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ABSTRACTS

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What have we learnt from anti-TNF therapy about the pathogenesis and treatment of rheumatoid arthritis?

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Anti-TNF therapy of rheumatoid arthritis (RA) with specific monoclonal antibody and soluble TNF receptor fusion proteins is rapidly becoming established as an advance in clinical practice.

The focus of this presentation is two fold: firstly, to examine the clinical significance of the alterations in biological responses mediated by TNFα blockade by infliximab, a monoclonal anti-TNFα-specific antibody. Ongoing studies have demonstrated that important mechanisms of action include de-activation of the pro-inflammatory cytokine and chemokine cascades, as well as the vascular endothelium. Thus, anti-TNF therapy results in a reduction in the retention in joints of circulating leukocytes, reduction in angiogenesis, and a reduction in connective tissue matrix degradation.

The second intention of this presentation is to discuss the likely future indications of the place of anti-TNF therapy in the management of RA.

The antiinflammatory actions of methotrexate: adenosinergic therapy

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Methotrexate remains among the most effective and widely used second-line agents for the treatment of rheumatoid arthritis and other forms of inflammatory arthritis. Methotrexate was originally developed as a folate antagonist that prevents the *de novo* synthesis of purines by rapidly dividing cells and has remained a mainstay of anti-neoplastic therapy since the late 1940s. In contrast to the high doses used to treat malignancies, methotrexate is administered in very low doses for the treatment of inflammatory diseases. Moreover, folic acid or folinic acid are usually co-administered with methotrexate to prevent its toxicity without interfering with its therapeutic actions. Thus, it is unlikely that the inhibition of cellular proliferation by inhibition of *de novo* purine and pyrimidine biosynthesis is the primary mechanism of action of methotrexate in the treatment of inflammatory arthritis. Results of recent studies in our laboratory reveal an alternative mechanism; the antiinflammatory actions of methotrexate are mediated by increased adenosine release at inflamed sites. The high levels of adenosine present in inflammatory exudates after methotrexate treatment interact with specific adenosine receptors on inflammatory cells to dampen the inflammatory response. Our data also suggest that adenosine receptor antagonists such as theophylline and caffeine can reverse the antiinflammatory actions of methotrexate. Sulfasalazine possesses a similar mechanism of action in the treatment of inflammation when studied in both *in vitro* and *in vivo* systems. Alternative agents that promote adenosine release at inflamed sites may be useful for the treatment of inflammatory diseases such as rheumatoid arthritis.

The human endoplasmic reticulum molecular chaperone bip is an autoantigen for rheumatoid arthritis and prevents the induction of experimental arthritis

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Introduction

Several lines of evidence suggest that rheumatoid arthritis (RA) is a T cell driven disease:[1]

— Presence of activated T cells in the synovium.
— Response to anti-T cell therapies.
— The association of rheumatoid arthritis (RA) with HLA-DR4 suggests that the disease is driven by antigenic peptides stimulating disease-specific T cells. Hitherto the nature of the antigens responsible has been difficult to define but has included bacterial antigens[2] and several glycoproteins.[3–5] A number of possible autoantigens have been proposed but there is no consensus; Suggested autoantigens include: heat shock proteins, type II collagen, cartilage proteoglycan, p205, p68, and HC gp-39.

Characterization of BiP as the rheumatoid autoantigen

Total condylar knee replacement in patients who have RA leads to cessation of inflammation in that joint despite ongoing synovitis in other joints.[6] This is in contrast to radical synovectomy, which only leads to temporary relief of synovitis. From this observation, one may hypothesise that RA may be an organ-specific autoimmune disease with immune reactivity directed against chondrocyte-specific antigens. Consequently, chondrocyte/chondrosarcoma extracts were Western blotted with RA sera. A reactivity against a p78 protein was detected in approximately 30% of RA sera. MALDI mass spectroscopy unambiguously identified p78 as BiP, a member of the p70 human chaperonin superfamily. BiP, or glucose regulated protein (GRP)78, has some interesting properties:

— it is an endoplasmic reticulum chaperone;
— it chaperones nascent proteins as they emerge from the ER;
— it promotes correct folding of Ig molecules;
— it is found in apoptotic blebs where it may be involved in breaking tolerance;
— hyperimmunization of mice with Ro proteins leads to development of anti-BiP antibodies.

