Cellular Immunity in Peyer's Patches of Rats Infected with *Trichinella spiralis*

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A rat model of *Trichinella spiralis* gut infection was used to observe the sequence of developing cellular immunity in Peyer's patches and other lymphoid tissues. Whereas cellular reactivity (lymphocyte blastogenesis) for worm antigens was evident in mesenteric lymph nodes draining the gastrointestinal tract within 3 days after infection, Peyer's patch lymphocytes developed maximal reactivity 2 to 3 weeks later at the same time as the spleen and other lymphoid tissues. Furthermore, the immune reactivity found in Peyer's patches was only transient. Thus, in this parasitic gut infection, the Peyer's patch lymphoid tissue does not appear to be the first site of cellular responsiveness but rather to acquire cellular reactivity only when other lymphoid elements in the infected host have also acquired similar antigen-induced reactivity.

The role of Peyer's patches in the immune response of the gastrointestinal tract has not been established. Recently, lymphocytes of both bone marrow (B) and thymus (T) origins were identified in this gut-associated lymphoid tissue (4, 13), and cellular functions usually attributed to B and T lymphocytes have been demonstrated with in vitro studies (4, 10, 13, 14). However, despite the presence and functional activity of lymphocytes within Peyer's patches, different immunization regimens have had varying success in stimulating Peyer's patch cells in situ. For example, Bienenstock and Dolezel (1) were unable to demonstrate stimulated cells in Peyer's patches of hamsters after vigorous oral and parenteral immunization with a soluble protein. Henry and co-workers (10) found only an occasional immune lymphocyte in the Peyer's patches of rabbits injected frequently with sheep erythrocytes, and immunization by the oral route was totally ineffective. In fact, only the direct insertion of antigen into Peyer's patches in vivo by Cooper and Turner (2) produced a primary immune response in situ. Thus, a number of authors (1, 10) have hypothesized that the Peyer's patches do not develop immune cells early in an immune response because antigens present in the gut cannot reach the potentially reactive cells within this tissue.

On the other hand, functional immune cells have been demonstrated in the Peyer's patches

of animals with gastrointestinal infections. For example, Dineen et al. (6) were able to transfer to recipient guinea pigs the ability to expel parasitic worms (*Trichostrongylus colubriformis*) by using Peyer's patch cells from multiply infected donors. Furthermore, in mice infected with another parasitic nematode, *Trichinella spiralis*, specific antigen-reactive cells have been observed in the Peyer's patch lymphoid tissue by Crandall and Crandall (5).

In an effort to study the temporal development of antigen-reactive cells in Peyer's patches, we used a model of trichinella infection in rats. In this model, the amount of specific antigen presented to the gut is not a limiting factor, and the development of specific immune responsiveness during infection has been well characterized (16). Our present study shows clearly that although antigen-reactive cells do populate the Peyer's patches of infected animals, they do so not during the earliest stages of infection, as in the mesenteric nodes, but rather only later, at a time when there is evidence of generalized systemic immunity.

MATERIALS AND METHODS

Animals. Adult male rats of the inbred Lewis strain were raised in the Laboratory Animals Division of Microbiological Associates, Bethesda, Md. They were 6 to 10 weeks old at the time of infection and weighed 150 to 225 g.

All animals were caged and maintained in accordance with the principles of the Committee on Laboratory Facilities and Care, Institute of Laboratory Animal Resources, National Research Coun-

cil. They had access to food (Wayne Lab-Blox, Allied Mills, Chicago, Ill.) and water ad libitum.

Trichinella infection. The parasitic nematode, *T. spiralis*, used in these studies was originally obtained from the Walter Reed Army Institute of Research and has been maintained in our laboratory by passage in Lewis rats for 3 years. Infective trichinella larvae were prepared from the skeletal muscle of animals infected for longer than 5 weeks as described previously (16). To infect rats experimentally, the concentration of worms was adjusted such that an infecting dose was suspended in 0.15 ml of saline and introduced into the esophagus of lightly ether-anesthetized animals via a blunted 18-gauge needle. The standard infecting dose was 1,200 larvae.

