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2-2). The Expression of LFA-1, ICAM-1, CD80 and CD86 Molecules in Lupus Patients: Implication for Immunotherapy

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Systemic lupus erythematosus (SLE) is a systemic disease characterized by generalized autoimmunity consisting of hyperactive T cells help and hyperactive B cell synthesis of immunoglobulins. We have detected activated T cells in peripheral blood lymphocytes (PBL) obtained from SLE patients by the expression of major histocompatibility complex (MHC) class II molecules, interleukin (IL)-2 receptor (IL-2R), serum soluble IL-2R and proliferating cell nuclear antigen in nuclei (1), and have been studying their immunologic function and association with disease activity. In this report, we studied the expression of costimulatory molecules, CD80 and CD86, and adhesion molecules, LFA-1 and ICAM-1 in lupus patients. In addition, we showed an application of anti-CD80 monoclonal antibodies in autoimmune diseases such as SLE and mixed connective tissue disease (MCTD).

Expression of LFA-1 and ICAM-1

The expression of LFA-1 and ICAM-1 on PBL in various connective tissue diseases was studied (Table 1). LFA-1 α and LFA-1 β were highly detected on CD4 and CD8 positive T cells in PBL obtained from patients with connective tissue diseases, and the expression of these molecules was significantly increased on T cells in lupus and MCTD patients compared with that of normals. In addition, the expression of ICAM-1 on B cells in lupus patients was significantly increased compared with that of normals.

Then, we studied the effect of the corticosteroid and intravenous cyclophosphamide pulse therapy (steroid pulse and CY

pulse, respectively) on the expression of LFA-1 and ICAM-1 on PBL in lupus patients. The expression of ICAM-1 on B cells decreased after steroid pulse, and the expression of LFA-1 on T cells decreased after CY pulse (Fig. 1A, B). The implication of these findings is that corticosteroid is effective for B cell abnormalities and cyclophosphamide is effective for T cell abnormalities. However, the elevation of the expression of LFA-1 on T cells and ICAM-1 on B cells was observed after CY and steroid pulse, respectively. Therefore, immunotherapies such as steroid and CY pulse are not ideal to normalize the expression of adhesion molecules on PBL in lupus patients.

Expression of costimulatory molecules, CD80 and CD86 on PBL in lupus patients

T cell activation requires at least two distinct signals (2). The first signal is interaction of the antigen-specific T cell receptor (TCR) with its antigen presented on MHC (3). The second signal is received from interaction with accessory or costimulatory molecules (4). Recently, the interaction between CD28 on T cells and CD80 (B7-1)/CD86 (B7-2) on antigen-presenting cells (APCs) was reported as the ligand/receptor pair, which can provide the necessary second signal to overcome the anergic state (5) (Fig. 1). CTLA-4, which has a homologous structure with CD28, can bind to CD80 and CD86, and cooperatively regulate T cell adhesion and activation. Although these data show CD80 and CD86 expressed on APCs play an important role in T cell activation, recent studies have revealed that CD80 can also be detected on activated T cell

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Table 1. CD80 Positive Lymphocytes in Patients with SLE and Other Connective Tissue Diseases

Disease	Number	CD80/ALL	CD80/CD3	CD80/CD19
SLE	26	11.70±6.77% ^{**}	4.15±3.53% ^{**}	67.75±17.93% ^{***}
MCTD	8	6.45±2.11	2.97±2.91	55.28±20.01
RA	18	6.15±3.97	2.61±1.38	48.82±25.45
Normal	22	4.82±1.86	1.41±0.66	35.43±11.74

*p<0.001 **p<0.05 ***p<0.03 SLE: systemic lupus erythematosus, MCTD: mixed connective tissue disease, RA: rheumatoid arthritis.

Figure 1. T cell proliferation and costimulatory molecules, CD80 and CD86.

clones and T cells activated repeatedly *in vitro*, and T cells infiltrated in skin lesion and rheumatoid synovial T cells *in vivo* (5). These data showed that CD80 was preferentially expressed on activated T cells infiltrating tissues *in vivo*, but it was not on T cells in the peripheral blood. Therefore, we attempted to study the expression of CD80 and CD86 on PBL including T cells in SLE patients (6).

