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REVIEW ARTICLE

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Diagnosis and therapy of rheumatoid arthritis in the future

Abstract Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic disease characterized by joint pain and destruction. We describe the importance of early diagnosis, recording magnetic resonance images images at an early stage, monitoring disease progression using gliostatin purified in our laboratory, and sugar-chain analysis of RA serum IgG. For treatment of RA, cyclooxygenase-2-selective inhibitors, disease-modifying antirheumatic drugs, biological products, and the possibility of gene therapy are discussed. The development of therapeutic methods based on the elucidation of the pathology of RA has progressed markedly in the past decade, and further progress and the development of an early diagnostic method are expected.

Key words Agalactosyl IgG · Cyclooxygenase (COX)-2 selective inhibitor · Gliostatin · Magnetic resonance imaging (MRI) · Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a systemic, chronic, inflammatory disease characterized by synovial hyperplasia and the deformity and destruction of joints and bone. Persistent arthritis impairs joint function, and leaves patients with marked social limitations and a financial burden. The objectives of therapy are inhibition of the inflammation to relieve pain, inhibition of the progression of joint destruction, and preservation of the function of the joint to maintain abatement. Rheumatologists, should seek methods for early diagnosis and explore the possibilities of new therapeutic methods.

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Importance of early diagnosis of RA

In RA therapy, it is very important to diagnose the RA before irreversible joint destruction occurs. However, there are various onset patterns of RA, making early diagnosis difficult. Since the pathogenesis is completed when the diagnosis of RA is made following the criteria for the classification of RA established by the American Rheumatism Association (ARA) in 1987,[1] the prevention of joint destruction is difficult. Thus, it is important to observe patients carefully, to record physical, hematological, and imaging findings, and to diagnose RA at an early stage to treat the patient before irreversible joint destruction occurs.

Usefulness of magnetic resonance imaging of the hand for early RA diagnosis

We studied the usefulness of T_1-weighted fat-suppression magnetic resonance imaging (MRI), by which the presence of synovitis in smaller regions is likely to be revealed.[2] Using T_1-weighted fat-suppression MRI and gadolinium (Gd)-enhancement, the frontal and axial planes of the region from the distal radioulnar joint to the metacarpal phalangeal (MCP) joint were imaged. Figure 1 shows the T_1-weighted fat-suppression image and its Gd-enhanced image. The arrows show the enhanced region due to synovitis. The regions evaluated were: (1) the radiocarpal joint; (2) the midcarpal joint; (3) the distal radioulnar joint; (4) the MCP joint; (5) the flexor tendon sheath of the intracarpal tunnel; (6) the flexor tendon sheath at the MCP joint; (7) the tendon sheath of the extensor digitorum communis; (8) the tendon sheath of the extensor carpi radialis; (9) the tendon sheath of the extensor carpi ulnaris. The study was performed in 16 joints in 16 patients with RA at an early stage within 1 year after onset. These patients underwent MRI examination of the wrist joint region between April 1998 and December 1999. High positivity rates were obtained (radiocarpal joint, midcarpal joint, carpal tunnel region, 100%; distal radioulnar joint, tendon sheath of the extensor digitorum communis, 93.8%), showing that

