Natural killer cells and γδ T cells in scleroderma: relationship to disease duration and anti-Scl-70 antibodies

Randall F Holcombe, Bruce A Baethge, Robert E Wolf, Kenneth W Betzing, Ruby M Stewart

Abstract
Objectives—To examine the expression of the natural killer (NK) antigen CD56, and T cell receptor δ chain antigen (TCRδ), expressed on the γδ T cell subset, in patients with scleroderma, and to correlate levels of expression with clinical characteristics.

Methods—Peripheral blood mononuclear cells (PBMCs) from 15 patients with scleroderma and 11 controls were obtained from heparinised blood on a ficoll/hyphaque gradient, stained with monoclonal antibodies, and examined by flow cytometry for expression of CD56 and TCRδ.

Results—Overall, the proportion of PBMCs expressing CD56 in the patient group (14-4 (SEM 2-6)%/ control 2-61 (0-46)%) was not significantly different from controls (8-75 (2-6)%). The greatest levels of expression were found in patients late (more than three years) in their disease course (18-1 (3-3)%) and in patients who did not express anti-Scl-70 antibodies (17-1 (3-5)%). The proportion of γδ T cells was significantly lower in the patient group (1-61 (0-52)% v control 2-61 (0-46)% (p < 0-05). Patients early in their disease or with anti-Scl-70 antibodies accounted for the reduction in γδ T cells (0-71 (0-29)% and 0-96 (0-41)% (p < 0-01 and p < 0-05, respectively).

Conclusions—This study emphasises that NK and γδ T cell numbers vary depending upon patient characteristics and may help explain prior contradictory reports. Decreased numbers of γδ T cells were seen in scleroderma patients, especially those with anti-Scl-70 antibodies and a disease duration of less than three years.

(Scl-70 antibodies)

Scleroderma is a collagen-vascular disease characterised by abnormalities in cellular and humoral immunity. Natural killer (NK) cells have been postulated to be important in the pathogenesis of scleroderma but have been variably reported to be present in peripheral blood in increased, decreased, and normal. Specific subtypes of γδ T cells, which exhibit NK activity and LAK activity, have also been reported to be increased in patients with scleroderma.10 Natural killer cells are able to kill target cells without major histocompatibility (MHC) restriction and without the requirement for prior sensitisation. LAK cells are a heterogeneous population of peripheral blood mononuclear cells (PBMCs) including NK and T cells which, upon stimulation with interleukin-2 (IL-2), acquire the ability to lyse tumour cells in a non-MHC-restricted fashion. γδ T cells comprise approximately 5% of CD3 PBMCs and their function in vivo is unknown. Freshly isolated γδ T cells exhibit NK-like activity, and clones of γδ T cells cultured with IL-2 in vitro exhibit LAK activity.

In this study, we have examined the proportion of PBMCs expressing NK and T cell receptor (TCR) γδ antigens in patients with scleroderma. Correlations were made with clinical characteristics and the presence of anti-topoisomerase I (Scl-70) antibodies. The literature relating to NK cells in scleroderma is also briefly reviewed.

Materials and methods
PATIENT POPULATION
Fifteen patients with scleroderma all met American College of Rheumatology guideline criteria for systemic sclerosis.11 Disease duration was determined from the onset of first symptoms referable to scleroderma and cutaneous involvement was characterised as limited or diffuse as defined by Medsger.12 Patients were receiving no medication, non-steroidal anti-inflammatory medication, or low dose prednisone (<10 mg/day). No patients were receiving penicillamine or immuno-suppressive chemotherapeutic agents. Controls were healthy individuals with no evidence of rheumatological disease. They were sex matched with the patient group, but were slightly younger (mean 34 years v 49 years). Seven control subjects were white and four were black.

SCL-70 ANTIBODIES
The presence of antibodies to topoisomerase I (anti-Scl-70 antibodies) was determined on patient serum (Therastest Laboratories, Inc., Chicago, IL).
patients having (Scl-70-) anti-topoisomerase 1 antibodies. <3 yr, >3 yr = disease duration from first symptoms. Mean values with SEM bars. *p < 0.05 v control.

**STATISTICAL ANALYSIS**

Comparisons between patient and control groups were performed using a non-parametric two-tailed Mann-Whitney test for independent variables.

**Results**

Five of the 15 patients had evidence of anti-Scl-70 antibodies.

**NATURAL KILLER CELLS**

The proportion of PBMCs expressing the NK antigen CD56 in this population of scleroderma patients was greater than, but not significantly different from controls (fig 1). The increase in NK cells was related primarily to large numbers in patients without anti-Scl-70 antibodies and patients with a disease duration greater than three years. Patients without anti-Scl-70 antibodies had an increased proportion of NK cells compared with control which reached marginal significance (p = 0.069). Similarly, NK cell numbers were increased in patients with disease duration greater than three years (p < 0.05 v control), while patients earlier in their disease course had NK cell numbers which were not statistically different from control.

Absolute numbers of CD56 and TCRβ1 cells were available for all patients. Values for CD56 were greater for patients with a disease duration greater than three years (377 (SEM 119) v 158 (67) cells/mm³), those without anti-Scl-70 antibodies (358 (113) v 153 (49) cells/mm³) and those with limited rather than diffuse disease (454 (273) v 230 (52) cells/mm³). These results are all consistent with data analysed for percent CD56 cells.

**γδ T CELLS**

The proportion of PBMCs expressing TCRδ was decreased in patients with scleroderma compared with controls (p < 0.05) (fig 2). Control values in this study corresponded with values reported previously. Patients with anti-Scl-70 antibodies and patients with shorter disease duration had smaller numbers of γδ T cells compared with controls (p < 0.05 and p < 0.01, respectively). Shorter disease duration and the presence of anti-Scl-70 antibodies were independent variables by multivariate analysis.

