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ABSTRACTS

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I: Synovial Pathology in Joint Destruction

I-1: Synovial-fibroblast mediated joint destruction

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The era of new biologics has brought a number of novel therapeutic options for the treatment of rheumatic diseases. More efficacious and safer drugs have been developed benefiting our patients tremendously. On the other hand, we cannot yet cure rheumatoid arthritis (RA).

One major reason appears to be the cytokine-independent activation of synovial fibroblasts (SF) in RA. Most interestingly, not only SF of the invasive growing synovial tissue are found at sites of cartilage and bone destruction, but also small groups of SF can be detected attached to articular cartilage. To explore the role of SF floating in the synovial fluid of RA patients, we cultured synovial fluid-derived cells and could show that these cells appear highly activated even in the absence of human immune cells. This observation indicates a major role of these cells contributing to joint destruction in RA.[1]

Because the germ-line encoded pattern-recognition receptors (PRRs) appear involved in the activation of SF in RA we explored the stimulation of Toll-like receptor (TLR-2) in RA-SF. We could show that microbial products induce not only NFkB, Cox-2, and matrix-degrading enzymes, like MMP-1, in RA-SF, but also large amounts of chemokines. The production of chemokines by activation of RA-SF via TLR-2 might contribute significantly to the formation of inflammatory infiltrates in the synovium.[2]

Based on the observation that synovial cell attachment to cartilage and bone is characterized by a strong expression of matrix-degrading enzymes and apoptosis-inhibiting molecules (SUMO-1 and FLIP), but a lack of the tumor suppressor PTEN at sites of destruction, we explored the contribution of hypoxia to the activation of SF in RA.

By using micro-arrays, we could identify novel genes in SF which are regulated by hypoxia in RA. Most interestingly, we could characterize the role of Id-2 (inhibitor of differentiation), a molecule related previously to malignant cellular phenotypes mediating migration and invasion. It was surprising that the expression of Id-2 appeared independent from hypoxia-induced factor (HIF)-1 and especially at sites of destruction. The selective induction of Id-2 in RA-SF, but not in skin fibroblasts, indicates that this protein induced in response to a hypoxic environment might play an important role in enhancing the invasive process.[3]

1) Neidhart M, Seemayer CA, Hummel KM, Michel BA, Gay RE, Gay S. Functional characterization of adherent synovial fluid cells in rheumatoid arthritis. Arthritis Rheum 2003;48:1873–80.
2) Pierer M, Rethage J, Seibl R, Lauener R, Wagner U, Hantzschel H, et al. Chemokine secretion of rheumatoid arthritis synovial fibroblasts stimulated by TLR-2 ligands. J Immun 2004; in press.
3) Kurowska-Stolarska M, Distler J, Moritz W, Marti H, Gay RE, Maslinski W, et al. The expression of inhibitor of differentiation (Id-2) is induced by hypoxia in synovial fibroblasts independently of HIF-1a. Arthritis Rheum 2003;48:S146.

I-2: Molecular mechanism of chondrogenesis via chromatin modifications

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Chondrocytes play an essential role in limb development via chondrogenesis, which is a multistep pathway during which multipotential mesenchymal stem cells differentiate into chondrocytes. Chondrocytes also form articular cartilage, which is a key component in biomechanical joint function and also the site of aberrant extracellular matrix remodeling in arthritis.

Sox9 high-mobility group (HMG) domain transcription factor is known to play an essential role in establishing the precartilaginous condensations and in initiating chondroblast differentiation. Specifically, Sox9 binds to a region within the first intron of the type II collagen gene (*Col2a1*) to regulate its transcription. Mutations in Sox9 underlie the rare congenital dwarfism syndrome, campomelic dysplasia. These genes contain a high mobility group domain and belong to the SOX family of proteins that are homologous to the protein encoded by Sry (sex-determining region of Y chromosome). Expression of Sox9 almost parallels that of *Col2a1*, which encodes type II collagen, during chondrogenesis; and analysis of mouse chimeras using Sox9(−/−) ES cells shows that Sox9(−/−) cells are excluded from cartilage tissues and are unable to express chondrocyte-specific extracellular matrix genes such as *Col2a1*. In the genital ridge, however, *Col2a1* is not expressed, despite abundant Sox9 expression, suggesting the existence of transcriptional coactivators for Sox9 to promote *Col2a1* gene expression in specific tissues.

In addition to sequence-specific factors, such as Sox9, various coactivators are involved in transcriptional activation. For example, the transcriptional coactivator CBP and its paralog p300 are recruited to promoter regions via direct interactions with various sequence-specific activators including CREB, AP-1, STATs, and nuclear hormone receptors. These coactivators facilitate transcription by promoting interactions between sequence-specific activators and the RNA polymerase II transcriptional machinery to initiate transcription.

We recently examined molecular mechanism of chondrogenesis via chromatin modification and have recently identified a Sox9 transcriptional co-activator, whose distribution is spatially and temporally restricted at the site of the chondrogenenic area in mouse embryos. This coactivator complex promotes chondrogenesis as well as Sox9-dependent gene expression. These data provide the first evidence that in addition to sequence-specific factors, developmental stage and tissue specific expression of co-activator tightly regulate cell differentiation and development as a novel molecular parameter. Elucidation of this pathway can reveal novel mechanisms that control cell differentiation and tissue development. This study will also provide new insight into arthritis pathogenesis and tissue engineering research.

I-3: BiP, the endoplasmic reticulum chaperone, as an immunomodulatory autoantigen in rheumatoid arthritis

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Introduction

The role of heat shock proteins (stress proteins) (HSP) or chaperonins (CN) in the pathogenesis of rheumatoid arthritis (RA) has long been investigated and has received intensive research scrutiny. There is, as yet, no consensus regarding the role of HSP or CN in the pathogenesis of the disease. This background review will focus on three areas.

Expression of HSP in the synovium

Extensive work has been carried out in many laboratories investigating the possibility that there is increased expression of HSP and CN in the RA synovial membrane (SM). These studies are difficult to compare because of the use of different methodologies. The studies can be grouped into those that show an increased expression and those that show no increase when compared either to osteoarthritic (OA) or normal synovial membrane. Indeed, many of these studies have assumed that the osteoarthritic synovial membrane is a reasonable control for the RA SM. However, this may be unwarranted as the OA synovial is under stress and frequently the material is obtained at joint replacement in which there is destruction of the joint and inflammatory changes as well as evidence of reperfusion injury which would increase the expression of HSP and CN. Increased expression is relevant since the assumption is made that through increased expression there will be increased antigenic drive, thus stimulating the pathogenic T-cells and contributing to the antibody responses to HSP and CN.

T-cell responses

The underlying assumption is that molecular mimicry between bacterial HSP and host HSP and CN could drive an autoimmune response against the latter. There is no doubt that there are robust T-cell responses, both proliferative and by the secretion of cytokines, by human T-cells to bacterial HSP. This phenomenon immediately introduces a complication into work involving human HSP. There are two sources for human HSP. The first is HSP extracted from human tissues or produced in mammalian expression systems. The second source is mammalian HSP expressed in bacterial and especially *E. coli* expression systems. Proteins prepared from bacterial expression systems may be contaminated not only by endotoxin but also with bacterial HSP such as GroEL. Thus lymphocyte responses may be the result of a response to the contaminating bacterial protein rather than to the mammalian HSP. This has undoubtedly been a major confounding factor in this work. Indeed, the consensus now appears to be that responses to bacterial HSP activate TH1 T-cells while there is increasing evidence that mammalian homologues of bacterial HSP activate predominantly a TH2 immune response.

B-cell responses

The same problem of specificity arises when looking at antibody responses to bacterial HSP and their mammalian or human homologues. Here, there is a consensus that at least a subset of patients with RA have elevated levels of antibodies to human HSP, particularly human HSP 60. There is a suggestion that patients with such elevations are more likely to have severe and/or active ongoing rheumatoid arthritis.

