

# Histological features of rheumatoid arthritis patients having antibodies to enterobacterial common antigens: Correlation of antibody levels with semiquantitative histologic scores and laboratory markers

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## **Abstract**

### **Objective**

*To ascertain whether clinically diagnosed rheumatoid arthritis (RA) patients having antibodies to enterobacterial common antigens (ECA) in synovial fluid (SF) have a histological appearance characteristic of RA synovitis.*

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### **Methods**

*Twenty five RA patients for which synovial biopsy specimens were preserved, were selected from 58 patients with RA tested for antibodies to ECA in SF. The synovial tissue specimens were examined histologically using a semiquantitative scoring system with quantitative counts. The correlation of anti-ECA antibody levels with total scores for synovitis and laboratory markers in three RA patient groups (markedly positive, moderately to slightly positive, and negative according to anti-ECA antibody levels) was analyzed statistically.*

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### **Results**

*Histologic examination in the markedly positive RA group (total score for synovitis, range 18~20 points) revealed typical histological features of rheumatoid synovitis. Total scores for synovitis were significantly higher in both the markedly positive and moderately to slightly positive RA groups than in the negative RA group. A comparison of anti-ECA antibody levels with total scores for synovitis revealed a strong and significant correlation. Furthermore, levels of anti-ECA antibodies were also correlated with rheumatoid factor and C-reactive protein.*

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### **Conclusion**

*Clinically diagnosed RA patients having anti-ECA antibodies in SF showed typical or characteristic histological features of RA synovitis. Our data suggest that a group of RA patients with an enterobacterial etiology exists in larger groups of patients with RA which is thought to be a heterogeneous disease.*

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### **Key words**

Rheumatoid arthritis, antibodies to enterobacterial common antigens, semiquantitative histologic scores, rheumatoid factor, C-reactive protein.

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## Introduction

Rheumatoid arthritis (RA) is an intractable systemic disorder in which a non-suppurative proliferative synovitis leads to destruction of the articular cartilage and bone (1,2). From a diagnostic standpoint, rheumatoid factors (RF) are the most characteristic autoantibodies in patients with RA. Therefore, RA is probably the best known of the autoimmune rheumatic diseases (3). Genetic factors, infectious agents and immune responses including type III and type IV hypersensitivity, and autoimmunity are clearly involved in the pathogenesis of RA, given recently reported evidence (4). Previously, we reported the induction of arthritis resembling RA in rabbits (5) by hyperimmunization with heat-killed *Escherichia coli* O:14 which contained large amounts of enterobacterial common antigens (ECA) (6, 7), and also showed that a high proportion of the animals with induced arthritis had high levels of antibodies to the *E. coli* antigen (8). In a recent study, we found that in patients with RA, levels of antibodies against heat-killed *E. coli* O:14 (anti-ECA antibodies) were significantly increased in 33 of 83 serum samples (39.8 %) and in 38 of 58 synovial fluid (SF) samples (65.5%), compared to control subjects (9).

It would be of interest in terms of the etiopathogenesis of RA to determine whether clinically diagnosed RA patients with anti-ECA antibodies in their SF as described above show a histological appearance characteristic of RA synovitis. Therefore, we studied the histological features of RA patients with anti-ECA antibodies in SF and the correlation of anti-ECA antibody levels with semiquantitative histologic scores for synovitis and laboratory markers including RF, C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR).

## Patients and methods

### Patients

Twenty-five RA patients (mean age 55.7 years, range 37-75; 6 men/19 women) for whom synovial biopsy specimens stained with hematoxylin and eosin (HE) and other stains were preserved at the Department of Pathology,

Aichi Medical University, were selected retrospectively from 58 patients with RA who had been chosen randomly and tested for antibodies to ECA in SF as previously described (9). The 22 knee specimens and 3 other specimens (2 hip and 1 wrist joints) had been collected over a period of several years. Laboratory tests were carried out, including IgM RF with degenerated human IgG-coated latex bead agglutination as detected by turbidimetric measurement, CRP and ESR. All patients with RA fulfilled the 1987 revised criteria of the American College of Rheumatology for the classification of RA (10). The joint disease was of 2 to 288 months' duration (median duration: 127 months). The study was approved by the local ethics committee.

