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An immunofluorescent study on anti-synovial tissue antibody in the body fluid from patients with rheumatoid arthritis*

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Abstract

There is as yet no plausible and convincing explanation for the etiology of rheumatoid arthritis. The authors investigated anti-synovial tissue antibody in the body fluid of rheumatoid arthritis by means of indirect immunofluorescent technic using non-affected synovial tissues as antigen. As the result the anti-synovial tissue antibody was detected in 7 cases of the 15 synovial fluid samples of rheumatoid arthritis and in two out of the six serum samples. The site of the localization of this antibody was demonstrated to be in the synovial membrane, especially in synovial cells and in the small blood vessel walls situated immediately adjacent to the synovial surface, but it was found in no connective tissues other than synovial membrane. It seems that this anti-synovial tissue antibody should be considered as an independent factor from rheumatoid factor, and that rather than the rheumatoid factor it is more actively associated with the localization and progression of chronic inflammation within the rheumatoid arthritis joint.

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**AN IMMUNOFLUORESCENT STUDY ON ANTI-SYNOVIAL
TISSUE ANTIBODY IN THE BODY FLUID FROM
PATIENTS WITH RHEUMATOID ARTHRITIS***

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The etiology of rheumatoid arthritis is not sufficiently clear at the present stage, but with recent advance in the concept of autoimmunization a great interest has been aroused on rheumatoid arthritis with emphasis on rheumatoid factor. It should be noted, however, that the so-called rheumatoid factor can often be encountered also in other diseases^{1,2}. It remains still unsolved problem as to whether the rheumatoid factor is only a secondary product in the rheumatoid arthritis, or it would play a certain etiological role, but it is generally considered that the rheumatoid factor does not play at least an active pathogenic role.

Independently of the rheumatoid factor STEFFEN and coworkers^{3,4} detected autoantibody to connective tissue in the patient sera of rheumatoid arthritis by means of anti-globulin consumption test. It is naturally considered that there are two kinds of antibody, namely, the cell bound antibody and the circulating antibody, concerned with such an autoimmune disease. Thereupon, on the assumption that the circulating antibody such as those mentioned by STEFFEN *et al.* also must be involved in the localization and progression of the lesion in rheumatoid arthritis, the authors conducted some basic experiments by COONS' indirect immunofluorescent technic⁵.

MATERIALS AND METHODS

The synovial fluid of 15 cases with rheumatoid arthritis and the sera from six patients served as materials. As for the control, 8 serum samples from chronic hepatitis cases, 5 synovial fluid samples from autopsy cases with no arthritis and 5 serum samples from normal persons were used. These synovial fluids, after being kept in the ice box at 0~4°C, were centrifuged at 3,000

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270 K. OGAWA, T. TSUJI, M. NAMBA, K. HAMAYA, T. OKAMOTO and Y. MIWA

r. p. m. for 30 minutes. The supernatant was then diluted with 0.01 M phosphate buffer saline (P. B. S.) to make it twice the original volume, and the sera were also diluted in the similar manner (twice its original volume).

For the purpose to locate the antibody binding site by the immunofluorescent technic, the synovia of patients with rheumatoid arthritis and these of non-rheumatic autopsy cases having each different blood types were prepared as tissue antigens. However, since the cytoplasm of plasma cells and the lymphoid cells as well as the site of fibrinoid degeneration in the synovial tissue of rheumatoid arthritis contained the positive fluorescence of gamma-globulin, the tissue of patient could not be used as antigen. Therefore, only the synovial tissue of non-rheumatic autopsy cases which did not contain gammaglobulin served for the purpose. As already described in a previous paper⁶, the tissues to be used were cut into the sections of 4 μ in thickness suitable for the immunofluorescent paraffin method.

The anti-human gamma-globulin goat globulin fluorescein isothiocyanate conjugated obtained from Baltimore Biological Laboratory and anti-human gamma-globulin rabbit globulin fluorescein conjugated with precipitin antibody titer of $\times 512 \sim \times 1024$ prepared in our laboratory were used as the fluorescent anti-human gamma-globulin solution.

For the staining, 3 or 4 drops of the synovial fluid from the patients, diluted two-fold, were dropped on the antigen section, incubated at 37°C for one hour, washed 5 times at the intervals of 15 minutes with P. B. S., onto which fluorescent anti-human gamma-globulin solution was dropped, incubated again at 37°C for 30 minutes, likewise washed 5 times with P.B.S., and embedded in Elbanol⁶ for the fluorescent microscopic observations. For each staining 4 serial synovial tissue sections from the autopsy cases were prepared.

