Antibody-dependent and phytohaemagglutinin-induced lymphocyte cytotoxicity in systemic lupus erythematosus

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Summary

An investigation of cell-mediated cytotoxicity in 22 patients with systemic lupus erythematosus (SLE), using both whole blood and purified peripheral blood mononuclear cells (PBM) to measure antibody-dependent (ADCC) and phytohaemagglutinin (PHA)-induced lymphocyte cytotoxicity for Chang liver cells, has revealed 2 distinct abnormalities in patients with active disease. PHA-induced cytotoxicity was found to be selectively reduced in whole blood assays only (P<0.05), whereas ADCC was impaired in both whole blood (P=0.02) and PBM (P<0.05) assays, when comparison was made with 52 normal controls. The addition of patients’ sera to corresponding assays utilising control PBM confirmed that the impaired PHA-induced cytotoxicity resulted from circulating inhibitory serum factors. Surprisingly little effect, however, was exerted on ADCC assays. These findings suggest that there is a reduction in numbers and/or functional capacity of Fc-receptor cells in active SLE, which may have pathogenetic implications.

Although peripheral blood lymphocyte subpopulations and mitogen responsiveness have been extensively studied in systemic lupus erythematosus (SLE), cell-mediated cytotoxic mechanisms have received comparatively little attention. The few studies of spontaneous lymphocyte cytotoxicity for cultured target cells have produced conflicting reports of both increased and normal cytotoxicity, while investigation of antibody-dependent cellular cytotoxicity (ADCC) has revealed the presence of impaired responses in patients with active disease when Chang liver cells were used as targets. In the present study we have extended investigation into both ADCC and phytohaemagglutinin (PHA)-induced lymphocyte cytotoxicity for Chang liver cells in SLE in relation to both the severity of the disease and the modifying effect of the patient’s serum.

Materials and methods

Patient and control groups

Twenty-two patients (21 women; 1 man; mean age 42·1, SD 11·8 years) with SLE who had either 4 criteria of the American Rheumatism Association or 3 criteria and a positive test for DNA antibodies were investigated. Untreated patients with clinically active disease were particularly selected for comparison with patients on treatment and in remission. Thirteen patients (12 women; 1 man; mean age 41·2, SD 13·2 years) were judged to have clinically active disease with varying combinations of fever, serositis, nephritis, arthritis, anaemia, and neurological manifestations, and only 4 of these were receiving treatment (prednisolone 5–15 mg/day in 4; azathioprine 100 mg/day in 1) at the time of their initial tests. Nine patients (all women; mean age 43·2, SD 9·9 years) were in remission, and all these were on treatment (prednisolone 5–15 mg/day in 8; azathioprine 25 mg/day in 1). Controls consisted of 52 healthy volunteers (25 women; 27 men; mean age 39·9, SD 14·7 years).

Accepted for publication 8 February 1980

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LABORATORY METHODS

Target cells. These consisted of Chang liver cells (Flow Laboratories, Irvine, Scotland) grown and harvested as previously described.11

Effector cells. Comparison of cytotoxic effector mechanisms in patients and controls was made by the simultaneous use of both heparinised (20 units/ml) whole blood (200 μl) and purified peripheral blood mononuclear cells (PBM) prepared as previously described.11

Measurement of ADCC and PHA-induced cytotoxicity. This was performed as described in detail by Wright et al.11

DNA antibodies. These were detected by a haemagglutination technique utilising stabilised chicken RBC coated with dsDNA.12

Lymphocytotoxins. These were detected by a microcytotoxicity test13 using B and T cells isolated by the differential centrifugation of SRBC-rosetted lymphocytes obtained from 4 normal donors of differing HLA type. Cytotoxicity was assessed after a 2-stage test, in which patients' sera and test lymphocytes were incubated for 1 h at 37°C, 18°C, and 4°C respectively, followed by the addition of rabbit complement and a further 2 h incubation at 25°C.

Lymphocyte subpopulations. T and B lymphocytes were estimated by the formation of T rosettes with SRBC and by immunofluorescence staining of surface immunoglobulin respectively as previously described.11

STATISTICAL METHODS

The cytotoxicity data were analysed by the Mann-Whitney ranking test.14 The relationship between cytotoxicity in whole blood and PBM assays was examined by regression analysis following logarithmic conversion of the data. Comparison of lymphocyte subpopulations in patients and controls was made by Student's t test.

Results

ANTIBODY-DEPENDENT CYTOTOXICITY

Patients with active SLE had reduced ADCC in both whole blood (P<0.02) and PBM assays (P<0.05) when compared with normal controls, whereas the corresponding cytotoxic responses of patients with inactive disease showed no such reduction (Fig. 1). There was a good correlation for ADCC between whole blood and PBM assays in controls (r=0.69, P<0.001, Fig. 2), and this relationship persisted in patients with SLE with both inactive (r=0.71, P<0.05, Fig. 3) and active disease (r=0.68, P<0.01, Fig. 4) despite the reduced cytotoxic responses of the latter subgroup.

