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Abstract

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Mast Cells in Osteoarthritic and Rheumatoid Arthritic Synovial Tissues of the Human Knee

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The distribution and density of mast cells in the normal and diseased synovial membranes were investigated. The mast cell count (MCC) in the osteoarthritic (OA) synovium (36.9 ± 26.9 cells/mm^2) was significantly higher than that in the rheumatoid arthritic (RA) synovium (18 ± 12.3 cells/mm^2). There was a marked positive correlation between the MCC and the volume of joint fluid in OA ($r = 0.544$). There was a marked negative correlation between the MCC and the volume of joint fluid in RA ($r = -0.478$). The synovial inflammatory score had a poor correlation with the MCC in OA ($r = 0.377$) and RA ($r = 0.305$). No correlation was noted between MCC and age, sex, roentgenographic grades, disease duration, C-reactive protein or leucocyte number in synovial fluid. Our data suggests, thus, that mast cells could be involved in the pathogenesis of inflammatory diseases of the synovium, especially in the mechanism of hydroarthrosis.

Key words: mast cell count, osteoarthritis, rheumatoid arthritis, hydroarthrosis, synovial membrane

Mast cells are present throughout normal connective tissue, but their number varies significantly among different species, as well as among different organs and tissues (1). They are characterized by their ability to release and synthesize a variety of molecules including proteases, vasoactive amines and chemotactic factors. These mediators can induce immediate and delayed-onset inflammatory reactions, increase vascular permeability and facilitate cellular infiltration into the tissue (2).

At the sites of T-cell aggregation, such as the synovial membrane in osteoarthritis (OA) or rheumatoid arthritis (RA) (2, 3), the number of mast cells is known to increase, a response attributable to the liberation by T-cells of lymphokine, a mast cell generating factor (1). To quantify the mast cell count (MCC) in joint capsules and joint fluids, several investigators have used various techniques (3, 4). However, few studies have demonstrated a significant difference in mast cell density between the OA and RA synovium.

In the present study, to identify mast cells, we used fine cationic colloidal iron staining at pH 1.5 which can detect strong negative charges in tissue. In addition, the derived MCC was compared with several factors such as roentgenographic grades, clinical findings, inflammatory parameters and the results were statistically analyzed.

Clinically, it is well known that a great number of patients with OA and RA suffer from hydroarthrosis which causes pain and joint disability. Here, for the first time, we have demonstrated a correlation between MCC, histological inflammatory score and the volume of synovial fluid. Our findings are an important step toward understanding the mechanism of synovial inflammation, especially hydroarthrosis.

Materials and Methods

Tissue preparation. Synovial tissues were obtained from 13 patients with OA (aged 43-88 years, average, 70 years) and 17 patients with RA (aged 39-77 years, average, 64 years) during total knee arthroplasty. Normal synovial tissues were excised during surgery from the amputated legs of 3 patients with malignant tumors (Table 1).

The specimens were cut into 5-10mm blocks and immersion-fixed with 4% paraformaldehyde in 0.1M cacodylate buffer (pH 7.3) for 24h at room temperature. Then, they were washed with cacodylate buffer, dehy-

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Table 1 Clinical data of patients

	Osteoarthritis	Rheumatoid arthritis	Control
Number of cases	13	17	3
Female/Male	9/4	14/3	0/3
Age in years	68.7 ± 11.48*	63.88 ± 9.51	62
Duration in years	4.2 ± 21.4	20.8 ± 9.41	—
ESR[a]	9/24/43	38/68/8	—
Leuko[b]	6.77 ± 1.47	13.14 ± 19.82	6.6
RF[c]	10.9 ± 22.73	163.17 ± 253.55	—
CRP[d]	3.4 ± 4.1	6.8 ± 5.88	0.8

a : Erythrocyte sedimentation rate at 0.5; 1; 2h
b : Number of leucocytes ($\times 10^3/\mu$l)
c : Rheumatoid factor (IU/ml)
d : C-reactive protein (mg/dl)
*: Mean ± SD

Table 2 The grading system of the synovial membrane

Score	Synovial lining cell hyperplasia	Cellular infiltration	Fibrosis of the lining cell layer
0	1-2 layers of cells	Not present	Normal
1	3-4 layers	Mild	Mild
2	5-6 layers	Moderate and focal	Moderate
3	Over 7 layers	Moderate and diffuse	Marked
4	Over 7 layers	Marked and diffuse	Marked

drated through a graded series of ethanol, and embedded in paraffin. Deparaffinized sections were stained by the fine cationic colloidal iron method (Murakami *et al.*, 1986) (5) at a pH value of 1.5, and observed with a light microscope (BH-2, Olympus, Tokyo, Japan).