In order to ascertain the antibody specificity, as to those samples from the cases having antibody in the body fluid, the tissue sections were treated with citric acid buffer solution (0.02 M), pH 3.2 for 90 minutes at room temperature⁷ prior to the fluorescent staining. As for the complement, the positive body fluid was incubated at 56°C for 30 minutes, while with reference to the organ specificity liver and kidney obtained from the autopsy cases that did not have any collagen disease, were used as the antigen.

RESULTS

It was found that by the indirect technic, circulating anti-synovial tissue antibody proved to be positive in the synovial fluid of 7 cases out of the 15 rheumatoid arthritis patients, and in the sera of 2 out of 6 cases. In the sera of other chronic hepatitis patients and in the synovial fluid as well as in the sera

of normal control, none proved to be positive (Table 1).

Table 1 Results of Immunofluorescent Staining
(circulating anti-synovial tissue antibody)

| Diagnosis | No. of cases | No. of binding |
|---------------------------------------|--------------|----------------|
| Rheumatoid arthritis (synovial fluid) | 15 | 7 |
| " (serum) | 6 | 2 |
| Chronic hepatitis (serum) | 8 | 0 |
| Normal control (synovial fluid) | 5 | 0 |
| " (serum) | 5 | 0 |

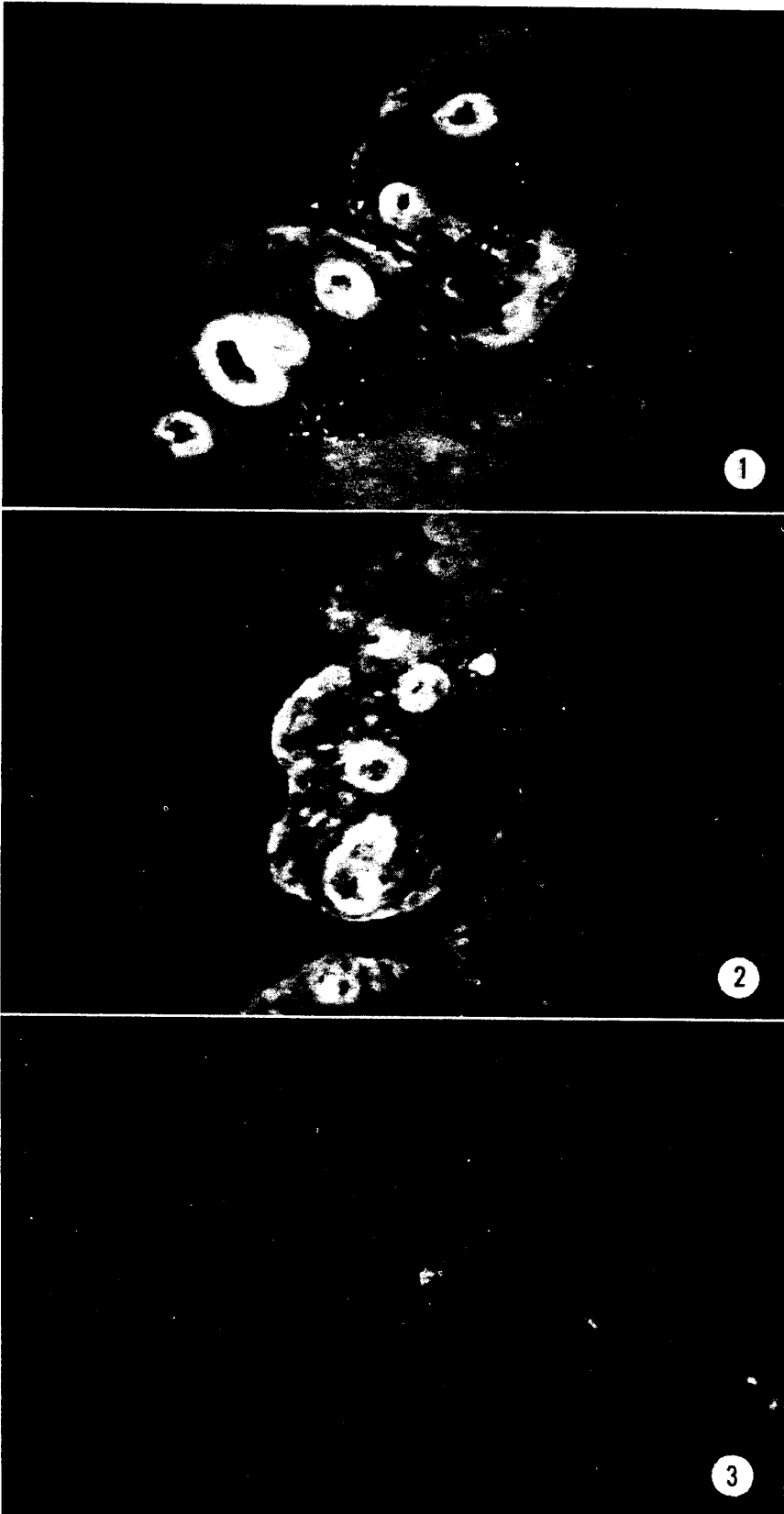
Among the six patients with rheumatoid arthritis 2 cases showed anti-synovial tissue antibody both in their synovial fluid and serum, whereas in the other two cases antibody could be detected only in the synovial fluid (Table 2).

