NOTES

Susceptibility of Rabbits Immunized with Mycobacterium bovis (BCG) or Mycobacterium phlei to Shigella Keratoconjunctivitis

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Vaccination of rabbits with mycobacteria increased their resistance to eye infection with Shigella flexneri. However, the severity of keratoconjunctivitis was not reduced in the vaccinated animals.

Mice vaccinated with Mycobacterium bovis strain BCG develop increased resistance to a variety of intracellular, pathogenic bacteria (1, 2, 4, 5, 9, 12). The mechanism of resistance is related to changes occurring in the reticuloendothelial system (1, 2, 5). However, the development of resistance is accompanied by increased reactivity of the host to bacterial endotoxin (5, 8, 10). Hyperreactivity is generally manifested by increased animal mortality, or by a decrease in the mean time of death following injection of purified endotoxin or large numbers of gram-negative bacilli (5, 8, 10).

We report here on the susceptibility of rabbits to infection of the eye with Shigella flexneri following vaccination with BCG or M. phlei. Infection of the rabbit eye (manifested by development of keratoconjunctivitis) has been shown to be a useful model in the study of Shigella pathogenicity (3).

M. bovis strain BCG (obtained from J. Bretey, Pasteur Institute, Paris, France) and M. phlei were grown at 37 C in Dubos Liquid Medium (BBL) containing 10% albumin. Rabbits were injected intravenously via the marginal ear vein with 0.4 ml of a 7-day-old culture of either BCG or M. phlei. Each rabbit received approximately $3 \times 10^7$ mycobacteria.

Three weeks after vaccination, the rabbits were tested for delayed hypersensitivity by injecting intradermally (ID) 10 $\mu$g of protoplasm prepared from disrupted cells of BCG or M. phlei. Reactivity to endotoxin was also determined at this time by injecting another skin site ID with 50 $\mu$g of endotoxin from Salmonella enteritidis (kindly supplied by K. C. Milner, Rocky Mountain Laboratory, Public Health Service, Hamilton, Mont.). The intensity of the skin reactions was determined by the method of Larson et al. (6). Volumes of the reactions at 24 hr were calculated according to the formula suggested by Waksman and Matoltsy (11).

Rabbits were challenged with S. flexneri 2 at 22 days after vaccination by dripping $7 \times 10^9$ washed, viable organisms onto the surface of the conjunctiva. Admittedly, this is a large infecting dose; however, our previous experience has been that a minimum inoculum size of $7 \times 10^9$ cells was required to consistently produced keratoconjunctivitis in our animals (Nakamura and Cross, unpublished data). Furthermore, only cultures of Shigella in the logarithmic stage of growth was used for eye inoculations because older cultures were not infective to the rabbit eye (Nakamura, Int. Symp. Intestinal Microecology, in press). Eyes were observed daily for signs of infection. Cultures for the isolation of S. flexneri were made daily from the eye as previously described (3). Eight rabbits were employed in each vaccinated and normal control group.

The volumes of the delayed reactions to specific protoplasm and of the reaction to endotoxin are shown in Table 1. The BCG-vaccinated
rabbits were highly reactive to *S. enteritidis* endotoxin. These animals also developed the most intense delayed reactions. Rabbits vaccinated with *M. phlei* were also considerably more reactive to the endotoxin than were the controls, although their delayed reactions to specific antigen were approximately one-tenth that of the BCG-vaccinated animals.

Table 2 shows the severity of keratoconjunctivitis and the recovery of *S. flexneri* from the vaccinated and control rabbits. Both vaccinated groups controlled the multiplication of *Shigella* organisms more effectively than did the normal animals; however, the severity of keratoconjunctivitis was not reduced in these animals. The number of *Shigella* organisms recovered from the rabbits immunized with acid-fast bacilli decreased during the 2nd and 3rd days after infection, whereas the control animals showed an increased number of shigellae during this time period. On the 6th day after infection, vaccinated rabbits had reduced the population of *Shigella* three- to fivefold.

The results indicate that prior infection with BCG or *M. phlei* (a nonpathogenic species of *Mycobacterium*) produces a certain degree of resistance to eye infections with *S. flexneri*. That resistance develops is best evidenced by the fact that reduced numbers of *S. flexneri* cells were recovered from the eyes of the vaccinated rabbits. BCG was most effective in inducing resistance and there was a direct correlation between the intensity of the delayed reactions to specific antigen and the ability to control the multiplication of *Shigella*. However, we feel that, in the model employed, the degree of bacteriological control induced by BCG vaccination is ineffective in reducing the symptoms of keratoconjunctivitis because the vaccinated rabbits are hyperreactive to *Shigella* endotoxin.

In a preliminary study, it was found that BCG-infected rabbits also react more severely than do normal rabbits to the intracorneal injection of *Shigella* endotoxin. It appears from our results that control of bacterial multiplication and reduction of the host's inflammatory response to bacterial products are equally important in lessening the pathological symptoms in the eye. Although it is tempting to speculate that the effects of BCG are nonspecific, from the data of Minden et al. (7), it would be wise not to overlook the possibilities that where related antigens occur among organisms classical immunological mechanisms may also be involved. Further studies of these parameters in the eyes of normal and BCG-infected rabbits are currently being conducted.

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**Literature Cited**


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**Table 1. Delayed hypersensitivity and endotoxin reactivity in vaccinated and normal rabbits**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Delayed reactions (mm)a</th>
<th>Reactions to endotoxin (mm)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>BCG-vaccinated</td>
<td>655</td>
<td>252–1,056</td>
</tr>
<tr>
<td><em>M. phlei</em>-vaccinated</td>
<td>61</td>
<td>8–170</td>
</tr>
<tr>
<td>Controls</td>
<td>4</td>
<td>0–9</td>
</tr>
</tbody>
</table>

a Volumes of reactions at 24 hr to the intradermal (ID) injection of 10 µg of protoplasm from *M. bovis* (BCG) or *M. phlei*.

b Volumes of reactions at 24 hr to the ID injection of 50 µg of endotoxin from *Salmonella enteritidis* strain 386.

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**Table 2. Isolation of *Shigella flexneri* 2 and severity of keratoconjunctivitis in vaccinated and normal rabbits**

<table>
<thead>
<tr>
<th>Animals</th>
<th>Mean no. of organisms recovered per ml³ (days)</th>
<th>Severityb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>BCG-vaccinated</td>
<td>1,460</td>
<td>1,070</td>
</tr>
<tr>
<td><em>M. phlei</em>-vaccinated</td>
<td>8,960</td>
<td>3,120</td>
</tr>
<tr>
<td>Controls</td>
<td>2,510</td>
<td>4,810</td>
</tr>
</tbody>
</table>

a Values reported are the number of colony-forming units of *S. flexneri* 2 in 1.0 ml of eye wash fluid.

b Average severity of the infection during the 6-day observation period; ++++, severe keratoconjunctivitis; ++++, severe keratoconjunctivitis with corneal necrosis.