原　　　著

Monoarticular Transfer of Adjuvant Arthritis by Intraarticular Injection with Sensitized Lymphocytes

Toyoji Uyeo, Yoshinori Tominaga,
Katsuyuki Kasahara and Seisuke Tanaka

Department of Orthopaedic Surgery Faculty of Medicine
Kyoto University (Director Prof. Dr. Tetsuo Ito)
Received for Publication Jan., 10, 1977

Although the immune theory in rheumatoid arthritis has been based on humoral factors, cellular immunity has been also suspected to play an important role in the pathogenesis of rheumatoid arthritis. Pearson (1964)[2] and Whitehous (1969)[8] reported that an adjuvant-induced arthritis was passively transferred in rats by intravenous infusions of viable lymph node cells, spleen cells or thoracic duct lymphocytes from sensitized adjuvant rats to normal rats. It has been shown in a previous report presented by one of us[4] that lymphocytes obtained from rheumatoid patients were not cytotoxic to rheumatoid synovial tissue cells *in vitro*, and rheumatoid lymphocytes significantly stimulated rheumatoid synovial cell proliferation in several cases examined. Lymphocyte-synovial cell interaction in rheumatoid arthritis is considered to be a factor in synovial cell proliferation.

In the present experiment, an attempt has been made to prove the monoarticular transfer of adjuvant arthritis by the intraarticular injection of sensitized lymphocytes, and also to clarify the mechanism for the transfer.

Methods

Animals

Sprague-Dawley rats cultivated in very closed colonies and weighing about 150g at the start of the experiment were used. Animals were healthy specific pathogen free males and were housed in clean conditions. The total number of rats used in this experiment were 115, which were separated into five groups for following experiments.

Preparation of sensitized lymphocytes.

Rats were immunized with 0.2ml of complete Freund's adjuvant (Difco) injected intra-

dermally into the dorsum of the tail root. Blood was collected from the donors on the 14th day after inoculation by cardiac puncture, and lymphocytes were separated by the density gradient centrifugation method using a Ficoll-Conray mixture.

Intraarticular injection of sensitized lymphocytes.

The sensitized lymphocytes were counted, washed three times and suspended in saline. Recipient rats were given an injection of 10^6, 5×10^6, or 10×10^6 lymphocytes in 0.2 ml saline into the right knee joint. As a control, normal unsensitized lymphocytes, which were separated from the peripheral blood of non-immunized donor rats, were injected into the knee joints of normal recipients. In order to attest the serum effect, 0.2 ml of the serum obtained from both adjuvant and normal rats was injected into the knee joints of normal rats respectively. Two weeks after inoculation, these rats were sacrificed and the paraffin sections of synovium in the knee joints were routinely stained with hematoxyline-eosine for histological examination.

Intraarticular injection of sensitized lymphocytes treated with rabbit anti-rat-thymus serum (RARTS).

Preparation of RARTS :

Thymic tissues removed aseptically from 3-week-old male rats were minced, passed through 160 gauge stainless steel wire mesh into saline, washed three times and resuspended in saline. Each of two albino rabbits weighing 3kg was given two intravenous injections of 5×10^8 thymic cells on the 1st and 21st days. On the 28th day, blood was collected by cardiac puncture. Sera was separated by centrifugation and incubated at 56°C for 30 minutes. The cytotoxicity of antiserum was tested *in vitro* against thymocytes from control rats using a trypan blue dye exclusion technique. The titers of thymocytotoxic activity of sera were 1:96 and 1:48, titrated to a level of 70% kill, and the former serum was used for the following experiment. After checking of the thymocytotoxic activity, the serum was stored at $-22°C$ untill used.

Inoculation of sensitized lymphocytes treated with RARTS :

A total number of 4×10^7 cells were obtained from seven rats. The cells were divided into two groups. Each group was incubated in 1.2 ml Hank's balanced solution and 0.06 ml RARTS with or without 0.12 ml of rabbit complement at 37°C for 45 minutes. It was shown by the trypan blue dye exclusions that the lymphocytes incubated without complement were all alive and about 50% of the lymphocytes incubated with complement were alive. Each of these treated lymphocytes were injected into the right knee joints of normal rats respectively. Two weeks after injection, the rats were sacrificed and the synoviums of the knee joints were examined histologically.

