T-Lymphocyte Involvement in Abscess Formation in Nonimmune Mice

MARY F. NULSEN,* JOHN J. FINLAY-JONES, AND PETER J. MCDONALD
School of Medicine, Flinders University of South Australia, Bedford Park, South Australia 5042, Australia

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Athymic mice formed significantly smaller abscesses than euthymic mice in response to the intraperitoneal inoculation of an abscess-inducing mixture of *Escherichia coli*, *Bacteroides fragilis*, and autoclaved colonic contents, an abscess-potentiating agent. Adoptive transfer of nonimmune, Thy-1-positive spleen cells to athymic mice restored their ability to make abscesses of sizes similar to those in controls, indicating that T lymphocytes contribute to abscess formation in normal mice.

Evidence of a role for T-lymphocyte-mediated immunity in abscess formation comes from experimental models in mice and rats. The basic model involves the implantation in the peritoneal cavities of rats of a gelatin capsule containing barium sulfate, autoclaved colonic contents, and *Bacteroides fragilis* (16). Rats immunized with the capsular polysaccharide of *B. fragilis* are protected from intra-abdominal abscess formation and the *B. fragilis* bacteremia also seen in rats given the abscess-inducing mixture (AIM) (12). This protection is specific for *B. fragilis*. Adoptive transfer of antibody protects against bacteremia but not abscess formation in unimmunized rats (17). Immunity to abscess formation is transferable with splenic T lymphocytes (17). The immune T lymphocytes have been further characterized in a similar model of abscess formation in mice. Protection is mediated by antigen-specific suppressor T lymphocytes (20) from which an antigen-specific suppressor factor can be prepared (23).

In addition to these studies demonstrating the role of T lymphocytes in immunity, it has been shown that athymic mice make fewer (3) or smaller (10) subcutaneous abscesses than controls. However, it was not clear whether this was due to a deficiency of T lymphocytes or to the presence of activated macrophages, which accounts for the enhanced resistance of athymic mice to bacterial infection in several models (1, 4, 5, 14, 19, 24). Here we present evidence with a murine model of intra-abdominal abscess formation (15) that normal spleen cells reconstitute the abscess-forming potential of athymic mice and that the reconstituting cell type is a T lymphocyte. Further, we show that the presence of activated macrophages is unlikely to account for the smaller abscesses seen in athymic mice.

The model used for the induction of intra-abdominal abscesses has been described previously (15). In brief, BALB/c mice and mice heterozygous (*nu*/*+*) and homozygous (*nu*/*nu*) for the nude mutation on a BALB/c background (the latter mice being a gift from G. Mitchell of The Walter and Eliza Hall Institute of Medical Research, Victoria, Australia), were given an intraperitoneal inoculation of an AIM containing 5 × 10⁹ *B. fragilis*, 1 × 10⁶ *Escherichia coli*, and 0.2 mg of autoclaved colonic contents in a volume of 0.05 ml. Abscesses were quantified 6 or 12 days after inoculation. The mice were killed by cervical dislocation; the intra-abdominal abscesses were removed and weighed, and their bacterial contents were determined by colony counts performed on dilutions of abscess homogenates.

For adoptive transfers of leukocytes, spleens and mesenteric lymph nodes were obtained from normal (+/+) mice and disrupted by repeated injections of phosphate-buffered saline and teasing. After the tissue fragments were allowed to settle at 1 × g for 10 min, the cells were washed three times and suspended in phosphate-buffered saline. For T-lymphocyte depletion, 2 × 10⁶ cells were incubated for 1 h at 4°C with a monoclonal anti-Thy-1.2 supernatant (final dilution 1/24, a gift from W. Thomas, The Walter and Eliza Hall Institute of Medical Research). After being washed, the cells were incubated for 1 h at 37°C in 10 ml of RPMI 1640 growth medium (Flow Laboratories, McLean, Va.) containing a 1/12 dilution of guinea pig serum as a source of complement. After being washed, the cells were suspended in phosphate-buffered saline. Viability, determined by the ability to exclude trypan blue, exceeded 90% in all cases. Control spleen cell suspensions were similarly treated with diluted guinea pig serum or RPMI 1640 growth medium only. Immunofluorescence with fluorescein-conjugated anti-Thy-1.2 antibody (Becton Dickinson and Co., Sunnyvale, Calif.) indicated that 33% of control spleen cells were Thy-1.2 (positive), whereas <1% of treated spleen cells were positive. Mice were each given 6 × 10⁷ cells intravenously less than 30 min before the inoculation of AIM. The data presented are pooled from two (day 6) or three (day 12) separate experiments.

By 12 days after the inoculation of AIM, control (+/+ and *nu*/*+*) mice had intra-abdominal abscesses significantly larger than those of unreconstituted athymic mice (*P* < 0.0001; Fig. 1A). Compared with the unreconstituted athymic mice, athymic mice reconstituted with syngeneic spleen cells had significantly larger abscesses (*P* < 0.007; Fig. 1A). Treatment of spleen cells with anti-Thy-1.2 supernatant and complement abrogated their ability to restore abscess formation by athymic mice. Compared with athymic mice given untreated spleen cells, those given anti-Thy-1.2-treated spleen cells formed abscesses that were significantly smaller on day 12 (*P* < 0.0001) and were similar in size to those found in athymic mice given no spleen cells.

