
</tr>
<tr>
<td>Osteo-arthritis</td>
<td>4</td>
<td>1.31±0.58</td>
<td>0.17±0.07</td>
<td>0.21±0.06</td>
</tr>
</tbody>
</table>

**Diffs significantly from the corresponding value of IPGF2* between rheumatoid arthritis and osteo-arthritis patients (student t).

**Diffs significantly from the corresponding value of IPGF2* between rheumatoid arthritis and osteo-arthritis patients (student t).

Fig. 3 Relationship between essential fatty acid levels and prostaglandin levels of synovial fluid in collagen disease

**DISCUSSION**

Essential fatty acids, such as linoleic, linolenic and arachidonic acids, are indispensable for the maintenance and renewal of organs.

In rats, the requirement for linoleate was estimated to be 1% of the calories. That for EPA varies depending on differences in age, sex and diet (number of calories and composition of lipids, protein and carbohydrates). At the same time, if this requirement increases due to pathological conditions, increased phospholipid turnover and partial disorganization of the enzymes on the lipoprotein membranes result. Abnormally lowered levels of linoleic and arachidonic acids in plasma and organs have been found in a number of disorders, including eczema, psoriasis, chronic cystic fibrosis, multiple sclerosis, infantile hyperalimentation, liver diseases and diabetes. The reason for this decrease of EFA has not yet been clarified, nor have studies determined how EFA act as precursors of prostaglandins. In our study, we found a decrease in plasma linoleic acid levels among collagen disease patients. In addition, we found that, among control subjects, males had higher levels of linoleic acid than females.

This shows that the decrease in plasma linoleic acid levels in collagen disease was of considerable clinical value and significance, because a large majority of our patients in this study were female.

Hagenfeld and others, following treatment with indomethacin, the plasma free arachidonic acid in male RA patients was increased, and we found that no correlation between the duration of the disease and plasma linoleic acid levels was observed usually, parallelism between the duration of the disease and the dose of drugs used for medication is observed. We also confirmed that the consumption rate of linoleic acid in tumor cells was unchanged with the addition of clinical doses of aspirin-like drugs and steroids, in vitro. These indicate that the decrease in plasma linoleic acid is not the result of the use of anti-inflammatory...
drugs in collagen disease patients. On the other hand, a decrease in proline acid levels in plasma was observed in 2 groups of collagen disease patients. RA patients with positive CRP test and collagen disease patients with anemia. We did not find a relationship between plasma proline acid levels and nutritional indicators such as plasma protein and lipid levels. Brown et al. reported that leucocytepenia was observed but anemia was not in adult patients with EFA deficiency induced by feeding low fat diets. Samson, et al, reported that anemia in RA patients might result from abnormalities of hemoglobin metabolism. Zierler et al. reported that PGE1 treated NZB/NZW F1 mice were protected against the development of anemia, clinical nephritis and death. The results of this study show that the anemia seen in collagen disease patients may be induced by inflammation. We found that there was a significant decrease in proline acid levels among RA patients with positive CRP and collagen disease patients with anemia. Thus, a decrease in plasma proline acid levels in total liquids may reflect an increased EFA requirement, which, in turn, is due to inflammation. However, in order to prove this hypothesis, the decreased levels of EFA in inflammatory organs must first be confirmed.

To this end, we studied EFA levels in platelets. The role of platelets in inflammation has yet to be clarified. However, platelets secrete inflammatory mediators and chemotaxic factors when stimulated. Moreover, due to their Fc receptors, platelets recognize an invasion and initiate phagocytosis. This suggests that platelets may play an important role in inflammation. Hutchinson et al. reported that shortened platelet survival and increased platelet turnover were observed in RA patients. This indicates that platelets may be injured in RA.

In this study, we observed a decrease in arachidonic acid levels and in the AL index in platelets. We have already reported that a decrease of the AL index was accompanied by increased PG generation when thrombin was added to platelet suspensions. Thus, the above-mentioned decrease in arachidonic acid and the AL index resulted from an increased requirement for arachidonic acid in RA patients. Lastly, we studied EFA and PG levels in the synovial fluid of RA and OA patients to confirm whether or not the decreased EFA levels resulted from an increased requirement for PG in RA patients. We then found that higher levels of iPGF2 and iPGF3 were in the synovial fluid were observed among the RA patients than the OA patients and that there is a correlation between the iPGF2 and EFA levels. This indicates that the decreased EFA levels in RA might be ascribable to an increased PG requirement.

Other workers reported that the biosynthesis of PGs is reduced in tissues from animals deficient in EFA. This leads to the possibility that EFA concentrations in a particular lipid, which is the precursor pool for PG synthesis, could determine the relative amounts of PGs generated by a given organ.

In this study, however, we found that there was a negative correlation between linoleic acid levels and iPGF2 levels in synovial fluid. Thus, PG generation would be increased in RA patients. In addition, decreased levels of linoleic acid in plasma might be caused by an increased PG requirement resulting from inflammation in collagen diseases.

REFERENCES
14) Goetz EG, Goldman DW : The role of lipoxy- genase products of arachidonic acid in the cellular component of the inflammatory reactions. Interna- tional symposium on leukotrienes and other lipoxy- genase products, abstract p.27, Florence, 1981
27) Bowes OY, Stally AV : Effects of age, sex, gastritis and the administration of tetanus and estradiol on level of serum cholesterol ester fatty acids in rats. Endocrinology 76 : 1047–1054, 1965
37) Tojiima T, Ueda N : In press.
40) Nachman RL, Wecker B, Ferris B : Character- ization of human platelet vascular permeability-
Immunological Studies in Juvenile Rheumatoid Arthritis

Yoshimasa ABE and Nobuo WATANABE

Department of Pediatrics, Kyorin University School of Medicine, Shinkawa, Mitaka-city, Tokyo 181

Immunoserological studies were performed on 21 patients with juvenile rheumatoid arthritis (JRA). Three patients were found to have hypogammaglobulinemia IgA, 2 patients were negative in the Candida skin test for delayed hypersensitivity and 2 others showed decreased E rosette-forming cells. The assay of PHA-induced lymphocyte blastogenesis revealed normal values in all the patients tested with the mitogen at a concentration of 15 μg/ml, whereas at reduced concentrations of 7.5 to 10 μg/ml, occasional patients showed low values. The K cell population of patients showed no significant difference from that in normal controls. However, of note, this immunologic parameter fluctuated with the course of the disease and markedly diminished shortly prior to relapse.

INTRODUCTION

In recent years, increasing attention has been directed toward the relationship of immune deficiency syndromes and rheumatic diseases, where a close bearing of abnormalities in humoral and cellular immune function upon those disease states has been suggested. As yet, the underlying immunopathogenic mechanism is not sufficiently clear. A considerable number of findings have been reported in the literature with respect to the interrelation between T and B lymphocytes in the peripheral blood and those in the synovial fluid, decreased complement titer in the synovial fluid and lymphocyte activation by phytohemagglutinin (PHA) with blastoid transformation of the cells in vitro in juvenile rheumatoid arthritis (JRA). This study was performed to investigate immunologic abnormalities in JRA by assessing the interrelation of PHA concentration and lymphocyte activation in the lymphocyte blastogenesis assay, reactions in the skin test with Candida antigen and the killer (K) cell population as determined by the hemolytic plaque assay as well as by methods employed in previously reported studies, i.e., determination of serum immunoglobulin levels, complement titration and estimation of T and B cell subpopulations of circulating lymphocytes.

PROCEDURES

Immunoglobulins. Serum levels of IgG, IgA and IgM were determined by the single radial immunodiffusion method.

Serum Complement Titer. The assay of complement function was carried out by the 50% hemolysis technique (CH50) of Mayer which is generally recognized as the test with the greatest reproducibility.

Candida Skin Test. Delayed hypersensitivity skin testing was performed using a Candida broth cul-
Lymphocyte Subpopulations. The subpopulation of T lymphocytes was measured by the assay for rosette-forming cells with sheep erythrocytes (E rosettes). The percentage of EA (erythrocyte-antibody) rosetting lymphocytes, a B cell marker, was also determined by a similar assay with sheep red cells.

Lymphocyte Blastogenesis with PHA. Cultures of lymphocytes purified from patient peripheral blood were set up in TC Medium 199 with added PHA-P at concentrations of 3.75, 7.5, 15.0 and 22.5 µg per ml and incubated. The rate of activated lymphocytes with blastoid transformation was determined in comparison with that in control cultures free of the mitogen.

K Cell Population. The conventional assay of antibody-dependent cell-mediated cytolysis primarily quantitates the activity of effector cells in vivo by measurement of the ^51Cr release. In 1975, Biberfeld et al.13 introduced a new method of direct K cell identification by observing hemolytic plaque-forming cells in lymphocytes with monolayer sheep erythrocytes as target cells. The K cell population was determined in this investigation by the plaque assay for ADCC in microtiter plates as modified by Zeniya et al.14 The brief outline of the procedure is illustrated in Fig. 1.

Sheep erythrocytes (SRBC), as target cells suspended in phosphate buffered saline (PBS) at a concentration of 1 x 10^5 cells per ml, were pipetted into wells of a microtiter plate (Falcon 3054), 10 µl in each well. After centrifugation at 350g for 10 minutes, the plate was inverted and left in that position for 1 hour to remove excessive SRBC and thus obtain monolayers of SRBC.

Patient lymphocytes were purified from 3 ml of heparinized peripheral blood which had been incubated at 37°C for 1 hour with added 0.3 ml of 5% carbonyl iron, by density gradient centrifugation on Ficoll-Conray. The cells were then washed three times with PBS and resuspended in RPMI 1640 containing Heps and supplemented with 10% fetal calf serum, at a concentration of 2 x 10^6 cells per ml, to be used as a lymphocyte suspension. One µl of the lymphocyte suspension and 1 µl of anti-SRBC rabbit IgG (0.5 µg/ml) were then added to each well and the plate was incubated at 37°C for 3 hours, followed by fixation and staining with glutaraldehyde and brilliant cresyl blue in agarose. The plaque-forming cells (PFC) were counted in each population of about 1,000 lymphocytes in wells under the microscope. The percentage of PFC in cultures of patient lymphocytes was calculated, from which the %PFC in control cultures, without addition of anti-SRBC rabbit IgG, was subtracted to estimate the K cell population.

Effects of JRA Serum, Systemic Lupus Erythematosus (SLE) Sera and Heat-Aggregated Human IgG Solution on Normal Lymphocytes. Peripheral blood lymphocytes from healthy subjects were incubated with the following at 37°C for 30 minutes and the per centages of PFC determined by the hemolytic plaque assay.

a. Serum from a JRA patient;

b. Sera from SLE patients (4 sera, inactive, immune complex concentration 7.8-215 µg/ml);

c. Solution in PBS of human IgG aggregated by heating at 63°C for 15 minutes (1,000 µg/ml);

d. Autologous sera pretreated by heating at 63°C for 10 minutes.

SUBJECTS

Twenty-one patients with JRA and 16 normal children, as controls in the assay of K cell population, were studied. The patients ranged in age from 16 months to 15 years and 9 months. Of the patients, 9 were tested for delayed hypersensitivity to intradermal injection of Candida and assessed as to subpopulations of lymphocytes and 15 examined for K cell population.

RESULTS

Immunoglobulin. The serum IgG concentration ranged between 610 and 1,520 mg/dl. Three patients were found to have hypogammaglobulinemia IgA, all of whom showed a gradual elevation of the immunoglobulin and therefore the patient of Case 3 was probably a slow starter rather than a true IgA deficiency. It is noteworthy that 3 children had hypogammaglobulinemia IgA among the 21 JRA patients examined.

Serum Complement Titer. The CHs ranged from 27.0 to 50.0 units per ml, with a mean value of 39.8 units/ml.

Candida Skin Test and Lymphocyte Subpopulations. Two patients were found negative in the Candida skin test and 2 others noted to have decreased E rosette-forming cells. As can be seen from Table 1, the patients of Cases 2 and 3 had hypogammaglobulinemia IgA and negative Candida skin tests and the patient of Case 3 had decreased E rosette-forming cells. Case 2 had been treated with corticosteroids until three years before and was on oral D-penicillamine, alone, at the time of this study, while Case 3 had received or was receiving neither cortico-steroids nor immunosuppressive therapy.

Lymphocyte Blastogenesis with PHA. In no patient was there evidence of abnormality in T cell population when the test was carried out with the usual PHA-P concentration of 15 µg/ml. With lower concentrations of this plant mitogen, however, the rate of activated lymphocytes with blastoid transformation declined significantly in occasional cases as compared to the normal controls (Cases 1 and 3). As for the interrelation between the changes in these immunological parameters and the activity of the disease, hypogammaglobulinemia IgA was observed during the active phase where the de
creased immunoglobulin showed a tendency to rise gradually up to the lower normal limit or slightly less. Depressed lymphocyte blastogenesis persisted over a long period in Case 1. This patient, who had developed the disease with onset of periartitis at 2 years of age, was virtually free from pyrexia and arthralgia and is leading an ordinary daily life though with some disability from joint rigidity and contractures, at present, more than 10 years after onset of the disease.

K Cell Population. In normal infants and children, the mean K cell population was 4.9±1.7% at 1-2 years (n=8) and 5.0±2.0% at 4-15 years (n=8), with an overall average of 5.0±1.9% (n=16).

Thirteen patients with JRA showed a mean K cell population of 4.8±2.4%; hence no significant difference from the normal controls (Fig. 2). However, there were 3 patients with values at or below the lower normal limit, i.e. 1.50, 1.82 and 2.36%; in one of these cases, of note, the value fell to 2.14% from 9.79%, shortly prior to relapse.

As for the effects of SLE sera, JRA serum and heat-aggregated human IgG on normal lymphocytes, assays by incubation with two isologous normal sera revealed values of 111 to 120% (mean: 115%) as distinguished from 100% of the K cell population in control cultures of untreated normal lymphocytes. With three autologous sera, heat-inactivated at 63°C for 10 minutes, the value ranged from 70 to 94% (mean: 83%).

Effect of SLE sera: When normal human lymphocytes were incubated with sera from four inactive SLE patients with circulating immune complex levels of 7.8 to 215 μg/ML IgM (by Clq solid-phase enzyme immunoassay), the K cell population value was found to be 97.5 to 113% with an average of 103.8% (Fig. 3).

Effect of JRA serum: The assay with normal human lymphocytes and a serum of a patient positive for rheumatoid factor (RAHA: 64X) showed a K cell population of 123% (Fig. 3).

Effect of heat-aggregated human IgG: Lymphocytes from three normal subjects were tested with 1,000 μg/ml solution of heat-aggregated human IgG in PBS. The assay revealed a marked reduction in plaque formation, the values found being 1.5, 1.1 and 2.2% as compared to 5.9, 5.4 and 7.1% in untreated controls, respectively. The mean value of the three specimens was 1.6% with heat-aggregated human IgG, compared to 6.1% in the untreated controls (Fig. 4).

**DISCUSSION**

IgA deficiency or hypogammaglobulinemia IgA is the most frequently seen immunologic abnormality in humans. Its incidence varies widely among studies, 0.097 to 0.25%. A report described that 14 out of 324 cases of JRA had hypogammaglobulinemia IgA, thus a noticeably high frequency as compared to controls. In children, arthritis is known to be associated with hypogammaglobulinemia IgA and the clinical features of this condition has been reported to be extremely difficult to distinguish from those of JRA. The patients found to have abnormally low serum levels of IgA in the present series showed a gradual restoration with the disease course and had no corresponding data at the onset of their disease; hence no evidence for its being primary hypogammaglobulinemia IgA. The condition appeared very likely to be hypogammaglobulinemia IgA associated with JRA in these cases. Exploration of the possibilities of defect of IgA-bearing lymphocytes and of the presence of anti-IgA antibody which could be important etiologic factors in these patients, failed to provide any clue to elucidation of the disease state. It is generally assumed that the pathogenesis of hypogammaglobulinemia IgA may consist in a disturbance somewhere in the process from differentiation to synthesis, secretion and catalysis of IgA-producing cells. It would be necessary, therefore, to assess the activity of helper T cells which participate in the differentiation of B lymphocytes into IgA-producing cells.

As for the relation of T cells to JRA, attention has been drawn to this possibility in view of the fact that arthritis is occasionally seen in such disorders as Wiskott-Aldrich syndrome and other T cell deficiencies. Cooper and coworkers observed repetitive arthritis in 4 out of 18 cases of T cell deficiencies examined. In the present series of 21 cases of JRA, 2 were found to show decreased percentages of E rosette-forming cells and the serum IgA concentration was as low as 20.9 mg/dl in one of these cases. This finding stresses the importance of the determination of the T cell subset in JRA patients with hypogammaglobulinemia IgA as well.

Reports of the serum complement titer in JRA, as yet, are few. Cases with CH50 or CH50 deficiency have been described. The serum CH50 is frequently higher than normal despite low complement activity in the synovial fluid.

The lymphocyte blastogenesis assay with PHA is usually performed at a mitogen concentration of 15 μg/ml, while there has been a report demonstrating the highest rate of lymphocyte activation at a PHA-P concentration of 2 μg/ml. In the present investigation, nevertheless, the stimulation of lymphocytes was optimal at mitogen concentrations of 7.5 to 15 μg/ml and, worthy of note, occasional patients displayed lower ratios of lymphocyte blastogenesis than normal controls when the assay was carried out with the plant mitogen at lower concentrations.

The previous data on the K cell population in the patients with JRA and normal children were determined by subtracting the %PFP found in controls without antibody from that in sample
lymphocytes. Since the PFC in the controls included natural killer (NK) cells, the distinction between NK and K cells presents a problem. The K cell population in adults, as determined by the hemolytic plaque assay, is 7.04 ± 1.71% (n = 38) according to Zeniya (1979)\(^{10}\) and 6.6 ± 2.4% (n = 16) according to Naoe et al (1980).\(^{10}\) These values, however, are without the subtraction of the PFC in the controls. In this way, for reference, the value was 5.3 ± 1.8% in normal infants and children and 5.6 ± 2.5% in the JRA patients in this present study.

Isturig (1976)\(^{10}\) and McGill et al. (1977)\(^{14}\) reported that, in adult patients with rheumatoid arthritis, the ADCC determined by the 9Cy release method diminished progressively with increasing activation of the disease. On the contrary, Daz-Jouanne (1976)\(^{10}\) and Panayi (1977)\(^{14}\) observed no difference in ADCC between adult rheumatoid arthritis and normal controls. It was noted by Daz-Jouanne that synovial fluid lymphocytes of RA had remarkably lowered ADCC which did not show any appreciable transient increase even after treatment by incubation for 12-18 hours at 37°C, thereby indicating the depletion of K cells in the synovial fluid lymphocytes possibly in consequence of K cell consumption and sequestration in damaged tissues. Abrahamsen et al. (1977)\(^{13}\) demonstrated a significantly lower ADCC of lymphocytes eluted from the synovial tissue than that of peripheral blood lymphocytes in JRA, as assayed by the 9Cy release method. There has been no report, to our knowledge, of any study of the immunological function of JRA patients in respect to their K cell population.

The experiments to explore the influence of pretreatment of normal lymphocytes on the hemolytic plaque assay of Zeniya et al. revealed some suppression of plaque formation by autologous sera previously heated at 65°C for 10 minutes. There was a marked depression of K cell population in the assay with incubation of normal lymphocytes with heat-aggregated human IgG. This might be due to blockade of the Fc receptor of K cells by the aggregated IgG. Kumagai (1979)\(^{16-17}\) described that sera from SLE patients and RA patients suppressed the ADCC of normal lymphocytes, probably as a result of K cell blockade by immune complexes. However, there was no evidence of inhibition of hemolytic plaque formation by SLE sera or JRA sera in this study. The SLE sera contained immune complexes, though from patients in an inactive phase. Comparative immunological assessments of the effect of immune complex in sera obtained during the active and inactive phases is necessary.

It seems irrational to make a direct comparison between the hemolytic plaque assay and the 9Cy release assay since the former is based on the enumeration of individual effector cells whereas the latter measures the sum of ADCC activity of effector cells. The hemolytic plaque assay is procedurally, fairly simple as it requires neither the use of any radioactive nor the application of cell culture techniques. The hemolytic plaque assay also has a great advantage in that almost a small amount of specimen suffices. A wide-spread clinical application of the K cell population assay by this technique is expected.

CONCLUSIONS

The immunological study of JRA has yielded the following results:

1. Of 24 patients with JRA studied, 3 patients (14%) were found to have hypogammaglobulinemia IgA.
2. The serum complement activity (CH50) ranged from 27.0 to 50.0 units/ml, being within or higher than normal limits for respective ages.
3. Candida skin testing was negative in 2 patients whose percentages of E rosette-forming cells were within normal limits.
4. There were 2 patients in whom decreased percentages of E rosette-forming cells were noted.
5. The lymphocyte blastogenesis assay with PHA did not reveal any abnormality when a usual mitogen concentration of 15 μg/ml was employed, whereas at 7.5-10 μg/ml, occasional cases showed a lowered stimulation of lymphocytes.
6. No significant difference in K cell population was observed between the JRA patients (4.8 ± 4.2%) and normal controls (infants and children; 5.0 ± 1.9%). It was noteworthy, however, that a marked diminution of K cell population occurred shortly prior to relapse of the disease in one of those cases in which this parameter could be serially followed during the course of the illness.
7. Neither sera from JRA patients seropositive for rheumatoid factor nor sera from SLE patients in the inactive phase with demonstrable circulating immune complex exerted any appreciable effect on the assay of K cell population. In contrast, heat-aggregated human IgG was found to markedly suppress hemolytic plaque formation in the assay.

REFERENCES

Extra-Articuler Manifestations of Juvenile Rheumatoid Arthritis

Satoshi FUJIKAWA, Katsutoshi YABANA, Chikara TONOUCHI*, Hiroyuki HIKITA* and Mashiko OHKUNI*

Department of Pediatrics, Tokyo Kyosai Hospital, Nakameguro, Meguro-ku, Tokyo 153
* Department of Pediatrics, Nihon University

The condition of patients with juvenile rheumatoid arthritis (JRA) is sometimes complicated by various extra-articular manifestations, including rheumatoid rash, iridocyclitis, pericarditis, myocarditis, pleural effusion, hepatitis, renal involvement and other problems.

In systemic type JRA, patients often lack articular signs other than slight arthralgia. In some cases, extra-articular signs precede the appearance of arthritis.

In the last 12 years, 49 cases of JRA were observed (systemic 21, polyarticular 19 and pauciarticular 9). Extra-articular manifestations observed among these patients were acute iridocyclitis 9, chronic iridocyclitis 4, pericarditis 7, pleural effusion 2, hepatitis 2, rheumatoid rash 17 and subcutaneous nodules 3.

No patient had nephropathy indicating amyloidosis. This complication is frequently seen in JRA patients in European countries.

Acute iridocyclitis was observed in 9 patients with the systemic type of the disease. This manifestation has not previously been discussed in the literature, but we consider it to be an important and diagnostic sign of systemic type JRA. The condition of the 9 patients with acute iridocyclitis improved with systemic and/or topical steroid therapy without severe sequelae. In the case of chronic and recurrent iridocyclitis, which was observed in the pauciarticular type of the disease, patients may have sequential ocular manifestations including cataracts and glaucoma, and so, there is a small possibility of blindness ensuing.

Pericardial and pleural effusions were observed in systemic cases of the disease and they improved with steroid administration. Histocompatibility antigens (HLA) were studied in these patients. Eighty-five percent of the patients with the polyarticular type had A9, which was a significantly higher percentage than in the control group (46.5%). B27 was not observed in patients nor controls.

INTRODUCTION

Patients with juvenile rheumatoid arthritis (JRA) show various extra-articular manifestations including rash, pericarditis, iridocyclitis, hepatitis, amyloidosis, HLA-antigen, iridocyclitis, juvenile rheumatoid arthritis, pericarditis

Keywords: amyloidosis, HLA-antigen, iridocyclitis, juvenile rheumatoid arthritis, pericarditis

† To whom reprint requests should be addressed.
appear before the onset of arthritis in the pauci-
articular type of JRA.6,7

Acute iridocyclitis was also confirmed in our
patients having systemic type JRA.

This paper presents the results of studies con-
ducted over the past 12 years with JRA patients
having extra-articular manifestations. Three short
of which show extra-articu-
lar signs without arthritis during a certain period
of an extended illness.

MATERIAL AND METHODS

Forty-nine patients with JRA had been receiv-
ing treatment and consultation at the Department
of Pediatrics of Niho University and at Tokyo
Kyorai Hospital between 1969 and 1981. The
diagnosis for this disease was based on the criteria
established by Groedest-Grossman.8 The pa-
patients were classified into 3 groups according to
the modes of onset of their disease based on varying
their degrees of systemic and articular involvement.
That is, (1) systemic type; characterized by high
fever, rash, lymphadenopathy and leukocytosis,
(2) polyarticular type; characterized by the simulta-
aneous involvement of four or more joints, and
(3) pauciarticular type; characterized by the in-
volve ment of a single or less than four joints.
The number of patients by type of JRA at the
onset of the disease were 21 systemic, 19 poly-
articular and 9 pauciarticular. Every patient,
even systemic cases at onset, underwent an oph-
thalmological examination during the first visit
to the hospital and received a regular microbiolo-
gical and hematologic test afterwards. Echocardigrams
were studied starting in 1975. Histocompati-
bility antigens of loci A, B, and DR were deter-
mcd in some of these patients. The determina-
tion of HLA antigens were performed by the NIH
lymphocytotoxicity inhibition test.

RESULTS

Extra-articular manifestations observed among
49 patients with JRA were as follows: acute
iridocyclitis, chronic iridocyclitis, pericarditis, pleu-

rity, hepatitis, rheumatoid rash and subcutaneous
nODULES (Table 1).

Acute iridocyclitis was recognized in 9 patients
with the systemic type of JRA. All of the pa-
tients with acute iridocyclitis were diagnosed by
the microbiological slit-lamp test. In all but one,
ophthalmo logical abnormalities were improved
with systemic and/or topical steroid therapy
without any subsequent sequelae (Table 2). Two
of the nine patients showed positive antinuclear
factor (ANF). Acute iridocyclitis was observed during
a period of poly cyclic episodes in one case of
the systemic type. Chronic and recurrent
iridocyclitis in the pauciarticular type of JRA have
been well discussed in previous reports, but no
literature has described acute iridocyclitis in the
systemic type of this disease. The authors believe
that this sign is an important and diagnostic mani-
festation of systemic JRA. Thus, regular oph-
thalmological examinations are required to see to
not overlook this manifestation in any patient with
any type of JRA.

