Infiltrate of syphilitic lesions before and after treatment

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SUMMARY An immunohistological study of skin biopsy specimens from patients with early syphilis was undertaken before and after treatment (one day after intramuscular administration of 2-4 MIU benzathine penicillin and eight days later, after a total administration of 3-6 MIU.

In chances from seronegative patients treatment with 3-6 MIU usually resulted in fewer immunocompetent cells in the infiltrate. In lesions of secondary syphilis treatment with 2-4 MIU benzathine penicillin produced a significant decrease in immunocompetent cells. After treatment with 3-6 MIU there was no further decrease. It was worth noticing that even eight to nine days after the initial pretreatment biopsy, when 3-6 MIU had been administered, the overall lymphohistiocytic infiltrate was not substantially diminished. Significantly more suppressor (T8+) cells were found in lesions of primary syphilis than of secondary syphilis, and they showed remarkable exocytosis. Activated local T8+ cells may release immunosuppressive lymphokines.

Abundant evidence suggests that Treponema pallidum escapes humoral immune defence, although the host produces antibodies early in the infection. Heterologous antibodies are diagnostic evidence of syphilis and indicate polyclonal activation of B lymphocytes. Conflicting results have been reported concerning cell mediated immunity status at different stages of the disease. Badanoiu et al,1 Janot et al,2 and Schell and Musher,3 are among the workers who have produced evidence of good in vitro cellular immune response against T pallidum. Other studies of delayed type hypersensitivity and inhibited leucocyte migration have indicated diminished cell mediated immunity to T pallidum in early syphilis.4-6 Gschnait et al have claimed that hitherto undetermined serum factors possibly block the immune system in the blood of patients with syphilis.8

In the study reported here we examined the identity of mononuclear cells and subsets that made up the lesional infiltrate at different stages of syphilis, both before and after penicillin administration, and speculated on the local pathogenetic mechanisms during the course of the disease.

Patients, materials, and methods
We studied skin biopsy specimens from 40 patients with early syphilitic lesions who were divided into four groups. Group 1 consisted of 11 with primary syphilis who were seronegative but dark field positive. Group 2a consisted of six similar patients 24 hours after they had received an intramuscular injection of 2-4 MIU benzathine penicillin. Group 2b consisted of four similar patients after intramuscular administration of a total of 3-6 MIU. Group 3 consisted of 11 patients with secondary syphilis (six with macular, and five with papular lesions) who were seropositive in the quantitative Venereal Disease Research Laboratory (VDRL), Wassermann, and fluorescent treponemal antibody absorption (FTA-ABS) (IgG and IgM) tests and the T pallidum haemagglutination assay (TPHA). Group 4a consisted of five patients similar to those in group 3 24 hours after receiving 2-4 MIU benzathine penicillin. Group 4b consisted of three similar patients after receiving 3-6 MIU. Both 2b and 4b groups were given 1-2 MIU one week after the 2-4 MIU penicillin. Patients with syphilis and positive for antibodies to HIV were excluded from the study.

The methods used were the indirect immunoperoxidase and the alkaline phosphatase anti-alkaline phosphatase (APAAP) techniques with monoclonal antibodies on 5μm cryostat sections fixed with acetone. The following antibodies were used: OKT3
Lymphocytes), OKT4/DAKO-T4 (predominantly for helper (inducer) cells), OKT8 (for suppressor (cytotoxic) T lymphocytes), Na,34 (for dendritic (mainly Langerhans') cells and activated T cells), B1 (for B cells), and OKM1 (for monocytes/macrophages). OKT3, OKT4, OKT8, and OKM1 were purchased from Ortho Pharmaceutical (Raritan, New York, USA), DAKO-T4 from Dakopatts (Denmark), Na,34 from Seralab (Sussex, England), and B1 from Coulter Electronics (Luton, England). The details of the indirect immunoperoxidase procedure have been described previously. APAAP was undertaken according to the protocol of Schaumburg-Lever. It was considered to be more sensitive and was used to check the results obtained with the indirect immunoperoxidase method. The results were always similar. OKT4 and DAKO-T4 gave similar results in the 12 specimens used. The percentage of cells staining positively with the "helper" antibodies was almost equal to that with peripheral T cell antibodies.