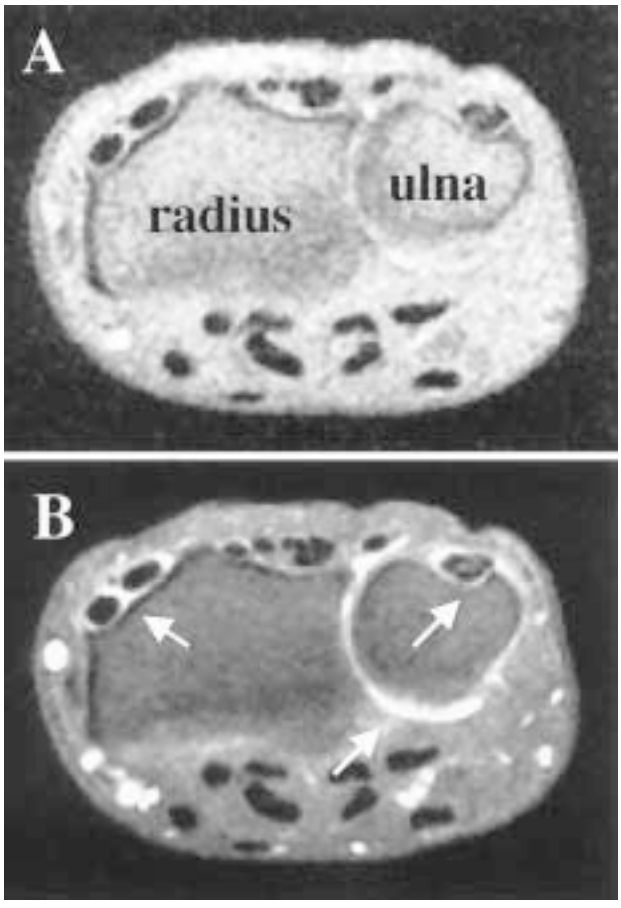

Fig. 1. Coronal images of the distal radioulnar joint. **A** T_1-weighted fat-suppression image. **B** Gadolinium (Gd)-enhanced image. The *arrows* indicate the regions of active synovitis

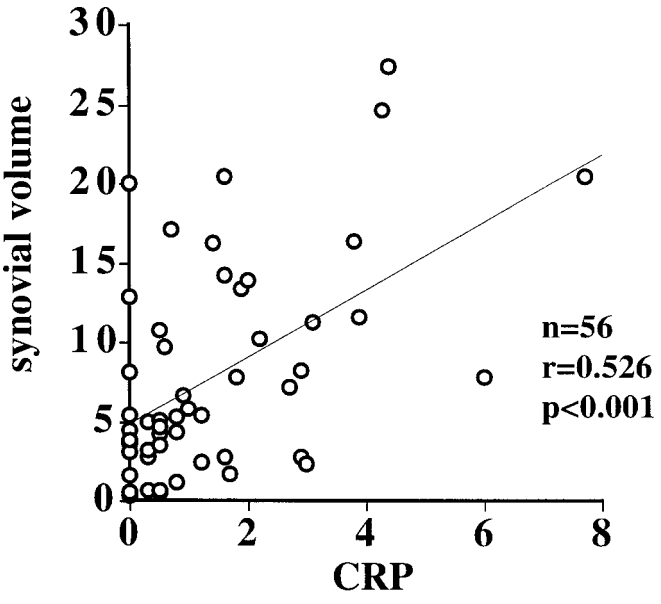

Fig. 2. Correlation between the amount of synovial membrane calculated from the Gd-enhanced T_1-weighted fat-suppression magnetic resonance image of the hand and serum C-reactive protein (*CRP*) (mg/dl)

MRI examination of the hand is useful for the early diagnosis of RA and that careful observation of these regions is necessary.

We have recently been quantifying the synovial membrane using Gd-contrast fat-suppression MRI of the hand. Excluding contrasted blood vessels and insufficiently suppressed subcutaneous fat tissues, the Gd-contrasted active synovial membrane was traced, each slice was integrated, and the amount of synovial membrane was calculated. In 65 RA patients with a mean age of 59.4 years (range 26–84 years) and with a mean disease duration of 7.3 years (range 1 month to 31 years), the amount of synovial membrane was highly correlated with the level of C-reactive protein (CRP) (Fig. 2). By semiquantifying the active synovial membrane, the activity of RA can be evaluated in relation to the disease course from the early stage. This information may also be useful for an assessment of drug effectiveness.

Monitoring RA course using gliostatin

Interleukin (IL)-1-β and tumor necrosis factor (TNF)-α are key elements in the destructive mechanism in RA. In addition, IL-6, granulocyte-macrophage colony stimulating factor, and chemokines are also detected in synovial fluid and serum in RA. We have reported that gliostatin (GLS), which is contained in RA synovial fluid at high concentrations, is closely involved in the pathology of RA.