Chronic and recurrent iridocyclitis was ob-
served in 3 patients with the pauciarticular type
of JRA and in one patient with the polyarticular
type. One of these patients had recurrent irido-
cyclitis for almost one year prior to the onset of her
pauciarticular type of arthritis (Case 1) (Table 3).
Pericarditis was observed in 7 cases of the sys-

temic type. Some of them were diagnosed only
by echocardigram. Two patients with peri-
carditis also had pleurisy. None of these patients
had cardiac tamponade. All of them improved
systemic steroid therapy (Table 4).

Hepatitis was observed in 2 systemic type pa-
tients. A boy who had a polycyclic systemic

Table 1 Extra-articular manifestations of patients
with JRA. Acute iridocyclitis, pericarditis, pleural
iffusion and hepatitis were complications found in
the systemic type of the disease.

<table>
<thead>
<tr>
<th>Ocular involvement</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute iridocyclitis</td>
<td>9</td>
</tr>
<tr>
<td>Chronic iridocyclitis</td>
<td>4</td>
</tr>
<tr>
<td>Cardiac involvement</td>
<td>7</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>7</td>
</tr>
<tr>
<td>Myocarditis</td>
<td>0</td>
</tr>
<tr>
<td>Pulmonary involvement</td>
<td>2</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>2</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>2</td>
</tr>
<tr>
<td>Rheumatoid rash</td>
<td>17</td>
</tr>
<tr>
<td>Rheumatoid nodules</td>
<td>3</td>
</tr>
<tr>
<td>Nephropathy</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 5 Frequency of HLA antigens in patients with JRA. HLA-A9 was observed in 85.5% of the patients the polyarticular type which was markedly higher than that of the controls (46.5%).

<table>
<thead>
<tr>
<th>HLA Antigens</th>
<th>Systemic type (N=13)</th>
<th>Polyarticular type (N=21)</th>
<th>Pauciartricular type (N=12)</th>
<th>Controls (N=187)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A locus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>5 38.5%</td>
<td>9 42.9%</td>
<td>8 66.7%</td>
<td>60 32.1%</td>
</tr>
<tr>
<td>A9</td>
<td>6 46.2%</td>
<td>18 85.8%</td>
<td>4 33.3%</td>
<td>87 46.5%</td>
</tr>
<tr>
<td>A10</td>
<td>4 30.8%</td>
<td>3 14.3%</td>
<td>3 25.0%</td>
<td>28 15.0%</td>
</tr>
<tr>
<td>A11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A23</td>
<td>1 7.7</td>
<td>1 4.8</td>
<td>1 8.3</td>
<td>18 9.6</td>
</tr>
<tr>
<td>B locus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bw21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bw35</td>
<td>1 7.7</td>
<td>1 4.8</td>
<td>1 8.3</td>
<td>2 1.1</td>
</tr>
<tr>
<td>Bw36</td>
<td>1 7.7</td>
<td>1 4.8</td>
<td>1 8.3</td>
<td>10 5.3</td>
</tr>
<tr>
<td>DR1</td>
<td>0</td>
<td>1 5.9%</td>
<td>0</td>
<td>1 2.9%</td>
</tr>
<tr>
<td>DR2</td>
<td>5 55.6%</td>
<td>7 41.2%</td>
<td>3 27.5%</td>
<td>15 36.1%</td>
</tr>
<tr>
<td>DR3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DR4</td>
<td>6 66.7%</td>
<td>11 64.4%</td>
<td>6 75.0%</td>
<td>21 58.5%</td>
</tr>
<tr>
<td>DR5</td>
<td>4 44.4%</td>
<td>8 47.1%</td>
<td>5 62.5%</td>
<td>13 62.5%</td>
</tr>
</tbody>
</table>

Table 6 Frequency of HLA-DR antigens in patients with JRA. No specific finding was observed concerning these antigens.

<table>
<thead>
<tr>
<th>HLA-DR Antigens</th>
<th>Systemic type (N=9)</th>
<th>Polyarticular type (N=17)</th>
<th>Pauciartricular type (N=8)</th>
<th>Controls (N=36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1</td>
<td>0</td>
<td>1 5.9%</td>
<td>0</td>
<td>1 2.9%</td>
</tr>
<tr>
<td>DR2</td>
<td>5 55.6%</td>
<td>7 41.2%</td>
<td>3 37.5%</td>
<td>15 36.1%</td>
</tr>
<tr>
<td>DR3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DR4</td>
<td>6 66.7%</td>
<td>11 64.4%</td>
<td>6 75.0%</td>
<td>21 58.5%</td>
</tr>
<tr>
<td>DR5</td>
<td>4 44.4%</td>
<td>8 47.1%</td>
<td>5 62.5%</td>
<td>13 62.5%</td>
</tr>
</tbody>
</table>

of the control group (p<0.005). HLA-B27 was not found in any patient nor in control children.

No common antigens of DR loci were confirmed (Table 7).

Three cases among the 49 patients showed the absence of any articular manifestation during a certain period of an extended illness.

CASE 1

T.M. (female) (Table 3, case No. 3) A 5 year 3 month old girl who had a first attack of iridocyclitis, and then within the following single year, she had five further episodes of irido-
cyclitis. She visited the Department of Pediatrics of Nihon University Hospital at the age of 6 when she had fever, iridocyclitis and bilateral knee and wrist joint swelling. A subcutaneous nodule was palpable at her right wrist.

Laboratory findings on admission revealed: CRP, +3; ESR, 27 mm/hr; WBCs, 4700/mm cu; RF, negative; ANF, negative; γ-globulin, 19.9%.

Her arthritis and iritis improved following prednisone 40mg/day. After this episode, she entered the pauciarticular course of the disease with recurrent iridocyclitis which did not parallel the activity of her arthritis.

CASE 2

T.T. (male) (Table 4, case No. 5)

His onset of the disease began with spiking fever and arthralgia at the age of 2 (Feb. 1971). Following that occasion, he had several episodes of polycyclic attacks. In May 1971, he had pyrexia, rheumatoid rash, cervical lymph nodes swelling and acute iridocyclitis.

Laboratory studies on that occasion: WBCs, 15,000/mm cu; ESR, 78 mm/hr; CRP, +6 and negative RF.

In July 1973, he was admitted to the Department of Pediatrics, Nihon University Hospital, because of intermittent fever and back pain. Pericardial and pleural effusions were observed on chest X-rays (CTR, 50%). An ECG showed elevated ST segment in leads 1, 11, V4, V5 and V6. Prednisone, 30mg/day, and diuretics were started, and his condition improved within several weeks. In March 1976, he had another episode of pericarditis with rheumatoid rash without any joint manifestation.

CASE 3

H.Y. (female) (Table 2, case No. 9)

This girl became ill at the age of 2 with fever, iridocyclitis and arthralgia, and she had recurrent episodes of fever, rheumatoid rash and arthritis. In March 1979, she visited the Department of Pediatrics, Nihon University Hospital, with complaints of recurrent but transient iridocyclitis, persistent rheumatoid rash and occasional arthralgia. Her arthritis and arthralgia was also transient and chiefly limited to the knee and ankle joints.

Her laboratory results were as follows: WBCs, 14,900/mm cu with 56%, PMN; hemoglobin, 10.1g/dl; ESR 50mm/hr; and negative RF and ANF.

Following this episode, she had recurrent attacks of spiking fever, arthralgia and rheumatoid rash. Her rheumatoid rash has continued for the past 3 years without arthritis. Her iridocyclitis was recurrent but transient and improved with local steroid therapy. No ocular sequela has been observed as yet.

DISCUSSION

Juvenile rheumatoid arthritis is a systemic disease and may affect many organs other than joints.1-3 In certain patients, however, extra-articular manifestations overshadow the articular signs and symptoms, and occasionally these manifestations are the only symptoms of juvenile rheumatoid arthritis. This phenomenon may chiefly be seen in patients with the systemic type of the disease.8 The typical complications of JRA are rheumatoid rash, rheumatoid nodules, cardiac findings (pericarditis and myocarditis), pulmonary (pleural effusion and fibrosis), ocular (iridocyclitis, band kerato-pathy and glaucoma), muscular disease, blood disease, hepatitis, renal involvement secondary to amyloidosis and other problems. Chronic and recurrent iridocyclitis is a common complication of the pauciarticular type of JRA, and this fact and the associated conditions have been well documented in many writings.4-6 The overall incidence of iridocyclitis reported for patients with JRA it is 5 to 15%, and its frequency is very high in pauciarticular patients. It may occur several years prior to articular manifestations and at any time during its course or during subsequent remissions.8-9 Almost all patients with iridocyclitis have no subjective complaints about their eyes when the inflammation is limited to the anterior uveal tract, posterior synchiae, irregular pupils or the delay of light reaction. These changes, however, may develop into glaucoma, cataracts and bank keratopathy. In our patients, chronic and recurrent iridocyclitis was observed in 4 of 9 patients with pauciarticular JRA. One girl had to undergo an operation for a cataract formed secondary to chronic iritis. Also, as shown in case 1, iridocyclitis occasionally precedes arthritis. During the period prior to the appearance of arthritis, iridocyclitis was the only manifestation of JRA in this patient. In spite of an extensive survey of the literature, the authors could not find any description of acute iridocyclitis in systemic type of JRA. In this series of 49 patients, 9 cases had acute iridocyclitis during the course of this disease. Almost all of these patients were of the systemic course or patients of the polyarticular type with severe systemic involvement including spiking fever, rash, pericarditis or other complications (case 2). All patients with acute iridocyclitis improved with systemic or topical steroid therapy without severe sequelae. From these results, we recommend ophthalmological examinations not only for patients with the pau-
ciarticular type but also for patients with systemic or polyarticular types with severe general complications, in order to avoid overlooking this ocular complication.

Pericarditis is also one of the common complications of JRA observed during the acute and active phase. Diagnosis of this condition is most readily made by echocardiography, which is more sensitive to the diagnosis as compared to chest X-rays or electrocardiography. B.H. Bernstein and others reported their results on echocardiography for the diagnosis of pericarditis in JRA patients. Almost 88% of their patients with the systemic type of JRA had pericardial effusion. In our series, 7 of 21 of the systemic type patients had pericardial effusion (33%). This, however, included patients who were examined before echocardiography was introduced for diagnosis.

Pleural effusion is occasionally associated with pericarditis in JRA. In our patients, 2 of 9 cases with pericarditis had pleural effusion.

Myocarditis is rarely recognized in patients with JRA and such cases usually develop congestive heart failure. In our series, no patient had myocarditis.

Hepatitis is an uncommon complication in patients with JRA. Two of 49 patients in our series had hepatic involvement, and one died in hepatic coma. Both of them has pyrexia, rash and other systemic complications. Neither had positive RF, ANF or HB antigen. A specific correlation between anti-inflammatory drugs and hepatic damage was not recognized. The boy who died of hepatic coma, in particular, had jaundice before drug therapy was given.

The etiology of hepatic damage in JRA is obscure as is the etiology of JRA itself. Amyloidosis should be considered as an etiologic factor, but liver involvement in JRA is observed chiefly in children with the systemic course; on the other hand, amyloidosis is frequently observed in the chronic polyarticular type. Histologic findings were not confirmed in our cases because of family objections. Amyloidosis is considered less frequent among Japanese children with JRA as compared to that of European or American patients. The data analyzed under the Research Program for Chronic Children’s Diseases of the Japanese Ministry of Health and Welfare in 1978, showed that 4 of 230 cases with JRA died during the one-year period studied. The cause of death of these patients were hypertensive congestive heart failure, intracranial bleeding, Waterhouse-Friderichen’s syndrome secondary to varicella infection and one obscure cause. Thus, only one case who died of hypertension suggested renal involvement due to amyloidosis. Research results concerning the cause of death of JRA patients reported in Japan are sporadic.

Recently, the study of histocompatibility antigens (HLA) on rheumatic disorders in children has produced good results. These disorders include ankylosing spondylitis (AS), Reiter’s syndrome, acute uveitis and inflammatory bowel disease with sarcoidosis. Brewerton et al. demonstrated B27 in 95% of the patients with AS and 55% of their asymptomatic first degree relatives. Concerning JRA, Raclelefsky et al. demonstrated B27 in 48% of the patients with JRA, but many reports have highly criticized their results. In our study, 85% of the patients with the polyarticular type of disease had HLA-DR, on the other hand, control children only had 46% (p<0.005). No patient, however, had B27 antigen. The study of HLA antigens seems important for the differential diagnosis of JRA and AS, especially for seronegative male patients with late onset pauciarticular disease.

REFERENCES
8. Jose DG: Irido-cyclitis and pauciarticular juven-

Studies on Lupus Nephritis

Yasu MATSUOKA, Michinori KOHNO, Shoichiro IRIMAI and Ippei FUJIMORI

Department of Medicine, Kawasaki Municipal Hospital, Shinkawa-dori, Kawasaki-ku, Kawasaki 211

Prognosis of 67 cases of systemic lupus erythematosus (SLE) was studied, based mainly on urinary findings and renal histology. The following results were obtained.

1. Diffuse glomerular changes such as diffuse proliferative lupus nephritis was found even in SLE patients without proteinuria.
2. Raynaud’s phenomenon and positive rheumatoid factors were more common among urornerative cases, especially among patients with minimal glomerular changes and mesangial lupus nephritis.
3. Uronegative patients never developed renal failure regardless of their renal histology, in contrast with urorpositive patients with diffuse proliferative lupus nephritis.

The authors emphasized the imprantance of urinary findings and renal histology as well as extrarenal symptoms and immunological tests in determining the prognosis of SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic disease with multiorgan involvement. Lupus nephritis is the most important complication that determines the prognosis of SLE, since uremia is the most frequent cause of death in this disease.

Lupus nephritis, in contrast with ordinary chronic glomerulonephritis, may respond to adrenocorticosteroids and immunosuppressants clinically as well as histopathologically (as proven by renal biopsy). Acquaintance with lupus nephritis and its management is therefore very important in the treatment of SLE.

There are many reports on the histopathology of lupus nephritis. Baldwin et al. examined its renal histology from the prognostic point of view.

It is not always easy to distinguish the prognosis from that of renal pathology alone. This report deals with lupus nephritis as stratified by urinary and renal histologic findings. Each group of lupus nephritis cases was correlated with renal and extrarenal involvement, immunological examination, prognosis and cause of death.

MATERIALS AND METHODS

Sixty-seven cases of SLE that satisfied at least four items of ARA preliminary criteria for SLE were subjected to renal examination by biopsy or necropsy. Forty-nine patients received renal biopsy between 1972 and 1978. Eighteen patients were autopsied between 1969 and 1978. Seven patients were male and 60 were female with an average age of onset 27.4 yrs and of the first diagnosis 29.8 yrs.

Urinary Findings. Positive proteinuria: Proteinuria (more than 300 mg/day or 30 mg/dl) persisted more than one month within one year after the first diagnosis was made.

Keywords: immunological test, lupus nephritis, prognosis/SLE, systemic lupus erythematosus (SLE), symptoms/extrarenal

† To whom reprint requests should be addressed.
Negative proteinuria: No proteinuria or proteinuria, including persistent proteinuria, appeared more than one year after the first diagnosis was made.

**Renal Histology.** Percutaneous renal biopsy was done under the X-ray fluorescence with a Tru-Cut Needle. Serum creatinine levels above 2.0 mg/dl were considered as contraindications of renal biopsy, and patients with these levels were excluded. Specimens from renal biopsy or autopsy were fixed with buffered 10% Formalin (also with Dulbecco-Brazil fixing solution in a few cases), and stained by hematoxylin-eosin, periodic acid-Schiff and periodic acid methenamin silver. Renal histology was classified according to the WHO classification of glomerular changes and also to Baldwin's classification with slight modification (i.e. we added "minimal change") (See Table 1).

**Clinical Symptoms.** Extraossal symptoms which appear in the ARA preliminary criteria for SLE were searched for in each case through the entire clinical course. Tests for renal symptoms included determination of the serum creatinine level and creatinine clearance rate. Data on creatinine clearance in patients with hyperaesthesia were discarded.

**Immunological Tests.** Antinuclear antibody, anitDNA antibody, complement and rheumatoid factors were determined at the first diagnosis. Antinuclear antibody was assayed by the indirect immunofluorescence technique using rat liver or chicken erythrocytes. Anti-DNA antibody was assayed by the passive hemagglutination test. Serum complement was represented by β2C globulin. Latex fixation test (LFT) and rheumatoid arthritis hemagglutination test (RAHA) were regarded as positive when the titer was above 80.

**Causa of Death.** Causes of death were classified as uremia, CVA, infection and others. In some fatal cases, uremia was not the direct cause of death, yet the serum creatinine level was above 2.0 mg/dl and hypertension was sustained. Such a case was recorded as "complicated with renal failure.”

## RESULTS

**Renal Involvement**

**Urinary Findings.** Urinary protein was negative in 35 cases and positive in 32 cases. Among 35 negative cases, 6 patients showed transient proteinuria i.e. within one month's duration) which was attributed to febrile aluminuria (3 cases), UTI (7), and cardiac failure (1). Five patients developed persistent proteinuria more than one year after the diagnosis was made. Among the 32 patients with proteinuria, 21 had a nephrotic syndrome and 26 showed urinary cell casts.

**Renal Histology.** All renal specimens from either biopsy or autopsy showed some abnormalities and none of them was intact. The results obtained were as follows: minimal change (Min) was found in 18 cases, mesangial lupus nephritis (Mes LN) in 16 cases, focal proliferative lupus nephritis (FPLN) in 6 cases, membranous lupus nephritis (Mem LN) in 10 cases, diffuse proliferative lupus nephritis (DPLN) in 17 cases.

**Relation between Urinary Findings and Renal Histology (Table 2).** Among the 35 cases without proteinuria, 15 cases of Min, 11 cases of Mes LN, 3 cases of Mem LN and 3 cases of DPLN were found. In contrast, 32 cases with proteinuria consisted of 14 cases of DPLN (12 of nephrotic syndrome), 7 cases of Mem LN (5 of nephrotic syndrome), 5 cases of Mes LN (1 of nephrotic syndrome), 3 cases of FPLN (1 of nephrotic syndrome) and 3 cases of Min (2 of nephrotic syndrome). Five patients who showed no proteinuria at the time of the first diagnosis but later developed proteinuria were divided into three types: two cases of Min, one case of Mes LN and two cases of DPLN. One of these two patients with Min later developed DPLN. As shown by the life table method, 87.1% of the patients without proteinuria showed persistent negative proteinuria in the 5th year, and 81.9% in the 10th year. Persistent negative proteinuria was seen in 33.8% of the DPLN cases, 83% of Mes LN and 92% of Min. Among the patients with proteinuria at the time SLE was diagnosed, four patients (Min 2, Mes LN 1, and FPLN 1) later became free of proteinuria. Seven cases of intermittent proteinuria were classified as Mes LN (1 case), FPLN (2), Mem LN (2), and DPLN (2). The remainder, i.e. patients with persistent proteinuria (21 cases), were defined as Min (1 case), Mes LN (3), Mem LN (5) and DPLN (12).

### Table 1: Classification of renal histologic findings

<table>
<thead>
<tr>
<th>WHO</th>
<th>Baldwin et al</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Minor lesions</td>
<td>Minimal change</td>
</tr>
<tr>
<td>B. Focal and/or segmental lesions</td>
<td>Focal proliferative lupus nephritis</td>
</tr>
<tr>
<td>C. Diffuse glomerulosclerosis</td>
<td>Mesangial lupus nephritis</td>
</tr>
<tr>
<td>1. proliferative mesangial glomerulosclerosis</td>
<td>Diffuse proliferative lupus nephritis</td>
</tr>
<tr>
<td>2. proliferative endocapillary glomerulosclerosis</td>
<td>Membranous lupus nephritis</td>
</tr>
<tr>
<td>3. mesangioproliferative glomerulosclerosis</td>
<td></td>
</tr>
<tr>
<td>4. membranous glomerulosclerosis</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Relationship of proteinuria to renal histologic findings

<table>
<thead>
<tr>
<th>Min</th>
<th>Mes LN</th>
<th>FPLN</th>
<th>Mem LN</th>
<th>DPLN</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>11</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Total (67 cases)</td>
<td>18</td>
<td>16</td>
<td>6</td>
<td>10</td>
</tr>
</tbody>
</table>

### Table 3: Frequency of each item of ARA preliminary criteria in patients with or without proteinuria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Cases without proteinuria</th>
<th>Cases with proteinuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discoïd</td>
<td>27.8%</td>
<td>6.5%</td>
</tr>
<tr>
<td>Raynaud's phenomenon</td>
<td>61.1*</td>
<td>22.6*</td>
</tr>
<tr>
<td>Arthritis</td>
<td>97.2</td>
<td>80.6</td>
</tr>
<tr>
<td>Pleuritis</td>
<td>2.8</td>
<td>19.4</td>
</tr>
<tr>
<td>Psychosis</td>
<td>11.1</td>
<td>29.0</td>
</tr>
<tr>
<td>Convolution</td>
<td>5.6</td>
<td>16.1</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>11.1</td>
<td>32.3</td>
</tr>
</tbody>
</table>

(*) : P<0.005

Sex
Six of the 7 male patients showed proteinuria (85.7%), while 26 of the 60 female patients had proteinuria (43.3%). Renal histology was classified as follows:

- **Males:** Min 1 case (14%), DPLN 3 cases (43%).
- **Females:** Min 17 cases (28%), DPLN 14 cases (23%).

Though the difference was statistically insignificant, male patients showed more advanced renal histopathology.

**Age**
There was no age difference among the SLE subgroups as stratified by urinary findings and renal histopathology.

**Extrarenal Symptoms**

**Urinary Findings and ARA Items of Extrarenal Symptoms (Table 3).** Raynaud's phenomenon was found in 61.1% of the patients without proteinuria and in 22.6% of the patients with proteinuria (p<0.005). Discoïd skin eruptions and arthropathy were more frequent among patients without proteinuria, and pleuritis, psychosis and thrombocytopenia were more frequent among patients with proteinuria, but these differences were not significant.
Renal and Arterial Lesions of Extraoral Symptoms. Raynaud’s phenomenon was found in 66.7% of the cases of Min and in 43.8% of Mes LN but in 11.8% of DPLN (p<0.005).

Urinary Findings: Renal Histology and Arterial Lesions of Extraoral Symptoms. Raynaud’s phenomenon was more common among the patients without proteinuria regardless of the renal histopathologic pattern. Frequency of Raynaud’s phenomenon among patients without proteinuria was 68.8% in Min and 54.5% in Mes LN, while among patients with proteinuria, it was 71.1% in DPLN (p<0.005). Psychosis was present in 50% of the positive proteinuric DPLN patients and in 6.7% of the negative albuminuric Min patients (p<0.05).

Renal Functions: Creatinine Clearance

Urinary Findings and Creatinine Clearance. Twenty-two patients without proteinuria showed a creatinine clearance of 60-135 ml/min and only 6 of them showed creatinine clearance of less than 80 ml/min. In contrast, patients with proteinuria showed a creatinine clearance of 31-130 ml/min and 12 of 23 such patients showed a creatinine clearance of less than 80 ml/min. Fig. 1 shows sequential changes in creatinine clearance to less than 50 ml/min among the patients with or without proteinuria. None of the patients without proteinuria showed a creatinine clearance of less than 50 ml/min over a 10-year period. However, 13.9% of the patients with proteinuria showed less than 50 ml/min clearance after 1 year, about 20% after 3 years and about 40% after 5 years.

Fig. 1 Change of creatinine clearance in patients with or without proteinuria (Endpoint: Ccr<30 ml/min.)

Renal Histology and Creatinine Clearance. None of the Min or FPLN patients showed a creatinine clearance of less than 50 ml/min, but about 30% of the DPLN patients showed such a clearance after one year and about 50% after 5 years.

Urinary Findings: Renal Histology and Creatinine Clearance. None of the patients without proteinuria, regardless of their renal histology, showed a creatinine clearance of less than 50 ml/min. Patients with proteinuria did show a creatinine clearance of less than 50 ml/min, 33.5% of these were classified as Mes LN, 50% as Membr LN and 67% as DPLN after 5 years.

Serum Creatinine Levels

Urinary Findings and Serum Creatinine Levels (Fig. 2). Sequential changes in serum creatinine levels according to the presence or absence of proteinuria are shown in Fig. 2. None of the patients without proteinuria developed serum creatinine levels of more than 1.5 mg/dl. Thirteen percent of the patients with proteinuria showed elevated creatinine levels after one year, about 20% after 2 years and about 50% after 5 years.

Renal Histology and Serum Creatinine Levels. None of the patients with Min, Mes LN and FPLN showed serum creatinine levels above 1.5 mg/dl. Serum creatinine levels were above 1.5 mg/dl in 55% of the DPLN patients and in 44% of the Memb LN patients 5 years after the first diagnosis of SLE.

Fig. 2 Incidence of renal failure in SLE (endpoint: serum creatinine>1.5 mg/dl)

Renal Histology and Serum Creatinine Levels (Fig. 2). Sequential changes in serum creatinine levels according to the presence or absence of proteinuria are shown in Fig. 2. None of the patients without proteinuria developed serum creatinine levels of more than 1.5 mg/dl. Thirteen percent of the patients with proteinuria showed elevated creatinine levels after one year, about 20% after 2 years and about 50% after 5 years.

Renal Histology and Serum Creatinine Levels. None of the patients with Min, Mes LN and FPLN showed serum creatinine levels above 1.5 mg/dl. Serum creatinine levels were above 1.5 mg/dl in 55% of the DPLN patients and in 44% of the Memb LN patients 5 years after the first diagnosis of SLE.

Complement (Fig. 3). Serum β2C levels were 15-124 mg/dl (average 54 mg/dl) in patients without proteinuria and 20-108 mg/dl (average 45 mg/dl) in patients with proteinuria. The frequency of the patients with β2C above 60 mg/dl was 12 of 31 patients without proteinuria and 1 of 19 patients with proteinuria (p<0.05). The serum β2C levels did not differ among the SLE subgroups classified by renal histology. It is interesting that DPLN patients without proteinuria showed β2C levels of 53-93 mg/dl (average 70.3 mg/dl), while DPLN patients with proteinuria showed 20-52 mg/dl (average 37 mg/dl).

Fig. 3 Renal findings and β2C globulin

Rheumatoid Factor (Table 1). Among negative proteinuric patients both RAHA and LFT were positive in 28.1%, only LFT was positive in 31.3%, and both were negative in 40.6%. Among proteinuric patients both were positive in 42.4%, only LFT was positive in 38% and both were negative in 62.5%. Positiveness of both RAHA and LFT was significantly higher in patients without proteinuria (p<0.05).