The cell subpopulations in the dermal infiltrate were given as percentages of lymphoid cells. Dendritic cells in the epidermis (Na,34+) were counted using an ocular micrometer (Zeiss, WKPL, 10×) on a light microscope (×400 magnification) and were expressed as cells/mm² epidermal cell surface.

The histological results were evaluated as intense disperse perivascular infiltrate, perivascular and in the dermal papillae, perivascular infiltrate only, or negative.

Cellular components were classified as (+ + +) if the cell type predominated in the infiltrate, (+ +) if there were many cells, (+) if they could be observed in adequate numbers, (±) if they were rarely observed, and (−) if there were none.

Statistical analyses were undertaken using the Wilcoxon rank sum test.

Results

From the histological study (table 1) vertical correla-

tion between groups did not show significant differences, except that significantly (p < 0.05) fewer plasma cells were found in group 2b than in group 1. More plasma cells were found in group 3, although not significantly more than in group 1.

The lymphohistiocytic infiltrate was mainly

<table>
<thead>
<tr>
<th>Group</th>
<th>No</th>
<th>Infiltrate</th>
<th>Lymphocytes</th>
<th>Macrophages (histiocytes)</th>
<th>Plasma cells</th>
<th>Polymorphs</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>3.00 (1.09)</td>
<td>2.73 (1.11)</td>
<td>2.09 (0.83)</td>
<td>2.18 (1.33)</td>
<td>0.18 (0.40)</td>
<td>0</td>
</tr>
<tr>
<td>2a</td>
<td>5</td>
<td>3.00 (0.89)</td>
<td>2.80 (0.98)</td>
<td>2.20 (0.40)</td>
<td>2.40 (0.80)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2b</td>
<td>4</td>
<td>2.50 (0.87)</td>
<td>2.00 (0.0)</td>
<td>2.20 (1.08)</td>
<td>1.00 (0.71)</td>
<td>0.25 (0.43)</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>13*</td>
<td>2.76 (1.09)</td>
<td>2.85 (0.97)</td>
<td>2.29 (0.72)</td>
<td>2.46 (0.96)</td>
<td>0.54 (0.66)</td>
<td>0.34 (0.49)</td>
</tr>
<tr>
<td>4a</td>
<td>5</td>
<td>2.00 (0.63)</td>
<td>1.80 (0.75)</td>
<td>1.80 (0.40)</td>
<td>1.30 (0.75)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4b</td>
<td>3</td>
<td>2.34 (0.47)</td>
<td>2.67 (0.47)</td>
<td>2.34 (0.47)</td>
<td>1.34 (0.47)</td>
<td>0.34 (0.47)</td>
<td>0</td>
</tr>
</tbody>
</table>

Groups 1 and 2 = patients with primary syphilis, group 2a 24 hours after receiving 2.4 MIU benzathine penicillin, group 2b after receiving 3.6 MIU; groups 3 and 4 = patients with secondary syphilis, group 4a 24 hours after receiving 2.4 MIU, group 4b after receiving 3.6 MIU.

The presence of polymorphs and eosinophils was not correlated.

*Two additional group 3 specimens included in histological study only.
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Table 2  
Semi quantitative immunohistochemical findings (figures show mean (SD) percentages of lymphoid cells except where shown)

<table>
<thead>
<tr>
<th>Group No.</th>
<th>T3⁺</th>
<th>T4⁺</th>
<th>T8⁺</th>
<th>B1⁺</th>
<th>OKM1⁺</th>
<th>Epidermal (cells/mm²)</th>
<th>Dermal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11*</td>
<td>31.78 (9.53)</td>
<td>41.36 (16.50)</td>
<td>29.20 (10.84)</td>
<td>19.04 (18.15)</td>
<td>24.34 (15.19)</td>
<td>269.05 (155.28)</td>
</tr>
<tr>
<td>2a</td>
<td>6</td>
<td>23.60 (9.62)</td>
<td>20.65 (9.36)</td>
<td>26.44 (11.08)</td>
<td>20.12 (7.75)</td>
<td>14.12 (10.61)</td>
<td>177.40 (187.30)</td>
</tr>
<tr>
<td>2b</td>
<td>4</td>
<td>13.87 (8.08)</td>
<td>16.28 (5.53)</td>
<td>7.49 (1.06)</td>
<td>3.26 (6.52)</td>
<td>19.85 (10.69)</td>
<td>197.67 (140.00)</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>37.99 (14.72)</td>
<td>34.46 (13.80)</td>
<td>44.84 (10.75)</td>
<td>39.50 (17.16)</td>
<td>21.56 (12.33)</td>
<td>341.80 (276.90)</td>
</tr>
<tr>
<td>4a</td>
<td>5</td>
<td>18.31 (11.00)</td>
<td>18.02 (15.64)</td>
<td>23.00 (14.89)</td>
<td>24.11 (18.15)</td>
<td>9.37 (9.76)</td>
<td>288.00 (202.79)</td>
</tr>
<tr>
<td>4b</td>
<td>3</td>
<td>22.00 (8.16)</td>
<td>25.53 (5.92)</td>
<td>26.89 (2.61)</td>
<td>8.33 (11.18)</td>
<td>14.97 (9.11)</td>
<td>195.40 (90.50)</td>
</tr>
</tbody>
</table>