GLS is a protein which was purified from neurofibroma by Asai et al. in 1991.[3] It has been identified as platelet-derived endothelial cell growth factor (PD–ECGF) and thymidine phosphorylase (dTPase) based on its physicochemical and biological properties. It has been reported to have a neurotrophic effect, to promote the growth and migration of vascular endothelial cells, and to promote neovascularization in vivo.[4,5]

Serum and synovial GLS concentrations are markedly higher in RA patients than in healthy individuals or in patients with osteoarthritis of the knee. The GLS concentration was also high in synovial tissue extracts from RA patients, and cells from the synovial surface and interstitial mesodermal cells were stained with anti-GLS antibody on immunostaining of synoviocytes in RA.[6] The synovial membrane may produce GLS in synovial fluid. The GLS concentration in synovial fluid was higher than the levels of inflammatory cytokines TNF-α, IL-1-α, IL-6, and IL-8.[7] The representative cytokines in RA synovial fluid are shown in Table 1.[7] Among these cytokines, the concentration of GLS is particularly high, and the serum concentration is also characteristically high.

The RA serum GLS level showed close correlations with indicators of inflammation such as erythrocyte sedimentation rate (ESR), CRP, and the Lansbury index. A typical case in which the serum GLS level, ESR, CRP, and activities of daily living (ADL) score were traced for more than 1 year is shown in Fig. 3. The patient was a 75-year-old woman with a disease duration of 12 years, who is currently at stage IV, class 2. The patient is receiving loxoprofen and

Table 1. Cytokine concentration in synovial fluid from knee joints of patients with rheumatoid arthritis ($n = 38$)

Cytokine	Concentration mean (SEM)
GLS	384.5 (42.9) ng/ml
TNF-α	237.6 (39.6) pg/ml
IL-1-α	1.437 (0.12) pg/ml
IL-6	8.303 (0.659) ng/ml
IL-8	1.320 (0.077) ng/ml

GLS, gliostatin; TNF, tumor necrosis factor; IL, interleukin; SEM, standard error of mean
Modified from ref. 7, with permission

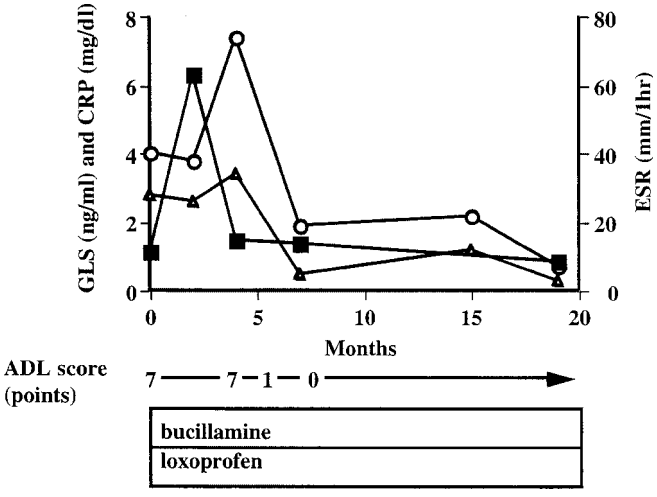

Fig. 3. Serially determined levels of serum gliostatin (*GLS*) and commonly used clinical markers of inflammation: *CRP*, erythrocyte sedimentation rate (*ESR*), and activities of daily living (*ADL*) score. The patient was a 75-year-old woman with a disease duration of 12 years. (From ref. 7, with permission). *Circles*, ESR (mm/h); *triangles*, CRP (mg/dl); *squares*, GLS (ng/ml)