T cell proliferation to BiP in patients with RA

BiP was expressed in *E. coli* with a HIS tag and purified on a nickel column. T cell proliferation studies showed that 6 of 10 patients with RA and 1 of 13 disease controls showed reactivity of synovial fluid (SF) T cells to BiP. The corresponding reactivity by peripheral blood T cells was weak. Proliferating SF T cells did not produce interferon-γ, suggesting that the cells did not belong to the classical TH1 subset. Proliferation could be inhibited by the addition of anti-HLA-DR monoclonal antibody to the cultures. Of the 6 RA responders, 5 were HLA-DR4-positive, and one of these was DR4-homozygous. The one responding disease control was DR4-negative. It is of interest to note that one disease control subject was homozygous-DR4, but SF T cells showed no proliferation to BiP. The failure of disease control SF T cells to proliferate to BiP was not due to technical factors, as they proliferated to tuberculin PPD commonly used as a recall antigen in the United Kingdom.

The role of BiP in experimental arthritis

Several strains of mice, including HLA-DR4+/− and HLA-DRl+/+ transgenic mice, and of rats did not develop arthritis following immunization with BiP in Freund's complete adjuvant. However, DBA/1 mice with type II collagen (CIA) or pristane arthritis (PIA) had elevated levels of IgG anti-BiP antibodies in their serum. This was particularly so for the PIA mice. Finally, intravenous administration of BiP one week before attempted induction of CIA completely prevented arthritis both clinically and histologically.

Summary and conclusion

In summary, we provide evidence that BiP is an antigen preferentially stimulating T cells from RA SF thus fulfilling two important requirements for an autoantigen, i.e., disease and tissue specificity. Furthermore, from the preliminary animal experiments, BiP may have important immunotherapeutic potential for the treatment of RA.

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Retroviral sequences in the activation of synovial cells in rheumatoid arthritis (RA)

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Based on the upregulation of proto-oncogenes in RA synovium and the detection of hitherto unclassified (retro)-viral-like particles in the synovial fluid of patients with RA, our laboratory searches for the presence of retroviral sequences. The detection of novel sequences encoded by highly conserved retroviral gene sequences has been used to search for endogenous and/or exogenous retroviral sequences in the joint of patients with RA.

In this regard we developed a RT-PCR-assay to detect retroviral sequences in pellets of synovial fluid derived from

patients with RA and other arthritidies. Cell-free, ultracentrifuged pellets are digested with protease K and DNase, RNA extracted and reverse-transcribed (RT) into cDNA using a retroviral pol-specific YMDD primer. PCR products were cloned into a sequencing vector and analyzed by sequencing and GenBank searches.

Numerous sequences were obtained. A 206 bp sequence with 98% homology to the retrotransposable L1 was obtained. Using an anti-sense probe for this sequence a subpopulation of RA fibroblasts at sites of invasion could be detected. It was shown further that L1 retrotransposons in RA are related to genomic DNA hypomethylation and effect gene expression.

Immune regulation by co-stimulatory signaling

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Cells involved in immune reactions display a variety of surface molecules that are responsible for specific effector of recognition functions. Besides the molecules of primary importance in the recognition of antigen, such as the T cell receptor complex, several accessory molecules as well as various kinds of lymphokine have been demonstrated to play an important role of in regulating immune response. The recently accumulating knowledge about the structure and function of those molecules, which has been obtained from molecular and cellular biological studies, provided several new approaches to understand the immunological disturbance accompanied with various kinds of disease.

The most extensively characterized costimulatory molecules are CD28 and CTLA-4 on T cells, and their ligands CD80 and CD86 on professional APC. To clarify the role of CD80 and CD86 in various immune responses, we examined the effects of treatment with anti-CD80 mAb anti-CD86 mAb in the prevention of autoimmune disease by using an autoimmune model of a mouse and in the induction of allospecific tolerance in organ transplantation.

Additionally, we have been focussing on the contribution of OX40/OX40L interaction in T cell activation, especially using RA model. Based on the experiments of these animal models, cellular and molecular mechanisms of autoimmune disease will be discussed.

Immune reactivity in rheumatoid arthritis

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The pathogenesis of rheumatoid synovitis involves the complex interplay of cells that are intrinsic components of the synovium, and immune and inflammatory cells that have migrated from the blood stream. Unique features of activated resident cells of the synovium, including their expression of adhesion molecules, and production of cytokines and chemokines facilitate the entry, activation and survival of lymphocytes in the synovium. The unique activities of these cells foster immunologic activity within the synovial tissue that serves to amplify and intensify chronic tissue inflammation and damage to cartilage and bone. Although effector molecules produced by immune and inflammatory cells, including TNFα and IL-1, play a major role in inducing signs and symptoms of rheumatoid arthritis, understanding the specific molecules that regulate immune responsiveness in the synovium may provide additional targets that might be useful in controlling rheumatoid inflammation.