Table 4 Rheumatoid factor in patients with or without proteinuria

<table>
<thead>
<tr>
<th>Proteinuria</th>
<th>Positive RAHA and LFT</th>
<th>Negative RAHA, positive LFT</th>
<th>Negative RAHA and LFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without proteinuria</td>
<td>9 (28.1%)</td>
<td>15 (31.3%)</td>
<td>15 (40.6%)</td>
</tr>
<tr>
<td>With proteinuria</td>
<td>1 (4.2%)</td>
<td>8 (33.3%)</td>
<td>15 (62.5%)</td>
</tr>
<tr>
<td><strong>: P&lt;0.05</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5 Causes of death

<table>
<thead>
<tr>
<th>Cause</th>
<th>Min</th>
<th>Mes LN</th>
<th>Memb LN</th>
<th>DPLN</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uremia</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Cardiovascular accident</td>
<td>1</td>
<td>1</td>
<td>4/3*</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Infection</td>
<td>1</td>
<td>1</td>
<td>30/2*</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Others</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td><strong>: cases with renal failure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
the patients with Min showed both positive RAHA and LFT, while both were positive in only 7% of the DPLN patients (not significant). RAHA and LFT were more frequently positive in negative albuminuric patients regardless of the type of renal histopathology.

**Survival Rate**

The survival rate for the present series of SLE was 76.7% at 5 years and 63% at 8 years after the first diagnosis of SLE.

**Urinary Findings and Survival Rate (Fig. 4)** The survival rate for the negative proteinuric group was 100% after one year and 97.2% after 5 years as well as 8 years. The survival rate for the positive proteinuric group was 80.6% after one year, 55.3% after 5 years and 23.8% after 8 years. The percentage of survivors for 8 years was significantly lower in the positive proteinuric group (p<0.005).

**Renal Histopathology and Survival Rate.** The Min group showed a survival rate of 94.5% after 5 and 8 years. In the Mes LN group, it was 76.5% after 5 and 8 years. None of the FPLN group died during the 8-year period. The Memb LN group showed a survival rate of 87.5% after 5 years, but it declined to 58.3% after 8 years. Seventy per cent of the DPLN group survived for one year, but the rate declined to 47.1% after 5 years and none of these survived after 8 years.

**Urinary Findings, Renal Histology and Causes of Death.** The number of deaths, causes of death and renal histopathology are shown in Table 5. The mean survival period after the first diagnosis was 5.4 years in the Min group, 2.1 years in the Mes LN group, 7 years in the Memb LN group and 2.1 years in the DPLN group.

**Discussion**

Pollock and Baldwin discussed the importance of renal histopathology for predicting the prognosis of lupus nephritis. Focal or segmental changes in the glomerulus means mild renal involvement and patients with such a change seldom develop uremia. On the other hand, diffuse glomerular changes indicate a serious condition and often later develop into uremia, making treatment difficult.

Recently, a discrepancy between urinary findings and renal histology, e.g., patients with DPLN yet without albuminuria, was suggested. Also sequential renal biopsy showed transition of Min, Mes LN, or FPLN into DPLN. Therefore no one can foretell the prognosis of lupus nephritis by a single renal biopsy.

The present series of SLE consisted of 67 cases, of which 32 showed clinical renal involvement. The frequency of renal involvement in our SLE patients corresponded well to that hitherto reported. But we must emphasize that we found in all cases of SLE some kind of renal histopathologic changes, regardless of the presence or absence of proteinuria. Renal histologic changes in the series were less severe than in Baldvin's. This is so because we did a renal biopsy soon after the diagnosis of SLE regardless of whether the patient had proteinuria or not. More than half of our patients were free of albuminuria. This fact implies that there must be a transition of renal histology from one type to another in the course of clinical aggravation. Sequential renal biopsy is, therefore, needed.

More than 50% of the SLE patients with proteinuria developed renal failure within 5 years after the diagnosis, but none of the patients without proteinuria showed renal failure. Histopathologically, only the patients with Mem LN or DPLN developed renal failure. Especially, albuminuric DPLN patients are prone to be really crippled within 1-5 years. The percentage of renal failures corresponds well with the percent survival. There were, however, a few patients in whom urinary findings did not correspond to the renal histology. For example, three patients in the Min group had proteinuria and two of them showed a nephrotic syndrome. In contrast, three patients with DPLN were free of proteinuria (2 of them, however, became later albuminuric, and one of them even developed a severe nephrotic syndrome). Mahajab reported that 12 out of 27 SLE patients without clinical renal involvement showed DPLN in the biopsy and one of them developed uremia.

Men are said to be less prone to suffer from SLE than women. In our series male patients with SLE tended to have more proteinuria and more advanced renal histopathology. This is in accord with Baldwin's report. Eiser examined renal histology in SLE patients without proteinuria and found an ageponderance for each histological type. According to this report, the mean age was 19 years in the DPLN group, and 41.8 years in the Min, Mes LN and FPLN groups. The authors of the present report could not confirm this age difference.

As for the extrarenal symptoms, Raynaud's phenomenon is much more common in the negative proteinuric Min and Mes LN groups; this is a clear contrast with the positive proteinuric DPLN group. Tominga reported the same observation. He also mentioned that arthritis is also common among proteinuric patients. Indeed, positive proteinuric DPLN patients showed less arthritis in our series.

There is no relationship between renal histology and serum antinuclear antibody or anti-DNA antibody as reported previously. Serum βC globulins were lower in patients with active lupus nephritis. This is also in accord with previous reports. But there are many exceptions, and βC alone is a weak predictor of prognosis in lupus nephritis. The fact that there are few RAHA-positive patients among positive proteinuric SLE patients as compared with those without proteinuria suggests a favorable effect of rheumatoid factors in prevention of immune-complex nephritis. Raynaud's phenomenon, specific antinuclear antibody and rheumatoid factors might have some interrelationship.

**Conclusion**

Sixty-seven patients with SLE were stratified according to urinary findings and renal histology. Clinical symptoms, laboratory findings, course and cause of death in each SLE-subgroup were ex-
Studies on Lupus Nephritis

Y. Matsuno et al.: Studies on Lupus Nephritis

amined and yielded the following results.
1) Thirty-two patients showed proteinuria and 35 patients were free of proteinuria within one year after the first diagnosis of SLE.
2) None of our 67 patients was free of renal histologic changes. Renal histology revealed:
   Min 18 cases, Mes LN 16 cases, FPLN 6 cases, Memb LN 10 cases, and DPLN 17 cases.
   Three patients with DPLN were proteinuria-free at first, but two of them later developed persistent proteinuria.
3) Patients without proteinuria, especially the Min and Mes LN groups, showed a higher incidence of Raynaud’s phenomenon than did the positive proteinuric DPLN group.
4) Patients without proteinuria showed less hypocomplementemia and more positive rheumatoid factors.
5) None of the patients without proteinuria became renally crippled, but many proteinuric DPLN patients developed renal failure.
6) The survival rate 5 years after the initial diagnosis of SLE was 97% in patients without proteinuria in contrast to 55% in patients with proteinuria. In particular, only 34.5% of the DPLN group survived for 5 years.
7) Renal failure plays an important role either directly or indirectly as a cause of death in SLE.

From these results the authors emphasize the importance of renal histologic examination in the treatment and determination of prognosis of SLE together with urinary findings, extrarenal symptoms and immunological examination.

REFERENCES


Support T Cell Activity in Rheumatoid Arthritis and Systemic Lupus Erythematosus

Tsuyoshi SAKANE, Yoshio TANIGUCHI, Masaki HONDA and Hiroyuki KOTANI

Department of Internal Medicine, Shisei Medical University, Enoya-cho, Isumo 693

Concanavalin A (Con A)-induced suppressor activity of peripheral blood T cells was in- vestigated in 15 patients with rheumatoid arthritis (RA), 19 patients with systemic lupus erythematosus (SLE) and 27 normal controls. Suppressor activity was generated by acti- vating peripheral blood T cells with Con A. These cells were added to assay cultures of responder cells and their ability to inhibit T cell proliferative response to allogeneic cells was assessed. Both cell proliferative response to pokeweed mitogen in the assay culture was evaluated. Impaired suppressor activity was observed in patients with active RA and active SLE.

Mixing experiments between the patient’s and normal cells were performed to further define the nature of the suppressor defect in these diseases. The results of such experi- ments demonstrated that 1) the impaired suppressor activity in patients with early active RA (duration of active disease <3 months) was due to a defect in the generation of suppressor T cells as well as a defect in the response of B cells to suppressor cell signals, that 2) lymphocytes from patients with chronic active RA (duration of active disease >12 months) were able to generate adequate suppressor T cells but defects in their suppressor function resulted from the failure of B cells to respond to normal suppressor T cells, and that 3) lymphocytes from patients with active SLE failed to generate normal suppressor T cell function but still retained the capacity to respond to normal suppressor cell signals.

INTRODUCTION

During the past several years there has been a prolif- eration of interest in defining the role of sup- pressor T cells in normal and abnormal immune regulation.1) The immune system requires checks and balances to prevent an excessive reaction by lymphocytes to exogenous antigens. It is probable that similar immunoregulatory influences play a crucial role in maintaining tolerance to auto- antigens. Thus, dysfunction of the suppressor T cells may be important in the pathogenesis of certain autoimmune diseases.2)–5) Support for this concept is provided by animal models of autoimmune disease, primarily the New Zealand mouse, where the loss of suppressor T cell function antedates increased autoantibody production.6) Similarly, impaired suppressor T cell function has been reported in patients with systemic lupus erythematosus (SLE).7)–9) Aberration of suppressor T cell function may also be of pathogenic importance in rheumatoid arthritis (RA), a disease which is often associated with hypergammaglobulinaemia and production of autoantibodies such as rheumatoid factor and antinuclear antibodies.
Indeed, some investigators have demonstrated a lack of suppressor T cell activity in peripheral blood lymphocytes from patients with RA, and in cells elicited from their synovial tissues. The present study represents a further extension of the previous work by studying the ability of lymphocytes from RA patients at various stages in their illness and duration, and from SLE patients at various phases of their illness to develop con- 

Canavalia A (Con A)-induced suppressor function. We have investigated whether the status of suppressor T cell function in RA is similar to that of patients with SLE. Our results provide evidence for a role of suppressor T cell dysfunction in the initiation and maintenance of active immune abnormalities in both SLE and RA patients.

MATERIALS AND METHODS

Patients and Source of Lymphocytes. Fifteen patients with classical or definite RA according to the ARA criteria and 19 patients with SLE according to the preliminary criteria of the ARA for the diagnosis of SLE were studied. The disease activity of RA was evaluated by clinical status (the number of actively inflamed joints, number of effusions, duration of morning stiffness, and extra-articular features) and laboratory findings (elevated blood sedimentation rate, positive CRP, and high titer of rheumatoid factors). Patients lacking these symptoms and findings of activity were categorized as inactive. Active patients were further divided on the basis of the duration of active disease into an early active RA group (<3 months) and a chronic active group (>12 months). It should be noted here that patients with both early active and chronic active RA had the active disease and thus no difference in clinical profile was found between them. All the RA patients were receiving salicylate and/or non- 

steroidal anti-inflammatory drugs at the time of study; drugs were withheld for 24 hours prior to the study. None of these RA patients were taking disease suppressants (gold and D-penicillamine) or immunosuppressive medications (misonidazole, cyclophosphamide, and corticosteroids) at the time of study. Blood samples were obtained at least three weeks after the last administration of these drugs.

The activity of the SLE was assessed on the basis of signs and symptoms (active rash, serositis, arthritis, active central nervous system disease, and active renal disease). The active patients in this study had at least three of the above criteria of activity. If patients had only mild subjective symptoms, they were included in the inactive group. Lymphocytes from the patients with active disease were obtained for in vitro studies prior to any treatment. Inactive patients had been previously treated with corticosteroids and occasionally with immunosuppressive drugs, but were not receiving such treatment at the time of the study. Normal suppressor function was obtained from 27 healthy controls who matched, as closely as possible, the sex and age of the patients tested.

Purification of T Cells, B Cells and Monocytes. Peripheral blood mononuclear cells were isolated by centrifugation on a Ficoll-Hypaque gradient. T cells, B cells and monocytes were separated from the mononuclear cells as previously described in detail. Briefly, spontaneously rosetted cells from mononuclear cells and sheep erythrocytes (SRBC) were performed with neuraminidase-treated SRBC. The rosetting cells were separated on another Ficoll-Hypaque gradient from the nonrosetting cells. Both rosetting and nonrosetting fractions were further purified by repeated rosetting with SRBC and sub- 

sequent density gradient centrifugation. The dou- 

bly purified rosetting cell population was recovered after lysis of SRBC by a Tris-buffered ammonium chloride solution. This population contained more than 90% T cells as determined by rosette 

forming. These will be referred to as T cells. The doubly purified rosetting population was depleted monocytes by the removal of cells adhering to the petri dishes. Monocytes were obtained by collecting the cells adhering firmly to the dishes. The percentage of T cell contamination in the nonadherent, nonrosetting cell population was less than 1% as determined by rosetting. We will refer to this population as B cells. More than 95% of monocytes were considered as judged by morphology after Giemsa staining.

Assay of Suppressor Cell Function. Suppressor T cells were generated by Con A activation in a first culture as previously described. These cells were added to responder cells in a second assay culture system that were stimulated with either pokeweed mitogen (PWM) or allogeneic cells (MLR). Responder cells in the assay cultures were obtained 3 days later from a new bleeding of the same individual who originally provided the suppressor cells, or from another individual. In detail, 3 x 10^6 T cells were incubated in 3 ml cul- 

ture medium, RPMI 1640 (Flow Laboratories Inc., Rockville, Md.) supplemented with 10% fetal bovine serum (Flow Laboratories Inc.) with 30 Con A, 100 U/ml (10 x 10^6 mitomycin (Sigma Chemical Co., St. Louis, Mo.))-treated monocytes were added. Sixty hours later, the cells were harvested, washed four times, treated with mito- 

mycin, and then tested for their suppressive ability in the second assay culture. 5 x 10^5 mi- 

tomycin-treated monocytes were added to the second assay cultures of 5 x 10^5 re- 

sponder cells plus 2,500 mitomycin-treated mono- 

cytes and stimulated with allogeneic cells or PWM. Where T cells were used as responder cells in the assay cultures, they were stimulated with 5 x 10^4 mitomycin-treated allogeneic cells. When B cells were used as responder cells, PWM (1 µg/ml; Grand Island Biological Co., Grand Island, N.Y.) was the stimulant in the culture. Because the B cell response to PWM is clearly T cell dependent, freshly prepared T cells, autologous to re- 

sponder B cells, that had been treated with mito- 

mycin were also added (5 x 10^4 per culture). All cultures were performed in triplicate with 0.2 ml in microtiter plates (Cooke Engineering Co., Alexandria, Va.) and incubated for 144 hours at 37°C in a 5% CO_2/95% air humidified environ- 

ment. The proliferative response was measured by the incorporation of [methyl-3H]thymidine (2 C/mumol; New England Nuclear, Boston, Mass.) during the last 20 hours of culture. The degree of suppression was determined by the fol- 

lowing formula: percent suppression = 1 - ([mean cpm of stimulated cultures containing Con A- 

activated T cells] - [mean cpm of unstimulated cultures containing Con A-activated T cells]) / [mean cpm of stimulated cultures containing nonactivated T cells] x 100.

RESULTS

Con A-Induced Suppressor Cell Activity

In the first series of experiments, the suppressor and the responder cells were obtained from the same individual. T lymphocytes from patients with either RA or with SLE, which had been precultured with Con A in a first culture system, were added to second assay cultures containing freshly prepared responder cells. These were then stimulated with allogeneic cells or PWM. The results of these experiments are summarized in Table 1.

T lymphocytes from the 4 patients with early active RA failed to manifest a normal degree of suppressor activity for both T cell proliferative response to MLR and B cell proliferative response to PWM. T cells from 6 chronic active patients showed a defect in the generation of suppressor function for the PWM response. The first culture was added to the second assay cultures of 5 x 10^4 re- 

sponder cells plus 2,500 mitomycin-treated mono- 

cytes and stimulated with allogeneic cells or PWM. Where T cells were used as responder cells in the assay cultures, they were stimulated with 5 x 10^4 mitomycin-treated allogeneic cells. When B cells were used as responder cells, PWM (1 µg/ml; Grand Island Biological Co., Grand Island, N.Y.) was the stimulant in the culture. Because the B cell response to PWM is clearly T cell dependent, freshly prepared T cells, autologous to re- 

sponder B cells, that had been treated with mito- 

mycin were also added (5 x 10^4 per culture). All cultures were performed in triplicate with 0.2 ml in microtiter plates (Cooke Engineering Co., Alexandria, Va.) and incubated for 144 hours at 37°C in a 5% CO_2/95% air humidified environ- 

ment. The proliferative response was measured by the incorporation of [methyl-3H]thymidine (2 C/mumol; New England Nuclear, Boston, Mass.) during the last 20 hours of culture. The degree of suppression was determined by the fol- 

lowing formula: percent suppression = 1 - ([mean cpm of stimulated cultures containing Con A- 

activated T cells] - [mean cpm of unstimulated cultures containing Con A-activated T cells]) / [mean cpm of stimulated cultures containing nonactivated T cells] x 100.

Cell Mixing Experiments

In the above experiments, the suppressor and the responder cells were from the same individual. These results, in general, showed that, after Con A activation, T cells from SLE or RA patients during the active phase of their disease did not suppress as well as did T cells from normal controls. In an attempt to determine whether or not the impaired suppressive ability of their T cells was in the generation of suppressor T cells or in the capacity to respond to negative regulatory signals produced by suppressor T cells, a series of reciprocal cell mixing experiments between normal and patient's lymphocytes was performed.
Table 1  Suppressor T cell activity in patients with RA and SLE*

<table>
<thead>
<tr>
<th>Source of suppressor T cells</th>
<th>Number tested</th>
<th>Con A-induced suppression</th>
<th>MLR</th>
<th>PWM</th>
<th>mean % ± SE</th>
<th>% MLR</th>
<th>% PWM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal RA</td>
<td>27</td>
<td>56.2 ± 2.4</td>
<td>53.1 ± 3.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>active</td>
<td>4</td>
<td>9.4 ± 5.0</td>
<td>5.2 ± 2.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>inactive</td>
<td>6</td>
<td>50.5 ± 3.3</td>
<td>7.2 ± 3.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLE</td>
<td>5</td>
<td>64.6 ± 6.5</td>
<td>46.3 ± 7.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>active</td>
<td>10</td>
<td>20.8 ± 6.4</td>
<td>9.3 ± 6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>inactive</td>
<td>9</td>
<td>53.6 ± 6.5</td>
<td>57.0 ± 9.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* T cells were incubated for 60 hours with Con A or without Con A. These cells were introduced into freshly prepared autologous responder cells in the assay culture which were stimulated with either allogeneic cells or PWM.

** T cells autologous with Con A-induced suppressor cells were used as responder cells in the assay culture.

† Significantly different from suppressor T cells activity obtained either from normals or from the other RA patient groups.

‡ Significantly different from suppressor T cell activity obtained either from normals or from the other SLE patient groups.

First we will show the results of the experiments of this type between normal and RA lymphocytes; the cells from one of each pair were used as responder cells and the cells from the other pair were used as suppressor cells (Table 2). T cells from patients with early active RA, preincubated with Con A, poorly suppressed the MLR and PWM responses of their own responder cells. Similarly, their Con A-activated T cells did not induce suppression of both reactions by normal responder cells in spite of the fact that normal responder cells could be consistently suppressed by Con A-activated normal T cells. Where normal cells were used as suppressor cells and RA cells as responder cells, Con A-activated normal T cells suppressed the proliferative response of RA T cells to MLR, but did not suppress the PWM response of RA B cells. It should be noted here that normal suppressor cells were consistently capable of causing suppression of the PWM response by normal responder B cells. Thus the failure of lymphocytes from early active RA patients to manifest normal suppressor activity appears to be due to defects: First, a defect in production of suppressor cells. Second, B cells of the patients with early active RA are defective in their ability to respond to suppressor cell signals produced by suppressor T cells.

Con A-activated T cells from patients with chronic active RA suppressed both the MLR and PWM responses of normal cells. In the PWM, but not MLR, response of responder RA B cells, normal suppressor cells did not induce suppression (Table 2). These results indicate that patients with chronic active RA are able to adequately generate suppressor T cells and that defects in their suppressor function reside in the failure of B cells to respond to suppressor cell signals. As expected, T cells from patients with inactive RA, preincubated with Con A, suppressed normal responder cells; adequate suppression generated by Con A-activated normal T cells was also obtained in the MLR and PWM responses of inactive RA responder cells (Table 2).

In parallel with the cell mixing experiments between normal and RA lymphocytes, we performed similar experiments between normal and SLE lymphocytes (Table 3). T cells from active SLE patients, preincubated with Con A, poorly suppressed the MLR and PWM responses of their own responder cells. In contrast, normal T cells preincubated with Con A suppressed these responses of SLE responder cells. Where SLE cells were used as suppressor cells and normal cells as responders, the suppression generated by Con A-activated SLE T cells was markedly impaired in the MLR and PWM responses of the normal responder cells. With regard to lymphocyte function of patients with inactive SLE, there was no abnormality in the generation of suppressor cells or in their capacity to respond to suppressor cells. Thus, the impaired suppressor T cell function observed in patients with active, but not inactive, SLE may reside in the generation of suppressor cells, and not in their response to suppressor cell signals.

Table 2  Cell mixing experiments between normal and RA lymphocytes: representative experiments*

<table>
<thead>
<tr>
<th>Patient</th>
<th>First culture</th>
<th>Second culture</th>
<th>Thyroidine incorporation in response to MLR</th>
<th>PWM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of suppressor cells</td>
<td>Con A</td>
<td>Source of responder cells</td>
<td>mean % ± SE</td>
<td>% MLR</td>
</tr>
<tr>
<td>chronic active RA</td>
<td>patient, S</td>
<td>normal</td>
<td>21.084</td>
<td>21.010</td>
</tr>
<tr>
<td>normal</td>
<td>patient, S</td>
<td>normal</td>
<td>24.875</td>
<td>24.875</td>
</tr>
<tr>
<td>normal</td>
<td>patient, S</td>
<td>normal</td>
<td>27.884</td>
<td>27.884</td>
</tr>
<tr>
<td>normal</td>
<td>patient, Y</td>
<td>normal</td>
<td>15.947</td>
<td>15.947</td>
</tr>
<tr>
<td>normal</td>
<td>patient, Y</td>
<td>normal</td>
<td>7.068</td>
<td>7.068</td>
</tr>
<tr>
<td>Inactive RA</td>
<td>patient, I</td>
<td>patient, I</td>
<td>46.140</td>
<td>46.140</td>
</tr>
<tr>
<td>normal</td>
<td>patient, I</td>
<td>normal</td>
<td>4.296</td>
<td>4.296</td>
</tr>
<tr>
<td>normal</td>
<td>patient, I</td>
<td>normal</td>
<td>12.941</td>
<td>12.941</td>
</tr>
<tr>
<td>normal</td>
<td>patient, I</td>
<td>normal</td>
<td>73.391</td>
<td>73.391</td>
</tr>
<tr>
<td>normal</td>
<td>patient, I</td>
<td>normal</td>
<td>80.183</td>
<td>80.183</td>
</tr>
</tbody>
</table>

* 5 × 10⁴ responder cells (second culture) either from a RA patient or from a normal individual were mixed with T cells whose suppressor activity was being tested. These suppressor T cells were obtained from this same patient or from the same normal individual.

† T cells were used as responder cells.

‡ B cells were used as responder cells.

§ Data are expressed as the difference between the cpm from stimulated cultures and the cpm from unstimulated cultures (2 cpm).

|| No significant suppression when compared to the response of responder cells that contained T cells preincubated without Con A (P > 0.05). Others show significant suppression.

Similar results were obtained in three additional sets of experiments.

DISCUSSION

Defects in suppressor T cell function have been shown in active SLE [5-8, 9] These defects, in some way, could be related to the pathogenesis of the disease. Indeed, our present [5, 6, 9] and present studies have demonstrated that patients with active SLE have a defect in Con A-induced suppressor T cell function and that this defect is related to the impaired generation of suppressor cells rather than in the response to suppressor signals (which was
Table 3  Cell mixing experiments between normal and SLE lymphocytes: representative experiments^a

<table>
<thead>
<tr>
<th>Patient</th>
<th>First culture</th>
<th>Second culture</th>
<th>Thyminde incorporation in response to</th>
<th>MLR^a</th>
<th>PWM^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of suppressor cells</td>
<td>Con A</td>
<td>Source of responder cells</td>
<td>d cpm</td>
<td>% suppression</td>
<td>d cpm</td>
</tr>
<tr>
<td>Active SLE</td>
<td></td>
<td></td>
<td>12,092</td>
<td>9.7%</td>
<td>7,036</td>
</tr>
<tr>
<td>patient, C</td>
<td></td>
<td></td>
<td>10,972</td>
<td>7.3%</td>
<td>7,310</td>
</tr>
<tr>
<td>patient, C</td>
<td></td>
<td></td>
<td>13,151</td>
<td>14.9%</td>
<td>14,846</td>
</tr>
<tr>
<td>normal</td>
<td></td>
<td></td>
<td>11,332</td>
<td>13.8%</td>
<td>15,203</td>
</tr>
<tr>
<td>normal</td>
<td></td>
<td></td>
<td>6,002</td>
<td>55.2%</td>
<td>5,098</td>
</tr>
<tr>
<td>normal</td>
<td></td>
<td></td>
<td>12,755</td>
<td>15.18%</td>
<td>15,180</td>
</tr>
<tr>
<td>normal</td>
<td></td>
<td></td>
<td>5,569</td>
<td>73.6%</td>
<td>6,491</td>
</tr>
<tr>
<td>Inactive SLE</td>
<td></td>
<td></td>
<td>9,966</td>
<td>22.70%</td>
<td>22,700</td>
</tr>
<tr>
<td>patient, N</td>
<td></td>
<td></td>
<td>4,226</td>
<td>55.8%</td>
<td>16,690</td>
</tr>
<tr>
<td>patient, N</td>
<td></td>
<td></td>
<td>13,361</td>
<td>27.53%</td>
<td>27,530</td>
</tr>
<tr>
<td>normal</td>
<td></td>
<td></td>
<td>8,908</td>
<td>45.9%</td>
<td>17,229</td>
</tr>
<tr>
<td>normal</td>
<td></td>
<td></td>
<td>8,400</td>
<td>21,562</td>
<td>21,562</td>
</tr>
<tr>
<td>normal</td>
<td></td>
<td></td>
<td>2,573</td>
<td>57.5%</td>
<td>10,941</td>
</tr>
<tr>
<td>normal</td>
<td></td>
<td></td>
<td>11,653</td>
<td>47,727</td>
<td>47,727</td>
</tr>
<tr>
<td>normal</td>
<td></td>
<td></td>
<td>5,599</td>
<td>52.0%</td>
<td>24,726</td>
</tr>
</tbody>
</table>

^a 5 x 10^4 responder cells (second culture) either from a SLE patient or from a normal individual were mixed with T cells whose suppressor activity was being tested. These suppressor T cells were obtained from the same patient or from the same normal individual.