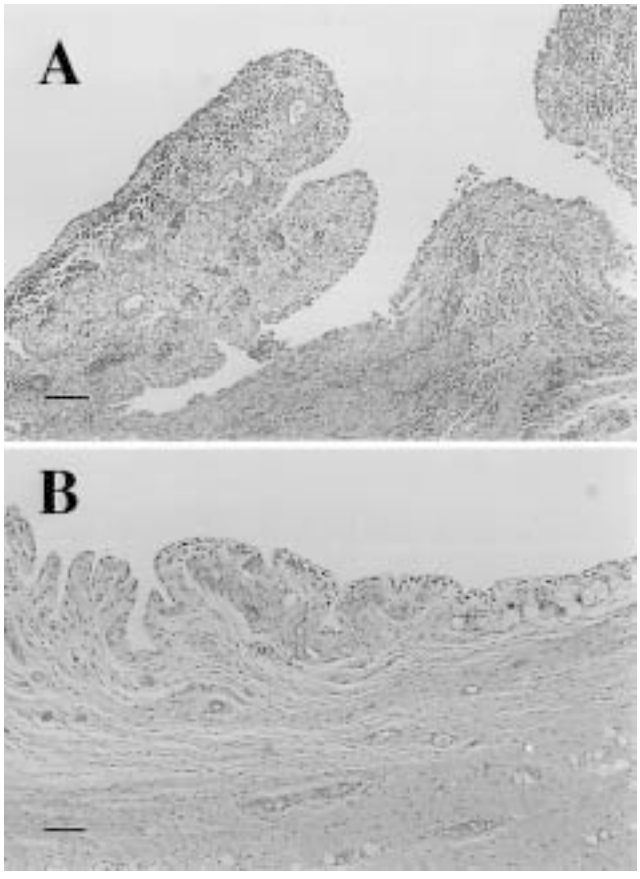

Fig. 4. Induction of synovitis by the injection of rGLS into the knee joint in rabbits. **A** The synovial membrane after 10 μg rGLS had been injected weekly for 3 months (H&E). Mononuclear cell infiltration, aggregation of lymphocytes, microvascular hyperplasia, and interstitial edema were marked. **B** The synovial membrane after the same amount of bovine serum albumin was injected into the other knee as the control (H&E). *Bar* 100 μm

Table 2. Induction of GLS by IL-1-α and TNF-α in fibroblast-like synoviocytes

Cytokine	(ng/ml)	GLS (ng/mg protein)
Control	0	ND
TNF-α	1	13.50 × 3.21
IL-1-α	1	14.13 × 3.26
IL-6	5	12.84 × 3.22
IL-8	5	14.67 × 2.56

Values are the mean × SEM ($n = 4$)
ND, not detected
Modified from ref. 7, with permission

bucillamine, and her GLS level, ESR, CRP level, and ADL score improved as the symptoms subsided.

Using the techniques of molecular biology, we investigated how synoviocytes produce GLS. Synovial specimens obtained during total knee joint replacement surgery were cultured and stimulated with cytokines TNF-α (1 ng/ml), IL-1-α (1 ng/ml), IL-6 (5 ng/ml), and IL-8 (5 ng/ml). GLS was not detected in the absence of cytokines, but GLS protein was induced in the presence of each of these inflammatory cytokines (Table 2).[7]

To investigate how GLS is involved in the destruction of the joint in RA, a large amount of recombinant GLS (rGLS) was purified using recombinant gene techniques, and the rGLS was injected into the articular cavity of the knee joint in rabbits. Ten micrograms of rGLS and 10 μg of bovine serum albumin as a control were injected into the right and left knee, respectively, weekly for 3 months. Mild synovitis occurred in the control knee. In the rGLS-injected knee, mononuclear cell infiltration, an aggregation of lymphocytes, microvascular hyperplasia, and interstitial edema were marked (Fig. 4).[8] No marked changes in the patellar cartilage were observed in the control knee, while the cartilage surface was rough and chondral necrosis in the deep layer was observed in the rGLS-injected knee (Fig. 5). These findings suggested that GLS may be involved in the production of matrix metalloproteinases (MMPs). When cultured synoviocytes were stimulated with rGLS at a concentration (300 ng/ml) comparable to the GLS level in the synovial fluid of RA patients, mRNA and protein expressions of MMP-1 and MMP-3 were induced.[9]

