Development of Macrophage Migration Inhibition in Rabbits Infected with Virulent *Treponema pallidum*

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Peritoneal exudate cells from rabbits infected with *Treponema pallidum* Nichols were used as indicators of macrophage migration inhibitory factor activity. Between 3 and 15 weeks after infection, the migration of peritoneal exudate cells was inhibited in the presence of 3 to 25 μg of *T. phagedenis* biovar Reiter protein per ml. Before this period, the migration patterns of peritoneal exudate cells from infected animals were uninhibited and similar to those from noninfected control rabbits. These observations were correlated with the development of active cell-mediated immunity during experimental *T. pallidum* infection.

The role of cell-mediated immunity in the host response to syphilis has not been clearly elucidated. Information provided by several reports suggests that cell-mediated immunity is suppressed in the early stages of the disease (2, 7, 12, 21). Consistent with these data is the observation that delayed-type skin reactions to *Treponema pallidum* are usually elicited in late, active syphilis, whereas negative reactions have been found in primary and secondary syphilis (21). Also, a depletion of lymphocytes in the paracortical areas of lymph nodes has been observed in patients with early syphilis (19). In addition, reduced levels of blastogenesis to phytohemagglutinin and treponemal and nontreponemal antigen have been described in cases of human syphilis to which the in vitro lymphocyte transformation test (3, 6, 10) had been applied.

In experimentally induced disease, a reduced number of lymphocytes was detected in the spleens of neonatal rabbits infected with *T. pallidum* (2). Poor in vitro responses to concavalin A (12) and phytohemagglutinin and pokeweed mitogen (11) have been reported in *T. pallidum*-infected rabbits. In two studies with peripheral blood leukocytes in the migration inhibition test, inhibition of migration occurred only in the later stages of *T. pallidum* infection (4, 20). In this current study, the macrophage migration inhibition test was used to assess the response of peritoneal exudate cells (PEC) from *T. pallidum*-infected rabbits.

MATERIALS AND METHODS

**Animals.** Outbred, New Zealand white male rabbits (Pel-Freez) with a negative fluorescent treponemal antibody absorption reaction were used. Animals weighing 7 to 8 pounds (ca. 3.18 to 3.63 kg) were kept at 16 to 18°C in individual isolation cages before and during infection.

**Bacteria and infections.** Rabbits were injected in each testis with 5 × 10⁷ virulent *T. pallidum* Nichols. Rabbits injected with an equal amount of heat-killed (56°C for 1 h) *T. pallidum* Nichols or saline were used as noninfected controls. *T. phagedenis* biovar Reiter was grown in spiroplor broth at 35°C for 3 days in an atmosphere of 10% H₂, 35% N₂, 5% CO₂, and 50% argon. After centrifugation of cultures at 30,000 × g for 15 min at 4°C, the isolated organisms were washed three times with phosphate-buffered saline and resuspended in it to yield 10⁷ spirochetes per ml. The cell suspensions were diluted, and the protein concentration of each was determined (8). Samples from each suspension were diluted in minimal essential medium (Grand Island Biological Co., Grand Island, N.Y.) containing 10% normal rabbit serum supplemented with 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 2 mmol of L-glutamine per ml and used in the test runs.