§§ 1 and § 2. Same as Table 2 respectively.

Similar results were obtained in six additional sets of experiments.

Consistent with the results presented here, impaired suppressor cell activity in patients with RA was previously reported to occur in similar and other suppressor systems.10-12 Abdou et al.40 demonstrated impaired suppressor function by Con A-activated T cells in a minority of RA patients with active arthritis early in their course of disease. Chattopadhyay et al.12 utilizing T cells generated spontaneously, demonstrated a lack of suppressor cell activity of synovial tissue lymphocytes. Keystone et al.,11 also showed impaired antigen-specific suppressor cell activity in RA peripheral blood lymphocytes. We have extended these observations to suppressor cells, in that T cells from a single patient were simultaneously studied with regard to their ability to suppress in vitro T cell and B cell functions. In addition, the present studies demonstrate that the defect of lymphocytes from patients with early active RA is in the generation of suppressor cells as well as in the response of their B cells to suppressor cells. Patients with chronic active RA were found to have only the latter immunologic aberration.

The mechanism of reduced suppressor cell generation in patients with RA remains unclear. A possible mechanism might be the presence of anti-T cell antibodies that were capable of inactivating suppressor cells or their precursors, as have been observed in patients with SLE.18,19,17,18 Several investigators have suggested the existence of anti-T cell antibodies in a segment of RA patients.18,19 Abdou et al.40 found antibodies to T cells only in early active RA and suggested that such antibodies could be responsible for the accelerated loss of suppressor cells or their precursors. Indeed, in preliminary experiments, we have observed that prior treatment of normal T lymphocytes with selected RA (early active RA) sera plus complement resulted in a significant reduction of numbers of T^+ cells (which have been shown to have suppressor/cytotoxic cell function).

It is unclear why the defect in the response to normal suppressor T cells exists in the B cells of RA, not in SLE patients. It is suggested, however, that B cell hyperactivity in RA may be independent from, and actually may precede, the T cell abnormality. In contrast, the results showing that SLE B cells were able to respond to suppressor cells in a normal fashion may indicate that B cell hyperactivity in SLE results from deficient suppressor cell activity. It is also unclear whether the defect in the ability of RA B cells to respond to suppressor cell signals could reside in the B cell defect, or alternatively, in a functional failure of helper T cells. Studies are currently under way to address this question.

In conclusion, the impaired suppressor cell activity in patients with RA and in those with SLE is consistent with the hypothesis that defective immunoregulation gives rise to the observed humoral immune abnormalities. Defects in the generation of suppressor T cells could be responsible for the initiation and for the maintenance of the autoimmune abnormalities in SLE. Defects in the suppressor cell generation as well as defects in the capacity of B cells to respond to suppressor cell signals could be responsible for the initiation of the autoimmune and inflammatory abnormalities in RA. The latter defects could also be related to the maintenance of these abnormalities.

REFERENCES

Anti-DNA Activity in Raji Cell Eluates of Sera from Patients with Systemic Lupus Erythematosus

Masakuni TOMII

Department of Internal Medicine, School of Medicine, Keio University
Shinano-machi, Shinjuku-ku, Tokyo 160

Circulating immune complexes were isolated depending upon thier size and were studied for their anti-DNA antibodies using Raji cells in 2 patients with systemic lupus erythematosus.

In a patient with lupus nephritis, anti-DNA antibody was found only in immune complexes which had a molecular weight between 150,000-900,000. On the other hand, in a patient without nephritis, anti-DNA antibody was found in immune complexes with an approximate molecular weight of more than 900,000. These results suggest that circulating immune complexes in systemic lupus erythematosus are partially composed of anti-DNA antibodies, and a relatively small size of DNA/anti-DNA complexes might be associated with lupus nephritis.

INTRODUCTION

Systemic lupus erythematosus (SLE) is thought to be one of the prototypes of immune complex (IC) disease because of the similarity of its clinical findings to those of experimental serum sickness and of the ICs deposition in glomeruli.\(^1\) The evidence that circulating DNA antigen appeared in a patient's serum and anti-DNA antibody disappeared just prior to the clinical flare in SLE\(^2\) also suggested that ICs might play a significant role in the pathogenesis of SLE. Recently, circulating ICs have been detected by a number of methods.\(^3,4\) However, the quantitative determination of the amount of circulating ICs using different techniques are still not well correlated.\(^5\) Moreover, even using the same method, some investigators demonstrate a positive relation between the serum IC level and the clinical activity but other investigators do not.\(^6,7\) The reasons for this discrepancy might partially come from the diversity of the ICs composition. Many autoantibodies have already been found in the serum of patients with SLE (13). Therefore, it is necessary to characterize the nature of the ICs found in these patients' sera. However, only limited numbers of papers concerned with the characterization of the specificities of circulating ICs have been published. The association between the nature of the ICs and the clinical findings has been unknown.

In this paper, we tried to isolate the ICs and to characterize DNA/anti-DNA antibody complex in the sera of patients with SLE in order to investigate the association between the size of the ICs and lupus nephritis.

MATERIALS AND METHODS

Selection of Sera. Two sera from 2 patients (YM and SR) with active SLE were selected for the study. The sera were drawn from patients during the active phase of their disease just prior to treatment. Although both of these patients had high

Keywords: anti-DNA antibody, immune complex, lupus nephritis, Raji cell, systemic lupus erythematosus
titers of anti-DNA antibodies and hypocomplementemia, lupus nephritis developed in only one patient (YM).

Preparation of Aggregated Human IgG (AHG). AHG was prepared from human IgG by heat treatment at 65°C for 30 min followed by gel filtration on a Sephacryl S-300 (Pharmacia Fine Chemicals, Uppsala) column (2.6 × 120 cm) chromatography. AHG (1.5 ml, 1000 μg/ml) was incubated with an equal volume of 1:20 diluted normal human serum as a source of complement (C). As a control, 1.5 ml of AHG was mixed with an equal volume of 1:20 diluted normal human serum which had already been inactivated.

Gel Filtration of Sera. Two sera were first added to a column of Sephacryl S-300, then fractions of the first 3 peaks were collected and concentrated with Minicon B-15 (Amicon Far East Ltd., Tokyo) to the original volume (6 ml). Half volumes of these fractions were precipitated at 56°C for 30 min in order to inactivate C which bound to the ICs as control.

Isolation of ICs Using Raji Cells. Circulating ICs in patients' sera were isolated using Raji cells according to the method of Theofilopoulos et al. with modifications. Briefly, 3 ml of a sample were incubated with 10⁶ Raji cells, which had been cultured in a medium of RPMI-1640 with 20% fetal bovine serum, in 50 μl of Dulbecco's phosphate buffered saline (PBS) at 37°C for 30 min. Afterward, the cells were washed with PBS, the ICs bound to cells were eluted by fresh citrate buffered saline, pH 5.2 at 37°C for 10 min. Eluates from Raji cells which had been incubated with column fractionated sera were added to a Sepharose CL-6B (Pharmacia) column (1 × 30 cm) to separate antibodies from antigen and then dialyzed against phosphate buffered saline (PBS), pH 7.4. Eluates from cells which had been incubated with AHG were directly dialyzed against PBS. The dialyzed materials were concentrated by Minicon B-15. The viability of the cells following elution was determined by trypan blue exclusion.

Other Immunological Methods. Circulating ICs were measured by the Raji cell radioimmune assay according to Theofilopoulos et al. Anti-DNA antibody was determined using H-DNA from E. coli by the method of Pincus et al. A binding value of more than 20% was considered positive in pathological sera. When negative values were calculated, they were regarded as 0%.

DNA was estimated by a counter immuno-electrophoresis (CIE) using a positive anti-DNA antibody serum as a standard.

RESULTS

Recovery Rate of AHG From Raji Cells

In order to confirm the validity of our ICs isolating system, the per cent IgG recovery rate using AHG from Raji cells was determined. The average recovery rate was approximately 5.4% of the total applied AHG when premixed with C. On the other hand, the per cent recovery of IgG was shown to be 0% when AHG without C was used.

The viability of cells was more than 94% in every experiment.

Anti-DNA Antibody in ICs Isolated From Fractionated Sera

Fig. 1 shows Sephacryl S-300 column chromatography of sera from YM and SR. Four major peaks of the absorption curve at 200 nm were seen in both column chromatography. Fractions from the first 3 peaks, tentatively designated as Fraction 1, 2 and 3 (Fr.1, 2 and 3) were pooled separately and concentrated to the original volume. The last peak was composed primarily of albumin so that this fraction was not used for the ICs isolating experiment. Fr.1, 2 and 3 were incubated with Raji cells and the ICs were eluted from cells with 5 ml of citrate buffered saline. From these eluates, monomeric IgG fractions were obtained through a Sepharose CL-6B column (Fig. 1) and concentrated 200-fold with Minicon B-15. Then concentration of IgG and %DNA binding were measured in each monomeric IgG fractions collected in eluates from Fr.1, 2 and 3 (Table 1). In the case of YM, the highest concentration of IgG was recovered in the eluate from Fr.2, while in the case of SR, the highest IgG recovery was found in Fr.1. The highest %DNA binding was also found in the eluate Fr.2 from YM and in Fr.1 from SR. Control experiments using inactivated Fr.1, 2 and 3 from both patients' sera were performed in the same manner. No IgG or anti-DNA antibodies were found in Fr.1, 2 and 3 in the case of YM. Only a trace amount of IgG was found in Fr.2 and 3 but no anti-DNA antibody was detected in them in the case of SR. Anti-DNA antibodies were positive in original sera, but became negative in diluted (1:20) sera from both patients.
Table 1 Recovered IgG and anti-DNA antibody in isolated ICs from fractionated sera

<table>
<thead>
<tr>
<th>Patient YM</th>
<th>Patient SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fr. 1</td>
<td>410</td>
</tr>
<tr>
<td>Fr. 2</td>
<td>600</td>
</tr>
<tr>
<td>Fr. 3</td>
<td>20</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>Inactivated Fr. 1</td>
<td>0</td>
</tr>
<tr>
<td>Inactivated Fr. 2</td>
<td>0</td>
</tr>
<tr>
<td>Inactivated Fr. 3</td>
<td>0</td>
</tr>
<tr>
<td>Original serum</td>
<td></td>
</tr>
<tr>
<td>1:1</td>
<td>29,000</td>
</tr>
<tr>
<td>1:20</td>
<td>1,460</td>
</tr>
</tbody>
</table>

In spite of the presence of anti-DNA antibody in eluates, DNA was not detected in any fraction obtained from a Sepharose CL-6B column by a method of CIE.

DISCUSSION

Raji cells were derived from a patient with Burkitt's lymphoma. Large numbers of C3b and C3d receptors and less frequent IgG Fe receptors are expressed on their surface about three days in vitro. It is of interest that the C3b receptors are increased in the Raji cell cultures. The ICs which are mainly bound with C are attached to the membrane of the Raji cells. It was supported by our experiment using AHG with or without C and allowed us to use this Raji cell method for the isolation of circulating ICs.

Anti-lymphocyte antibodies, especially those of the IgG type, might react with Raji cells. It is important to determine the reactivity of inactivated serum to the cells. In our experiment, we always prepared inactivated samples as controls to compare the results with samples that had not been inactivated. The viability of the Raji cells in the method of isolating the ICs is also important. It is known that dead cells might react with antibodies, including anti-DNA antibody. Large numbers of dead cells which bind considerable amount of antibody may give us non-IC antibodies in their eluate. However, the viability of the cells was always good and no or minimum amount of IgG recovery from cells using inactivated samples negates this kind of interference.

In this paper, anti-DNA antibodies were found in eluates from fractionated sera. Whether these antibodies were components of the ICs and only DNA/anti-DNA antibody complexes composed IC is difficult to ascertain. It is widely accepted that DNA/anti-DNA antibody complexes may play a significant pathogenic role in SLE. However, the demonstration of DNA in sera of SLE patients has been reported by only a few investigators. We failed to demonstrate DNA antigen in the ICs. This might be partially due to the method of CIE. Not only its sensitivity but also the size of the DNA might be too small to be detected by this technique.

Haakenstad et al. stated that 11S ICs persisted in the circulation much longer than larger or smaller ICs and were associated with glomerular deposits in experimental mice. In patients with SLE, the association between the size of the ICs and lupus nephritis was studied by Levinsky et al. They suggested that ICs with molecular weight of 300,000–1,000,000 were associated with nephritis. They also suggested that various sizes of ICs were present in sera from SLE. We showed anti-DNA antibodies in the eluates from fractionated sera. In a patient with lupus nephritis, anti-DNA antibody was found in the eluate from the fraction which might be composed of relatively small ICs. On the other hand, anti-DNA antibody was found in fractions which might be composed of larger ICs in a patient without nephritis. These results seem to be similar to previous reports but further controlled experiments are required to solve this interesting problem.

ACKNOWLEDGMENTS

The author wishes to express his gratitude to Professor M. Honna for his continuous interest and valuable advice throughout the work. He is further indebted to Drs. T. Tojo and M. Takano for their helpful suggestions in the preparation of this manuscript.

REFERENCES

13) Tojo T, Tomii M, Okamoto T: Clinical characteristics of patients with antibodies to nuclear ribonucleoprotein (RNP) in SLE. Systemic Lupus Erythematosus. (Tokase S ed) p 209–221 Tokyo, Univ of Tokyo Press 1980
20) Haakonstad AO, Manaliak M: The appearance kinetics of soluble immune complexes prepared with reduced and alkylated antibodies and with intact antibodies in mice. Lab Invest 35: 283–293, 1976
Precipitating Antibody to a Soluble Nuclear Antigen "Ki" with Specificity for Systemic Lupus Erythematosus

Takeshi TOJOI, Junichi KABURAKI, Masakatsu HAYAKAWA,*
Takashi OKAMOTO, Masakuni TOMII and Mitsuo HOMMA

Department of Medicine, Keio University School of Medicine, Shinjuku-ku, Tokyo 160
*Kasumigaura National Hospital

A distinction of the precipitating antibodies to the Ki antigen from other precipitating antibody systems to nonhistone nuclear proteins by using known reference sera is described. Sera from 449 patients with various connective tissue diseases were screened for antibodies to soluble nuclear antigens by an immunodiffusion test. Anti-Ki antibodies were positive in 37 patients, all but two of them were patients with SLE or SLE overlap syndrome. The frequency of anti-Ki antibodies was 11.8% in 253 patients with SLE. The maximum reciprocals of precipitating antibody titer to Ki antigen exceeded 2. Antibody titers tended to decrease with signs of clinical improvement in SLE. Anti-Ki antibodies showed a positive correlation with anti-Sm antibodies and positive LE cells. The clinical findings associated with anti-Ki antibodies and other ANA in 282 patients with SLE or SLE overlap syndrome were analyzed using a computer. A possible clincio-serological association was observed between positive anti-Ki antibodies and the presence of persistent arthritis or pericarditis. However these clinical items were also associated, statistically, with the presence of other ANA with different antigen specificities. These data indicate that a minor group of antibodies to soluble nuclear antigens, including the Ki system, may also have a close link with the diagnostic clinical features of patients with connective tissue diseases.

It has been increasingly apparent that certain types of antinuclear antibodies (ANA) are associated with particular types of connective tissue diseases and that they have diagnostic and prognostic importance. Saline soluble nuclear proteins have been shown to contain a variety of minor antigens other than the Sm, ribonucleoprotein (RNP) or Sjögren's syndrome B (SS-B) antigen, which are reactive with sera from patients with these diseases.1-6 The minor group of antigens, including the Ki antigen, reacted with sera from patients with systemic lupus erythematosus (SLE) giving speckled antinuclear staining in immunofluorescence.6

In this report, we describe a distinction of the precipitating antibodies to the Ki antigen from other precipitating systems to non-histone nuclear proteins (NHP) by using known reference sera. The clinical characteristics of patients with these antibodies are also described.

MATERIALS AND METHODS

Patients Studied. Sera were obtained from 449 patients with various connective tissue diseases.
These included 255 patients with SLE, 90 with scleroderma, 33 with rheumatoid arthritis (RA), 29 with polymyositis or dermatomyositis (PM-DM), 6 with psoriatic arthritis (PN) and 36 with overlap syndrome. They were diagnosed by the definite criteria of the American Rheumatism Association for SLE, scleroderma and RA.10-11 Patients with PM-DM were diagnosed by the definite criteria of Bohan et al.12 Patients with PN were diagnosed by a confirming biopsy. Patients with features fully satisfying two or more definite criteria for SLE, scleroderma and PM-DM were diagnosed as overlap syndrome. However, those with only incomplete features of other diseases were not included in the overlap syndrome.9-11 An serum sample was used from each patient. All sera were inactivated at 50°C for 30 min before testing.

The Source of Soluble Nuclear Antigens. Lyophilized powder of an acetone extract of young rabbit thymus (Pel-Freeze Biologicals, Ark., USA) was used as the nuclear antigen source for screening precipitating antibodies with soluble nuclear antigens in this study. For each 100 mg of the powder, 1.0 ml of 50 mM Tris/HCl buffer pH 7.2 containing 0.14 M NaCl was added. Soluble antigens were extracted by gently stirring at 4°C for 6 hr. Insoluble materials were removed by centrifugation at 3,000 rpm for 10 min. The extract was digested by deoxyribonuclease (free of RNAse; Sigma, 200 µg/ml) in 25°C water bath with gentle shaking for 60 min. after adding MgCl2 to make a final concentration of 5 mM. The digested extract was clarified by centrifugation at 3,000 rpm for 10 min. at 4°C. The concentration of the supernatant was adjusted to contain 20 µg of protein per ml as the soluble nuclear antigen for the screening test. Antigens were kept at -80°C until use.

Lyophilized powder of this antigen was also used in this study when concentrated antigen was needed. Protein concentrations were determined by the Bio-Rad protein assay procedure (Bio-Rad Technical Bulletin 1031 E, 1991, Calif., USA). To test the anti-Mi and PM-1 reference sera,11 lyophilized powder of whole calf thymus extract prepared by the method of Masiotti & Reichlin was used.13-14 Whole human liver extract prepared by the method of Clark14 was also used in this study as the antigen for the anti-anti-SS-A system.

Double Immunodiffusion. Plastic petri dishes containing 20 ml of 0.4% agarose (Seakem, Maine, USA) dissolved in 50 mM Tris/HCl buffer pH 7.2 containing 0.14 M NaCl and 0.01% sodium azide were used for double immunodiffusion. Six peripheral wells 6 mm in diameter were placed 4 mm apart from a center well. Reactions were allowed to proceed at room temperature and precipitin lines were observed after 24, 48 and 72 hrs. Sera were screened, in dilutions for the presence of precipitating antibodies to the soluble nuclear antigens by the double immunodiffusion test. The specificities of the precipitating antibodies were analysed and identified by use of the reference sera containing specific antibodies to known antigenic NHP.

Reference Sera. The anti-Sm, -RNP and -SS-B reference sera used in this study had been standardized internationally as previously described.10 Ne serum and Og serum were used as the prototype sera for anti-Ne5 and anti-Og6 systems. Ne serum was the protosera which was used by Drs. Miyachi and Tan in describing the anti-NCNA (proliferating cell nuclear antigen) system.15 The immunological identity between the anti-Og system and the anti-Scl-1 or scl-70 systems17 has been confirmed, as described elsewhere.6 The anti-SS-A reference sera was also identical to the standard sera used in Dr. E.M. Tan's laboratory.6 The anti-FM-1 reference serum12 was kindly supplied by Dr. M. Takano who has been using this serum as the standard serum for the FM-1 system in Dr. G. Sharp's laboratory. Protosera Mi and anti-Jo-1 reference sera were kindly provided by Dr. M. Nishikai.11,13 The Ki protosera used in this study was taken from a male patient with non-renal SLE fulfilling five items in the ARA's criteria for SLE; ic, polyarthritis, facial erythema, cytopenia, alopecia and positive LE cells.

Anti-DNA Antibodies. Anti-DNA antibodies were detected by the 3H-DNA binding assay according to the method of Pincus et al.10 Greater than 20%, binding of 3H-DNA was labelled positive, and greater than 50% as positive in high titer.

Clinical Analysis. Medical records of the 255 patients with SLE and 27 patients with SLE overlap syndrome were reviewed. Eighty items of clinical and laboratory findings, including the result of ANA determinations, were recorded in each of these patients. Statistical analysis of these data was performed with the z2 test using a FACOM OS IV computer.

RESULTS

A Distinction of Precipitating Antibodies to the Ki Antigen from Known Antibodies to NHP.

Initial double immunodiffusion studies were designed to demonstrate that anti-Ki antibodies had different antigen specificities from those of antibodies to known NHP, including recently described systems. As shown in Fig. 1, the anti-Ki precipitin lines clearly crossed the anti-RNP, -Sm and -SS-B precipitin lines. Also in Fig. 2, 3 and 4, the non-identity of the anti-Ki precipitin lines from those of known precipitating antibodies is shown.

The Frequency of Precipitating Antibodies to the Ki Antigen.

Table I shows the frequency of precipitating antibodies to the Ki antigen in 499 patients with various connective tissue diseases. Thirty of 255 patients with SLE (11.8%) and 2 of 36 patients with...
T. Tojo et al: Precipitating Antibody to the Ki Antigen

Table 3 Clinical findings associated with precipitating antibodies to the Ki antigen in 228 patients with SLE including 27 with SLE overlap syndrome

<table>
<thead>
<tr>
<th></th>
<th>Persistent arthritis</th>
<th>Pericarditis</th>
<th>Pulmonary hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Ki positive</td>
<td>11/35</td>
<td>6/35</td>
<td>4/35</td>
</tr>
<tr>
<td>Anti-Ki negative</td>
<td>34/247</td>
<td>16/243</td>
<td>5/237</td>
</tr>
<tr>
<td>F' value</td>
<td>7.13</td>
<td>6.68</td>
<td>8.28</td>
</tr>
</tbody>
</table>

*Only the significant difference (p<0.05) are shown

Table 2 Association of other ANA antibodies with LE cells and anti-Ki antibodies occurring in SLE sera

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of patients</th>
<th>Number positive</th>
<th>Percent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>255</td>
<td>30</td>
<td>11.8</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PM DM</td>
<td>29</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Overlap syndrome</td>
<td>36</td>
<td>7</td>
<td>19.4</td>
</tr>
<tr>
<td>PN</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RA</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

SLE: systemic lupus erythematosus
PM:DM: polymyositis or dermatomyositis
PN: periarteritis nodosa
RA: rheumatoid arthritis

with overlap syndrome (19.4%) had precipitating antibodies to the Ki antigen. Five of these 7 patients with these antibodies were diagnosed as SLE overlap syndrome. In the present study, none of the patients with other diseases had antibodies to the Ki antigen.

Figure 5 shows the precipitating antibody titers to the Ki antigen and other known NHP in sera from 255 patients with SLE.

Fig. 4 Precipitin reaction showing the non-identity of the anti-Ki precipitin line from the lines formed with the anti PM-1 and anti-SS-A reference sera. Well Ag contained calf thymus extract (100 mg/ml) in the upper fugue and human liver extract (40 mg/ml) in the lower figure. The Ki serum contained antibodies to the SS-A antigen.
the Ki antigen appeared to be specific for patients with SLE. The frequency of these antibodies was as high as that of anti-Sm antibodies. A positive correlation was observed between anti-Ki- and anti-Sm antibodies. These data indicate that antibodies to the Ki antigen may also have a close link with the diagnostic clinical features of patients with SLE.

Most lupus sera contain more than one type of ANA. Because of this complexity, clinical correlation with individual ANA is often hard to determine. In this study the clinical characteristics of lupus patients with anti-Ki antibodies were not conclusive as yet. However the clinic-serological analysis of ANA may help to determine the role of these antibodies in the pathogenesis of the different tissue lesions that are seen in patients with SLE and other connective tissue diseases.

REFERENCES
11) Nishikai M, Reichlin M: Purification and characterization of a nuclear non-histone basic protein (M-I) which reacts with anti-immunoglobulin sera and the sera of patients with dermatomyositis. Molecular Immunol 17: 1129-1141, 1980

The Rymachi 21 (Suppl.): 185-190, 1981

Immunogenetic Studies of Sjögren’s Syndrome

Yoshinobu NAKAO, Takuji FUJITA, Yasutaka YAMAI, Hiashi ISHIKAWA, Kazunori HIROYATA, Roji MIYAIWA, Toshihiko SUGINOHITA, Hideo MATSUMOTO and Kimiyo TSUJI

The Third Division, Department of Medicine, Kobe University School of Medicine, Kusunoki-cho, Chuo-ku, Kobe 650
# Department of Medicine, Shinnan Medical College
# Department of Orthopedics, Kobe University School of Medicine
# The Third Division, Department of Medicine, Okayama University School of Medicine
# Department of Medicine, Utano National Hospital
# Department of Legal Medicine, Osaka Medical College
# Department of Transplantation Immunology, Tokai University School of Medicine

Fifty-four unrelated patients with Sjögren’s syndrome, including 47 sicca alone (SS) and 7 SS with rheumatoid arthritis (SS-RA), were studied for HLA typing. Individually, serum samples from 86 patients with Sjögren’s syndrome, 154 with RA and 132 with SLE were examined for IgG heavy chain allotypes (Gm).

The frequency of HLA-DR5 was significantly increased in SS patients compared to the controls (p<0.01, corrected P<0.05, RR=5.32). In patients with SS-RA, none of them was HLA-DR5 positive, while 4 out of 7 patients (57.1%) were HLA-DR4 positive as compared to 52 out of 110 controls (47.2%). The sera from patients with SS and with RA did not show different Gm phenotype frequencies as compared to normal controls. Our data, however, suggest that the immunogenetic background of SS and SS-RA is different and SS-RA shows a resemblance to RA in HLA-DR antigen frequencies.

INTRODUCTION

Sjögren’s syndrome (SS; sicca syndrome) is an autoimmune disease characterized by lymphocytic infiltration of exocrine glands, particularly the lacrimal and salivary glands, resulting in xerostomia and xerophthalmia. Recent studies have called attention to the possible existence of subtypes of Sjögren’s syndrome. Moulopoulos and co-workers proposed that Sjögren’s syndrome be termed primary when it occurs alone and secondary when it is associated with another systemic autoimmune disease, especially with rheumatoid arthritis. In addition to attempting to divide this disorder into distinct clinical or serological subgroups, recent studies have examined the presence or absence of genetically homogenous subgroups based on the presence or association with certain HLA antigens in Caucasian patients. The present study was undertaken to determine whether the same immunogenetic background is present in Japanese patients.

