Resistance to *Mycobacterium leprae* in Mice Infected with *Toxoplasma gondii* and Besnoitia jellisoni

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Mice chronically infected with the intracellular protozoan *Toxoplasma gondii* or *Besnoitia jellisoni* were resistant to footpad challenge with *Mycobacterium leprae*. Resistance was manifested by lower numbers of recoverable *M. leprae* in the footpads of protozoal-infected mice and was enhanced in *Toxoplasma*-infected mice by a booster injection of *Toxoplasma* antigen in the infected footpad. The results suggest a major role for the activated macrophage in the control of *M. leprae* infection.

Infection of the mouse footpad with *Mycobacterium leprae* provides a model suitable for studying some aspects of the host-parasite relationship in leprosy. After the inoculation of a small number of viable *M. leprae* into the footpad of an immunologically competent mouse, there is a lag phase lasting 30 to 60 days, during which there is no detectable increase in the number of organisms (21). There follows a logarithmic phase of growth during which the number of organisms increases with an average doubling time of about 12.5 days until, at 100 to 150 days, a plateau of approximately $10^6$ organisms per footpad is reached and multiplication of *M. leprae* essentially ceases. Histopathological observations reveal that during this stationary phase the infected footpad tissue displays a low-grade inflammatory process characterized by the presence of a mononuclear cell infiltrate, and death of the organisms is demonstrated upon subinoculation into mice (9, 22). The growth of organisms beyond the level of $10^6$ in footpads of immunologically compromised mice (23), and the demonstration that an established stationary phase infection, or one late in the logarithmic phase, protects against challenge with *M. leprae* in the opposite footpad (4), suggests that immunological factors are responsible for this cessation of growth and subsequent death of *M. leprae*. Moreover, ultrastructural observations of macrophages present in the infected footpad during the stationary phase revealed changes in these cells consistent with those seen in activated macrophages (1).

Infection with *Toxoplasma gondii* or *Besnoitia jellisoni* stimulates a population of activated macrophages that persist for prolonged periods, probably for the life of the host. Such macrophages have been shown to be the effector cells of nonspecific resistance to such intracellular pathogens as *Listeria monocytogenes*, *Salmonella typhimurium*, *Brucella abortus*, *Cryptococcus neoformans*, and Mengo virus in these protozoal-infected mice (3, 17, 18-20). The purpose of the present study was to determine whether chronic infection of mice with *Toxoplasma* or *Besnoitia* also protects against infection with *M. leprae*.

**MATERIALS AND METHODS**

Protozoal infection. Chronic infection was produced in female BALB/c mice (bred at the Public Health Service Hospital, San Francisco) weighing 22 to 25 g by intraperitoneal inoculation of $10^5$ trophozoites of *Toxoplasma gondii* (C56 strain) or $5 	imes 10^6$ trophozoites of *Besnoitia jellisoni* as previously described (18).

*M. leprae* infection and harvest. Experimental and control mice were inoculated into both hind footpads with approximately $5 	imes 10^5$ *M. leprae* of a strain originally isolated from a patient with lepromatous leprosy by C. C. Shepard, Center for Disease Control, Atlanta, Ga., and subsequently carried in mouse passage. The course of the *M. leprae* infection in the footpads was followed at intervals by harvest and enumeration of the acid-fast bacilli (AFB), according to methods described by Shepard (21, 24). The time required for the number of *M. leprae* to reach the plateau value was calculated from the regression of the logarithm of the number of AFB harvested per footpad on the time elapsed between inoculation and harvest. To determine the proportion of viable *M. leprae* in the footpads of normal and protozoal-infected mice, normal mice ("passage mice") were inoculated into the footpads with $5 	imes 10^6$ *M. leprae* harvested from the footpads of these mice.
and the multiplication of *M. leprae* was evaluated at intervals by harvesting the footpad tissues of the passage mice. Assuming a constant doubling time during logarithmic multiplication and a log phase of constant duration, the time from passage to multiplication to the plateau level is directly related to the proportion of viable *M. leprae* in the passage inoculum. An analysis of 71 growth curves, each based on two harvests, demonstrated that the standard deviation of the time-to-plateau calculated from a single harvest is 8 days (10).

