Production of Specific Agglutinins for Mycobacteria in Peritoneal Fluid of Mice

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Specific agglutinins were produced in ascitic fluid of mice by intraperitoneal injection of certain mycobacteria suspended in Freund's incomplete adjuvant.

The agglutination reaction has been used as a method for the identification of mycobacteria (3, 5–7). Although antisera of satisfactory titer for this purpose can be obtained from rabbits, it is an expensive procedure. Some rabbits die during the process of hyperimmunization, and other animals often fail to produce acceptable serum titers. Also, the initial cost of rabbits as well as the maintenance expense makes it desirable to develop an alternative procedure for the production of antisera to be used in the agglutination test.

It has been shown that, in mice injected repeatedly by the intraperitoneal route, large volumes of ascitic fluid containing specific antibodies may develop (1, 2, 4). We have found it of practical importance to use mice rather than rabbits in the production of agglutinins for mycobacteria, and herein present a report of preliminary observations.

Albino mice (Ha/ICR) weighing from 18 to 22 g were used. Mycobacterial isolates of known serotype were selected and subcultured on Lowenstein-Jensen medium. Serotypes included in this study were Mycobacterium avium I, M. avium II, Boone, Watson, Wilson, and Davis (7). (Known serotypes were kindly furnished by Werner B. Schaefer, National Jewish Hospital, Denver, Colo.) One milligram of cells (wet weight) was suspended in 2.5 ml of sterile water to which was added 2.5 ml of Freund's incomplete adjuvant. The suspension was emulsified by shaking on a vortex blender for 5 min. The mixture contained 0.2 mg (wet weight) of cell suspension per milliliter. Cell suspensions were prepared by using 2-week-old growth from these cultures.

Four mice were used for each culture. Each animal was injected intraperitoneally, twice weekly at intervals of 3 or 4 days for 3 weeks, and then 30 days later with 1 ml of the mixture.

Seven days after the last injection, the ascitic fluid was collected by tapping the distended peritoneal cavity with an 18-gauge needle and a 30-ml syringe containing 0.05 ml of 0.1% heparin. The fluid was centrifuged to remove cells. The titer of agglutinating antibody was determined by serial dilutions of homologous cell suspension. The bacterial cell suspensions used for agglutination tests were prepared by subculturing each of the isolates on 7H10 liquid medium (Difco). After incubation for 1 week, the cells were harvested and suspended in 0.01 M phosphate buffer containing 0.8% NaCl and 0.5% phenol (pH 7.0) as described by Schaefer (5). The density of the cell suspension was adjusted to optical density of 0.3 on the Coleman Junior spectrophotometer by using a wavelength of 525 nm. This is about equivalent to McFarland nephelometer no. 2.

The titer for the ascitic fluid ranged from 1:40 to 1:80. The specificity of the antibody produced to each of the serotypes is shown in the Table 1.

Of the six strains included in the study, four produced specific agglutination of homologous cell suspension only. No cross-reactions were observed with the other strains at the given dilution. M. avium I cell suspension was partially agglutinated by M. avium II immune ascitic fluid; however, M. avium I immune ascitic fluid did not agglutinate the other serotypes. Serotype Davis, which was used in low dilutions (1:10), cross-reacted with M. avium II and Boone cell suspensions.

The results show that peritoneal fluid of mice immunized with mycobacterial cells in Freund's incomplete antigen is a good source of agglutinating antibody. The use of mice rather than rabbits for the production of antibody is of practical value because, in comparison to rabbits, mice are less expensive to purchase and to maintain. Furthermore, one may use viable
Table 1. Agglutination reactions of cell suspensions

| Cell suspension | Ascites a | c. avium I | c. avium II | Boone | Watson | Wilson | Davis |
|-----------------|-----------|------------|-------------|-------|--------|--------|-------|
| c. avium I      | +         | +          | -           | -     | -      | -      | -     |
| Boone           | -         | -          | -           | -     | -      | -      | +     |
| Watson          | -         | +          | +           | +     | +      | -      | -     |
| Wilson          | -         | +          | -           | +     | -      | -      | +     |
| Davis           | -         | -          | -           | -     | -      | -      | +     |

a Dilutions of 1:40 were used for Boone, Watson, and Wilson ascites, 1:20 for c. avium I and c. avium II, and 1:10 for the Davis cell suspensions.

b +, Complete agglutination; ±, partial agglutination; -, no agglutination.

organisms in some instances because the required doses of certain mycobacteria such as c. avium do not produce progressive fatal disease in mice as they do in rabbits. This eliminates the possible alteration of antigenic determinants when organisms are heat-treated.

In mice injected intraperitoneally with six different serotypes of mycobacteria suspended in Freund’s incomplete adjuvant, large amounts of ascitic fluid developed with agglutinins for homologous strains. About 50% of the mice responded with accumulation of ascitic fluid in amounts varying from 1.0 to 26 ml. This preliminary study indicates that ascitic fluid of mice does provide a practical source of specific antimycobacterial agglutinins for use in tests for identification.

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