MATERIALS AND METHODS

HLA Typing. Fifty-four unrelated Japanese patients with Sjögren’s syndrome, including 47 pri-
mary (sickle alone; SS) and 7 secondary type (SS with systemic autoimmune disease) were studied for HLA typing. Seven secondary Sjögren’s syndrome patients were in conjunction with definite or classic rheumatoid arthritis (SS-RA) diagnosed according to the preliminary American Rheumatism Association criteria. The diagnosis of Sjögren’s syndrome was based on xerostomia (decreased parotid flow rate, abnormal results on parotid scintigraphy, pathological changes in the biopsy specimen of the parotid gland) and keratoconjunctivitis sicca (punctate corneal ulcers on slitlamp examination and abnormal results in Schirmer’s test). The group with SS contained 4 men and 43 women 18 to 60 years old. The group with SS-RA were all women 21 to 56 years old. One-hundred and ten unrelated healthy men and women served as controls.

Microcytotoxicity tests for HLA-A, -B, and -DR specificities were carried out according to the standard techniques described at the 8th International Histocompatibility Workshop. To define HLA-A and -B specificities, 96 anti sera which covered 8 HLA-A, 18 HLA-B antigens were used. Sixty-one sera for defining 9 HLA-DR specificities were of local origin and were obtained through exchange with other investigators. All sera for HLA-DR typing were absorbed with pooled platelets and were confirmed as not reacting with T lymphocytes. The rabbit complement was carefully selected not to have cytotoxicity against B-lymphocytes. Comparisons between groups were made with Halden’s modification of the chi-square method.

### RESULTS

#### Frequencies of HLA-A and -B Antigens

All 54 patients with Sjögren’s syndrome were typed for HLA-A and -B antigens (Table 1, 2). A borderline increase of HLA-Bw60+61 was found in patients with SS as compared to controls. However, this increase, in the patients, was not statistically significant ($\chi^2=4.17$, p<0.05, corrected $p=0.05$, RR=2.05).

#### Frequencies of HLA-DR Antigens

Fourteen-nine patients with Sjögren’s syndrome were tested for HLA-DR antigens (Table 3). The frequency of HLA-DR3 was significantly increased in SS patients as compared to controls ($\chi^2=11.44$, p<0.005, corrected $p=0.05$, RR=3.52). In patients with SS-RA alone, HLA-DR5 positive, while 4 out of 7 patients (57.1%) were HLA-DR4 positive as compared to 52 out of 110 controls (47.2%).

### Table 1 HLA-A antigen frequencies in patients with Sjögren’s syndrome

<table>
<thead>
<tr>
<th>HLA-A Controls (N=110)</th>
<th>Sjögren’s syndrome (N=54)</th>
<th>SS-RA (N=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>A1 4  3.6</td>
<td>0  0</td>
<td>0  0</td>
</tr>
<tr>
<td>A2 49  44.5</td>
<td>51  54.7</td>
<td>30  63.01</td>
</tr>
<tr>
<td>A3 1  0.9</td>
<td>1  2.1</td>
<td>0  0</td>
</tr>
<tr>
<td>A2w24 62  56.5</td>
<td>32  59.2</td>
<td>26  55.3</td>
</tr>
<tr>
<td>A26 26  26.5</td>
<td>11  20.3</td>
<td>11  23.4</td>
</tr>
<tr>
<td>A11 20  18.2</td>
<td>7  12.9</td>
<td>6  12.7</td>
</tr>
<tr>
<td>A3 1  0.9</td>
<td>1  2.1</td>
<td>0  0</td>
</tr>
<tr>
<td>A1 16  14.5</td>
<td>1  1.8</td>
<td>0  0</td>
</tr>
</tbody>
</table>

1) $\chi^2=4.889$, P<0.05, Corrected $p=0.05$, Relative risk=2.19

### Table 2 HLA-B antigen frequencies in patients with Sjögren’s syndrome

<table>
<thead>
<tr>
<th>HLA-B Controls (N=110)</th>
<th>Sjögren’s syndrome (N=54)</th>
<th>SS-RA (N=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>B7 13  11.8</td>
<td>5  9.2</td>
<td>4  8.5</td>
</tr>
<tr>
<td>B8 0  0</td>
<td>0  0</td>
<td>0  0</td>
</tr>
<tr>
<td>B13 4  3.6</td>
<td>5  9.2</td>
<td>2  4.2</td>
</tr>
<tr>
<td>B13 23  20.9</td>
<td>13  24.0</td>
<td>11  23.4</td>
</tr>
<tr>
<td>B27 0  0</td>
<td>0  1.8</td>
<td>1  2.1</td>
</tr>
<tr>
<td>B27 0  0</td>
<td>0  3.5</td>
<td>5  6.3</td>
</tr>
<tr>
<td>B35 22  20.0</td>
<td>5  9.2</td>
<td>5  10.6</td>
</tr>
<tr>
<td>B35 2  1.8</td>
<td>0  0</td>
<td>0  0</td>
</tr>
<tr>
<td>B50 0  0</td>
<td>0  0</td>
<td>0  0</td>
</tr>
<tr>
<td>B50(16) 4  3.6</td>
<td>5  9.2</td>
<td>5  6.3</td>
</tr>
<tr>
<td>B50(12) 15  13.6</td>
<td>6  11.1</td>
<td>6  12.8</td>
</tr>
<tr>
<td>B66 5  4.3</td>
<td>1  1.8</td>
<td>1  2.1</td>
</tr>
<tr>
<td>B66 5  4.3</td>
<td>1  1.8</td>
<td>1  2.1</td>
</tr>
<tr>
<td>B66 5  4.3</td>
<td>1  1.8</td>
<td>1  2.1</td>
</tr>
<tr>
<td>B66 5  4.3</td>
<td>1  1.8</td>
<td>1  2.1</td>
</tr>
</tbody>
</table>

1) $\chi^2=5.09$, P<0.05, Corrected $p=0.05$, Relative risk=2.14

### Table 3 HLA-DR antigen frequencies in patients with Sjögren’s syndrome

<table>
<thead>
<tr>
<th>HLA-DR Controls (N=110)</th>
<th>Sjögren’s syndrome (N=49)</th>
<th>SS-RA (N=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>DR1 14  12.7</td>
<td>4  8.2</td>
<td>3  7.1</td>
</tr>
<tr>
<td>DR2 36  32.7</td>
<td>11  25.2</td>
<td>11  19.0</td>
</tr>
<tr>
<td>DR3 3  0.9</td>
<td>3  6.1</td>
<td>3  7.1</td>
</tr>
<tr>
<td>DR4 22  17.2</td>
<td>16  22.6</td>
<td>12  28.5</td>
</tr>
<tr>
<td>DR5 7  6.3</td>
<td>11  22.41</td>
<td>11  25.1</td>
</tr>
<tr>
<td>DRw6 21  19.1</td>
<td>5  10.2</td>
<td>4  9.5</td>
</tr>
<tr>
<td>DRw9 3  0.9</td>
<td>0  0</td>
<td>0  0</td>
</tr>
<tr>
<td>DRw9 13  11.8</td>
<td>3  10.2</td>
<td>4  9.5</td>
</tr>
<tr>
<td>DRw9 30  27.2</td>
<td>3  10.2</td>
<td>4  9.5</td>
</tr>
</tbody>
</table>

1) $\chi^2=8.41$, P<0.005, Corrected $p=0.05$, Relative risk=4.15

2) $\chi^2=11.44$, P<0.005, Corrected $p=0.05$, Relative risk=3.52

3) $\chi^2=0.25$, P<0.05, Relative risk=1.48
Table 4 Gm phenotype frequencies in patients with Sjögren's syndrome

<table>
<thead>
<tr>
<th>Gm phenotypes</th>
<th>Normal controls (N=135)</th>
<th>Classical Sjögren’s syndrome (N=86)</th>
<th>Rheumatoid arthritis (N=135)</th>
<th>Classical Sjögren’s (N=86)</th>
<th>Rheumatoid arthritis (N=135)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>1, 21</td>
<td>72 (21.0)</td>
<td>20 (23.2)</td>
<td>32 (23.7)</td>
<td>5 (36.3)</td>
<td>7 (5.0)</td>
</tr>
<tr>
<td>1, 13, 15, 16</td>
<td>82 (25.3)</td>
<td>24 (27.9)</td>
<td>26 (21.9)</td>
<td>4 (21.0)</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>1, 2, 21</td>
<td>54 (15.7)</td>
<td>11 (12.8)</td>
<td>25 (18.5)</td>
<td>4 (21.0)</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>1, 3, 5, 13, 21</td>
<td>37 (10.8)</td>
<td>6 (6.9)</td>
<td>7 (5.2)</td>
<td>2 (15.0)</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>1, 2, 15, 16, 21</td>
<td>54 (9.0)</td>
<td>9 (18.4)</td>
<td>12 (22.8)</td>
<td>2 (15.0)</td>
<td>2 (1.5)</td>
</tr>
<tr>
<td>1, 3, 3, 5, 15</td>
<td>75 (12.5)</td>
<td>2 (2.3)</td>
<td>15 (2.3)</td>
<td>2 (15.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>1, 15, 16</td>
<td>19 (5.5)</td>
<td>8 (9.3)</td>
<td>15 (9.6)</td>
<td>2 (10.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>1, 2, 5, 13, 21</td>
<td>13 (3.5)</td>
<td>6 (6.9)</td>
<td>6 (4.4)</td>
<td>1 (5.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>1, 3, 5, 15</td>
<td>7 (2.0)</td>
<td>0 (0.0)</td>
<td>1 (0.7)</td>
<td>0 (0.0)</td>
<td>2 (1.5)</td>
</tr>
</tbody>
</table>

1) \( Z^2 \geq 4.18, P < 0.05, \) Corrected \( P>0.05, \) Relative risk = 1.82

* JRA: juvenile rheumatoid arthritis

** SLE: systemic lupus erythematosus

Table 5 Gm haplotype frequencies in patients with Sjögren's syndrome

<table>
<thead>
<tr>
<th>Gm haplotype</th>
<th>Normal controls (N=133)</th>
<th>Classical Sjögren’s syndrome (N=135)</th>
<th>Rheumatoid arthritis (N=135)</th>
<th>Classical Sjögren’s (N=86)</th>
<th>Rheumatoid arthritis (N=135)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>Gm( ^{1,2} )</td>
<td>55.4 43.5</td>
<td>55.4 39.4</td>
<td>55.4 39.4</td>
<td>55.4 39.4</td>
<td>55.4 39.4</td>
</tr>
<tr>
<td>Gm( ^{1,1} )</td>
<td>54.3 13.9</td>
<td>54.3 17.9</td>
<td>54.3 17.9</td>
<td>54.3 17.9</td>
<td>54.3 17.9</td>
</tr>
<tr>
<td>Gm( ^{1,3,5,15} )</td>
<td>22.5 25.3</td>
<td>22.5 25.3</td>
<td>22.5 25.3</td>
<td>22.5 25.3</td>
<td>22.5 25.3</td>
</tr>
<tr>
<td>Gm( ^{1,3,5} )</td>
<td>42.2 12.9</td>
<td>42.2 12.9</td>
<td>42.2 12.9</td>
<td>42.2 12.9</td>
<td>42.2 12.9</td>
</tr>
</tbody>
</table>

* JRA: juvenile rheumatoid arthritis

** SLE: systemic lupus erythematosus

1) \( Z^2 \geq 6.41, P < 0.025, \) Corrected \( P>0.05, \) Relative risk = 1.57

**DISCUSSION**

The earliest studies of HLA-A and -B antigens in Sjögren's syndrome included patients with and without rheumatoid arthritis and an increased prevalence of HLA-Bw8 was noted in Caucasians. In 1977, Chused et al.\(^{112}\) reported an increased prevalence of HDL-Dw5 in SLE and noted that 5 of their 6 SS-RPs lacked this antigen. On the other hand, HLA-DDR-3 was increased in patients with SS-RP. This observation is in keeping with the reported increase of HDL-DR4 in rheumatoid arthritis. Thus, Moutopoulos and coworkers proposed that there are genetic differences between SS and SS-RP patients. They suggested that SS is a distinct, genetically determined, immunologic disorder, whereas SS-RP is a secondary, glanular response to chronic lymphocytic activation elsewhere.\(^{7}\)

In our present study, an increased prevalence of HLA-DR3 was demonstrated in SS patients but not in SS-RP patients. In contrast, the distribution of HLA-DR antigens in SS-RP patients showed a similarity to that of RA patients. Both testing of HLA and Gm is underway in our laboratories. We may, however, suggest that the immunogenetic background of SS and SS-RP is different in Japanese patients as in Caucasians.

This work was supported in part by a Grant-in-Aid for Specific Diseases from the Ministry of Health and Welfare of Japan.

Acknowledgments. We thank Dr. T. Miyazaki, Mrs. K. Sugata, and Miss Y. Maruyama for their technical assistance.

**REFERENCES**


A positive level of circulating immune complexes was demonstrated in about 30% of patients with Behçet’s syndrome. The investigation of immunochemical properties of circulating immune complexes was performed, in Behçet’s syndrome, by analysis of cryoproteins. The results obtained were as follows.

1. A positive level of cryoproteins was recognized in 8 of 50 patients with Behçet’s syndrome. An erythema nodosum-like eruption and articular symptoms were found to be strongly associated with cryoglobulinemia.

2. A heterogeneity in the size of the immune complexes was found in sera and cryoproteins from 3 cryoprotein positive patients, using sucrose density gradient ultracentrifugation.

3. Upon examination of rheumatoid factor activity in the fractions obtained by sucrose density gradient ultracentrifugation and acidic treatment of complexed rheumatoid factor it was noted that rheumatoid factor bound immune complexes existed in sera and cryoproteins of patients with Behçet’s syndrome.

4. The ability of solubilized cryoproteins to activate complement by the classical or alternative pathways was examined by in vitro assays. Seven of eight cryoproteins activated by the classical pathway. Five of eight cryoproteins also activated by the alternative complement pathway.

INTRODUCTION

Since the report by Behçet in 1937 of the clinical triad of recurrent aphthous oral ulcerations, genital ulcerations and ocular inflammation, many other manifestations of Behçet’s syndrome including skin lesions, arthritis, thrombophlebitis, arteritis, colonic ulcerations, and involvement of the central nervous system have been recognized. The etiology and pathogenesis of Behçet’s syndrome are unknown. The demonstration of both antibodies to oral mucosa1-7 and cellular immune response to tissue homogenate8,9 suggest an immunologic pathogenesis for the disease. Activation of complement preceding acute uveitis,9,10 and deposition of immunoglobulins and C3 in the wall of venules and capillaries11,12 have been noted. These observations suggest that immune complexes may be contributing to the systemic inflammatory process. Several reports13-19 have demonstrated the presence of circulating immune complexes in Behçet’s syndrome. Previous reports from our laboratory19 have shown that a positive level of circulating immune complexes is recognized in about 30% of patients with Behçet’s syndrome and are frequently observed in the active stage of articular lesions and an
erythema nodosum-like eruption. Though most immune complexes-composing antibodies have been shown to belong to IgG.\textsuperscript{10} The size and the ability of immune complexes to activate complement and bind rheumatoid factor are unknown. The purpose of this study was designed to evaluate these immunological properties of immune complexes by analysis of cryoproteins in Bechet's syndrome.

**MATERIALS AND METHODS**

**Patients and Controls.** The fifty patients included in the present study fulfilled the criteria (Ministry of Welfare, Japan 1972) for the diagnosis of Bechet's syndrome. All patients were seen and followed at Teikyo University Hospital. 20 patients were females and 30 were males. Their ages ranged between 27 years and 64 years with a mean of 40.7 years. Eighteen normal healthy adults were used as controls.

**Collection of Sera and Isolation of Cryoprecipitates.** Blood was withdrawn from patients into warmed sterile tubes and allowed to clot at 37°C in a water bath for 2 hours. Serum was separated from the clot by centrifugation at 3,000rpm for 10 minutes at 37°C. 3 ml sera were kept at 4°C for 72 hours. The precipitates which formed were isolated by centrifugation at 15,000 rpm for 20 minutes at 4°C and washed three times with 3 ml cold 0.01M phosphate buffered 0.15M sodium chloride (PBS) (pH 7.2) and were solubilized in 3 ml PBS (pH 7.2) at 37°C. The isolated cryoprecipitates showed no reaction in double immunodiffusion against antihuman serum albumin, showing no detectable cryoprecipitation of serum proteins. To detect activation of the classical and alternative complement pathways, the cryoprecipitates were prepared from fresh serum or serum that had been stored at −70°C. The protein concentration in the cryoprecipitates was measured by the Lowry method using a standard protein curve from purified IgG.

**Analysis of Cryoprecipitates.** The amount of immunoglobulins and complement proteins in the isolated cryoprecipitates was measured by single radial immunodiffusion. Rheumatoid factor activity in the isolated cryoprecipitates and serum was examined by the latex fixation test.

**Solute Density Gradient Ultracentrifugation Studies.** The isolated cryoprecipitates were dissolved in equal volumes of PBS (pH 7.2) at the same serum. After solubilization, a 0.25 ml sample was layered on to a 5 ml linear 10–40% sucrose gradient made in 0.06M citrate buffer (pH 7.2). The gradients were formed by using an automatic density gradient maker (Atto instruments, Tokyo). A 0.25 ml serum sample was examined by the same technique. Samples were centrifuged at 35,000 rpm for 20 hours at 4°C using an ultracentrifuge of type 65P (Hitachi instruments, Tokyo). Marker proteins of 4.3S (BSA), 7.3S (alcohol dehydrogenase), 11.3S (catalase) and 16S (β-galactosidase) were centrifuged separately with each experiment. The sucrose density gradient was separated into 0.2 ml fractions by collecting it drop by drop from the bottom of the tube.

**Assays of Immune Complexes.** Determination of immune complexes was performed by the platelet aggregation test according to Pentillen's method\textsuperscript{11} and the polyethylene glycol test according to Dignon's method.\textsuperscript{12}

**Determination of Complexed Rheumatoid Factor.** The presence of complexed rheumatoid factor (RF) was investigated in sera and immune complexes enriched serum fractions of cryoprotein positive patients. Serum was inactivated at 56°C for 30 minutes. The immune complexes enriched fraction was isolated from native serum by precipitation with 3.5% polyethylene glycol according to Dignon's method.\textsuperscript{12} These aliquots were diluted 4-fold with 1/15M PBS (pH 7.2) or 0.2M glycine-HCl buffer (pH 3.2) and incubated overnight at 4°C. The RF titers of the diluted samples were determined using a Kit of Rheumatoid Arthritis Haemagglutination test. Neutralization of acidified samples was done in the 1st row of microplates by means of 0.4 M Na₂HPO₄, immediately before titration of RF activity. An at least 4-fold increase in RF titer, after acidic treatment, was accepted as criterion for positivity of complex RF.\textsuperscript{13}

**Activation of the Classical Complement Pathway by Isolated Cryoprecipitates.** Activation was determined by the percent of haemolytic C4 consumed when normal human serum was incubated with isolated cryoprotein.\textsuperscript{14} 1 volume of cryoprotein was added to 2 volumes of fresh normal human serum and incubated at 37°C for 60 minutes. The effective molecule titration of C4 remaining was measured by adding dilutions of this serum to EAC1 human cells (2.0 × 10⁹/ml) and fresh C4-deficient guinea pig serum (final dilution 1:40) to provide the terminal complement proteins. The percentage of haemolytic C4 consumed was determined by comparing the cryoprecipitate-activated fresh normal serum with a control containing only PBS and fresh normal serum. Any cryoprecipitates which produced >10%, consumption of C4 were considered positive for activation of the classical complement pathway.\textsuperscript{14} All assays were performed in duplicate on the same day.

**Activation of the Alternative Complement Pathway by Isolated Cryoprecipitates.** Activation of the alternative complement pathway was examined by factor B conversion into split products.\textsuperscript{14,16} 5 µl fresh normal human serum in the presence of EGTA (10mMol/l) and MgCl₂ (0.5mMol/l) was permitted to react at 37°C for 30 minutes. The mixture was then electrophoresed on a microscope slide coated with 1.5% agarose using veronal buffer (pH 8.6, isotonic strength 0.05) and EDTA (0.02mMol/l). The slide was developed with monospecific antiserum to factor B. Conversion of factor B, indicating alternative complement pathway activation, was detected by the appearance of split products with alpha and gamma mobility.

**RESULTS**

**Frequency of Positive Level of Cryoproteins.** The cryoprotein concentrations of 18 sera from normal subjects were in the range 0.38–7.57mg/dl, mean 3.53mg/dl. The threshold of positivity was 0.05mg/dl which represents the mean increased by two standard deviations. Cryoproteins above 0.05mg/dl were detected in 8 of 50 patients with Bechet's syndrome, 4 of 7 patients with systemic lupus erythematosus, 2 of 5 patients with progressive systemic sclerosis, 2 of 9 patients with rheumatoid arthritis, and none of 18 normal healthy subjects (Fig. 1).

**Analysis of Cryoprecipitates and Sera from Cryoprotein Positive Patients.** The immunoglobulin classes detected in the cryoprecipitates from B cryoprotein positive patients with Bechet's syndrome were IgG and IgM in 4, IgG and IgA in 1, and IgG alone in 1. The complement proteins detected in the cryoprecipitates in those patients were C3 and C4 in 2, and C3 alone in 1. Six of 8 cryoprecipitates and 3 of 8 sera from cryoprotein positive patients contained RF activity. RF activity in cryoprecipitate was inhibited in vitro by the same density of purified IgG of serum. Circulating immune complexes were detected in all 8 patients using the platelet aggregation test and in 4 of 8 patients using the polyethylene glycol test (Table1).

**Clinical Manifestations of Cryoprotein Positive Patients**

The clinical manifestations of cryoprotein positive patients with Bechet's syndrome are shown in Table 2. An erythema nodosum-like eruption and articular symptoms appeared, most frequently, within 3 months prior to examination and constituted the chief complaint in cryoprotein positive patients.
Table 1 Laboratory data of cryoprotein positive patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Cryoprotein (mg/dl)</th>
<th>PEG test</th>
<th>Pi a. test</th>
<th>RF activity in serum</th>
<th>RF activity in cryoprecipitates</th>
<th>Immunoglobulins in cryoprecipitates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y.M.</td>
<td>15.00</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>IgA, IgG</td>
</tr>
<tr>
<td>Y.Y.</td>
<td>12.27</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>IgG, IgM</td>
</tr>
<tr>
<td>S.K.</td>
<td>11.10</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>IgG, IgM</td>
</tr>
<tr>
<td>H.T.</td>
<td>32.93</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>IgG, IgM</td>
</tr>
<tr>
<td>K.Y.</td>
<td>10.50</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>IgG</td>
</tr>
<tr>
<td>K.H.</td>
<td>11.33</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>IgG</td>
</tr>
<tr>
<td>Y.Y.</td>
<td>8.07</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>IgG</td>
</tr>
</tbody>
</table>

Table 2 Clinical manifestations of cryoprotein positive patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Oral ulcers</th>
<th>Ocular lesions</th>
<th>Erythema nodosum</th>
<th>Other skin lesions</th>
<th>Genital ulcer</th>
<th>Articular lesions</th>
<th>Vasculo-Behçet</th>
<th>Neuro-Behçet</th>
<th>Enema-Behçet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y.M.</td>
<td>+</td>
<td>+</td>
<td>☀</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>☀</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y.Y.</td>
<td>+</td>
<td>☀</td>
<td>☀</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>☀</td>
<td></td>
<td>☀</td>
</tr>
<tr>
<td>S.K.</td>
<td>+</td>
<td>☀</td>
<td>☀</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>☀</td>
<td></td>
<td>☀</td>
</tr>
<tr>
<td>H.T.</td>
<td>+</td>
<td>+</td>
<td>☀</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>☀</td>
<td>☀</td>
<td>☀</td>
</tr>
<tr>
<td>K.Y.</td>
<td>+</td>
<td>+</td>
<td>☀</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>☀</td>
<td>☀</td>
<td>☀</td>
</tr>
<tr>
<td>K.H.</td>
<td>+</td>
<td>+</td>
<td>☀</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>☀</td>
<td>☀</td>
<td>☀</td>
</tr>
<tr>
<td>Y.Y.</td>
<td>+</td>
<td>☀</td>
<td>☀</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>☀</td>
<td>☀</td>
<td>☀</td>
</tr>
<tr>
<td>K.H.</td>
<td>+</td>
<td>+</td>
<td>☀</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>☀</td>
<td>☀</td>
<td>☀</td>
</tr>
</tbody>
</table>

 (): The chiefly complained symptoms
 (): The symptoms which appeared within the past three months

Determination of the Size and Rheumatoid Factor Binding Ability of Immune Complexes by Sucrose Density Gradient Ultracentrifugation

Cryoprecipitates and sera from 3 cryoprotein positive patients with Behçet's syndrome were fractionated by sucrose density gradient ultracentrifugation and determination of the approximate molecular size and rheumatoid factor binding ability of the immune complexes was performed. The results of the platelet aggregation test and the latex fixation test performed on the sucrose density gradient fractions in case 1 are presented in Fig. 2. Heterogeneity in the size of immune complexes was found in both sera and cryoprecipitates of all three cases. The bulk of immune complex activity existed in fractions 78-198 and above 198. In case 1, RF activity of serum was found in fractions 78-198 and at 198, and also that of the cryoprecipitates was found in fractions above 198. In case 2, RF activity of serum was recognized in fractions above 198, and that of the cryoprecipitates in fractions 78-198. These findings suggest the presence of RF bound immune complexes and IgG RF in Behçet's syndrome.

Complexed Rheumatoid Factor Measurement

The presence of complexed RF was investigated by acidic treatment of serum and immune complex enriched serum fractions by 3.5% polyethylene glycol from 8 cryoprotein positive patients with Behçet's syndrome. An at least 4-fold increase in RF titers after acidic treatment was recognized in 3 of 8 sera and 2 of 8 immune complex enriched serum fractions (Table 3).

Activation of the Complement System

The ability of the isolated cryoprecipitates to activate the classical complement pathway was determined by the percentage consumption of haemolytic C4 when the cryoprecipitates were incubated with fresh normal human serum. Activation of the alternative complement pathway by isolated cryoprecipitate was determined by immunoelectrophoretic conversion of factor B when the cryoprecipitates were incubated with fresh normal human serum under conditions where C1 and C4 activation was inhibited. Seven of 8 cryoprecipitates from cryoprotein positive patients with Behçet's syndrome were activated by the classical complement pathway. Five of 8 cryoprecipitates from cryoprotein positive patients with Behçet's syndrome produced conversion of factor B into split products of alpha and gamma mobilities (Table 4).