### ELISA tests for antibodies to ECA

Titers of the antibody to *Escherichia coli* O:14 containing large amounts of ECA were determined by the enzyme-linked immunosorbent assay (ELISA). *E. coli* O:14 was kindly supplied by the Research Institute for Medical Diseases, Osaka University. ELISA plates were coated with 15 µg/ml of heat-killed *E. coli* O:14. After the remaining non-specific sites were blocked, test SF diluted to 1:20 was distributed into each of three wells. Then, goat anti-human immunoglobulin (polyvalent) conjugated with horseradish peroxidase (TAGO, Inc, Burlingame, USA) was added to each well. The amount of enzyme bound to the wells was assayed using o-phenyldiamine dihydrochloride solution. The enzyme reaction was stopped and the optical density (OD) at 492 nm was measured with an ELISA reader. Samples were regarded as positive when the OD was at least 2 standard deviations above the mean value in patients with osteoarthritis (OA) as controls ( $0.144 + [2 \times 0.067] = 0.278$ ) (9).

### Histologic evaluation

The HE-stained specimens were each analyzed with regard to seven histologic criteria characteristic of RA synovitis (11). All fields of the sections were examined and evaluated carefully with double checks by two pathologists. The degree of histologic change was graded

semiquantitatively on a scale of 0-3 points (12), 0 corresponding to "not present, i.e. normal". These synovial membrane (SM) characteristics included:

- 1) Synovial lining cell (SLC) hyperplasia. The grading scale used: 0 = one to two SLCs; 1 = three to four SLCs; 2 = five to seven SLCs, 3 = seven SLCs. The SLCs were counted from the edge of the SM to the subsynovial tissue.
- 2) Palisading appearance of SLCs. The grading: 1 = non-typical, 2 = typical.
- 3) Synovial giant cells. The grading: 1 = foreign body type, 2 = non-foreign body type.
- 4) Inflammatory cell infiltration. The grading of lymphocytes: 1 = diffuse infiltrate, 2 = lymphoid follicle, 3 = formation of germinal center. The grading of plasma cells: 1 = mild, 2 = moderate, 3 = severe.
- 5) Proliferation of granulation tissue [mesenchymoid transformation (13)] including destruction of the articular cartilage and bone due to pannus. The grading: 1 = mild, 2 = moderate, 3 = marked.
- 6) Surface fibrin deposits or fibrinoid necrosis (14) was graded on a scale of 1 to 3.
- 7) Synovial hemosiderosis. The grading: 1 = either cellular or stromal, 2 = both cellular and stromal.

The changes in each diagnostic category were then summed.

#### Statistical analysis

Comparisons of the histologic semiquantitative scores with the quantitative counts for synovitis in the different patient groups were analyzed using the Mann-Whitney U test. The correlation of ECA antibody levels with histologic scores for synovitis and laboratory markers was assessed using Spearman's rank correlation coefficient ( $r$ ). Values of  $P < 0.05$  were considered significant.

#### Results

According to the anti-ECA antibody levels expressed as OD in SF (cut-off value, 0.278), three RA groups were established: markedly positive (OD value: 0.859 – 2.343), moderately to slightly positive (OD value: 0.281 –

**Table I.** Comparison of total scores for synovitis and laboratory markers in ECA antibody-positive and -negative RA groups.

Anti-ECA antibodies	Markedly positive RA group (n=5)	Moderately to slightly positive RA group (n=12)	Negative RA group (n=8)
*OD values in SF	0.859 – 2.343	0.281 – 0.780	0.119 – 0.277
Mean $\pm$ SD	1.596 $\pm$ 0.599	0.423 $\pm$ 0.146	0.174 $\pm$ 0.054
Total scores for synovitis	18 – 20	10 – 18	10 – 13
Mean $\pm$ SD	19 $\pm$ 1	14 $\pm$ 2.59	11.1 $\pm$ 1.36
Mean RF (IU/ml) $\pm$ SD	548.6 $\pm$ 417.1	148.6 $\pm$ 157.3	125.4 $\pm$ 189.3
Mean CRP (mg/dl) $\pm$ SD	7.11 $\pm$ 4.86	4.78 $\pm$ 4.07	2.94 $\pm$ 1.60
Mean ESR (mm/hr)	61.8 $\pm$ 26.5	73.4 $\pm$ 33.8	67.5 $\pm$ 29.3
*cut off value = 0.278			

0.780) and negative (OD value: 0.119 – 0.277). Total scores for synovitis and laboratory markers in each RA group are shown in Table I. Histological examinations in the markedly positive RA group (total score for synovitis, range 18-20 points, mean  $\pm$  SD 19  $\pm$  1) revealed rheumatoid synovitis with hypertrophied villi of the SM due to chronic inflammation with lymphoid follicle formation (Fig.1). Moreover, a marked proliferation of the synovial lining cells showing an arrangement of elongated cylindrical synoviocytes in the palisade and multinucleated synovial giant cells at the synovial surface was seen (Fig. 2). Mesenchymoid transformation of the synovial stroma was also recognized (Fig.2). In the moderately to slightly positive RA group, the total score for synovitis ranged from 10 to 18 points (mean  $\pm$  SD 14  $\pm$  2.59).