**Protozoal antigens.** Soluble *T. gondii* antigen was prepared from purified and osmotically lysed trophozoites of the RH strain as previously described (7). Soluble *B. jellisoni* antigen was prepared in a similar manner from trophozoites obtained from macroscopic cysts harvested from the omentum and serosal lining of the peritoneum of chronically infected mice. Protein content of the antigens was determined by the method of Lowry (11).

**RESULTS**

To determine whether infection with *T. gondii* induces increased resistance to multiplication of *M. leprae*, uninfected control mice and mice infected intraperitoneally with *T. gondii* 40 days earlier were challenged with *M. leprae* in both hind footpads. At intervals after challenge, harvests of the inoculated footpads and enumeration of AFB revealed significantly fewer *M. leprae* in the footpads of the *T. gondii*-infected mice than in those of control mice (Table 1). At the time of the first harvests, 120 days after inoculation with *M. leprae*, the number of organisms per footpad of the *T. gondii*-infected mice was only about 20% of that found in control mice; 210 days after challenge, the number of AFB present in footpads of *Toxoplasma*-infected mice was only 61% of the number present in control animals 70 days earlier. The calculated time-to-plateau was longer in *Toxoplasma*-infected than in control mice by 88 days (P < 0.001).

**Table 1. Growth of *M. leprae* in the footpads of *T. gondii*-infected mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of harvest</th>
<th>No. of <em>M. leprae</em> per footpad (×10^9)</th>
<th>Time to 10^9 <em>M. leprae</em> per footpad (days)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>120</td>
<td>8.9</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td><em>Toxoplasma</em></td>
<td>120</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>infected</td>
<td>169</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>185</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>210</td>
<td>10.2</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated from the number of organisms harvested and the time required from inoculation to harvest.

To determine whether a local booster injection of *Toxoplasma* antigen would enhance resistance to *M. leprae* in *Toxoplasma*-infected mice, 10 μg of protein of soluble *Toxoplasma* antigen was injected into the right hind footpad of mice chronically infected with *Toxoplasma* that also harbored approximately 10^6 *M. leprae* from a previous infection in both hind footpads. As a control, a similar group of mice was injected in both hind footpads with Hanks balanced salt solution (BSS). Harvests were performed on all footpads 13 days after the booster injection of *Toxoplasma* antigen, and the number of AFB in each footpad was counted. A dilution made to provide an inoculum of 5 × 10^3 organisms per footpad was passaged into the footpads of normal mice. At intervals thereafter, the footpads of the passage mice were harvested and the number of AFB were counted (Table 2). At the first harvest (128 days), the antigen-boosted right hind footpads of the *T. gondii-M. leprae*-infected mice yielded only 6% of the number of AFB present in the footpads of the *T. gondii-M. leprae*-infected mice injected locally with BSS; the local injection of antigen had produced a marked loss of viable *M. leprae*. This conclusion is supported by the calculations of the time-to-plateau in the passage mice—156 days for the passage mice that received 5 × 10^6 AFB from the boosted footpads, compared with 117 days for the passage mice that received AFB from the BSS-injected footpads (P < 0.001). The results presented in Table 2 also show that there was killing of *M. leprae* in the opposite unboosted left hind footpads of the mice receiving *Toxoplasma* antigen in the right hind footpad. At 128 days after passage, the footpads of the mice to which these organisms were passaged contained only 39% of the number of AFB present in passage mice inoculated with AFB.