Intraarticular injection of both sensitized and non-sensitized lymphocytes killed by repeated freezing and thawing.

Lymphocytes obtained from adjuvant rats or normal donors as described before were killed by repeated freezing and thawing, and the dead cells were injected into the knee joints of normal rats. The number of the injected cells were 10×10^6 for each joint. Two weeks after injection, the rats were sacrificed and synoviums of the knee joints were examined histologically.

Result

Effect of intraarticular injection of sensitized lymphocytes.

Two weeks after injection of the sensitized lymphocytes, marked proliferation of the lining cell and fibrin deposition of the sublining layer were observed. Marked subsynovial fibrosis and infiltration of the chronic inflammatory cells were also observed in such cases. When the increased number of sensitized lymphocytes was injected intraarticularly, the synovial changes of recipient normal rats were more dominant. In the cases injected with 10^6 sensitized lymphocytes, proliferation of the lining cell layer and infiltration in the sublining layer were relatively mild (Fig. 1). Hyperplasia of the lining cell layer was observed locally or widely in rats injected with 5×10^6 lymphocytes. The perivascular aggregations of chronic inflammatory cells were seen in several cases of this group (Fig. 2). Furthermore, fibrosis of the subsynovial layer was observed in most of the cases. When 10×10^6 of sensitized lymphocytes were injected into the normal knee joint, the pathologic changes of the synoviums were much more severe. Hyperplasia of the lining layer with large lining cells in a number of layers was present and diffuse fibrosis of the sublining layer was observed (Fig. 3). When the same number of normal lymphocytes was injected into the normal knee joints, such histological changes of synovitis were not observed except very slightly in the lining cell proliferation (Fig. 4).

Effect of intraarticular injection of adjuvant serum.

Intraarticular injection of adjuvant serum into normal rats produced a slight proliferation of the lining cell layer 14 days after injection, but there was no infiltration of chronic inflammatory cells or fibrosis of the sublining layer (Fig. 5). Injection of normal serum into the normal knee joints did not induce inflammatory changes.

*Effect of RARTS on sensitized lymphocytes for inducing arthritis.*

The pathologic changes of the synovium induced by the antiserum-treated adjuvant lymphocytes were weaker than the synovitis induced by the non-treated adjuvant lymphocytes. This suppressive effect of RARTS was more marked in the cases treated with RARTS with complement than RARTS alone. Although the synovitis induced by sensitized lymphocytes was suppressed by the treatment with antiserum, mild proliferation of the lining cell layer was still observed (Figs. 6, 7).

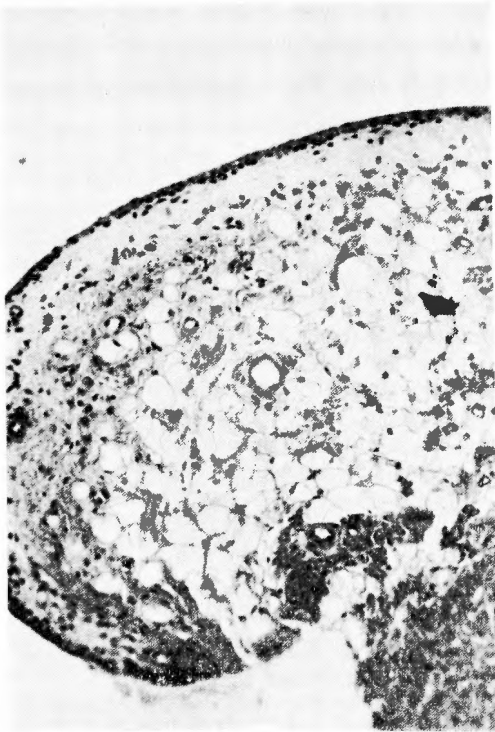*Effect of intraarticular injection of lymphocytes killed by freezing and thawing.*