Fig. 1 Antibody-dependent lymphocyte cytotoxicity (mean ± SEM) for Chang liver cells (20 000/ml) using both whole blood 200 μl (□) and peripheral blood mononuclear cells, 500 000/ml (■) in patients with active (n=13) and inactive (n=9) systemic lupus erythematosus (SLE), and normal controls (n=31). P values refer to comparisons with the corresponding control data.

PHYTOHAEMAGGLUTININ-INDUCED CYTOTOXICITY

By contrast, patients with active SLE had reduced PHA-induced cytotoxicity in whole blood assays only (P<0.005) when compared with both patients with inactive SLE and normal controls (Fig. 5). Cytotoxic responses in PBM assays, however, showed no such reduction. As a result of this selective reduction of PHA-induced cytotoxicity in the whole blood assay in active SLE there was no longer any correlation between the results of the two assays in these patients (r=0.02, not significant (NS), Fig. 6). However, patients with inactive SLE continued to show a good correlation between the 2 assays (r=0.69, P<0.05, Fig. 7), which was also present to a less extent in the control group (r=0.36, P<0.01, Fig. 8).
Lymphocyte cytotoxicity in SLE

**Fig. 2** The relationship between antibody-dependent lymphocyte cytotoxicity for Chang liver cells (20 000/ml) using both whole blood (200 µl) and peripheral blood mononuclear cells (500 000/ml) in normal controls ($r=0.69, P<0.001$).

**Fig. 3** The relationship between antibody-dependent lymphocyte cytotoxicity for Chang liver cells (20 000/ml) using both whole blood (200 µl) and peripheral blood mononuclear cells (500 000/ml) in patients with inactive systemic lupus erythematosus ($r=0.71, P<0.05$). (○) Patient on immunosuppressive drugs.

**Fig. 4** The relationship between antibody-dependent lymphocyte cytotoxicity for Chang liver cells (20 000/ml) using both whole blood (200 µl) and peripheral blood mononuclear cells (500 000/ml) in patients with active systemic lupus erythematosus ($r=0.68, P<0.01$). (○) Patient on immunosuppressive drugs.

**SERUM ADDITION EXPERIMENTS**

The effect of sera from patients with both active and inactive SLE and normal controls on ADCC and PHA-induced cytotoxicity is summarised in Figs 9 and 10.

In ADCC the addition of lupus sera had, surprisingly, little effect on the cytotoxicity produced by normal control lymphocytes; only 1 serum from a patient with active disease produced a marked reduction in cytotoxic response (Fig. 9).

By contrast, in PHA-induced cytotoxicity 8 of the 14 lupus sera tested produced a marked reduction in the response of normal control lymphocytes. The effect was particularly noticeable in the sera of patients with active disease, as 6 of the 9 patients in this subgroup had sera that produced marked inhibition of PHA-induced cytotoxicity (Fig. 10).

**DNA ANTIBODIES AND LYMPHOCYTOTOXINS**

The incidence of DNA antibodies and cold-reactive (4°C) lymphocytotoxins in patients with SLE is summarised in Table 1. The accuracy of the clinical assessment of disease activity was confirmed by the finding that 11 of the 13 patients with active disease had a significantly raised titre of antibody against ds DNA ($\geq 1/80$). By contrast, only 1 of the 9 patients with inactive disease had detectable DNA antibody.
Cold reactive (4°C) lymphocytotoxins also showed some correlation with disease activity. Four of the active disease subgroup had such lymphocytotoxins, whereas there was no activity in the sera of any of the patients with inactive disease.

**Lymphocyte Subpopulations**

T lymphocytes were found to be significantly reduced in patients with active SLE (P<0.02) when compared with normal controls (Fig. 11). There was also a significant reduction of B lymphocytes, which was again, restricted to patients with active disease (P<0.05).

Table 1 The incidence of DNA antibodies and cold-reactive lymphocytotoxins in patients with active and inactive SLE

<table>
<thead>
<tr>
<th>No. DNA antibodies (titre &gt; 1:80)</th>
<th>Lymphocytotoxins (4°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active SLE 13</td>
<td>11</td>
</tr>
<tr>
<td>Inactive SLE 9</td>
<td>1</td>
</tr>
</tbody>
</table>

P values refer to comparisons with the corresponding control data.

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**Fig. 5** Phytohaemagglutinin-induced lymphocyte cytotoxicity (mean ± SEM) for Chang liver cells (20 000/ml) using both whole blood, 200 µl (□□□) and peripheral blood mononuclear cells, 500 000/ml (□□□□) in patients with active (n=13) and inactive (n=9) systemic lupus erythematosus and normal controls (n=51). P values refer to comparisons with the corresponding control data.

**Fig. 6** The relationship between phytohaemagglutinin-induced lymphocyte cytotoxicity for Chang liver cells (20 000/ml) using both whole blood (200 µl) and peripheral blood mononuclear cells (500 000/ml) in patients with active systemic lupus erythematosus (r=0.02, P=NS). (0) Patient on immunosuppressive drugs.

