Expression of Costimulatory Molecules CD80 and CD86 in T Cells from Patients With Systemic Lupus Erythematosus

Chung-Ming Huang, Tsai-Hsiu Lin[1], Hsiu-Jain Wang[1], Chi-Lan Chen[2]

Division of Immunology and Rheumatology, Department of Internal Medicine,

[1]Department of Clinical Laboratory, China Medical College Hospital,

Taichung; [2]Foo Yin Institute of Technology, Kaohsiung, Taiwan, R.O.C.

Objective. The aim of this study was to assess the effects of costimulatory molecules CD80 and CD86 expression in T cell subsets of peripheral blood mononuclear cells (PBMCs) obtained from both patients with systemic lupus erythematosus (SLE) and healthy subjects.

Methods. CD80 and CD86 expressions in PBMCs were quantified by flow cytometry and monoclonal antibodies.

Results. Expressions of CD80 and CD86 were higher in both $CD4^+$ and $CD8^+$ T cell subsets from SLE patients when compared with those from healthy subjects, although the differences were significant only in the $CD4^+$ $CD80^+$, $CD4^+$ $CD86^+$, and $CD8^+$ $CD86^+$ groups. After stimulation with PHA, the percentages of CD80 and CD86 in T-cell subsets were significantly lower in SLE patients compared with the control subjects for $CD4^+$ $CD80^+$, $CD4^+$ $CD86^+$ and $CD8^+$ $CD86^+$. There were no significant correlations between T cell subset expressions of CD80 and CD86 and disease activity parameters which included Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), C3, C4 and anti-dsDNA.

Conclusions. The results suggest that $CD4^+$ $CD80^+$, $CD4^+$ $CD86^+$, and $CD8^+$ $CD86^+$ are expressed in PBMCs of patients with SLE in vivo, but are not associated with the disease activity of SLE. PBMCs in patients with SLE may have an intrinsic defect that alters their activator process which might explain some of the T-cell immunoregulatory abnormalities observed in these patients. **(Mid Taiwan J Med 2002;7:7-13)**

Key words

activated T cell, CD80, CD86, systemic lupus erythematosus

INTRODUCTION

Optimal T cell activation requires two distinct signals. One is antigen specific, and is provided by the interaction between major histocompatibility complex (MHC)-peptide complexes and T-cell receptors (TCR). The other signal is non-specific, and is produced by the interaction of accessory or costimulatory molecules of the B7 family, namely B7-1 (CD80) and B7-2 (CD86) expressed in antigen-presenting cells (APC) [1-3]. CD80 and CD86 are members of the immunoglobulin (Ig) supergene family. They are expressed in activated B cells, macrophages and dendritic cells, and bind to two counter-receptors, CD28 and CTLA-4, expressed in T lymphocytes and thymocytes. CD28-B7 (CD80 and CD86) interaction prevents the induction of energy in antigen-stimulated T cells, whereas

Received : September 5, 2001. Revised : December 6, 2001.
Accepted : January 2, 2001.

Address reprint requests to : Chung-Ming Huang, Department of Internal Medicine, Division of Immunology and Rheumatology, China Medical College Hospital, No 2, Yuh-Der Road, Taichung 404, Taiwan, R.O.C.

blockage of this pathway results in tolerance [4-6].

In vitro and in vivo experiments have shown that activated human T cells express CD80 and CD86 [7-9]. Sansom and Hall demonstrated that B7 was found in T cell clones and in repeatedly activated peripheral T cells [8]. Moreover, CD80 and CD86 have been detected in T cells in rheumatoid synovial fluid and membranes [10,11]. Recently, it was found that CD80 and CD86 were expressed in circulating T cells in the peripheral blood of patients with systemic lupus erythematosus (SLE) [12].