The role of BiP

BiP is an endoplasmic reticulum CN. It was originally recognized because it is the primary CN involved in the correct folding of heavy and light chains to form the complete immunoglobulin molecule. We and the Burmester group in Berlin have shown that patients with rheumatoid arthritis have anti-BiP responses both in terms of antibodies and T-cells. We have gone on to show that BiP cannot induce arthritis in a number of mouse and rat strains. It is thus possible that BiP may have a role in the pathogenesis of rheumatoid arthritis which is not pathogenic. We have shown that BiP given intravenously to DBA/1 or HLADR1 transgenic mice will prevent the development of collagen-induced arthritis. Subsequent work has shown that BiP given either intravenously or subcutaneously will treat ongoing collagen-induced arthritis in these two mouse strains. The mechanism of action of BiP in this system is presently unknown. It is unlikely to be due to sequence or conformational similarity between BiP and collagen. In work using human peripheral blood mononuclear cells we have shown that BiP will stimulate the secretion of large amounts of interleukin-10 from human peripheral blood monocytes. This could be one of its mechanisms in preventing and treating collagen-induced arthritis in mice. We have also shown that BiP will induce the generation of CD4 or CD8 T-cells that have a TH2 or anti-inflammatory cytokine profile and which in vitro, can downregulate immune T-cell responses. Thus BiP could be involved in regulating immune-mediated inflammation, as happens in the joint, by at least two distinct processes. We have shown that there is cell-free BiP within the rheumatoid synovial fluid so that these mechanisms could be occurring in the patients with rheumatoid but at a level insufficient to switch off the synovitis. Clearly BiP is a promising and interesting candidate for the immunotherapy of rheumatoid arthritis.

I-4: Proteomic surveillance of autoimmunity in osteoarthritis

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Objective: To understand immunological aspects of osteoarthritis (OA), which has been considered a degenerative disease, we compared profiles of autoimmunity comprehensively between OA and rheumatoid arthritis (RA) by using analysis of chondrocytes proteome.
Methods: Proteins extracted from normal articular chondrocytes were separated by 2-dimensional electrophoresis (2DE). Then western blotting (WB) was used to detect antigenic protein spots, using 20 serum samples from either OA or RA patients. Then mass fingerprinting was used for identification of the detected autoantigens. The identified proteins were prepared as recombinant fusion proteins with maltose binding protein (TPI-MBP) to confirm their antigenicity and investigate frequency of the autoantibodies, epitope localization, and clinical significance by ELISA and WB, using serum samples obtained from 93 patients with OA, 54 patients with RA, and 43 patients with SLE.
Results: Sixty-two autoantigens were detected in responses to OA and RA serum samples, in which 19 protein spots were detected

only in the OA group. One such apparently OA-specific protein spot, detected in 4 out of 20 OA but not in RA, was identified as human triose phosphate isomerase (TPI). All four positive sera against this spot reacted to a fusion protein of TPI-MBP but not MBP alone. Also TPI-MBP affinity-purified antibodies from these sera only reacted to the spot which was identified as TPI in the 2-DE membrane. The frequency of the anti-TPI IgG in the sera from OA, RA, and SLE patients, was 24.5%, 5.6%, and 4.7%, respectively. Further frequency of the autoantibody in synovial fluid from 29 OA and 19 RA patients was found to be 24.1% and 0%, respectively. All the positive samples were further confirmed by western blotting. In the epitope mapping using 8 truncated recombinant TPI proteins, multiple epitopes were identified, one of which was recognized in more than 90% of the positive serum samples. Clinically, X-ray grading was lower in the anti-TPI positive OA group than in anti-TPI negative one.

Conclusion: The overall profile of autoimmunity in OA differs from that in RA, which may reflect OA-specific pathological roles of autoimmunity. The autoantibodies to TPI, detected in OA predominantly and produced by the antigen-driven mechanism, would have potential as a diagnostic marker for OA.

I-5: Synoviolin, an E3 ubiquitin ligase, as a novel pathogenic factor for arthropathy

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Rheumatoid arthritis (RA) is one of the most critical articular diseases, with synovial hyperplasia followed by impairment of quality of life. However, the mechanism(s) that regulates synovial cell outgrowth is not fully understood. To clarify its mechanism(s), we carried out immunoscreening by using antirheumatoid synovial cell antibody and identified and cloned Synoviolin, an E3 ubiquitin ligase. Synoviolin was highly expressed in the rheumatoid synovium, and mice overexpressing this enzyme developed spontaneous arthropathy. Conversely, synoviolin+/− mice were resistant to collagen-induced arthritis by enhanced apoptosis of synovial cells. We conclude that Synoviolin is a novel causative factor for arthropathy by triggering synovial cell outgrowth through its antiapoptotic effects. Our findings provide a new pathogenetic model of RA and suggest that Synoviolin could be targeted as a therapeutic strategy for RA.

II: Pros and Cons in Anti-cytokine Therapy for RA

II-1: Anti-TNF therapy: pros and cons

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Experimental analysis revealed that TNF is at the apex of a pro-inflammatory pre-destructive cascade of importance not only in rheumatoid arthritis but also in other chronic inflammatory diseases. This pathway is operative even in later-stage disease and so provides therapy for patients resistant to all other therapy. The benefit is in signs, symptoms, physical functions, joint protection, and promotion of joint repair. In early patients (e.g., 1st year) there is evidence that remissions can be induced in a significant percentage. In late patients (e.g., 10 years) there are 30% or so poor and nonresponders to TNF blockade. Approaches to maximize the benefit of anti-TNF will be discussed.

II-2: The pros and cons of cytokine inhibition for RA

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The inflammatory process plays a critical role in diseases such as rheumatoid arthritis (RA), psoriatic arthritis (PA), ankylosing spondylitis (AS), Crohn's disease, and scleroderma (PSS). Over the last 10 years, the development of specific cytokine antagonists has greatly improved the outcome of patients with RA.

The beneficial effects of agents that inhibit macrophage-derived cytokines have demonstrated the role of the macrophage in the pathogenesis of the inflammatory disorders. The TNF blockers etanercept, infliximab, and adalimumab, as well as the interleukin-1 receptor antagonist anakinra, have been successful at improving the signs and symptoms of RA with ACR 20 responses in some case of over 70%. The TNF blockers are approved for inhibiting the progression of joint destruction. Infliximab inhibits both erosions and joint space narrowing at 2 years in established RA (ATTRACT Trial) and at 1 year in early RA (ASPIRE Trial). Similarly, etanercept inhibits radiologic progression in early RA (ERA Trial) and adalimumab is effective in established RA. These agents have had dramatic effects on the quality of life for patients with RA.

Etanercept is also approved for the treatment of psoriatic arthritis and ankylosing spondylitis (AS). Trials with infliximab demonstrate efficacy in both of these diseases. Similarly, both look promising for reactive arthritis and may be beneficial in PSS as data from acute and chronic graft versus host disease (GVHD) demonstrated benefit with infliximab. One difference between these two agents has been their effectiveness in Crohn's disease and ulcerative colitis, where only infliximab has been effective.

With all cytokine inhibitors, the primary toxicity appears to be infections. For the TNF blockers, the incidence of infections is not increased, but the severity may be increased with patients needing to be screened for tuberculosis and other occult fungal infections. The incidence of pneumonia is increased with anakinra. While animal arthritis data show excellent results by blocking both TNF and IL-1, the human data suggest an increased incidence of serious infections.

The FDA addressed the issue of lymphoma with the TNF blockers in March 2003. Patients with severe RA are more likely to get lymphoma, and these are the patients who would receive the most aggressive agents such as the TNF blockers. The FDA concluded to continue to observe closely this rare but serious disease.

In order to effectively evaluate the side effects of a group of agents, one also needs to evaluate the side effects of the disease, in this case RA. As of 1994, patients with RA were still dying younger, unchanged from 1955. The causes of death are heart disease, infections, and lymphoma. Only early intensive therapy (Saw-tooth with continuous DMARDs or methotrexate) has demonstrated a change in this "ultimate" outcome.

To date, over 700000 patients have been treated with the TNF blockers. Together, fewer than 600 patients have developed TB or lymphoma, with far fewer dying as a result of these diseases. Recent data suggest the remission rates with TNF blockers in RA approach 40% (ASPIRE and TEMPO Trials), far greater than the

previous rates of 5%. Therefore, the benefits of such cytokine therapy far outweigh the potential risks, most of which can be readily monitored in the clinic.

II-3: Pros and cons of anti-TNF therapy for RA

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Treatment of RA in the 21st century is now directed towards trying to reach complete remission. The rational for aggressive therapy is that the side effects of RA are severe for the patient, including severe functional decline and early mortality. RA progresses rapidly in the first few years and few patients go into spontaneous remission. For this reason, there have been a number of new therapies discovered whose goal is to obtain better control of disease. The most effective of these are the TNF-blocking agents.

