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The role of cell-mediated immune mechanisms in syphilis in Ethiopia

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SUMMARY

The lymphocyte transformation test was used to assess cell-mediated immune reactivity in 107 Ethiopian patients with syphilis. Lymphocytes from patients with early syphilis were unreactive to the Nichols strain of *Treponema pallidum*, which was in marked contrast to previous findings in similar patients in England. Lymphocytes obtained from patients with late syphilis, however, were reactive. The responses elicited by a strain of *T. pallidum* isolated from an Ethiopian with early syphilis did not differ from those with the usual Nichols strain. About half the patients with early syphilis who had received antibiotic treatment for 8 days showed an increase of lymphocyte reactivity towards *T. pallidum*, although responses to PPD and PHA were unchanged.

Plasma from patients with syphilis was examined for its capacity to inhibit lymphocyte responses *in vitro*. Although plasma from people with late (cardiovascular) syphilis did not differ from controls, plasma from patients with early syphilis inhibited the responses of their own cells to both PPD and PHA. The inhibitory effect on PHA responses was abrogated after the patients had received antibiotic treatment for 1 week.

The significance of the differences in lymphocyte reactivity observed between Ethiopians with syphilis and their counterparts in England is discussed with regard to a possible explanation of the differences in the natural history of the disease in the two countries.

INTRODUCTION

The relative contributions of humoral and cellular mechanisms of immunity in determining the manifestations and outcome of many infectious diseases have become the subject of much research in recent years. In many infections, antibodies alone confer only partial protection, and this has been shown to be the case in experimental syphilis of rabbits (Bishop & Miller, 1976; Weiser *et al.*, 1976). Since *Treponema pallidum* can enter cells, it may escape the action of circulating antibody (Sykes & Miller, 1971). As with other intracellular organisms such as *Mycobacteria* (Rees *et al.*, 1967) and *Leishmania* (Bryceson *et. al.* 1970), cell-mediated effector systems probably play a major role in overcoming the infection. Evidence in support of this proposition was provided by work in which rabbits with experimental syphilis were treated with immunosuppressive drugs, which diminished their cell-mediated immune mechanisms without affecting antibody production (Metzger, 1976). Lesions, which would have otherwise remained localized at the site of inoculation, became much more severe and spread confluently over the skin of the back.

Several studies of cell-mediated reactivity in human syphilis have been made *in vitro*, using both the lymphocyte transformation test (Friedmann & Turk, 1975; From, Thestrup-Pedersen & Thulin, 1976; Musher *et al.*, 1975) and the leucocyte migration inhibition test (Fulford & Brostoff, 1972; From *et al.*, 1976). They showed that cellular reactivity towards *T. pallidum* was present in the early stages of the disease. Lymphocytes from people with well-established seropositive primary syphilis, and also the

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later papular forms of secondary syphilis, were reactive, although cells from people with the macular type of secondary syphilis were not (Friedmann & Turk, 1975). Moreover, the reactivity of lymphocytes of most patients, including those that were unreactive, increased dramatically shortly after treatment with antibiotics was started (Friedmann & Turk, 1975; From *et al.*, 1976).

This study was conducted in Ethiopia, in order to extend the observations of lymphocyte reactivity to include people with late forms of the disease. It became apparent, however, that although early syphilis was highly prevalent, late forms were very uncommon. This finding was unexpected, since in Europe before effective treatment became available, about a third of people with the disease had ultimately developed late manifestations (Gjestland, 1955). The difference in the natural history of syphilis in Ethiopia and Europe might be accounted for by differences in the immune responses of the two populations.

MATERIALS AND METHODS

Patients. Blood samples were obtained from 139 adult Ethiopians, comprising 107 people with syphilis and thirty-two healthy controls (Table 1). Serological tests were positive on all patients with syphilis, and negative in control subjects. Those with early syphilis were classified according to established criteria, while those classified as latent syphilis had positive serological tests in the absence of clinical signs of disease. Cardiovascular syphilis was diagnosed when patients were found by miniature chest radiography to have a widened ascending aorta, sometimes accompanied by clinical signs of aortic valve incompetence or a resonant aortic second heart sound.