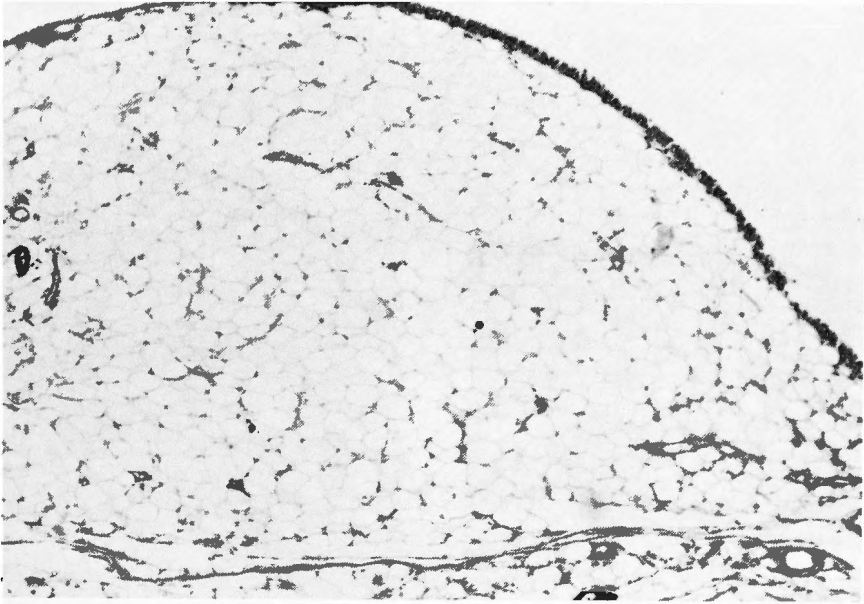The histological change of synovitis induced by the killed sensitized lymphocytes was

1
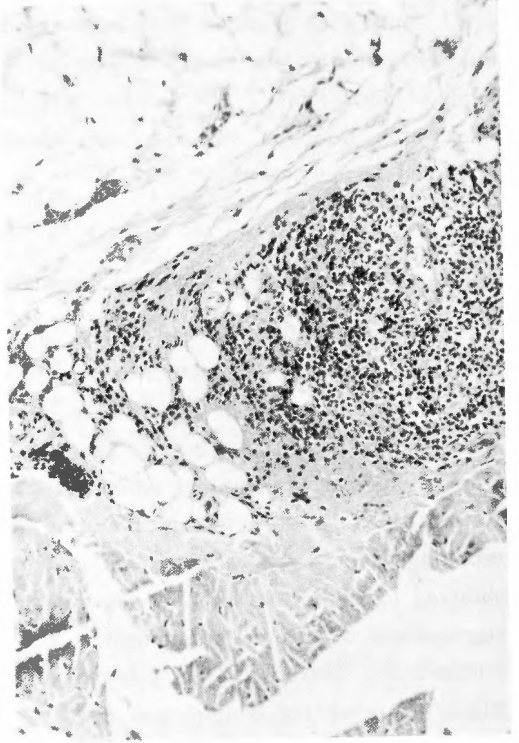
2

3

4

5

Fig. 1. Passively induced arthritis by intraarticular injection of 10^6 cells. Synovitis is relatively mild. (H&E). $100\times$

Fig. 2. 5×10^6 of sensitized lymphocytes were injected into the knee joint of the normal rat. An aggregation of chronic inflammatory cells is seen in the sublining layer. (H&E). $100\times$.

Fig. 3. 10×10^6 of sensitized lymphocytes were injected intraarticularly. Note hyperplasia of the lining layer, infiltration of inflammatory cells and fibrosis in the subling layer. (H&E). $100\times$.

Fig. 4. 10×10^6 of normal lymphocytes were injected intraarticularly. Histological changes of synovitis are not observed except for very slight lining cell proliferation. (H&E). $100\times$.