Most RA patients in the antibody-positive groups showed a marked perivascular inflammatory cell infiltration with lymphocytes and plasma cells in the subsynovial tissue and an abundance of eosinophilic fibrin at the synovial surface. Furthermore, an extension of the proliferating granulation tissue (pannus) containing hyperplastic synoviocytes and inflammatory cells at the articular cartilage margin, and cartilage-bone fragments destroyed by pannus were visible (Fig. 3).

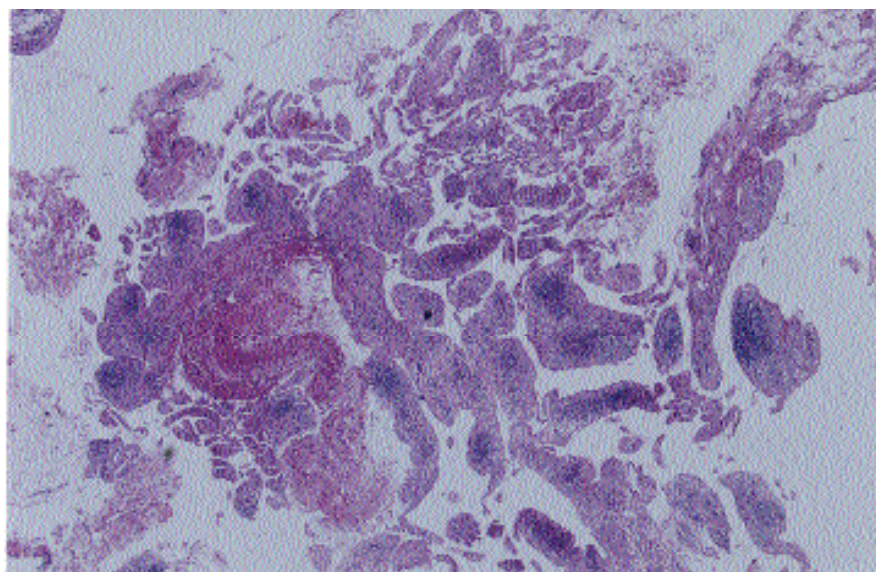
On the other hand, the total score for synovitis in the negative RA group without anti-ECA antibodies ranged from 10 to 13 points (mean  $\pm$  SD, 11.1  $\pm$  1.36). Most of the negative RA pa-

tients showed histological evidence of synovitis with comparatively low scores for the seven items. Total histological scores for synovitis were significantly higher in both the markedly positive and in the moderately to slightly positive RA groups than in the negative RA group ( $P < 0.05$ , Mann-Whitney U test). Concerning the association between ECA antibody levels and the total score for synovitis from biopsy and laboratory markers, a strong and highly significant correlation between OD values for anti-ECA antibodies in SF and the total score for synovitis was found using the Spearman's rank correlation coefficient ( $r$ ) ( $r = 0.926$ ,  $p < 0.05$ ). Furthermore, OD values for anti-ECA antibodies were also correlated with the RF ( $r = 0.408$ ,  $p < 0.05$ ) and CRP ( $r = 0.469$ ,  $p < 0.05$ ) respectively, but not with ESR ( $r = 0.021$ ).

