T lymphoid cells in primary syphilis
Quantitative studies

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SUMMARY In previous studies to assess the role of cell-mediated immunity in Treponema pallidum infection investigations of delayed type skin hypersensitivity tests, lymphocyte transformation test, leucocyte migration inhibition tests, and histological studies of the reticulo-endothelial system have been performed.

In this study, the numbers of T (thymus-dependent) lymphoid cells in 10 patients with primary syphilis (eight untreated) were estimated by the rosette technique. Results indicate a significant decrease in the number of T lymphoid cells in patients with primary syphilis.

Introduction

The results of studies to assess the role of cell-mediated immunity in Treponema pallidum infection are inconsistent and therefore difficult to interpret, although the general impression that cell-mediated immunity has a protective function in syphilis—but is somehow adversely affected during the early and infectious stages of the disease—still remains.

Studies of delayed type skin hypersensitivity towards T pallidum antigens indicate that a specific anergy to antigens of T pallidum exists in the primary and secondary stage, as well as in general paralysis of the insane, without impairment of responses to other unrelated antigens.

The lymphocyte transformation test in human and experimental rabbit syphilis has been studied, but the results are conflicting; in some studies, a normal lymphoblastic response to T-cell mitogen phytohaemagglutinin (PHA), but reduced proliferation to treponemal antigens without suppression by syphilitic sera, was observed, whereas in other studies a diminished lymphoblastic response to T-cell mitogens (concanavalin-A (con-A) and PHA) but normal proliferation to T pallidum antigens was found, although the latter could be suppressed by syphilitic sera.

The results of studies using the leucocyte migration inhibition test are more consistent and agree with the results of delayed type skin hypersensitivity tests, indicating a diminished cell-mediated immunity towards T pallidum in primary and secondary syphilis.

Investigations of the histological appearance of lymphoid tissues in syphilis have shown that thymus-dependent areas are adversely affected in early acquired syphilis and in congenital syphilis.

Another method of assessing the cellular limb of immunity in syphilis is by determining the number of circulating T lymphoid cells in relation to the general distribution of lymphoid cells in patients with syphilis. In the present study, the number of circulating T lymphoid cells was estimated in 10 patients with primary syphilis by the rosette technique and compared with that in a healthy control group.

Patients and methods

STUDY GROUP
The patients included in this study were referred from the outpatient department of our hospital. A total of 10 patients was studied (the same as in a previous study).

All patients were male, and a diagnosis of primary syphilis was based on the existence of an indurated ulcer from which T pallidum could be demonstrated by darkfield microscopy and on a positive result to a treponemal serological test at the initial or at a subsequent examination.

Eight of the patients were untreated at the time of investigation whereas two had been treated for less than one week.

HEALTHY CONTROLS
Ten healthy staff members served as controls for the quantitative determination of T lymphoid cells. All were male and of the same age range as the study group.
T lymphoid cells in primary syphilis

ISOLATION OF LYMPHOID CELLS

Lymphoid cell separation was carried out as described previously20 according to the method of Boyum,25 with some minor modifications as described below.

Ten millilitres of peripheral blood was collected aseptically by venepuncture in Erlenmeyer bats containing 10 glass beads and shaken for 10 minutes for defibrination. To this was added 30 ml of phosphate-buffered saline (PBS). Portions of 10 ml of this suspension were layered on top of portions of 2·5 ml Ficoll-Isopaque mixture and centrifuged at 400 × g for 10 minutes. The resulting lymphoid-cell-rich layer between the Ficoll-Isopaque and the supernatant was aspirated and washed three times in PBS. After each washing, the lymphoid-cell suspension was centrifuged at 200 × g for five minutes. The lymphoid-cell-rich layer was composed of about 90-95% lymphoid cells and 5-10% monocytes and neutrophils. Viability of the isolated cells was over 98% as determined by trypan blue exclusion.

DETERMINATION OF T LYMPHOCYTES

Isolated lymphoid cells (1 × 10⁹) were resuspended in 0·3 ml minimal essential medium (MEM); to this was added 0·3 ml of fresh sheep red blood cell (SRBC) suspension containing a total of 50 × 10⁶ SRBC: this suspension of SRBC had previously been washed four times in MEM, each time at 200 × g for 10 minutes. The whole suspension of lymphoid cells and SRBC was centrifuged at 200 × g for five minutes and then incubated for one hour at 4°C. It was resuspended carefully. Only those lymphoid cells which had attracted at least three SRBC to form a rosette were counted (by light microscopy); these were T lymphoid cells and their number was expressed as a percentage of the total lymphoid cells in suspension.

PERIPHERAL BLOOD COUNTS

Total peripheral leucocyte counts and their differentiation were estimated to relate percentage figures to the absolute numbers of lymphoid cells.

STATISTICAL METHOD

The non-parametric Wilcoxon's test,26 with the usual correction for ties and the normal approximation to obtain the P value, was used for statistical analysis of the numbers of T lymphoid cells in the study group and in controls.

MATERIALS

Isopaque (Natrium N-, ethyl-3,5-diacetamido-2,4,6-trijodbenzoas) solution: 20 ml Isopaque (Nyegaard and Co, Oslo, Norway) was diluted with 25 ml distilled water.

Isopaque-Ficoll mixture: 10 parts Isopaque solution were mixed with 24 parts of Ficoll (9% in distilled water) (Pharmacia, Uppsala, Sweden).

Phosphate-buffered saline (PBS), pH 7·4: 8·2 g NaCl + 1·6 g Na₂HPO₄·2H₂O + 0·2 g NaH₂PO₄·2H₂O dissolved in 1 litre distilled water.

Minimal essential medium (MEM): A modified Eagle's solution containing 2 g/l sodium bicarbonate without glutamine (Flow Laboratories, Irvine, Scotland).

Sheep red blood cells (SRBC): Fresh sheep red blood cell suspension in Alsevers medium (Central Laboratory of the Red Cross Blood Transfusion Services, Amsterdam, the Netherlands).

Trypan blue: 0·05% in PBS.

RESULTS

The results of T lymphoid cell determination in 10 patients with primary syphilis are given in the Table, together with those in the 10 healthy controls. Absolute numbers of lymphoid cells in the study group did not differ from normal (mean: 2·35 × 10⁹/l; range 1·66–2·95 × 10⁹/l), and statistical analysis was performed on percentage figures.

<table>
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<th>Control No</th>
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Range  45-63  Range  54·5-69·0
Median 52·0  Median 62·5

*Patients who had been treated for less than a week
Standardised W = −2·9593
P value = 0·0035

The exact values of standardised W and two-sided tail probability, after Wilcoxon's test had been applied to the percentages of T lymphoid cells, are given in the Table. The number of T lymphoid cells in the study group and the control group was significantly different (P = 0·0035), with a difference in medians of −10·5%, indicating a decrease in T lymphoid cells in primary syphilis.
References