Reports

Ascites Induced in Mice by Staphylococcus

Abstract. Ascitic fluid containing high titers of antibody was induced in mice by *Staphylococcus*. Two to three intraperitoneal inoculations of 0.3 ml of a killed suspension of *S. aureus* mixed with complete Freund's adjuvant, given at 5-day intervals, produced ascites in 100 percent of the mice tested.

An adequate supply of potent antibody is indispensable for studies in basic immunology. This report describes a method for producing ascitic fluid containing high titers of antibody against *Staphylococcus aureus* strain 18 in mice (*1*).

Munoz, using paraffin oil mixed with *Mycobacterium phlei* as an adjuvant and egg albumin or bovine serum albumin as the immunizing agent, produced ascitic fluid containing good antibody titers in 50 percent of the mice tested (*2*). Herrmann and Engle, using sarcoma 180 cells and influenza A and Newcastle disease viruses, produced ascitic fluid in mice containing antibodies against these viruses (*3*).

Male mice of the National Institutes of Health general-purpose Swiss stock, weighing 20 to 25 g when used, were given two to three injections of antigen intraperitoneally at 5-day intervals. For the preparation of the antigens, a 20-hour culture of *Staphylococcus aureus* 18 grown in trypticase soy broth was treated in three different ways. (i) Cultures of live organisms were adjusted to give a concentration of 1×10^4 cells per milliliter. (ii) Cultures of organisms were concentrated to a level of 4×10^9 cells per milliliter and exposed to a temperature of 60°C for 1 hour on two successive days. (iii) Cultures were adjusted to deliver 4×10^9 cells per milliliter, formaldehyde was added, to a final concentration of 0.5 percent, and then the cultures were allowed to stand for 24 hours prior to use.

Each antigen preparation was inoculated into 16 mice, with each mouse receiving 0.3 ml of a mixture of equal parts of the prepared *Staphylococcus* antigen and Freund's complete adjuvant. The total amount of antigen inoculated into each mouse varied from 0.6 to 0.9 ml. Ascites appeared in 100 percent of the mice irrespective of the antigen preparation used. Repetition of this experiment yielded similar results in a separate investigation involving 60 additional mice.

In the mice receiving live antigen, ascites appeared during the third or fourth week after the primary inoculation. Formaldehyde and heat-killed antigens produced ascites in 1½ to 2 weeks, after the first injection. In each instance, the quantity of ascitic fluid obtained with an 18-gage needle varied from 2 to 12 ml per mouse. The total yield of ascitic fluid for each group of 16 mice after two to three taps at weekly intervals was 50 to 60 ml with the live organisms, 110 to 120 ml with the heat-killed antigen, and 90 to 100 ml with formaldehyde-treated culture. Freund's complete adjuvant, when mixed with uninoculated sterile trypticase soy broth, produced ascites in four of 16 mice 4 weeks after the primary inoculation.

When no further ascitic fluid could be obtained from the mice, the animals were exsanguinated. The titer of antibody in the serum was determined and compared with the titers of antibody contained in the ascitic fluid at successive taps. Antibody titers measured by the slide agglutination method were essentially the same for the ascitic fluid and the blood serum of each group of mice and varied from 1/2000 to 1/4000. There was no increase in the titer of antibodies of the ascitic fluid following booster injections given 6 to 7 weeks after the primary inoculation.

Another experiment, with a different strain, *S. aureus* 2 (*4*), showed that it, too, can induce the production of ascitic fluid containing antibodies in mice. In addition, the elimination of *Mycobacterium butyricum* in Freund's adjuvant had no effect on the number of mice affected with ascites, the volume of ascitic fluid formed, the level of antibody produced, or the duration of antibody response.

Abdominal adhesions may occur after two or three withdrawals of ascitic fluid, and difficulty in "tapping" the mouse may be encountered. These adhesions appear to be less severe if ascites is produced after two to three injections and may be dependent upon the quantity of the inoculum used and the time interval between doses.

Further studies are in progress to determine the effect of various staphylococcal components; the importance of strain, age, and sex in mice; the optimal immunization regimen; and the role of different adjuvants in the production of ascitic fluid containing antibodies in mice.

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References and Notes

1. *Staphylococcus aureus* strain 18 was recently isolated from an abscess in a patient at the National Naval Medical Center, Bethesda, Md.
2. J. Munoz, *Proc. Soc. Exptl. Biol. Med.* **95**, 757 (1957).
3. E. C. Herrmann, Jr., and C. Engle, *ibid.* **98**, 257 (1958).
4. *Staphylococcus aureus* strain 2 was isolated from sputum from a patient with pneumonia at the U.S. Public Health Hospital, Staten Island, New York.

16 October 1958

Phototropic Equilibrium in Phycomyces

Abstract. When illuminated by two light sources, the positively phototropic sporangiophores of *Phycomyces blakesleeanus* come into a state of phototropic equilibrium. In this state, the sporangiophores assume an orientation determined by the light fluxes falling upon opposite sides of their light-sensitive zones. Under certain conditions, the sporangiophores show tropic oscillations about the equilibrium direction.

Sporangiophores in their mature fast-growing stage were illuminated by two horizontal (*1*) light beams which made angles with each other of from 180° to 30°. When the intensities of the beams are equal, the sporangiophores assume a direction of growth that bisects the angle between the beams. Such a stable equilibrium position is often maintained for more than 12 hours, during which time the sporangiophores grow a distance of more than 3 cm. If, however, the beams

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].