ALTERATIONS IN T LYMPHOCYTES AND T-LYMPHOCYTE SUBPOPULATIONS IN PATIENTS WITH SYPHILIS

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SUMMARY The distribution of T-lymphocyte subpopulations was studied in 34 patients with primary or secondary syphilis before and after treatment. An absolute and relative T lymphopenia was found in all patients. In primary syphilis the concentration of helper cells—T cells with Fc receptors for IgM (Tμ)—was low whereas in secondary syphilis the suppressor cell concentration—T cells with Fc receptors for IgG (Tγ)—was reduced. Using lymphocytes from healthy subjects this could be imitated in vitro by the addition of serum from patients with secondary syphilis. In many autoimmune diseases a low concentration of Tγ cells may be a primary factor in the production of autoantibodies. The occurrence of similar changes in patients with secondary syphilis, however, indicates that such fluctuations in the T-cell subpopulations may take place during a strong immune response.

Introduction

In syphilis the appearance of heterologous antibodies is diagnostic of the disease and indicates polyclonal activation of B lymphocytes. At the same time various cell-mediated immune functions seem to be reduced. These findings indicate that patients with syphilis have an imbalance between B-cell activation and T cells with immunoregulatory functions.

In the present investigation of patients with syphilis, characteristic changes were observed in the subpopulations of T lymphocytes. These changes were related to the presence of antibodies and could be reproduced in vitro. The implication of these findings is discussed in relation to similar observations in patients with autoimmune diseases.

Patients and methods

Study population

Thirty-four patients with syphilis were studied. Their ages ranged from 16 to 44 years (mean 27.2 years). Seventeen had primary and 17 secondary syphilis. All had positive serological test results for syphilis. After syphilis had been diagnosed clinically, blood was collected for immunological investigations. The patients were then treated with procaine penicillin retard (PAM) 600 000 IU daily for either 10 days (primary syphilis) or 14 days (secondary syphilis). The immunological investigations were repeated one and two months after treatment.

Ninety-eight controls were studied, ranging in age from 16 to 60 years (mean 29 years); these were staff members and other individuals who had been screened for venereal disease and were without actual disease at the time of the study.

Isolation of mononuclear cells

Lymphocytes from heparinised blood were isolated on Ficoll-Hypaque gradients and phagocytic cells removed by the carbonyl iron incubation method. After separation, lymphocytes were washed three times in Hank's balanced salt solution (HBSS) with 2% fetal calf serum (FCS) and adjusted to 1 × 10⁶/ml.

Enumeration of T lymphocytes

One hundred microlitres of lymphocytes (1 × 10⁶/ml) and 100 μl of 2-aminoethylisothiouronium bromide hydrobromide-treated sheep erythrocytes (E-AET) were incubated at 37°C for 15 minutes in RPMI-1640 with 20% FCS in a ratio of 1:100. The mixture was spun at 150 × g for five minutes and incubated at 4°C for at least one hour. After 10 μl of 0.1% acridine orange had been added the pellet was carefully resuspended, and 200 lymphocytes were counted with a fluorescence microscope. Lymphocytes with three or more erythrocytes bound to their surface were counted as rosette-forming cells.

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The subfraction of T lymphocytes with high affinity to sheep erythrocytes form rosettes with untreated sheep erythrocytes (E-active or E-a rosettes). One hundred microlitres of lymphocytes suspended in RPMI-1640 without FCS were incubated with 100 μl untreated sheep erythrocytes in a ratio of 1:10 for five minutes at 37°C. The mixture was spun for five minutes at 150 × g, incubated at 4°C for five minutes, and resuspended for rosette enumeration.

PURIFICATION OF T LYMPHOCYTES
Five millilitres of E-AET and 5 ml of lymphocytes were mixed and incubated as described above. After resuspension, Ficoll-Hypaque flotation was performed (600 × g for 15 minutes). The pellet of E-AET-rosetting T lymphocytes was resuspended in 0.83% TRIS NH₄C1 (10 minutes) to lyse the erythrocytes. After three washes with HBSS without serum the T lymphocytes were adjusted to a concentration of 1 × 10⁶/ml. Purity, as determined by E-AET rosetting of the T-lymphocyte suspensions, was between 91% and 98%. Lymphocytes with surface immunoglobulin (B lymphocytes) were not found in the purified T-lymphocyte suspensions.