Fig. 5. Intraarticular injection of adjuvant serum into normal rats produced a slight proliferation of the lining cell layer 14 days after injection. But there is no infiltration of chronic inflammatory cells or fibrosis of the sublining layer. (H&E). $50\times$

observed extensively. Not only the killed adjuvant lymphocytes but also the killed normal lymphocytes induced synovitis, although pathologic change was mild in the knee joints treated with the killed normal lymphocyte as compared with the sensitized lymphocyte (Figs. 8, 9).

Discussion

An adjuvant-induced arthritis has been passively transferred in rats by intravenous infusions of viable lymph node or spleen cells (PEARSON, 1964)[2] or thoracic duct lymphocytes from donor adjuvant rats to normal recipients (WHITEHOUSE, 1969)[8]. In the present investigation, lymphocytes sensitized by Freund's adjuvant were injected intraarticularly and could successfully transfer the arthritis monoarticularly to the injected joint. This approach was to exclude many factors which should be in consideration when lymphocytes
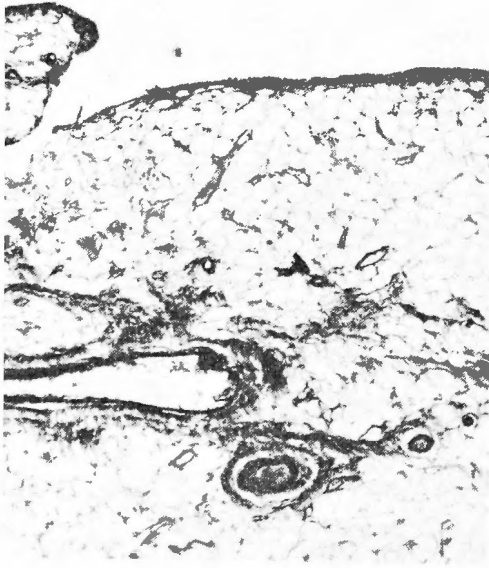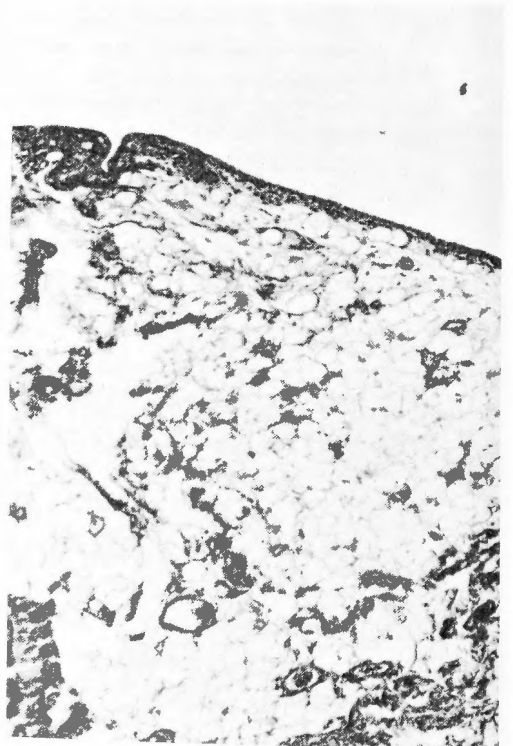
6
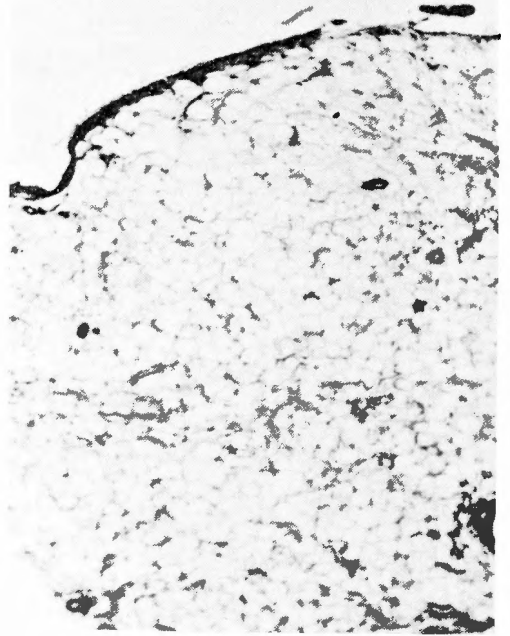
7

8

9

Fig. 6. 10×10^6 of adjuvant lymphocytes were treated with antiserum and injected into the knee joint of the normal rat. The pathologic changes are weaker than the one induced by the non-treated adjuvant lymphocytes. (H&E). 50×.