Table 2 Results of Body Fluid from Patients
with Rheumatoid Arthritis

| No. of patients | Synovial fluid | Serum |
|-----------------|----------------|-------|
| 1 | - | - |
| 2 | ++ | ++ |
| 3 | + | + |
| 4 | + | - |
| 5 | - | - |
| 6 | + | - |
| 7 | - | / |
| 8 | - | / |
| 9 | + | / |
| 10 | + | / |
| 11 | -- | / |
| 12 | + | / |
| 13 | - | / |
| 14 | - | / |
| 15 | - | / |

The localization of the antigen against circulating antisynovial tissue antibody was detected in the synovial layer, especially in the synovial cells and in the small blood vessel walls immediately adjacent to the synovial surface (Figs. 1 and 2). In contrast, the sera from chronic hepatitis patients and those from normal, used as the control, all proved to be negative to the same fluorescent staining (Fig. 3). All the samples from the positive cases, after the treatment with citric acid buffer solution, lost their specific fluorescence.

The difference in blood type of autopsy cases whose synovial tissues were used as antigen is not in any way involved in the localization of the specific fluorescence in these normal tissues positive to the antigen-antibody reaction. In addition, the control tests using liver and kidney tissues from non-rheumatic autopsy cases all proved to be negative to the antigen-antibody reaction. The finding of specific fluorescence was not changed by incubation of the body fluid at 56°C for 30 minutes.



DISCUSSION

Rheumatoid arthritis is a somatic lesion, which stubbornly persists for a long period of time as a chronic inflammation of entire joints of the body. Furthermore, it is not infrequent that the infiltration of a considerable number of lymphoid cells and plasma cells or the formation of germinal center, which might be regarded as an autoimmune phenomenon, can be observed in histological changes.

CECIL *et al.*⁸, WAALER⁹, ROSE *et al.*¹⁰ have already done considerable works on the rheumatoid factor involved in rheumatoid arthritis¹¹⁻¹⁷. As for the studies on the rheumatoid factor concerned with rheumatoid arthritis by the immunofluorescent technic, MELLORS *et al.*^{18,19} and McCORMICK²⁰ examined it by fluorescent aggregated gamma-globulin or fluorescent anti-7S gamma-globulin and anti-19S gamma-globulin solutions, and found the rheumatoid factor to be located in the part of fibrinoid degeneration and the plasma cells in synovial membrane.

The rheumatoid factor, however, can be observed also in non-rheumatic diseases such as systemic lupus erythematoses, chronic liver diseases, syphilis, diseases of viral origin and macroglobulinemia^{1,2,21}, although the positive rate of the rheumatoid factor is distinctly high in rheumatoid arthritis. Since the rheumatoid factor responds especially well to aggregated gamma-globulin, the aggregated gamma-globulin *in vivo* is thought to compete against such an antibody (rheumatoid factor) as its antigen. Therefore, there is a great possibility of rheumatoid factor being an autoantibody to IgG (7S gamma-globulin) as mentioned in recent reviews^{22,23}.

As STEFFEN *et al.*⁸ have pointed out, the immunological study on rheumatoid arthritis is at present mainly directed toward three objectives: 1) antibody against Streptococcus-O-antigen, 2) rheumatoid factor, and 3) autoantibody to tissues such as connective tissue and joint capsular tissue. In their investigations on these objectives by means of anti-globulin consumption test STEFFEN *et al.* have demonstrated that out of 340 cases tested to the autoantibody to connective tissue 233 cases (66%) proved to be positive and they consider it to be as a factor independent of the rheumatoid factor. They have further biochemically analyzed the homogenates of joint capsular tissues and periarticular connective tissues of

Fig. 1 Section of synovial tissue from non-rheumatic autopsy case treated with synovial fluid of rheumatoid arthritis (Case 2) followed by fluorescent anti-human gamma-globulin solution (F. A. H. G.). The section was specially prepared by the paraffin embedding method for immunofluorescent technic. Note staining of the synovial tissue, especially the synovial cells and the blood vessel walls ($\times 400$).