Antigen. A soluble, saline-extract larval antigen was prepared (16) similar to that described by Kagan (11) and shown previously to share antigenic determinants with adult worm preparations (17). In brief, washed muscle-stage larvae were isolated and stored at -20 C until approximately 2×10^6 larvae had been collected. Then larvae were pulverized in a microhomogenizer (Omni-Mixer with micro-adapter; Ivan Sorvall Co., Newtown, Conn.) set to run at 16,000 rpm at 4 C. The pulverized material was extracted into saline at 37 C for 4 h and then overnight at 4 C. After centrifugation at $400 \times g$, the supernatant material was heated at 56 C for 1 h. High-speed centrifugation at $20,000 \times g$ for 30 min yielded a soluble supernatant material, which was passed through a 0.45-μm filter (Millipore Corp., Bedford, Mass.) before being divided into aliquots and frozen for storage at -20 C. Standardization of this antigenic material was by the protein determination of Lowry et al. (15). Approximately 150 mg of protein was derived from 2×10^6 larvae.

Cell suspension. Rats to be tested for antigen-reactive cells were sacrificed with ether anesthesia. Peyer's patches were obtained as described (13, 14). They were cut out from the serosal side of the small bowel, washed free of debris with Hanks balanced salt solution (prepared by the NIH Media Unit), and teased apart with needles, and the resulting cell suspension was passed rapidly through a 1-cm rayon wool column. Effluent cells were pelleted ($500 \times g$) and washed several times in cold Hanks balanced salt solution. In each experiment, Peyer's patch cells from two to three animals were pooled in order to obtain at least 8×10^6 cells needed for various cell stimulation studies. Viability of each cell preparation, determined by dye exclusion, was 75% or greater.

Single-cell suspensions were prepared from mesenteric nodes by sterile passage of these tissues through 60-mesh stainless-steel screens. Cells washed through the screen with Hanks balanced salt solution were passed through a rayon wool column and washed as described above.

Lymphocyte transformation. Cells to be cultured were suspended in RPMI-1640 medium (Grand Island Biological, Grand Island, N.Y.) supplemented with 10% serum from Brown Norway rats (16), glutamine (300 μg/ml), gentamicin sulfate (10 μg/ml), and penicillin (1,000 units/ml). Two-tenths milliliter of culture medium containing 4×10^5 nucleated cells was dispensed into wells of a plastic microtray (Cooke Engineering, Alexandria, Va.).

For each set of experimental or control observations, quadruplicate cultures were prepared. In vitro stimulation of the experimental cell cultures was performed with a concentration of trichinella antigen (100 μg/ml) (16). In all instances the "functional viability" of the cultured cells was monitored by stimulation of other aliquots of these cells with the mitogen concanavalin A (Calbiochem, La Jolla, Calif.) at 5 μg/ml. After 3 days of culture at 37 C in an atmosphere of 5% CO_2 and moist air, each culture well was labeled with 1 μCi of [^{3}H]thymidine (specific activity, 5.0 Ci/mmol; New England Nuclear Corp., Boston, Mass.) for 4 h. A semi-automated harvesting technique was used to collect cells (9). Incorporation of [^{3}H]TdR into nucleoprotein material was counted in a liquid scintillation spectrometer (Packard model 3375, Packard Instrument Co., Downers Grove, Ill.). Counts per minute were used to determine a stimulation index of experimental counts per minute divided by control nonstimulated counts per minute.

RESULTS

Approximately 50 rats were orally infected with trichinella larvae and sacrificed at intervals during the next 50 days. In Fig. 1, the in vitro reactivity to trichinella antigen, as measured by the lymphocyte transformation response, is compared for lymphocytes cultured from Peyer's patches and mesenteric lymph nodes of these infected animals. With respect to antigen reactivity in Peyer's patch cells, the sample intervals after infection on day 0 could be divided into three relatively uniform periods. Three to 11 days after infection, Peyer's patch cells responded no differently from uninfected controls ($n = 6$). Between days 12 to 21, these cells were clearly reactive to antigen with mean experimental/control stimulation indexes of approximately 4 ($n = 10$), significantly greater than those found in the earlier intervals (Student's t test; $t = 2.4$, $P < 0.05$). Peak values were found in experimental Peyer's patch cell collections made on days 13, 14, and 16 after infection. During the next interval, 3 to 7 weeks after infection, Peyer's patch activity declined ($n = 9$) and stimulation was not detectable after day 35. The mean stimulation (experimental/control) of about two ($n = 9$) during this period was approximately half of that found in the preceding interval and not significantly different from the preinfection values.

The pattern of antigen reactivity by mesenteric lymph node cells was different from that of the Peyer's patch cells. Within 3 days after infection, mesenteric node cells could be stimulated with trichinella antigen, and maximum

E/C indexes were obtained between days 7 and 21. The reactivity persisted in these nodes for at least 40 days after infection. Antigen responsiveness of splenic lymphocytes is not shown in Fig. 1 but was similar to that previously obtained in this rat model (16). Spleen cell stimulation was minimal 3 to 11 days after infection but increased thereafter, and mean E/C indexes of 7 ($n = 5$) and 5 ($n = 7$) were measured during the 12- to 21-day and 22- to 50-day intervals, respectively.