Pro: In the clinical trials which have assessed the clinical efficacy of traditional DMARDs (MTX, sulfasalazin, leflunomide, hydroxychloroquine, etc.), the best ACR 20 seen have not been as robust as those seen with anti-TNF therapy, especially when the anti-TNF was started early in disease and given in combination with aggressively dosed MTX. Similarly, combination DMARDs have not reached the same degree of clinical response as the combination of anti-TNF and aggressively dosed MTX. In addition, the depth of response (proportion of patients reaching ACR 50 and 70 as well as EULAR remission) is clearly much higher in patients treated with anti-TNF agents and aggressively dosed MTX as is improvement in patient function measured by decreased HAQ and prevention of X-ray progression measured by Sharp scores. Anti-TNF agents are tolerated for a much longer period of time than traditional DMARDs, probably because of their better efficacy as well as the fact that they do not cause significant organ damage (hematologic, hepatic, pulmonary, renal, etc.) when compared with traditional DMARDs

Con: In the United States, the cost of MTX is less than $1000 per year while the anti-TNFs can range from $16 000 to more than $50 000 per year. Increased incidence of tuberculosis has been seen with all anti-TNF agents compared with RA patients. Most of these reports have occurred post-marketing and have occurred with infliximab rather than with etanercept. The cases with infliximab occur within the first 6 weeks, suggesting reactivation of latent tuberculosis infection, while most of the reports with etanercept have occurred after months of therapy. With both agents, however, most of the infections are extra-pulmonary. Fortunately, however, with proper assessment of risk of latent tuberculosis, this problem can be avoided so that either of these medications can be utilized successfully, as recently demonstrated in Spain. The situation is not as clear with other opportunistic infections which cannot be screened for as easily, but the numbers, fortunately, are rather small. There is no increased incidence of malignancy with either agent. Lymphoma does occur more frequently than in the non-RA population with both agents, but it is not yet clear whether lymphoma occurs more frequently with these agents than in the RA population, whether treated with these agents or not. Neither of these agents should be used in patients with class III or IV congestive heart failure, with demyelinating diseases, or with chronic infections. Both agents should be temporarily discontinued if the patient develops a serious infection.

In conclusion, anti-TNF therapy, especially in combination with MTX, is the most likely therapy to provide major clinical response, improve patient function and slow X-ray progression without producing major organ damage or annoying side effects. The cost of medication as well as untreated or latent Tbc without proper therapy is a concern as well as class III and IV CHF, chronic infection, and demyelinating disease.

II-4: A closer look on joint destruction

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II-5: Effect of conventional DMARDs and anti-cytokine therapy on radiographs in RA: What did we learn?

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Structural damage as assessed on conventional radiographs is an important outcome measure in clinical trials with antirheumatic treatment. There are several sensitive scoring methods available to quantify the progression over time. These are the Larsen and the Sharp scoring methods with several modifications and they have been used in clinical trials performed since the eighties. Applying these methods in various observational cohorts have shown that the annual progression rate is between 1.6% and 1.9% of the maximal scoring range of the scoring methods. This gives an annual progression rate around 3.5 units if assessed by the (modified) Larsen method, around 5 if assessed by the Sharp method, and around 7.5 if assessed by the Sharp–van der Heijde modified method. This can serve as background information to judge the progression rate in the various trials.

However, the actual numbers of the various trials cannot be compared across trials. By comparing one trial arm with a trial arm from another trial, the randomization is lost. This means that the prognostic similarity can no longer be ensured. Also the patient characteristics might be quite different. Another problem is that this way patients with early disease are being compared with patients with long-standing disease, or patients who are being treated with the first DMARD, with patients who already failed many DMARDs. All these factors make a direct comparison unjustified. Other complicating factors are that there are differences in trial designs. Some trials use a parallel design with a start of a certain DMARD in all patients (e.g. MTX) and other trials use a nonresponder design with patients nonresponding to the drug (e.g. MTX) and thereafter continue with the same drug either with placebo or another intervention added. The two MTX arms from these trials are by definition completely incomparable. Other difficulties in comparing numbers from different trials arise from the differences in radiographic scoring. This applies to the scoring method used, the readers involved, and the order in which the films are being scored. Although there is some comparability between the scoring methods, the scores are based on different abnormalities and have various scoring ranges (maximum score ranges from 190 to 448). It is well known that readers have their own levels of reading. This is not important if trial arms within a trial are being compared, but it is if you compare data across trials. Research has also shown that if films are read in pairs with known chronology, the assessed progression rate is higher, compared to the situation in which this chronology is unknown.

Given all these limitations of comparing the actual numbers across trials, we can still reach a few conclusions from the data we have seen from recent trials. In general, most of the older trials with conventional DMARDs are scored with known chronology of

the films and the recent trials with anti-cytokine therapy all with unknown chronology. Nevertheless, there is a general impression that the progression rate seen under conventional DMARDs is higher as compared to anti-cytokine therapy. Treatment with sulfasalazine, MTX, cyclosporine, leflunomide, intramuscular gold, and corticosteroids have all been shown to retard radiographic progression. It is also apparent from recent trials that high doses of MTX (about 20–25 mg weekly) are very effective in reduction of radiographic progression. Moreover, these high doses are tolerated well and cause relatively low safety issues. A general trend that can be deduced from the various trials is that combination therapy is often more effective than single drug therapy. This can be seen for example in the COBRA trial and the FIN-RACO trial.

The anti-cytokine treatment with infliximab, etanercept, and adalimumab are very effective in reducing radiographic progression. This has been defined in patients with early and late disease, in patients who start with this therapy as the first drug, and in patients who already failed treatment with MTX before. However, the relative efficacy of these various anti-cytokine treatments cannot be given. On a group level, the progression rate can be brought back (close) to zero in most of the trials. Also more trials are presenting negative progression scores. It still needs to be determined to what extent this also indicates the possibility of repair of already existing damage. In one of the trials it was also clear that the parallel start of MTX and etanercept provide stronger inhibition of radiographic damage compared to either MTX or etanercept alone.

For clinical practice it will be important to compare various treatment strategies. For example, should we start with single drugs and change in case of inefficacy, or add other drugs? Should we start with a combination from the onset? Should anti-cytokine treatment be included immediately? The first data on comparing treatment strategies are available and show that the start with a combination (with or without anti-cytokine therapy) is more effective as compared to single and step-up therapy. More data, especially with longer follow-up, are needed to be able to draw firm conclusions.

II-6: Do we need new treatment principles for RA patients beyond anti-TNF medication?

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Over the past decade significant progress has been made with regard to more effective treatment principles of RA patients, using biologics blocking TNF-alpha. However, with the available TNF-alpha antagonists, only 60%–65% of patients respond. Furthermore, a majority of so-called responders show improvement only to a limited extent. Therefore, it appears obvious that new targets and the effects of their blockade will not only give more insight into disease-specific pathogenic mechanisms, but might also help to develop further and even more specific targeted therapies.

Patient-tailored treatment could include combination therapy rolling over therapeutic devices or the definition of new therapeutic targets. With regard to combination therapy, combination of cytokine antagonists with DMARDs and combination of different cytokine antagonists with cytokine antagonists and/or anti-inflammatory cytokines will be introduced into clinical practice.

Rolling over therapeutic strategies imply the switch from one TNF-alpha antagonist to another. Data from open trials suggest that patients not responding to one anti-TNFalpha medication might benefit from the switch to another.

New targets have been defined within the innate immune system as well as the adaptive immune system. Within the innate immune system chemokines and chemokine receptors are presently being explored as targets for new medications in RA patients. Within the adoptive immune system, T cells, regulatory T cells, and a shift from the Th1 to the Th2 subsets are currently being explored for anti-T cell-directed medications. Also B cells using an anti-CD20-monoclonal antibody are used in treating RA patients and patients suffering from other rheumatic diseases. In addition, monoclonal antibodies as well as fusion proteins are already being employed for treating RA patients using different cytokines or adhesion molecules as targets.

Looking at novel treatment principles for rheumatic diseases, anti-IL15 and anti-IL18 principles will soon reach clinical practice. The same is true for the blockade of co-stimulating molecules such as CTLA V Ig. Also adhesion molecule blocking principles will soon be routinely used for treating chronic inflammatory diseases.

Beside the necessity for the development of new treatment modalities for RA patients, of equal importance is the development of tools that will enable us to separate responders from so-called nonresponders for a given targeted therapy.

III: New Frontier Drug Therapy for OA

III-1: Identification of susceptibility gene(s) for osteoarthritis

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OA (osteoarthrosis, or osteoarthritis; MIM 165720) is a crippling disease characterized by loss of articular cartilage and formation of osteophytes. OA is a major problem in current and future society: More than 7 million patients are suffering from the disorder in Japan and the incidence increases with age. OA causes pain, deformity, and loss of joint function, leading to a decrease of activity of daily life and quality of life in elderly people. Thus, OA is certain to be a serious problem in the coming "old-aged" society. However, currently there is no fundamental treatment for OA, because its pathogenesis and etiology are unclear.