Fig. 7. 10×10^6 of adjuvant lymphocytes were treated with both antiserum and complement, and injected into the knee joint of normal rats. The synovitis is weaker than the ones induced by the lymphocytes that were treated with antiserum alone. (H&E). 50×.

Fig. 8. 10×10^6 of adjuvant lymphocytes were frozen and thawed three times, and injected into the knee joint of normal rats. The synovitis is observed extensively. (H&E). 50×.

Fig. 9. After 10×10^6 of normal lymphocytes were frozen and thawed three times, they were injected into the knee joints of normal rats. Mild synovitis was observed. (H&E). 50×.

were injected intravenously, and is considered to be useful to clarify the mechanism of the synovial proliferation induced by sensitized lymphocytes. The reaction of the synovium was dose dependent and 10×10^6 lymphocytes injected intraarticularly produced severe histopathological changes in the synovium. Comparing to this, in the intravenous route for administration of sensitized cells to recipients, the minimal number of viable cells necessary for transfer of adjuvant arthritis was 2.0 to 2.3×10^8 [2].

In the present study, the induced synovitis was suppressed by the treatment of adjuvant lymphocytes with RARTS or both RARTS and complement. By trypan blue exclusion, the lymphocytes treated with RARTS without complement were viable in 100% and the ones treated with both RARTS and complement were killed in 50% of the original lymphocytes. Because RARTS used in this experiment was antithymic, presumably T-cells were coated by antibody when treated with the antiserum, and were killed when treated with serum and complement. B-cells were supposed to remained unchanged. The synovitis induced by lymphocytes which were treated with both antiserum and complement, was milder than that induced by lymphocytes which were treated with antiserum alone. These findings suggest the involvement of T-cells being sensitized rather than B-cells as causing the development of passively transferred arthritis, though the B-cell etiology is not definitely excluded.

WAKSMAN et al. (1963) [5] reported that sensitized lymphoid cells killed by heating failed to produce arthritis. In this investigation, however, killed lymphocytes by freezing and thawing induced very severe synovitis in the knee joint injected. Because even killed normal lymphocytes produced typical synovitis, there is a possibility that enzymic factors, especially lysosomal enzymes, released from the destructed cell are a factor of pathologic changes in the synovium [7]. However, the differences in the grade of lining layer hyperplasia, cellular infiltration and sublining fibrosis were observed between synovitis induced by the killed sensitized lymphocyte and that by the killed normal lymphocyte. This findings suggests that adjuvant itself or the immunological factors are also concerned in these reactions. ANDREIS et al. (1974) [1] reported that arthritis was produced in the rabbit by intraarticular injection of a preparation of mediators of cellular immunity (lymphokines)

obtained from the lymphocytes sensitized with keyhole limpet hemocyanin. They suggested that since the mononuclear chemotactic and migration inhibitory factors were present in the supernatants injected, mononuclear cells might be attracted to the lining layer from the blood by the mononuclear chemotactic factor[6] and immobilized at the surface by the migration inhibitory factor[3]. Mediators which induced synovitis in the present experiment may be heat labile intracellular components including lymphokine in immune lymphocytes.

Summary

Monoarticular synovitis was produced in the rat by the intraarticular injection of sensitized lymphocytes obtained from the rats with adjuvant arthritis. The induced synovitis was dose dependent and suppressed by the immunological treatment of sensitized lymphocytes with rabbit anti-rat-thymus serum plus complement. T-cells may be implicated in the pathogenesis of the transfered arthritis. The adjuvant lymphocytes killed by the physical, freezing and thawing methods, produced severe synovitis monoarticularly when injection into the knee joint of normal rats. Intracellular components, including lymphokines, in the sensitized lymphocytes may produce synovitis. Cellular immunity may play an important role in the development of the passive transfer of adjuvant arthritis.

