DECREASED T CELL RESPONSE TO ANTI-CD2 IN SYSTEMIC LUPUS ERYTHEMATOSUS AND REVERSAL BY ANTI-CD28

Evidence for Impaired T Cell–Accessory Cell Interaction

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Objective. To assess the ability of T cells from patients with systemic lupus erythematosus (SLE) to respond to a mitogenic combination of anti-CD2 monoclonal antibodies (Mab), and to learn the molecular basis of the documented defect.

Methods. Peripheral blood mononuclear cell (PBMC) populations from individuals with SLE and paired controls were stimulated in vitro with anti-CD2, and the proliferative response was compared with that evoked by stimulation with phytohemagglutinin (PHA) and anti-CD3. Surface markers on lymphocyte populations were assessed by flow cytometry after staining with specific MAb.

Results. The proliferative response to anti-CD2 was decreased to a greater extent than was the response to anti-CD3 or PHA in SLE patients. This defect was found in approximately one-half of the patients examined, was not associated with disease activity, and was maintained upon repeated testing. Since either monocytes or resting B cells can serve as accessory cells for T cells following activation by anti-CD2, we examined the T cell response after depletion of adherent cells. In approximately two-thirds of the individuals with a decreased response, depletion of monocytes or substitution of monocytes with allogeneic, resting B cells from normal donors corrected the defect. The addition to PBMC of anti-CD28, but not of a neutralizing antibody to interleukin-10, largely reversed the anti-CD2 proliferative defect. Significantly fewer CD8+ T cells expressed CD28 in SLE, and this defect was also documented, to a lesser extent, in CD4+ cells.

Conclusion. This study provides evidence that some functional T cell defects in SLE may be due, at least in part, to decreased CD28-mediated costimulatory activity following the interaction of T cells with conventional accessory cells.

Systemic lupus erythematosus (SLE) is characterized by B cell hyperactivity and a panoply of autoantibodies against nuclear and cell surface antigens. IgG pathogenic autoantibodies in SLE are T cell dependent (1–3). Although there are many examples of T cell dysfunction in SLE (4–11), those that contribute to B cell hyperactivity are poorly understood. Possible explanations include an intrinsic abnormality of CD4+ T cells in SLE (12,13), abnormal CD8+ regulatory T cells (14), or inhibitory serum factors (15,16). In addition, inhibition or lack of costimulation by antigen-presenting cells can lead to T cell dysfunction (17–19). Many of the abnormalities described may correlate with the severity of disease and, therefore, reflect a secondary event (5,6). However, defects such as decreased interleukin-2 (IL-2) production have been described in subjects with inactive as well as active disease (20).

While T cell activation through the antigen receptor, the CD3/T cell receptor (TCR) complex, has been studied extensively in SLE (4–6), cell surface CD2 molecules also have an important role in the adhesion of T cells to accessory cells and in T cell signaling. These 50-kd glycoprotein molecules are expressed on almost all peripheral T cells (21). Although T cell activation via CD2 was first described as an “alternate pathway” (22),
it has become evident that signaling through CD2 can enhance or inhibit T cell-B cell interactions. Costimulatory signals induced by perturbation of CD2 have an obligatory role in T cell–dependent activation of resting B cells (23) and enhancement of B cell function (24). However, anti-CD2 monoclonal antibodies (MAb) also have inhibitory effects on T cell function both in vitro (25) and in vivo (26). One recently documented inhibitory effect of anti-CD2 is the transforming growth factor β (TGFβ)–dependent induction of T suppressor cells (27–29).

To date, however, information concerning T lymphocyte responsiveness to anti-CD2 in human autoimmune diseases is scanty. There are single reports of decreased proliferative response of peripheral blood mononuclear cells (PBMC) to anti-CD2 in multiple sclerosis (30), Sjögren’s syndrome (31), and SLE (32). In SLE, the decreased response to anti-CD2 has been explained by both T cell and accessory cell defects. We have confirmed this finding and propose that this defect can be explained in large part by inadequate costimulatory signals generated by the ligation of CD28 on T cells with B7 co-receptors on monocytes. Both T cells and accessory cells appear to contribute to this defect.