PREPARATION OF ANTI-OX-RBC ANTIBODIES
Rabbits were immunised by weekly subcutaneous injections of 1 ml of 10% ox red cells (ORBC) in Freund's incomplete adjuvant. Bleedings were performed weekly starting one week after the first immunisation. Serum was inactivated at 56°C for 30 minutes and IgG was purified by DEAE-cellulose ion-exchange chromatography. IgM was obtained by gel filtration (Ultrogel AcA 22, LKB). Both the IgG and IgM suspensions gave single peaks in a crossed immunoelectrophoresis against swine anti-rabbit immunoglobulins. Purity of the IgM suspension was further indicated by the lack of rosetting capacity of monocytes with ORBC coated with IgM antibodies; all monocytes with ORBC coated with IgG antibody, however, formed rosettes.

ERYTHROCYTE-ANTIBODY (EA) SUSPENSIONS
ORBC-antibody complexes were prepared from ox erythrocytes in Alsevers solution, washed five times in HBSS, and resuspended to a concentration of 2%. Subagglutinating concentrations of IgG or IgM anti-ORBC antibody were mixed with equal volumes of 2% ORBC and kept for 90 minutes at room temperature. Three washes of the ORBC-antibody complexes were done in HBSS with 2% FCS. Finally, 10% FCS was added. These erythrocyte-antibody (EA) suspensions were kept at 4°C and used during the next two days.

ENUMERATION OF T-LYMPHOCYTE SUBPOPULATIONS
Purified T lymphocytes were incubated in RPMI-1640 with 20% FCS at 37°C in a humidified CO₂ atmosphere. After 24 hours' (day 1) and 48 hours' (day 2) incubation appropriate amounts of T lymphocytes were washed three times in HBSS with 2% FCS. The T lymphocytes (0.01 ml) were mixed with ORBC coated with subagglutinating doses of either IgG (0.01 ml) or IgM (0.01 ml), spun immediately at 150 × g for five minutes, and incubated at 4°C for three hours. The percentage of cells forming rosettes was counted as described above.

SERUM ASSAYS
Immunoglobulins in serum from each of three patients with secondary syphilis were removed by ammonium sulphate precipitation. Sera from three controls were treated similarly. T lymphocytes from the three controls were cultured for 24 hours and incubated for 60 minutes at 37°C with autologous serum, autologous serum without immunoglobulins, syphilitic serum, and syphilitic serum without immunoglobulins. T lymphocytes without addition of serum acted as controls. Serum was not inactivated by heat. Cell numbers and cell deaths were controlled before and after serum treatment. Three washes with HBSS with 2% FCS were done before rosette enumeration.

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

Results
The total number of T lymphocytes (E-AET rosette-forming lymphocytes) was significantly reduced during both primary and secondary syphilis (table I). This was due to lymphopenia, since the percentage of T lymphocytes was within the normal range although slightly reduced (table II).

The subfraction of T lymphocytes with high affinity for sheep erythrocytes (E-a rosette-forming lymphocytes) was also reduced (table I). In patients with primary syphilis both an absolute and relative reduction of E-a T lymphocytes was found, whereas in patients with secondary syphilis only the absolute number of E-a T cells was reduced (table II).

During primary syphilis the percentage of T lymphocytes with Fc receptors for IgM (Tµ) was significantly reduced. T lymphocytes with Fc receptors for IgG (Tγ) were slightly increased (t₉₀=1.50, 0.1<0.05; fig I). In patients with secondary syphilis this was almost reversed. The
### TABLE I  
**Total number of T lymphocytes in peripheral blood from patients with syphilis and controls**

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>One month after treatment</th>
<th>Two months after treatment</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E-AET</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary syphilis</td>
<td>1479±213(9)*</td>
<td>2052±293(6)</td>
<td>2045±228(7)</td>
<td>2089±73·11(87)</td>
</tr>
<tr>
<td>Secondary syphilis</td>
<td>1579±183(13)†</td>
<td>2204±224(10)</td>
<td>2651±352(9)</td>
<td></td>
</tr>
<tr>
<td><strong>E-a</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary syphilis</td>
<td>829±63(9)‡</td>
<td>1175±157(7)</td>
<td>1061±229(6)</td>
<td>1179±47·24(84)</td>
</tr>
<tr>
<td>Secondary syphilis</td>
<td>882±83(12.§</td>
<td>1302±130(12)</td>
<td>1477±245(8)</td>
<td></td>
</tr>
</tbody>
</table>

SEM = standard error of mean; n = No of patients or controls; E-AET = 2-aminoethylisothiouonium bromide hydrobromide-treated sheep erythrocytes; E-a = untreated sheep erythrocytes

