Spontaneous lymphocyte-mediated (NK cell) cytotoxicity in systemic sclerosis: a comparison with antibody-dependent lymphocyte (K cell) cytotoxicity

J. K. Wright, P. Hughes, and N. R. Rowell

From the University Department of Medicine, Northern General Hospital, Sheffield, and the University Department of Dermatology, the General Infirmary at Leeds

Summary

Spontaneous (NK cell) and antibody-dependent (K cell) cytotoxicity were investigated in 39 patients with systemic sclerosis (SS) and compared with that found in 52 normal controls. $^{51}$Cr-labelled Chang liver cells were used as targets in assays utilising both whole blood (WB) and peripheral blood mononuclear cells (PBM) as effectors. Patients with SS, who were severely affected by extensive visceral disease, were found to have significant impairment of both NK (p<0.005; p<0.05) and K (p<0.001; p<0.05) cell cytotoxicity by both effector systems, when compared with normal controls. These findings, which seem to be part of a wider defect in cell-mediated immunity, may provide a possible explanation for the described association of malignancy with systemic sclerosis.

Materials and methods

Thirty-nine patients with systemic sclerosis (34 women, 5 men; mean age 54.8 ± 12.7 years) were studied. Five of the patients were receiving treatment with immunosuppressive drugs (prednisolone, penicillamine, or azathioprine). Raynaud's phenomenon and acrosclerosis were constant features, and all patients were assessed for the extent of systemic involvement by the disease using previously described investigational criteria to allocate points and so produce a 'disease score' for each patient. On this basis patients with systemic sclerosis were divided into categories of mild (disease score 0–5; 23 women, 3 men; mean age 56.9 ± 11.8 years) and severe (disease score 6–17; 11 women, 2 men; mean age 50.4 ± 14.5 years) disease.

The controls consisted of 52 normal healthy volunteers (25 women, 27 men; mean age 39.8 ± 14.8 years).

Spontaneous (NK cell) and antibody-dependent lymphocyte (K cell) cytotoxicity. These assays were performed as previously described with $^{51}$Cr

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Correlation between whole blood (WB) and peripheral blood mononuclear cell (PBM) assays for NK and K cell cytotoxicity in patients with systemic sclerosis (n = 39) and controls (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of assay</td>
<td>Group studied</td>
</tr>
<tr>
<td>NK cell</td>
<td>Systemic sclerosis</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>K cell</td>
<td>Systemic sclerosis</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
</tbody>
</table>
Fig. 1  Spontaneous lymphocyte-mediated (NK cell) 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 mild (n = 26) and severe (n = 13) systemic sclerosis (SS) and normal controls (n = 52).

Fig. 2  Antibody-dependent lymphocyte (K cell) 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 mild (n = 26) and severe (n = 13) systemic sclerosis (SS) and normal controls (n = 52).

Fig. 3  The relationship between spontaneous lymphocyte-mediated (NK cell) cytotoxicity and antibody-dependent lymphocyte (K cell) cytotoxicity for Chang liver cells (20 000/ml) using whole blood (200 µl) in normal controls (r = 0.70, p<0.001).

Fig. 4  The relationship between spontaneous lymphocyte-mediated (NK cell) cytotoxicity and antibody-dependent lymphocyte (K cell) cytotoxicity for Chang liver cells (20 000/ml) using peripheral blood mononuclear cells (500 000/ml) in normal controls (r = 0.64, p<0.001).
labelled Chang liver cells as targets and both heparinised whole blood and purified peripheral blood mononuclear cells (PBM) as effector systems. NK cell cytotoxicity was expressed as percentage specific cytotoxicity after the 18 hour incubation of otherwise unmodified triplicate cultures of target and effector cells, while K cell cytotoxicity was similarly calculated for cultures to which had been added an optimal amount of rabbit anti-Chang cell serum.\(^{12}\)

The cytotoxicity data were analysed by the Mann-Whitney ranking test. The relationship between NK cell and K cell cytotoxicity in both whole blood and PBM assays was examined by regression analysis following \(\log_{10}\) conversion of the data.

**Results**

**Spontaneous (NK cell) cytotoxicity.** Comparison of NK cell cytotoxicity in both whole blood and PBM assays revealed some correlation which was more evident in patients with systemic sclerosis (Table 1, \(r = 0.44, p < 0.01\)) than in the controls (Table 1, \(r = 0.27, p = 0.05\)). Nevertheless, patients with severe systemic sclerosis showed reduced NK cell cytotoxicity in both whole blood (\(p = 0.005\)) and PBM assays (\(p < 0.05\)) when compared with the normal controls, whereas the corresponding cytotoxic responses of mildly affected patients showed no such reduction (Fig. 1).