According to Feldmann and Maini,[10] an imbalance of cytokines may occur in RA joints, as shown in Fig. 6, and

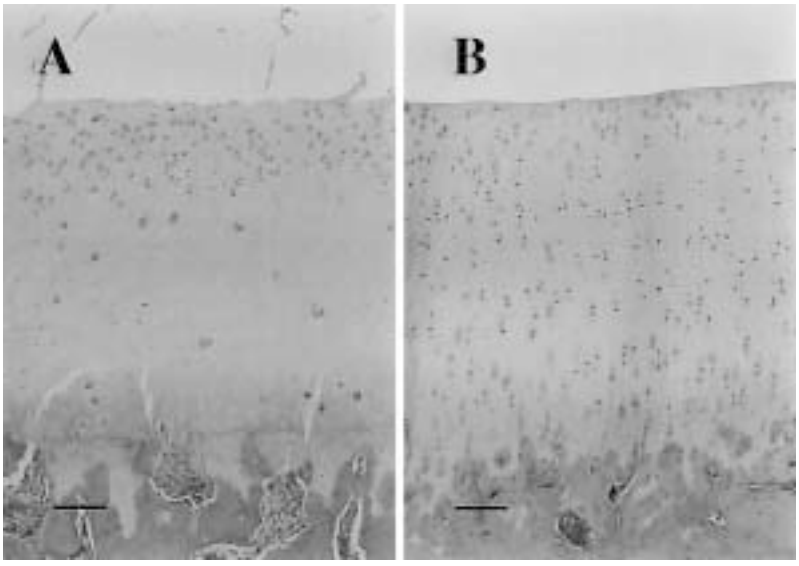

Fig. 5. Induction of patellar cartilage destruction by the injection of rGLS into the knee joint in rabbits. **A** The cartilage after 10 μg rGLS had been injected weekly for 3 months (H&E). **B** The cartilage after the same amount of bovine serum albumin was injected into the other knee as the control (H&E). *Bar* 100 μm

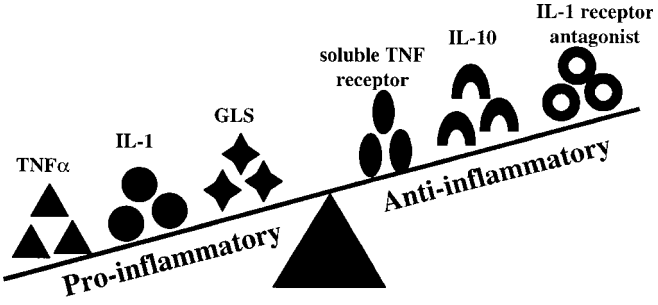

Fig. 6. Cytokine disequilibrium in RA. *TNF* tumor necrosis factor; *IL* interleukin; *GLS*, gliostatin (Modified from ref. 10, with permission)

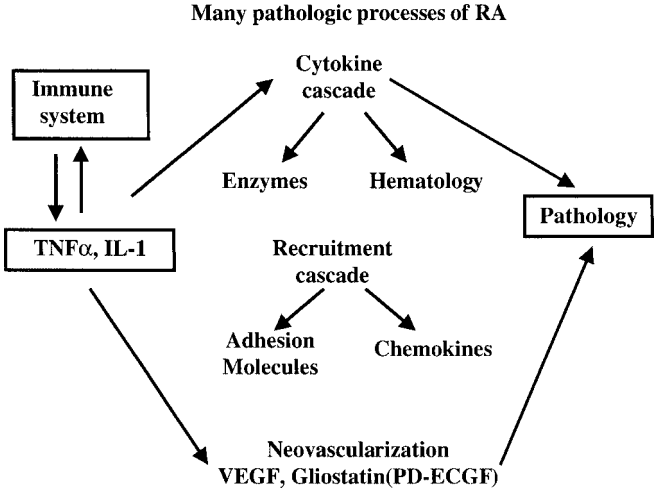

Fig. 7. Position of GLS in the cytokine network in RA. *VEGF* vascular endothelial growth factor; *PD-ECGF* platelet-derived endothelial cell growth factor (Modified from ref. 10, with permission)