Engagement of CD28 on T cells by its counter-receptors B7-1 (CD80) and B7-2 (CD86) on accessory cells is pivotal for T cell activation (33,34). CD28 is a 44-kd homodimeric receptor expressed by CD4+ cells and most CD8+ cells. Ligation of the T cell membrane antigen CD28 strongly enhances cytokine secretion in human T lymphocytes that are activated via the CD3/TCR complex or CD2 molecules. CD28 regulates IL-2 gene expression by stabilizing messenger RNA synthesis (33). Anti-CD28 MAb have been reported to substitute for accessory cells in providing the costimulatory signals required for T cell activation (35). We report here that the expression of CD28 on SLE T cells, especially the CD8+ population, is significantly decreased.

**Patients and Methods**

**Antibodies.** The 2 anti-CD2 MAb used in this study were GT2 (IgG1; provided by Dr. Alain Bernard, Nice, France [36]) and OKT11 (IgG1; American Type Culture Collection, Bethesda, MD). Other MAb used were anti-CD3 (454, IgG2a; a gift from Dr. W. Stohl, Los Angeles, CA [37] and UCHT1, IgG1; a gift from Dr. Peter Beverley, London, UK [38]), anti-CD28 (9.3, IgG2a; a gift from Dr. John Hansen, Seattle, WA [39]), anti-CD74 (L243, IgG2a; American Type Culture Collection [40]), anti-CD8 (284, IgG2a; a gift from Dr. W. Stohl), anti-CD16 (3G8, IgG1; provided by Dr. Jay Unkeless, New York, NY), and anti-CD11b (OKM1, IgG1; American Type Culture Collection).

SLE patients and controls. Between 1992 and 1996, 52 patients with SLE that fulfilled the American College of Rheumatology revised criteria for the classification of the disease (41) were studied. The group consisted of 47 women and 5 men, most of whom were Hispanic. The mean age was 34 years (range 19–60 years), and the mean disease duration was 6 years (range 0–30 years). The study group included 6 hospitalized individuals with newly diagnosed, untreated SLE; the remainder were ambulatory and were receiving prednisone, nonsteroidal antiinflammatory drugs, or hydroxychloroquine, but not cytotoxic drugs. PBMC from each patient were studied in parallel with cells from a paired control matched as closely as possible for age, sex, and ethnic group.

In 31 patients, SLE activity was assessed retrospectively by chart review. Disease activity in the remaining 21 patients was determined prospectively by one of the authors (MMS), who examined the patient at the time the blood was drawn and obtained information needed for the Systemic Lupus Activity Measure (SLAM) (42) and SLE Disease Activity Index (SLEDAI) (43) instruments. These subjects were divided into 2 groups on the basis of disease activity as determined by clinical manifestations, the need for change in the drug therapy regimen, and the results of the SLAM and SLEDAI.

Preparation of PBMC, CD4+ T cells, and a standard source of B cells. Thirty milliliters of blood was obtained from donors by venipuncture, and the PBMC were isolated by density centrifugation using a Ficoll-containing preparation (Ficoll-Hypaque; Atlanta Biologicals, Norcross, GA). The PBMC were incubated at 37°C for 30 minutes, then washed 3 times in warmed medium to elute cytotoxic antibodies. To deplete adherent cells, PBMC were added to a plastic petri dish, and after 1 hour at 37°C, the nonadherent cells were recovered.

To obtain CD4+ T cells, plastic-nonadherent cells were stained with anti-CD8, anti-CD74 (HLA-DR), anti-CD16, and anti-CD11b MAb. The reactive lymphocytes were removed by adding anti-mouse IgG–coated immunomagnetic beads (Dynabeads; Dynal, Lake Success, NY) followed by passage over a magnet. The remaining cells were >90% CD4+, >98% CD3+, <1% CD20+, and <1% CD14+.

Lymphocytes from a tonsil obtained from a child undergoing tonsillectomy were made into a single cell suspension and added to a Ficoll density gradient. The interface cells were then incubated with AET-treated sheep red blood cells. After 1 hour at 4°C, the cells were gently layered onto a Ficoll gradient and centrifuged. The nonrosetting B cells were separated into high-density cells by Percoll gradient centrifugation and then frozen in aliquots at −70°C for use as a standard source of accessory cells in some experiments. The B cells (>98% CD20+) were irradiated with 3,000 rads from a cesium source prior to use.