DISCUSSION

Currently, the most important issue in the study of Peyer's patch lymphoid tissue is to determine what role these collections of lymphocytes play in immunity in vivo, i.e., whether they serve as a site of differentiation for immunological stem cells (3) or whether they participate more directly in immune responsiveness either as part of the afferent limb, the efferent limb, or both limbs of the immune response. By virtue of their unique physical proximity to the extensive mucosal surface of the gastrointestinal tract, these lymphoid aggregations are in a seemingly ideal position both to process gastrointestinally presented antigen and to participate in the immune rejection of gastrointestinal allergens or pathogens.
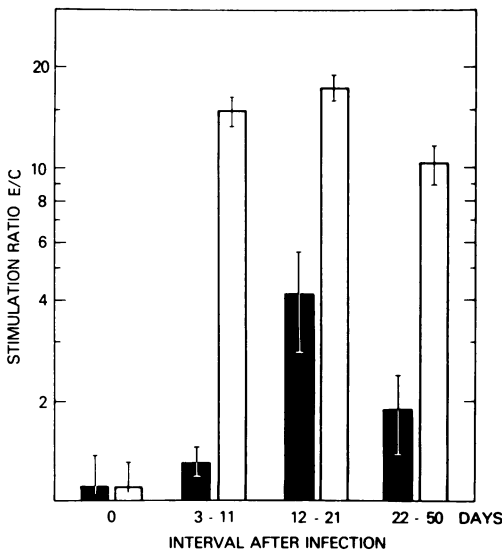
In the present study, it was our primary pur-

FIG. 1. *Comparison of the blastogenic response (ratio of experimental to control counts of [³H]thymidine incorporation) to trichinella antigen by Peyer's patch (solid bars) and mesenteric lymph node (open bars) cells from rats infected with T. spiralis. Base-line activity of all control cultures ranged from 300 to 400 counts/min.*

pose to determine just how early in an immune response specifically committed cells could be identified among the lymphocytes of the Peyer's patches with the hope that some inference might be made as to the importance of these structures in the afferent limb of immune responsiveness to antigens presented by the gastrointestinal route.

In the rat model of parasitic helminth infection which we used, antigen-specific cellular reactivity develops quickly in mesenteric lymph nodes and is followed within 1 to 3 weeks by increasing cellular activity both in peripheral blood lymphocytes and in distal lymphoid tissue such as the spleen and popliteal nodes (16). When Peyer's patch cells were studied in a similar fashion, rather than becoming antigen reactive very early in the infection as the mesenteric node cells had, their pattern of responsiveness more closely paralleled that of the remote or distal lymphoid tissue, whose activity generally reflects or defines the degree of systemic immunity.

Although these observations suggest that the Peyer's patches are not involved in the afferent limb of the immune response, they still do not prove it. First, although the trichinella-infected rat model should be particularly suited to study this question, the blastogenic assay may not be the most appropriate one, for if antigen-committed cells are present in Peyer's patches in the first several days of infection, they may be so activated by their differentiative process that they are "unresponsive" in the blastogenic assay to further antigenic stimulation. Second, since it has been shown that specific inhibitor or suppressor cells characteristically appear early in an immune response (8), it is also possible that our failure to see early antigenic responsiveness by Peyer's patch cells is the result of a masking of this responsiveness by simultaneously developing suppressor cells. Finally, it has been suggested (4) that the efferent traffic of committed lymphocytes from the Peyer's patches is so great that one cannot demonstrate the presence of specifically immune cells in these structures but, in fact, can find them only "downstream" in the draining mesenteric lymph nodes or among the thoracic duct cells. Thus, if this hypothesis is true, the antigen-reactive cells we demonstrated early in the infection in the mesenteric nodes and among the peripheral blood lymphocytes may well be the Peyer's patch cells (or their progeny) that were committed to antigen specificity several days earlier.

It is clear that our data show no antigen responsiveness among Peyer's patch lympho-

cytes early in this gastrointestinal parasitic infection. What is not clear, however, is whether or not this observation means that the Peyer's patch lymphoid tissue is not, in reality, a part of the afferent loop of the gastrointestinal immune response. Other interpretations of our findings are possible, and it is these alternatives that must be pursued in subsequent studies.

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