Expression of costimulatory molecules on peripheral blood lymphocytes of patients with systemic lupus erythematosus

M Bijl, G Horst, P C Limburg, C G M Kallenberg

Abstract

Objective—In systemic lupus erythematosus (SLE) autoantibody production is T cell dependent. For a proper T and B cell interaction, signalling of costimulatory molecules on these cells is necessary. The expression of costimulatory molecules on peripheral blood lymphocytes in patients with SLE in conjunction with disease activity was measured to evaluate whether expression of costimulatory molecules in SLE is increased.

Methods—Thirteen patients with SLE with active disease, 10 patients with inactive disease, and 14 controls entered the study. In addition, samples from 10 of the 13 patients with active disease could be studied at a moment of inactive disease as well. Isolated peripheral blood lymphocytes were stained for the lymphocyte subset markers CD4, CD8, CD19, their respective activation markers CD25, HLA-DR, CD38, and the costimulatory molecules CD40L, CD28, CD40, CD80, and CD86. Expression was measured by flow cytometry.

Results—Peripheral blood lymphocytes of patients with SLE showed signs of increased activation at the moment of active disease. Almost all CD4+ T cells expressed CD28, both in patients and in controls. CD80 expression on CD19+ B cells was low in both groups and did not correlate with disease activity. In contrast, the percentage of CD19+ B cells expressing CD86 was increased in patients with SLE even in patients with inactive disease (p=0.04) and correlated with the SLEDAI score (p=0.0005) and levels of anti-dsDNA (p=0.006). No changes in CD40 or CD40L expression were found in the patients with SLE.

Conclusion—In patients with SLE the expression of CD86 on CD19+ B cells is increased and is associated with disease activity, B cell activation, and levels of anti-dsDNA. The increased CD86 expression will render (autoreactive) B cells more susceptible for T cells. This can facilitate autoantibody production and might be a target for immunosuppressive treatments.

Subjects and methods

PATIENTS AND BLOOD SAMPLES
Outpatients eligible for this study fulfilled at least four American College of Rheumatology criteria for SLE, were non-smokers, and had either active or inactive disease. Patients with active disease had to fulfill predefined criteria. Inactive disease was defined as the persistent absence of clinical disease activity for at least four months while patients were without, or
Table 1  Expression of activation markers and costimulatory molecules on peripheral blood lymphocytes. Results are given as mean (SD).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Inactive</th>
<th>Active</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4/CD25+ (%)</td>
<td>4.0 (1.4)</td>
<td>4.4 (3.2)</td>
<td>8.5 (6.3)*</td>
</tr>
<tr>
<td>CD8/HLA-DR+ (%)</td>
<td>5.6 (2.5)</td>
<td>8.1 (5.3)</td>
<td>10.6 (8.3)</td>
</tr>
<tr>
<td>CD19/CD38+ (%)</td>
<td>79.7 (8.7)</td>
<td>84.3 (7.7)</td>
<td>86.3 (9.8)*</td>
</tr>
<tr>
<td>CD4/CD28+ (%)</td>
<td>95.3 (3.8)</td>
<td>93.6 (7.9)</td>
<td>95.0 (3.5)</td>
</tr>
<tr>
<td>CD18/CD40+ (%)</td>
<td>85.0 (9.3)</td>
<td>81.3 (11.2)</td>
<td>81.9 (12.4)</td>
</tr>
<tr>
<td>CD18/CD86+ (%)</td>
<td>1.7 (0.9)</td>
<td>3.1 (5.7)</td>
<td>4.4 (3.8)</td>
</tr>
<tr>
<td>CD19/CD86+ (%)</td>
<td>2.9 (1.4)</td>
<td>6.9 (7.2)*</td>
<td>12.5 (8.9)**</td>
</tr>
</tbody>
</table>

*p<0.05, ***p<0.001 compared with controls. Percentages are expressed as proportion of total CD4+, CD8+, and CD19+ subpopulations from healthy controls (n=10), patients with inactive disease (n=20), and patients with active disease (n=13).

Results

Ten patients (two male, eight female) with inactive SLE entered the study. Their mean (SD) age was 48.5 (1.8) years. Of 13 different patients (1 male, 12 female) aged 39.2 (16.7) years, blood samples could be drawn at the moment of active disease. In addition, from 10 of these 13 patients with active disease, samples could be analysed at a moment of inactive disease as well. This resulted in a total of 20 patients evaluated at the moment of inactive disease.