Reagents. Phytohemagglutinin (PHA; Difco, Detroit, MI) was added at 1 µg/ml. Phorbol myristate acetate (Sigma, St. Louis, MO) was added at 0.1 ng/ml. Cell-free hybridoma supernatants of the anti-CD2 MAb (GT2 and OKT11) were added at dilutions of 1:20 and 1:40, respectively. These concentrations were found to be optimal for mitogenesis. Similarly, an optimal concentration of anti-CD3–purified IgG (100 ng/ml) was added to the cultures.
Table 1. Response of control and systemic lupus erythematosus (SLE) patient peripheral blood mononuclear cells to phytohemagglutinin (PHA), anti-CD2, and anti-CD3

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SLE</th>
<th>Control</th>
<th>SLE</th>
<th>Control</th>
<th>SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (n = 18)</td>
<td>69,645 ± 11,632</td>
<td>62,828 ± 13,740</td>
<td>20,619 ± 3,605</td>
<td>9,257 ± 1,960†</td>
<td>28,893 ± 5,625</td>
<td>21,371 ± 4,789</td>
</tr>
<tr>
<td>Group A: normal</td>
<td>67,042 ± 17,002</td>
<td>109,263 ± 24,671</td>
<td>16,489 ± 3,883</td>
<td>22,021 ± 4,652</td>
<td>35,610 ± 13,092</td>
<td>29,540 ± 6,197</td>
</tr>
<tr>
<td>Group B: decreased response to anti-CD2 (n = 5)§</td>
<td>76,890 ± 24,188</td>
<td>50,884 ± 11,276</td>
<td>21,870 ± 10,173</td>
<td>8,483 ± 3,659¶</td>
<td>24,510 ± 11,377</td>
<td>36,411 ± 13,292</td>
</tr>
<tr>
<td>Group C: decreased response to PHA, anti-CD2, and anti-CD3 (n = 6)¶</td>
<td>56,941 ± 9,037</td>
<td>19,111 ± 6,634‡</td>
<td>20,174 ± 8,074</td>
<td>2,857 ± 1,031¶</td>
<td>23,164 ± 4,863</td>
<td>5,866 ± 3,580¶</td>
</tr>
</tbody>
</table>

* Values are the mean ± SEM cpm.
† P < 0.01 versus controls, by t-test or Wilcoxon rank sum test.
‡ Six female, 1 male; mean age 27 years; mean disease duration 2 years; mean physician assessment of global disease activity (0–4 scale) 1.6; 43% with anti-DNA antibodies; 28% with decreased C3 and C4 values; 14% untreated; 29% receiving prednisone (mean 10 mg/day); 43% receiving nonsteroidal antiinflammatory drugs (NSAIDs); 43% receiving hydroxychloroquine.
§ Five female, 0 male; mean age 29 years; mean disease duration 2 years; mean physician assessment of global disease activity (0–4 scale) 2.7; 80% with anti-DNA antibodies; 60% with decreased C3 and C4 values; 60% untreated; 40% receiving prednisone (mean 20 mg/day); 40% receiving NSAIDs; 0 receiving hydroxychloroquine.
¶ P < 0.05 versus controls, by t-test or Wilcoxon rank sum test.
# Five female, 1 male; mean age 46 years; mean disease duration 7 years; mean physician assessment of global disease activity (0–4 scale) 1.5; 33% with anti-DNA antibodies; 17% with decreased C3 and C4 values; 33% untreated; 50% receiving prednisone (mean 12.5 mg/day); 33% receiving NSAIDs; 33% receiving hydroxychloroquine.

Proliferation assay. Cells in complete medium (RPMI 1640) supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, and 25 μg/ml gentamicin were added to the wells of a flat-bottomed microtiter plate, at 1 × 10⁷ cells/well unless indicated otherwise. MAb and/or other reagents were also added to these wells. Proliferative activity was measured after the cells were cultured for 72 hours; 1 μCi ³H-thymidine (6.7 Ci/mM; ICN Radiochemicals, Irvine, CA) was added for the final 18 hours of culture.