Serological tests. Sera were screened by the slide VDRL test (USPHS Manual, 1969), in which microparticulate carbon-labelled cardiolipin antigen (Searle) was used. Positive results were confirmed by the fluorescent treponemal antibody test with serum diluted 1/200 (FTA_{200}) (USPHS Manual, 1969).

Lymphocyte transformation (LT) tests. Tests were performed by a micromethod (Friedmann & Turk, 1975). Lymphocyte cultures were supplemented either with 10% pooled normal human serum obtained from healthy European donors and stored at −70°C, or plasma recovered from the lymphocyte separation procedure, or serum obtained from an aliquot of the same blood. Some cultures received phytohaemagglutinin (PHA, Wellcome, reagent grade) at a final dilution of 1/100 and were incubated for 3 days; others received appropriate concentrations of antigens and were incubated for 5 days. Cultures were given a 4 hr pulse of [^{3}H]thymidine (0·2 μCi, sp. act. 2 Ci/mmol) before aspiration onto glass fibre paper with an automatic harvester. [^{3}H]thymidine uptake was determined by liquid scintillation counting (Intertechnique SL-30).

Antigens. A washed saline suspension of Nichols strain *Treponema pallidum* grown in rabbit testicles (kindly provided by Dr Nafra Johnston, Venereal Disease Reference Laboratory, London) was stored frozen at −70°C. It was used at a final concentration of 10^5–10^6 organisms per ml, which was the same as that used in the previous study (Friedmann & Turk, 1975).

A fresh isolate of *T. pallidum* was obtained by aspiration from an inguinal lymph node of an Ethiopian patient with secondary syphilis. The aspirate was enriched by three passages through rabbits previously shown to be free from other treponemes. The separation procedure was the same as that used for the Nichols strain. They were used at a concentration of 10^5–10^6 organisms per ml.

A saline extract of normal rabbit testis treated as for the extraction of *T. pallidum* was used as control.

Purified protein derivative of tuberculin (PPD), free of preservative (Statens serum Institut, Copenhagen), was used at a final concentration of 10 μg/ml.

The results of lymphocyte transformation, as shown by incorporation of [^{3}H]thymidine, are expressed as net counts per minute (ct/min) per culture, obtained by subtracting the mean ct/min of control cultures from that of stimulated cultures. Lymphocyte responses in autologous plasma or serum are expressed as a percentage of their response in NHS. Experiments that tested the effect of plasma or serum from patients with syphilis upon normal lymphocytes were performed with cells from one European control subject.

Statistical comparisons of results were made by the Mann–Whitney 'U' test and the Wilcoxon matched-pairs signed-ranks test for paired observations (Siegel, 1956).

RESULTS

Responses to T. pallidum

When cultured with *T. pallidum*, lymphocytes from control subjects and those with early syphilis were unresponsive (Fig. 1, Table 1). However, cells from some patients with latent syphilis responded to *T. pallidum*, while those from most patients with cardiovascular syphilis did so.

The lack of response of cells from Ethiopian patients with early syphilis was in marked contrast to that

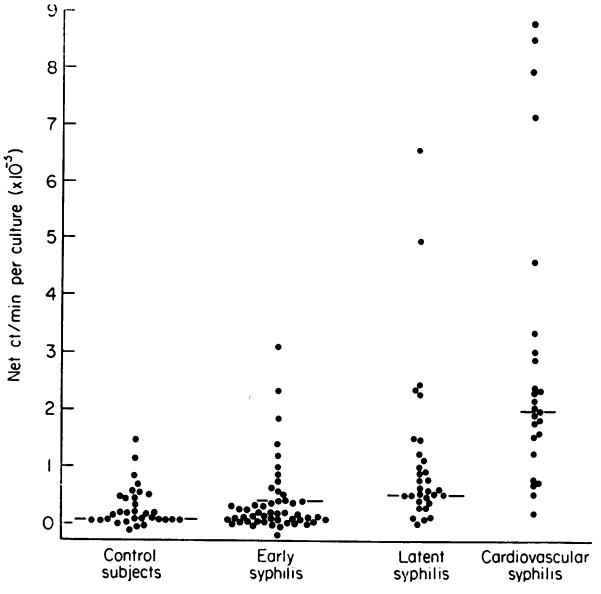

FIG. 1. Thymidine uptake by lymphocytes cultured with *T. pallidum*. Each point is the result from one subject. Median values are indicated with bars.