*Significantly reduced ($t_{94} = 2.71$, $P<0.01$)
†Significantly reduced ($t_{98} = 2.58$, $P<0.02$)
‡Significantly reduced ($t_{91} = 4.45$, $P<0.005$)
§Significantly reduced ($t_{94} = 3.12$, $P<0.005$)

### TABLE II  
**Percentage of T lymphocytes in peripheral blood from patients with syphilis and controls**

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>One month after treatment</th>
<th>Two months after treatment</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E-AET</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary syphilis</td>
<td>77·4±2·5(15)</td>
<td>76·7±1·2(10)</td>
<td>78·7±1·4(7)</td>
<td>80·5±0·55(98)</td>
</tr>
<tr>
<td>Secondary syphilis</td>
<td>77·6±2·0(16)</td>
<td>81·4±1·2(14)</td>
<td>78·3±1·5(11)</td>
<td></td>
</tr>
<tr>
<td><strong>E-a</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary syphilis</td>
<td>40·4±1·8(14)*</td>
<td>41·7±1·7(9)†</td>
<td>38·3±3·3(7)‡</td>
<td>45·9±0·93(95)</td>
</tr>
<tr>
<td>Secondary syphilis</td>
<td>44·1±2·4(15)§</td>
<td>46·7±3·8(14)§</td>
<td>41·0±2·1(10)§</td>
<td></td>
</tr>
</tbody>
</table>

SEM = standard error of mean; n = No of patients or controls; E-AET = 2-aminoethylisothiouonium bromide hydrobromide-treated sheep erythrocytes; E-a = untreated sheep erythrocytes

*Significantly reduced ($t_{107} = 2·7$, $P<0.01$)
†Significantly reduced ($t_{102} = 2·15$, $P<0.05$)
‡Significantly reduced ($t_{100} = 2·23$, $P<0.05$)

**FIG 1**  
The percentage of Ty and T$\mu$ rosettes in patients with primary syphilis before treatment (A, $n=14$), one month after (B, $n=7$), and two months after treatment (C, $n=7$) in T cells cultured for 24 hours and 48 hours compared with controls ($n=84$). Results represent mean ± SEM (Student’s t test).

**FIG 2**  
The percentage of Ty and T$\mu$ rosettes in patients with secondary syphilis before treatment (A, $n=9$), one month after (B, $n=11$), and two months after treatment (C, $n=12$) in T cells cultured for 24 hours and 48 hours compared with controls ($n=84$). Results represent mean ± SEM (Student’s t test).
percentage of T\(\mu\) lymphocytes was normal whereas that of Ty cells was significantly reduced (fig 2).

The reduced percentage of Ty cells during the secondary stage was investigated in vitro by adding serum from each of three patients with secondary syphilis to T lymphocytes from three healthy donors. Complement was not inactivated by heat. The expression of the Fc-IgG receptor on the T lymphocytes was significantly reduced without selective killing of Ty cells \((t_2=6.48, P<0.025)\). When the immunoglobulin in serum was removed through ammonium sulphate precipitation this effect disappeared (table III). No reduction of the expression of the Fc-IgM receptor was observed in similar experiments using serum from a patient with primary syphilis (results are not shown).

**Discussion**

The importance of cell-mediated immunity (CMI) in syphilis is suggested by T-lymphocyte depletion in the paracortical areas of lymph nodes and the perirenal area of the spleen in children with fatal congenital syphilis.\(^9\)\(^10\) In rabbits, the infection takes a severe course if T lymphocytes are destroyed by cyclophosphamide treatment.\(^11\) In patients with syphilis evidence has been found for a depression in CMI, which involves cutaneous reactivity, low T-cell numbers,\(^6\) low in-vitro reactivity to *Treponema pallidum*,\(^1\)\(^3\)\(^5\) and immunosuppressive factors in serum.\(^1\)\(^2\)

This study demonstrates that the total number of T lymphocytes are reduced in patients with early syphilis. The T lymphopenia may be due to an unequal distribution of lymphocytes in the lymphoid system, although a depletion of lymphocytes is also seen in histological examinations of enlarged lymph nodes during early syphilis.\(^12\) Another possibility is that cytotoxic antibodies to T lymphocytes may occur, although lymphocytotoxic antibodies were shown to be active only at 15°C.\(^13\) Also, the T lymphopenia disappears rapidly after treatment and the serum assays do not show any enhanced cell death. A third possibility is that *T pallidum* itself is directly toxic to T lymphocytes.