As for the etiology of OA, the presence of genetic factors has been implicated for more than 60 years and is supported by recent large cohort and genetic studies. OA is a polygenic disease controlled by genetic and environmental factors. Recent advances in molecular genetics and high-throughput technology have enabled us to tackle genetic factors of common diseases including OA. Identification of its susceptibility genes (the genetic factor) will open a new path for better treatment. The genes must be hidden somewhere in the genome.

To identify the susceptibility genes, there are two approaches. One is the knowledge-based approach (candidate gene approach), and the other is the position-based approach (genome scan). The knowledge-based approach starts from a priori knowledge, or information on the disease. Based on this knowledge, one reasons and determines a candidate and examines its association with a particular disease. The position-based approach (genome scan) is a statistical method that uses genetic markers and examines transmission of the markers in health and disease. Infrastructures for the genome-wide study are rapidly and extensively being constructed through international collaboration, including a high-throughput genotyping system and database for the human genome sequence, SNP, and haplotype.

We are employing both approaches for OA: For a knowledge-based approach, we are using the skeletal dysplasia, a group of heritable, monogenic diseases affecting the skeleton. It is a genetic

model of common bone and joint diseases. There are many skeletal dysplasias complicated by OA. These diseases are nothing but severe, early-onset, genetic forms of OA. Then, disease genes of these diseases are already proven to cause OA in vivo, in humans. Therefore, these genes are good candidates for idiopathic, common, polygenic OA. For a position-based approach, we are performing a genome-wide association study for hip and knee OAs by screening for 100000 SNPs (single nucleotide polymorphisms) on the genome. We are adopting the step-wise screening strategy to reduce labor, cost, and time. With these approaches, we have identified several promising candidate genes for OA.

III-2: COX-2 regulation in synoviocytes of patients from rheumatoid arthritis

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Prostanoid, as in prostaglandins (PGs) and thromboxane (Tx), is implicated in the pathogenesis of rheumatoid arthritis (RA). Nonsteroidal antiinflammatory drugs (NSAIDs) are thought to achieve their antiinflammatory efficacy by inhibition of PG production, especially PGE_2, which induces joint destruction in RA patients by inflammatory effects such as increased vasopermeability, production of metalloproteinases, proliferation of synovial tissues, and angiogenesis. Moreover, PGE_2 has the effects of bone resorption by the induction of RANKL into osteoblasts and differentiation or activation of osteoclasts.

Cyclooxygenase (COX), the first enzyme of the pathway in which arachidonic acid is oxidized to PGs, is usually described as the rate-limiting step in PG production. COX is found in two isoforms: COX-1, which is expressed constitutively in all cells, and COX-2, which is inducible following an inflammatory or mitogenic stimulus. COX-2-derived PGs involve inflammation, angiogenesis, and carcinogenesis. COX-1-derived PGs involve homeostasis such as gastroprotection, inhibition of gastric acid secretion, and regulation of renal blood flow.

There are several selective COX-2 inhibitors known as the "coxibs". Inflammatory cytokines such as TNF-α and IL-1 induce COX-2 in synoviocytes as well as LPS. COX-2 is continuously upregulated in RA synovia including synovial lining cell layers, inflammatory monocytes, fibroblast-like cells, and vascular endothelial cells without down-regulation of COX-2 by glucocorticoid. COX-2 is rarely expressed in OA or normal synovia. COX-2 is a biochemical correlate of disease activity in RA patients and adjuvant-induced arthritis in rats. COX-2 inhibitors such as celecoxib, rofecoxib, and meloxicam are demonstrated to convey the same therapeutic benefit of traditional NSAIDs with significantly less gastrointestinal toxicity due to inhibition of COX-1.

COX-2 inhibition by celecoxib, glucocorticoid, and T-614 induces the suppression of joint destruction in adjuvant-induced arthritis in rats. COX-2 antisense DNA and 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15-d-PGJ_2) , a ligand of PPAR-γ, also suppress the joint destruction of adjuvant-induced rats by inhibition of PGE_2 production. COX-2 antisense DNA and 15-d-PGJ_2 induce apoptosis into synoviocytes as well as celecoxib. Auranofin and NF-κB antisense suppress PGE_2 production by inhibition of NF-κB binding to COX-2 promotor.

Sphingosine-1-phosphate (S-1-P), a lipid mediator, is a ligand of edg-1 and is implicated in angiogenesis and carcinogenesis. S-1-P induces the elevation of PGE_2 production by IL-1β stimulation in RA synoviocytes, but induces apoptosis in high concentration of S-1-P.

Upregulation of COX-2 in synoviocytes plays a role in the pathogenesis of RA.

III-3: Glucosamine sulfate as a structure-modifying agent in osteoarthritis

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From the perspective of the laboratory, recent studies have shown that glucosamine sulfate (GS) antagonizes IL-1b mediated responses possibly through reducing NF-κB activation.

We examined the potential structure-modifying effects of orally administered glucosamine sulfate in 6-month-old New Zealand white rabbits following medial hemimeniscectomy (Moskowitz model). Cartilage was nearly normal by both gross and microscopic examination for those animals receiving GS 100 or 200 mg/day per os. Immunohistochemistry revealed a dose-response reduction in MMP-1 and MMP-3 in the GS-treated animals. Substrate gel analysis revealed increased MMP-2 and MMP-9 in all operated groups. MMP-3 was less elevated in a dose response in the GS groups than the OA group. RT-PCR revealed no difference in MMP-1, MMP-3, and MMP-13 in all the operated groups.

Although most of the sulfate is hydrolyzed from glucosamine before absorption, some GS is absorbed intact. Independently, the hydrolyzed sulfate appears to have an independent beneficial effect on cartilage. Once in the system, GS penetrates synovium and cartilage.

There have been more than 10 clinical trials supporting a symptom (mostly pain)-modifying role for GS in OA. In addition, two nearly identical 3-year clinical trials demonstrated less progression of joint space narrowing in OA of the knee than in a control population. One of the trials has provided further support for their conclusion with a 5-year followup.

Data from preclinical and clinical studies support a role for orally administered GS as a structure-modifying agent for OA.

III-4: Disease modification in osteoarthritis: What is the goal and what is the evidence?

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Available treatments for osteoarthritis (OA) focus on decreasing pain and improving function. When this fails, destroyed joints can be replaced by arthroplasty with good long-term outcome. However, we still lack reliable means of intervening in the OA disease process in order to stop or slow down the gradual destruction of articular cartilage and other joint tissues that is a hallmark of this disease. We could summarize the current situation such that we now (a) treat the pain and improve the function, and (b) replace the destroyed joint when needed. However, being able to (c) detect cartilage loss before it is too severe and prevent further loss or (d) help grow back lost cartilage largely eludes us.

The few long-term studies available on the natural history of OA suggest that many patients with mild or moderate OA do not progress much in their disease, but remain on the same level of pain and function for years, or even in some cases improve. For

these patients, pharmacological prevention of joint breakdown may not be a relevant treatment choice.

On the other hand, there is enough progressive OA around to generate a yearly rate of more than a million joint replacements for OA in the world. In addition, several studies have highlighted the risk of severe OA at a young age following joint injuries. These young patients with OA have high expectations of physical activity and ability to work, while the surgeon is reluctant to replace their failed joint due to the significantly increased risk of implant wear and loosening in these young patients. These facts provide a strong rationale for treatments that would stop or slow joint destruction in OA, and provide relevant benefit for the patients.

There is evidence that pharmacological modification of joint breakdown in OA is a reality in animal models. There are also early results from human clinical trials, which suggest that pharmacological modification of joint destruction in osteoarthritis may be possible. However, most of the agents tested so far have been either ineffective or associated with significant side effects, or both. OA is a chronic but not life-threatening disease. The acceptance level for side effects of disease-modifying pharmacological treatment of OA will likely be low.

A major problem facing the introduction of any disease-modifying OA therapy is the lack of reliable and convenient outcome measures to document changes in joint structure, function, or metabolism resulting from the treatment. It may even be argued that until such measures are developed, it will be difficult to prove the efficacy of even the most promising drug candidate. The indirect measurement of joint cartilage thickness by plain radiography as a clinically relevant outcome is yet unproven in the context of the clinical trial. MRI shows promise as an alternative method to monitor joint structure but is equally unvalidated in regard to its relationship with clinical outcome. Biomarkers for OA are being developed and show promise, but for lack of an agent with proven disease modifying effect, it remains difficult to validate biomarkers for OA as outcome measures.

