ADOPTIVE SUPPRESSION OF GRANULOMA FORMATION*

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Previous studies have demonstrated that the anti-egg granuloma formation, which occurs during infection with Schistosoma mansoni, is an immune response (1). This reaction is, in large measure, attributable to cell-mediated immune functions which occur in response to a soluble schistosomal egg antigen preparation (SEA) (2). A number of immune reactions to SEA have been reported, including lymphocyte blastogenesis (3), the production of migration inhibitory factor (4, 5), intradermal responses (2, 5, 6), the production of agglutinating and reaginic antibodies (2, 7), and the production of eosinophil stimulation promoter (8). Several of these responses have been observed to vary independently during the course of chronic S. mansoni infection (5, 7, 9). Granuloma formation, which represents the sum phenotypic expression of these various anti-SEA responses, has been reported to become less florid as chronic infection progresses (10-12). This diminution in the size of newly formed granulomas, late in infection, has been sequentially termed endogenous desensitization (12) and spontaneous modulation (5). A variety of immunoregulatory mechanisms have been suggested as candidates to explain this phenomenon (5, 7, 9). However, none of these have been substantiated by experimental data.

In this study, passive transfers of lymphoid cells, either lymph node (LN) or spleen (Spl) cells, from chronically infected mice to syngeneic mice in the early stages of infection, actively and effectively suppressed granuloma formation. In contrast, passive transfers of serum from chronically infected mice had no effect upon active granuloma formation.

**Materials and Methods**

*Schistosomal Infections.* CBA/J mice (The Jackson Laboratories, Bar Harbor, Maine) were infected with 30 S. mansoni cercariae (13) and maintained with access to rodent chow and water during their infection.

*Serum and Lymphoid Cell Preparation.* 7, 20, or 30 wk after infection, 4-6 mice were anaesthetized, exsanguinated, their blood pooled and allowed to clot, and serum collected by centrifugation at 2,500 g for 15 min at 4°C. Spleens and mesenteric lymph nodes were removed from these mice, placed on ice in RPMI 1640 (Flow Laboratories, Rockville, Md.), and prepared separately. These tissues were teased with fine forceps, passed through two layers of sterile gauze, centrifuged (190 g; 10 min; 4°C), and resuspended at concentrations of either 20 x 10^6 or 50 x 10^6 per 0.5 ml. Spleen cell preparations were pooled according to the age of the infection of the donor, as were LN cells.

*Passive Transfers.* A vol of 0.5 ml of serum from 7- or 20-wk-infected mice was injected once.

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intravenously, into each recipient CBA/J mouse. These mice had been infected with 30 cercariae of *S. mansoni* 6 wk before the time of transfer. Either Spl cells or LN cells from 7- or 20-wk-infected mice were transferred once to similar recipients. The cell dose was $20 \times 10^6$ lymphoid cells, intravenously, in a vol of 0.5 ml. Similar transfers of serum, Spl cells, or LN cells were performed using materials from 30-wk-infected mice; however, recipients received from same type of material twice, once at 6 wk and again at 7 wk of their infection. In this experiment the cell doses used were $50 \times 10^6$ cells per injection. Control 6-wk-infected mice received intravenous injections of 0.5 ml of normal saline. One control group received another injection of saline at 7 wk after infection.

**Granuloma Size Assay.** 8 wk after initiation of their *S. mansoni* infection, passive transfer recipients were killed. Their livers were removed and fixed in neutral formalin. Tissues were processed and stained with hematoxylin and eosin for histopathologic analysis. Newly formed granulomas (those that contain a central, still eosinophilic, schistosome egg) (10) were located and their diameters measured across opposing axes using an ocular micrometer. Based upon the generally spherical nature of the schistosome egg granuloma (11), the mean diameter and volume of each granuloma was calculated. Between 75 and 125 such granulomas were measured in the liver of three to five separate mice to give the group means reported. Data were analyzed by comparing the mean volumes of the experimental groups to the mean volume of their respective control (saline injected) group, using Student's *t* test. Granulomas were also measured in the livers of the donor 7, 20, and 30-wk-infected mice.

**Results**

The data from two complete passive transfer experiments are presented in Table I. Passive transfer of serum from mice with either chronic (20 or 30 wk) or early (7 wk) infections into 6-wk-infected mice had no effect upon the size of the granulomas that these recipients were making at 8 wk of infection. Likewise, double administration of "chronic serum", at 6 and 7 wk of infection, did not alter granuloma formation.

However, either spleen or lymph node cells from chronically infected (20 or 30

**TABLE I**

*Passive Transfer of Lymphoid Suppression of Active Granuloma Formation*  

<table>
<thead>
<tr>
<th>Source of transferred material</th>
<th>Mean volumes ± SEM of newly formed anti-<em>S. mansoni egg granulomas</em></th>
<th>Materials passively transferred</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Serum</td>
</tr>
<tr>
<td>Exp. 1 Control</td>
<td>215 ± 14</td>
<td>-</td>
</tr>
<tr>
<td>30 wk infected</td>
<td>-</td>
<td>204 ± 14</td>
</tr>
<tr>
<td></td>
<td>(NS)</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>Exp. 2 Control</td>
<td>176 ± 13</td>
<td>-</td>
</tr>
<tr>
<td>20 wk infected</td>
<td>-</td>
<td>171 ± 9</td>
</tr>
<tr>
<td></td>
<td>(NS)</td>
<td>(P &lt; 0.005)</td>
</tr>
<tr>
<td>7 wk infected</td>
<td>-</td>
<td>171 ± 10</td>
</tr>
<tr>
<td></td>
<td>(NS)</td>
<td>(NS)</td>
</tr>
</tbody>
</table>

* Volumes given in mm$^2 \times 10^4$. All mean values determined from 75–100 granuloma measurements from three to five individual mice.