Signal transduction and transcription factors in rheumatoid arthritis

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The ability of transcription factors to bind DNA and modulate gene transcription is tightly regulated in normal cells. Specific intracellular signal transduction pathways regulate transcription factor activity through modification of key residues and domains within the transcription factor. These intracellular signal transduction systems transduce extracellular signals from the cell surface to the nucleus, where they are integrated at the level of transcription factor activity. The signaling pathways are highly diverse, yet display an extraordinary degree of specificity for a given transcription factor or transcription factor family. The identification and molecular cloning of multiple regulatory enzymes that control key steps of these signal transduction pathways have sparked a search for novel therapeutic agents.

NF-κB plays a key role in the expression of many genes central to the rheumatic diseases. Participation of this transcription factor in the expression of IL-1β in monocytes, and ICAM-1, TNFα and IL-6 in rheumatoid synoviocytes, has been demonstrated. Also, NF-κB regulates inducible expression of the cell adhesion molecules E-selectin, VCAM-1 and ICAM-1 in vascular endothelial cells, and NF-κB inhibition results in reduced leukocyte adhesion and transmigration. The efficacy of aspirin, gold compounds such as aurothioglucose, sulfasalazine and corticosteroids in the treatment of rheumatoid arthritis may be related to their inhibitory effects on NF-κB.

Intra-articular injection of adenoviral gene constructs holds potential as an efficient method for the delivery of therapeutic proteins in the treatment of rheumatoid arthritis. We have utilized this system to explore the effects of inhibiting the cytokine-activated IκB kinase IKK-2, a key

regulator of the nuclear transcription factor NF-κB. NF-κB regulates transcription of an array of inflammatory genes, and has been implicated in the pathogenesis of rheumatoid arthritis. We have previously reported that IKK-2 but not IKK-1, regulates NF-κB activation and inflammatory gene transcription in fibroblast-like synoviocytes from RA patients, in human monocytes, and in human CD4+ T lymphocytes. These cells are variably implicated in the pathogenesis of rheumatoid arthritis. In vitro infection of fibroblast-like synoviocytes with adenoviruses expressing wild-type IKK-2 (Ad.IKK-2 wt) led to spontaneous activation of NF-κB. Infection of the same cells with adenovirus encoding a kinase-inactive mutant of IKK-2 mutant (Ad. IKK-2-dn) led to potent inhibition of TNF-alpha-induced nuclear translocation of NF-κB.

Intraarticular gene transfer of IKK-2wt into the joints of normal rats resulted in signs and symptoms of arthritis, including significant paw swelling and histologic evidence of synovial inflammation. Increased IKK activity was detectable in the Ad.IKK-2wt-injected ankle joints, coincident with enhanced nuclear NF-κB DNA binding activity. Intraarticular gene transfer of Ad.IKK-2-dn significantly ameliorated the severity of adjuvant arthritis, as evidenced by decreased paw swelling. These effects were accompanied by a significant decrease in levels of nuclear NF-kB DNA binding activity in the joints of Ad.IKK-2-dn-treated animals. These data substantiate a key role for the IkB kinase IKK-2 in synovial inflammation. Intra-articular gene therapy to inhibit IKK-2 activity represents an attractive strategy for the treatment of severe rheumatoid arthritis.

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Werner syndrome: more than systemic sclerosis?

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Werner syndrome (WS), a natural model of human aging, is an autosomal recessively transmitted disease with a wide variety of premature aging phenotypes.

The gene responsible for this unique syndrome is RecQ type DNA/RNA helicase located at the short arm of chromosome 8p12. Since the first description of WS by Otto Werner in 1904, over 1200 cases have been reported worldwide; among these, 850 were of Japanese origin. As WS has been sometimes misdiagnosed as systemic sclerosis in Japan, the patients with WS manifest generalized skin sclerosis, skin ulcers, autoantibodies as well as early growth spurt failure, early graying of the hair, hoarseness, cataracts, diabetes mellitus, hypogonadism, osteoporosis, atherosclerosis, multiple cancers followed by death at an average of 47 years old. Although there are many overlapping phenotypes between WS and systemic sclerosis, the patients with WS do not show signs of severe lung fibrosis and esophageal dilatation. In contrast, the patients with systemic sclerosis usually do not have early gray hair, cataracts, hoarseness, diabetes mellitus, hypogonadism, athrosclerosis and multiple cancers. Although the function of the WS gene (RecQ helicase) is under investigation, I will review the clinical and biological aspects of WS in comparison with those observed in normal aging and systemic sclerosis.

Molecular characterization of RUNX2/PEBP2αA/CBFA1 essential for osteogenesis

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The transcription factor PEBP2/CBF is a heterodimer composed of α and β subunits. There are three α subunit genes, RUNX1/PEBP2αB/CBFA2, RUNX2/PEBP2αA/CBFA1, and RUNX3/PEBP2αC in mammals which are homologous to *Drosophila runt* and *lozenge*, required for segmentation and eye development, respectively. These genes encode a highly conserved region termed the Runt domain which is essential for binding to DNA and heterodimerization with the β subunit. Gene disruption studies revealed that RUNX1 is essential for hematopoiesis and RUNX2 is a global regulator of osteogenesis.