Monoclonal antibodies against CD80 and CD86 were used for flow cytometry and the expression of those molecules on PBL was studied. CD80 was detected on CD3⁺ and CD19⁺ cells in patients with SLE and it was significantly higher than that of normals (Table 1). Since CD80 was detected not only on B cells but also on T cells, the expression of CD86 on T cells was also examined. As shown in Fig. 2, CD86 was also highly detected on T cells in lupus patients, and it was shown that patients with a higher expression of CD86 were distinct from the patients with higher expression of CD80. Then we studied the subset of T cells positive for CD80 and CD86, found that CD80 was mainly detected on CD4 positive cells and CD86 was mainly detected on CD8 positive cells in SLE. When the phenotype of CD80⁺CD4⁺ and CD86⁺CD8⁺ T cells was analyzed, they were CD25 or HLA-DR positive CD45RA⁻ and CD45RO⁺ activated memory T lymphocytes. The percentage of CD80⁺ cells in CD4⁺ cells increased in the active stage of SLE, and it

Figure 2. Expression of CD80 and CD86 on peripheral blood T cells obtained from SLE patients. **Figure 3. Relationship between CD80⁺CD4⁺ T cells and SLE disease activity index (SLEDAI).**

Table 2. Inhibition of Production of Immunoglobulin by Anti-CD80 in SCID Mice

Disease	Name	Human IgG Kchain (μg/ml)		Human IgM (μg/ml)	
		PBS	anti-CD80	PBS	anti-CD80
SLE	K. S.	0	0	0	0
	K. H.	1,480	0	1.6	0
	S. S.	0	0	0	0

SLE: systemic lupus erythematosus, IgG: immunoglobulin G, IgM: immunoglobulin M.

was significantly correlated with the SLE disease activity index (Fig. 3).

Application of anti-CD80 monoclonal antibody in autoimmune diseases

Folzenlogen et al reported that CD80 and CD86 are highly expressed on B cells on PBL in SLE, and suggested that the overexpression of the costimulatory molecules on circulating B cells in patients with SLE plays a major role in the production of continuous autoreactive T cell help for B cells, leading to the production of autoantigens (7). Our results also support this speculation showing the significantly higher expression of those molecules on B cells and T cells. These data suggest that the immunotherapy to inhibit the binding of CD28/CTLA-4 and the costimulatory molecules, CD80/CD86 is useful to treat patients with SLE. In fact, Nakajima et al showed that treatment with a combination of anti-CD80 and CD86 monoclonals before onset of lupus completely prevented autoantibody production and nephritis, and prolonged survival in NZB/W F1 lupus mice (8).

We then attempted to study the effect of anti-CD80 monoclonal antibodies in autoimmune disease using SCID mice that received an intraperitoneal injection of PBL obtained from patients with SLE, MCTD and Sjögren's syndrome. Two out of 4 SCID mice which received PBL from SLE and 1 out of 2 SCID mice which received PBL from MCTD produced human γ -globulin and antibodies to U1 ribonucleoprotein (RNP). Using this system, anti-CD80 monoclonals were injected every week for 4 months intravenously, and the production of human γ -globulin and autoantibodies was tested. One out of 3 SCID mice which received PBL from lupus patients produced human immunoglobulin (Ig) G and IgM, and it was completely inhibited by anti-CD80 (Table 2). Since the specific autoantibodies were not detected in the SCID mouse that received PBL

from the patient, we could not show the inhibition of autoantibody production by anti-CD80 in this mouse. But these data suggest that the application of anti-CD80 is useful to treat patients with SLE.

In summary, we showed the increased level of adhesion molecules, LFA-1 and ICAM-1, and costimulatory molecules, CD80 and CD86 on PBL including T cells in lupus patients. Our data suggest that the application of monoclonal antibodies to these costimulatory molecules is useful for the treatment of SLE by inducing an anergic state on T cells in SLE patients.

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