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Depression of Lymphocyte Response to Concanavalin A in Rabbits Infected with *Treponema pallidum* (Nichols Strain)

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Peripheral blood lymphocytes isolated from rabbits in the early stages of *Treponema pallidum* infection responded poorly when exposed to concanavalin A in vitro. Maximal depression of blastogenesis occurred when lymphocytes were cultured in the presence of autologous serum in comparison with fetal calf or normal homologous rabbit serum.

The role of cell-mediated immunity in the host defense against syphilis remains unclear. There is substantial evidence that a state of anergy or immunosuppression exists in early syphilis (7, 8, 10, 11, 18), which is consistent with negative skin reactions to *Treponema pallidum* in primary and secondary syphilitics (6, 9). A decreased number of lymphocytes in the paracortical areas of lymph nodes has also been found in early disease (15). In vitro tests for cell-mediated immunity such as lymphocyte transformation have indicated reduced levels of blastogenesis to phytohemagglutinin (7) and treponemal or nontreponemal antigens (10, 11). In experimental rabbit syphilis, a partial depletion of lymphocytes was found in the spleens of neonatal rabbits infected with *T. pallidum* (2). Nevertheless, nonspecific resistance to *Listeria monocytogenes* was induced in rabbits 3 to 6 weeks after treponemal infection (13). Attempts to protect rabbits against syphilitic infection by vaccination with BCG, a known inducer of cell-mediated immunity, were unsuccessful (3, 14). Because of these inconsistencies we investigated the in vitro response of lymphocytes derived from *T. pallidum*-infected rabbits to concanavalin A (ConA).

Outbred New Zealand white male rabbits (Pel-Freeze) with a negative fluorescent treponemal antibody absorption reaction were injected intratesticularly with 5×10^7 virulent *T. pallidum* (Nichols strain; Center for Disease Control, Atlanta, Ga.). Control rabbits were inoculated in each testis with heat-killed *T. pallidum* (Nichols, 56 C for 1 h) or with saline. Rabbits were bled at weekly intervals for the purpose of obtaining autologous serum and peripheral blood lymphocytes. Using the modified separation technique of Boyum (1), heparinized blood was centrifuged at $650 \times g$ for 20 min in a Methocel-Hypaque gradient. After removing the lymphocyte band at the gradient interface, the cells were washed twice in minimal essential medium (GIBCO) supplemented with 2 mmol of L-glutamine, 100 U of penicillin, and 100 μg of streptomycin per ml and resuspended to a concentration of 5×10^6 cells/ml. A Wright stain indicated that 95% of the cells were lymphocytes; 92 to 98% of the cells were viable as seen by exclusion of trypan blue. Lymphocytes were cultured in flat-bottomed microtiter plates (Linbro) as described by Williams (16) at a cell concentration of 5×10^5 cells/0.1 ml in minimal essential medium with 10% fetal calf serum, autologous serum, or normal homologous rabbit serum. Autologous serum cultures are defined as cultures established with lymphocytes and serum obtained from an individual rabbit on the same day. Sera were inactivated by heating at 56 C for 30 min, and triplicate cultures were established for each serum. ConA (Miles Laboratories) was dissolved in minimum essential medium and added to selected lymphocyte cultures established in individual microtiter wells at a concentration of 2.5 μg/0.1 ml. Controls received an equal volume of minimal essential medium without mitogen. The plates were covered with sterile lids and incubated for 60 h at 37 C in a humidified chamber of 95% air and 5% CO_2. The cultures were pulsed for an additional 18 h with 0.5 μCi of [^{3}H]thymidine (2 Ci/mmol; Nuclear Dynamics). Lymphocytes were harvested as described by Williams (16) except that Econofluor (New England Nuclear Corp.) was used as scintillation fluid. Samples were counted for radioactivity in a liquid scintillation spectrometer. The data in counts per minute are presented as group means and standard deviation of the mean of triplicate samples. This value is obtained by subtracting the mean counts per minute of unstimulated cultures from the mean

counts per minute of ConA-stimulated cultures. The data were analyzed using Student's t test.

Rabbits injected with virulent *T. pallidum* developed an orchitis 7 to 12 days after infection. For the next 2 to 3 weeks, the rabbits exhibited ulcerative lesions and subsequent scarring. Finally, 33 to 34 days after infection, spontaneous regression occurred in these animals with no overt evidence of disseminated external lesions. Figure 1 shows the in vitro response to ConA of lymphocytes isolated from *T. pallidum*-infected rabbits during infection. During the first 3 weeks of infection the uptake of [^{3}H]thymidine was markedly depressed in cells cultured in fetal calf serum and normal homologous rabbit serum ($P < 0.005$), and maximal depression occurred when lymphocytes were cultured in autologous serum ($P < 0.001$). In contrast, preinfected autologous lymphocytes cultured in the preinfection autologous serum demonstrated normal blastogenesis. Although the stimulatory effect of ConA on lymphocytes was slightly below normal 4 and 5 weeks after infection, normal or elevated blastogenesis as measured by uptake of [^{3}H]thymidine was soon reached (Fig. 1).