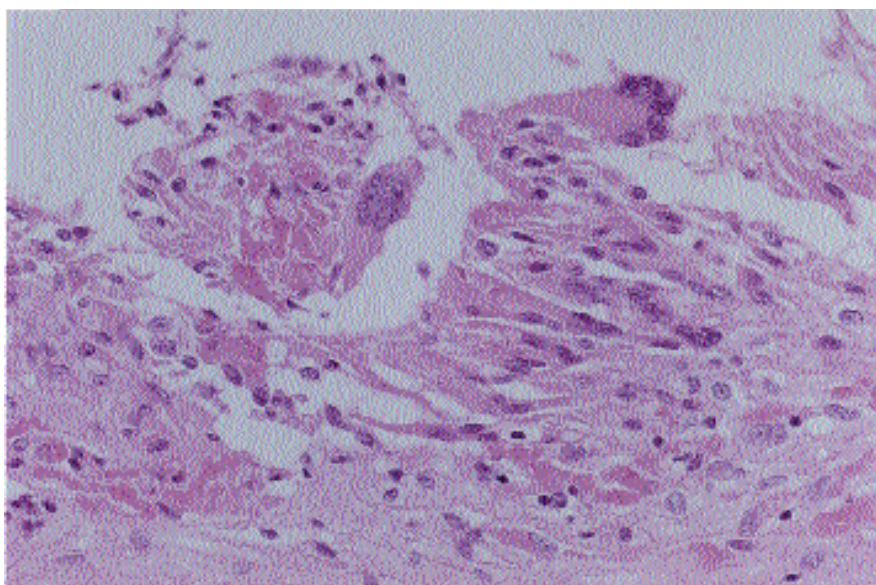
#### Discussion

This study has demonstrated that clinically diagnosed RA patients with anti-ECA antibodies in their SF show histological features typical or characteristic of RA synovitis and a significant correlation of anti-ECA antibody levels with the semiquantitative histologic scores for synovitis and laboratory markers including RF and CRP.

The reason why anti-ECA antibodies in the SF and not the serum were used in the present study is that SF showed a higher positive frequency of antibodies than did the serum (9). Furthermore, since the SF is in direct contact with the rheumatoid synovium, the SF is consi-



**Fig. 1.** Synovial tissue (total score for synovitis, 19 points) from a 46-year-old woman with clinically active seropositive RA and markedly positive anti-ECA antibodies (OD value 2.000): synovial villous hyperplasia with lymphoid follicle formation (dark blue areas) in the stroma of villi is seen (HE stain, x10).



**Fig. 2.** Synovial tissue (total score for synovitis, 20 points) from a 39-year-old woman with clinically active seropositive RA and markedly positive anti-ECA antibodies (OD value 0.859): palisading of synovial lining cells accompanied by remnants of fibrin and stromal mesenchymoid transformation and multinucleated synovial giant cells can be seen at the synovial surface zone (HE stain, x200).

dered to be closely related to the synovial lesions of RA patients.

With regard to the histopathological changes observed in the synovial tissue of RA patients, there have been many reports (11-20). Well known histopathological features of RA synovitis may be summarized as follows: synovial hypertrophy with formation of villi; synoviocyte hyperplasia, often accom-

panied by palisading and the appearance of synovial giant cells; surface fibrin deposition or fibrinoid necrosis; proliferation of blood vessels (neovascularization); frequent formation of lymphoid follicles, occasionally with germinal center formation and dense plasma cell infiltration in the synovial villi; hemosiderosis in the synovial tissue; and the proliferation of granula-

tion tissue (mesenchymoid transformation) developing to destroy the articular cartilage and bone by pannus.

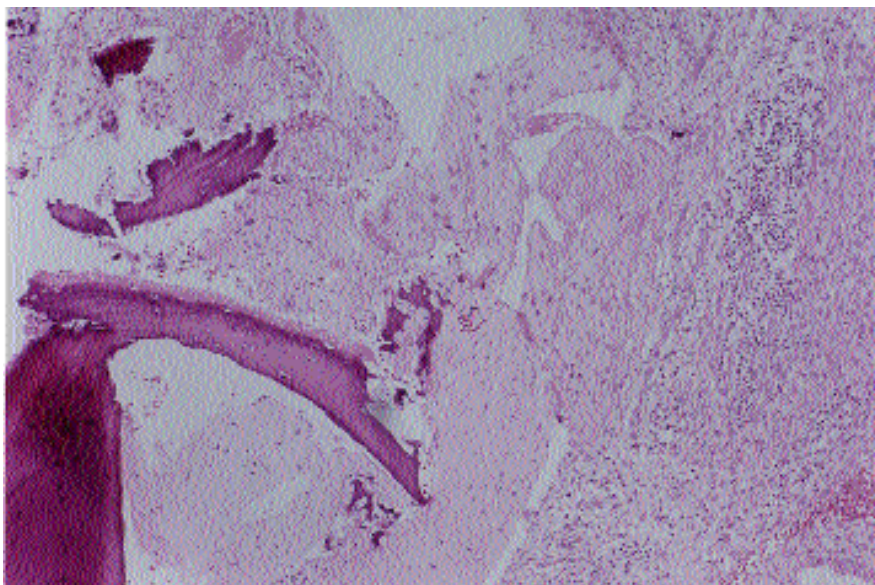
Koizumi *et al.* (10) reported that between RA and OA there were significant differences with regard to the presence of lymphoid follicles ( $p < 0.01$ ), formation of a germinal center ( $p < 0.05$ ), the infiltration of plasma cells ( $p < 0.001$ ), and the presence of granulation tissue ( $p < 0.001$ ), fibrin ( $p < 0.01$ ) and hemosiderosis in the synovial stroma ( $P < 0.01$ ). Fonseca *et al.* (21) also reported a significant difference in the mean of 6 histological scores between RA and OA ( $p < 0.01$ ) in a semiquantitative analysis of synovial biopsy specimens and suggested the intensity of inflammatory histological features to be of prognostic value in RA. Grimley *et al.* (15) reported that giant cells were present in synovium from the knee in 9 of 19 patients with active, seropositive RA, though none were found in 9 seronegative RA patients. Gardner mentioned that synovial giant cell formation was characteristic but not pathognomonic of rheumatoid synovitis (12). Pap *et al.* (2) stated that the destruction of articular cartilage and bone represented a unique and prominent feature of RA, as it clearly distinguished RA from the other arthritides, as well as determining its outcome.

