

VARIATION OF LYMPHOCYTES IN PERIPHERAL BLOOD AND BONE MARROW IN COLLAGEN-INDUCED ARTHRITIS

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Collagen-induced arthritis (CIA) was found to be useful as an animal model to investigate the mechanism by which rheumatoid arthritis is developed. We therefore studied variations of T and B lymphocytes in peripheral blood and bone marrow in order to ascertain the importance of T and B lymphocytes in the development of CIA. T lymphocyte, which is considered to be associated with the onset and deterioration of CIA, was not changed during the course of CIA, whereas B lymphocyte, which produces anti-type II collagen antibody regarded as an indispensable factor in CIA induction, was significantly decreased in peripheral blood and bone marrow at 10 days after the 2nd immunization when arthritis was able to be detected. In bone marrow, although two cell populations of Ly5/B220-positive cells were detected, only the cell population with lower fluorescence intensity was decreased transiently. These results suggest that B lymphocyte in both bone marrow and peripheral blood is intimately involved in the course of CIA.

KEYWORDS collagen-induced arthritis; lymphocyte; bone marrow; flow cytometry

Collagen-induced arthritis was developed by Trentham¹⁾ as an animal model of polyarthritis that can be induced in susceptible rats, mice and monkeys by immunization with native type II collagen and as one of the models with a similarity to rheumatoid arthritis (RA).²⁾ Studies thus far have indicated that the development and regulation of arthritis are associated with humoral and cellular immunity to collagen, as evidenced by the induction of the arthritis by passive transfer of antibody against type II collagen³⁾ or sensitized T lymphocytes.⁴⁾ In addition, CIA is an attractive model because anti-type II collagen antibody is detected in serum and synovial fluid of rheumatoid patients at a considerable rate of incidence.^{5,6)} Histopathological observation indicates that CIA is similar to human RA in which the lesion is one of synovial proliferation that progresses to pannus formation and results in marginal erosions with extensive destruction of cartilage.⁷⁾ Pathological speculation, that a specific cell may play a critical role in the cause of the arthritis, has been confirmed by the results that CD8+, Leu-7+ cells,⁸⁾ and CD45RBdim memory T cells⁹⁾ increase in synovial fluid in RA patient joint and that the expression of CD7 in T cells decreases in the peripheral blood and in the synovium.¹⁰⁾ Furthermore, in bone marrow adjacent to joints affected by RA, abnormal myelopoiesis^{11,12)} and elevated activity of myeloid growth factor¹³⁾ were observed, thus suggesting that bone marrow cells were involved in the pathogenesis of RA. We therefore investigated variations of mononuclear cells in peripheral blood and bone marrow in mouse CIA in order to clarify the role of lymphocytes in the course of CIA.

MATERIALS AND METHODS

Animal Male DBA/1J mice were purchased from Seiwa experimental animal laboratory, Fukuoka, Japan. All mice were used at 8-12 weeks of age.

Collagen Bovine type II collagen was obtained from Cosmo-Bio.Co, Tokyo, Japan.

Immunization Collagen for immunization was dissolved in 0.1M acetic acid at a concentration of 2mg/ml by stirring at 4°C. Mice were primed by injection at the base of the tail with 100µg of collagen emulsified in complete Freund's adjuvant (Difco Laboratory). Three weeks later, the mice received a booster injection of 100µg of collagen emulsified in incomplete Freund's adjuvant (Difco Laboratory). Mice were observed daily for the presence of arthritis, and severity was graded visually according to the method of Wood.¹⁴⁾

Flow Cytometric Analysis Prepared cells were treated with lysing solution (Becton Dickinson Co.) for the purpose of removal of erythrocytes and fixation. After 10 min incubation at room temperature, the cells were washed with phosphate-buffered saline (PBS) and incubated with FITC- or PE-conjugated monoclonal antibody (anti-Thy1,2, anti-Ly5/B220, anti-L3T4, and anti-Lyt2 antibodies) at an appropriate concentration at 4°C for 1h. The cells were then

washed twice, resuspended in PBS, and analyzed by a FACSscan (Becton Dickinson). Data were analyzed using Consort 30 and "paint a gate" software.