TGF-β and BMP are each recognized by specific receptor complexes which have serine/threonine kinase domains. Upon ligand binding, receptors transmit signals via Smads. Smads1, 5, and 8 are receptor-regulated smads (R-Smads) which are specifically activated through phosphorylation by BMP receptors, and Smads2 and 3 are R-Smads which are specifically activated by the TGF-β and activin receptors. When R-Smads are activated, they associate with Smad4, a common mediator Smad (Co-Smad), and translocate into the nucleus. We found that three Runx proteins form complexes with R-smads that act in TGF-β/activin pathways as well as those acting in BMP pathways. Since virtually all transcription factors shown to interact with Smads so far described do so with R-Smads specific to TGF-β only, PEBP2 seems to have a very special relationship with TGF-β superfamily.

We have identified several mutations in RUNX2 in Japanese cleidocranial dysplasia (CCD) patients. One of them, CCDαA376, truncates the C-terminal region of RUNX2, including a part of the transcription activation domain, but leaving the Runt domain intact. CCDαA376 fails to interacts with R-Smads and to respond to TGF-β and BMP signaling. We propose that CCD is caused by haplo-insufficiency of the ability of RUNX2 to transmit TGF-β/BMP signaling to target genes.

We have evidence that TGF-β and BMP induce *de novo* synthesis of Runx proteins at the transcriptional level. At least some of the varieties of biological activities elicited by TGF-β and BMP appear to be mediated by coordinated actions of TGF-β/BMP-induced RUNX proteins and TGF-β/BMP-activated Smads.

Cathepsin K and pycnodysostosis

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Pycnodysostosis (Pycno) is a rare sclerosing skeletal dysplasia inherited as an autosomal recessive trait. Features include short stature, osteosclerosis, and numerous abnormalities of the cranial and facial bones. Using a positional cloning/candidacy approach, we identified the disease gene as the lysosomal cysteine protease, cathepsin K. This protease has substantial collagenase activity, is present at high levels in osteoclasts, and is secreted into the subosteoclastic space where bone matrix is degraded. Molecular heterogeneity of genetic lesions causing Pycno was documented with mutation analysis on 21 Pycno families. Thirteen mutations have been identified, including three nonsense, a stop codon (X330W), and nine missense mutations. Missense mutations have occurred in the signal peptide, pro region, and mature form of the cathepsin K prepropeptide. Molecular modeling of the first pro region missense mutation in a cysteine protease (G79E) indicated deformation of a conserved seven-residue motif; expression of G79E in *Pichia pastoris* resulted in an unstable precursor protein, consistent with misfolding of the proenzyme. Expression of five mature region missense defects revealed that G146R, A277E, A277V, and R312G precursors were unstable, and no mature proteins or protease activity were detected. Y212C precursor was activated to mature enzyme which retained its dipeptide substrate specificity and gelatinolytic activity, but had markedly decreased activity toward type I collagen and cathepsin K-specific tripeptide substrate, indicating that it was unable to bind collagen triple helix.

To determine the *in vivo* effects of cathepsin K mutations on bone metabolism in general and osteoclast-mediated bone resorption specifically, several bone metabolism markers were assayed in serum and urine from seven Pycno patients. Two markers that detect type I collagen telopeptide crosslinks from the *N*- and *C*-termini, NTx and CTx respectively, were low in Pycno. A third marker which detects a more proximal portion of the *C*-terminus of type I collagen in serum, ICTP, was elevated in Pycno, a seemingly paradoxical result. The finding of decreased osteoclast-mediated type I collagen degradation as well as the use of alternative collagen cleavage sites by other proteases, and the accumulation of larger *C*-terminal fragments containing the ICTP epitope, established a unique biochemical phenotype for Pycno.

In addition to documenting the critical role of cathepsin K in osteoclast-mediated bone resorption, ongoing studies are delineating a role for this protease in rheumatoid arthritis (RA). In situ hybridization analysis of revealed that cathepsin K message is upregulated in RA synovium, with expression mainly in synovial fibroblasts. *In vitro* biochemical enzymatic studies demonstrated that cathepsin K is capable of degrading insoluble collagen and intact cartilage, whereas cathepsin L and matrix metalloproteinase I had minimal degradative activity. Chondroitin-4-sulfate increased cathepsin K activity 200-fold by stabilizing the enzyme. Thus, data are accumulating implicating cathepsin K in the pathogenesis of RA.