Clinical findings and laboratory data. The volume of joint fluid was measured before or during total knee arthroplasty. The erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), and C-reactive protein (CRP) were estimated within the two weeks before surgery. The Japanese Orthopaedic Association (JOA) score, sex, age, duration of disease, and roentgenographic grades [Koshino's grade in OA (6); Larsen's grade in RA (7)] were also determined within the two weeks before surgery. The inflammatory score of the synovial tissue was evaluated according to the grading system of the synovial membrane (8) (Table 2). According to this method, synovial lining cell hyperplasia, cellular infiltration (degree of infiltration by lymphocytes and mononuclear and polymorphonuclear leucocytes) and fibrosis of the lining cell layer were used as histopathological parameters.

Counting of mast cells. The number of mast cells was accurately counted under the light microscope, which was fitted with a special eyepiece containing a 1 square cm grid. The mean cell numbers in 10 randomly selected areas ($250 \times 250\,\mu$m) which were located a $750\,\mu$m distance from surface of the synovial surface were used for statistical analysis.

Statistical analysis. Statistical analyses were performed using linear regression analyses and unpaired 2-tailed Mann-Witney U-tests as appropriate.

Results

Mast cells were easily identified as their cytoplasm showed a strong Prussian blue reaction to the colloidal iron stain at pH 1.5 in light microscopy (Fig. 1). Most of them were distributed in the subsynovial tissues; few were found between synovial lining cells. Under closer examination, mast cells were found to be most abundant in the alveolar synovium. In the fibrous synovium, mast cells were less frequently noted than in the alveolar synovium. In the adipose synovium, the cells were sparsely spread among the fat cells. In all layers of the synovium, the mast cells were usually located along or near small blood vessels, including arterioles.

The mean MCC was 36.9 ± 26.9 (range, 6-92) cells/mm^2 in OA and 18 ± 12.3 (range, 2-38) cells/mm^2 in the RA synovium (Fig. 2). Compared with normal syovial tissues, synovial membranes from patients with active RA and OA contained significantly more numerous mast cells. Statistically, the MCC in the OA synovium was higher than that in the RA synovium ($P < 0.05$).

Relationship between MCC and clinical findings. In the synovial membranes with hydroarthrosis, the MCC was elevated in OA and lowered in RA. That is, the MCC in OA showed a positive correlation with the volume of joint fluid (Fig. 3, $r = 0.544$, $n = 13$, $P < 0.05$), while it showed an inverse correlation in RA (Fig. 3, $r = -0.478$, $n = 14$, $P < 0.05$). Moreover, the MCC was significantly positively correlated with ESR in RA ($r = 0.791$, $n = 17$, $P < 0.01$) but not in OA. The MCC was weakly positively correlated with CRP in RA ($r = 0.377$, $n = 17$, $P = 0.1382$) but not in OA. The inflammatory score of synovial tissue showed a correlation with MCC in OA ($r = 0.377$, $n = 13$, $P < 0.05$) and in RA ($r = 0.305$, $n = 17$, $P < 0.05$), with joint fluid in RA ($r = -0.39$, $n = 14$, $P < 0.05$) and with CRP in RA ($r = 0.42$, $n = 17$, $P < 0.05$).

