Fluctuations in natural killer cell activity in early syphilis

JØRGEN R JENSEN, KRISTIAN THESTRUP-PEDERSEN, AND ELLIS FROM
From the Department of Dermatology and Venereology, University of Aarhus, Marselisborg Hospital, Aarhus, Denmark

SUMMARY The natural killer cell activity was studied in 25 patients with primary, secondary, or latent syphilis before and after treatment. In primary syphilis natural killer cell activity was increased, especially in patients lacking circulating lipoidal antibodies. In patients who had become seroreactive in the lipoidal tests it was depressed in those with secondary and latent syphilis. The natural killer cell activity thus becomes activated by the syphilitic infection but is significantly reduced during progression of the disease. The importance of the natural killer cell activity in controlling syphilitic infection is questionable.

Introduction

Host defence against infection with Treponema pallidum is primarily dependent on cell-mediated immunity (CMI). Previous studies of CMI in syphilis have shown reductions of both in-vivo and in-vitro immune reactions. Recently, we reported a reduction in natural killer (NK) cell activity in patients with secondary syphilis and obtained evidence for the depressive role of circulating immune complexes. The present study extends our previous findings and reports activation of NK cell activity in early primary syphilis. The increased NK cell activity found at this stage of the disease is later reduced, possibly owing to the action of circulating immune complexes.

Patients and methods

Twenty-five patients with syphilis were studied; their ages ranged from 18 to 62 years (mean 39·4 years). Eight had primary, 17 secondary, and two latent syphilis; two of the secondary cases also had syphilitic hepatitis. All but four primary cases had positive results to lipoidal serological tests for syphilis. After confirmation of the clinical diagnosis blood was collected for immunological investigation. The patients were treated with procaine penicillin (PAM) 600 000 IU daily for either 10 days (primary syphilis) or 14 days (secondary and latent syphilis). The immunological investigations were repeated one and two months after treatment.

Address for reprints: Dr J R Jensen, Department of Dermatology and Venereology, Marselisborg Hospital, Dk-8000 Aarhus C, Denmark

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Sixty-five control subjects, ranging in age from 20 to 56 years (mean 33·1 years), were studied in parallel.

ISOLATION OF LYMPHOCYTES

Twenty millilitres of heparinised blood (20 IU/ml heparin) was obtained from patients and controls. Phagocytic cells were removed by the addition of 0·2% (w/v) carbonyl iron to the blood, and lymphocytes were isolated using Ficoll-Hypaque gradients. After separation the lymphocytes were washed three times in Hanks' balanced salt solution (HBSS) with 2·5% (v/v) fetal calf serum (FCS) and resuspended in RPMI 1640 with 2·5% FCS to a density of 1 × 10^6/ml.

NATURAL KILLER CYTOTOXICITY ASSAY

Target cells were the human myeloid cell line K-562, clone 6, mycoplasma-free. The cell line was kindly donated by S Bisballe, Institute of Human Genetics, University of Aarhus. K-562 cells were maintained in RPMI 1640 with 2·5% FCS, penicillin (100 IU/ml), streptomycin (100 μg/ml), and gentamicin (1·5 μg/ml) in a humidified 5% CO₂ atmosphere at 37°C. Target cells (1 × 10^6 cells) were washed once with HBSS and 0·1 ml was labelled with 0·1 ml (100 μCi) of chromium-51 (³¹Cr) (Radiochemical Centre, Amersham, UK) for 60 minutes at 37°C. Labelled target cells were washed three times in cold HBSS with 2·5% FCS and resuspended in RPMI 1640 with 2·5% FCS to a density of 1 × 10^6 cells/ml. Effector cells (lymphocytes) were then mixed with 1 × 10^4 target cells in three effector-target (E:T) cell ratios (10:1, 25:1, and 100:1) in a final volume of 1·1 ml in 11 × 70 mm polystyrene tubes, centrifuged at 150 × g for five...
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minutes and incubated for four hours at 37°C in a humidified 5% CO₂ atmosphere. After resuspension and recentrifugation 0.55 ml of the supernates were withdrawn and the release of ⁵¹Cr determined in a gammacounter (LKB, Ultragammacounter). Tests were set up in triplicate. Spontaneous release (SR) was determined from target cells without effector cells and the maximal release (MR) of ⁵¹Cr was determined from target cells in medium after hypotonic lysis. SR never exceeded 20% of MR. The following formula was used to calculate the percentage of specific lysis:

\[
\frac{cpm\ \text{Exp} - cpm\ SR}{cpm\ MR - cpm\ SR} \times 100%\]

where cpm Exp refers to ⁵¹Cr release from admixtures with effector cells.

Effector titration graphs were made for each experiment with specific lysis as a function of log E:T ratio. The number of effector cells required to give 50% specific lysis was determined from the titration curves. The results were expressed as lytic units: 1·0 lytic unit represented 50% lysis with 10⁶ effector cells.

STATISTICAL ANALYSIS
Student’s t test (two-tailed) was used.

Results

In patients with primary syphilis the NK cell activity was increased compared with both the controls (t₁₅₀ = 3·93, p<0·001) and the lytic activity of their lymphocytes one month after treatment of the disease (t₇ = 3·33, p<0·02) (table). Patients with secondary and latent syphilis had significantly reduced NK cell activity compared with the controls (t₁₅₅ = 3·88, p<0·001). After treatment the NK cell activity became normal. The increase in NK cell activity seemed to be an early event in primary syphilis and was found in the absence of circulating lipoidal antibodies (figure). Once patients with primary syphilis had become seroreactive in the lipoidal tests the NK cell activity declined. In secondary syphilis the decline in NK cell activity became even more pronounced (figure).

Discussion

In a previous study we found a slight increase of NK cell activity in some patients with primary syphilis, all of whom had positive serological test results for syphilis. In this study we were able to investigate four patients with syphilis before their serum showed positive results in the lipoidal test; these patients clearly had increased NK cell activity. Thus NK cell activity may play a part in the body’s defence against infection with T. pallidum similar to that which occurs against viral infections and cancer.

The possible defence mechanism, however, seems to be unsuccessful, as determined by the reduced NK cell activity found in patients with later stages of the disease — that is, secondary and latent syphilis. We have reported previously that the depression of NK cell activity in secondary syphilis may be due to

<table>
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<tr>
<th>Stage of disease</th>
<th>Mean lytic units ± SD in relation to treatment:</th>
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<tbody>
<tr>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>Primary</td>
<td>5·81±1·42(8)+</td>
</tr>
<tr>
<td>Secondary and latent</td>
<td>1·35±1·32(16)+</td>
</tr>
</tbody>
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*See text
†Significantly increased compared with controls (Student’s t test: t₁₅₀ = 3·93, p<0·001) and with post-treatment level (paired t test: t₇ = 3·33, p<0·02)
‡Significantly reduced compared with controls (Student’s t test: t₁₅₅ = 3·88, p<0·001).
circulating immune complexes. Suppressive serum factors were found in secondary syphilitic serum more than six weeks after treatment, and this may explain the slightly reduced NK cell activity presently found two months after treatment. Also, persistence of Treponema pallidum and circulating immune complexes have been reported in patients after treatment of their disease.

NK cells are Fcγ-receptor-bearing lymphocytes. Some are contained within the Ty cell population. The Ty cell population is slightly increased in patients with primary syphilis and significantly reduced in secondary syphilis, probably owing to an in-vivo blockage by circulating immune complexes.

The present study provides evidence of an activation of NK cell activity induced by infection with Treponema pallidum. Probably, the presence of circulating immune complexes inhibits the NK cell activation through blocking of Fcγ-receptor-bearing lymphocytes. This may be yet another explanation for the prolonged clinical course of syphilis.

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References