RESULTS

Proliferative response of PBMC to anti-CD2, anti-CD3, and PHA in SLE. Our first objective was to examine the proliferative response of SLE PBMC to anti-CD2 in comparison with the response to PHA and anti-CD3. The initial study group consisted of 18 patients, 16 women and 2 men, with a mean age of 34 years (range 20–56 years). Sixteen were Hispanic and 2 were white. All were receiving <20 mg prednisone/day. Table 1 shows that the response to anti-CD2, but not to anti-CD3 or PHA, was decreased in the total patient group. There was, however, considerable heterogeneity of responsiveness within this group. Three patterns of lymphocyte reactivity could be discerned: In 7 patients (group A), the responses to PHA, anti-CD2, and anti-CD3 were similar to those observed in paired controls; in 5 patients (group B), the response to only anti-CD2 was significantly decreased; and in 6 patients (group C), the response to all 3 mitogens was significantly decreased. Demographic and clinical data for each subgroup are reported in Table 1, but the numbers in each subgroup are too small for comment except that there was heterogeneity in both the response to these mitogens and the clinical profile.

The response to anti-CD2 appeared to be relatively constant in individual patients who were tested serially. Table 2 describes studies with PBMC from 8 separate patients and paired controls studied at intervals of up to 15 months. Three of these patients demonstrated normal responsiveness upon repeated testing. Four others revealed decreased responses. Another patient also exhibited a poor response on 2 occasions; however, because the paired control also demonstrated a poor response on 1 occasion, the anti-CD2 response of the SLE patient varied considerably when expressed as a percent of that of the paired control.

Another group of 21 patients with SLE was prospectively evaluated for disease activity to determine whether there were possible correlations with decreased T cell anti-CD2 response. One of the authors (MMS), who was unaware of the T cell data, divided the patients into 2 groups based on clinical manifestations, medication adjustments, and disease activity scores (Table 3). The dosage of corticosteroids was not restricted in this series of experiments. Even though the prednisone
DECREASED RESPONSE TO ANTI-CD2 IN SLE

Table 2. Stability of the T cell response to anti-CD2 in individual systemic lupus erythematosus patients*

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Patient</th>
<th>Control</th>
<th>% of control</th>
<th>Interval (months)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient A</td>
<td>27,955</td>
<td>3,709</td>
<td>740</td>
<td>–</td>
</tr>
<tr>
<td>Patient B</td>
<td>30,646</td>
<td>3,050</td>
<td>775</td>
<td>4</td>
</tr>
<tr>
<td>Patient C</td>
<td>5,636</td>
<td>5,658</td>
<td>100</td>
<td>14</td>
</tr>
<tr>
<td>Patient D</td>
<td>3,914</td>
<td>4,525</td>
<td>86</td>
<td>–</td>
</tr>
<tr>
<td>Patient E</td>
<td>6,209</td>
<td>5,658</td>
<td>109</td>
<td>9</td>
</tr>
<tr>
<td>Patient F</td>
<td>23,802</td>
<td>26,739</td>
<td>89</td>
<td>–</td>
</tr>
<tr>
<td>Patient G</td>
<td>6,349</td>
<td>5,658</td>
<td>112</td>
<td>11</td>
</tr>
<tr>
<td>Patient H</td>
<td>1,791</td>
<td>2,136</td>
<td>84</td>
<td>–</td>
</tr>
<tr>
<td>Group 2</td>
<td>Patient I</td>
<td>1,655</td>
<td>5,658</td>
<td>29</td>
</tr>
<tr>
<td>Patient J</td>
<td>3,023</td>
<td>3,983</td>
<td>27</td>
<td>–</td>
</tr>
<tr>
<td>Patient K</td>
<td>2,670</td>
<td>23,213</td>
<td>12</td>
<td>–</td>
</tr>
<tr>
<td>Patient L</td>
<td>5,596</td>
<td>21,612</td>
<td>25</td>
<td>14</td>
</tr>
<tr>
<td>Patient M</td>
<td>6,499</td>
<td>12,540</td>
<td>51</td>
<td>–</td>
</tr>
<tr>
<td>Patient N</td>
<td>812</td>
<td>5,658</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Patient O</td>
<td>1,430</td>
<td>13,411</td>
<td>11</td>
<td>–</td>
</tr>
</tbody>
</table>

* Patients were arbitrarily divided into 2 groups on the basis of the proliferative response to GT2 and T11 compared with a paired control. † After subtraction of values from studies of unstimulated cultures. ‡ Time since the first study in the indicated patient.

dosage was higher in the active disease group, there was no difference in the lymphocyte response to anti-CD2 between the active disease and inactive disease groups.

