74. B Cell Stimulatory Factor 2 (BSF2/IL-6) and Rheumatoid Arthritis

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(Systemic autoimmune diseases represented by systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are characterized by hyperactivation of B cells and the production of various autoantibodies. Several studies in murine systems suggest that the deregulation of lymphokine production or the production of abnormal lymphokines could be responsible for hyperactivation of autoantibody-producing B cells.1,2)

Several interleukins are involved in the regulation of the B cell response into antibody-producing cells. These interleukins are divided into three categories, (1) a factor mainly involved in the activation of resting B cells (BSF-1, IL-4), (2) a factor for the growth of activated B cells (BCGF-II/IL-5) and (3) a factor for the final differentiation of B cells into antibody-secreting cells (BSF-2/IL-5).3 The cDNAs for these three interleukins have been cloned4~6 and the mechanisms regulating antibody production in B cells have become amenable to investigation at the molecular level. BSF-2 is involved in the final maturation of B cells into antibody-producing cells as well as in the growth of plasmacytoma cells.7,8 A previous study10 demonstrated that several tumor cells, such as cardiac myxomas and cervical cancer cells, aberrantly produced BSF-2 and patients with such tumors showed autoantibody production and autoimmune symptoms. The application of the cDNA, recombinant molecules and the antibody against BSF-2 has made it possible to examine the relationship between the abnormal regulation of the BSF-2 production and autoimmune diseases. This report demonstrates an abnormal increase in BSF-2 production by synovial tissues from active rheumatoid arthritis (RA) patients.

The concentration of BSF-2 in the synovial fluid from the affected joints was measured by a radioimmunoassay (RIA) utilizing polyclonal anti-BSF-2 antibody. Patients with RA satisfied more than 5 points of the diagnostic criteria determined by the American Rheumatism Association. Patients with osteoarthritis (OA) showed monoarthritis with hydrops, no erosive changes of bone and negative RA test. Twenty two out of 25 specimens (92%) from RA patients showed detectable levels of BSF-2. The mean value of BSF-2 in these 22 samples was

* This study was supported by a Grant-in-Aid to T. K. for the special project research from the Ministry of Education, Science and Culture, Japan.
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15.6±13 ng/ml (2.6~59 ng/ml). However, only 4 out of 17 specimens (24%) obtained from OA patients displayed detectable but low level of BSF-2 (3.4~5.8 ng/ml). The same synovial fluids of RA patients were tested for the levels of IL-1. Only 3 out of 25 specimens displayed a detectable level of IL-1 and the level of IL-1 was not correlated with that of BSF-2; a sample (#116) did not include any BSF-2 but showed the highest level of IL-1α and β. In the other 22 specimens, IL-1 was not detectable either by ELISA assay or by thymocyte co-stimulatory assay. In order to test whether synovial fluids from RA patients actually contain biologically active BSF-2, several samples were examined for BSF-2 activity utilizing murine hybridoma clone, MH60.BSF2, which depends on BSF-2 for its growth. Data showed that synovial fluid, in fact induced the uptake of 3H-TdR in MH60.BSF2 cells. Furthermore, one of the BSF-2 positive synovial fluids (patient #110) was affinity-purified using an anti-BSF-2-conjugated Sepharose CL 4B column and it was demonstrated that the eluate induced IgM-production in another BSF-2-responsive B cell line, SKW6-Cl-4 cells. These results indicated that biologically active BSF-2 was present in the synovial fluids from RA patients.

In order to examine whether synovial fluid cells produce BSF-2 in vivo, the expression of the BSF-2 mRNA in freshly obtained synovial fluid mononuclear cells (MNCs) was examined. A variety of level of the BSF-2 mRNA was detected in all synovial fluid MNCs examined and several cases showed a striking increase in the mRNA. Furthermore, the synovial tissues (18 samples) obtained from the active RA patients by joint biopsy, when incubated for 24 hr, produced large amounts of BSF-2 (mean value was 212±165 u/ml). The culture supernatant induced IgM-production in SKW6-Cl-4 cells and 3H-TdR uptake in MH60.BSF2 cells and both activities were completely neutralized with anti-BSF-2 antibody, indicating that the active molecule was indeed BSF-2.

Previously, Al-Balaghi et al. reported that a B cell differentiation factor (BCDF) activity, which could induce Ig-secretion in activated B cells, was present in synovial fluids of RA patients. By employing anti-BSF-2 antibody, we have demonstrated that all BCDF activity can be attributed to BSF-2. Therefore, it is very likely that the BCDF previously detected in the synovial fluids of RA patients was BSF-2. It has been reported that T cells of MRL/lpr mouse constitutively produce a kind of BCDF (I-BCDF) and B cells of Motheaten mouse spontaneously produce B cell maturation factor(s). The constitutive production of these factors could be related to the genetically determined autoimmune diseases. Furthermore, we previously demonstrated the results suggesting that unregulated production of BSF-2 might be responsible for the autoimmune conditions in patients with cardiac myxoma and uterine cervical cancer. The present result further demonstrated an abnormal increase in BSF-2 production in the affected joints of RA patients. Taken all results into accounts, it may be concluded that the unregulated productions of BSF-2 may play an important role in the pathogenesis of RA: the infiltration of a large number of plasma cells into synovial tissues and the production of various autoantibodies including rheumatoid factor.

Recent studies indicate that recombinant BSF-2 can stimulate hepatocytes to produce acute phase reactants such as β-fibrinogen, α2-macroglobulin and C-reactive protein. Therefore, several generalized symptoms in RA patients could also be explained by the overproduction of BSF-2. It is interesting that MRL/lpr mice show an increase in CRP but its level is normal in NZB mice. This observation might suggest the involvement of BSF-2 in the autoimmune
disease of MRL/1pr mice. BSF-2 gene expression can be induced by IL-1. Therefore, the question to be asked is whether the abnormal production of BSF-2 in synovial cells was due to IL-1 stimulation. However, we could not detect IL-1 activity in synovial fluid in most of those specimens tested. Therefore, IL-1 is unlikely as causing agents of BSF-2 production and the molecular mechanism causing the unregulated expression of the BSF-2 gene in synovial tissues is the target for the study which reveals the pathogenesis of RA.

References

7) Hirano, T. et al. (1986): ibid., 324, 73–76.