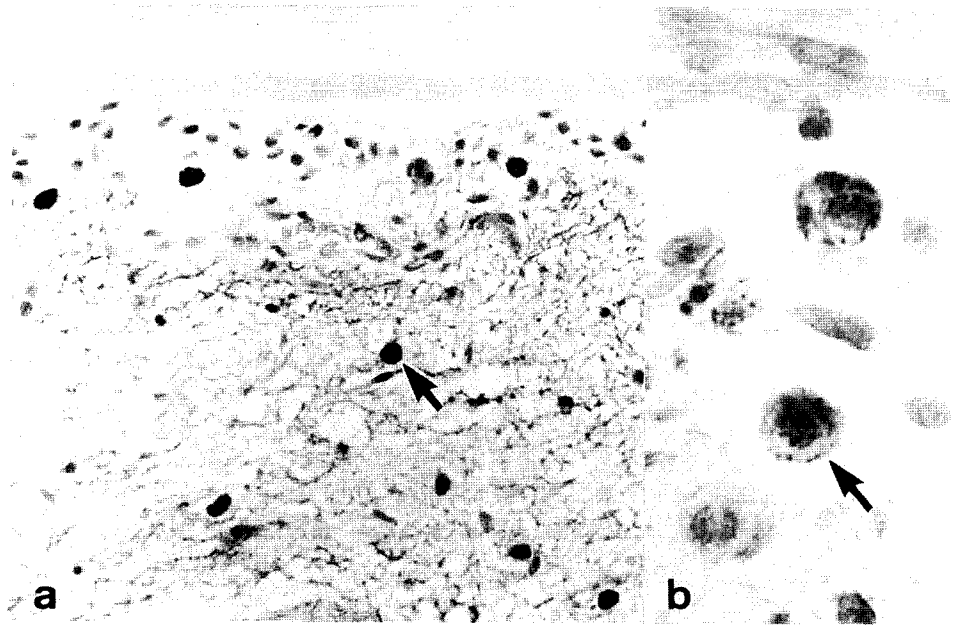

Fig. 1 Light micrographs of osteoarthritis (OA) synovial tissues stained with cationic colloidal iron at pH 1.5. Mast cells (arrows) showed an intense Prussian blue reaction (original magnification **a**: × 400, **b**: × 1000).

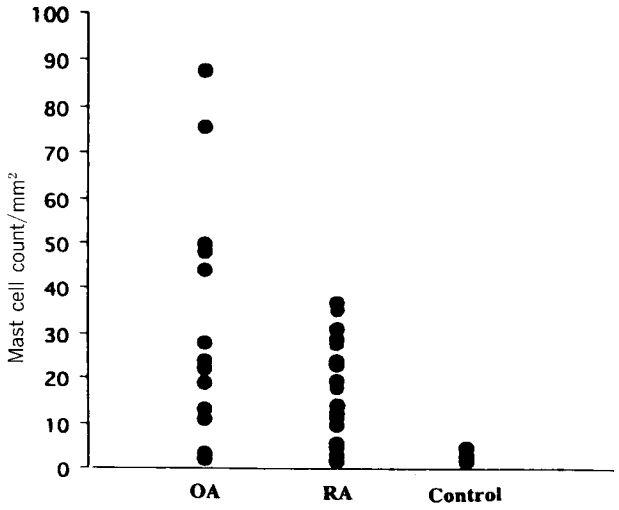

Fig. 2 Mast cell count (MCC) in the synovium of OA and rheumatoid arthritis (RA) knees. $P < 0.05$ (OA *vs* RA, Mann-Whitney U-test). OA: See legend to Fig. 1.
OA: n = 13; RA: n = 17; Control: n = 3.

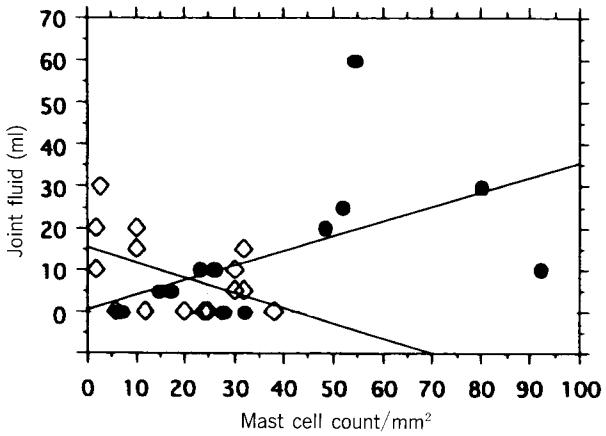

Fig. 3 Correlations between the MCC and the volume of joint fluid in each case of OA and RA (OA: $r = 0.544$, n = 13, $P < 0.05$; RA: $r = -0.478$, n = 17, $P < 0.05$). MCC, OA, RA: See legends to Figs. 1, 2.
●: OA joint fluid; ◇: RA joint fluid.