Statistical Analysis Statistical significance was determined by Student's t test, and each value was given at mean \pm S.E.M.

RESULTS AND DISCUSSION

Inflammation of the rheumatoid joint is considered to start with infiltration of polymorphonuclear cells and mononuclear cells, resulting in proliferation of the synovial membrane and eventually degradation of the articular cartilage.

In addition, in epiphyseal bone marrow adjacent to the affected joints of patient with RA, abnormal myeloid cells and their growth factors were observed; high levels of interleukin 1 and 6 were also detected in bone marrow of rat with adjuvant-induced and collagen-induced arthritis, thus indicating that bone marrow could play a significant role in the development of arthritis. We therefore investigated variation of the cell population in peripheral blood and bone marrow using several kinds of antibody against mononuclear cell surface antigen during the course of CIA. In mouse CIA, two immunizations are necessary to induce arthritis. Therefore, we primed mice with 100 μ g of type II collagen at day 0 and then boosted mice with same amount of collagen at day 20. Flow cytometric analyses indicated that anti-Ly5/B220 antibody recognized one cell population in peripheral blood but two cell populations in bone marrow (data not shown). The number of Ly5/B220-positive cells in peripheral blood was not changed after the primary immunization, whereas it decreased more than 15% at 10 days after the 2nd immunization when the signs of arthritis began to be detected (Fig. 1a). At day 40, the Ly5/B220 positive cells recovered to control level.

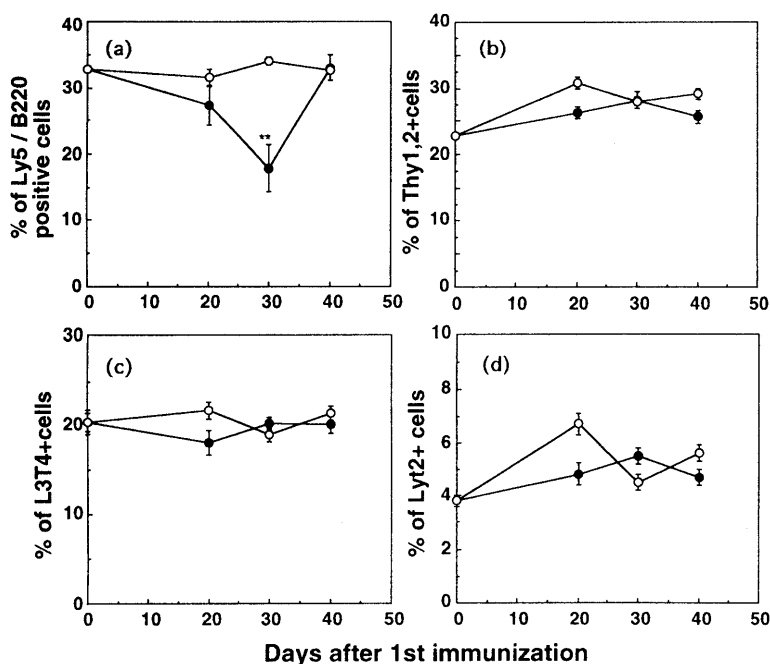


Fig. 1. Variation of Ly5/B220, Thy1,2, L3T4, Lyt2-Positive Cells in Mouse Peripheral Blood

Mice were injected intradermally with 100 μ g of type II collagen at day 0 and then received a booster injection of same amount of collagen at day 20. Blood collected in tubes containing 10mM EDTA in PBS was stained with fluorescein isothiocyanate- or phycoerythrin- conjugated anti-Thy 1,2, anti-Lyt 2, anti-Ly5/B220 or anti-L3T4 antibodies after erythrocytes were lysed with lysing solution and analyzed with FACSscan. (a) Ly5/B220⁺, (b) Thy1,2⁺, (c) L3T4⁺, (d) Lyt2⁺ cells. Open circle: normal mouse, Closed circle: mouse immunized with collagen.