Some consensus has been reached on clinical trial methodology to evaluate structure modification. However, it remains to be proven that structure modification such as slowing or reversal of joint structural change in OA actually translates into a relevant and measurable patient benefit, and with which delay, if any. Such benefit could be improvement as determined by patient-relevant outcome questionnaires, health-related quality of life, and/or a delay of need for joint replacement.

III-5: The role of cleavage of type II collagen by collagenases in cartilage destruction in osteoarthritis

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Damage to type II collagen, because of its enhanced denaturation and cleavage by collagenase, are principal features of the articular cartilage matrix breakdown in both osteoarthritis (OA) and rheumatoid arthritis (RA).

Recently there has been increased interest in trying to measure matrix remodeling and pathologic changes in arthritis involving bone and cartilage in vivo. Many of these bone and cartilage matrix molecules, or their degradation products as well as C2C, are released from cartilage and bone and can be detected biochemically and immunologically in body fluids. An example of the value of this biomarker approach has come from the study of the excessive resorption of bone seen in osteoporosis. The cleavage of type II collagen by collagenases is a critical step in degradation of type II collagen. Recently we developed a new monoclonal antibody to detect and quantify the cleavage neoepitope of type II collagen by collagenases (C2C). We demonstrated that its release into culture media, synovial fluid, and serum was increased on induction of degradation in explant cultures and in experimental arthritis. We measured the serum levels of C2C in RA patients and showed that it may reflect RA disease activity more precisely, compared to CRP, MMP-3, and COMP. In the therapeutically uncontrolled group, elevated levels of C2C suggested severe and prolonged cartilage damage based on our observations in experimental models. A reduction of serum levels of C2C in the well–control group suggested that treatment (mainly with methotrexate) could prevent cartilage damage.

Aging and sex are important factors of the progression of cartilage damage in OA. We showed that the serum content of the collagenase-generated cleavage epitope in type II collagen also changes with age, sex, and menopause in healthy persons. The changes in serum levels of C2C may reflect changes in normal cartilage metabolism that may precede the onset of OA. Mechanical stress also plays important roles in cartilage degradation. Rupture of the anterior cruciate ligament (ACL) causes instability or abnormal loading on the knee and results in OA. We analyzed synovial fluid of patients with an injured ACL and compared it to the macroscopic damage of cartilage and menisci. Furthermore, we prepared a torn-ACL rabbit model. In this model, serum levels of C2C were strongly associated with cartilage damage and progression of OA. Interestingly, we found excessive athletic stress (running 42 km) caused increased urine levels of C2C in another study.

C2C may reflect enzymatic cartilage matrix degradation by chondrocytes and mechanical damage of collagen molecules cleaved by collagenases. C2C could be useful as a tool monitoring for cartilage degradation in OA. And a specific inhibitor of collagenase could be a therapeutic target. Loss of aggrecan is suggested to occur earlier than damage of collagen in OA. Aggrecan protects the collagen network. Therefore, inhibitor aggrecans also could have an effect in treatment of OA.

IV: Up-to-date Indications in Rheuma-Surgery

IV-1: Locking compression plating (LCP): a new solution for fractures in rheumatoid patients

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In China, rheumatoid arthritis affects about 0.32%–0.34% of the population, which means more than 4.1 million people. Osteoporosis is a well-known phenomenon in RA. Unlike postmenopausal osteoporosis, it is characterized by relatively preserved bone mass in the axial bone and marked loss in the peripheral bone. It was found that the frequency of osteoporosis in RA can be increased twofold compared with the reference population.

At the same time, osteoporosis increases the risk of fracture in RA patients; the rate of fracture can be as high as 2.7%. It is reported that RA patients accounted for 5.6% of all hip fractures, which increased threefold compared with the normal population. Several factors, including the inflammatory process itself, treatment with glucocorticoids, and physical inactivity have been suggested as being implicated in the etiology.

Osteoporosis makes the treatment of fracture in rheumatoid patients more challenging. The osteoclast cell was proved to have a crucial role in RA in inducing structural joint damage (erosion)

and high-turnover osteoporosis. The latter may lead to difficulty in fracture fixation and delay of fracture union. The risks, such as secondary loss of reduction or fixation failure, are also increased in this group of patients.

The locking compression plate (LCP) is designed under the concept of "internal fixator," which provides a new solution for fixation in osteoporotic fracture. The fixed angle between the screw and plate increases the pull-out strength of the system, and the stability of the fixation system no longer totally depends on the quality of the bone. The other benefits of this system include no accurate plate contouring required, protection of local blood supply, and supporting minimal invasive plate osteosynthesis.

IV-2: Simultaneous bilateral total knee replacement for patients with rheumatoid arthritis

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In rheumatoid arthritis and osteoarthritis, not only pain caused by destructive change of bilateral knee joints but severe walking disability will also occur by deformity, limitation of motion, and instability, to which replacement arthroplasty of bilateral knee joints is applied. In cases of bilateral TKA, even though one knee joint is surgically treated, walking disability remains because the other is impaired, and rehabilitation cannot be adequately performed. We have performed simultaneous TKA on both knees at an interval of 20–30 min since April 1984. There have been a number of studies on simultaneous bilateral TKA, and generally there are no differences in the results between unilateral and bilateral replacement.

We report the application of simultaneous bilateral TKA, its surgical procedures, postoperative results, and advantages and disadvantages of this method.

Application of simultaneous bilateral TKA is possible under the following conditions: (1) patients with pain in the bilateral knees, walking disability, loss of joint space, deformity, or bone deficiency observed on an A-P radiogram of one knee in the standing position, and severe limitation of motion, in whom TKA can be applied to both knee joints, (2) those without severe complications, such as severe dysfunction of the heart, lungs, and kidneys, or severe anemia at an Hb level of less than 8.0 mg/dl, (3) those wishing or consenting to undergo simultaneous bilateral TKA, (4) those motivated to improve dysfunction in the lower limbs, to relieve pain in the knee joints in particular, and to improve walking disability, (5) cooperation of four or more orthopedists including two orthopedists who have experience in performing TKA, and (6) cooperation with an anesthesiologist.

Simultaneous bilateral TKA is not indicated for patients under the following conditions: (1) sufficient understanding of replacement of bilateral knee joints is impossible, (2) severe complications in the heart, lungs, and kidneys, (3) systemic infection, (4) uncontrollable diabetes, (5) infected knees, and (6) severe obesity (over 100 kg).

Surgery is started using a tourniquet by two surgeons on both sides and one nurse. Surgical treatment is first performed in the more severe knee joint, and after osteotomy of the femur on that side is completed, surgery of the other side is started. TKA is performed on each side at intervals of about 20 min. To prevent hemorrhage, hemostasis is sufficiently performed during surgery and is treated by the drain-clamp method after surgery.

We performed simultaneous bilateral TKA in 1048 knee joints of 524 patients between January 1984 and December 2002. Since July 1995, surgery has been performed using the same type of FNK developed in our department in 982 knee joints of 491 patients, which was 59% of all 865 patients (1356 knee joints) in whom FNK was used. The mean age at the time of surgery was 69.8 years (26–90 years), and the disorders consisted of RA in 102 patients (20.8%) and OA in 389 patients (79.2%). The patients consisted of 62 men (12.6%) and 429 women (87.4%). The mean follow-up observation period was 4 years and 3 months.

Postoperative results did not differ from those of unilateral replacement, and good results were obtained in both OA and RA patients. Knee function was markedly improved. The incidence of complications did not differ from that after unilateral replacement, and no complications causing problems were noted with this method. Whenever possible, 400 ml blood was preoperatively collected for autotransfusion, and homologous transfusion was performed in 14 patients (3%), indicating that the transfusion avoidance rate was 97%.

The advantages of simultaneous bilateral TKA are: (1) requires only one surgery and anesthesia, (2) reduces the admission period, (3) requires only one postoperative rehabilitation, (4) is economical due to single anesthesia, surgery, and postoperative rehabilition during an admission period, (5) uses the same surgical procedures on both sides, and (6) reduces the surgery time.

The disadvantages of this method are: (1) is more invasive due to surgery on both sides, (2) there is a slightly larger amount of bleeding than that with unilateral replacement, (3) requires four or more orthopedists and an anesthesiologist, and (4) there is risk of infection due to the difficulty in keeping the surgery table organized if the surgeons are not well experienced.