See footnote of table 1 for descriptions of groups.

Results not available from one group 1 patient for B1⁺ and Na₃⁴⁺.

Subepidermal and perivascular, and it persisted even after treatment with 3-6 MIU penicillin.

The immunohistochemical study showed a characteristic exocytosis of T8⁺ cells (fig 1) that was prominent mainly before treatment. Minor exocytosis of T3⁺, T4⁺, and OKM1⁺ cells could be observed in some cases. Dermal Na₃⁴⁺ cells were predominantly lymphoid, although dendritic cells could also be observed in the upper dermis.

Table 2 shows semiquantitative analysis of the data obtained. Differences between groups 1 and 2a were significant regarding T4⁺ cells (p < 0.05) and showed a trend towards significance for T3⁺ and OKM1⁺ cells and Na₃⁴⁺ epidermal dendritic cells. Group 2b had significantly fewer T3⁺ and T4⁺ (p < 0.05), T8⁺ (p = 0.05), and B1⁺ (p < 0.01) cells than group 1, and Na₃⁴⁺ dermal cells showed a trend towards decrease without reaching significance. The decrease in T8⁺ and B1⁺ cells in group 2b was significant even compared with group 2a (p = 0.05 and p < 0.01, respectively).

Comparison between (untreated) groups 1 and 3 showed significantly more T8⁺ cells (p < 0.01) (fig 2) and B1⁺ cells (p < 0.05) in group 3 and a trend towards more dendritic epidermal cells.

Significantly fewer T3⁺ (p = 0.05), T8⁺ (p < 0.05), and Na₃⁴⁺ dermal cells (p < 0.01) were seen in group

![Figure 2](http://sti.bmj.com/GenitourinMed: first published as 10.1136/sti.64.5.289 on 1 October 1988. Downloaded from http://sti.bmj.com/ on September 15, 2023 by guest. Protected by copyright.)

Fig 2  Disperse T8⁺ cells in dermal infiltrate of syphilitic papule. (Alkaline phosphatase anti-alkaline phosphatase.)
This study showed significantly fewer epidermal dendritic cells in group 1 skin specimens than in normal skin, which is compatible with the hypothesis that reduced numbers of Langerhans' cells in the skin might enhance local multiplication of treponemes. Significantly smaller percentages of T4+ and T8+ cells in the inflammatory infiltrate of group 1 and 3 specimens than in that of patients with lichen planus favours the hypothesis of somehow decreased local cellular immune events in the inflammatory infiltrate of lesions of early syphilis. Treating patients with primary syphilis with 3·6 MIU benzathine penicillin usually reduced immunocompetent cells in the infiltrate. Treatment of patients with secondary syphilis showed an earlier response, producing an appreciable decrease after 2·4 MIU benzathine penicillin. Therafter, however, the rate of decrease appeared to be slow.

Correlating the histological with the immunohistochemical findings we can see that, although the percentages of B and T lymphoid cell subpopulations of the infiltrate changed after treatment and were decreasing at their own particular rates, even after a total of 3·6 MIU penicillin had been administered eight to nine days after the initial pretreatment biopsy, the overall lymphohistiocytic infiltrate was not substantially reduced.

T cells and subsets have been considered to be locally important in the early stages of syphilis, as Bjerke et al postulated for the interaction of host and parasite at that stage of the infectious process, despite the fact that systemically their function might be impaired by serum factors (such as spirochaete products, immune complexes or host immunosuppressive substances).