**Table 2. Growth of *M. leprae* in footpads of passage mice receiving *M. leprae* from *Toxoplasma*-infected mice boosted locally with *Toxoplasma* antigen**

<table>
<thead>
<tr>
<th>Booster treatment</th>
<th>Source of passed <em>M. leprae</em></th>
<th>Day of harvest</th>
<th>No. of <em>M. leprae</em> per footpad (×10^9)</th>
<th>Time to 10^9 <em>M. leprae</em> per footpad (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>RHF, LHF</td>
<td>128</td>
<td>22.2</td>
<td>117</td>
</tr>
<tr>
<td><em>Toxoplasma</em></td>
<td>RHF, LHF</td>
<td>128</td>
<td>1.3</td>
<td>156</td>
</tr>
<tr>
<td>Antigen in RHF</td>
<td>RHF, LHF</td>
<td>128</td>
<td>8.7</td>
<td>130</td>
</tr>
<tr>
<td>Antigen in RHF</td>
<td>RHF, LHF</td>
<td>128</td>
<td>27.0</td>
<td></td>
</tr>
</tbody>
</table>

* RHF, Right hind footpad; LHF, left hind footpad.
from the BSS-injected controls ($P < 0.05$). That the booster effect of Toxoplasma antigen did not result from the toxicity of the preparation was shown in a separate but similar experiment using mice infected only with *M. lepraec*. The organisms from the antigen-injected animals multiplied in passage at the same rate as did the *M. lepraec* recovered from mice injected with BSS.

To determine whether infection with *B. jellisoni* also induces resistance to challenge with *M. lepraec*, mice infected 40 days earlier with *Besnoitia* were challenged with *M. lepraec* in both hind footpads. As shown in Table 3, fewer organisms were observed in the footpads of the *Besnoitia*-infected than in those of the control mice. At 109 days after challenge, the number of *M. lepraec* harvested from the *Besnoitia*-infected mice was only about 5% of the number present in the control footpads, and the time-to-plateau was 73 days longer in the mice to which organisms from the *Besnoitia*-infected mice were passaged ($P < 0.001$). Unlike the results of the experiment in which a booster injection of Toxoplasma antigen was administered, there was no delay of multiplication of *M. lepraec* passaged from *B. jellisoni*- *M. lepraec*-infected mice that had been injected into the footpad with *Besnoitia* antigen.

**DISCUSSION**

The results of these experiments demonstrate that prior infection of mice with *T. gondii* or *B. jellisoni* confers protection against challenge in the footpad with *M. lepraec*. The results of the initial studies, showing that in protozoal-infected mice there were smaller numbers of *M. lepraec* at each harvest, could have resulted either from killing or from inhibition of multiplication of *M. lepraec*. The results of the experiments in which a booster injection of Toxoplasma antigen was administered into the *M. lepraec*-infected footpads of normal and Toxoplasma-infected animals bear on this point. The multiplication of *M. lepraec* varied greatly among the groups of passage mice, and the differences among the groups resulted solely from differences in the source of inoculum. Significantly less multiplication occurred in the footpads of the recipients of $5 \times 10^9$ *M. lepraec* from antigen-boosted mice than occurred in passage mice receiving a similar inoculum from the footpads of unboosted mice. These results suggest that the local injection of specific antigen enhanced the resistance already present in Toxoplasma-infected mice by a mechanism which, during the 2-week interval between boost and passage, appears to have resulted in the killing of *M. lepraec*. It is possible that our *Besnoitia* antigen preparation was more labile or less active than the Toxoplasma antigen. Whereas we are certain of the stability and activity of soluble Toxoplasma antigen in cell-mediated immune reactions (5–8), similar experiments have not been performed with *Besnoitia* antigen.

The mechanisms underlying the apparent nonspecific resistance to *M. lepraec* observed in the present studies are unknown. Chronic infections of mice with *T. gondii* and *B. jellisoni* have been shown to induce nonspecific resistance to a variety of phylogenetically unrelated facultative or obligate intracellular pathogens including bacteria (18, 19), fungi (3), protozoa (18), and viruses (17). In many of these examples of nonspecific resistance, the effector cell was shown to be the activated macrophage, which possessed enhanced microbicidal capacities. Thus, it is interesting to speculate on the role of the activated macrophage in the experiments described in the present report.