‡ Mean granuloma volumes in livers of donor mice were: 30 wk, 64 ± 6 and 81 ± 7; 20 wk, 67 ± 5; 7 wk, 153 ± 16.

§ NS, not significant, *P > 0.05*. Other *P* values given in parenthesis derived by comparing each group to their control group.
wk) mice, administered once or twice (total dose of $20 \times 10^6$ or $100 \times 10^6$), were seen to effectively diminish the size of the granulomas formed at 8 wk of infection in the recipients of these cells. It should be reclarified that as previously described (7) both 20- and 30-wk donor mice exhibit equally depressed granuloma formation (legend of Table I) and are considered together as mice harboring chronic *S. mansoni* infection. The actual size of their newly formed granulomas were comparable to those previously reported (7, 11). Spleen cells, but not LN cells, from 7-wk-infected mice also had an effect upon granuloma formation, causing a partial, but significant, suppression of this response.

The mean lesion diameters ± SEM of saline-injected mice (331 ± 8) (four animals) and chronic spleen cell-injected mice (242 ± 5) (five animals), in Experiment I, were not as dramatically disparate as the mean lesion volumes for these groups presented in Table I. However, the mean diameters were statistically different at a level comparable to the volume data ($P < 0.001$). The results have been reported as lesion volumes because this presentation may convey the space occupying nature of the lesion and thereby more correctly reflect the phenomenon.

**Discussion**

Egg production by adult *S. mansoni* worms begins 4½ wk after cercarial infection and continues throughout the chronic phases of disease. Between 5 and 7 wk after infection, host responses to SEA, from this egg stage, are detectable, and periovum, hepatic granulomas begin to occur. These reactions are produced at maximal size during the 8th wk of infection, and subsequently, newly formed granulomas are generally smaller (5, 7, 10-12). This diminution was not based upon any diminished antigenic nature of eggs produced late in infection, because early (8 wk) eggs elicited the same reduced granuloma response as those eggs that occur in chronic infection. Thus, it has been speculated that the immune responsiveness of the host to SEA, or its expression, is regulated (5, 7, 9).

The current study demonstrates that this regulation is mediated by lymphoid cells present in both the spleens and mesenteric lymph nodes of chronically infected mice which are expressing this diminished ability to form anti-egg granulomas. Boros et al. (5) have shown that anti-PPD responsiveness remains constant in such mice. Although 7-wk-infected mice are progressing to the period of maximal granuloma formation, their spleens, but not LN, are seen to contain cells that partially lower granuloma formation. In some experiments the spleen has been observed to be preferentially rich in suppressor T lymphocytes (14). However, it remains to be elucidated whether this is the explanation of this activity and whether then 7-wk-infected spleen cells already contain a significant population of suppressor cells which manifest themselves upon passive transfer into 6-wk-infected mice.

Serum from chronic mice (even two doses, totaling 1.0 ml) failed to diminish or alter granuloma formation in recipient mice. This inability to affect the granulomatous process appears to preclude those theories that would evoke a blocking or enhancing process (5, 7), mediated by serum antibody or antigen-antibody complexes (15). However, it should be made clear that this passive transfer
system is affected at the systemic level. Therefore, the possibility remains that the adoptively transferred cell suspensions could contain antibody-producing cells which might take up residence within the developing lesion and thereby influence the granulomatous process at the local level.

This study does not define the actual mechanism(s) of the diminution observed. It does, however, indicate the requisite role of adoptively transferred cells from lymphoid tissues of chronically infected mice. The chronic antigen-specific stimulation situation which induces this immunoregulatory phenomenon would still allow for continued speculation as to the mechanism(s) involved (5, 7, 9). However, it now appears that investigations that will focus upon the specificity of the suppression and the actual cell type which mediates it, as well as possible in vitro correlates based upon these cell types, will be most informative. It has been shown that the diminution of active granuloma formation observed during schistosomiasis can be passively transferred with cells from lymphoid tissues. This passive transfer of a suppressive effect operates even during the most active phase of granuloma formation and results in the occurrence of smaller granulomas that occupy less hepatic space. This system is offered as a potentially useful model for studying the kinetics and mechanisms of immunoregulatory situations involving a wide variety of granulomatous processes.

Summary

Anti-egg granulomas formed in mice with chronic S. mansoni infection are smaller than those formed early (8 wk) after infection. Passive transfer of serum from mice with chronic infections to recipient mice with developing (6 wk) infections did not affect hepatic granuloma size at 8 wk of infection. In contrast, either spleen cells or lymph node cells from mice with chronic infections strongly suppressed the granulomatous process in recipient mice. Spleen cells, but not lymph node cells, of early-(7 wk) infected mice exhibited some ability to diminish granuloma formation in recipients. It appeared that the use of two sequential, weekly passive transfers of spleen or lymph node cells from chronic mice was even more effective in this suppressive capacity than a single transfer.

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References


