ABSTRACT—Rheumatoid arthritis (RA) is a chronic inflammatory disease and its exact cause and pathophysiological process remain unclear. Fibroblast-like synoviocytes, macrophages and T lymphocytes are considered to be the major contributors in the pathophysiological process of RA; however, an increasing number of papers have drawn attention to the potential role of mast cells (MCs) in the process. In an animal model of RA, we reported an increase in MC numbers in the arthritic region, which agreed with the observation in human RA. In addition, a good correlation between the number of MCs and the development of disease was observed. However, there has been little experimental or clinical evidence of the beneficial effects of the modification of MC activity on the pathogenesis of RA and this is the weak point of the hypothesis. We therefore studied the effects of a MC-stabilizing compound, cromoglicate lisetil (CL), which is an orally deliverable prodrug of cromolyn sodium, on the RA disease model. The MC-stabilizer had efficacy in a mouse model. The beneficial effects of CL in this animal model further suggested the contribution of MCs in the pathophysiological process of RA. Concerning the contributive mechanism of MC on the pathogenesis of RA, our results using a disease model suggested that activation of MC chymase and matrix metalloproteinases might be involved. MC is now considered to be one of the targets of RA treatment.

Keywords: Mast cell, Rheumatoid arthritis, Collagen arthritis, Cromolyn, Matrix metalloproteinases

Introduction
Rheumatoid arthritis (RA) is an autoimmune disease in which persistent inflammatory synovitis, then cartilage destruction, bone erosions, and finally changes in joint integrity are observed (1). Genetic risk factors for RA such as HLA-DR4 alleles are known; however, they do not fully account for the incidence of RA, and environmental factors also play a role in the etiology of the disease (1). The exact cause and pathophysiological process of RA remain unclear.

Various drugs such as steroids, non-steroidal anti-inflammatory drugs, and disease-modifying anti-rheumatic drugs are prescribed for RA patients. However, all these drugs have the limitation of insufficient clinical remission, especially for joint damage, and considerable risk of adverse effects has been known (2, 3). Although combination therapies of agents have been developed (3), some RA patients are still not satisfactorily palliated. The development of novel drugs for RA from new aspects is desired.

Pathologically, fibroblast-like synoviocytes, macrophages and T lymphocytes have been considered to be the three major contributors among a number of cells thought to be involved in the initiation and development of RA (4) (Fig. 1). T lymphocyte is the predominant infiltrating cell in the rheumatoid synovium. Activated synoviocytes are particularly prominent in the lining layer and at the interface with bone and cartilage. Activated synoviocyte is considered as a kind of fibroblast and it is called the fibroblast-like synoviocyte (4). The rheumatoid synovium is characterized by the presence of a number of secreted products of activated T cells, macrophages and fibroblast-like synoviocytes.

Mast cells (MCs) are multifunctional cells of the immune system and the role of MCs in the acute inflammation process has been well established. In addition, an increasing body of evidence has drawn attention to the possible contribution of MCs in the pathophysiological process of RA (for review see refs. 5 and 6) (Fig. 1). In this review, we will discuss the role of MC on the pathogenesis of RA based on our recent results.

Mast cells in rheumatoid arthritis and its models
MCs are usually distributed throughout the normal
connective tissues including the synovium (5). Numerous potent proinflammatory and fibrogenic mediators and various cytokines are expressed in MCs and their release may affect the pathogenesis of RA (for review see refs. 6 and 7). Various studies have suggested the contribution of MCs on the process of chronic inflammation and matrix degradation involved in the pathogenesis of RA (8–15, for review see refs. 5–7). In a significant proportion of rheumatoid specimens, MC activation or degranulation has been demonstrated at sites of cartilage erosion (8, 9). Most quantitative studies have reported an increase in the number of MCs in the synovium and fluids from RA patient joints compared with that from normal joints (8, 10, 11), especially at sites of cartilage erosion (12). A positive correlation was noted between clinical parameters of disease activity and the number of MCs in the synovial tissue (10, 11). Clinical improvement with some drug therapy can be accompanied by a decrease in MC numbers (11). An increase in the levels of MC-derived mediators, including histamine, heparin, chymase, and tryptase, has been observed in the inflamed synovial fluid (9, 12, 13). Thus, an increase in MC numbers is considered to be a symptom of the disease, although degeneration of some MCs occurred in parallel during the pathological process and MC-derived mediators should be important for the process.

Collagen-induced arthritis (CIA) in experimental animals is a disease model of human RA (16, 17). Chronic joint inflammation was observed in bovine type II collagen (CII)-immunized mice and this model has also been demonstrated to share many characteristics with human RA (2, 17, 18).

We reported an increase in MC numbers in the arthritic region of the CII-immunized mice (19). Then, we compared CIA of 3 strains of rats among which the severity of arthritic response was different (20). The numbers of MCs in the arthritic region of the CII-immunized rats increased; however, those in the non-arthritic region of the same animals did not change. MC numbers in the arthritic region and arthritic scores showed a good correlation in the 3 strains of CII-immunized rats. Thus, similar changes in MC numbers in the arthritic region of the disease model and RA patients were observed.

**Mast cells as a target of rheumatoid arthritis treatment**

A possible role of MC in the pathophysiological processes of RA has been suggested as above. However, there has been little experimental or clinical evidence of the beneficial effects of the modification of MC activity on the pathogenesis of RA or its animal models and this is the weak point of the hypothesis. We therefore studied the effects of MC-stabilizing compounds on the RA disease model to clarify the role of MC in the pathogenesis of RA.