Table 3. Lack of correlation between the T cell proliferative response to anti-CD2 and disease activity in systemic lupus erythematosus (SLE)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Active disease (n = 12)</th>
<th>Inactive disease (n = 9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>29.5</td>
<td>44.9</td>
<td>0.008</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>2.2</td>
<td>12.1</td>
<td>0.024</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>12.7</td>
<td>2.2</td>
<td>0.001</td>
</tr>
<tr>
<td>SLAM</td>
<td>9.1</td>
<td>2.8</td>
<td>0.004</td>
</tr>
<tr>
<td>Anti-DNA titer</td>
<td>1:212</td>
<td>1:20</td>
<td>0.172</td>
</tr>
<tr>
<td>C3 level</td>
<td>67.5</td>
<td>93.4</td>
<td>0.053</td>
</tr>
<tr>
<td>Prednisone dosage, mg/day</td>
<td>34.2</td>
<td>3.1</td>
<td>0.007</td>
</tr>
<tr>
<td>ESR, mm/hour</td>
<td>50.6</td>
<td>23.7</td>
<td>0.02</td>
</tr>
<tr>
<td>Response to anti-CD2, cpm</td>
<td>8,646</td>
<td>5,445</td>
<td>0.30</td>
</tr>
<tr>
<td>% of response in cells from paired control</td>
<td>56.2</td>
<td>54.0</td>
<td>0.30</td>
</tr>
</tbody>
</table>

* Values are the means. SLEDAI = SLE Disease Activity Index; SLAM = Systemic Lupus Activity Measure; ESR = erythrocyte sedimentation rate.

A role for adherent cells in the anti-CD2 proliferative defect. The decreased T cell proliferative response to anti-CD2 could be explained by a defect in T cells and/or accessory cells. Since resting B cells can substitute for monocytes as accessory cells for T cell proliferation induced by signaling through the CD2 pathway (44), we could assess the proliferative response of PBMC after adherent monocytes had been depleted. In 9 SLE patients and paired controls, the response of each individual before and after monocyte depletion was studied (Figure 1). Whereas before depletion the response was significantly decreased in the SLE patients (mean ± SEM 9.6 ± 2.1 counts per minute × 10^{-3} versus 4.1 ± 0.8 cpm × 10^{-3} in controls and patients, respectively; P = 0.022 by Student’s t-test), this difference disappeared following depletion of monocytes (14.1 ± 3.4 cpm × 10^{-3} versus 16.1 ± 1.6 cpm × 10^{-3}, respectively).

Because of well-established differences in B cell
function between patients with SLE and normal individuals (1), we also used an alternative approach to document an accessory cell defect. Instead of autologous B cells, we prepared resting (high-density) B cells from a single tonsil and froze them at –70°C. Previous studies from this laboratory had indicated that high-density B cells were poor stimulators of an allogeneic mixed lymphocyte reaction (45). Aliquots of these B cells were irradiated and added to CD4+ cells from patients with SLE and controls as a standard source of accessory cells. Figure 2 shows striking differences between the SLE group and the control group. In this study, which included 6 healthy controls, the response of purified control CD4+ T cells to anti-CD2 was similar to or slightly less than the response of control PBMC (CD4+ cells 22.0 ± 6 cpm × 10^{-3}, PBMC 15.3 ± 4.3 cpm × 10^{-3}). In contrast, the response of SLE CD4+ T cells was markedly higher than the response of SLE PBMC (19.9 ± 5.7 cpm × 10^{-3} versus 5.1 ± 1.5 cpm × 10^{-3}; P < 0.001 by Student’s t-test).

Failure of IL-10 antagonism to correct the anti-CD2 proliferative defect. Constitutive production of IL-10 is increased in SLE, and monocytes appear to be a principal source of this cytokine (46–48). Besides enhancing B cell function, IL-10 has well-known immunosuppressive activities (49,50) including inhibition of IL-2 production (51). We considered the possibility that IL-10 produced by SLE monocytes might be a factor in the decreased proliferative response to anti-CD2. In a study of 8 patients with SLE and 7 paired controls, the addition of anti–IL-10 did result in a 2-fold increase in the T lymphocyte response to anti-CD2. Since antagonism of IL-10 also increased the response in controls to a similar extent, however (Figure 3), the proliferative abnormality in SLE was not corrected. Thus, elevated levels of IL-10 could not explain the anti-CD2 proliferative defect in SLE.