Absolute values of TCRβ1 cells were smaller for patients with a disease duration less than three years (14.2 (5.9) v 37.5 (12.5)
cells/mm³), those with anti-Scl-70 antibodies (23.9 v 10.4 v 30.3 (11.5) cells/mm³) and those with diffuse disease (23.7 v 7.2 v 40.3 (25.1) cells/mm³). Again, all these results are consistent with data for patients with cutaneous CD56+ and CD57+ T cells between patients receiving or not receiving steroid medications.

Discussion
As summarised in the table, NK cells have been reported to be present in increased, decreased and normal numbers in patients with scleroderma. Previous studies have utilised antibodies directed against CD56, 2 CD16, 3,6 and CD57, 5 each of which identifies potentially different populations of PBMCs with NK activity. Differences in the antibodies utilised to identify NK cells contribute to the significant variations between studies. Additionally, control groups in earlier studies have been reported to have NK cells ranging from as few as 5–7% of PBMCs to 21–0%. 2 Values for controls in the current study lie within this range, though the control group was not age matched to the patient group. Differences in reported values for control groups and the use of controls not closely matched to the patient group studied also contributed to the apparent contradictions in the literature as to the proportion of NK cells present in scleroderma patients. Most importantly, differences in patient characteristics in the study groups have a critical influence on the reported numbers of NK cells.

Anti-Scl-70 antibodies are typically associated with diffuse cutaneous disease. The comparatively smaller numbers of CD56 cells seen here in patients with anti-Scl-70 antibodies are consistent with several previous reports demonstrating the smaller proportions of NK cells in patients with diffuse disease. 5, 14 The greater proportions of NK cells in patients with a disease duration greater than three years is also consistent with previous reports which have demonstrated lower levels in patients early in the course of their disease. 5, 7 Lower NK activity has also been reported to be correlated with early and diffuse disease. 5, 8 In one previous report, 29 of 40 scleroderma patients with decreased NK activity had evidence of anti-Scl-70 antibodies. 15 Diminished NK activity in patients early in their disease who express anti-Scl-70 antibodies may result from diminished numbers of specific subpopulations of NK cells or smaller numbers of NK cells and γδ T cells. 3, 7 The normal NK activity seen by Gonzalez et al. 8 may reflect a different patient group composition.

The decreased proportion of γδ T cells found in this study contrasts with the only previous investigation of these cells in patients with scleroderma, which revealed a statistically significant increase in non-disulphide linked γδ T cell receptor polypeptide chains, a small but non-significant decrease in the expression of the disulphide linked form, and overall normal levels. 10 All seven of the patients studied had a disease duration less than five years. The extent of cutaneous involvement and the presence or absence of anti-Scl-70 antibodies were not reported. The current study is the first to report a statistically significant decrease in γδ T cells in a subgroup of patients with scleroderma. Tissue recruitment of γδ T cells in patients with active disease may explain the reduction of these cells in the circulation.

Overall, the proportion of NK cells and γδ T cells in the peripheral blood of scleroderma patients depends upon the specific characteristics of the patient group studied and the specific monoclonal antibodies utilised to define a population of PBMCs as NK cells. Conclusions regarding increases or decreases in these cell types also depend in part upon values determined for a particular control group. Based on this study and a review of other studies reporting NK numbers and activities, it appears that the smallest proportions are seen in patients early (less than three years) in the course of their disease and in patients with cutaneous disease, especially if they express anti-Scl-70 antibodies. Decreased numbers of γδ T cells are also seen in patients early in disease and in those with anti-Scl-70 antibodies. The decrease in NK and LAK activity variably reported in scleroderma patients may reflect a relative decrease in NK or γδ T cell number in specific subgroups, or may reflect an as yet undefined defect in the function of PBMCs mediating NK and LAK activity.

Flow cytometric analysis of the proportion of PBMCs expressing natural killer antigens: comparison of current study with previously reported values for scleroderma patients and controls

<table>
<thead>
<tr>
<th>MAb</th>
<th>Controls</th>
<th>Patients</th>
<th>Conclusion</th>
<th>Reference</th>
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<tr>
<td></td>
<td>mean (SEM) %a</td>
<td>n</td>
<td>mean (SEM) %a</td>
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<tr>
<td>CD56+</td>
<td>21-0 (2-1)</td>
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<td>9-5 (1-5)</td>
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<tr>
<td>CD16+</td>
<td>193 (14°)</td>
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<td>197 (16°)</td>
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<td>74 (10-5)</td>
<td>21</td>
<td>20-8 (1-2)</td>
<td>16</td>
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<tr>
<td></td>
<td>12-7°</td>
<td>18</td>
<td>10-2°</td>
<td>50</td>
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<td>15-9 (1-2)</td>
<td>23</td>
<td>10-4 (1-0)</td>
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<td>171 (18°)</td>
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<td>147 (15°)</td>
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<td>218 (17°)</td>
<td>29</td>
<td>91 (0°)</td>
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<tr>
<td>CD57+</td>
<td>57 (5-0)</td>
<td>14</td>
<td>10-5 (1-5)</td>
<td>15</td>
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<tr>
<td></td>
<td>10-7°</td>
<td>18</td>
<td>8-7°</td>
<td>50</td>
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MAb = Monoclonal antibody; n = number of subjects; NS = not significantly different from control.

1 Except those indicated thus, which are absolute number of cells/mm³.
2 Conclusion of authors as to level of (++) staining in scleroderma patients ± control.

All scleroderma patients: increased in certain subgroups

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