SLE is an autoimmune disease characterized by functional alteration of both T and B lymphocytes. Peripheral blood lymphocytes obtained from lupus patients often contain T cells that exhibit signs of activation in vivo as demonstrated by increased expressions of MHC class II molecules [13] and serum-soluble interleukin-2 receptors (IL-2R) [14]. Several previous reports have revealed that cells from SLE patients display lower responses to stimulation assays when compared with cells obtained from healthy donors [15,16]. Therefore, the aim of this study was to investigate the expression of CD80 and CD86 in the T cell subsets of peripheral blood mononuclear cells (PBMCs) obtained from lupus patients and healthy donors. We also evaluated the differential expressions in lymphoid subpopulations at rest and after stimulation with phytohaemagglutinin (PHA).

MATERIALS AND METHODS

Subjects

Samples of peripheral blood were obtained from 22 patients with SLE (1 male and 21 females; mean age: 30.2 years; range: 16–56 years), diagnosed according to the American College of Rheumatology criteria [17]. Twenty gender- and age-matched healthy hospital employees (1 male and 19 females; mean age: 29.1 years; range: 19–53 years) served as the control group. Lupus disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [18]. Fifteen of the 22 SLE patients received prednisolone (5–60 mg) and/or immunosuppressive drugs (hydroxychroloquine 200–400 mg and/or azathioprine 50–100 mg) at the time of the study.

Isolation of Mononuclear Cells

PBMCs were isolated from heparinized blood by a Ficoll-Hypaque gradient. After three washes in HBSS (Hank's balanced salt solution), cells were adjusted to 1×10^6/mL for the following experiment.

Monoclonal Antibodies and Immunofluorescence Analysis of Lymphocytes

The following monoclonal antibodies (MoAbs) were used in this study: phycoerythrin (PE)-labeled anti-CD86 (B70/B7-2, FUN-1, IgG-K; Phamingen, San Diego, California) and PE-labeled anti-CD80 (L307.4, murine IgG1; Becton Dickinson, San Jose, California). In this experiment, the following reagents were also used: fluoresein isothiocyanate (FITC)-labeled anti-CD3 (SK7, IgG1), FITC-labeled anti-CD4 (SK3, IgG1), and FITC-labeled anti-CD8 (SK1, IgG1). Fluorescence-conjugated antihuman MoAbs of FITC were purchased from Becton Dickinson. FITC- and PE- conjugated mouse IgM or IgG served as isotype controls. PBMCs (1×10^6/mL) were stained immediately after isolation with FITC-labeled antibodies, PE-labeled anti-CD80 and PE-labeled anti-CD86. After two-color staining, the cells were analyzed by flow cytometry (FACScar plus: Becton Dickinson).

PHA Activated Peripheral Blood

Freshly isolated PBMCs were cultured in RPM1 containing 10% fetal calf serum, 1% L-glutamin, 1% penicillin-streptomycin and PHA (5 μg/mL) on 96-well plates for 2 days. Then the cells were stained with antibodies as described above.

Statistical Analysis

The nonparametric Mann-Whitney U test was used for the statistical analysis of the

Table 1. Expressions of CD80 and CD86 in freshly isolated and activated T-cell subsets from SLE patients and healthy control subjects

Cell subset	Condition	Mean ± SD	
		SLE (n = 22)	Control (n = 20)
$CD3^+CD80^+$	Medium only	3.27 ± 4.37	2.55 ±3.44
	+PHA	7.64 ±4.84	8.05 ±3.52
$CD4^+CD80^+$	Medium only	1.09 ±1.15*	0.35 ± 0.59
	+PHA	2.50 ± 1.68‡	4.65 ± 2.11
$CD8^+CD80^+$	Medium only	2.59 ± 4.66	1.00 ± 0.92
	+PHA	7.18 ± 5.34	7.80 ± 3.46
$CD3^+CD86^+$	Medium only	9.50 ±5.77*	5.00 ±3.21
	+PHA	11.0 ±5.52‡	17.25 ± 6.84
$CD4^+CD86^+$	Medium only	5.55 ±3.89*	3.10 ± 2.07
	+PHA	3.86 ± 2.53†	10.40 ± 5.07
$CD8^+CD86^+$	Medium only	6.86 ± 5.46†	2.45 ±1.28
	+PHA	8.27 ± 4.48*	12.65 ± 5.47