Our results showed a delayed influence of treatment on locally distributed immunocompetent cells. This is
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consistent with the results of Friedman and Türk who
found that people undergoing treatment with drugs
showed pronounced lymphocytic reactivity to the T
pallidum antigen.16

In syphilis, T pallidum stimulates local cell mediated
immunity that leads to its elimination.3 Macrophages
helped by Langerhans’ cells undertake phagocytosis
and process the antigen, which has specific biological
properties. Moreover the spirochaete is protected by a
mucoid envelope that prevents its being engulfed by
phagocytic cells or those presenting antigen. Jensen
and From have suggested that macrophages are
inhibited by infected T cells, which render C3β recep-
tors unavailable for processing antibody complement
opsonised treponemes.17

More Na,34+ epidermal dendritic cells (fig 3) were
found in patients with secondary than primary
syphilis. This was consistent with the findings of
Mittag and Klingmüller, who also found several
Langerhans’ cells in one patient with granulomatous
syphilis (a transitional stage between early and late
syphilis).14 They considered that the interaction of
Langerhans’ cells with intraepidermal T pallidum
might be important for the epidermotropic infiltr-
ation.

Decreased numbers of circulating suppressor cells
have been reported in secondary syphilis,18 which may
partly account for the increased amount of heterologous antibodies during this stage. The relative
increase in locally distributed T8+ cells at this stage (fig
2) might be related to the decrease in blood, because of
the peripheral distribution of lymphocytes in selective
tissues.19 Increased T8+ cells possibly release
immunosuppressive lymphokines20 locally and into
the blood stream, giving the invading micro-organism
an advantage for long term survival.4 This, in conjunc-
tion with local antibody releasing cells (B cells and
plasma cells) might explain the serological findings in
advanced primary or secondary syphilis.

References

1 Badanoiu A, Gavricesco M, Nicolau G, Circiumaresco T. Lymphoblastogenèse immunoallergique et antigène trepon-
2 Janot C, Grandidier M, Pupil P, Thomas JL, Berney J, de
Lavergne E. Le test de transformation lymphoblastique au
3 Schell RF, Muscher DM. Detection of non-specific resistance to
Listeria monocytogenes in rabbits infected with Treponema
4 Wright DJM, Grimble AGS. Why is the infectious stage of syphilis
5 From E, Thstrup-Pedersen K, Thulin H. Reactivity of
lymphocytes from patients with syphilis towards Treponema
pallidum antigen in the leucocyte migration and lymphocyte
migration tests. British Journal of Venereal Diseases
6 Fulford KWM, Brotstoff J. Leucocyte migration and cell mediated
immunity in syphilis. British Journal of Venereal Diseases
7 Pavia CS, Folds JD, Baseman JB. Cell-mediated immunity during
8 Gschnait F, Schoenwald F, Schidt BL, Lugcr A. Laboratory
evidence for impaired cellular immunity in different stages of
9 Tosca AD, Varelitzidis AG, Economidou J, Stratigos JD. Mucosis
fungoides: evaluation of immunohistochemical criteria for the
early diagnosis of the disease and differentiation between stages.
10 Schaubmq-Lever G. Immunoenzyme techniques in dermatopa-
11 McMillan EM, Wainik R, Beeman K, Everett MA. In situ
immunologic phenotyping of mucosis fungoides. J Am Acad
12 Tosca A, Pantelallos D, Varelitzidis A, Stratigos JD. Dendritic cells
13 Tosca A, Varelitzidis A, Michalopoulos M, Georgala S, Stratigos
JD. In situ identification of mononuclear cells in lichen planus.
14 Mittag H, Klingmüller G. Langerhans cells in granulomatous
15 Bjerke JR, Krogh HK, Matre R. In situ identification of mono-
nuclear cells in cutaneous infiltrates in discoid lupus
erythematosus, sarcoidosis and secondary syphilis. Acta Derm
16 Friedman PS, Türk JL. A spectrum of lymphocyte responsiveness
17 Tabor DR, Azadegan AA, Schell RF, Lefrock JL. Inhibition of
macrophage C3β-mediated ingestion by syphilitic hamster T
18 Jensen JR, From E. Alterations in T lymphocytes and
T-lymphocyte subpopulations in patients with syphilis. British
19 Turner DR, Wright DJM. Lymphadenopathy in early syphilis.
20 Moretta M, Mingari MC, Santali D, Perlman P, Moretta L.
Human T-lymphocyte subpopulations: alterations in systemic