The molecular mechanism of osteoclastogenesis: ODF/RANKL-dependent and independent pathways

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It is well established that osteoblasts and bone marrow stromal cells express osteoclast differentiation factor (ODF, also called RANKL) in response to several bone-resorbing factors to support osteoclast differentiation from their precursors. Osteoclast precursors which express RANK, a TNF receptor family member, recognize ODF/RANKL through cell-to-cell interaction with osteoblasts/stromal cells, and differentiate into osteoclasts in the presence of M-CSF. Osteoclastogenesis inhibitory factor (OCIF, also called OPG) acts as a decoy receptor for ODF/RANKL. ODF/RANKL is responsible for inducing not only differentiation, but also survival and activation of osteoclasts. IL-1α can be substituted for ODF/RANKL in inducing the survival and activation of osteoclasts.

More recently, we found that mouse TNFα strongly stimulated the differentiation of M-CSF-dependent bone marrow macrophages (M-BMMø) into osteoclasts in the presence of M-CSF without any help of osteoblasts/stromal cells. Osteoclast formation by TNFα was inhibited by respective antibodies against TNF receptor 1 (TNFR l) or TNFR 2, but not by OCIF/OPG. Experiments using M-BMMø prepared from TNFR1- or TNFR2-knockout mice showed that both TNFR1- and TNFR2-induced signals were important for osteoclast formation induced by TNFα. Osteoclasts induced by TNFα did not form resorption pits

on dentine slices, but they formed pits when IL-1α was added simultaneously.

These results demonstrate that TNFα directly acts on osteoclast progenitors and stimulates their differentiation into osteoclasts in the presence of M-CSF through a mechanism independent of the ODF/RANKL-RANK system. It is likely that physiological osteoclast formation is strictly regulated by the ODF/RANKL-RANK system. The TNFα-TNFRl/TNFR2 interaction may be involved in pathologically occurring bone resorption observed in inflammatory and metabolic bone diseases.

Osteoclast-like cell established from bone marrow blood in RA patients

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Hyperactivated immune responses are observed in joints of RA patients. Contribution of lymphocytes, granulocytes and macrophages for RA pathogenesis have been well investigated in past 10 years, however, the cells with a key role for joint/bone destruction are not defined. Osteoclasts were believed to be a professional cells for bone resorption solely, and osteoclast-like cells are observed in the synovial tissues of patients with RA suggesting activated bone resorption inside joints. Osteoclasts (osteoclast-like cells) are large, multinucleated cells, and are derived from pluripotent $CD34^+$ hematopoietic stem cells. Osteoclast progenitors are in the monocyte/macrophage lineage, and differentiated into mononuclear preosteoclasts. Mature osteoclasts are generated by the fusion of these preosteoclasts in close physical contact with bone marrow stromal cells. These bone marrow stromal cells facilitate osteoclastogenesis by producing soluble and membrane-associated factors. To elucidate the mechanisms for joint/bone destruction of RA, we examined whether osteoclast-like cells were also present in joint fluid and peripheral blood of RA patients.

First, we found that $CD14^+$ monocyte-like cells isolated from synovial fluid (SF-$CD14^+$ cells) of RA patients differentiated into osteoclast-like cells in the absence of accessory cells.[1] These osteoclast-like cells formed resorption pits on dentine slices, indicating that these cells were osteoclasts. SF-$CD14^+$ cells grew and were maintained in the presence of nurse-like stromal cells which were isolated simultaneously from RA synovial fluids. As described below, the nurse cells from synovia and bone marrows of RA patients sustained lymphocytes for a long period, and produced large amounts of cytokines.

The differentiation of SF-$CD14^+$ cells required interleukin (IL)-3, IL-7, and/or granulocyte–macrophage colony stimulating factor (GM-CSF). IL-5 and osteoclast-differentiating factor showed weak activity for the differentiation than those induced by IL-3, IL-7, and/or GM-CSF. On the other hand, IL-1, IL-4, IL-6, soluble IL-6 receptor, macrophage colony stimulating factor, tumor necrosis factor α, 1,25-dihydroxyvitamin D_3, or dexamethasone did not induce differentiation of SF-$CD14^+$ cells into osteoclasts in our experiments. These results suggest that there are abundant precursor of osteoclast(-like cells), and accessory cells are not essential in the final stage of osteoclast differentiation.