Up to 3 weeks after infection the ability of lymphocytes from *T. pallidum*-infected rabbits to be transformed by ConA (Fig. 1) was suppressed when compared with the lymphocyte response of the noninfected control rabbits (Fig. 2 and 3). This observation was significant ($P < 0.01$) for cells cultured in fetal calf serum and normal homologous rabbit serum. The variance was maximal for cells grown in the presence of autologous serum ($P < 0.001$). After this 3-week period, differences in the lymphocyte response between infected and noninfected animals were generally similar.

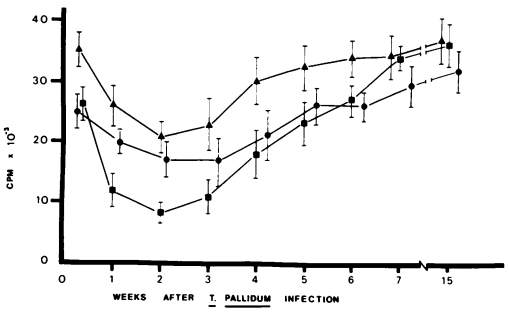

FIG. 1. *Deoxyribonucleic acid synthesis represented by uptake of [^{3}H]thymidine in rabbit peripheral blood lymphocytes before and after T. pallidum infection. Cells were cultured in fetal calf serum (▲), normal homologous rabbit serum (●), and autologous serum (■). The data, representative of four separate experiments using duplicate rabbits, are reported as group mean counts per minute (CPM) of ConA-stimulated cultures after subtraction of unstimulated controls. The standard deviation is shown by the brackets.*

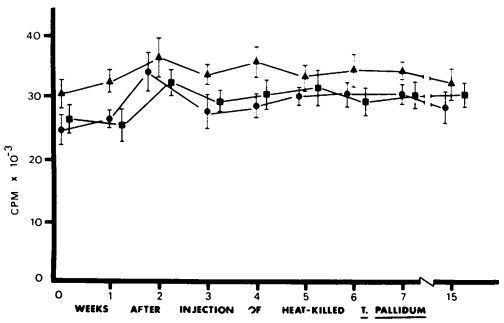

FIG. 2. *Deoxyribonucleic acid synthesis represented by uptake of [^{3}H]thymidine in rabbit peripheral blood lymphocytes before and after injection of heat-killed treponemes. Cells were cultured in fetal calf serum (▲), normal homologous rabbit serum (●), and autologous serum (■). Experimental conditions were as reported in the legend to Fig. 1.*

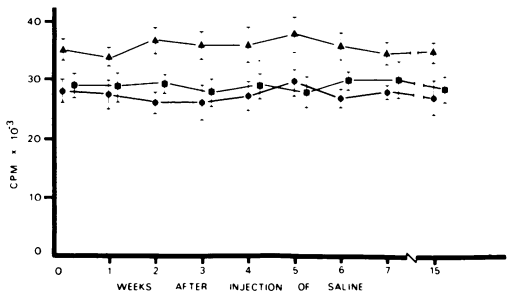

FIG. 3. *Deoxyribonucleic acid synthesis represented by uptake of [^{3}H]thymidine in rabbit peripheral blood lymphocytes before and after saline injection. Cells were cultured in fetal calf serum (▲), normal homologous rabbit serum (●), and autologous serum (■). Experimental conditions were as reported in the legend to Fig. 1.*

These results demonstrate a significant reduction in the ability of lymphocytes from rabbits in the early stages of syphilis to be transformed by ConA. The relative deficiency in blastogenesis correlates with the findings of Levene et al. (7) of reduced levels of lymphocyte transformation to phytohemagglutinin in patients with primary and secondary syphilis. Also, these data may be related to the depressed lymphoblastic response of subjects during early disease to treponemal antigens, such as *T. refringens*, *T. reiteri*, and *T. pallidum*, and nontreponemal antigens including candida and trichophytins (10, 11).

Our data indicate an interrelationship between the appearance and regression of orchitis

and the in vitro response to ConA by the infected rabbits. At the height of orchitis and ulceration (1 to 4 weeks) the rabbits manifested poor lymphocyte reactivity, whereas during remission (after 4 weeks) blastogenesis returned to normal or higher levels.

In vitro lymphocyte transformation is considered to be an indicator of cell-mediated immunity (12) and has been used to monitor the cellular immune capability of humans and other animals to various diseases (5). Since T-cell immunity may be involved in host resistance to bacterial infections (17), the reduced lymphocyte response to ConA in rabbits infected with *T. pallidum* may reflect ineffectual clearance of the spirochete and relate directly to the pathogenesis of the disease.

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