this imbalance may facilitate the chronic progression of inflammation and destruction of the joint tissue. Although the position of GLS in the cytokine network in RA may be equivalent to that of vascular endothelial growth factor, which has a neovascularization effect (Fig. 7), because GLS destroys cartilage, it is more likely to be involved in the pathogenesis of RA. Future research will investigate whether the measurement of serum GLS levels at an early stage of RA would facilitate early diagnosis.

Sugar-chain analysis of serum IgG in RA

IgG contains sugar chains bound to asparagine at 297 in the CH_2 domain of each H chain. These sugar chains are involved in maintaining the tertiary structure of the Fc region, and have several biological functions. Figure 8 shows a typical sugar-chain structure. In 1985,[11] it was reported in *Nature* that this IgG sugar-chain structure has an abnormality, and that galactose-deficient sugar chains increased in patients with RA.

We analyzed the sugar chains of serum IgG from RA patients using high-performance liquid chromatography (HPLC). The sugar-chain structure was determined by the

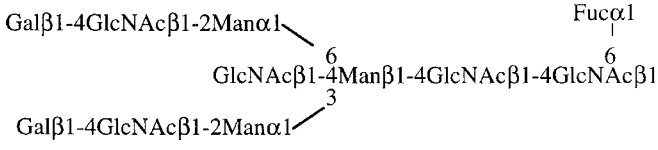

Fig. 8. A typical IgG sugar-chain structure

two-dimensional mapping method reported by Takahashi et al.[12] The sugar chains were roughly divided into three groups: group I (peaks A, B, C, and D) lacking both fucose and bisect GlcNAc residues, group II (peaks E, F, G, H) containing Fuc residue but lacking GlcNAc residue, and group III (peaks M, N, O, and P) containing both fucose and bisect GlcNAc residues. These sugar-chain patterns were almost constant in healthy individuals, and group II

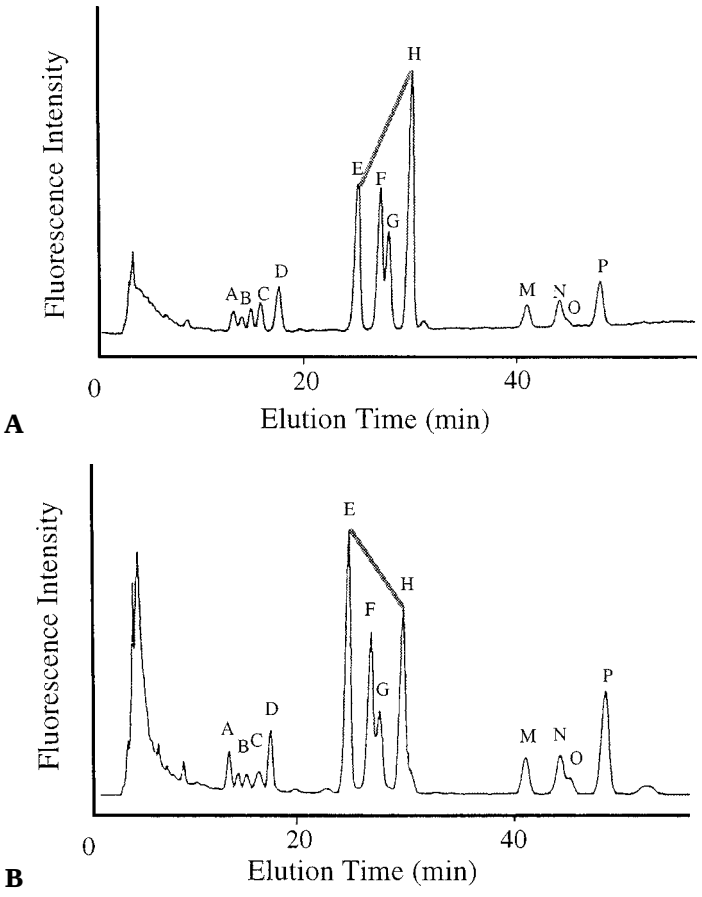

Fig. 9. Typical sugar-chain patterns separated by HPLC. **A** Healthy subject (40-year-old woman); **B** RA patient, a 50-year-old woman with a disease duration of 4 years