Further, we examined the origin of the SF-$CD14^+$ cells. We isolated $CD14^+$ cells from peripheral blood (PB-$CD14^+$ cells) of healthy volunteers. These PB-$CD14^+$ cells were defined as monocytes by the analysis of cell-surface markers, and were cocultured with RA synovial nurse cells. After 3–4 weeks, the PB-$CD14^+$ cells were examined whether differentiated into osteclast-like cells or not, by stimulating with various cytokines. PB-$CD14^+$ cells cocultured with RA synovial nurse cells differentiated into osteoclast-like cells by the stimulation with IL-3, IL-5, IL-7, and/or GM-CSF. The differentiated cells formed resorption pits as well as osteoclast-like cells from RA joints did. Few freshly isolated PB-$CD14^+$ cells differentiated into osteoclast-like cells, thus, close contact with RA nurse cells is essential for PB-$CD14^+$ cells for differentiation into osteoclast(-like cells) precursor.

We recently reported that there are nurse (or nurse-like) cells in RA synovia or bone marrow.[2–4] Nurse cells were first isolated from thymus and reported as mescenchymal cells with a unique characteristic, called pseudoemperipolesis. Pseudoemperipolesis is a phenomenon whereby lymphocytes crawl beneath the nurse cells following initial adhesion in vitro, and is believed to contribute for maturation, including positive/negative selection of thymocytes. Thus, it has been assumed that the nurse cells in RA synovia and bone marrows contribute to local immune responses and play an important role in the pathogenesis of RA. Furthermore, in this review, we provide the evidence for commitment of RA nurse cells for maintenance of activated osteoclast(-like cells) precursor. Peripheral blood monocytes recruited into synovia may be stimulated by RA nurse cells via close cell–cell contact, and differentiate into SF-$CD14^+$ cells (osteoclast precursor) in the joint. The SF-$CD14^+$ cells can differentiate into osteoclast-like cells with bone resorbing activity, and contribute joint/bone destruction in RA. Our findings will provide new insights into RA pathogenesis.

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Mechanisms of cartilage degradation

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Articular cartilage is the central tissue in the pathogenesis of osteoarthritis (OA), the major aging-associated musculoskeletal disorder. The major physiologic function of articular cartilage is to provide a surface for joint movement and to absorb mechanical stress. This is reflected in cartilage architecture and structure. Most of the molecular components of cartilage are involved in endowing the tissue with its biomechanical properties. Cartilage is devoid of anatomical structures such as blood vessels and nerve fibers. Cartilage also does not contain mononuclear phagocytes, and mature tissue appears to contain only differentiated chondrocytes but no mesenchymal stem cells.

Cumulative joint injury, age-associated matrix and cellular changes in context of individual disposition lead to the initiation of the OA process. This process is characterized by aberrant remodeling of the cartilage extracellular matrix with degradation of existing proteins and proteoglycans and the production of matrix components that are not part of normal cartilage. The outcome of this process is loss of cells and degradation of extracellular matrix, biomechanical dysfunction and the generation of pain as the major subjective symptom.

Cartilage degradation involves both loss of cells and extracellular matrix, phenomena which are mechanistically related. Chondrocyte survival is supported by cytokines, growth factors and extracellular matrix. Chondrocyte apoptosis is induced by specific cell death stimuli, by mechanical stress and oxidant stress. Chondrocyte apoptosis is a feature of human osteoarthritic cartilage and of tissue from animals with experimentally induced disease. Areas of cartilage that contain apoptotic cell are characterized by loss of proteoglycans. The potential value of preventing chondrocyte apoptosis (cytoprotection) to maintain tissue integrity (chondroprotection) will be demonstrated with cartilage cultures and animal models of cartilage injury.

The process of cartilage matrix degradation will be reviewed with respect to the extracellular stimuli, intracellular signals and effector molecules. Extracellular stimuli of matrix degradation are cytokines, degradation products of cartilage and mechanical stress. Signal transduction pathways activated by these distinct types of catabolic stimuli will be compared to identify critical intracellular events that can serve as targets for pharmacologic intervention. Glycosaminoglycan degrading lysosomal enzymes will be presented as a new class of effectors of cartilage destruction and therapeutic efficacy of specific inhibitors of these enzymes in models of cartilage degradation will be reported.

Causes and regulation of damage to articular cartilage in arthritis

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Understanding how articular cartilage is damaged and lost in arthritis has occupied the attention of many researchers in recent years. In this presentation new insights into this process will be presented focussing on damage to the collagen fibril in both inflammatory (rheumatoid) and degenerative (osteo) arthritis.

Recent work will be described which has identified new mechanisms that cause this damage and inhibitors that can prevent it. Moreover, new technology is being developed to detect damage in patients so that effects of treatment can be assessed more quickly and effectively.