Reversal of the anti-CD2 proliferative defect by addition of anti-CD28. We considered the possibility that costimulatory signals provided by the interaction of T cells and accessory cells might be decreased in SLE. Since anti-CD28 MAb can substitute for accessory cells in providing the costimulatory signals required for T cell activation (35), we added anti-CD28 to anti-CD2 or
DECREASED RESPONSE TO ANTI-CD2 IN SLE

Figure 4. Normalization of the proliferative response of SLE PBMC to anti-CD2 by addition of anti-CD28. PBMC (1 \times 10^6/well) from 13 patients with SLE and 10 healthy controls were stimulated with either a mitogenic combination of anti-CD2 antibodies (GT2 and OKT11) or anti-CD3 (UCHT1) in the presence or absence of anti-CD28 (9.3). Proliferative activity was determined after 72 hours. Values shown are the mean and SEM of all samples. The proliferative activity of PBMC cultured with medium alone was 824 ± 223 cpm in controls and 340 ± 77 cpm in SLE patients; that of PBMC cultured with anti-CD28 only was 1,415 ± 193 cpm in controls and 750 ± 193 cpm in SLE patients. Since the values obtained in each group were not normally distributed, the data were log transformed for this purpose. See Figure 1 for definitions.

anti-CD3 MAb in the stimulation of PBMC from 13 patients with SLE and 10 controls. As before, the defect in the SLE T cell response to anti-CD2 was clearly seen (Figure 4). When anti-CD28 was added to anti-CD2, the enhancement of proliferation in SLE was markedly greater than that of controls, so the mean differences between these groups were no longer significant. In this experiment, we substituted the anti-CD3 MAb UCHT1 (IgG1) for 454 (IgG2a). Notwithstanding the possible heterogeneity of responsiveness to this IgG1 MAb because of monocyte IgG Fcγ receptor II polymorphism (52), the relative proliferative response in SLE and controls was similar to the results with the IgG2a anti-CD3 MAb. There was modest enhancement of proliferation after the addition of anti-CD28 to anti-CD3 in both groups, and these increases were equivalent.

T cell phenotype abnormalities in SLE. We and others have previously reported decreased ratios of CD4+ to CD8+ T cells in SLE (53,54). In the present study, the percentage of T cells displaying various surface markers was examined by flow cytometry in a sample of 13 SLE patients and 6 controls. As before, we observed decreased percentages of CD4+ T cells and increased percentages of CD8+ T cells in the SLE patients (Table 4). The decreased percentages of CD4+ T cells were not associated with steroid therapy. Although the percentage of lymphocytes stained by GT2 was slightly higher than that stained by T11, the values for each were similar in SLE patients and normal subjects in this sample.

The percentage of lymphocytes expressing CD28 was, surprisingly, decreased in SLE. Consistent with the findings of other studies (55), 96.8 ± 0.5% (mean ± SEM) of CD4+ cells in controls co-expressed CD28. In SLE patients, however, the mean percentage of CD4+CD28+ cells was 84.8 ± 4.4% (P = 0.086 by Student's t-test). In the control group, 67.7 ± 3.5% of CD8+ cells co-expressed CD28, while in the SLE patients, this subset was markedly decreased 44.4 ± 4.2% (P = 0.003) (Figure 5).

Two examples of CD28 expression on SLE lymphocytes are shown in Figure 6. SLE patient 1 was representative of most patients, in whom the percentage of CD8+ cells expressing CD28 was moderately decreased. In others, as shown by the findings in SLE patient 2, this decrease was even more marked, and percentages of CD4+CD28+ cells were also decreased.

**DISCUSSION**

We have demonstrated a defect in the T cell proliferative response to anti-CD2 in SLE that can primarily be attributed to accessory cells and/or costimulatory signals generated by T cell-accessory cell interactions. This conclusion was suggested by studies with monocyte depletion, the use of high-density (resting) B cells from normal subjects as accessory cells, the use of anti-CD28 to bypass accessory cell effects, and also by decreased expression of CD28 on SLE T cells.