*$p < 0.005$ when compared with controls; †$p < 0.01$ when compared with controls; ‡$p < 0.05$ when compared with controls.

groups. The correlations between B7 in T lymphocytes and SLE disease activity indices, including SLEDAI, C3, C4 and anti-dsDNA, were analyzed by the Spearman correlation coefficients test. A p value less than 0.05 was considered significant. All statistical calculations were performed by the SAS program (version 6.12 for Windows 95).

RESULTS

CD80 in $CD3^+$ cells was detected in higher amounts in patients with SLE than in $CD3^+$ cells of healthy subjects. However, the differences were not statistically significant. The mean percentage of CD86 in $CD3^+$ cells was significantly higher in SLE patients compared with the control subjects (9.50 ± 5.77 *vs* 5.00 ± 3.21%, $p < 0.005$). The mean percentage of $CD80^+$ cells in $CD4^+$ cells was significantly higher in the SLE patients than in the control subjects (1.09 ± 1.15 *vs* 0.35 ± 0.59%, $p < 0.005$) (Fig. 1). CD86 was highly expressed in the $CD4^+$ and $CD8^+$ T-cell subsets of the SLE patients. There were significant differences in the mean percentages of $CD4^+$and $CD8^+$ cells expressing CD86 between the SLE patients and healthy controls (5.55 ± 3.89 *vs* 3.10 ± 2.07%, $p < 0.005$ and 6.86 ± 5.46 *vs* 2.45 ± 1.28%, $p < 0.01$, respectively), (Table 1).

Stimulation with PHA increased the expressions of CD80 and CD86 in T cell subsets ($CD3^+$, $CD4^+$ and $CD8^+$) of the SLE and control groups, except in the SLE of $CD4^+CD86^+$ groups. The mean percentage of $CD80^+$ expression in CD4 cells was significantly lower in the SLE patients than in the control group (2.50 ± 1.86 *vs* 4.65 ± 2.11%, $p < 0.05$) following PHA stimulation (Table 1). Similarly, there were significantly lower levels of $CD3^+CD86^+$ (11.05 ± 5.52 *vs* 17.25 ± 6.84%, $p <$

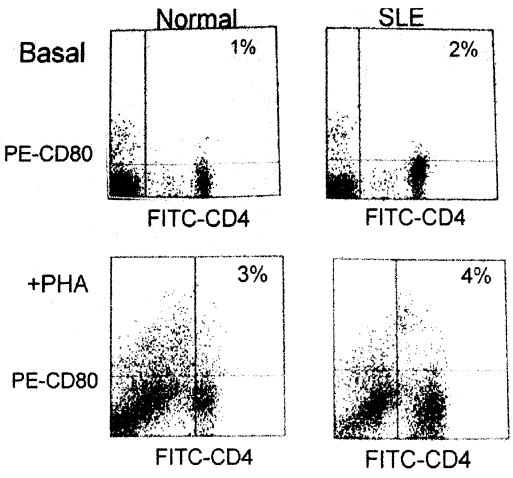

Fig. 1 Expression of CD80 on $CD4^+$ T cells of the peripheral blood mononuclear cells. A two-color analysis of unstimulated (upper panels) and PHA-activated PBMCs of a SLE patient (right side) and a normal control (left side). Cells were stained as described under Materials and Methods.

0.05), $CD4^+CD86^+$ (3.86 ± 2.53 *vs* $10.40 \pm 5.07\%$, $p < 0.01$) and $CD8^+CD86^+$ (8.27 ± 4.48 *vs* $12.65 \pm 5.47\%$, $p < 0.005$) in SLE patients after PHA stimulation, when compared with healthy subjects. There were no significant differences in expressions of CD80 and CD86 in T-cell subsets before or after PHA stimulation between SLE patients with and without prednisolone (data not shown).