We concluded that simultaneous bilateral TKA is a beneficial procedure for patients with destructive bilateral knees in RA and OA.

IV-3: Indication of mobile-type total knee arthroplasty for rheumatoid arthritis

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Introduction

TKA is a successful procedure for the relief of pain and restoration of walking ability. However, the range of motion after TKA is not satisfactory for an Asian lifestyle. Recently, there have been many studies on the improvement of the range of motion after TKA in Japan. In 1999, Dr. Insall developed a new total knee system, LPS-Flex, with the Zimmer corporation. This artificial knee joint has thicker posterior condyles, and has a polyethylene mobile bearing to prevent patellar tendon impingement. It allows an enlarged flexion angle, with a maximum of 150°. This prosthesis has a mobile bearing, but it does not have a mechanism to prevent bearing lift-off. Strict control of soft tissue balance is required for this TKA system. The decision to use this TKA is made during the operation according to the soft-tissue balance. This study has focused on the indication of the mobile-type TKA for rheumatoid arthritis.

Operation procedure

The distal femur is osteotomized at 6° valgus and the proximal tibia is osteotomized perpendicularly. Medial soft tissue is released to achieve a rectangular extension gap using a tension meter. Then a flexion gap equal to the extension gap is made by cutting the posterior condyles of the femur using a tension meter. This procedure enables creation of a gap of the same size in extension and

flexion, conceptually. However, it is difficult to achieve a perfect soft-tissue balance. The mobile-type TKA was not used in cases without a perfect balance and/or an interchangeability between femoral and tibial components.

Patients and method

Between January 2000 and December 2002, 86 knees in 75 patients underwent total knee arthroplasty. (RA: 29 knees in 24 patients, OA: 57 knees in 51 patients.) Average age of patients was 68 years. Twenty patients were men and 55 were women. In addition to the ordinary clinical results, the relationships between the types of TKA and preoperative alignment were examined.

Results

The mobile-type implants were used in 19 knees. In RA, 5 knees underwent mobile-type implants (17%), while 14 knees (25%) underwent mobile-type implants in OA. The improvement of alignment was from 183.1° ± 7.3° to 173.1° ± 2.2° in FTA of OA. In RA, the change of FTA between pre- and postoperation was small. (FTA was about 173°.) In 31 knees with over 185° in FTA (RA: 2 knees, OA: 29 knees), the mobile-type implants were used in 9 knees, all of which were OA.

Discussion

Deformity of knees is not extreme in most cases of RA. However, the usage rate of mobile-type implants for RA is lower than that for OA. It was found that a good soft-tissue balance of the osteoarthritis knee could be achieved even when the deformity was extreme. The conclusion is that the indication of mobile-type implants does not depend on the degree of deformity, but on the laxity of the joint due to RA.

IV-4: Orthopedic surgery in rheumatoid arthritis

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The history of operative treatment of disorders caused by rheumatoid arthritis is short and hardly more than 50 years old. One of the pioneer institutes to develop orthopedic surgery for RA is definitely the Hospital of Rheumatoid Arthritis in Heinola, Finland. This hospital was constructed in the middle of a rural pine forest, near big lakes, and in the period just after the Second World War. This landscape was considered healthy for the RA patient. Hundreds of foreign orthopedic surgeons have been trained during short or long visits to Heinola. Gradually such training has changed the attitudes and treatment protocols, and today operations for RA disorders can skillfully be performed in most major orthopedic centers. One of the messages learned from Heinola is that cooperation between the orthopedic surgeon, the rheumatologist, and rehabilitation professionals is essential. The main goals are to retain or restore mobility and to treat pain and inflammation.

For the rheumatoid patient, one of the most handicap-causing problems involves motility of the hand. In fact, the training of an orthopedic surgeon specializing in RA treatment must include good training in hand surgery. Such training includes learning several techniques to perform synovectomies, tendon transpositions, arthrodeses, and today, increasingly, total joint replacements, e.g., of the MCP joints. Here I want to point out the Finnish innovation, where biodegradable MCP prostheses are succesfully used. The implant gradually vanishes and is replaced by a fibrous and well-functioning pseudojoint. More than 200 patients are currently being followed up in Finland and the results are very promising.

Probably the most difficult joint to treat surgically in the RA patient is the elbow. For a long time, the Vainio arthroplasty, developed in Heinola and which includes interposition of skin to replace the destroyed cartilage, was, additionally to routine synovectomies, the state-of-the-art operation. Now for some time, development of elbow joint replacements has been successful and especially the Kudo arthroplasty is popular worldwide. Also for the shoulder, the Neer prosthesis and recent modifications have become popular.

My personal role in the development of surgery for RA was during a couple of decades focused on the cervical spine. For the RA patient, severe cervical spine disorders are often terrible and massively reduce the quality of life. As is commonly known today, most patients with long-lasting and severe RA develop atlantoaxial subluxation of some degree. The worst forms of it can lead to cranial subluxation of the odontoid or also posterior subluxations. Subaxial subluxations must also be recognized and the subluxation can be combined with intraspinal formation of pannus and synovitis of the facet joints. Recent literature provides advice for operative treatment of all the different categories of rheumatoid cervical spine disorders. Because of good education, one hardly sees anymore the most serious forms of cervical disorders. Also, modern fixation techniques usually allow immediate mobility.

For any orthopedic surgeon, treatment of RA destruction of the hip or knee joint is familiar. Maybe the most recent development has been to increasingly perform bilateral replacements to reduce the total load for the patient.

Similarly to the hand, the foot and ankle are common sites for RA destruction. Very good results are achieved by arthrodesis of the subtalar joints. The case with the talocrural joint is more difficult. After several previous failures, the most recent prostheses show good 3- to 5-year results. At the current stage we are waiting for more long-term experiences of the TC arthroplasties.

IV-5: Current surgical indication for ulnar drift in the rheumatoid patient

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Although ulnar drift of the MP joint is common in the rheumatoid patient, the etiologies of the deformity are not well understood. Anatomical structures, gravity, mechanical stress from the thumb, longitudinal malalignment, and synovitis of the MP joints have all been thought to cause of the deformity. Ulnar deformities vary in severity, and mild to severe ulnar deviation of the MP joints can be associated with various degrees of extension loss with/without volar subluxation of the MP joints. While development of the deformity is different in each case, volar subluxation of the MP joints and intrinsic contractures tend to increase the rate of associated swan-neck deformity.

Treatment of ulnar drift differs depending on the severity of the deformity. Synovectomy (if required) and extensor centralization are the basic procedures, and suture or reconstruction of the radial collateral ligaments is an option when ulnar deviation is severe. Ulnar intrinsic release is necessary if intrinsic contracture and/or swan-neck deformity are combined. Crossed intrinsic transfer may also be added for severe ulnar deviation. The choice of arthro-

plasty of the MP joint is determined by whether subluxation of the joint is reducible or not. When the joints are reducible after volar release, a reefing procedure of the dorsal capsule to maintain the reduced position as described by Wood is used. Resection of the metacarpal head is required for irreducible joints. Volar plate arthroplasty (Tupper's) is one of the choices, but long-term outcomes are unpredictable. Silicone implants have been used for 20 years and remain indicated for relatively elderly patients. Cementless surface implants (Self-Locking Finger Joint, SLFJ) have been developed and used for 5 years. Although further follow-up is required, the results so far have been satisfactory, and no major complications have been found in over 50 patients. We therefore use the SLFJ for younger patients.

Since recurrence of the deformity and/or progressive stiffness are the major concerns in long-term outcomes for ulnar drift, every chosen procedure must be carefully evaluated. Soft-tissue reconstruction should be chosen especially for young patients. If needed, arthroplasty can be done later on. Some recurrence of the deformity may occur, but results have been improved with meticulous soft-tissue reconstruction and postoperative therapy. Reconstruction of the radial collateral ligament in all joints, complete release of ulnar intrinsic muscles in the little finger, and postoperative alignment of the wrist are considered to be the most important factors to prevent recurrence. Suturing the long extensor to the basal phalanx and suturing the flexor pulley in good alignment are used in selective cases to improve stability and correct deformity. Our current indication and techniques for various degree of ulnar drift will be presented.

V: Regulation of Osteoporosis in Rheumatic Diseases

V-1: Osteoclast formation and bone loss in rheumatoid arthritis (RA)

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Understanding the factors causing pathological bone loss is essential in order to find treatments for osteolytic diseases. Joint destruction due to bone loss in the arthritic joint is prevalent in patients with severe rheumatoid arthritis (RA). We have investigated the molecular mechanisms that stimulate osteoclast formation and activation in this disease.