Initially, we stimulated PBMC from SLE patients

**Table 4.** Surface markers on control and systemic lupus erythematosus (SLE) patient T cells*

<table>
<thead>
<tr>
<th>Marker</th>
<th>Control (n = 6)</th>
<th>SLE (n = 13)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4</td>
<td>42.8 ± 3.2</td>
<td>32.5 ± 2.3</td>
<td>0.019</td>
</tr>
<tr>
<td>CD8</td>
<td>25.6 ± 2.4</td>
<td>37.2 ± 3.3</td>
<td>0.039</td>
</tr>
<tr>
<td>CD28</td>
<td>59.6 ± 3.5</td>
<td>44.1 ± 2.8</td>
<td>0.006</td>
</tr>
<tr>
<td>CD2 (T11)</td>
<td>78.7 ± 3.2</td>
<td>78.4 ± 3.4</td>
<td>0.900</td>
</tr>
<tr>
<td>CD2 (GT2)</td>
<td>85.2 ± 3.5</td>
<td>85.6 ± 2.8</td>
<td>0.934</td>
</tr>
</tbody>
</table>

* Lymphocytes from SLE patients and controls were stained with monoclonal antibodies against the surface antigens indicated and examined by flow cytometry. Values are the mean ± SEM. P values were determined by Student's t-test.
and paired controls with PHA, anti-CD2, and anti-CD3 MAb. In a group of 18 individuals, only the response to anti-CD2 was significantly decreased. Within this group, however, 3 response patterns could be discerned: the T cell response was normal, was decreased only with anti-CD2, or was decreased with all 3 mitogens. This heterogeneity is important in that other investigators have reported significantly decreased proliferative responses to PHA and anti-CD3 in SLE (for review, see refs. 4-6). It is quite likely that with larger numbers of study subjects, we also would have found significant differences. Although the clinical significance of this heterogeneity is not immediately apparent, the finding of a selective decrease in the CD2 pathway in some patients may be important for reasons given below.

Only one group has previously studied the response of SLE T cells to anti-CD2. Following pulse cyclophosphamide treatment, the response to anti-CD2, but not to other T cell mitogens, was decreased in SLE (56). Using an accessory cell–independent combination of mitogenic anti-CD2 MAb, those investigators reported that the decreased T cell proliferative response was not related to disease activity and could be explained by T cell or accessory cell defects (32). Using an accessory cell–dependent combination of anti-CD2 MAb, we have confirmed and extended these observations. We also could not find a relationship with disease activity or treatment. Moreover, the responses detected in individual patients were relatively stable in repeated studies performed during a 1-year period.

In the majority of SLE patients, monocyte depletion normalized the proliferative response to anti-CD2. Although the T cell response to GT2 and T11 is accessory cell dependent, resting B cells, as well as monocytes, function as accessory cells with this mitogenic combination of anti-CD2 MAb (44). Therefore, because of well-known abnormalities of SLE B cells (3,57), additional experiments were performed in which CD4+ T cells from SLE patients and controls were cultured with a standard source of allogeneic resting B cells. These studies confirmed one or more accessory cell abnormalities in the majority of patients. Others have previously reported that SLE monocytes can inhibit T cell proliferative response.

**Figure 5.** Decreased expression of CD28 on systemic lupus erythematosus (SLE) T cells. Lymphocytes from 13 SLE patients and 6 controls were labeled with anti-CD4 or anti-CD8, and anti-CD28. Values shown are the mean and SEM. Significance of the differences between the SLE group and the control group was determined by Student's t-test.

**Figure 6.** Expression of CD28 on CD4+ or CD8-gated lymphocytes from a healthy control and from 2 SLE patients. PBMC were stained with anti-CD28 (fluorescein isothiocyanate) and anti-CD4 (phycoerythrin) or anti-CD8 (phycoerythrin). The percentages of CD4+ or CD8+ cells stained with anti-CD28 are indicated. See Figure 1 for definitions.
when graded amounts of prostaglandin E2 were added to complexes (69,70), which are characteristically increased in SLE. Moreover, IL-10 can decrease B7 expression on monocytes (71), and expression of B7.1 (CD80) is decreased in SLE monocytes (72,73). However, the addition of CD11a–CD54 interactions can explain decreased T cell proliferation in SLE since expression of these adhesion molecules is increased in this disease (81,82), and increased T cell CD11a expression has been associated with development of autoreactivity (82). We and others (32) have been unable to document impaired cell surface expression of CD2 on SLE T cells that could explain the proliferative defect.

