Lymphocyte Transformation in Syphilis: an In Vitro Correlate of Immune Suppression In Vivo?

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Suppression of cellular immunity during primary and secondary infection may explain, in part, the unusual clinical evolution of syphilis. We have previously shown that lymphocytes from normal subjects undergo blastic transformation when exposed in vitro to *Treponema refringens*. This response was suppressed in patients with syphilis, the suppression being unrelated to serum factors. In the present paper we studied lymphocyte response in vitro to *T. refringens*, *T. reiter*, and *T. pallidum* as well as to monilia and trychophytins. The response to these antigens was suppressed in patients with syphilis although the response to phytohemagglutinin, pokeweed mitogen, and streptolysin was normal. These data support the hypothesis that human infection with *T. pallidum* is followed by a complex interaction between cellular and humoral immunity, the former being suppressed in primary and secondary stages.

One explanation for the unusual clinical progression of syphilis in which secondary (disseminated) disease waxes and wanes for weeks to months before culminating in latency is that there is an unusual balance between development and suppression of immunity (9, 17, 20, 21). Circulating antibodies that specifically adhere to *Treponema pallidum* (FTA-ABS) or immobilize it are present throughout, suggesting that defective humoral immunity is not responsible. Cellular immunity to treponemal antigens as manifested by delayed hypersensitivity does not appear until late in secondary syphilis, just before the onset of latency (2, 16). We have recently shown that lymphocytes from patients with primary and secondary syphilis have a suppressed response when exposed to *Treponema refringens* in vitro (9), and we proposed that this finding might support the hypothesis that a mechanism(s) of cellular immunity is inhibited during the early stages of syphilitic infection (17, 20, 21). We now present data showing that this suppression of lymphocyte responsiveness is more generalized, involving treponemal and nontreponemal antigens as well.

**MATERIALS AND METHODS**

**Patients.** Patients were identified at the Venereal Disease Clinic of the Houston City Health Department and blood was drawn before antibiotics were given. Primary syphilitics had characteristic chancres, which were shown by dark-field examination to contain treponemes. Patients with secondary syphilis had a characteristic rash and a positive FTA-ABS; late chancres containing treponemes and other manifestations of secondary syphilis were frequently present. All patients had reactive VDRL test. Control subjects were matched for age, sex, race, and approximate socioeconomic status; their VDRL and FTA tests were nonreactive.

**Lymphocyte transformation.** Lymphocyte transformation was studied by using previously described techniques (3, 9). Lymphocytes (5 \times 10⁶) obtained from defibrinated blood were cultured in 1 ml of minimal essential medium that contained 30% autologous serum and was supplemented with glutamine and neomycin. On the 5th day of culture in a CO₂-enriched atmosphere at 37°C, 1 μCi of [³H]thymidine was added. Two hours later the reaction was terminated. Deoxyribonucleic acid was extracted by precipitation, washed, and solubilized. The amount of [³H]thymidine was determined as counts per minute by using a liquid scintillation spectrophotometer.

**Mitogens and nontreponemal antigen.** The following mitogens in 0.05-ml quantities were added to lymphocyte cultures: phytohemagglutinin M (PHA; Difco), pokeweed mitogen (PWM; GIBCO), and streptolysin (SLO; Difco). Antigens included: mumps skin testing antigen (Lilly), dermatophytin O (Derm O; Hollister-Stier) and monilia skin test antigen (Lilly).

**Treponemal antigens.** Cultivable treponemes were prepared as follows: *T. refringens* and *T. reiter* were grown in spriolate broth (6) at 35°C for 5 days; the final concentration was 2 \times 10⁶ to 3 \times 10⁶ per ml.

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T. pallidum (Nichols strain), maintained in the laboratory by intratesticular passage, was extracted from testicular material of rabbits that had syphilitic orchitis as follows: gross cellular debris was separated by two centrifugations at 120 × g for 10 min, and the supernatant was fixed in buffered glutaraldehyde (1% vol/vol) and washed three times with phosphate-buffered saline. The final yield was 5 × 10^6 nonviable T. pallidum per ml in a relatively pure suspension.