DISCUSSION

The presence of immune complexes in the cryoprecipitates of patients with immunological diseases has been demonstrated. In this study immunochromic properties of circulating immune complexes in Behçet's syndrome was investigated by analysis of cryoproteins. Two cases of mixed cryoglobulinemia associated with Behçet's syndrome have been described previously. Clinical manifestations such as peripheral oedema, lymphoproliferative disorder, peripheral neuropathy and immune complex mediated vasculitis have been recognized in those
cases. A positive level of cryoproteins was recognized in 8 of 50 patients (16%) with Behçet's syndrome, a lower frequency as compared to systemic lupus erythematosus and progressive systemic sclerosis and at the same frequency as compared to rheumatoid arthritis, in our study. An erythema nodosum-like eruption and articular symptoms have been found to be strongly associated with cryoglobulinemia in Behçet's syndrome. These clinical manifestations also have been frequently noted in circulating immune complex positive patients.19

The heterogeneity in the size of the immune complexes has been reported in Behçet's syndrome.7,20 This study had also provided evidence of heterogeneity in the size of the immune complexes using sucrose density gradient ultracentrifugation. The bulk of immune complex activity existed in zones 7S-19S and above 19S. The presence of RF bound immune complexes was suggested in Behçet's syndrome by the evidence that RF activity existed in cryoprecipitates more frequently than in serum. Further investigations of RF binding ability of immune complexes were performed in Behçet's disease. RF activity of serum and cryoprecipitates was often revealed in zones 7S-19S and above 19S by sucrose density gradient ultracentrifugation, indicating the presence of RF bound immune complex and IgG RF. The existence of 'hidden' RF proved by acidic treatment in serum and immune complex enriched fractions from cryoprotein positive patients has also supported the RF binding ability of immune complex in Behçet's syndrome.

Activation of complement preceding acute uveitis6 and deposition of immunoglobulins and C3 in the walls of venules and capillaries10 have been already recognized. Circulating immune complexes in Behçet's syndrome have been suggested as contributing to the systemic inflammatory process. It was disclosed in our study, by in vitro assays, that the cryoproteins from patients with Behçet's syndrome not only activated the complement system via the classical pathway but also via the alternative pathway.

Though we do not know, at present, whether immune complexes may be responsible for any of the manifestations of Behçet's syndrome or whether their presence reflects a response to the pathologic process, the existence of circulating immune complexes with ability to bind complement and RF may play an important role in the pathogenesis of multiple organ involvements, especially of an erythema nodosum-like eruption and articular lesions in Behçet's syndrome.

REFERENCES
6) Hashimoto T: Articular lesion in Behçet's disease. Ryuunchi 17: 271-275, 1973
A New Model of Experimental Arthritis Induced by an Aqueous Form of Synthetic Adjuvant in Immunodeficient Rats (SHR and Nude Rats)

Osamu KOHASHI, Yukiko KOHASHI, Shojo KOTANI and Atushi OSAWA

Department of Microbiology, Tokai University School of Medicine, Isehara, Kanagawa 259-11
* Department of Microbiology, Osaka University Dental School

A water-in-oil emulsion containing a synthetic adjuvant, N-acetylmuramyl-L-alanyl-D-isoglutamine (MDP) produced polyarthritis in euthymic rats, clinically and histologically indistinguishable from classic adjuvant-induced arthritis. MDP did not induce any disease in immunodeficient rats such as congenitally athymic nude rats and spontaneous hypertensive rats, suggesting an important role of the thymus in the development of MDP-induced arthritis. We, however, found that these immunodeficient rats, as well as immunologically normal rats, developed polyarthritis with a 100% incidence after consecutive intravenous administration of an aqueous form of lipid-conjugated MDP instead of its water-in-oil emulsion. The histological features of this new model of arthritis revealed acute inflammatory reactions evidenced as acute exudative synovitis, subsynovitis and tendinitis which were comparable to those of the acute stage of classic adjuvant-induced arthritis.

This acute polyarthritis rapidly subsided after stopping the injection. The acute swelling flared up within 24 hours after each booster injection. The acute swelling following booster injections revealed a very enlarged synovial cavity with the proliferation of synovial lining cells and relatively few polymorphonuclear leukocytes, suggesting an increased vascular permeability.

It was hypothesized that MDP-induced arthritis may consist of two stages: one, an acute inflammatory reaction which may be T cell independent and the other, a chronic one which may be T cell dependent and possibly related to some kind of immune response.

INTRODUCTION

Adjuvant-induced arthritis is regularly produced by a single intradermal injection of complete Freund's adjuvant (CFA) containing Mycobacterium or with tubercle bacilli or their components in mineral oil alone. Tubercle bacilli in saline did not induce adjuvant disease and actually prevented subsequent induction of arthritis by CFA. This disease is believed to be one manifestation of delayed type hypersensitivity to disseminated mycobacterial fragments. Mycobacterium can be replaced by many kinds of bacterial cell walls and their cell wall peptidoglycans. One of the most important arthritogenic components shared among these bacterial cell walls was found to be a peptidoglycan moiety which consists of two or more glycan units. There was, however, no direct correlation between the development of adjuvant disease and delayed type hypersensitivity to these peptidoglycans. Most recently it was

Keywords: animal model, arthritis, lipid-conjugated MDP, SHR, nude rats

† To whom reprint requests should be addressed.
found that N-acetylmuramyl-L-alanyl-D-isoglutamic acid (MDP) is one of the minimal structures responsible for development of adjuvant-induced arthritis.\(^9\) MDP is well known as an essential structure of CFA in terms of enhancing humoral immunity, induction of cell-mediated immunity and enhancing tumor immunity or protective immunity to infectious disease.\(^{10}\)

It is thus possible that MDP-induced arthritis is the same disease as classic adjuvant-induced arthritis.\(^11\) In addition to a previous report,\(^1\) our previous findings\(^9\) demonstrated that MDP as well as CFA failed to produce arthritis in congenitally athymic nude rats, suggesting an important role of the thymus in the production of this disease.

Our preliminary findings revealed that spontaneous hypertensive rats (SHR), T cell deficient strain,\(^12\) resisted disease development whereas they developed the disease after consecutive intravenous administration of lipid-conjugated MDP instead of MDP itself.\(^12\) This finding prompted us to investigate whether congenitally athymic nude rats can also develop the disease after administration of lipid-conjugated MDP in aqueous form in order to elucidate the involvement of thymus function in promoting the development of adjuvant-induced arthritis. The present studies thus demonstrated that an aqueous form of lipid-conjugated MDP but not MDP itself induces polyarthritis in immunodeficient rats such as congenitally athymic nude rats and SHR. Histological features of this new model of arthritis are also described as representing an acute stage of classic adjuvant-induced arthritis.

**MATERIALS AND METHODS**

**Animals.** Spontaneous hypertensive rats (SHR/NCr) were kindly obtained from Japan Charles River Inc. and were used at 6 to 8 weeks of age. Congenitally athymic nude rats (rmu/nu) and their littermates of euthymic rats (rmu/+) were obtained from The Central Institute for Experimental Animals, Kawasaki, Japan. Lewis and Sprague-Dawley rats (SD) were obtained from Japan Charles River Inc.. All of these rats were housed under specific pathogen-free condition in the animal center of Tokai University.

**Adjuvants.** A synthetic adjuvant, N-acetylmuramyl-L-alanyl-D-isoglutaminic acid (MDP) and its analogs were kindly obtained from Daichi Pharmaceutical Co., Tokyo. Lipid-conjugated MDP analogs were 6-O-stearyl MDP (LS304MDP) and 6-O-(2-tetracetylhexadecanoyl) MDP (B305MDP).

MDP and lipid-conjugated MDP were synthesized as previously described.\(^13\) Heat-killed Mycobacterium bovis (BCG) was completely crushed with a mortar and pestle in squelane (Wako Pure Chemicals, Japan) to make complete Freund's adjuvant.

**The Induction of Adjuvant-induced Arthritis.** MDP and lipid-conjugated MDP were dissolved in sterile phosphate buffered saline (PBS; 0.01M, pH 7.2). 6-O-stearyl MDP (LS304MDP) was very soluble in PBS but B305MDP was not soluble in PBS. B305MDP was thus suspended in PBS by means of a Teflon Homogenizer. A water-in-oil (w/o) emulsion was prepared by dropwise addition of PBS containing MDP or lipid-conjugated MDP to an equal volume of mineral oil (incomplete Freund's adjuvant: Difco). Each rat was injected intradermally by means of a tail vein or intradermally into the left hind foot pad, with an indicated amount of adjuvant under ether anesthesia. The intravenous injections were carried out by daily administration of the adjuvant at about the same time in the morning on the indicated days. After inoculation, the rats were examined daily to evaluate the time of onset of polyarthritis and they were graded 0 to 4 for each appendage except for the injected paw, as in a previous paper.\(^14\)

**Histologic Studies.** Rats were sacrificed at various intervals after adjuvant inoculation. Tissue sections were fixed in 10% formalin, decalcified in formic acid and stained with hematoxylin and eosin.

**Leukocyte Counts.** Thirty SD rats were divided into three groups; the rats were intravenously injected with lipid-conjugated MDP (2 mg/kg), MDP (2 mg/kg) and PBS, respectively. Five rats from each group were bled by means of a tail vein at 3, 6, 12, 24, 48 and 72 hours after the injection to make blood smears and to measure peripheral blood leukocyte counts with a hemocytometer. Blood smears were stained by May-Grünwald stain and observed under the microscope (1000 x).

**RESULTS**

The Susceptibility of Rat Strains to Adjuvant-Induced Arthritis

As shown in Table 1, euthymic rmu/+ rats and Lewis rats were most susceptible to BCG-induced arthritis, SHR was very resistant and congenitally athymic nude rats were nonresponders. After inoculation of MDP in a w/o emulsion, all the rmu/+ rats developed severe arthritis comparable to that of BCG-induced arthritis. Most of the Lewis rats also developed the disease with less severity than the rmu/+ rats. SHR and nude rats did not develop any disease even after administration of higher doses of MDP.

**Table 1 The susceptibility of rat strains to adjuvant-induced arthritis**

<table>
<thead>
<tr>
<th>Rat Strains</th>
<th>Adjuvant*</th>
<th>Polyarthritis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCG</td>
<td>Type</td>
</tr>
<tr>
<td>rmu/+</td>
<td>BCG</td>
<td>w/o</td>
</tr>
<tr>
<td></td>
<td>MDP</td>
<td>w/o</td>
</tr>
<tr>
<td>Lewis</td>
<td>BCG</td>
<td>w/o</td>
</tr>
<tr>
<td></td>
<td>MDP</td>
<td>w/o</td>
</tr>
<tr>
<td>SHR/NGj</td>
<td>BCG</td>
<td>w/o</td>
</tr>
<tr>
<td></td>
<td>MDP</td>
<td>w/o</td>
</tr>
<tr>
<td>rmu/nu</td>
<td>BCG</td>
<td>w/o</td>
</tr>
<tr>
<td></td>
<td>MDP</td>
<td>w/o</td>
</tr>
<tr>
<td>rmu/+</td>
<td>BCG</td>
<td>w/o</td>
</tr>
</tbody>
</table>

* Each rat was injected intradermally into its left hind foot pad 0.05 ml of a water-in-oil (w/o) emulsion containing the indicated amounts of various adjuvants.

a Severity was calculated as an arithmetic mean of the highest score of each rat per group.

**Table 2 The successful induction of adjuvant-induced arthritis with an aqueous form of lipid-conjugated MDP in immunologically deficient rats**

<table>
<thead>
<tr>
<th>Rat Strains</th>
<th>Adjuvant*</th>
<th>Polyarthritis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type</td>
<td>Dose(µg/kg)</td>
</tr>
<tr>
<td>SHR/NGj</td>
<td>Lip-MDP</td>
<td>2 mg/kg</td>
</tr>
<tr>
<td></td>
<td>MDP</td>
<td>2 mg/kg</td>
</tr>
<tr>
<td></td>
<td>MDP</td>
<td>5 mg/kg</td>
</tr>
<tr>
<td>rmu/nu</td>
<td>Lip-MDP</td>
<td>2 mg/kg</td>
</tr>
<tr>
<td></td>
<td>MDP</td>
<td>2 mg/kg</td>
</tr>
<tr>
<td>Lewis</td>
<td>Lip-MDP</td>
<td>2 mg/kg</td>
</tr>
<tr>
<td></td>
<td>[B305]-MDP</td>
<td>6X</td>
</tr>
</tbody>
</table>

a Each rat was injected intradermally with an aqueous solution containing the indicated amounts of adjuvant on the indicated days.
joints which already had the disease. This occurred as early as 24 hours following the booster injection. A similar phenomenon was observed in congenitally athymic nude rats. This was a rather unexpected finding, namely, seven consecutive intravenous injections of lipid-conjugated MDP in aqueous form induced acute swellings around joints in all the nude rats slightly earlier than the 2 to 4 days than that of SHR after the first inoculation. MDP, in aqueous form, did not induce any disease even with higher doses. It was also surprising that the consecutive injections of an aqueous form of lipid-conjugated MDP induced acute swellings around joints in +/+ rats as early as 24 hours following the first injection. Seven consecutive injections of PBS did not induce any disease in these rats.

**Histological Studies of the New Model of Arthritis**
A total of 60 sections were obtained from 40 affected animals. Two or three animals were sacrificed for histological studies at 1, 2, 3, and 4 weeks after the administration of the adjuvants.

The microscopic changes started as an acute inflammatory reaction of synovial and sub synovial tissues, along the peristeme and around the tendon sheaths and occasionally between muscle bundles. This acute inflammatory reaction consisted of an acute exudative synovitis, sub synovitis and tendinitis infiltrated with massive numbers of polymorphonuclear leukocytes (PMNs) (Fig. 1). Several days after the clinical signs disappeared, all the SHR rats were boosted with 2 mg/kg of lipid-conjugated MDP in aqueous form and sacrificed 24 hours later for histological studies. Histological features of these acute swellings were marked by a very enlarged synovial cavity with relatively few PMNs in the diseased joints (Fig. 2). This suggests increased vascular permeability and vasodilatation. These same acute reactions were also observed in other rat strains such as Lewis, SD and +/+ rats. These changes are comparable to the early changes of MDP-induced arthritis (Fig. 3).

**Fig. 1** Acute exudative inflammatory reaction of the tarsal joint a few days after the clinical onset of SHR induced by seven consecutive intravenous injection of 1 µg MDP (2 mg/kg). Hyper trophy of the synovial villi is associated with the proliferation of synovial lining cells and hyperplasia of the connective tissue infiltrated with massive numbers of PMNs. (×60)

**Fig. 2** A very enlarged synovial cavity with acute exudative inflammatory reaction which consists of relatively few PMNs and of extensive exudation. Note the hypocellular responses around the synovial membrane and the synovial stroma. (×60)

**Fig. 3** Acute exudative inflammatory reaction of the tarsal joint 14 days after injection of a w/o emulsion containing 0.1 mg of MDP which was inoculated intradermally into the left hind footpad. Note the hypertrophy of the synovial villi, the hypercellular responses with massive PMN infiltration and fibrin deposition in the joint space. (×60)
Fig. 4 A typical kinetic of peripheral blood leukocytes after a single injection of adjuvant. MDP (1 to 2 mg/kg) and LPS-MDP (0.1 to 2 mg/kg) showed almost similar kinetics. (2), total leukocyte count in the peripheral blood, after injection of 2 mg/kg of LPS-MDP; (2), total leukocyte count after injection of PBS as a control (Ly); lymphocyte; (PMN); polymorphonuclear leukocyte; (4), percentage of lymphocytes in the blood smear; (5), percent of PMN in the blood smear.

The Kinetics of Peripheral Blood Leukocytes

A typical kinetic of peripheral blood leukocytes after a single injection of adjuvant is shown in Fig. 4. All three adjuvants such as MDP, LPS-MDP and [B30]-MDP markedly increased the number of PMNs in either euthymic or athymic rats to a maximum at about 3 to 6 hours after the injection and all diminished within 24 hours. The lymphocytes decreased in an opposite fashion and monocytes tended to increase more than 48 hours later (Data not shown). As a control, PBS did not show any changes in leukocyte count and showed the normal leukocyte-lymphocyte ratio.

DISCUSSION

The present studies confirmed our previous report and extended it to that a synthetic adjuvant, N-acetylneuraminy-L-sphingosine-diphosphate (MDP) produced polymorphonuclear leukocytes in euthymic rats but not in immunodeficient rats such as congenitally athymic nude rats and spontaneous hypertensive rats (SHR). The most important finding from the present studies was that these immunodeficient rats did not develop the disease after the usual subcutaneous injection of w/o emulsion containing MDP whereas consecutive intravenous injections of an aqueous form of lipid-conjugated MDP such as LPS-MDP and [B30]-MDP did induce polymorphonuclear leukocytes in these immunodeficient rats as well as in immunologically normal rats.

Histologic features of this polyarthritis were acute inflammatory responses around the joints, characteristic of acute exudative synovitis, sub-synovitis and tenosynovitis with a massive infiltration of polymorphonuclear leukocytes (PMNs). These inflammatory responses were comparable to those in the acute stage of classic adjuvant-induced arthritis.

It is well known that adjuvant-induced arthritis can easily be affected by the adjuvant composition, the composition of oil vehicles and the route of injection. It was, however, rather an unexpected finding that few or no susceptible strains to this disease such as congenitally athymic nude rats and SHR were able to develop the disease with 100% incidence by systemic administration of an aqueous form of adjuvant. This finding is the first success for lipid-conjugated MDP in aqueous form, not in w/o emulsion, inducing polymorphonuclear leukocytes not only in immunological normal rats but also in immunodeficient rats. Our previous finding that MDP in w/o emulsion induced polymorphonuclear leukocytes in euthymic rats but not in athymic nude rats strongly suggested a very important role of the thymus in promoting the development of MDP-induced arthritis. Since congenitally athymic nude rats and SHR were T cell deficient animals in terms of little or no response to PHA and Con A, very low antibody response to sheep red cells and impaired cell-mediated immunity, it is possible that T cell functions may be majorly involved in this new model of arthritis. This model appeared to represent an acute stage of MDP-induced arthritis in terms of clinical and histologic similarities. It may thus be reasonable to consider that MDP-induced arthritis consists of at least two stages: one an acute stage which may be T cell independent and the other a chronic stage which may be T cell dependent.

The present studies also demonstrated that a single intravenous injection of lipid-conjugated MDP in aqueous form markedly increased the number of PMNs in either euthymic or athymic rats. This leukocytosis rapidly diminished to normal levels within 24 hours. By this time acute swellings were observed around the joints in euthymic rats which histologically showed acute exudative synovitis involved with a massive number of PMNs (Fig. 1 and 2). These findings suggest that lipid-conjugated MDP induces leukocytosis in peripheral blood, these may be localized within the joints and persistently exist in the joints, possibly because of its resistance to biodegradation (unpublished data), eventually resulting in the induction and persistence of acute inflammatory responses, possibly through the release of lysosomal enzymes.

In the present studies, the onset, severity and duration of the clinical signs was strongly related to the dose of lipid-conjugated MDP, suggesting that an appropriate concentration accumulating in the joints is needed to produce the clinically recognizable swelling and histopathologic lesions around the joints. In other words, there exists a threshold of material deposited in the tissue to cause distinct swelling and inflammatory reaction around the tissues. Very low doses of 0.1 mg/kg of lipid-conjugated MDP did not produce any disease in spite of a marked increase in the PMNs in the peripheral blood.

The reason why the arthritis could be induced with lipid-conjugated MDP but not MDP itself in aqueous form is not known but it may be explained by differences in degradability and persistence within the joint tissue. In this connection, Crotarci et al. reported that very the resistant Buffalo strain to classic adjuvant-induced arthritis developed the disease with a 100% incidence when they were injected intraperitoneally with very large amounts of peptidoglycan-polysaccharide complex derived from Streptococcus pyogenes cell walls in an aqueous form instead of w/o emulsion.

It will be also very interesting to know whether or not an immune response either to an autoantigen such as collagen or to MDP or lipid-conjugated MDP may be involved in promoting the development of our new model of arthritis. Since MDP is not an immunogen, an immune response either to MDP or possibly also to lipid-conjugated MDP may not be involved in the development of this disease. We can not rule out autoimmune mechanisms as responsible for the production of this disease.

The use of lipid-conjugated MDP in addition to MDP itself would provide very useful tools to elucidate the underlying mechanisms of adjuvant-induced arthritis and mimicking T cell dependent and independent stages of this disease process, or in studying the involvement of acute and chronic inflammatory reactions in the pathogenesis of this disease.

REFERENCES

2. Wikman BH; Pearson CM, Sharp JT: Studies of arthritis and other lesions induced in rats by injection of Mycobacterial adjuvant, II. Evidence that the disease is a disseminated immunologic response to exogenous antigen. J Immunol 85: 403-417, 1960
11. Kayashima K, Koga T, Onoue K: Role of T

A New Breed of Mouse Showing Multiple Osteochondral Lesions — twy Mouse —
Yasuhiro HOSODAI, Yukio YOSHIMURA* and Shozo HIGAKI**
Departments of Pathology and Clinical Laboratories, Keio University School of Medicine, Shinano-cho, Shinjuku-ku, Tokyo 160
*Central Institute for Experimental Animals
**Department of Orthopedic Surgery, Faculty of Medicine, University of Tokyo

In Japan, a new breed of mouse showing multiple osteochondral lesions was discovered and was named the twy mouse (tiptoe walking-Yoshimura). This mouse line is characterized by tiptoe walking, stiffness of the vertebral column and limb joints, and later motor paresis. Calcification and ossification bridging adjacent vertebral bodies are seen in association with degeneration and destruction of the intervertebral discs. In limb joints, destruction of articular cartilage and synovial cell proliferation with or without inflammatory cell infiltration are observed. Calcification in and around the joint capsule is also noticed. Serum calcium level is within normal limits.

These characters are inherited through an autosomal recessive single gene. This model may be a useful model simulating various human diseases including ankylosing spondylitis, osteoarthrosis and others.

The etiology of most osteochondral diseases in man is still obscure, and difficulty in obtaining lesions of the early stage in these human diseases has limited the elucidation of their pathogenesis.

For these reasons, animal models simulating human osteochondral diseases are desired and many attempts have been made to establish suitable animal models.1–3 Many of these animal models need the administration of chemical, physical or biological agents or surgical manipulations to produce lesions of bone, cartilage or synovia,1–4 although several spontaneous animal models have been described.3–4

Some of the osteochondral diseases are known to be hereditary diseases or at least to have some hereditary background. In Japan, a new animal model simulating osteoarthrosis or ankylosing spondylitis or idiopathic osteoporosis was discovered at the Central Institute for Experimental Animals. Protein manifestations in this animal open the possibility of its being a valuable model for investigating various human osteochondral diseases.

BACKGROUND OF THE TYW MOUSE

In 1978, a line of mice that show tiptoe walking, stiffness of the vertebral column and exaggeration of the vertebral curve, and later develop motor disturbance of the limbs was discovered during brother-sister mating in order to establish a homogeneous strain from ICR strain mice. Further studies revealed that these abnormalities
are inherited through an autosomal single recessive gene (Fig. 1). This gene was named twy (tip toe walking-Yoshimura). The twy mouse shows no definite skeletal abnormalities or abnormally low weight at weaning. At the 8th week of age, cessation of increase in body weight, abnormal gait, rigidity of the vertebral column and stiffness of limb joints develop. The mice begin to die at 18 weeks of age, and most of them die before 30 weeks of age (Fig. 2). The abnormal findings occur in both sexes at the same frequency. A precise report on the process of discovery and our genetic analysis will appear in another paper.  

**Lineage of twy Mouse**

<table>
<thead>
<tr>
<th>Generation</th>
<th>Pedigree</th>
<th>Sex</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>×</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F1</td>
<td>×</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F2</td>
<td>×</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F3</td>
<td>×</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F4</td>
<td>×</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F5</td>
<td>×</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F6</td>
<td>×</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F7</td>
<td>×</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F8</td>
<td>×</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F9</td>
<td>×</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F10</td>
<td>×</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F11</td>
<td>×</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F12</td>
<td>×</td>
<td></td>
<td>Normal</td>
</tr>
</tbody>
</table>

**Fig. 1** Lineage of the twy mouse. ( ) means percentage of incidence of abnormal offspring from the parent.

**Body Weight Change**

<table>
<thead>
<tr>
<th>Week of Age</th>
<th>Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>34</td>
<td>34</td>
</tr>
</tbody>
</table>

**Fig. 2** Body weight changes in twy mouse and the control.

**MATERIALS AND METHODS**

In order to ascertain the full-blown changes in the twy mouse, mice were sacrificed at 28 weeks of age. As the control, mice of the original ICR strain of the same age were used.

A radiological study using a soft X-ray apparatus (SOFTEX EMB type) was carried out on some twy and some control mice.

For histology, vertebral columns were bisected sagittally in the median plane. Fore- and hind legs were bisected along their long axis. The specimens were decalcified, embedded in paraffin, and routinely stained with hematoxylin and eosin, Mallory's trichrome, and alcian blue-PAS. Alcian blue stain, luxol fast blue-hematoxylin-eosin stain, and toluidine blue metachromasia were added as needed.

**RESULTS**

**Findings of Soft X-ray Examination**

In the vertebral spaces, deposition of calcium was noticed at the intervertebral spaces, especially at both edges of the vertebral bodies. The calcification connected the adjacent vertebral bodies. These changes somewhat resemble the "bamboo-spine" of ankylosing spondylitis or spur formation in osteoarthritis (Fig. 3).

In limb joints, calcification was demonstrated in and around the joints, and in proximal joints roughening of the articular surface was observed (Fig. 4). At the same time, osteoporosis in a moderate degree was noticed in the whole skeletal system.

Calcification of the Achilles tendon and of hair follicles of the whiskers was also seen on soft X-ray films.

**Findings of Histological Examination**

In the intervertebral disc, degeneration and destruction of the cartilage matrix with lateral protrusion were observed (Fig. 5). Unorganized proliferation of chondrocytes and irregular calcification were seen in the protruded discs. Over the protruded discs, proliferation of fibrous tissue from the peristium and irregular calcium deposition were present. Although a minimal to slight cellular reaction consisting of macrophages and small round cells was usually noted, no pronounced inflammatory cell infiltration was observed. The protruded discs occasionally pushed into the spinal cord (Fig. 6).