Accordingly, the findings demonstrated in Figures 1, 2 and 3 are considered to be representative histological features which characterize RA synovitis. In the present study of RA patients with antibodies to ECA, it may be said that all of those in the markedly positive group had typical histological features of RA synovitis and most of those in the moderately to slightly positive group also showed characteristic synovitis.

With regard to the association of anti-ECA antibodies with the semiquantitative histologic scores, a strong and highly significant correlation between the OD values for ECA antibodies in the SF and the total scores for synovitis was found. In addition, anti-ECA antibody levels were also correlated with RF and CRP, respectively, but not with ESR.

An experimental arthritis due to *Entero-bacteriaceae* – i.e., chronic polyarth-





**Fig. 3.** Synovial tissue (total score for synovitis, 14 points) from a 56-year-old man with clinically active seropositive RA and slightly positive anti-ECA antibodies (OD value 0.367): destruction of articular cartilage and bone by pannus containing hyperplastic synoviocytes and inflammatory cells is visible (HE stain, x50).

ritis in rabbits induced by hyperimmunization with heat-killed *E. coli* O:14 containing large amounts of ECA—has been reported to have a morphologic, serologic and immunohistologic resemblance to human RA(5,8,22) and it was shown that a high proportion of the animals with induced arthritis also had high levels of antibodies to the *E. coli* antigen containing ECA (8). Because of similarities between the arthritic conditions in human RA and those we were able to induce in rabbits experimentally, it is suggested that the anti-ECA antibodies in rheumatoid SF described here may play a role in the pathogenetic mechanism of RA synovitis and are a useful index of the extent of RA in patients with anti-ECA antibodies.

Persistent microorganisms, such as the bacteria present in the environment (i.e., enterobacteria), offer an explanation for the sustained high titers of RF seen in the pathologic conditions of RA, because structural analysis indicates that organisms with multiple epitopes spatially arrayed in a confined area and coated with IgG are efficient in triggering RF (23). Accordingly, it is suggested that enterobacterial sensitization is associated with the production of RF, similar to the rheumatoid factor-

like substance described previously in our animal model (8). CRP is generally accepted to be the most accurate measure of the acute phase response and hence of tissue inflammation. In response to inflammation, its concentration can rapidly increase up to 1,000-fold (24). A significant correlation between OD values for anti-ECA antibodies in SF and CRP is considered to reflect the intensity of synovial inflammation induced by enterobacterial sensitization, similar to the chronic polyarthritis in rabbits evoked by hyperimmunization with heat-killed *E. coli* O:14 containing EAC (8).

In conclusion, RA patients with an excess of antibodies to ECA in their SF showed the typical or characteristic histological features of seropositive RA synovitis. A significant correlation of anti-ECA antibodies with the semi-quantitative scores for synovitis and laboratory markers including RF and CRP, but not ESR, was observed. Our data suggest that a subgroup of RA patients with an enterobacterial etiology exists within larger groups of patients with RA, which is thought to be a heterogeneous disease. However, further studies in this direction will be needed on a worldwide scale to obtain confirmation of our hypothesis.

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