Lymphocytes of patients with SLE showed signs of increased activation at the moment of active disease. The percentage of CD4+ lymphocytes expressing CD25 was higher (p=0.03) in patients with active disease than in controls (table 1). Furthermore, the percentage of activated (CD38+) B lymphocytes was raised in patients with active disease (p=0.04 v controls). Expression of costimulatory molecules was measured on CD4+ T cells and on CD19+ B cells. Almost all CD4+ T cells expressed CD28 on their surface, which was comparable between controls and patients with SLE (table 1). CD40L was hardly detectable on CD4+ T cells of controls and patients (data not shown). Regarding B cells, the percentage of cells expressing CD40 was comparable between controls and patients. The percentage of CD19+ B cells expressing CD80 was low in controls as well as patients. A correlation between CD80 expression and the disease activity of patients with SLE was not found. In contrast, CD86 expression differed between the groups investigated. Compared with controls the percentage of CD19+ B cells expressing CD86 was increased in patients with SLE with inactive disease (p=0.04) and increased even more in patients with active disease (p=0.0003 v controls). Furthermore, CD86 expression correlated with the SLEDAI score (fig 1) and levels of antibodies to dsDNA (r=0.47, p=0.006). To analyse the relation between CD86 expression and cell activation we concentrated on activated—that is, CD38+ B cells. Indeed, the percentage of B cells positive for both markers, though low, was substantially increased among patients with active disease (p=0.006 v controls).

For all costimulatory molecules paired samples from patients with active and inactive disease were analysed separately. This analysis
Costimulatory molecules in SLE

of dsDNA antibodies and prolonged life.3 In globulin in NZB/W F1 mice, which neutralises costimulation. Injection of CTLA-4 immunization seems to be dependent on CD86 interaction. Indeed, in murine lupus the production of changes may facilitate autoantibody production. These cells in mice.10 Therefore, increased expression of CD86 might promote lymphocyte survival involving with the elimination of autoreactive lymphocytes. Furthermore, it may render B cells more susceptible for T cell help. These changes may facilitate autoantibody production. Indeed, in murine lupus the production of anti-dsDNA antibodies and prolonged life.1 In MRL-lpr/lpr mice treatment with anti-CD86 alone inhibited anti-dsDNA expression while treatment with anti-CD80 did not change anti-dsDNA expression.1 Furthermore, it was shown that the presence of costimulatory molecules influenced the course of the disease. CD86 deficient MRL-lpr/lpr mice had significantly milder glomerulonephritis than wild-type mice.4

Aberrant CD86 expression on B lymphocytes has been reported in SLE. Folz en logen et al. analysed CD80 and CD86 expression on B cells of 13 patients with inactive SLE and found CD86 increased in patients compared with controls.11 We confirm these data and show that during disease activity CD86 is further up regulated. In addition, our data suggest a discrepancy in the expression of CD80 and CD86. Costimulatory signals of CD80 and CD86 lead to differential polarisation of T helper cell responses. There is evidence that CD80 preferentially acts as a costimulus for the generation of Th1 cells, whereas CD86 costimulates and induces Th2 cells.12 Our data, showing a difference in the expression of CD80 and CD86, are in line with the concept of SLE being a predominantly Th2 mediated disease.

In T cells, ligation of CD28 stimulates membrane expression of another accessory molecule, CD40 ligand (CD40L).10 CD40L is necessary for T cell help for B cells as shown by defective antigen-specific T cell responses in CD40L deficient mice.13 For example, CD40L influences apoptosis when ligation with its counter receptor CD40 takes place. Ligation of CD40 by CD40L can block B cell apoptosis induced by antigen receptor cross linking.14 In line with the CD28/CD86 pathway, aberrant expression of CD40 or CD40L may be a relevant factor in the development of autoimmune disease. Indeed, in murine lupus, treatment with anti-CD40L reduced the level of antibodies to dsDNA, and delayed the development of nephritis.7 Furthermore, a dysregulation of CD40L expression in human lupus has been described.15 We did not find changes in CD40L expression on CD4+ T lymphocytes in patients with SLE. CD40L expression was low in patients with inactive disease and controls and, unlike the results of others, did not increase during active disease. In experiments in which we stimulated isolated peripheral blood mononuclear cells with anti-CD3, we detected proper increase of CD40L expression in conjunction with T cell activation, excluding the possibility that our CD40L monoclonal antibody was inappropriate (data not shown). Also, CD40 expression on B cells, being high in lupus patients irrespective of disease activity, did not differ from that in healthy controls.

In conclusion, our data clearly show that in patients with SLE, even at the moment of inactive disease, the expression of CD86 on CD19+ B cells is increased, and is associated with B cell activation and levels of antibodies to dsDNA. Whether aberrant expression of CD86 in patients with SLE is constitutive or merely results from increased lymphocyte activation needs further investigation. Nevertheless, our data provide increasing evidence that manipulating costimulatory pathways in lupus may be a potentially beneficial therapeutic strategy.

This study was supported by the Dutch Kidney Foundation (grant NSN 1482).


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Ann Rheum Dis 2001 60: 523-526
doi: 10.1136/ard.60.5.523

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