was the major fraction. Figure 9 shows typical patterns of sugar chains isolated from healthy individuals and RA patients by HPLC. In RA patients, group II (peaks E, F, G, and H) sugar chains lacking galactose increased.[13] HPLC is a highly reproducible analytical method and is advantageous in that it detects the presence or absence of bisecting N-acetylglucosamine (bisec GlcNAc) and fucose residues in addition to galactose. However, the analysis procedure is complex, and a kit for the measurement of serum anti-agalactosyl IgG antibody (Eitest CA–RF, Eisai, Tokyo, Japan) has been produced as a simpler method that is covered by health insurance in Japan. Takubo et al.[14] compared the clinical usefulness of Eitest CA–RF with the previous rheumatoid factor (RF) measurement method (LN–RF). They tested 180 RA patients at the early stage within 1 year after onset. These patients had not received treatment with oral steroids or disease-modifying antirheumatic drugs (DMARDs) and could be followed for 2 years after the first examination. The positivity rates obtained using Eitest CA–RF and the RF measurement method were 90.6% and 70.6%, respectively. In our measurement of RF in 60 patients meeting the ARA diagnostic criteria, the positivity rate obtained using Eitest CA–RF was 82.5%, while the rate obtained by the previous RF method was 69.8%, showing that the Eitest CA–RF method is more sensitive.

Newest drug therapy

In recent years, cyclooxygenase (COX)-2-selective inhibitors have attracted attention in relation to nonsteroidal anti-inflammatory drugs (NSAIDs). In 1971, Vane reported that NSAIDs such as aspirin and indomethacin inhibit prostaglandin production by inhibiting COX activity, and exhibit an antiinflammatory effect.[15] In 1990, the presence of at least two isozymes, COX-1 and COX-2, was shown.[16] In regard to the genetic characteristics, COX-1 is a constitutive enzyme, while COX-2 is an inducible enzyme. Available NSAIDs that selectively inhibit COX-2 include etodolac, meloxicam, and nimesulide. In the United States, the COX-2-selective inhibitors celecoxib and refecoxib were rapidly approved and have been launched.

Drugs used for the treatment of RA include DMARDs that may correct the abnormal immunity and inhibit the progression of joint destruction. Nine types of DMARDs are used in Japan, and methotrexate (MTX), which is the drug of first choice in Western countries, became covered by health insurance in August 1999. The limitations of the current therapy with DMARDs include the following problems: (1) they are slow acting; (2) some patients are responders, but some are nonresponders; (3) the escape phenomenon; (4) the relatively high frequency of adverse side effects. Ideal DMARDs completely inhibit arthritis and stop joint destruction. However, although currently used DMARDs can delay bone destruction in RA, they cannot completely stop it. The drugs reported to inhibit bone destruction in RA include gold sodium thiomalate (GST) and MTX. Although cyclosporin A is not indicated in Japan, this drug has been reported to inhibit bone destruction. New DMARDs have also been developed, such as the pyrimidine synthesis inhibitor leflunomide, which was approved by the Food and Drug Administration (FDA) in the USA in 1998.

Biological products that are different from currently used DMARDs have also been developed. The involvement of various cytokines in the pathology of RA has been shown, and IL-1 and TNF-α play important roles. Accordingly, methods targeting these cytokines have been developed as new therapeutic strategies. In particular, TNF-α is considered to be located upstream in the cytokine network, and biological products targeting TNF-α were approved by the FDA in the USA in 1998 and 1999.