Destruction mechanism of articular cartilage in collagen induced arthritis in monkey: comparison with that of RA patients

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In 1977, Trentham et al.[1] reported that chronic inflammatory arthritis develops in rats injected intradermally on the back with heterogenous or homologenous cartilage-derived Type II collagen and either CFA or ICFA. Histopathologic studies showed that the primary lesion provoked by Type II collagen was a chronic proliferative synovitis. Articular cartilage and bone destruction appeared to be sequelae of synovial inflammation. Mononuclear cells invaded and persisted in the synovium, suggesting that immune processes may be important in the pathogenesis of the disease. Since the characteristics of collagen-induced arthritis (CIA) resemble those of rheumatoid arthritis (RA), CIA was proposed to be an appropriate animal model for the human disease. However, there are difficulties to translate experimental findings, regarding the etiology of arthritis induced in rats or mice, to human disease states. Therefore, we developed CIA in monkey (a primate that is evolutionary closer to human than rodents, both anatomically and immunologically), and the clinical and histological investigations were performed.

The experimental animals consisted of 6 female crab-eating monkeys weighing 2.5 kg. Monkeys were injected intradermally in multiple sites on the back with 4 mg of bovine Type II collagen emulsified in CFA. Serum antibodies to collagen were detected and quantified by ELISA.[2] At necropsy, collected tissues were fixed in 4% paraformaldehyde, decalcified with EDTA, embedded in paraffin, stained with hematoxin and eosin, alcian blue and safranin

0. The joint tissues were also subjected to immunohistochemical analysis. A chronic inflammatory arthritis developed in 6 out of 6 animals (100%), around 4 weeks after immunization. Early-stage polyarthritis involved DIp, PIp, MCP and MTP joints of the upper and lower limbs in a symmetrical fashion. All monkeys with arthritis were found to develop IgM and IgG class antibodies to native Type II collagen at 2 and 4 weeks after immunization, respectively. Histological studies revealed remarkable hypertrophic changes in synovium 2 weeks after arthritis onset that occurred 4 weeks after immunization. Subsequently, fibrinoid necrosis accompanied by diffused granulating inflammation, and inflammatory cells infiltration into the pannus tissue, which extended into the bone and cartilage, were observed. Immunohistochemical analysis demonstrated significant 1L-1α and TNFα expression in both articular cartilage and subchondral bone 2 weeks after immunization. In contrast, these cytokines were not detected in synovial tissue. Following the onset of arthritis, TRAP positive multinucleated cells, osteoclastic cells, were found to migrate into the subchondral bone. The above results indicated that pathological changes in the subchondral bone and articular cartilage initiate arthritic joint destruction rather than inflammatory changes in synovial tissue, as it has been postulated before.

Parallel to the experimental observations described above, we have previously found that anti-Type II collagen IgG antibody appears in high incidence during the early phase of RA. The antibody was all negative in sera from patients with gout, osteoarthritis (OA) and non-arthritic diseases.[3] In order to impart an approach for understanding the pathogenesis of RA, we have performed histological and immunohistochemical studies on the arthritic joints of RA patients.[4] Although histologically, the surface of these cartilages appeared smooth and undamaged, immunohistochemical analysis showed less staining for Type II collagen and intense staining for CNBr-derived peptides of Type II collagen in the deep zone matrix than anywhere else. In contrast, the deep zone of articular cartilage from OA patients was stained with antibody against Type II collagen. A wholly different mechanism may be the bases of joint destruction in RA and OA. Type II collagen breakdown peptides in the deep zone of rheumatoid arthritic cartilage may become epitopes. Interestingly, there was a considerable formation of islands that invaded into the deep zone of articular cartilage through the calcified cartilage from below, where the subchondral bone was located. These islands were not in contact with the articular margins and contained a significant amount of TRAP positive multinucleated cells. Inflammation was also confirmed in the underlying subchondral bone, and CD68 positive mononuclear cells, MT-1 positive cells, and HLA-DR-positive cells were detected. The presence of T cells (MT-1 positive cells) may be crucial for anti-Type II collagen IgG antibody production in this region. Pathological changes described above may produce subchondral cysts that are not related to the synovial granulation tissue.

Thus, we propose the possibility that initiation of the rheumatoid process is triggered by the autoimmune reaction involving Type II collagen in the articular cartilage, as consequence of an unknown etiologic agent. Synovitis and other extra-articular features may be induced secondary to the immune complex formation in the subchondral area. In fact, a previous study showed that insoluble IgG antibody–antigen complexes are capable of initiating the release of inflammatory mediators from isolated macrophages in vivo.[5] There are some important clinical evidences to support our hypothesis, as below: (1) Even after surgical synovectomy, most cases develop varing degrees of recurrent synovitis with time, and the progression in joint destruction and deformity cannot be prevented. (2) Synovitis progression tends to gradually decrease when articular cartilage and bone are severely destroyed in the advanced stages. (3) Active sinovitis remarkably diminish after excision of articular cartilage and subchondral bone during prosthetic joint replacement or joint fusion even if the hypertrophid and inflamed synovium remains unremoved. If the proposed sequence of events is proven, it will be ideal to direct our focus to medicinal treatment that will suppress the invasion of osteoclastic cells and immunocytes into the subchondral bone rather than the inflammation of the synovial membrane.