We studied the relationships between the percentages of different T-cell subsets in the SLE patients and the disease activity parameters, including SLEDAI, C3, C4 and anti-dsDNA. There was no significant correlation between T cell subsets of CD80, CD86 and SLEDAI. Likewise, there was no significant correlation between C3, C4, anti-dsDNA and expressions of CD80 and CD86 in T cell subsets of the SLE patients.

DISCUSSION

B7 molecules (including CD80 and CD86) are expressed in antigen-presenting cells (APC) including monocytes, macrophages, B cells, dendritic cells, and T cells [9]. A recent study showed that B7 was expressed in activated human peripheral blood T cells, CD4 T cell clones, CD8 T cell clones, and natural killer cell clones [7]. Sansom and Hall detected CD80 in T cell clones and in repeatedly activated, but not freshly isolated, peripheral blood T cells [8]. Verwilphen and co-workers detected CD80 in 30% of T cells in synovial membranes and 20% of T cells in synovial fluid of patients with RA [10]. In contrast, no CD80 expression was detected in peripheral blood T cells in their patients [10]. Furthermore, Nickloff et al demonstrated the expression of CD80 in T cells from skin lesions of patients with psoriasis and mycosis fungoides [19]. Although CD80 and CD86 expressions in activated T cells in peripheral blood and infiltrating tissues have been clearly demonstrated, the in vivo expressions of CD80 and CD86 in T cells from peripheral blood have not been well defined in patients with autoimmune diseases.

Recently, Takasaki et al showed that CD80 and CD86 were expressed in T cells of peripheral blood lymphocytes obtained from patients with SLE [12,20]. They reported that CD80-expressing T cells were mainly CD4 cells and CD86-expressing T cells. Our data also revealed that patients with SLE had CD80- and CD86-expressing T cells in their peripheral blood. However, in our lupus patients, the amounts of $CD4^+CD80^+$ and CD86 in all T cell subsets including CD3, CD4 and CD8 T cells were significantly higher than that in normal controls. It has been shown that CD86 molecules were constitutionally expressed in unstimulated monocytes, while CD80 increased after stimulation. Furthermore, Abe et al demonstrated that $CD4^+CD80^+$ and $CD8^+CD86^+$ were markers for disease activity in patients with SLE using the longitudinal method [12]. In the present study, we failed to show associations between $CD4^+CD80^+$, $CD4^+CD86^+$ and $CD8^+CD86^+$ and disease parameters including SLEDAI, C3, C4 and anti-dsDNA. The discrepancy may be due to differences in the methods used.

To the best of our knowledge, this is the first study of the expression of both CD80 and CD86 in SLE T cells stimulated by PHA. In the present report, percentages of CD86 were significantly lower in patients with SLE than in healthy subjects following PHA stimulation. The results support previous findings of suboptimal proliferation in response to multiple stimuli in lymphocytes of SLE patients [23]. The fact that stimulated T cells were unable to upregulate the expression of costimulatory molecules represented a defect intrinsic to the lymphoid function in SLE patients. The defective proliferation after PHA stimulation in SLE cells was mirrored by IL-2 production, and was directly correlated with low levels of IL-2 mRNA [24]. This abnormal expression following PHA stimulation of PBMCs for 48 h, the same conditions used in proliferation experiments, was surprising. The underlying causes of defective up-regulation

of B7 expression in SLE APC remain unclear, but alternation in cytokine production by SLE T cells must be considered [25]. Increased IL-10 production and diminished IFN-γ synthesis have been demonstrated in patients with SLE [26,27]. This may account for the lower amounts of CD86 molecules since IL-10 can inhibit B7 expression in APC [28] and IFN-γ strongly regulates expression of B7 molecules in various cells.