Cromolyn sodium is widely used for prophylactic treatment of allergic diseases as a prototype of MC-stabilizing compounds (21). However, it is hardly absorbed from the digestive tract. Then, an orally deliverable prodrug, cromoglicate lisetil (CL), was designed (22). So we studied the effects of orally applied CL on arthritis in CII-immunized mice (23).

CL was applied to CIA mice for 6 weeks, starting after the apparent occurrence of arthritis. The arthritis scores increased significantly in the non drug-treated CIA mice, whereas elevation of the scores was suppressed by CL-treatment, indicating the beneficial effects of the produg of cromolyn sodium, CL. The drug also improved the radiographic scores of phalangeal destruction. The decrease in the radiographic scores and pathohistological observations indicated the efficacy of CL on the improvement of joint destruction in CIA mice. Thus, CL evidently inhibited the arthritis development including phalangeal destruction. The increase of the number of MCs in the arthritic region of the non-treated CII-immunized mice was suppressed by CL-treatment (Fig. 2). Furthermore, we found a correlation between the reduction in MC numbers in the arthritic region and the improvement of arthritis by the drug.

Efficacious treatment with CL on CIA may support the hypothesis that MCs are important in the pathogenesis of the RA model. However, pemirolast, another MC-stabilizing drug, was not effective in this disease model, indicating that not all MC-stabilizing drugs may have the same anti-RA effect.

There is a possibility that cromolyn sodium affects other systems as well as MCs directly (for review, see ref. 24). For example, IgE synthesis in human B cells was blocked.
Mast Cells in Rheumatoid Arthritis

The pathophysiological role of MCs in RA is probably very complex and is just starting to be outlined. Because large amounts of various proinflammatory mediator substances and cytokines, such as tumor necrosis factor (TNF)-\(\alpha\), were produced and stored by MCs, they can release such compounds in response to cell activation (5, 9). Their activation affects the matrix turnover as well as the neighboring cells.

Possible mechanism of the contribution of mast cells on the pathogenesis of rheumatoid arthritis

The pathophysiological role of MCs in RA is probably very complex and is just starting to be outlined. Because large amounts of various proinflammatory mediator substances and cytokines, such as tumor necrosis factor (TNF)-\(\alpha\), were produced and stored by MCs, they can release such compounds in response to cell activation (5, 9). Their activation affects the matrix turnover as well as the neighboring cells.

Further study is necessary to clarify whether the direct target of CL in the present disease model is MCs and/or other cells, although the increase in MC numbers at the arthritic site was suppressed by CL. Cromolyn has been used clinically with relative safety. Further studies on the mechanism of action of CL may contribute to the development of novel drugs for controlling RA in the future.

In the case of cyclosporin A (CsA) treatment in the disease model mice, the number of MCs in the CsA-treated CIA mice was lower than that in the normal control mice. However, suppressed but apparent arthritis was observed in the CsA-treated CIA mice. These results indicate that MC is not the sole factor in the pathogenesis of the present disease model (Y. Kobayashi et al., unpublished observation).

Human MCs can synthesize a variety of immuno-regulatory cytokines and chemokines. Some MC-derived cytokines and chemokines are lymphocyte chemotactic factors and they may contribute to lymphocytes, especially T lymphocytes infiltration at sites of tissue damage in RA (6). The activation of MCs also induced macrophages to increase interleukin (IL)-1 production (26). Increased IL-1 may affect fibroblasts. The induction of migration and proliferation of fibroblasts by MCs has been demonstrated and results in the acceleration of fibrosis (27). Such a complex interaction among synovial cells including MCs may be important in the pathogenesis of RA (Fig. 1).

Synovial MCs may also be important in cartilage destruction and bone remodeling (5). MC products stimulated fibroblast-like synoviocytes or chondrocytes to produce increased amounts of the matrix metalloproteinases (MMPs) (Fig. 1) (14, 28). MC tryptase and/or chymase have been...
shown to destroy the matrix by activating MMPs directly or indirectly (15, 29). We have already reported the increase of chymase activity with arthritis in the animal model (19). Furthermore, we have recently observed immunohistochemically that MMPs such as MMP-2, -3 and -9 also showed parallel changes with the severity of arthritis in the animal model (Fig. 3). The measurement of the expression of mRNAs for MMP-2, -3 and -9 in the disease model by competitive RT-PCR is now in progress. The importance of MMPs activation on RA pathogenesis has also been reported in humans (14, 15). The activation of MMPs may be one of the possible mechanisms of the contribution of MCs to the arthritic process of RA.

In contrast, MCs may mediate matrix regeneration and pathological fibrosis as they contain a variety of profibrotic cytokines and growth factors (5, 9, 30). Histamine is synthesized and released in large quantities by MCs and its effects are mostly suppressive for acute inflammation (5). Thus, MCs may exert bi-directional effects on the matrix turnover.

Closing remarks

There is an increasing body of evidence that MCs represent one of the central components in the inflammatory and immune responses in RA. The beneficial effects of a MC-stabilizer, CL, in the CIA model further suggested the potential contribution of MCs to the pathophysiological process of RA. Thus, MCs are now considered to be one of the targets of RA treatment.

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