Treponemes were diluted in saline and the concentrations were adjusted so that 0.05 ml contained 5 × 10^10 to 3 × 10^10 organisms. These treponemal suspensions were divided into samples and stored at −70°C; one sample was used for each study. For each treponemal preparation, a dose response curve was described as described previously (9); for convenience, concentrations that gave maximal stimulation of lymphocytes from normal subjects are presented.

Statistical analysis. All studies were carried out in triplicate. Values for each tube in counts per minute after subtraction for background were converted to log_{10} and averaged. The analysis of variance (15) was used to examine pairs of means when a significant F ratio indicated reliable mean differences. The alpha level was set at 0.05 before beginning the experiment.

RESULTS

The purpose of the first stage of this investigation was to extend earlier studies (9) which suggested that inhibition of lymphocyte response to nontreponemal antigens may be generalized during syphilitic infection. Nine patients who had syphilis (five primary, four secondary) and nine control subjects were studied. Results for primary and secondary syphilitics were combined and are presented in Table 1. Log_{10} [3H]thymidine incorporation values by unstimulated lymphocytes from control subjects and syphilitics were nearly identical (2.793 and 2.808, respectively). There were no significant differences between these two groups after stimulation with PHA, PWM, SLO, or mumps. Differences between the two groups were significant (P < 0.05) for dermatophytin (Derm) and two preparations of monilia antigens (Derm O and monilia). A partial replication of this experiment (see Derm O column, Table 2) confirmed that secondary syphilitics had a suppressed response compared with control subjects (P < 0.001).

In the second stage of these studies we sought to determine the extent of suppression of lymphocyte responsiveness to treponemal antigens other than T. refringens in patients who had syphilis (Table 2). T. reiter, another cultivable treponeme, and a relatively pure preparation of the virulent T. pallidum were used. There were nine subjects in each group. Unstimulated [3H]thymidine uptake by lymphocytes from control subjects and patients with primary syphilis was virtually identical (2.892 and 2.849, respectively). The degree of stimulation by all three treponemes was two- to threefold less in primary syphilitics than in control subjects; these differences were statistically significant (P < 0.05).

In the case of patients with secondary syphilis, unstimulated base-line counts appeared to be lower than those for control subjects, but this difference was not statistically significant (P > 0.05). Differences between [3H]thymidine uptake by lymphocytes from secondary syphilitics and controls were significant for several concentrations of T. pallidum (P < 0.01), T. refringens (P < 0.001), and T. reiter (P < 0.01). Because there appeared to be differences in thymidine uptake by unstimulated lymphocytes from controls and secondary syphilitics, even though these differences were not statistically significant, the stimulation index was derived by subtracting log_{10} [3H]thymidine incorporation in resting from that in stimulated lymphocytes.

Results are shown in the second line for each group of subjects in Table 2; they indicate that when compared with the unstimulated base line, lymphocyte stimulation was two- to threefold greater in normal than in syphilitic subjects.

DISCUSSION

These results show that lymphocytes from syphilitic patients do not respond normally in vitro to stimulation with treponemal (T. pallidum, T. refringens, T. reiter) and nontreponemal (trichophytin and monilia) antigens. They confirm our previous findings (9) that the response to mitogens (PHA, PWM, and SLO) is identical in lymphocytes from syphilitic and control subjects. We have previously shown that serum factors are not responsible for the observed suppression that appears to be a property of the lymphocytes themselves (9).

What is the cause of this suppression of lymphocyte reactivity and what might be its pathogenic significance? Suppression of lymphocyte response to antigens related to the infecting organism and/or to mitogens such as PHA has been demonstrated in fungal (1, 4, 5) and mycobacterial (19) but not in acute bacterial (10, 22) infections. In viral infections (7, 10), decreased lymphocyte response to PHA has been noted and this finding has correlated, in preliminary studies, with decreased numbers of thymus-dependent (T) lymphocytes in peripheral blood (10). An alteration in the ratio of circulating T and bursa-dependent (B) lymphocytes in syphilis may offer a partial explanation for our results; this has not, to date, been studied in patients with syphilis. That this
alteration is not entirely responsible for our findings is suggested by the fact that responses both to PHA and PWM were virtually identical in syphilitic and normal subjects; significant alterations in the ratio of B and T lymphocytes would presumably affect these results, to some extent, as well (8).