**Migration inhibition factor assay.** The direct macrophage migration inhibition test was performed as previously described (1) with minor modifications. At biweekly intervals, PEC were obtained by injecting rabbits intraperitoneally with 30 to 50 ml of sterile, light mineral oil. Three to four days later, the animals were anesthetized and injected with 200 to 250 ml of heparinized saline (5 μl/ml). The abdomen was kneaded gently, and the liquid contents was collected via syringe and placed into a separatory funnel. The oil and aqueous phases were allowed to separate, and the aqueous phase was transferred to centrifuge tubes and spun at 400 × g for 10 min at 4°C. The pelleted cells were washed twice in Hanks balanced salt solution and finally suspended at a concentration of 2 × 10⁴ cells per ml in serum-enriched minimal essential medium. A Wright stain indicated that 70 to 75% of the PEC were macrophages, 15 to 20% were lymphocytes, and the remainder were granulocytes. Small capillary tubes (1.3 to 1.5 by 85 mm) were filled with
the cell suspension and sealed at one end with Vaspar. The tubes were then centrifuged at 180 X g for 5 min and cut at the cell-fluid interphase, and the portion containing the cells was incubated in Sykes-Moore chambers. Two tubes were placed in each chamber and secured with a small amount of silicone. Chambers were sealed and then filled with the enriched medium alone or enriched medium containing antigen. In most cases, three control chambers were established along with three test chambers containing each antigen concentration. Chambers were placed in a humidified CO2 incubator (5% CO2, 95% air) at 37°C and examined at 24 and 48 h. The area of migration was photographed, and the images produced by the capillaries were measured with a planimeter. The following formula was used to assess the extent of migration inhibition from the PEC:

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\% \text{ Migration inhibition} = \frac{\text{average area of control sample} - \text{average area of test sample}}{\text{area of control sample}} \times 100
\]

Negative values would indicate stimulation of migration.

RESULTS

Dose response. Several concentrations of Reiter treponemal antigen were tested against PEC from six normal rabbits. Severe levels of migration inhibition occurred at concentrations of 50 to 100 \(\mu g\) of Reiter protein per ml (Fig. 1), suggesting a nonspecific effect on PEC similar to that reported by Fulford and Brostoff (4). Therefore, concentrations of Reiter protein below these values were employed in the migration inhibition factor assay.

Migration inhibition factor production from control rabbits. Figure 2 illustrates the biweekly response of rabbits injected with heat-killed \(T. pallidum\) Nichols. During the 15-week period, macrophages from these animals were not significantly inhibited when cultured and exposed to 3, 10, or 25 \(\mu g\) of Reiter protein per ml. At the same time, PEC from rabbits injected with saline were incubated with each of the three concentrations of the Reiter preparation. No significant inhibitory effect on the macrophages was observed at each test interval (Fig. 3).

Migration inhibition factor production from \(T. pallidum\)-infected rabbits. Between 3 and 15 weeks after infection, an inhibitory effect occurred when PEC from \(T. pallidum\)-infected rabbits were exposed to either 10 or 25 \(\mu g\) of Reiter protein per ml (Fig. 4). Before this period, the response of these cells generally remained unchanged. At a concentration of 3 \(\mu g\) of Reiter protein per ml, macrophage migration was inhibited 5 weeks after infection, and inhibition continued for the duration of the experiment. For the first 3 weeks of infection, this reduced concentration of Reiter protein did not exert an inhibitory effect, and a low level of stimulation of migration was observed after the first week.

DISCUSSION

\(T. pallidum\) infection in rabbits has been an experimental model of choice for elucidating the course of syphilis in humans. We have found that 3 to 5 weeks after rabbits were infected with \(T. pallidum\), inhibition of migration occurred when their PEC were cultured with Reiter protein. Migration inhibition factor production appears to be an in vitro indicator of active cell-mediated immunity (13). Presumably this reactivity is due to the presence of an enriched population of antigen-reactive lymphocytes in
the PEC and/or antigen-binding activity of macrophages (14). After incubation with the appropriate antigen, immunocompetent cells release soluble mediators known as lymphokines, one of which has been shown to inhibit macrophage migration (13). The inhibitory effect observed in our infected animals can be interpreted as being a response by sensitized cells to antigenic determinants of *T. phagedenis* biovar Reiter, which are shared with the virulent *T. pallidum* Nichols (9).

Our findings parallel the results of others. When the leukocyte migration test was employed with the Reiter treponeme as antigen, inhibition of migration occurred only in patients with late, active syphilis (4). A similar pattern has been reported with peripheral blood leukocytes from rabbits several weeks after *T. pallidum* infection (20). However, unlike the previous studies, we were unable to demonstrate a significant degree of enhanced migration of PEC in the early stages of *T. pallidum* infection. For the most part, PEC remained unresponsive during this period. In addition, the levels of inhibition detected with PEC from infected rabbits in this study were more severe than responses reported by others. These inconsistencies may be due, in part, to different preparations or concentrations of antigen used. Also, variations in the sensitivities of macrophages used in our study and of peripheral blood leukocytes employed by other investigators as indicator cells could account for these discrepancies.