* Corresponding author.
† Present address: Department of Microbiology and Genetics, Massey University, Palmerston North, New Zealand.
Athymic mice examined 6 days after inoculation of AIM also had significantly smaller abscesses than those of control animals (7 \pm 6 and 47 \pm 12 mg per mouse, respectively). At this stage, however, the reconstituted athymic mice had abscesses similar in weight to those of the nonreconstituted animals (8 \pm 6 mg per mouse).

The number of viable bacteria in abscesses found in the day 12 mice followed a pattern similar to that of the abscess weight (Fig. 1B). There were significantly lower numbers of *E. coli* and *B. fragilis* in abscesses from hypothymic mice on day 12 when compared with the numbers in control mice (*P* < 0.03). By day 12, the numbers of *E. coli* and *B. fragilis* in the reconstituted athymic mice were similar to those of the control mice and significantly greater than those of the unreconstituted mice (*P* = 0.002).

Although the incidence of abscesses found in the reconstituted athymic mice doubled from day 6 to 12 (Table 1) and there was a significant increase in abscess weight, there was no significant change in the bacterial content of these abscesses over this period. This was also the case for the macroscopic lesions of the unreconstituted athymic mice.

Hematoxylin-eosin-stained sections of abscesses from control, heterozygous, and athymic mice revealed no significant differences among these groups.

Microorganisms present in peritoneal cavity washouts were quantified over 6 days after AIM inoculation into the peritoneal cavity on day 0. There was no significant difference in the clearance of either *E. coli* or *B. fragilis* between control and athymic mice (Fig. 2). In addition, the total number of bacteria that could be cultured from the peritoneal cavity, liver, spleen, and macroscopic abscesses did not differ significantly between the two groups until day 6, when the reduced incidence of abscesses and the lower bacterial content of those abscesses was found in athymic mice. Macroscopic abscesses did not develop in either control or athymic mice until 3 to 4 days after the inoculation of AIM.

We conclude that nonimmune T lymphocytes are involved in abscess formation. This is based on the observation that a Thy-1.2-bearing lymphocyte population was able to transfer to athymic mice the ability to make abscesses of significantly increased size (Fig. 1A). Although this ability was not evident 6 days after reconstitution, a delay in recovery of a normal response in reconstituted athymic mice has been reported previously (13). The participation in abscess formation of other cell types (e.g., B lymphocytes, and macrophages) also present in the reconstituting cell population is not excluded in our experimental protocol.

The smaller size of intra-abdominal abscesses observed in athymic mice (Fig. 1A) is consistent with the findings of two groups who examined subcutaneous abscess formation in athymic mice. They also found smaller lesions in athymic mice than in controls (3, 10).

A possible explanation for these observations in athymic mice, in particular the significantly fewer bacteria found in their abscesses, is that the formation of smaller abscesses in athymic mice may be the result of the presence of non-specifically activated macrophages. Non-specifically activated macrophages have been reported to cause enhanced clearance of, and consequently higher resistance to, several bacterial pathogens in athymic mice. These include *Listeria monocytogenes* (1, 4, 5, 25), *Brucella abortus* (1), *Staphylococcus aureus* and *Salmonella typhimurium* (14), and *Candida albicans* (2, 19). However, most of these pathogens are intracellular parasites and normally reside within macrophages, whereas *B. fragilis* and *E. coli* are extracellular pathogens, phagocytosed mainly by neutrophils (6). Furthermore, failure to observe any difference between control and athymic mice in the clearance of *E. coli* and *B. fragilis* from the peritoneal cavity over 6 days (Fig. 2) suggests that activated macrophages were not important in inhibiting abscess formation in athymic mice.

The smaller abscess size in athymic mice may have been due to the absence of a T-lymphocyte-dependent mechanism required to encapsulate an area of infection adequately. A discrete capsule or wall of granulation tissue which contains collagen develops around abscesses after 4 to 6 days (11, 15). Studies in athymic mice have shown that the deposition of fibrous tissue and collagen around the parasite *Mesorhizobium croti* is T-cell dependent (18). Others have noted that athymic mice have a reduced capacity to encapsulate microbes within granulomas (9, 21, 22). The inability of athymic mice to form abscesses of normal size (Fig. 1A) and incidence (Table 1) may be due to a deficiency in T cells.

**Table 1. Incidence of abscess formation in control and athymic mice**

<table>
<thead>
<tr>
<th>Micea and reconstituting cells</th>
<th>No. of mice with abscesses/</th>
<th>% of mice examined (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control (+/+)</td>
<td>23/25 (92)</td>
</tr>
<tr>
<td></td>
<td>heterozygous (nu/nu)</td>
<td>NDb</td>
</tr>
<tr>
<td></td>
<td>athymic (nu/nu)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>none</td>
<td>5/13 (38)c</td>
</tr>
<tr>
<td></td>
<td>spleen cells (+/+)</td>
<td>5/13 (38)c</td>
</tr>
<tr>
<td></td>
<td>spleen cells (+/+) (anti-Thy-1.2 + complement treated)</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Mice were inoculated intraperitoneally with AIM on day 0.  
b ND, Not done.  
c This value is significantly different from the control (*P* < 0.005 by the chi-square test with Yates' correction).
lymphocyte activities in abscess formation and the activities of abscess-derived neutrophils may help to clarify this.

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FIG. 2. The clearance of B. fragilis (A) and E. coli (B) from the peritoneal cavities of control and athymic mice inoculated with AIM on day 0. The bacterial inoculum is indicated by the arrow. The means ± 2 standard deviations for the control mice (●) were derived from three separate experiments, with four to eight mice per group. Each point for the athymic mice (▲) represents the result from an individual animal.


