NOTES

STUDY OF UNCLASSIFIED ACID-FAST BACILLI AND STRAINS OF MYCOBACTERIUM TUBERCULOSIS BY INOCULATION OF THE CISTERNA MAGNA OF GUINEA PIGS

CHARLES D. BROWN AND MARY E. CLARK

New York State Department of Health, Division of Laboratories and Research, Albany, New York

Received for publication September 11, 1961

The basal cistern is a responsive testing ground for virulent Mycobacterium tuberculosis when the organism is injected in saline culture suspension (Brown and Harris, Am. Rev. Tuberc. 76:426, 1957). Strains of acid-fast bacilli not previously studied were investigated to determine the pattern of tissue response, survival time, viability of the acid-fast bacilli in vivo, and relationship of these factors to virulence.

Inoculum. Cultures grown for 3 to 4 weeks were harvested and homogenized by shaking with glass beads for 15 min, then were diluted with physiological salt solution to make approximately 1 mg per ml. The leptomeninges of guinea pigs weighing from 180 to 200 g were infected with 0.1 ml of this culture suspension.

Animal experiments. The experiments were designed to determine the physiological response of the animals to different strains of acid-fast bacilli isolated at this laboratory and cultures received from other institutions; 36 strains of M. tuberculosis, 78 scotochromogens, and 50 nonphotochromogens, 27 of which definitely belonged to Runyon's group III, were studied.

The 36 specimens of M. tuberculosis were tested in 1955-1960 in 51 animals; 25 proved to be virulent when injected by both the intracisternal and intramuscular routes. Six were tested by the former route only. These animals all developed tuberculous meningitis. Five tested by both routes induced equivocal response by the intracisternal route and definite tuberculosis via the intramuscular route. Since the granulomatous lesions did not contain acid-fast bacilli, these five strains were reinjected intracisternally; all animals developed tuberculous leptomeningitis.

The 78 scotochromogens were tested in 170 animals. These strains usually are not significantly active on intramuscular inoculation of guinea pigs. Abscesses have been found occasionally in the regional lymph nodes, but more often at the site of injection. The animals live the full 8 weeks of observation, usually gaining weight. The 78 strains were injected intracisternally to determine whether a lesion of the leptomeninges would be induced, which might be confused with the pathological changes induced by low-virulence strains of M. tuberculosis (Brown and Clark, N. Y. State Dept. Health, Ann. Rept. Div. Labs. and Research, 1959, p. 8).

TABLE 1. Reaction of guinea pigs to intracisternal inoculation of scotochromogens

<table>
<thead>
<tr>
<th>Grade of reaction</th>
<th>Guinea pigs</th>
<th>Survival time in days*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Per cent</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>8.2</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>4.7</td>
</tr>
<tr>
<td>2-3</td>
<td>84</td>
<td>49.4</td>
</tr>
<tr>
<td>0-1</td>
<td>64</td>
<td>37.7</td>
</tr>
<tr>
<td>Total</td>
<td>170</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* Animals surviving over 50 days were all gaining weight and appeared to be in a good state of health. These animals were killed at 56 days.

These tests were assessed histopathologically and graded for convenience, using arbitrary numbers from 0 to 5: 0 = negative findings; 1 = slight tissue reaction; 2 = minimal granulomatous meningitis; 3 = granulomatous meningitis; 4 = tuberculoid meningitis; and 5 = tuberculoid meningitis with occasional acid-fast bacilli in the lesions (Table 1). Of the nonphotochromogens, 18% were grade 5, 12% grade 4, 46% grades 2-3, and 24% grades 0-1. These findings are similar to those with the scotochromogens.
The granulomatous lesions induced by the scotochromogens differ cytologically from those induced by virulent *M. tuberculosis*, and resemble lesions induced by some strains of tubercle bacilli of low virulence associated with an extended survival time. Exudative cells are few and lymphocytes are relatively increased, usually in aggregations; epithelioid cells are not predominant. Occasional acid-fast bacilli are found in a matrix of epithelioid cells. In such lesions, the acid-fast bacilli have been removed from the point of inoculation early in the disease.

The findings indicate that with careful screening there should be no conflict in interpretation of the histopathological changes induced by the scotochromogens and those of virulent *M. tuberculosis*, but strains of *M. tuberculosis* eliciting low-grade tissue response in the meninges present patterns that would in some instances be difficult to differentiate from those caused by scotochromogens.

ACTIVITY OF LYSOZYME IN INBRED MICE

HANS MEIER and WARREN G. HOAG

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

Received for publication September 14, 1961

In the last decade, defense mechanisms that cannot be associated with specific antibody have been uncovered and rediscovered. Among the nonspecific humoral factors recognized or suspected of participating in both natural and specific immunity are complement, properdin, and lysozyme; endotoxins may stimulate, either specifically or nonspecifically, the production of one or more of these factors. We have been concerned with lysozyme activity in blood and tissues of inbred strains of mice. Originally, we sought to correlate bacterial infection in mice with levels of lysozyme. The significance and protective action of lysozyme has been reviewed (Thompson, Arch. Pathol. 30:1096, 1940).

Lysozyme was determined by measuring the amount of lysis of suspended Bacto-Lysozyme Substrate (Difco) and interpolation with a standard curve. Lysis was measured at 540 μμ by the amount of light transmission through a solution containing both Substrate and Lysozyme (Difco crystalline egg-white enzyme) after 20 min of incubation (Smolelis and Hartsell, J. Bacteriol. 58:731, 1949). The original macro-procedure, requiring several milliliters of sample, was adapted to a microprocedure using only 200 μlitters of unknown in a Spinco Ultramicroanalytical system (instead of a Coleman spectrophotometer); this allowed determination of serum levels from individual mice. Prior to studying the influence of a variety of experimental procedures on serum lysozyme, we determined the resulting lysozyme concentration of healthy mice from 13 different inbred strains (Table 1). In some strains, essentially no lysozyme was observed; others had appreciable amounts. Interestingly, the distribution of lysozyme levels among these strains correlates with the history of their development, suggesting that there may be a genetic basis for normal lysozyme content. Measurements in F₁ hybrids seemed to indicate a simple dominant inheritance pattern (Table 2).

In further testing of this hypothesis, we set up breeding experiments involving strain and hybrid