It is now clear that receptor activator for NFκB (RANK), its ligand, RANKL, and its natural antagonist, osteoprotegerin (OPG), appear to be central factors influencing physiological osteoclast formation. There is growing evidence that disregulation of RANKL and/or OPG can cause excessive osteoclast formation in a number of bone-loss pathologies. Additional cytokines, such as monocyte-colony stimulating factor (M-CSF), are also required for osteoclast formation, and inflammatory mediators, such as TNFα, can both induce RANKL and produce exaggerated responses to it. We have shown that RANK, RANKL, TNFα, and M-CSF, are all expressed abundantly by cells in the pannus tissues adjacent to osteolytic zones in RA. Using RT-PCR and in situ hybridization, we found that RANK, RANKL, and M-CSF were all expressed strongly in tissues from active RA disease, compared with osteoarthritic (OA) tissues used as controls. Higher ratios of RANKL:OPG were observed in the rheumatoid tissues compared with the controls. Furthermore, the propensity of cells isolated from pannus tissue to form active osteoclasts associated strongly with the RANKL:OPG mRNA ratio in those tissues.

OPG was primarily localized to endothelial cells in the joint tissues, and, interestingly, we found that active rheumatoid arthritis (RA) pannus tissue revealed a dramatic decrease in endothelial cell OPG protein content, compared with control tissue or similar tissue sampled from patients with inactive RA. Although the role of OPG in preventing osteoclast formation and activity is well documented, a potential role of OPG in endothelial cell survival has recently been described. We have now found that vascular endothelial cells in situ, and human umbilical vein endothelial cells (HUVEC) in vitro, express abundant OPG, co-localized with P-selectin, within the Weibel-Palade bodies. Treatment of HUVEC with the inflammatory cytokines, TNFα and IL-1β, resulted in a rapid secretion of OPG protein accompanied by a sustained increase in OPG mRNA and protein expression. Similar mobilization of OPG was observed after treatment of human osteoblasts with TNFα and IL-1β. Taken together, these results support a modulatory role for OPG in inflammation, which may have important consequences for signaling between vascular elements and osteoblasts, the net result of which is potentially pro-osteoclastogenic. In support of this notion, in investigating the effects of inflammatory cytokines on the phenotype of human osteoblasts we have found a dramatic increase in proliferation and a delay of maturation of these cells. We have recently shown that it is the immature fraction of human osteoblasts in which RANKL can be induced by agents such as 1,25(OH)2vitamin D. These potential mechanisms for increased osteoclast activity in RA, uncovered from cell and tissue studies, will be discussed in some detail.

V-2: Mechanisms of bone destruction in experimental arthritis

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Bone erosion is a crucial element of chronic destructive arthritis, and understanding of mechanisms and mediators involved in this process may provide novel therapeutic options.

There is no doubt that RANKL is a pivotal mediator in bone erosion in a range of murine arthritis models. The cytokines TNF and IL-1 play an important role in upregulation of RANKL, and stimulation of osteoclast maturation and activation occurs in the inflamed synovial tissue, in particular at sites close to the bone. In fact, RANK expression demonstrated a gradient pattern with increased numbers of RANK-positive cells in areas close to periosteum and cortical bone, shortly after onset of experimental murine collagen arthritis. This coincided with RANKL expression at similar sites. The pattern of bone erosion sites includes synovial activation, but also marginal zones of bone marrow cavities as well as transport caniculi between marrow and synovium.

Apart from TNF and IL-1, Th1 cell derived IL-17 is a major promoter of bone erosion, whereas Th2 derived IL-4 is a strong inhibitor. Effects of these cytokines translate in dramatic shifts in the balance of RANKL and OPG (osteoprotegerin), the natural inhibitor of RANKL. Therapeutic approaches with adenoviral overexpression of OPG reveal that both systemic and local treatment with OPG markedly suppresses bone erosion in various forms of murine arthritis models.

Intriguingly, OPG treatment not only reduced bone erosion, but also markedly inhibited cartilage erosion in collagen arthritis, ap-

parently through impressive downregulation of synovial activation and local levels of destructive cytokines. Although chondrocytes express RANK, a catabolic effect of RANKL on these cells is not clear, and a direct blocking effect of OPG on this RANKL-RANK interaction is unlikely.

V-3: Pro-inflammatory cytokines and osteoporosis in rheumatoid arthritis

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Osteoporosis is a major unsolved problem in rheumatoid arthritis (RA). The skeletal complications of RA consist of focal bone erosions and juxtaarticular osteopenia at sites of active inflammation and generalized bone loss with reduced bone mass. The mechanism of bone loss in these conditions remains unclear, but previous studies have indicated that osteoclasts play a crucial role in the development of not only focal bone erosions but also juxtaarticular and generalized osteoporosis. Furthermore, experimental data also suggest that pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), interleukin 1 (IL-1) and interleukin 6 (IL-6) are important in the pathogenesis of osteoporosis in RA.

Osteoclasts are highly specialized multinucleated cells which are uniquely capable of lacunar bone resorption. Mononuclear osteoclast precursors are bone marrow-derived cells which circulate in the monocyte fraction and differentiate into functional osteoclasts at the bone surface. Osteoclast formation requires the presence of macrophage colony-stimulating factor (M-CSF) and involves an interaction between the receptor activator for nuclear factor-B (RANK), expressed on osteoclast precursors, and RANK ligand (RANKL), which is expressed by several cell types, including osteoblasts. Both TNF-α and IL-1 have been shown to regulate RANKL and osteoprotegerin (OPG), the decoy receptor for RANKL, gene expression in osteoblastic cells, and are strongly synergized with RANKL. It has also recently been reported that TNF-α can directly stimulate osteoclast differentiation in mouse bone marrow cell cultures.

Using human monocytes, we have shown that in the presence of M-CSF, TNF-α stimulates differentiation of osteoclast progenitors into mature osteoclasts, and IL-1 acts directly on osteoclasts to increase the bone-resorbing capacity of these cells. Recently we have reported that the other pro-inflammatory cytokine, IL-6, is also sufficient for inducing human osteoclast differentiation from circulating precursors by a process which is distinct from the RANK/RANKL signalling pathway. Osteoclastogenesis induced by pro-inflammatory cytokines was inhibited by the addition of dexamethasone but not by osteoprotegerin. These findings suggest that pro-inflammatory cytokines play a pivotal role in pathogenesis of pathological bone resorption not only in local erosions but also in generalized osteoporosis.

Biologic agents aimed at inhibiting the pro-inflammatory cytokines which influence osteoclastogenesis are under development. Among various therapies for generalized osteoporosis in RA, anti-cytokine therapy is promising.

V-4: Regulation of synovial fibroblast function by adenovirus vector-mediated gene transduction

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Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown etiology characterized by invasive synovial hyperplasia leading to progressive joint destruction. Rheumatoid synovial cells not only are morphologically characterized by their transformed appearance but also are phenotypically transformed to proliferate abnormally. They invade bone and cartilage by producing an elevated amount of proinflammatory cytokines and metalloproteinases and by inducing differentiation and activation of osteoclasts, multinucleated cells exclusively responsible for bone resorption. On the other hand, regarding catabolic action of synovium in RA, there is accumulating evidence that synovial tissues contain multipotent cells with chondrogenic potential, which might be involved in the repair process of articular cartilage defects and therefore provide a good source for engineering articular cartilage. In the present study, we examined the effect of adenovirus vector-mediated gene transduction in regulating SF function.

Small GTPase Ras, the protein product of proto-oncogene ras, is ubiquitously found in eukaryotic organisms. Ras is known to function as a downstream effector of cell surface receptor tyrosine kinases and leads to activation of mitogen-activated kinase (MAPK) pathways, which in turn regulates the activities of nuclear transcription factors and gene transcriptions. Although increased expression and mutations of Ras in RA synovial tissue have been reported, the function of Ras in RA pathology remains to be clarified. We utilized a replication-deficient adenovirus vector carrying the dominant negative mutant of *ras* gene (Ras^{DN}) to investigate the role of Ras in RA synovial fibroblasts (SFs) in vitro and in vivo. Adenovirus-mediated overexpression of Ras^{DN} dramatically decreased the proliferation rate of SFs and inhibited IL-1-induced MAPK activation and IL-6 production. Importantly, injection of Ras^{DN} virus into ankle joints of adjuvant arthritis rats not only ameliorated the inflammatory reactions but also suppressed bone destruction in arthritic joints.