Ackrowledgments

Refere ces

1) Andreis M, Stastny P, Ziff M. Experimental arthritis produced by injection of mediators of delayed hypersensitivity. Arthritis Rheum, **17** : 537-551, 1974.
2) Pearson C.M, Wood F.D. . Passive transfer of adjuvant arthritis by lymph node or spleen cells. J. Exp. Med., **120** : 547-559, 1964.
3) Nathan C.F, Karnovsky M.L, David JR. : Alterations of macrophage functions by mediators from lymphocytes. J. Exp. Med., **133** : 1356-1376, 1971.
4) Uyeo T, McMaster J.H. : Lymphocyte-target cell interaction in various combinations of lymphocytes, synovial cells, skin tissue cells and serum obtained from rheumatoid arthritis and non-rheumatoid controls. Ryumachi, **15** : 476, 1975 (abstract)
5) Waksman B.H, Wennersten C. : Passive transfer of adjuvant arthritis in rats with living lymphoid cells of sensitized donors. Int. Arch. Allerg., **23** : 129-139, 1963.
6) Ward P.A, Remold H.G, David J.R. The production by antigen stimulated lymphocytes of a leucotactic factor distinct from migration inhibitory factor. Cellular Immunol., **1** : 162-174, 1970.
7) Weisman G, Spilberg I, Krahauer K. : Arthritis induced in rabbits by lysates of granulocyte lysosomes. Arthritis Rheum., **12** : 103-116, 1969.
8) Whitehouse D.J, Whitehouse M.W, Pearson C.M. : Passive transfer of adjuvant-induced arthritis and allergic encephalomyelitis in rats using thoracic duct lymphocytes. Nature, **225**, 1322, 1969.

和文抄録

感作リンパ球関節内注入による
アジュバント関節炎の transfer

京都大学医学部整形外科学教室（主任：伊藤鉄夫教授）

上 尾 豊 二, 冨 永 芳 徳
笠 原 勝 幸, 田 中 清 介

　これまで慢性関節リウマチの免疫は主に液性因子の関与が考えられていたが, 近年, 細胞性免疫が重要な役割をしていることが判明している. 我々は, ラットのアジュバント関節炎をモデルとして, 感作リンパ球が関節炎発症にしめる役割を明らかとする目的で, 実験を行った.

　実験は, 体重150gの Sprague-Dawley ラットを使用し, これを Freund's complete adjuvant 0.2ml を尾根部皮内に注入して感作した. 2週後に採血し, Ficoll-Conray 法にてリンパ球を分離した. 100万から1,000万個のリンパ球を正常ラットの膝関節に注入し, 2週後に標本を採取した. 同様に, 正常ラットのリンパ球を採取して, 正常ラットに注入した. 両者を比較すると, アジュバントリンパ球注入群は著明な滑膜表層の肥厚, フィブリン沈着, 炎症細胞の滲潤をみとめ, 正常リンパ球注入群では軽度の滑膜表層の肥厚以外には変化をみとめなかった. 又, アジュバントリンパ球の注入量が多い程, 滑膜炎の程度は高度であった. アジュバントリンパ球を rabbit anti-rat-thymus serum で処置して, 膝関節に注入すると, 滑膜炎の発症は著明に抑制された. しかし, アジュバントリンパ球を繰り返し凍結融解して細胞破壊の上で膝関節に注入しても, 滑膜炎の発症は抑制されなかった. 尚, 血清を分離して膝関節に注入した群では, アジュバント血清でわずかに滑膜表層の肥厚を生じた以外に変化はなかった.

　以上の結果から次の結論が考えられる.

　1) 感作リンパ球の関節内注入により滑膜炎が発症し, この反応は dose-dependent である. 2) T-cell を抑制することにより, 滑膜炎の発症は抑制される.

　3) 物理的にリンパ球を破壊しても, 関節炎は発症することから, lymphokine を含めて何か細胞内の物質が滑膜炎発症因子として働くものと考えられる.