TABLE 1. Results of LT tests with three different reagents on lymphocytes from 107 patients with syphilis

Stage of disease	*T. pallidum*	PPD	PHA
Control	130 (−177–1455)*	1500 (−170–20442)	12000 (1399–47117)
Number tested	32	32	30
Early syphilis	148 (−250–3069)	2870 (−40–19316)	19017 (2291–47867)
Number tested	51	49	51
Latent syphilis	500 (−43–6540)†	2239 (0–12384)	13000 (1895–42415)
Number tested	31	31	30
Cardiovascular syphilis	1971 (141–8764)‡	3200 (502–15810)	6500 (1054–28323)
Number tested	25	25	24

* Figures are median net ct/min, range in brackets.
† $P < 0{\cdot}001$ compared with controls.
‡ $P < 0{\cdot}001$ compared with latent syphilis.

of their counterparts in England (Friedmann & Turk, 1975). Since this difference might reflect antigenic differences between the Nichols strain of *T. pallidum* and that which caused the disease in Ethiopia, a strain of *T. pallidum* isolated from a patient in Ethiopia was used as test antigen in parallel with the Nichols strain. There were no differences in lymphocyte responses to the two antigens. Cells from ten patients with latent syphilis gave a median response to the Nichols strain of 2728 ct/min (range 1195–6540); while that to the human isolate was 2239 ct/min (range 534–6591).

Responses to control rabbit extract, PPD and PHA

Lymphocytes from controls and patients were unresponsive when cultured with control rabbit extract. When cultured with PPD, cells from all groups gave similar responses (Table 1). When cultured with PHA, cells from patients with latent syphilis gave similar responses to those from control subjects

(Table 1). Cells from patients with early syphilis gave higher responses than controls ($P<0{\cdot}05$), whereas those from patients with cardiovascular syphilis were less responsive ($P<0{\cdot}02$). Patients in the latter group, however, were on average 20 years older than the controls and responsiveness to PHA diminishes as age increases (Hallgren *et al.*, 1973).

Effect of treatment on lymphocyte responses

Preliminary observations in England had shown that when patients with early syphilis were treated with antibiotics, the responses of their lymphocytes to *T. pallidum* increased dramatically (Friedmann & Turk, 1975). Since Ethiopian patients with early syphilis were unresponsive before they had received treatment, their lymphocytes were examined again at various times during, or after, the period of treatment. In the case of early syphilis, although the median response rose from 98 ct/min (range −176–962) to 2200 ct/min (range 374–12,933), after 7–8 days of treatment ($P<0{\cdot}005$ by Wilcoxon test) (Table 2), the responses of half the patients did not change (Fig. 2). With latent syphilis, the responses increased after 10 days of treatment (Table 2). The median response of cells from eight patients who returned for follow-up after 4–6 weeks had fallen below the value obtained before treatment. In the case of patients with cardiovascular syphilis, after 14 days of treatment the cells lost their reactivity. The responses to PPD and PHA of cells from all three groups of patients did not change after treatment.

Effect of autologous plasma on lymphocyte responses

A previous report showed that plasma from patients with early syphilis inhibited lymphocyte responses to PHA (Levene *et al.*, 1969). Results presented here compare the responses of lymphocytes to *T. pallidum*, PPD and PHA in autologous plasma with those obtained in pooled NHS (Table 3). As inhibitory serum factors have been described in pregnancy (Gatti, 1971), results from fourteen control subjects and sixteen with latent syphilis, who were women who had given birth in the previous 24 hours, were excluded.

The effects of plasma on lymphocyte responses to *T. pallidum* or PPD could only be evaluated when cells gave a positive response to the test antigen in pooled NHS. With *T. pallidum*, such positive responses were given only by cells from patients with cardiovascular syphilis.

Autologous plasma from patients with early syphilis inhibited the responses of their own lymphocytes to both PPD and PHA (Table 3). By contrast, plasma from control subjects and those with cardiovascular syphilis augmented the responses of their own lymphocytes to PPD and PHA. Furthermore, the responses to *T. pallidum* of cells from patients with late syphilis were not inhibited by autologous plasma (Table 3).