**Fig. 7** The relationship between phytohaemagglutinin-induced lymphocyte cytotoxicity for Chang liver cells (20 000/ml) using both whole blood (200 µl) and peripheral blood mononuclear cells (500 000/ml) in patients with inactive systemic lupus erythematosus (r=0.69, P<0.05). (0) Patient on immunosuppressive drugs.
Discussion

This investigation has shown that patients with active SLE have abnormalities of both ADCC and PHA-induced lymphocyte cytotoxicity for cultured Chang liver cells. The simultaneous use of both whole blood and PBM as effector systems in these assays has been important in revealing the quite distinctive nature of these 2 defects of lymphocyte function.

In PHA-induced lymphocyte cytotoxicity, which is mediated predominantly by T lymphocytes, the impaired responses found in whole blood assays in patients with active disease are likely to result from the presence of inhibitory serum factors, as the corresponding assays using PBM showed no parallel reduction of cytotoxicity. Further confirmation of the presence of circulating inhibitory factors was provided by the finding that a high proportion of sera from patients with active SLE also produced a marked reduction in PHA-induced cytotoxicity in control PBM.

Although the origin of these serum effects requires further investigations, sera from patients with SLE frequently contain factors capable of inhibiting various aspects of lymphocyte function in vitro. A lymphokine-like substance which inhibited PHA-induced lymphocyte cytotoxicity in a chicken red blood cell system has been described previously in the sera of patients with active disease. The possibility of antilymphocyte antibodies causing such inhibition also exists as lymphocytotoxins, some of which may show a relative specificity for T lymphocytes, have been described in SLE. In the present study such lymphocytotoxins showed an association with disease activity but did not correlate with the inhibition of PHA-induced lymphocyte cytotoxicity detected either in whole blood assays or in the serum addition experiments. However, the sera of patients with active SLE may also contain...

![Graph showing correlation between whole blood specific cytotoxicity and peripheral blood mononuclear cells specific cytotoxicity](image)

**Fig. 8** The relationship between phytohaemagglutinin-induced lymphocyte cytotoxicity for Chang liver cells (20,000/ml) using both whole blood (200 µl) and peripheral blood mononuclear cells (500,000/ml) in normal controls (r=0.36, P<0.01).

![Bar chart showing percentage of control cytotoxicity](image)

**Fig. 9** The effect of serum (100 µl) from patients with active and inactive systemic lupus erythematosus (SLE) and controls on antibody-dependent lymphocyte cytotoxicity for Chang liver cells using peripheral blood mononuclear cells from 3 normal controls. Enclosed area indicates 95% confidence limits for normal control sera. (○) Patient on immunosuppressive drugs.
other noncytotoxic antilymphocyte antibodies which have been shown to be capable of inhibiting suppressor T lymphocytes. Although the lymphocytes mediating PHA-induced cytotoxicity are not known to have any suppressor function, the possibility of a further circulating antibody with the capability of also inhibiting this particular T lymphocyte subpopulation is, nevertheless, a very real one. The alternative explanation that the inhibitory effect resulted from circulating immune complexes seems unlikely in view of the lack of any appreciable inhibitory effect of the lupus sera on the ADCC produced by control lymphocytes. Finally, there remains the possibility that the inhibitory effect could have been caused by a nonimmunological mechanism. PHA binding factors, identifiable in macroglobulin fractions, have been detected in the sera of patients with Hodgkin's disease but have not, as yet, been reported in SLE.

By contrast, ADCC, which is mediated by Fc-receptor bearing cells, was found to be reduced in both the whole blood and PBM assays during active phases of SLE. The preservation of a good correlation between the whole blood and PBM assays makes it unlikely that the reduced ADCC in patients with active SLE is the result of inhibitory or 'blocking' serum factors such as antilymphocyte antibodies or immune complexes. These initial,
rather surprising, conclusions are supported by the almost complete absence of any effect that sera from these patients had on the ADCC responses of control PBM, even though they certainly contained lymphocytotoxins and are likely to have contained immune complexes. Our findings confirm the earlier reports of Schneider et al.8 and Cooper et al.,9 who used an assay based solely on PBM, and clearly establish that the reduced ADCC is not the result of concomitant steroid therapy. The use of DNA antibody titres in this series also provides improved objective assessment of disease activity in the patient subgroups.

Our observations suggest that there is a real reduction in either the cytotoxic capacity and/or the numbers of circulating Fc-receptor cells during active phases of SLE. Such a reduction in numbers could occur through the sequestration of these cells in the tissues either as a result of interaction with locally bound autoantibody or as a result of the 'arming' of Fc-receptor cells by immune complexes. In this context it may be relevant that the tissues of patients with SLE can contain substantial numbers of lymphoid cells even in the presence of a well marked lymphopenia.22 In the light of our present findings further characterisation of both Fc-receptor cells in peripheral blood at all stages of the disease and detailed investigation of ADCC for cultured target cells could reveal additional pathogenetic mechanisms in patients with SLE.

We thank Mr I. D. Allonby for excellent technical assistance. J. K. Wright is recipient of a Medical Research Council research studentship.

References

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doi: 10.1136/ard.40.1.11