In some animals, intracartilaginous ossification bridging adjacent vertebrae was seen in the pro-

**Fig. 3** Soft X-ray film of the vertebral column of twy mouse. Note calcification connecting adjacent vertebrae.
Fig. 4 Soft X-ray film of the knee joint of tay mouse. Roughing of the articular surface is noted.

Fig. 5 Vertebral column of tay mouse showing lateral protrusion of the intervertebral regions. Orig. mag. ×22

Fig. 6 Intervertebral disc of tay mouse protruding into the spinal cord. Orig. mag. ×60

Fig. 7 Ossification at the margin of the intervertebral disc. Orig. mag. ×99
Fig. 8 Focal destruction of articular cartilage and synovial cell proliferation in a limb joint of twy mouse. Orig. mag. ×60

Fig. 9 Limb joint of twy mouse showing inflammatory reaction. Orig. mag. ×150

Fig. 10 Pronounced cellular reaction in a limb joint of twy mouse. Orig. mag. ×300

Fig. 11 Perifollicular calcification of whiskers of twy mouse. Orig. mag. ×150
truded disc (Fig. 7).

In limb joints, erosion and destruction of articular cartilage with synovial cell proliferation were observed. This was not usually accompanied by active inflammatory cell infiltration (Fig. 8). In some cases, however, severe inflammatory cell infiltration was marked in association with destruction of articular cartilage and synovial proliferation (Figs. 9). On rare occasions, proliferating cells showed an abortive palisade arrangement mixed with inflammatory cell infiltration (Fig. 10). Subchondral bone absorption beneath the joint surface was also noticed.

Calcification of the Achilles tendon, of hair follicles of the whiskers (Fig. 11), and of the aorta were observed.

Marked calcification was not present in the kidney. Accumulation of acidic glucosaminoglycans was not found in the liver, heart, spleen or kidney. The serum calcium level of the two mice was 5.3 mEq/l. The value was similar to that of the controls (4.9 mEq/l).

**DISCUSSION**

Morphological examination of the two mouse is still in progress. Therefore, precise evaluation of this animal model must wait for further studies. The results presented here, however, indicate that this animal model apparently resembles some osteoarthritic diseases.

Changes in the vertebral column simulate, at least in some aspects, ankylosing spondylitis or osteoarthritis. Calcification and ossification connecting adjacent vertebræ is one of the characteristics of these diseases. But insignificant inflammatory cell infiltration and lack of male propon- derness appear to be unsatisfactory aspects in relation to ankylosing spondylitis.

In the limb joints, thinning and erosion of the articular cartilage, subchondral bone absorption, and synovial cell proliferation corresponded fairly well with changes seen in human osteoarthritis. Superimposition of inflammatory cell infiltration on these changes gives some resemblance to destruc- tive arthritis.

Similar morphological changes can be shown to be reproducible in other spontaneous or experimental animal models. In most of them, however, significant differences from the two mouse are noticed. Changes simulating ankylosing spondylitis spontaneity occur in larger animals, for example, dogs, bulls, cats, horses, swine, deer and monkeys. Models of ankylosing spondylitis in laboratory animals have not been described. To develop experimental osteoarthritis, antecedent manipulations have been necessary in many animals. Spontaneous osteo- arthritis in laboratory rodents was observed in mice, Syrian hamsters, guinea pigs, and Prasongs (Mustonse) natalensis, but the lesions develop in a later stage of their lifespan.

Although the pathogenesis of changes found in the two mouse is not yet clarified, analysis of the heredity, chemistry and morphology of this mouse line should contribute to the investigation of osteochondral diseases.

At the same, it must be emphasized that strict evaluation and cautious interpretation are essential for studies of animal models of human diseases.

**REFERENCES**

Histopathological Study. The mice were anesthetized with ether and bled to death. Then various organs, including lymphoma, were taken and fixed in neutralized formalin. The front and hind paws were further decalcified by the Plank-Rychlo method. All the specimens were cut into sections and stained with hematoxylin and eosin (HE), periodic acid Schiff (PAS), and some other special stains. In the kidney study, 20 glomeruli from each mouse were observed and classified histopathologically. The kidney and synovial tissues were fixed in glutaldehyde and observed under the electron microscope.

RESULTS

Clinical Findings

Two out of 7 control mice had ascites due to lupus nephritis, but no animal treated with CCA developed any evidence of anasarca. All of the control mice developed visible and palpable lymphoma. Treated mice had smaller sized lymphoma which were sometimes palpable but not visible. Two out of 7 control mice had hematomae in their eardrums due to vasculitis. Three out of 7 nontreated control MRL/1 mice developed clinical polyarthritis. One of them had very severe arthritis and crawled about to get feedings. The treated mice had no deforming clinical arthritis at 20 weeks of age. Some treated mice might develop some swelling of the peripheral joints, but it was not possible to recognize the actual lesion.

Histopathological Findings

Nontreated male MRL/1 mice developed lupus nephritis, which had membranous or membrano-proliferative changes dominant, histopathologically. CCA protected against progression of glomerular changes (Fig. 1). In the preliminary experiment, chronological analysis was performed. Progression of glomerular involvement correlated well with

![Fig. 1: Glomerular changes of MRL/1 mice (male, 20 week old). A nontreated mouse developed profound glomerulonephritis with lobulation (A: HE, ×400), to wire loop formation with PAS positive substance deposition in the endothelial side and spike formation in the epithelial side of the glomerular basement membrane (C: PAS, ×400). Progression of the lesion was protected against by CCA administration (B: HE, ×250, D: ×250), showing slight increase of cellularity in the glomeruli.](image)

![Fig. 2: Histopathological changes of renal glomeruli in male MRL/1 mice. Chronological analysis and long term therapy with CCA](image)

![Fig. 3: A nontreated MRL/1 mouse (male, 20 week old) had a huge amount of electron dense deposits in the mesangial matrix and endothelial side of the basement membrane (original magnification ×5840).](image)
age. Comparing glomerular changes of age matched groups, at 20 weeks of age, CCA had a beneficial effect on the lesion at a significance level of 0.01 (Ridit analysis method) (Fig. 2). Electron microscopic study revealed that non-treated control mice had electron dense deposits in the mesangial matrix and at the endothelial side of the basement membrane. They had proliferation of endothelial cells and mesangial cells (Fig. 3). CCA treated MRL/1 mice had less electron dense deposits and less proliferation of mesangial cells (Fig. 4). CCA also suppressed growth of lymphoma.

Profuse vasculitis was characterized by granulation, proliferation of media cells and their penetration through the intima and adventitia. The lesion was observed, in kidneys, in 5 out of 7 non-treated mice, and was noted in 1 out of 10 treated mice. The $\chi^2$ test disclosed a statistical difference between the two groups ($p<0.01$).

Figure 4: A CCA treated MRL/1 mouse (male, 20 week old) had less electron dense deposits (original magnification $\times 0310$)

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control (n=8)</th>
<th>CCA treated (n=6)</th>
<th>Ridit analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edema of subcapsular soft tissues</td>
<td>intact</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>mild</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>severe</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$p&lt;0.01$</td>
</tr>
<tr>
<td>Multiplication of synovial lining cells</td>
<td>intact</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>mild</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>severe</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$p&lt;0.01$</td>
</tr>
<tr>
<td>Destructive changes in the cartilage</td>
<td>intact</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>mild</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>severe</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Granulation in the cartilage</td>
<td>intact</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>mild</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>severe</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The present investigation is the first attempt made to analyze the effect of a synthetic immunomodulator on the autoimmune disorders in male MRL/1 mice. Originally MRL/1 mice were known to develop lupus nephritis and lymphoma. The mice have serological abnormalities characteristic of human systemic lupus erythematosus such as antinuclear antibodies, anti-DNA, anti-Sm antibodies, and ANA. They also show a high incidence of antithrombocyte antibody in the lymphoma, 68.2% were Thyl-2 positive T cells and 69.9% were B cells. MRL/1 mice develop arthritis resembling human rheumatoid arthritis. Histopathologically it was defined as “lympho-plasmocytes reaction with connective tissue proliferation”. The animals have hypergammaglobulinemia, rheumatoid factor (IgM and IgG), cryoglobulinemia, and immune complexes.

CCA, an immunomodulator, has protective and suppressive effect on adjuvant arthritis in rats, even it has no effect on experimental acute inflammation. Lower doses of CCA had suppressive effects and higher doses had augmentative effects on hemolytic plaque formation in vivo. The agent suppressed or augmented number of rosette forming cells and of anti-sheep red blood cell antibody titer assayed by the hemagglutination method. (NZB×NZW)F hybrid mice treated with CCA had lowered titer of antinuclear antibodies. CCA induced Thyl-2 positive cells in the spleen of nude mice.

In the present investigation, male MRL/1 mice were treated with CCA from 8 to 20 weeks of age. CCA protected against progression of lupus nephritis, lymphoma, vasculitis and arthritis. In general, the immunomodulator appeared to mediate diverse influences acting on development, function and proliferation of immune responsive cells such as macrophages, granulocytes, and lymphocytes. CCA suppressed the proliferation of lymphoma cells and deceased the amount of immune complex deposition in the glomerulus and articular synovium.

**ACKNOWLEDGMENTS**

This experiment was partially supported by the Research Committee of Experimental Models for Intractable Diseases, the Ministry of Health and Welfare, Japan. The authors thank Professor Yoshio Fukuoka, M.D., and Kiyoaki Kamiu, M.D. for their helpful advice.

**REFERENCES**

2) Abe C, Shiokawa Y: Spontaneous polyarthritis in MRL/1 mice. New Horizons in Rheumatoid
Mixed Connective Tissue Disease after Breast Augmentation which Terminated in Scleroderma Kidney

An Autopsy Case Report of Human Adjuvant Disease

Yasuo KUMAGAI, Chiyuki ABE, Takao HIRANO, Yoshiro FUKUDA and Yuichi SHIOKAWA

The Division of Rheumatology, Department of Medicine, and Department of Pathology*, School of Medicine, Juntendo University, Hongo, Bunkyo-ku, Tokyo 113

We report a case of mixed connective tissue disease (MCTD) with serum anti-ribonucleoprotein antibodies in high titers which developed 22 years after breast augmentation using silicone injection. The autopsy disclosed systemic vascular lesions characteristic of progressive systemic sclerosis (PSS) including kidney involvement. This patient might illustrate the close relationship between MCTD and PSS with vascular lesions as well with a foreign substance injected in cosmetic surgery.

In 1964, Miyoshi and co-workers reported the occurrence of undifferentiated connective tissue disease in two women following cosmetic surgery with injections of foreign substance. They believed that the adjuvant effect of these materials contributed to the induction of the disease as in the case of adjuvant arthritis in rats. The illness resembling a connective tissue disease following the injection of foreign materials has been known for some time as human adjuvant disease in Japan, and has been the subject of over 30 case reports. Included among these have been four cases of systemic lupus erythematosus (SLE), one of rheumatoid arthritis, and seven of scleroderma, (six of progressive systemic sclerosis (PSS) and one of morphea). We describe a case of mixed connective tissue disease (MCTD) with serum anti-ribonucleoprotein antibodies of high titer which developed after breast augmentation using silicone injection.

CASE REPORT

The patient was a 44-year old Japanese woman who underwent breast augmentation using silicone injection in 1954. In June 1976, she noted fever, polyarthralgia, Raynaud’s phenomenon, and puffiness of the fingers, and six months later her physician suspected PSS. In August 1977, after exposure to the sun during a vacation, she developed a high fever (40°C), fatigue, prolonged polyarthralgia, photosensitivity, headache, nausea and vomiting. Her past history included a history of chronic otitis media at 36 years of age, and two induced abortions.

Physical examination on admission to Juntendo University Hospital later that month revealed a

Keywords: augmentation, mammoplasty, adjuvant disease, mixed connective tissue disease, scleroderma kidney, silicone

1 To whom reprint requests should be addressed.
middle-aged woman whose blood pressure was 182/110 mmHg, pulse 88/min., respiration 21/min., and temperature 37.3°C. She had Raynaud’s phenomenon of the fingers and toes, sclerodactyly without other sclerodermatous skin changes, proximal muscle weakness and tenderness, and pitting edema of the feet. There were large, indurated and irregularly surfaced masses in the breasts secondary to her breast augmentation.

Laboratory tests disclosed a mild anemia, elevated erythrocyte sedimentation rate (105 mm/hour, Westergren), hypergammaglobulinemia (2244 mg/dl), and elevated serum muscle enzymes with creatin phosphokinase (305 IU), aldolase (8.3 mU), and lactic dehydrogenase (1450 IU). She had mild hyponatremia (129 mEq/l), normal serum potassium (4.1 mEq/l), BUN (15 mg/dl), creatinine (0.9 mg/dl) and mild hyperreninemia (2.8 ng/ml/hr). The urinalysis was unremarkable except for a trace protein; no red blood cells were seen in the urinary sediment. Serum antinuclear antibody was positive at a titer of 1:640 with a speckled staining pattern. Hemagglutinating serum antibodies to RNAse-sensitive components of extractable nuclear antigen (ENA) was positive, 1:32,000, while no antibody to RNAse-resistant components of ENA was detected. A chest roentgenogram showed mild bibilar pulmonary fibrosis, and the electrocardiogram was within normal limits.

After admission, the patient repeatedly complained of headache, nausea and vomiting which were felt to be symptoms of hypertensive encephalopathy, dysuria, drowsiness, and microangiopathic hemolytic anemia also developed. Her hypertension was resistant to 250 mg/day of alpha methyldopa, and her blood pressure rose to 220/140 mmHg the day prior to her death. On the 16th hospital day, 40 mg/day of prednisolone was administered intravenously. She expired suddenly on the 15th hospital day for unknown reasons although her acute renal failure had been obvious. Her clinical course is illustrated in Fig. 1.

Autopsy revealed that the injected substance was a transparent liquid. On histopathological examination of the breast tissue, this material was seen as numerous large extracellular droplets. Also noted were numerous macrophages with foamy cytoplasm which had phagocytosed the injected material. These macrophages extended beyond the implant and into part of the pectoralis major muscle (Fig. 2). This foreign substance identified as silicone in the form of dimethyldiphenylsiloxane by infrared spectrophotometry and gel permeation chromatography. The molecular weight ranged between 20,000 and 30,000 daltons. The renal intraluminal arterioles had fibrinoid necrosis with severe intimal proliferation and marked luminal narrowing (Fig. 3). Trapped red blood cells were seen in the intima. Afferent arterioles were also narrowed because of intimal thickening and fibroinoid degeneration (Fig. 4). Numerous glomerular capillaries were necrotic due to ischemia. This type of vascular change was also found in the pancreas (Fig. 5), ovaries, and lymph nodes. Mild fibrosis was seen in both lower lung fields with no intimal thickening of the pulmonary arteries. A moderate degree of interstitial fibrosis with irregular atrophy of the myocardial muscle fibers was found in the heart. Skeletal muscle fibers were also atrophic and vacuolated although infiltration of lymphocytes was not remarkable. The skin over the breasts was normal. No PSS-like fibrosis was noted in the esophagus and gastrointestinal tract. No foreign substance was identified in any section examined except at the injection site.

**DISCUSSION**

We have previously described several characteristics of connective tissue disease following cosmetic surgery (human adjuvant disease). Patients had a past history of cosmetic surgery, most often breast augmentation, with injection of a foreign substance such as paraffin or silicone. Foreign body granu-
The relationship between the foreign substance injection for cosmetic purposes and the development of classical connective tissue diseases was not seriously considered until our report in 1979 of four cases of scleroderma following cosmetic surgery. It was hypothesized that these cases might be analogous to PSS associated with occupational exposure to silica dust. An additional case has been seen at our hospital and three more have been reported by other investigators in Japan.

Our patient had Raynaud’s phenomenon, polyarthralgia, myopathy, and swelling of the hands leading to a sausage-like appearance of the fingers. Antibody to RNase-sensitive ENA was positive to a high titer in hemagglutination, whereas antibody to RNase-resistant ENA was negative, which was compatible with MCTD. The patient was treated, only briefly, with corticosteroid therapy. She expired with persistent hypertension and acute renal failure, findings highly compatible with PSS.

Histopathologically, marked intimal thickening and fibrinoid necrosis were observed in the interlobular arteries and the afferent arterioles to glomeruli, which were typical of a “sclerodermia kidney.” Such arterial changes were not confined to the kidneys, but were widespread including vessels in the pancreas, ovaries, and lymph nodes. It is generally accepted that MCTD is less frequently accompanied by vascular involvement than PSS. However, thickening in medium to large sized arteries in MCTD has been reported.

Recently, Nisenstein and co-workers reported a subsequent evaluation of the original 25 patients with MCTD. According to their article, a total of 6 of 14 surviving patients had PSS as their exclusive or predominant clinical disease. One of 8 patients who died had scleroderma kidney. Although at present, it is not clear whether this finding was due to the natural course of the MCTD or to modification of it with corticosteroid therapy, they suggested the close relationship of MCTD to PSS.

Our case report might illustrate such a relationship not only with vascular involvement, but with a foreign substance injection in cosmetic surgery. Another patient who was suspected of having MCTD after breast augmentation was reported, although the anti-ENA titer was not described. She developed fever, arthralgia, sausage-like swelling of the fingers, dermatomyositis-like manifestations, pulmonary fibrosis, esophageal dilatation, leukopenia and positive ANA, several years after breast augmentation.

In contrast to our previous patients, this individual received silicone rather than paraffin injections. The former has been used more widely for plastic or cosmetic purposes in Japan. Although silicone oil is generally thought to be biologically inert, it may interact with the host. Several adverse local reactions have been noted after silicone injection and silicone elastomer prostheses. Although the full spectrum of problems associated with foreign substance injection is not known, this case report points out one potential hazard of this type of surgical intervention.

ACKNOWLEDGMENTS

We are indebted to Drs. Y. Ohashi and T. Takagi of Juntendo University and T. Inoshita of the East Tennessee State University for review of the pathological materials, to Dr. S. Matsumiya of the Biological Medical Laboratory in Tokyo for identification of the injected substance, and to Drs. G.P. Rodnan and T.A. Medsger Jr. of the University of Pittsburgh for their thoughtful advice.

REFERENCES


Systemic Lupus Erythematosus or Mixed Connective Tissue Disease? -- A Case of 14 Year Old Girl with Viral Myocarditis

Toshiko IIDAI, Hirohisa EGUCHI, Koji ODAIRA, Hiroyuki HIKITA and Masahiko OHKUNI

Department of Pediatrics, Nihon University School of Medicine, Ohyoguchi, Itabashi-ku, Tokyo 173

This is a case report of a 14 year old girl who was suspected of having systemic lupus erythematosus (SLE) on her first admission and had two episodes of myocarditis with a high antibody titer to Coxsackie B group virus, during the course of her disease. In addition, she exhibited sclerodermatous skin changes on her fingers and face. Though she had no episode of polymyositis (PM) clinically, her serum GOT, GPT, CPT and aldolase were all elevated before the onset of her myocarditis. This case showed an extremely high titer of the antibody to ribonucleoprotein (RNP). These findings suggest that she may develop into a definite mixed connective tissue disease (MCTD) patient in the future.

INTRODUCTION

Mixed connective tissue disease (MCTD) was first described as a new and apparently distinct rheumatic disease syndrome by Sharp et al. in 1972. Patients with MCTD are characterized by the clinical features of systemic lupus erythematosus (SLE), progressive systemic sclerosis (PSS) and polymyositis (PM) and serologically by antibodies to the ribonuclease (R Nase) sensitive component of an extractable nuclear antigen (ENA). 1-10

Many adult cases have been reported but, as yet, only a few pediatric cases have been described. 11-10

This is a case report of a 14 year old girl who had two episodes of myocarditis with a high viral antibody titer and showed a high ENA antibody titer. Recently, the relationships between viral infection and so-called connective tissue disease have been discussed. 11-14 In this case MCTD with viral myocarditis was suspected.

CASE REPORT

A 14 year old girl was admitted to the Nihon University Hospital in October 1978, with the chief complaints of arthralgia and Raynaud's phenomenon. Since April 1978, she experienced anorexia, weight loss, Raynaud's phenomenon, arthralgia and swelling of the fingers and of the joints of the foot (Fig. 1).

Her past history was noncontributory. Her family history revealed that her brother died of acute lymphoblastic leukemia at the age of four. On admission, her body temperature was 36.4°C. Arthralgia and spindle shaped glossy swelling of the finger joints and right retroauricular lymphadenopathy were observed. A facial butterfly rash and photosensitivity were not noted. Laboratory findings on admission are shown in Table 1. Thrombocytopenia was recognized but

Keywords: extractable nuclear antigen, Coxsackie B group virus, mixed connective tissue disease, myocarditis

1 To whom reprint requests should be addressed.
T. Iida et al.: A Case of SLE or MCTD in Childhood with Viral Myocarditis

Cardiology revealed pericardial effusion and the electrocardiogram (ECG) showed low voltage. She recovered from the acute episode in a short period of time with increased dosage of prednisolone. She was readmitted to the hospital with the chief complaints of dyspnea and chest pain again several days after her initial discharge. On that occasion, chest X-ray revealed an enlarged heart silhouette with a cardiothoracic ratio of 0.56. ECG showed an inversion of the T waves in leads II, III, AVF and the chest leads (Fig. 2). Two dimensional echocardiogram revealed an enlarged right ventricle and a narrow left ventricular cavity but pericardial effusion was not demonstrated.

About one month after this series of cardiac symptoms, high complement fixation (CF) antibody titers to Coxsackie B3 and B5 virus were recognized but the results of the neutralization (NT) test to the two viruses were not elevated. An elevation of titer to Coxsackie B4 virus was recognized, but the elevated titer did not persist. Viral myocarditis was suspected because the elevated viral antibody titers were observed.

The changes in serum enzymes during the clinical course are shown on Fig. 3. In June 1980, before the onset of her myocarditis, the levels of serum GOT, GPT, and LDH began to rise and reached their maximum levels during August to October 1980. Two months before the onset of myocarditis, the levels of serum creatinine phosphokinase (CPK) and aldolase were elevated (Fig. 3). From these laboratory findings, we suspected the complication of polymyositis (PM), though the patient did not complain of muscle weakness, myalgia nor exhibit a heliotropic skin rash.

In November 1980, ESR, CRP, ANA, anti-DNA antibody worsened (Table 3). Antibody to ribonucleoprotein (RNP) showed an extremely high titer (1:165,840), and antibody to Sm was positive (1:320). ANA showed a peripheral pattern at that time. Her pulmonary function and esophagram were normal. The patient is well controlled and in favorable condition as of October 1981.

DISCUSSION

MCTD is an inflammatory disease of unknown etiology which may have overlapping characteristics of SLE, PSS and PM. Commonly, patients with MCTD have arthritis, swollen hands and fingers, abnormal esophageal motility, Raynaud’s phenomenon, and active myositis. They may also experience lymphadenopathy, fever, hepatomegaly, serositis, and splenomegaly. Renal disease is rare in MCTD. Sera from these patients give positive indirect immunofluorescent tests for ANA with a characteristic coarse speckled pattern.

Table 2: Anti-Coxsackie virus antibody titer

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>6.24</td>
</tr>
<tr>
<td></td>
<td>8.26</td>
</tr>
<tr>
<td></td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>12.12</td>
</tr>
<tr>
<td></td>
<td>5.36</td>
</tr>
<tr>
<td>B1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>64</td>
</tr>
<tr>
<td>B2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>129</td>
</tr>
<tr>
<td></td>
<td>2048</td>
</tr>
<tr>
<td></td>
<td>4096</td>
</tr>
<tr>
<td></td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>64</td>
</tr>
<tr>
<td>B3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>16</td>
</tr>
</tbody>
</table>

Table 1: Laboratory findings (first admission)

<table>
<thead>
<tr>
<th>WBC</th>
<th>6500/mm³</th>
<th>RBC</th>
<th>456 x 10⁶/mm³</th>
<th>Ht</th>
<th>36.1 %</th>
<th>Hb</th>
<th>12.2 g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMC</td>
<td></td>
<td>Hct</td>
<td>11.3 %</td>
<td>Plt</td>
<td>9.1 x 10⁹/mm³</td>
<td>Ret</td>
<td>11 %</td>
</tr>
<tr>
<td>ESR</td>
<td>57 mm/hr</td>
<td>CRP</td>
<td>1+</td>
<td>RA</td>
<td>1+</td>
<td>RAHA</td>
<td></td>
</tr>
<tr>
<td>ANA</td>
<td>1+</td>
<td>Anti-DNA</td>
<td>2+</td>
<td>Diffuse</td>
<td>LE cell</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>BFF</td>
<td>negative</td>
<td>C₃</td>
<td>74 mg/dl</td>
<td>C₄</td>
<td>9.4 mg/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C₁₉</td>
<td>13.5 mg/dl</td>
<td>C₂₂₀</td>
<td>24.3 u/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-Protein</td>
<td>9.6 g/dl</td>
<td>Ab</td>
<td>45.3 %</td>
<td>α1</td>
<td>2.5 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>α2</td>
<td>11.5 %</td>
<td>β</td>
<td>5.8 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>γ</td>
<td>36.5 %</td>
<td>GGT</td>
<td>88.2 mg/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GPT</td>
<td>1200.6 mg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>LDH</td>
<td>465.6 mg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>BUN</td>
<td>9.7 mg/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>(-)</td>
<td>Sugar</td>
<td>(-)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occlot</td>
<td>(-)</td>
<td>SRCS</td>
<td>0.1/F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>WBCS</td>
<td>1.2/F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coombs</td>
<td>test</td>
<td>negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HB Ag, Ab</td>
<td>negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The diagnosis is confirmed by finding serum antibodies directed against RNP, a ribonucleoprotein sensitive component of ENA.

We considered that she was taken ill with Raynaud's phenomenon and arthritis at the age of fourteen. Subsequently the exhibited pyrexia, lymphadenopathy, myocarditis, thrombocytopenia, hypergammaglobulinemia and a transient butterfly rash, but nephropathy was not recognized clinically. Moreover she demonstrated spindle-shaped glossy swelling of the finger joints and Raynaud's phenomenon as symptoms of PSS. As for symptoms of PM, muscle weakness and a heliotropic skin rash was not recognized but her levels of CPK and aldolase were elevated two months before the onset of the myocarditis, which suggested asymptomatic muscular involvement. It is also possible that myocarditis may be an accidental complication. Sharp and associates\(^1\) have reported that clinical symptoms of myositis, and elevated serum levels of CPK and aldolase were observed in 72% of patients with MCTD. In this case, a positive speckled fluorescence pattern for antinuclear antibody, with a titer of 1:320 was present serologically, the ribonucleoprotein sensitive ENA was present in a high titer (>1:163,840) and antibody to Sm was negative, in March 1981. High titers of antibody to RNP, and little or no antibody to Sm, are usually found in the sera of patients with MCTD.