We next examined the role of TGF-b/BMP pathways in SFs. Adenovirus vector-mediated expression of constitutively active activin receptor-like kinase 3 ($ALK3^{CA}$), also known as BMP type IA receptor, induced chondrocyte-specific gene expression such as type II procollagen and aggrecan in SFs cultured in pellets, in which both Smad and p38 pathways are essential. Similar chondrogenic phenotypes were also observed in SF pellets transplanted into nude mice. To analyze the downstream of ALK3 signaling, we utilized adenovirus vectors carrying either Smad1 to stimulate Smad pathways or constitutively active MKK6 ($MKK6^{CA}$) to activate p38 pathways. Smad1 expression had a synergistic effect on $ALK3^{CA}$, while activating pMAP kinase pathways alone by transducing $MKK6^{CA}$ accelerated the terminal chondrocytic differentiation, leading to type X collagen expression and enhanced mineralization. Overexpression of Smad1 prevented $MKK6^{CA}$-induced type X collagen expression and maintained type II collagen expression. In osteoarthritis model mice, activated p38 expression was detected in osteochondrophytes and marginal synovial cells.

In conclusion, suppression of the catabolic action of SFs by inhibiting Ras activity and promoting their chondrogenic

differentiation by stimulating ALK3 pathways can be a good therapeutic strategy for regulating arthritic bone and joint destruction.

V-5: The coupling mechanism of bone resorption and formation

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The discovery of RANKL elucidates the mechanism of osteoclast differentiation and function regulated by osteoblasts. Osteoprotegerin (OPG), a soluble decoy receptor of RANKL, inhibits both differentiation and function of osteoclasts. OPG-deficient (OPG-/-) mice exhibited severe osteoporosis caused by enhanced osteoclastic bone resorption. Deficiency of OPG in humans has been shown to result in juvenile Paget's disease. The previous morphological study showed that osteoblastic bone formation was activated in OPG-/- mice. Blood alkaline phosphatase activity of OPG-/- mice was about four times as high as that of wild-type mice. These results suggest that osteoclastic bone resorption coincidentally induces osteoblastic bone formation by an unknown factor (called coupling factor). Using a bone morphometry technique, we explored whether such a coupling factor is present in bone in OPG-/- mice. When bisphosphonate (risedronate) was injected into OPG-/- and wild-type mice every day for 30 days, bone resorption-related parameters were sharply decreased in both OPG-/- and wild-type mice. Treatment of OPG-/- mice with bisphosphonate induced complete disappearance of activated cuboidal osteoblasts which was often observed in untreated OPG-/- mice. All bone formation-related parameters as well as bone resorption-related parameters were significantly decreased in bisphosphonate-treated OPG-/- mice. Medication of bisphosphonate in OPG-/- mice decreased serum alkaline phosphatase activity to a level lower than that of wild-type mice. Serum levels of RANKL were markedly elevated in OPG-/- mice, but were unaffected by risedronate. We next investigated whether ectopic bone formation induced by BMP is also accelerated in OPG-/- mice. Collagen sponge disks containing bone morphogenetic protein-2 (rhBMP-2) were implanted into the dorsal muscle pouches in OPG-/- mice and wild-type mice, and bone mineral density (BMD) of the collagen sponge disks was determined every week for 3 weeks. No significant difference in BMD of the disc was observed between OPG-/- mice and wild-type mice. These results suggests that bone formation is accurately coupled with bone resorption at local sites in OPG-/- mice, and that serum RANKL levels do not reflect this coupling.

V-6: Isolation and characterization of osteoclast precursor cells from rheumatoid arthritis synovial fluid: two-step differentiation induced by nurse-like stromal cells and cytokines

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Objective

To elucidate the mechanism of joint destruction in patients with rheumatoid arthritis (RA), we conducted detection and characterization of osteoclast precursor cells (pOC) isolated from joints of patients with RA.

Methods

Joint-infiltrating cells were collected from joint fluids of RA patients under informed consent. The cells were cultured at 37°C in DMEM 10% FCS in 5%–7.5% CO_2 in air. After 2 to 3 weeks, CD14-positive mononuclear cells were collected and stained for tartrate-resistant acid phosphatase (TRAP) to confirm that the cells were pOC. The pOC were then purified with anti-CD14 monoclonal antibody, and were stimulated with various cytokines to find cytokines which can induce osteoclast (OC) differentiation. Differentiated OC were examined for bone-resorbing activity on bovine bone slices.

Results

The pOC were maintained for more than 6 months in DMEM 10% FCS in the presence of nurse-like stromal cells (RA-NSC) obtained simultaneously from joint fluids. The pOC were differentiated into multinucleated giant cells (MGC) in the presence of IL-3, IL-5, IL-7, GM-CSF, or RANKL in combination with M-CSF. The MGC expressed TRAP, carbonic anhydrase II, and calcitonin receptor, and formed resorption pits on bovine bone slices, indicating that they were mature osteoclasts (OC). Peripheral blood monocytes from healthy donors were also differentiated into pOC when they were cocultured with RA-NSC, suggesting that pOC in RA joint fluid were induced peripherally. Using the differential display method, we detected a novel gene that encodes a cell surface molecule specifically expressed in OC. Polyclonal antibodies specific to the protein-stained human and mouse OC differentiated in vivo and in vitro.

Discussion

We found that RA-NLC play an important role inducing pOC in joints of RA patients. In addition, to our knowledge, this is the first method for generation of human pOC in vitro. Regulation of bone resorption is to be studied with this method.

V-7: Role of signal transduction pathways in joint destruction

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Signal transduction pathways regulate cellular responses to stress and play a critical role in inflammation. The complexity and specificity of signaling mechanisms represent major hurdles for developing effective, safe therapeutic interventions that target specific molecules. One approach is to dissect the pathways methodically to determine their hierarchy in various cell types and diseases. This approach contributed to the identification and prioritization of specific kinases that regulate NF-κB and the mitogen-activated protein (MAP) kinase cascade as especially attractive targets. Al-

though significant issues remain with regard to the discovery of truly selective kinase inhibitors, the risks that accompany inhibition of fundamental signal transduction mechanisms can potentially be decreased by careful dissection of the pathways and rational target selection.

Careful dissection of the NF-κB pathway in RA has focused on a signal complex that includes two IkB kinases, IKK1 and IKK2. Both are constitutively expressed by synoviocytes, and IKK functional activity increases 10- to 20-fold within minutes of cytokine exposure. Using dominant negative and constitutively active adenoviral constructs, IKK2 was identified as the primary kinase for NF-κB activation in cytokine-stimulated synoviocytes. IKK2 blockade in vitro with a dominant negative adenoviral construct inhibits induction of IL-6, IL-8, and ICAM-1 after stimulation with IL-1 or TNF-α. Intra-articular decoy oligonucleotides or gene therapy using the dominant negative IKK2 adenovirus suppresses joint inflammation in rat models of inflammatory arthritis. More recently, selective IKK2 inhibitors have demonstrated efficacy in arthritis models.

The MAP kinases represent another attractive target for RA because they can regulate cell proliferation, apoptosis, cytokine expression, and metalloproteinase production. The three major MAP kinase families, c-Jun-N-terminal kinase (JNK), extracellular regulating kinase (ERK) and p38 kinase, differ in their substrate specificity and subsequent responses to stress depending on the cell type and the environmental influences. The MAP kinases regulate various genes via both transcriptional and post-transcriptional mechanisms. The upstream MAP kinase kinases (MAPKK) serve as regulators of MAP kinase activity by phosphorylating specific threonine and tyrosine residues. MAPKKs are, in turn, regulated, by MAPKK kinases (MAPKKK or MAP3K).

ERK, JNK, and p38 are expressed in synovial tissue, and the active phosphorylated forms can be detected by immmunohistochemistry and western blot analysis in RA. They are also expressed by cultured fibroblast-like synoviocytes, and treatment of cells with pro-inflammatory cytokines leads to rapid activation. Cytokine gene expression, especially IL-1 and TNF-α, is induced by p38 through both pre- and post-transcriptional mechanisms. One of the primary substrates for JNK is c-Jun, which can increase metalloproteinase gene expression. p38 and JNK inhibitors suppress joint destruction in several mouse and rat models of joint inflammation, perhaps due to a combination of effects on cytokine expression and a direct effect on metalloproteinase production.

Careful evaluation of the signaling networks in RA can identify key pathways and provide organizational structure. It is not yet possible to determine whether blockade of the targets identified by this approach will be safe and/or effective in chronic inflammatory diseases. However, the systematic dissection of signaling networks can at least contribute to the rational target selection for therapeutic interventions.