** p<0.01 vs control group (n=5).

However, T lymphocyte population in peripheral blood, for instance, Thy1,2, L3T4, Lyt1,2-positive cells, did not change during the course of CIA (Fig. 1b-d), though many investigators have reported variation of T lymphocytes in inflamed joint. We next examined the cell population in bone marrow. In analysis using parameter of forward scatter and side scatter, no change was observed. However, Ly5/B220-positive cells, especially those with lower fluorescence intensity, decreased by about 5% at 10 days after the 2nd immunization, in a similar fashion to peripheral blood (Fig. 2). During the course of CIA, fluorescence intensity in Ly5/B220-positive cells was not changed in any cell population, which suggested that CIA development did not influence the expression of Ly5/B220 but did decrease the Ly5/B220-positive cell number. There are few reports that examine cell population in the course of initiation and deterioration of CIA. Hayashida¹⁵⁾ has reported that the number of myeloid cells increases progressively in bone marrow of rats afflicted with CIA. We also determined the increment of myeloid cells in rat bone marrow during the course of CIA, but not that in mouse bone marrow.

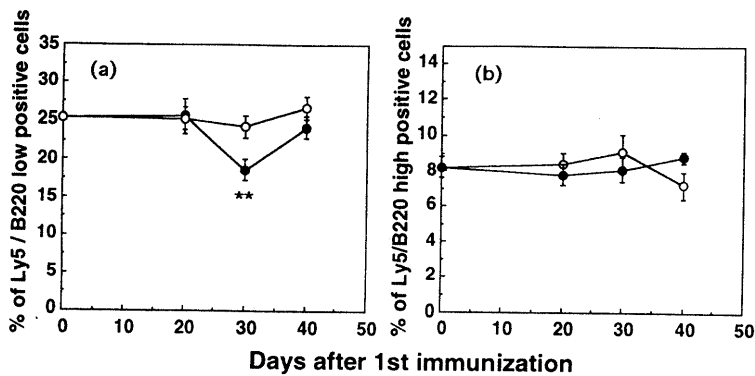


Fig. 2. Variation of Ly5/B220-Positive Cells in Bone Marrow

(a) Ly5/B220⁺ cells with high fluorescence intensity, (b) Ly5/B220⁺ cells with low fluorescence intensity.

Open circle: normal mouse, Closed circle: mouse immunized with collagen. ** $p < 0.01$ vs control group ($n=5$).

The reason a different result was observed is that the immune response to collagen depends on animal species, evidenced by using different methods in order to induce arthritis in rat or mouse. The activation of humoral and cellular immunity is essential for the onset of CIA, and polyclonal activation of B lymphocytes in autoimmune diseases produces autoantibodies.¹⁶⁾ Thus the elevation of immunity appears to be necessary to induce CIA. However, we detected that Ly5/B220-positive cell number decreased around the day of onset of CIA and that Ly5/B220-positive cells in both peripheral blood and bone marrow had a tendency to decrease more in mice with severe arthritis than in those with slight arthritis, thus indicating that B lymphocytes would be closely associated with the pathogenesis of CIA. In RA patient, B lymphocytes are found to appear in the synovium around the time when angiogenesis begins to be observed in the depth of the synovium. Thus there is a possibility that B lymphocytes accumulate in a part of the synovium at a stage of CIA, resulting in antibody production being significantly accelerated in the synovium. At present, we don't know whether the decrement of Ly5/B220-positive cells results from the transfer of the cells to the synovium or to some lymph node. However, since antibody against type II collagen is known to be essential for prolonged arthritis, the phenomena that we found in this experiment probably support the idea that B lymphocytes move to the site where arthritis will occur, and induce abnormal local antibody production in concert with T lymphocytes. The variation of Ly5/B220-positive cells not only in peripheral blood but also in bone marrow further supports the possibility that some humoral factor may play an important role in the onset of CIA. To clarify the mechanism by which CIA is developed, further detailed study is underway.

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