Fig. 2 Same section of synovial tissue treated with serum of rheumatoid arthritis (Case 3) followed by F. A. H. G. Note same specific fluorescence ($\times 400$).

Fig. 3 Control section treated with synovial fluid from autopsy case with no arthritis ($\times 400$).

274 K. OGAWA, T. TSUJI, M. NANBA, K. HAMAYA, T. OKAMOTO and Y. MIWA

man, ox and calf and also performed immunologically the anti-globulin consumption test with such materials⁴. As the result, each of these materials gave positive reaction to patient sera. Therefore, they conclude that this antigen has no species specificity, and the soluble collagen and the collagen from articular capsule homogenizations show a stronger positive reaction than insoluble collagen, homogenizations from connective tissue and skin collagen.

Our results of the study conducted by the indirect immunofluorescent technic, in which we used the non-affected synovial membrane from non-rheumatic case as antigen, have demonstrated that the synovial membrane, especially the synovial cells and the small blood vessel walls situated immediately adjacent to the synovial surface show specific fluorescence. However, as most of the connective tissues of fibrous layer show only a strong white autofluorescence, it was impossible to obtain accurate positive results by this immunofluorescent technic.

In our control examination with the use of the liver and kidneys from non-rheumatic autopsy cases as tissue antigen we found such a circulating antibody does not react with parenchymal cells nor with interstitial tissues, suggesting that the specific fluorescence can be detected only in the synovial membrane. In addition, as the gamma-globulin could not be detected in the synovial membrane used as antigen by the direct immunofluorescent technic and as the findings on the rheumatoid factor by using fluorescent aggregated human gamma-globulin^{18,19,24} also differed, it is obvious that this circulating antibody is neither usual serum globulin nor rheumatoid factor. From the findings that specific fluorescence disappears when the tissues to be tested are pretreated with citric acid buffer solution, that any complement is not concerned with the reaction, because of the body fluid previously incubated at 56°C for 30 minutes did not change the fluorescence, and that the blood type of antigen is in no way involved in the fluorescence, it is reasonable to assume that the substance in the serum or in the synovial fluid, combining with synovial tissue antigen, is a circulating anti-synovial tissue antibody.

There are already many demonstrations of autoantibody to tissue by indirect immunofluorescent technic; HUNTER and others²⁶, PARONETTO and coworkers²⁶, TSUJI and colleagues⁶ found it in chronic hepatic disease, KAPLAN²⁷ in rheumatic fever, KLAVINS²⁸ in ulcerative colitis, BEUTNER *et al.*²⁹ in chronic thyroiditis, and WISE and others³⁰ in chronic dermatitis, etc.

From these investigations, there seems to be a possibility that the tissue is first destroyed due to some initiating factors and then secondarily acquires an antigenicity, and the anti-tissue antibody might be produced against this newly formed antigen. In the rheumatoid arthritis, it also appears that such an antibody has no significance in the primary response, but when it is once formed, it

continues vicious circles of antibody formation by persistently repeating antigen-antibody reaction circumscribed in the affected joint, perhaps in cooperation with the cell bound antibody situated in the lesion. The relation between the circulating anti-synovial tissue antibody and the rheumatoid factor must be resolved in the further studies.

SUMMARY

There is as yet no plausible and convincing explanation for the etiology of rheumatoid arthritis. The authors investigated anti-synovial tissue antibody in the body fluid of rheumatoid arthritis by means of indirect immunofluorescent technic using non-affected synovial tissues as antigen.

As the result the anti-synovial tissue antibody was detected in 7 cases of the 15 synovial fluid samples of rheumatoid arthritis and in two out of the six serum samples. The site of the localization of this antibody was demonstrated to be in the synovial membrane, especially in synovial cells and in the small blood vessel walls situated immediately adjacent to the synovial surface, but it was found in no connective tissues other than synovial membrane.

It seems that this anti-synovial tissue antibody should be considered as an independent factor from rheumatoid factor, and that rather than the rheumatoid factor it is more actively associated with the localization and progression of chronic inflammation within the rheumatoid arthritis joint.

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276 K. OGAWA, T. TSUJI, M. NAMBA, K. HAMAYA, T. OKAMOTO and Y. MIWA

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