It has been suggested that there may be a relationship between the degree of sensitivity of PEC and the concentration of antigen used in the cell migration test (18). This is reflected in our findings, since the inhibition phenomenon occurring several weeks after infection was maximal when cells were incubated with the higher concentrations of antigen (Fig. 4). Lymphoid cells in the early stages of syphilis may be, at best, weakly sensitized to treponemal antigen(s) and, thus, remain unaffected when cultured in the presence of 10 to 25 μg of protein per ml of Reiter protein. Then, in the later stages, the cells become strongly activated and are capable of responding well in vitro, especially to higher doses of antigen.

How are the cells of the immune system involved in the host reaction and how is this related to the pathogenesis of syphilitic infection? Although antibody formation appears to progress unimpaired, it is becoming increasingly apparent that the cellular limb of the immune response is defective in the early stages of syphilis and is mobilized at other stages. The in vitro mitogenic response of lymphocytes from patients with primary and secondary syphilis is depressed when these cells are exposed to phytohemagglutinin (6), treponemal (3), and nontreponemal antigens (10). Plasma or serum from patients with the early stages of the disease was found to cause reduced levels of blastogenesis of normal lymphocytes (5, 6). During the first few weeks of infection, lymphocytes from *T. pallidum*-infected rabbits respond poorly after being cultured with concanavalin A (12) or phytohemagglutinin and pokeweed mitogen (11). However, normal lymphoblastic responses were reached 5 to 6 weeks after initial infection in these animals. Some success has been obtained in inducing nonspecific resistance to *Listeria monocytogenes* in rabbits 3 to 5 weeks after a previous infection with *T. pallidum* (15). By adoptive transfer techniques, it has been suggested that T-cells and possibly macrophages

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**Fig. 3.** Biweekly response of PEC from rabbits injected with saline to Reiter antigen. Experimental conditions are as reported in the legend to Fig. 2.

**Fig. 4.** Biweekly response of PEC from rabbits infected with virulent *T. pallidum* to Reiter antigen. Experimental conditions are as reported in the legend to Fig. 2.
are involved (16). Nonetheless, attempts to protect rabbits from T. pallidum infection by vaccination with BCG, a known stimulator of the reticuloendothelial system, have been unsuccessful (17).

In comparing the different in vitro parameters used to assess the presence or absence of cell-mediated immunity during T. pallidum infection, the data obtained in the migration assay correlate with the findings reported in our earlier transformation experiments (11, 12). We have demonstrated an abnormality in the responsiveness of lymphocytes from T. pallidum-infected rabbits to T-cell mitogens 1 to 4 weeks after infection that, in turn, could account for the delay in the onset of significant migration inhibition factor production during the same period. In a similar manner, when T-lymphocyte reactivity was restored, as reflected by a return to normal levels of blastogenesis (usually after 5 weeks), maximal levels of migration inhibition were observed (Fig. 4).

It is possible that the transient period of immunosuppression in syphilis could be the result of a modification of T-cell and macrophage function by blood factor or other cellular components of host or treponemal origin generated during treponemal infection. This condition, in turn, could account for the impairment of the release of soluble mediators (lymphokines) necessary for the activation and/or migration inhibition of macrophages and other phagocytes during early T. pallidum infection. The inability of the host to call forth effector cells such as macrophages to specific tissues and/or the capacity of T. pallidum to resist phagocytosis due to innate biological properties of the spirochete may delay the early destruction of virulent treponemes and contribute to widespread dissemination and establishment of the microorganisms in selected host sites. Then, as the infection progresses to later stages, a transition in the immune status of the host may occur. Normal T-lymphocyte function is restored (11, 12), and host defense mechanisms may then participate in treponemical activities as reflected by inhibition of macrophage migration several weeks after T. pallidum infection.

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