Although the persistence of activated macrophages is quite transient following infection with certain intracellular pathogens (i.e., *Listeria monocytogenes, Brucella abortus* [8, 12]), mice infected with Toxoplasma or *Besnoitia* remain infected for life and are continuously being re-exposed to antigen (14, 16). Infection with *T. gondii* and *B. jellisoni* may therefore stimulate a population of activated macrophages for prolonged periods, probably for the life of the host. Such persistence of a population of activated macrophages and the concurrent state of nonspecific resistance appear to depend on the continued interaction of host lymphocytes with the antigens of the infecting organism (13). The activation of normal macrophages in vitro following incubation with Toxoplasma antigen and sensitized lymphocytes from Toxoplasma-infected guinea pigs has been reported (7).

The *M. lepraec* in the mouse footpad infection have been shown to reside free in the cytoplasm of macrophages until toward the end of the

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**Table 3. Growth of *M. leprae* in footpads of *B. jellisoni*-infected mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of harvest</th>
<th>No. of <em>M. lepraec</em> per footpad ($\times 10^9$)</th>
<th>Time to $10^4$ <em>M. lepraec</em> per footpad (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>109</td>
<td>4.3</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>134</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>199</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>155</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>203</td>
<td>13.3</td>
<td>197</td>
</tr>
<tr>
<td><em>Besnoitia</em>-infected</td>
<td>109</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>134</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>199</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>155</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>203</td>
<td>13.3</td>
<td>197</td>
</tr>
</tbody>
</table>
logarithmic phase of multiplication, after which time the organisms may be found to be degener-
vating within the phagolyssomes of activated macrophages (1). The peritoneal macrophages as well as those of the liver and spleen of the protozoal-infected mice are known to be ac-
vatated at the time of M. leprae challenge in the present experiments (40 days after infection). It is possible that, as a result of the protozoal infection, macrophages normally residing in the footpad were also activated, or that those mi-
grating into the footpad as a result of the M. leprae infection included activated macro-
phages and thus killed a large fraction of the inoculated M. leprae prior to the beginning of the phase of logarithmic multiplication. Whereas killing of M. leprae appears to have occurred in the booster experiments, in the experiments in which antigen boosting was not employed, killing of M. leprae may also have occurred, or alternatively, activated macro-
phages in the infected footpads may have pro-
vided an intracellular environment unfavorable to the multiplication of the phagocytized M. leprae. Either of these mechanisms, or both, could have produced the results observed.

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LITERATURE CITED

1. Evans, M. J., and L. Levy. 1972. Ultrastructural changes in cells of the mouse footpad infected with Mycobacteri-
Randall. 1951. Protein measurement with the Folin
committed lymphoid cells on macrophage activation in
immunity. Prog. Allergy 11:89–140.
encysted form of Toxoplasma gondii from human skeletal
15. Remington, J. S., J. L. Krahenbuhl, and J. W. Menden-
hall. 1972. A role for activated macrophages in resist-
ance to infection with Toxoplasma. Infect. Immun.
6:829–834.
chronic infections with avirulent strains of Toxoplasma
virus challenge in mice infected with protozoa or
Studies on the mechanisms of resistance to phyloge-
intracellular infection: resistance to bacteria in mice
macrophage in acquired immunity to phylogenetically
unrelated intracellular organisms, p. 414–422. Antimi-
follows the injection of human leprosy bacilli into
established infections with Mycobacterium leprae in
23. Shepard, C. C., and C. C. Congdon. 1968. Increased
growth of Mycobacterium leprae in the thymecto-
mized-irradiated mice after footpad inoculation. Int. J.
counting acid-fast bacteria. Int. J. Lepr. 36:78–82.

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