In conclusion, expressions of $CD4^+$ $CD80^+$, $CD4^+$ $CD86^+$ and $CD8^+$ $CD86^+$ in PBMC are not associated with the disease activity of SLE. Furthermore, PBMCs from patients with SLE may have intrinsic defects that alter their activator process, which might explain the T-cell immunoregulatory abnormalities in SLE.

ACKNOWLEDGMENT

This study was supported by a grant (DMR-89-034) from China Medical College Hospital, Taiwan, R.O.C.

REFERENCES

1. Linsley PS, Ledbetter JA. The role of the CD28 receptor during T cell responses to antigen. [Review] *Annu Rev Immunol* 1993;11:191-212.
2. Linsley PS, Brady W, Grosmaire L, et al. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. *J Exp Med* 1991;173:721-30.
3. Weaver CT, Unanue ER. The costimulatory functions of antigen-presenting cells. [Review] *Immunol Today* 1990;11:49-55.
4. June CH, Bluestone JA, Nadler LM, et al. The B7 and CD28 receptor families. [Review] *Immunol Today* 1994;15:321-31.
5. June CH, Ledbetter JA, Linsley PS, et al. Role of the CD28 receptor in T-cell activation. [Review] *Immunol Today* 1990;11:211-6.
6. Guinan EC, Gribben JG, Boussiotis VA, et al. Pivotal role of the B7:CD28 pathway in transplantation tolerance and tumor immunity. [Review] *Blood* 1994;84:3261-82.
7. Azuma M, Yssel H, Phillips JH, et al. Functional expression of B7/BB1 on activated T lymphocytes. *J Exp Med* 1993;177:845-50.
8. Sansom DM, Hall ND. B7/BB1, the ligand for CD28, is expressed on repeatedly activated human T cells in vitro. *Eur J Immunol* 1993;23:295-8.
9. Wyss-Coray T, Mauri-Hellweg D, Baumann K, et al. The B7 adhesion molecule is expressed on activated human T cells: functional involvement in T-T cell interaction. *Eur J Immunol* 1993;23:2175-80.
10. Verwilphen J, Lovis R, De Boer M, et al. Expression of functional B7 and CTLA4 on rheumatoid synovial T cells. *J Immunol* 1994;153:1378-85.
11. Ranheim EA, Kipps TJ. Elevated expression of CD80 (B7/BB1) and other accessory molecules on synovial fluid mononuclear cell subsets in rheumatoid arthritis. *Arthritis Rheum* 1994;37:1637-46.
12. Abe K, Takasaki Y, Ushiyama C, et al. Expression of CD80 and CD86 on peripheral blood T lymphocytes in patients with systemic lupus erythematosus. *J Clin Immunol* 1999;19:58-66.
13. Fu SM, Chiorazzi N, Wang CY, et al. Ia-bearing T lymphocytes in man. Their identification and role in the generation of allogeneic helper activity. *J Exp Med* 1978;148:1423-8.
14. Uchiyama T, Broder S, Waldmann TA. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac (+) cells. *J Immunol* 1981;126:1393-7.
15. Crispin JC, Martinez A, de Pablo P, et al. Participation of the CD69 antigen in the T-cell activation process of patients with systemic lupus erythematosus. *Scand J Immunol* 1998;48:196-200.
16. Garcia-Cozar FJ, Molina IJ, Cuadrado MJ, et al. Defective B7 expression on antigen-presenting cells underlying T cell activation abnormalities in systemic lupus erythematosus (SLE) patients. *Clin Exp Immunol* 1996;104:72-9.
17. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271-7.
18. Bombardier C, Gladman DD, Urowitz MB, et al. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992;35:630-40.
19. Nickoloff BJ, Mitra RS, Lee K, et al. Discordant expression of CD28 ligands, BB-1 and B7 on kerotinocytes in vitro and psoriatic cells in vivo. *Am J Pathol* 1993;142:1029-40.
20. Takasaki Y, Ogaki M, Abe K, et al. Expression of costimulatory molecule CD80 on peripheral blood T cells in patients with systemic lupus erythematosus. *J Rheumatol* 1998;25:1085-91.
21. Hathcock KS, Laszlo G, Dicker HB, et al. Identification of an alternation CTLA-4 ligand costimulatory for T cell activation. *Science* 1993;262:905-7.
22. Alcocer-Varela J, Alarcon-Riquelme M, Laffon A, et al. Activation markers on peripheral blood T cells from patients with active or inactive systemic lupus erythematosus. Correlation with proliferative responses and production of IL-2. *J Autoimmunity* 1991;4:935-45.