One of the principal findings of this study was that the defect in the anti-CD2 proliferative response was essentially reversed by the addition of anti-CD28. Cross-linking of cell surface CD28 molecules provides a helper signal that bypasses the need for accessory cells in T cell activation (35). The natural ligands for CD28 are the B7 family of molecules, which are generally expressed on activated monocytes and B cells (83,84). SLE T cells have been reported to respond normally to B7 (85). As stated above, however, up-regulation of B7 expression on antigen-presenting cells as detected by CTLA-4 ligand and anti-B7.1 is decreased in SLE (72,73). Thus, the cross-linking of CD28 could counteract the decreased expression of the ligands for CD28 on monocytes, and normalize proliferation.

An important observation was the decreased expression of CD28 on SLE T cells, especially CD8+ T cells. A similar observation has been made in patients with human immunodeficiency virus infection (86,87). Since CD28− T cells do not proliferate in response to mitogens (86), the increased numbers of these cells could contribute to the proliferative defect observed. Perturbation of CD28 by its ligand on target cells appears to protect T cells from activation-induced cell death (88). This is of interest in view of the recent finding of increased apoptosis in SLE (89). Signaling through CD28 does not require the activation of protein kinase C and is cyclosporine resistant (90,91). Moreover, there are significant differences between the synergistic effects of anti-CD28 with anti-CD2 and with anti-CD3. The combination of anti-CD28 and anti-CD2 MAb leads to prolonged expression of IL-2 receptor, while anti-CD28 and anti-CD3 produces only a transient expression of this receptor (34). This is of interest since the synergism between anti-CD28 and anti-CD2 in SLE patients was much greater than the modest synergism of anti-CD28 and anti-CD3 in SLE patients and normal subjects (Figure 4). Others have reported that the...
addition of anti-CD28 to anti-CD2 markedly increased IL-2 production by SLE lymphocytes in comparison with lymphocytes from normal controls (92). It is well established that IL-2 production by mitogen-stimulated T cells is decreased in SLE (13,20,93).

This report raises several questions that will require further study for definitive answers. If the decreased response to anti-CD2 can be explained by inadequate costimulation from the CD28 pathway, why is the response normalized following monocyte depletion, since B cells also express the B7 complex? Previous studies from this laboratory have revealed that MAb against LFA-1 or ICAM-1 inhibited the response to anti-CD2 more strongly than did anti-B7 MAb (44). In addition, we have identified certain SLE patients with markedly decreased CD28 expression on their CD4+ T cells. One of these individuals is SLE patient 2 represented in Figure 6. PBMC from this individual responded poorly to anti-CD2. Her purified CD4+ T cells, however, responded to anti-CD2 even better than did those of her paired control, when cultured with allogeneic B cells (results not shown). Thus, ligation of CD28 may not be essential for T cell proliferation when B cells function as accessory cells.

If the numbers of T cells expressing CD28 are decreased in SLE, why does the addition of anti-CD28 generally reverse the defect? In most patients, defective expression of CD28 was observed in predominantly CD8+ rather than CD4+ cells. Cross-linking of CD28 on CD4+ cells, therefore, provided the strong costimulatory signal that bypassed the need for other accessory cells.

What is responsible for the relative selectivity of the proliferative defective to anti-CD2 in comparison with anti-CD3? This is an interesting question since activation signals transduced by CD2 are transmitted by CD3 zeta molecules (94). Spatially, the CD3 zeta chains are closely linked with other components of CD3. This, however, is not the case with CD2. On this basis, it is reasonable to suggest that signaling through CD2 might be easier to inhibit than signaling through the CD3/TCR complex.

Finally, can a decreased response to anti-CD2 MAb in SLE be important in the pathogenesis or perpetuation of this disease? In addition to providing costimulatory signals that promote T cell activation and B cell differentiation (23,24), signaling through CD2 has complex immunosuppressive effects both in vitro and in vivo (25,26,95,96). Treatment of mice with anti-CD2 prevents the onset of autoimmune diabetes mellitus and allergic experimental encephalomyelitis (97,98). Moreover, anti-CD2 significantly inhibits T cell-dependent B cell differentiation induced by anti-CD3 (95). We have recently observed that, unlike anti-CD3, anti-CD2 can induce CD8+ regulatory cells that suppress antibody production (28). This regulatory cell activity depends upon the ability of anti-CD2 to upregulate TGFβ, and anti-CD2–induced active TGFβ is decreased in SLE (99). Therefore, induction of regulatory cell activity by signaling through the CD2 pathway may also be decreased in this disease.


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