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Novel paradigm in the pathogenesis and treatment of osteoarthritis

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Background

Osteoarthritis (OA) is one of the most frequent rheumatic diseases observed mainly in elderly people. The clinical findings of an inflammatory process such as joint swelling or synovial effusions in the affected joints are one of the most common clinical features in primary OA. Based on conventional paradigm, OA has been considered as degenerative joint disorder. However, the dominant clinical symptom has been characterized by a non-infectious chronic inflamma-

tory condition with infiltration of inflammatory cells in the synovial tissue or synovial fluid, especially in the early stage of the disease. The inflammatory process appeared to develop degeneration of chondrocytes and/or formation of osteophytes. In contrast with rheumatoid arthritis (RA), little attention has been paid to the inflammatory process of OA. However, several lines of evidence suggest the presence of inflammatory process, such as increased serum C-reactive protein levels, the presence of immune complexes in the superficial articular cartilage, and inflammatory cells in synovial immunohistochemical staining of synovial tissue with OA. Moreover, in the early stage of disease, presence of T-cell infiltration in the perivascular area, some of which were CD4 positive T cells. Recently, we identified the clonality of restricted TCR usage of Vβ chain by single-strand conformation polymorphism (SSCP) method on T-cell repertoire in OA synovium. These data strongly suggest the contribution of T cell impairment in the pathogenic process of OA.

Role of insult T cell in OA synovitis

Immunohistochemical analysis of primary osteoarthritis synovium revealed dominant infiltration mild or moderate infiltration of CD4 positive T cells. Immunophenotypical analysis of synovial T cell profile of activated T cell in tissue lesion has dominant to Th1 type disease in rheumatoid synovium as well. Among of the T cells, we identified the clonality of restricted TCR usage of Vβ chain by single strand in the SSCP image. As a result, mono or oligo positive bands were observed in some of the Vβ genes, whereas almost all Vβ genes from PBL were smeared. From the results of SSCP, we chose Vβ4–Vβ9 for further sequencing. The sequences of TCR Vβ CDR3 regions were analyzed. If at least two clones had an identical sequence, we defined the clone as dominant. Several dominant clones were found in each sample and common amino acid motifs in the CDR3 regions (LRGS, LAGG, LQG, GTG, GTS, GSA, QVG and TGG) were conserved in different samples. These data suggest that an antigen-driven immune reaction exist in the OA synovium with insult of T cell like as RA as well.

Novel animal model by cartilage-driven peptide

To identified T cell mediated osteoarthritis cartilage damage, we established animal models of osteoarthritis by cartilage-driven peptides YKL-39 and related peptides. As YKL-39 is a chrondrocytes secretory protein family, we seek whether immunity to YKL-39 induces arthritis in mice. YKL-39 was prepared as a fusion protein with maltose binding protein (MBP). To incomplete adjuvant (FLA) was injected into BALB/C mice. Control group was administered with the fusion partner, MBP (50 mg) conjugated with FLA. The mice were immunized by intradermal injection in the base of tail 10 days after the first immunization in the right footpad. Severity of arthritis was assessed by scoring each paw and by X-ray analysis. Fifty-three days after the first immunization, the hindfoot sections were analyzed histochemically by staining with hematoxylin and eosin. The titers of anti-YKL-39 antibody and anti-C II antibody were measured with the control group. By histochemical analysis, inflammatory cells were infiltrated synovial tissue. Moreover not only anti-YKL-39 antibody but also anti C II antibodies were detected in sera of the mice immunized with YKL-39. Accordingly immunity to YKL-39 lead to autoimmunity to C II, and these spreading of immunity may lead to severe arthritis. In conclusion, YKL-39 may be a novel arthritogenic nature. Furthermore, current our findings (Masuko-Hong K. et al.) YKL-39 and related peptides appeared to be excluding from apoptotic chrondrocytes.

Novel concept for treatment of OA by termed DMCD

Based on these findings, we postulated the clinical efficiency of several DMARDs or immunosuppose agents which are available in the treatment stratage. We proposed these drugs as a category of disease modifying cartilage destruction (DMCD). First of all, we have administrated 100 mg–200 mg of bucillamine to the 25 patients with early stage of OA as an open trial manner. Improvement of arthritis symptom has been recognized. Furthermore, we have evaluated several DMARDS as DMCD in OA. Based on these data, we have proposed a novel concept in the pathogenesis of OA by means of intrarticular immune response, especially, chondrocytes derived peptide.

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