The inhibitory effect of plasma from thirteen patients with early syphilis was retested after the patients

TABLE 2. Lymphocyte responses to *T. pallidum* of patients who received penicillin

Stage of disease	Number of patients	Time after starting treatment			
		Day 0	7–8 days	10–14 days	4–6 weeks
Early syphilis	15	98 (−176–962)*	2200 (347–12933) [$P<0{\cdot}005$]†	n.d.	n.d.
Latent syphilis	12	1028 (62–6540)	n.d.	2095 (324–7790) [$P<0{\cdot}05$]	702 (−289–1512)‡ [n.s.]
Cardiovascular syphilis	8	2003 (647–7944)	n.d.	338 (60–1745) [$P<0{\cdot}01$]	n.d.

n.d. = Not done; n.s. = not significant.

* Figures are median net ct/min, range in brackets.

† Paired data compared by Wilcoxon test.

‡ Only eight patients returned for follow-up.

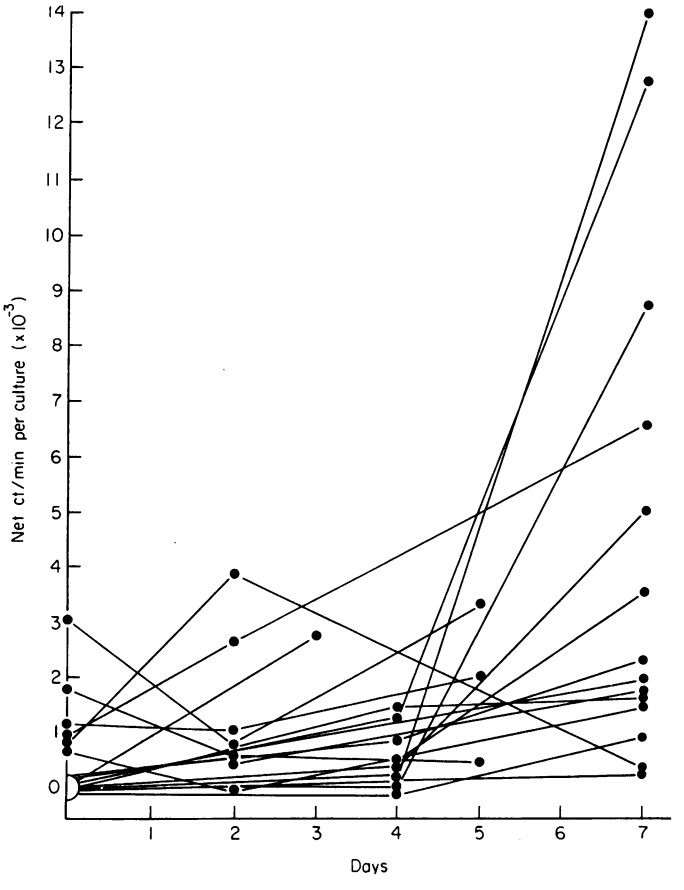

FIG. 2. Change in lymphocyte responses to *T. pallidum* when patients with early syphilis received penicillin. Sequential results from each patient are linked. Horizontal axis shows days of penicillin treatment.

TABLE 3. Results of LT tests with three different reagents done in autologous plasma compared with those done in pooled serum

Stage of disease	*T. pallidum*	PPD	PHA
Controls	—*	135 (11–571)†	104 (61–263)
Number tested	—	13	17
Early syphilis	—	57 (2–271)‡	67 (3–201)‡
Number tested	—	34	51
Latent syphilis	—	137 (67–468)	75 (25–136)
Number tested	—	11	14
Cardiovascular syphilis	109 (38–390)	168 (40–422)	133 (28–621)
Number tested	18	20	23

* No positive responses obtained with cells cultured in pooled NHS.

† Response in autologous plasma expressed as percentage of that in pooled serum, figures are medians with range in brackets.

‡ $P < 0{\cdot}001$ compared with control subjects.

had received 6–8 days of penicillin treatment. The median percentage inhibition of their own lymphocyte responses to PHA was 42% (range 22–81) before treatment and 74% (range 32–122) after treatment ($P<0{\cdot}01$ by Wilcoxon). There was, however, no significant change in the inhibitory effect on responses to PPD after this short period of treatment.