Discussion

The cationic colloidal iron used in this study is finer in size and more stable over a wider pH range (0.8–7.6) than other agents. It has a tendency to bind to negatively charged groups in the tissue (5). Ohtsuka *et al.* (9) previously reported that the cytoplasm of the mast cell was distinctly and selectively stained with the cationic colloidal iron at pH 1.5. In this study, mast cells in the synovial tissues were clearly visible and easily distinguished because of their stainability, facilitating counting by light microscopy.

The present study proves that the MCC in the synovial tissues from patients with OA and RA is elevated above normal. Similar findings have been reported by other authors (3, 10). Malone *et al.* (11) examined the MCC in synovial specimens from RA patient and found a strong positive correlation between their histological inflammatory index and MCC. They also described an interesting finding, namely, that mast cells decreased after the administration of an intraarticular steroid injection in all three patients studied. Based on this they concluded that some of the benefits derived from steroid therapy arise in part from a blockage of mast cell-dependent inflammatory mechanisms. Our results in the present study showed positive correlations of the inflammatory score with MCC both in the OA and the RA synovium. We also showed that the MCC is positively correlated with the ESR in RA. Taken together with our histological findings, this findings suggest that the MCC is likely to increase along the activity of synovitis.

A higher density of mast cells in the OA as compared with the RA synovium has been reported by Fritz *et al.* (3), whereas a lower density was reported by Gotis-Graham *et al.* (12). It is generally accepted that the pathologic mechanisms in the OA synovium differ markedly from those in RA. Gotis-Graham *et al.* (12) have distinguished mast cells as positive for tryptase only (MC_T) or for tryptase and chymase both (MC_{TC}) using the double-immunohistochemical staining technique, and reported an increased number of mast cells in RA (60.9 cells/mm^2) as compared with OA (21.7 cells/mm^2) and the normal (9.4 cells/mm^2) synovium. There was a selective increase in the MC_T subset in OA, and an increase in both subsets in RA synovium associated with infiltrating inflammatory cells or with regions of highly cellular fibrous tissues. In our study, MCC in OA synovium was greater than in RA. In the RA synovium, mast cells were not often seen in the region of massive infiltration of inflammatory cells, fibrous tissues and granulation. This inconsistency might partly result from differences in the histological activity of synovitis in the tissue examined by Godfrey *et al.* (10). They have mentioned that patients with active RA have more synovial mast cells than those with end-stage diseases.

Mast cells are known to be activated by some antigen through IgE which can bind to Fc ε RI receptors on the cell surface (13). Activated mast cells can release various kinds of chemical mediators such as histamines, tryptases, chymases, heparins and chondroitine sulfates (4). In the present study, colloidal iron particles effectively bound to the negatively charged molecules contained by mast cells. These molecules are believed to be heparins and chondroitine sulfates, whose roles of which in inflammatory synovitis are not fully understood. Heparin has the ability to bind and stimulate migration of endothelial cells (14). One of the potential effects of heparin released from synovial mast cells is to sustain angiogenesis which is an integral feature of inflammatory synovitis. In addition, activated mast cells can produce leukotorienes and platelet activating factors. These chemical mediators are known to increase vascular permeability (15). Interestingly, mast cells showed a positive immunoreaction to vascular permeability factor (VPF, also known as vascular endothelial cell growth factor or VEGF) (not shown). Increased permeability can cause the infiltration of inflammatory cells and fluid to flow out of vessels resulting the hydroarthrosis of the joint. Our findings, of perivascular distribution of mast cells and the close relationship of the MCC to the volume of joint fluid, also indicate that mast cells can play an important role in the pathogenesis of hydroarthrosis.

Human mast cells are known to express mRNA for TNF-α, IL-4 and IL-8 and can produce TNF-α and IL-4 (16, 17). More recently, mast cells have been reported to express the CD40 ligand (18). These facts indicate that mast cells can directly lead to the synthesis of IgE by B lymphocytes binding to the CD40 expressed on B lymphocytes. Ultimately, mast cells can be activated by some antigen through IgE and variety of immunoreactions can be introduced. In the complicated synovial inflammatory reactions, mast cells seem to play a key function by acting upon various kinds of cells relevant to the synovial structure and immune function.

In summary, we have demonstrated the increase in number of mast cells in the synovium of patients with OA and RA. Mast cells seem to play a role in the initial phases of flare-ups of disease activity and could produce hydroarthrosis in the joint.

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