Gene therapy for RA has also been developed. In May 1999, RA was included in the indications for gene therapy in Japan. Therapeutic strategies such as immunosuppressive gene therapy, antiinflammatory gene therapy, transcriptional factor-regulating gene therapy, and synoviocyte-regulating gene therapy are considered to correspond to the pathology of RA.

References

1. Arnett FC, Edworthy SM, Bloch DA, Mcshane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987

revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
2. Taneda Y, Matsui N, Yoshida Y, Nagaya Y, Mukofujiwara Y. MRI diagnosis of early rheumatoid arthritis (in Japanese). Riumachi-ka 1999;21:122–9.
3. Asai K, Hirano T, Kaneko S, Moriyama A, Nakanishi K, Isobe I, et al. A novel glial growth inhibitory factor, gliostatin, derived from neurofibroma. J Neurochem 1992;59:307–17.
4. Asai K, Nakanishi K, Isobe I, Eksioglu YZ, Hirano A, Hama K, et al. Neurotrophic action of gliostatin on cortical neurons: identity of gliostatin and platelet-derived endothelial cell growth factor. J Biol Chem 1992;267:20311–6.
5. Ishikawa F, Miyazono K, Hellman U, Drexler D, Wernstedt C, Hagiwara K, et al. Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. Nature 1989;338:557–61.
6. Takeuchi M, Otsuka T, Matsui N, Asai K, Kato T, Tada T, et al. Aberrant production of gliostatin/platelet-derived endothelial cell growth factor in rheumatoid arthritis. Arthritis Rheum 1994;37: 662–72.
7. Waguri Y, Otsuka T, Sugimura I, Matsui N, Asai K, Moriyama A, et al. Gliostatin/platelet-derived endothelial cell growth factor as a clinical marker of rheumatoid arthritis and its regulation in fibroblast-like synoviocytes. Br J Rheumatol 1997;36:315–21.
8. Waguri-Nagaya Y, Otsuka T, Sugimura I, Matsui N, Asai K, Nakajima K, et al. Synovial inflammation and hyperplasia induced by gliostatin/platelet-derived endothelial cell growth factor in rabbit knees. Rheumatol Int 2000;20:13–9.
9. Muro H, Waguri-Nagaya Y, Mukofujiwara Y, Iwahashi T, Otsuka T, Matsui N, et al. Autocrine induction of gliostatin/platelet-derived endothelial cell growth factor (GLS/PD–ECGF) and GLS-induced expression of matrix metalloproteinases in rheumatoid arthritis synoviocytes. Rheumatology 1999;38:1195–202.
10. Feldmann M, Maini RN. The role of cytokines in the pathogenesis of rheumatoid arthritis. Rheumatology 1999;38 Suppl 2:3–7.
11. Parekh RB, Dwek RA, Sutton BJ, Fernandes DL, Leung A, Stanworth D, et al. Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. Nature 1985;310:452–7.
12. Takahashi N, Ishi I, Ishihara H, Mori M, Tejima S, Jefferis R, et al. Comparative structural study of N-linked oligosaccharides of human normal and pathological immunoglobulin G. Biochemistry 1987;26:1137–44.
13. Mukofujiwara Y, Otsuka T, Waguri Y, Matsui N, Asai K, Kato T, et al. Abnormal glycosylation of IgG as a clinical parameter in patients with rheumatoid arthritis: its constitutional analysis by HPLC. J Clin Biochem Nutr 1998;25:131–42.
14. Takubo N, Yamamoto T, Nakata S, Yamada Y. The clinical usefulness of measurement of the serum anti-agalactosyl IgG antibody values in the early onset RA (in Japanese). Riumachi-ka 1997;17:228–34.
15. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for the aspirin-like drugs. Nature 1971;231:232–5.
16. Vane JR, Botting RM. Mechanism of action of anti-inflammatory drugs. Scand J Rheumatol 1996;25 Suppl 102:9–12.