In sera of patients with SLE, antibody to Sm is specific and is commonly found in the active stage.

With these findings, we had strong doubts as to MCTD. Singsen and associates\(^9\) have reported that cardiac involvement in MCTD appears to be more common in children than in adults. The present case had two episodes of myocarditis with high antibody titers to Coxsackie B group virus.

Many reports concerning the relationship between viral infection and so-called connective tissue diseases have been published. As for SLE and rheumatoid arthritis, the pathogenic roles of certain viruses have been discussed. The immune response of the host, the role of the immune complex and the activity of the reticuloendothelial system may play roles in the immune reactions of these patients to viral infections.\(^12,14\)

Sawada, et al.\(^12\) reported that cases with high antibody titers to ENA tended to show high antibody titers to viruses. So the high CF titers to Coxsackie viruses in our case is considered similar to the reported cases.

It is said that an elevation of CF titers to Coxsackie virus does not necessarily give proof of actual Coxsackie virus infection, and that the elevation of neutralization antibody titers to Coxsackie virus is considered as proof of actual virus infection.

The rise of neutralization antibody titer to Coxsackie B4 virus was noted in our case but the elevated titer did not persist and the clinical course of the neutralization antibody was considered to be atypical. As for the appearance of cardiac symptoms in our case, however, we believe that the Coxsackie B4 virus played a pathogenic role, possibly as the virus which caused the myocarditis.

As for the elevation of viral antibody in patients with SLE, Homma et al.\(^14\) reported that patients with SLE tended to be affected by viral infections and to show antibody overproduction to virus.

Our case suffered from herpes zoster two years after the onset of her disease. So, she might produce viral antibodies as well as autoantibodies to viral infections. We consider the investigation of viral antibodies in cases with SLE and MCTD as important for clarifying the relationship between these diseases and viral infections.

It has been reported that patients with MCTD usually show a good response to corticosteroid therapy and that the prognosis in these patients is considered relatively favorable.\(^17\) However, a fatal case of MCTD who had both cardiac and pulmonary involvement was reported in which the patient suddenly died.\(^18\) In our case, cardiac signs and symptoms appeared twice during the clinical course, but she recovered nicely and her ECG findings have returned to normal. Careful observation and follow up of this case will be needed.

REFERENCES


Polymyositis Associated with Interstitial Pulmonary Fibrosis and Glomerulonephritis

A Report of Two Autopsy Cases

Yasuhiro HOSODAI*, Shunji YOSHIDA*, Tsuneyo MIMORI*, Takashi TOGUCHI*, Shinichi INADA*, Matsumi TOMII*, Hajime YAMAGATA*, Masashi AKIZUKI* and Tohru ABE*

Departments of Pathology and Clinical Laboratories, and *the Department of Internal Medicine, Keio University School of Medicine, Shinjuku-ku, Shinjuku-ku, Tokyo 160

Two autopsy cases (a 65-year-old female and a 54-year-old male) of polymyositis associated with interstitial pulmonary fibrosis and glomerulonephritis were reported. These two cases showed positive Jo-1 antibody. The significance of these cases is discussed.

Dermatomyositis (DM) and polymyositis (PM) are categorized as among the main disorders of collagen disease which have common target organs, for example, joints, lungs, kidneys or blood vessels. As in other diseases, interstitial pulmonary fibrosis is occasionally observed in DM and PM. 

Renal lesions, however, are rarely present in DM or PM, in contrast to systemic lupus erythematosus or polyarteritis nodosa.

We have observed two cases of PM which were complicated by interstitial pulmonary fibrosis and glomerulonephritis, and positive Jo-1 antibody.

REPORT OF CASES

Case 1
A 65-year-old Japanese housewife had been in good health until 56 years of age when she developed bilateral shoulder pain and myalgia in both her upper and lower extremities. At the age of 56, she first experienced Raynaud’s phenomenon and proteinuria. Upon admission, fine dry rales at both lung bases and slight tenderness of the proximal muscles of the upper and lower extremities were present, but there was no muscular weakness. There were no skin rashes or sclerodematous skin changes. Serum creatinine phosphokinase (CPK) was elevated to 262 IU/l, the titer of Jo-1 antibody was 128 x by immunodiffusion. When corticosteroid was diminished, the CPK was further elevated to 732 IU/l. Then, polymyositis was confirmed by a myogenic pattern on EMG and degeneration of muscle fibers and inflammatory cell infiltrates on deltoid muscle biopsy. Her muscle symptoms responded well to an increased dose of corticosteroid (20 mg/day of prednisolone) and she was discharged on the 30th hospital day. However, her renal symptoms continued and gradually became worse. Sixteen months later she was readmitted because of profuse proteinuria (5.4 g/day) and orthopnea. No sign of myositis were found. After admission her renal function diminished and two months later she died of heart failure.

At autopsy, skeletal muscles showed generalized atrophy. Biventricular cardiac hypertrophy with dilatation (433 g) was observed. Alveolar septa were grossly prominent in the lower lobes of the
lungs (650g, 330g). The surface of both kidneys were finely granular and the cortices were reduced (140g, 140g). Histologic examination revealed atrophy and lipid infiltration with diffuse or focal inflammatory infiltrate of mononuclear cells in variously located striated muscles (Fig. 1). In the lungs, severe alveolar fibrosis resulted in obliteration of the alveolar spaces and a few epithelialized alveolar remnants and bronchiectatic bronchioles were present in the fibrotic tissue. There was a moderate interstitial chronic inflammatory cell infiltration (Fig. 2). The kidneys showed diffuse mesangial proliferation with focal segmental sclerosis and hyalnosis (Fig. 3).

Case 2
A 54-year-old male engineer was referred to our Hospital because of muscle weakness, myalgia and productive cough. One year prior to admission he developed high fever, pitting edema, dyspnea and muscle weakness. He was admitted to another hospital and diagnosed as “collagen disease”. During that period, pulmonary tuberculosis was discovered and antibiotics were started. One month prior to admission to our hospital, the tuberculosis relapsed and cessation of his corticosteroids aggravated the muscular symptoms at which time he was referred to this hospital. Upon admission, fine dry rales in the right lung base, hepatomegaly and muscle weakness of the lower extremities were noted. Neither skin rashes or sclerodermatomous skin changes were found. Besides the active tuberculosis, polymyositis was diagnosed, from the findings of proximal muscle weakness, myalgia, elevated serum CPK (2716 IU/l) and a myogenic pattern on EMG. The titer of Jo-1 antibody was 32× by the immunodiffusion method. The myositis was inactivated with prednisolone and cyclophosphamide, but relapsed three times during his hospital course. After nineteen months, severe muscle weakness was the sole muscular symptom. Upon admission, a small quantity of urinary protein was found, which increased and reached 15.8g/day after ten months, and then decreased. His respiratory function worsened and dyspnea appeared. Two and a half years after his admission, he died of right ventricular failure.

On autopsy, proximal and distal limb muscles showed remarkable atrophy. Both lungs (480g, 700g) were diffusely fibrotic, especially in the lower lobes. Cystic dilatation of the terminal air spaces were scattered in fibrotic lung tissue. Biven-
tricular cardiac hypertrophy with dilatation was present (490g). The kidneys (155g, 150g) were slightly enlarged, but no significant macroscopic changes were noted. Histological changes in the skeletal muscles were extensive but variable. In general, atrophy and fibrosis were prominent. Focal infiltration of mononuclear cells and degeneration and regeneration of muscle fibers were also observed in many muscles (Fig. 4). In the lungs, diffuse fibrous thickening of the alveolar septa with small round cell infiltration was observed. Interstitial fibrosis was more pronounced in the subpleural portions (Fig. 5). In the kidneys, diffuse thickening of the glomerular capillary walls with numerous small pores in the basement membrane were observed following PAM staining. These changes were indicative of membranous glomerulonephritis (Fig. 6).

**DISCUSSION**

The two cases presented here feature the association of interstitial pulmonary fibrosis and glomerulonephritis. As far as we know, this type of association is exceptional in DM and PM.1-3

According to Frazier and Miller,2 the incidence of interstitial pulmonary fibrosis is 5 percent in PM. As in our cases, the interstitial lung disease of DM and PM usually resembles that of progressive systemic sclerosis.3 Gross or microscopic hematuria, mild to moderate albuminuria and azotemia may be present in PM.4 As a rule, however, the kidneys are generally spared in PM and DM.4 In our cases, the changes in the glomeruli were indistinguishable from those in other collagen diseases. Immunological abnormalities and glomerulonephritis in our cases analogous to other collagen diseases suggest the existence of some common background between PM and other collagen diseases in their ability to elaborate renal lesions. At the same time, we must accept the possibility of a coincidental association of the pulmonary and renal lesions in our cases.

Concerning PM and DM, some significant abnormalities in cellular immunity were reported by many investigators, and, also, various noteworthy abnormalities of humoral immunity were recently recognized. These abnormal immunologic conditions including humoral antibodies,
Two Cases of Ascites Due to Lupus Peritonitis

Takashi OKAMOTO!, Yutaka OKANO, Mitsuhiro KAWAGOE* and Masako HARA*

Department of Internal Medicine, School of Medicine, Keio University,
Shinjuku-ku, Shinjuku-ku, Tokyo 160
*First Department of Internal Medicine, National Defense Medical College

The authors observed two cases of systemic lupus erythematosus (SLE) with massive ascites as the major clinical manifestation. In both cases the ascites resolved completely with large doses of prednisolone. The peritoneal fluid was exudative in type with a markedly depressed complement level. In one case, the titers of anti-RNP and anti-DNA antibodies were much higher in the peritoneal fluid than in the serum. The concentration of immune complexes was detected by the precipitation method using polyethylene glycol. The concentration of immune complexes was much greater in the peritoneal fluid than in the serum. Anti-RNP antibody activity could be detected in the IgG recovered from the immune complexes in the peritoneal fluid. The specific activity for the anti-RNP antibody of this IgG was higher than that of the free IgG in the peritoneal fluid. The possible role of immune complexes in lupus peritonitis is discussed.

INTRODUCTION

In SLE patients, peritoneal effusion is not unusual. Usually it is associated with the nephrotic syndrome, constrictive pericarditis or portal hypertension due to coincidental liver disease.1-3 However, recently, some cases, with massive ascites due to peritonitis as the major clinical presentation of SLE have been reported.4-6 Immune complexes mediated tissue injury has been suggested as a possible cause of the lupus peritonitis, because immunoglobulins and complement deposits were demonstrated in vessels of the peritoneum by indirect immunofluorescent studies.4,5,6 The authors recently evaluated two patients with massive ascites which we considered as due to lupus peritonitis. The results of the immunological studies of the peritoneal fluid from such a patient suggest a role of local immune complex formation in the development of lupus peritonitis.

CASE REPORTS

Case 1 (Fig. 1)
The patient, a 42 year-old woman, was admitted to Keio University Hospital on November 15, 1980 because of colicky abdominal pain, vomiting, diarrhea and massive ascites.

The patient was in good health until February 1979, when fever, polyarthralgia and skin rashes of the face and extremities developed. She was then referred to Keio University Hospital and admitted for further evaluation. Laboratory examinations revealed positive anti-nuclear antibody, leukopenia and hypocomplementemia. The diagnosis of SLE was suspected but she recovered without corticosteroid therapy and was discharged after three weeks. In April 1980, because of the development of low grade fever and abdominal pain associated with
diarrhea, she was admitted again to Keio University Hospital. After admission, the abdominal symptoms became much worse, a high fever (up to 39°C) occurred, polyarthralgia and pleural effusion also appeared. At this time, she was diagnosed as probable lupus peritonitis and 50 mg of prednisolone was administered daily. Within forty-eight hours she became afebrile and her complaints completely disappeared. She was discharged on 10 mg of prednisolone daily, on June 28, 1980.

In August 1980, when her prednisolone was decreased to 5 mg, low grade fever, abdominal pain, vomiting and frequent diarrhea developed and she was admitted to Keio University Hospital for the third time, in September. On admission abdominal tenderness with muscle guarding and a small amount of ascites were noticed. On this occasion she became well on 20 mg of prednisolone. However, one week after discharge her abdominal symptoms recurred. She was then readmitted to Keio University Hospital for the fourth time, on November 15, 1980.

On physical examination, temperature was 36.4°C, blood pressure 120/80 mmHg, pulse 112 and respirations 26. Her abdomen was distended and there were decreased bowel sounds and guarding with direct tenderness from epigastrium to the left flank. In spite of diuretics and intravenous albumin infusion her abdominal symptoms continued and the peritoneal fluid rapidly increased. Repeated paracenteses of over 1.0 liter daily were needed because of respiratory embarrassment. The fluid was an exudate with a few cells. Cultures for fungi and bacteria were negative. The cytology was class I. There was no laboratory finding to suggest the nephrotic syndrome, constricutive pericarditis or liver disease. Pelvic examination was also normal. At this time her WBC was of 3500/mm³ and the C3 was 30 mg/dl. Prednisolone was increased to 60 mg a day. The ascites gradually regressed and the other gastrointestinal symptoms resolved. After 10 days the ascites completely disappeared. Subsequently she has remained well after tapering of her prednisolone to 10 mg daily.

The immunological characteristics of the patient’s serum and peritoneal fluid on the same date of the fourth admission are summarized in the Table. The total protein concentration of the serum and the peritoneal fluid was 5.7 and 3.1 g/dl, respectively. While the IgG concentration of the serum and the peritoneal fluid was 1105 and 730 mg/dl, respectively. The peritoneal IgG concentration was greater than that estimated from the total protein concentration of the peritoneal fluid considering the IgG/total protein ratio of the serum (730 versus 601 mg/dl). Conversely, the complement (C3 or C4) content of the peritoneal fluid was lower than the serum. In addition, we tested anti-DNA and anti-RNP antibody both in the serum and the peritoneal fluid. Anti-DNA antibody was detected by radioimmunoassay and antinuclear acidic protein antigen (NAPA) antibodies including anti-RNP antibody were tested by double immunodiffusion using rabbit thymus extract as an antigen. The titers of antibodies to DNA and RNP in the peritoneal fluid were
greater than in the serum. Furthermore, we detected immune complexes according to the method of Digeon et al.10 The concentration of immune complexes, which was represented by the IgG content of the immune complexes,10 was much higher in the peritoneal fluid than in the serum (1050 versus 160 μg IgG/ml). Furthermore, the immune complexes of the peritoneal fluid were treated with 0.02 M citrate buffer, pH 3.210 and the monomeric IgG was separated by gel filtration (Fig. 2a). On the other hand, the IgG of the unfractuated original peritoneal fluid was separated by DEAE cellulose ion exchange chromatography.11 Figure 2b shows the specific activity for anti-RNP antibody of these IgG. Compared with the IgG purified from the original peritoneal fluid, the immune complexes had IgG with a much higher specific activity to RNP.

Case 2 (Fig. 3)
The patient, a forty-year-old woman, was transferred to National Defense Medical College Hospital on October 10, 1978 because of massive ascites.

She had been well until June 1978, when fever, lid edema and abdominal distention developed and she visited a nearby hospital. On admission, she was noted to have a skin rash resembling erythema exudativum multiforme on finger tips, knees and both sides of the abdomen. Laboratory tests at this time revealed trace proteinuria and decreased serum protein of 5.7 g/dl (serum albumin 2.9 g/dl).

In July 1978, the patient’s ascites rapidly increased and frequent paracenteses were needed. She was then receiving 30 mg of prednisolone daily. Her fever and rash disappeared but the ascites remained unchanged. She was then referred and admitted to the National Defense Medical College Hospital.

On admission, alopecia, a butterfly rash and finger tip erythema suggestive of SLE were found. A small amount of pleural effusion was noted on chest roentgenogram. Laboratory evaluation revealed leukopenia, hypocomplementemia, anti-nuclear antibody of speckled pattern with a titer of 1:40. Anti-RNP antibody was positive by double immunodiffusion.

The diagnosis of SLE was made. On paracentesis, the fluid was a sterile exudate with a few cells. Extensive work-up failed to find any other cause for the ascites other than the patient’s systemic disease. Her dose of prednisolone was then increased to 50 mg daily and then the ascites, together with her other clinical symptoms gradually started to resolve. The immunological characteristics of the serum and the peritoneal fluid are summarized in the Table.

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(serum)</td>
<td>(ascites)</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>5.7</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.4</td>
</tr>
<tr>
<td>IgG (mg/dl)</td>
<td>1105</td>
</tr>
<tr>
<td>CH 47</td>
<td>25</td>
</tr>
<tr>
<td>CH 2</td>
<td>21</td>
</tr>
<tr>
<td>CH 50 (unit/ml)</td>
<td>N.T.</td>
</tr>
<tr>
<td>Anti-DNA antibody</td>
<td>6.75*</td>
</tr>
<tr>
<td>Anti-RNP antibody</td>
<td>(+):1:64</td>
</tr>
<tr>
<td>Anti-sm antibody</td>
<td>(−)</td>
</tr>
<tr>
<td>Immune complex (IgG μE/ml)</td>
<td>160</td>
</tr>
</tbody>
</table>

* +H-DNA binding. ** RIA unit. ‡ measured by single radial immunodiffusion. † detected by 3.5% polyethylene glycol precipitation as described in the text. N.T. not tested

** COMMENTS**

The two patients presented are rare cases of systemic lupus erythematosus which involved the peritoneum and which was manifest as massive ascites with severe gastrointestinal symptoms resembling acute surgical conditions of other diseases. They did not have severe nephrotic syndrome, constrictive pericarditis or liver disease. Bacterial, fungal, tuberculous and neoplastic conditions were also excluded. According to the results of Dubois14 with 520 cases of SLE, inflammation of the abdominal lining membrane alone had not been noted to produce ascites without other significant causes.

However, in 1974, Metzger et al.10 first reported a case of lupus peritonitis with ascites. They demonstrated the presence of visceral vasculitis and in vivo LE cell formation, positive antinuclear antibody and depressed complement level in the peritoneal fluid. Afterwards, Birtan et al.15 and Shocket et al.16 demonstrated similar cases. They showed the deposition of immunoglobulins and complement in the mesothelial layer and in the visceral arteries of the involved peritoneum. All these findings suggest that immune complex mediated pathological processes may take part in lupus peritonitis as well as in other organs involved in SLE.14-16

The immunological findings obtained from one of our patients (case 1) suggest that immune complexes, especially those consisting of RNP-anti-RNP antibody, in this case, might participate in the development of lupus peritonitis, although it may be possible that the high concentration of immune complexes might be due to detecting aggregated IgG. In this patient, the relative IgG content and the titers of anti-DNA and anti-RNP antibodies were elevated in the peritoneal fluid. The complement level, which was detected by single radial immunodiffusion, was markedly depressed. In addition, the concentration of the immune complexes was much higher in the peritoneal fluid than in the serum. Finally, the IgG obtained from the immune complexes fraction of the peritoneal fluid was shown to have a higher specific activity for anti-RNP antibody than that obtained from the unfractuated peritoneal fluid.

However, the high concentration of immune complexes shown in this study may participate in or result from some secondary processes in the development of lupus peritonitis. Many publications have indicated that the vasculitis of the peritoneal vessels may be the major cause of lupus peritonitis. It has also been suggested that circulating immune complexes may cause the vasculitis. In this circumstance, the immune complexes in the peritoneal fluid may be an aggravating factor of the peritonitis. Further study is needed to detect the time course of the immune complexes both in serum and in peritoneal fluid.
ACKNOWLEDGEMENTS

We thank Dr. Masakuni Tomii for his helpful advice in the immune complex studies. We also thank Drs. Mitsuo Homma and Makoto Takano for their critical reading of the manuscript.

REFERENCES

1) Dubois EL: Systemic Lupus Erythematosus. 2nd ed. p 284, Los Angeles, University of Southern California Press, 1974

The List of Papers Rewarded by the Japan Rheumatism Association

The Japan Rheumatism Association has awarded his prize for the scientific excellent research since 1962. The prize winners and their co-workers are listed below. Most of their papers were published in English, with several exceptions. For the convenience of overseas researchers, summaries of those papers which have appeared in Japanese were translated in English by the authors.

1962 Tamotsu TERAWAKI

1963 Tamotsu SHIMIZU

Shoichiro IRIMAJIRI

To clarify the difference between agglutinating factors (RA factors) in sera from patients with rheumatoid arthritis (RA) and those in nonrheumatic diseases, especially in liver diseases, latex fixation test (LFT) and benzentine flocculation test (BFT) were performed on whole serum and globulin fraction, the latter reacted positively with higher percentage compared with whole serum.

In liver diseases, positive reactions were obtained 74.4% in LFT and 72.9% in BFT respectively, and they were almost as high as in RA patients.

In LFT and BFT, sera from liver diseases reacted positively with lower agglutination titer compared with those from RA. In SSCA, 42.3% of RA sera showed positive in DAT but none of from liver diseases showed positive. A positive correlation was noted between TTT and BFT titer in RA, which was not in liver diseases.

Ultracontrastifugal study of sera from RA and liver cirrhosis failed to demonstrate any direct relationship between BFT titer and quantity of macroglobulin. Continuous zone electrophoresis on paper revealed the presence of agglutinating fractions in β1-γ1-globulin area mainly in γ1-globulin with RA serum and β2 with liver diseases.

The above results suggested that those two agglutinating factors belonged to macroglobulin but qualitative difference beyond quantitative one did firmly existed between two factors.

1964 Hiroshi AZAWA

Although acute phase reactants, particularly CRP, rapidly become negative when rheumatic fever is treated with anti-inflammatory drugs, the symptoms often recur when treatment is discontinued. Since normalized reactive protein levels do not always mean abatement of activity, there exist no indications for discontinuing treatment. Thus, chondroitin-sulfate tolerance tests (CST) were examined as a possible clinical indicator. Contrasting with healthy adults, CST often proved positive when such symptoms were present, but became negative around the 10th week after onset. Regardless of the abnormal levels noted in the negative stage, relapse was observed when treatment was discontinued. However, when discontinued, recurrence was noted in all cases in the positive stage with abnormal ECG and heart rate findings. Thus, it was clinically
confirmed that CST is probably useful as an indicator. Worsened results in the positive stage implied relapse because their different aspects were definitely distinguishable from rebound. Prednisolone in large doses proved effective in an early stage of inflammation. This reaction was also confirmed in rabbits given allylamine. The in vitro positive results of CST are briefly discussed.

Keiji UDAKA

1965 Toshio NISHIMURA

1966 Yutaka MIZUSHIMA

1969 Masahisa KYOGOKU

Terunobu SAITO

I. Studies on the changes of gastric mucosa and hemorrhagic tendency in massive aspirin therapy
In aspirin therapy of rheumatic diseases, maintenance of an effective blood level of aspirin is essential for a successful result, and this necessitates a long-term treatment with massive doses. Although disorder of the digestive tract and tinnitus are known as the two major side-effects of such a massive treatment, very few fundamental and clinical studies have been devoted in Japan to the former subject.

By gastrocamera or gastrofiberscope, the author investigated the change of gastric mucosa in massive aspirin therapy of rheumatic diseases. Relationship was analyzed between the extent of hemorrhage of the gastric mucosa and various, probable influential factors including the acidity of gastric content and the chemical structure and pharmaceutical type of aspirin drug. Consequently, it was revealed that the intragastric pH and the concentration of dissolved aspirin (strictly speaking, the concentration in the field of action) are most closely concerned with the development of hemorrhage; the lower the pH and the higher the local concentration of aspirin, the more frequent and more pronounced the mucosal hemorrhage.

II. Experimental studies on the cause of gastric hemorrhage in aspirin therapy
The effect of aspirin on the rabbit gastric mucosa was studied. Gross and microscopic examinations of this treated specimen revealed that pH of the gastric mucosa and the concentration of aspirin at the site play the most important roles in the development of hemorrhagic lesions of the stomach. On the basis of these experimental facts, the author does not support the presently prevailing view of A. Muir and others that firmly adhere particles of aspirin are chiefly responsible for the development of gastric hemorrhage.

1971 Masaki WATANABE

Takuro Sugano

Bone atrophy associated with RA (rheumatoid arthritis) was biochemically studied and the following results were obtained:

(1) The composition of the organic and inorganic substances in the affected bone was not different from that of normal bones.

(2) Decrease in mucopolysaccharide was generally evident in RA-affected bones. Particularly among younger patients, this reduction was markedly greater than that among normal subjects.

(3) The separative assay of hexosamine into galacosamine and glucosamine revealed that these had undergone qualitative changes. The alterations similar to senile degenerations were seen even in younger RA patients.

(4) As for collagen content in the bone, there was no difference between RA and control groups. In RA group, however, abnormality was seen in collagen dissolution with a certain type of reagent.

(5) These findings suggest that RA has influence not only on the bone of RA-affected site but also on the bone of the whole body.

(6) Urinary calcium and hydroxyproline levels tended to increase in the active phase of RA. However, blood biochemical findings were, generally, unchanged.

1972 Kazushi HIROHATA

Shigehis AOKI

1973 Hajime INOUE

1974 Kyioaki TANIMOTO

Kiyonobu MIKANAGI

1976 Tatsuo SUZUTA

Koshibiro MIYATA, Masahiko HOKONOHARA and Yoshitaka NAGATA

1977 Taro OKAZAKI

1979 Shunichi HIROSE

Immunological phenomenon caused by antigen-antibody reaction has well correlated with various biological activities of immune complex which was confirmed with various kinds of antigens and antibodies. However, neither the actual nature of antigen molecule and antibody molecule modified by the antigen-antibody binding nor the pathogenic role of such immune complex and the course of the diseases resulting from these reactions has been made clear enough.
Though the immunological phenomenon is almost doubtless the cause for the diseases, so called immune complex diseases, there still remains a room to doubt that immune complex might be a direct cause for the diseases. Therefore, we must take a step forward for further research to define whether immune complex has a role as a certain pathogene for a disease. I, hereto, start with a basic understanding of immune complex and consider a relationship between such immunological phenomenon and the appearance of related diseases.

For a true understanding of immune complex, especially in biological sense, each factor related to immunological phenomenon is discussed as follows:

Masashi AKIZUKI

1980  Takeshi SAKANE

Motomichi TANAKA

1980  Hisashi IWATA

This book contains general and pathological biochemistry about macromolecules of extracellular matrix, especially proteoglycan and associated subject—bone morphogenetic protein of bone matrix gelatin, and its clinical application. The contents were divided into three parts.
I: Structure and function of extracellular macromolecules.
II: Extracellular macromolecules and biological events
III: Extracellular macromolecules and clinical significance