A second possibility, although much less likely, is worthy of note. Graybill and Alford (5) recently showed that even after their patients who had cryptococcosis were completely cured their lymphocytes did not respond normally in vitro to cryptococcal antigens. These differences were reflected in the stimulation index but not in absolute values of [3H]thymidine incorporation. Differences in the ratio of T and B lymphocytes were not responsible. These authors suggested that an underlying lack of immune competence may have been responsible for the initial acquisition of active cryptococcosis by their subjects. This explanation seems to be far more tenable in the case of cryptococcosis, where the infecting organism is ubiquitous and the attack rate miniscule, than in the case of syphilis, where a relatively high proportion of contacts are thought to become infected. Moreover, once cured of their syphilis, our patients had normal lymphocyte responsiveness to T. refringens (9).

Another possibility is that, in fact, some degree of immunosuppression is caused by infection with T. pallidum. Favoring this hypothesis are several observations: (i) syphilitic infection progresses despite the presence of antibodies that immobilize the infecting organism in vitro (20) and suppress the development of infection after passive immunization in vivo (11, 18); and (ii) the onset of latency is associated with the development of delayed hypersensitivity of treponemal antigens (16). These observations suggest that cellular immunity fails to develop or, perhaps, is suppressed until unknown factors supervene, leading to an effective cell-mediated response that ushers in the latent period. Moreover, in contrast to human syphilis, infection caused by T. pallidum in rabbits is acute and self-limited. Preliminary studies have shown that lymphocyte response in syphilitic rabbits is not suppressed (D. Musher and R. Schell, unpublished observations). Perhaps the observed suppression of lymphocyte reactivity in human patients is consistent with the protracted nature of syphilis in human beings, in contrast to this same infection in rabbits.

Our present concept of the immune response to syphilis is that both humoral and cellular factors are involved. Partial protection has been induced in rabbits by transfusing immune serum (11, 18). Using as an index the ability of rabbits to suppress the growth of Listeria, we have shown that acquired cellular resistance is enhanced in syphilis (12) and that this resist-

### Table 1. Response of lymphocytes from control subjects and syphilitic patients to stimulation by nontreponemal antigens

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Unstimulated</th>
<th>PHA</th>
<th>PWM</th>
<th>SLO</th>
<th>Derm</th>
<th>Derm O</th>
<th>Monilia</th>
<th>Mumps</th>
</tr>
</thead>
</table>

* Differences between control subjects and syphilitic patients are statistically significant (P < 0.05).

### Table 2. Response of lymphocytes from control subjects and syphilitic patients to stimulation by treponemal antigens

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Resting</th>
<th>PHA</th>
<th>Derm O</th>
<th>T. pallidum (3 x 10^4)*</th>
<th>T. refringens</th>
<th>T. reiter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.893</td>
<td>4.547</td>
<td>4.383</td>
<td>3.353</td>
<td>3.558</td>
<td>3.245</td>
</tr>
<tr>
<td>Primary syphilitic</td>
<td>2.849</td>
<td>4.658</td>
<td>4.137*</td>
<td>3.075*</td>
<td>3.058c</td>
<td>2.904c</td>
</tr>
<tr>
<td>Secondary syphilitic</td>
<td>2.479</td>
<td>4.392</td>
<td>3.550*</td>
<td>2.688*</td>
<td>2.795c</td>
<td>2.453c</td>
</tr>
</tbody>
</table>

* For each group of subjects, results in the upper line indicated log10 counts per minute and those in the lower line the stimulation index.

* Denotes number of treponemes per milliliter of tissue culture medium.
* Significant difference when compared with control subjects (P < 0.05).
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* Significant difference when compared with control subjects (P < 0.01).
ance can be transferred by using T lymphocytes (13, 14). These observations, which document activation of both humoral and cellular immunity, may explain why syphilis in rabbits is an acute, self-limited infection. By contrast, for the reasons noted above, cell-mediated immunity may be suppressed early, in human syphilis, thereby explaining the progression of primary and secondary disease despite the presence of a seemingly effective humoral response.

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LITERATURE CITED