23. Umland S, Lee R, Howard M, et al. Expression of lymphokines genes in splenic lymphocytes of autoimmune mice. *Mol Immunol* 1989;26:649-56.
24. Cuadrado MJ, Marubayashi M, Ortega C, et al. Relationship of IL-2, IL-2R (CD25+), soluble IL-2R and IL-4 with disease activity in SLE patients. *Lupus* 1993;2:257-60.
25. Hagiwara E, Gourley MF, Lee S, et al. Disease severity in patients with systemic lupus erythematosus correlates with an increased ratio of interleukin-10: interferon-gamma-secreting cells in the peripheral blood. *Arthritis Rheum* 1996;39:379-85.
26. Huang CM, Chen CL. Elevated IL-10 levels correlate with disease activity in SLE. *Mid Taiwan J Med* 2000;5:37-42.
27. Willems F, Marchant A, Delville JP, et al. Interleukin-10 inhibits B7 and intracellular adhesion molecule-1 expression on human monocytes. *Eur J Immunol* 1994;24:1007-9.

系統性紅斑性狼瘡患者共同刺激因子CD80和CD86在T細胞之表現

黃春明 林彩秀[1] 王秀娟[1] 陳季蘭[2]

中國醫藥學院附設醫院 內科部免疫風濕科 檢驗部[1]

高雄輔英技術學院[2]

背景 本篇研究在探討系統性紅斑性狼瘡患者及正常人其共同刺激分子CD80和CD86在T細胞之表現。

方法 使用流式細胞儀(Flow cytometry)和單株抗體(monoclonal antibodies)，去測量狼瘡患者及正常人週邊血T細胞未經及經由PHA刺激後CD80和CD86之表現。

結果 系統性紅斑性狼瘡患者其週邊血$CD4^+$和$CD8^+$T細胞之CD80和CD86表現較正常人來得高。然而，只有$CD4^+CD80^+$、$CD4^+CD86^+$和$CD8^+CD86^+$三組有統計學上意義。經由PHA刺激後，$CD4^+CD80^+$、$CD4^+CD86^+$和$CD8^+CD86^+$三組在系統性紅斑性狼瘡患者其上升百分比在統計學上均較正常人來得低。T細胞之CD80和CD86表現和系統性紅斑性狼瘡疾病活動度指標，如活動性指數（SLEDAI）、C3、C4及雙股核糖核酸（dsDNA），皆無相關性。

結論 本篇研究顯示系統性紅斑性狼瘡患者體內之週邊單核球可表現出$CD4^+CD80^+$、$CD4^+CD86^+$和$CD8^+CD86^+$，但和系統性紅斑性狼瘡疾病活動度並不相關。而在PHA刺激後, 狼瘡患者之$CD4^+CD80^+$、$CD4^+CD86^+$和$CD8^+CD86^+$明顯較正常人爲低，這可能因狼瘡病人其週邊單核球有內生性缺陷，導致其活化過程改變。（中台灣醫誌 **2002;7:7-13**）

關鍵詞

活化T細胞，CD80，CD86，系統性紅斑性狼瘡

聯絡作者：黃春明

地 址：404台中市北區育德路2號

中國醫藥學院附設醫院 內科部免疫風濕科

收文日期：9/5/2001 修改日期：12/6/2001

接受日期：1/2/2001