It has been proposed that T lymphocytes with Fc receptors may function as helper (T\(\mu\)) or suppressor (Ty) cells.\(^14\) In primary syphilis the T\(\mu\)-cell concentration was low, consistent with compartmentalisation of helper cells—that is, an unequal distribution of lymphocytes in the body during an immune response.\(^15\) Compartmentalisation of helper cells occurs during early antibody production.\(^16\)\(^17\) The Ty-cell concentration was increased, perhaps as a result of suppressor-cell activation.\(^18\)

In patients with secondary syphilis the T\(\mu\)-cell concentration was normal until two months after treatment when a fall occurred. The reason for this fall is obscure. During active secondary syphilis the Ty-cell concentration was significantly reduced. Explanations for this may be similar to those for the T lymphopenia. Furthermore, during secondary syphilis immune complex formation takes place,\(^19\) and it is well known that immune complexes interact in vitro with Ty cells leading to an irreversible loss of the Fc receptor for IgG.\(^20\) This mechanism may explain the reduction of the Ty cells in peripheral blood as indicated by the results from the serum assays (table III). No similar mechanism seemed to operate in primary syphilis.

It has been found that Ty cells activated by pokeweed mitogen and immune complexes release immunosuppressive factors.\(^21\) *T pallidum* may give an in-vivo stimulation, which in conjunction with immune complexes stimulates Ty cells to release immunosuppressive factors. This may explain the

**Table III** Effect of secondary syphilitic serum on homologous T lymphocyte subpopulations in three controls

<table>
<thead>
<tr>
<th>Donor serum (40%)</th>
<th>Subpopulations</th>
<th>Percentage of T lymphocyte subpopulations in controls:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 1</td>
<td>Control 2</td>
</tr>
<tr>
<td>Autologous serum</td>
<td>Ty</td>
<td>9·0</td>
</tr>
<tr>
<td></td>
<td>T(\mu)</td>
<td>31·5</td>
</tr>
<tr>
<td>Autologous serum without Ig</td>
<td>Ty</td>
<td>9·5</td>
</tr>
<tr>
<td></td>
<td>T(\mu)</td>
<td>29·5</td>
</tr>
<tr>
<td>Secondary syphilitic serum</td>
<td>Ty*</td>
<td>3·0</td>
</tr>
<tr>
<td></td>
<td>T(\mu)</td>
<td>33·0</td>
</tr>
<tr>
<td>Secondary syphilitic serum without Ig</td>
<td>Ty</td>
<td>8·5</td>
</tr>
<tr>
<td></td>
<td>T(\mu)</td>
<td>33·0</td>
</tr>
<tr>
<td>Controls without serum</td>
<td>Ty</td>
<td>11·0</td>
</tr>
<tr>
<td></td>
<td>T(\mu)</td>
<td>33·0</td>
</tr>
</tbody>
</table>

*Significant reduction (78%; \(t_2=6·48, P<0.025\))
reduced cutaneous reactivity in syphilitic patients\(^1\) and the depressed in-vitro functions of lymphocytes from peripheral blood.\(^1\)\(^3\)-\(^5\)

Reduction of the T\(^\text{y}\) cell concentration has been observed in various autoimmune diseases and in other diseases with increased antibody production.\(^2\)-\(^8\) The increased antibody production may be caused by direct polyclonal B cell activation or indirectly by a defective suppressor-cell mechanism, which may be a primary disorder or secondary to the occurrence of lymphocytotoxic antibodies or immune complexes. In systemic lupus erythematosus (SLE) the suppressor-cell defect is mainly a quantitative rather than a qualitative disorder.\(^29\)

Immunosuppressive factors, which depress non-specific suppressor-cell activity, have also been found in SLE.\(^30\)\(^31\)

The changes observed in the subpopulations of T lymphocytes occurred during an infectious disease in young subjects who were not suspected of having any immune deficiencies. These changes are similar to those found in various diseases with an imbalance between an increased B cell activity and signs of reduced immunoregulatory T cells.\(^22\)-\(^28\) Our findings seem to show that the changes in immunoregulatory T cells may be secondary to a strong immune reaction — that is, treponemal infection.

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17. Hahn H, Kaufmann SHE, Falkenberg F, Chahin M, Horn W. Peritoneal exudate T lymphocytes with specificity to sheep red blood cells. II Inflammatory helper T cells and effecter T cells in mice with delayed type hypersensitivity and in suppressed mice. Immunology 1979; 38: 51-5.