In some experiments, parallel cultures supplemented with fresh autologous serum were included. It appeared that when lymphocyte responses to PPD or PHA were inhibited, plasma was more inhibitory than was serum. When lymphocytes from a single healthy European control subject were cultured in plasma or serum from patients with early syphilis, there was a tendency for responses which were inhibited to be affected more by plasma than by serum.

DISCUSSION

The finding that lymphocytes from Ethiopian patients with early syphilis, even of a florid papular type, did not respond to *T. pallidum* in the LT test was quite unexpected, since such patients in England were usually responsive (Friedmann & Turk, 1975). That this difference was not merely a reflection of antigenic differences between the Nichols strain of *T. pallidum* and the actual pathogen found in Ethiopia was demonstrated in tests with lymphocytes from reactive patients, both in Ethiopia and subsequently in England (Friedmann, 1977). Similar results were obtained using the Nichols strain and one isolate from an Ethiopian patient.

The natural history of syphilis in Ethiopia is clearly different from that in Europe. Before effective treatment was available, about a third of people in Europe who contracted syphilis developed late complications (Gjestland, 1955). In Ethiopia, acquired syphilis is still highly prevalent (Ferreira-Marques, 1964; Friedmann & Wright, 1977), and congenital syphilis caused 8% of perinatal deaths in 1975 (Demussie, personal communication). Late syphilis, whether congenital or acquired, is very uncommon, however, and is certainly not found in proportion to the prevalence of the early disease. This was true even before 1953 when penicillin first became available (Ferreira-Marques, 1964). Differences in the immunological responses of Ethiopians and Europeans may account in part for these variations in the pattern of syphilis.

Evidence is accumulating which suggests that the results of the LT test correlate with the existence of delayed hypersensitivity rather than with protective immunity (Bjune *et al.*, 1976; Fleer *et al.*, 1976). In England, lymphocytes from patients with clear-cut syphilitic re-infections were highly reactive in the LT test during the earliest manifestations of primary lesions, and in most cases before serum antibody was detectable (Friedmann, 1977). The patients clearly had no protective immunity.

By contrast with findings in English patients, Ethiopians with syphilis do not usually develop cellular hypersensitivity to *T. pallidum*, as detected by the LT test. They are also spared the late complications of the disease. Moreover, lymphocytes reactive to *T. pallidum* were obtained regularly only from Ethiopian patients with cardiovascular syphilis. It seems likely, therefore, that late complications are caused by the tissue-damaging effects of delayed hypersensitivity reactions.

There is evidence which suggests that other diseases in Ethiopians show a similar dissociation between immune and hypersensitive reactions. For instance, leprosy occurs mainly as the 'borderline' form, usually of the more tuberculoid type which is associated with fairly competent host defences. Delayed hypersensitivity as detected by the LT test was, however, only present in a minority of patients (Bjune *et al.*, 1976). Again, onchocerciasis is highly prevalent in parts of Ethiopia, but the associated blindness, which is thought to be produced by hypersensitivity reactions, was found to be very rare in one endemic area in which it was sought (Oomen, 1967). There are many factors which may contribute to the different patterns of disease found in Ethiopia. These include concomitant infection with other organisms, and the effects of various dietary factors derived from the staple cereal food 'Teff' and other herbal medications taken on a wide scale. It is unlikely that purely racial factors are important, since the people of Ethiopia comprise several tribes of different origin.

It is not clear why lymphocyte responses to *T. pallidum* increase in patients given antibiotics. Possible explanations include the generation of increased numbers of specifically sensitized cells by the release of

spirochaetal antigens, the abrogation of immunological paralysis by reduction of antigen load or the appearance in the circulation of lymphocytes that had been trapped in lesions.

The nature of the inhibitory factors present in the plasma of patients with syphilis is far from clear. The factor is non-specific and appears to be labile, since attempts to preserve inhibitory activity by keeping plasma samples frozen at −70°C were unsuccessful. Other workers should be alerted to the possibility that different results may be obtained when plasma is used rather than serum.

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