**Antibody-dependent lymphocyte (K cell) cytotoxicity.** Comparison of K cell cytotoxicity in both assays revealed better correlations in both patients (Table 1, \(r = 0.64, p < 0.001\)) and controls (Table 1, \(r = 0.69, p < 0.001\)) than in the case of NK cell cytotoxicity. Patients with severe systemic sclerosis again showed reduced K cell cytotoxicity in both whole blood (\(p = 0.001\)) and PBM assays (\(p < 0.05\)) when compared with normal controls, in contrast, once more, to the normal responses of the mildly affected patients (Fig. 2).

**Correlation of spontaneous (NK cell) and antibody-dependent lymphocyte (K cell) cytotoxicity.** Comparison of NK cell and K cell cytotoxicity in both whole blood and PBM assays revealed good correlations in both controls (Fig. 3, \(r = 0.70, p < 0.001\); Fig. 4, \(r = 0.64, p < 0.001\)) and patients with systemic sclerosis (Fig. 5, \(r = 0.82, p < 0.001\); Fig. 6, \(r = 0.58, p < 0.001\)).

**Discussion**

There is increasing evidence that spontaneous lymphocyte-mediated cytotoxicity against allogeneic target cells, both neoplastic and nonneoplastic, is produced by a heterologous group of lymphocytes (natural killer or NK cells) which are now regarded as predominantly T cell in origin.\(^{13}\) Fractionation studies have shown that this ‘natural killing’ is carried...
out most efficiently by T cells lacking sheep red blood cell (SRBC) receptors and that increasing avidity of the SRBC receptor is associated with decreasing natural killer activity of the lymphocyte. In this spectrum of NK cell activity Fc receptor positive cells are generally more active than those lymphocytes lacking this receptor.\textsuperscript{14}

With this background the findings of the present investigation are of 3-fold interest. Firstly, the conclusion that patients severely affected by systemic sclerosis have reduced NK cell activity is in accord with earlier observations of reduced T cell numbers and mitogen responsiveness in the disease.\textsuperscript{4} The maintenance of a good correlation between NK and K cell cytotoxicity in this study is in keeping with previous findings in normal controls, even though there is now evidence that the lymphocyte subpopulations mediating these 2 types of cytotoxicity are to some extent distinct\textsuperscript{15} and can be distinguished in some disease states.\textsuperscript{15}

Secondly, the finding of impaired NK cell cytotoxicity in systemic sclerosis may also have a practical relevance. It has been suggested that NK cells are an important component of immune surveillance mechanisms against the development of tumours.\textsuperscript{16} There is growing support for this hypothesis in animal models,\textsuperscript{17} 18 while in man depressed NK cell function (as well as other immunological abnormalities) and an increased incidence of malignancy are found in diseases such as Wiskott-Aldrich syndrome, ataxia telangiectasia,\textsuperscript{19} and kidney allograft recipients on immunosuppressive therapy.\textsuperscript{15} An increased incidence of pulmonary tumours\textsuperscript{20} and perhaps more general malignancy\textsuperscript{21} has also been reported in systemic sclerosis, and our current findings provide a possible explanation for these associations.

Finally, the observation of low NK cell activity in patients severely affected by systemic sclerosis may also be important in relation to assays for antigen specific T cell cytotoxicity. Failure to take into account the "background" effect of NK cell cytotoxicity may mask the detection of more specific T cell meditated effects. In illustration of this point Svedmyr and Jondal\textsuperscript{22} found that it was necessary to remove a proportion of the NK cells before it was possible to detect antigen specific T cell cytotoxicity to EB virus infected cells in patients with infectious mononucleosis. It is therefore possible that the failure to take into account this masking effect of NK cell activity may account for the failure of recent studies to confirm the earlier reports\textsuperscript{4} of increased lymphocyte cytotoxicity in systemic sclerosis. As both experimental\textsuperscript{2} and human graft-versus-host disease\textsuperscript{9} 8 may be complicated by sclerodermatous changes, further investigation of cytotoxic mechanisms in systemic sclerosis using fractionated lymphocyte subpopulations would certainly seem to be justified and could shed further light on the pathogenesis of the disorder.

This study was supported by a project grant from the Wellcome Trust.

References


32 Svedmyr E, Jondal M. Cytotoxic effector cells specific for B cell lines transformed by Epstein-Barr virus are present in patients with infectious mononucleosis. *Proc Natl Acad Sci USA* 1975; 72: 1622–6.
Spontaneous lymphocyte-mediated (NK cell) cytotoxicity in systemic sclerosis: a comparison with antibody-dependent lymphocyte (K cell) cytotoxicity.

J K Wright, P Hughes and N R Rowell

Ann Rheum Dis 1982 41: 409-413
doi: 10.1136/ard.41.4.409

Email alerting service

These include:

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/