INFECTION AND IMMUNITY, Jan. 1973, p. 35-38
Vol. 7, No. 1


# Immunity to *Vibrio cholerae* in the Mouse

## II. Effect of a Cell-Adherent Immune Factor

PAUL ACTOR AND DONALD PITKIN
*Smith Kline & French Laboratories, Philadelphia, Pennsylvania 19101*

Received for publication 23 August 1972

Serum, peritoneal exudate cells, or spleen cells were obtained from donor mice immunized with *Vibrio cholerae* Ogawa 41. Normal recipients were protected from lethal *Vibrio* infection when challenged one day after transfer of immune serum or peritoneal cells or normal peritoneal cells exposed in vitro to immune serum. Protection of recipients of immune spleen cells was evident when the cell transfer-challenge interval was 14 days but not when it was 1 day. Transfer of immunity with peritoneal cells from actively immunized donors was long lasting, whereas that derived from in vitro treatment of normal cells was of short duration. Both a cell-adherent and a nonadhering immune factor appear to be important in this immunity.

The mouse protection test has served as the system for assessing the value of cholera vaccines (5, 10). Although mice immunized with cholera vaccine prior to intraperitoneal challenge with *Vibrio cholerae* are protected in this system, relatively little is known about the mechanism of immunity or the course of infection. Protection in the mouse is apparently linked to the production of an antibody in response to cell wall antigens (15). A preliminary report on the dynamics of intraperitoneal infection in the mouse reveals that the infection is essentially limited to the peritoneum with peak numbers of organisms observed at 12 hr (O. A. Lukasewycz and L. J. Berry, Bacteriol. Proc., p. 102, 1971).

We have reported the passive transfer of protection via immune milk or serum (9). In this study, protection was long lasting and related to the antigenic dose given the mother. Protection was not due to transfer of antigen for active immunity could not be induced in immunized newborn mice. It has been reported that macrophages from immunized mice exposed to immune serum resist in vitro challenge with *V. cholerae* (4). We have described our initial efforts which were designed to determine the effectiveness of serum or cells in transferring resistance to recipient mice (Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 102, 1972). A cell-adherent immune factor, possibly a cytophilic antibody, appeared to play a role in immunity to *V. cholerae*. The present communication describes our work with the passive transfer of protection with serum and cells.

## MATERIALS AND METHODS

The culture of *V. cholerae* Ogawa 41 used was generously provided by R. A. Finkelstein, Department of Microbiology, University of Texas Medical School, Dallas. Mice used were either CFW or A/JAX strain adult males purchased from Carworth Farms, New City, New York, or Jackson Laboratories, Bar Harbor, Maine, respectively. The mice were housed in disposable plastic cages. Commercial lab chow and water were supplied ad lib. Challenge bioassays with a death end point were carried out in recipient mice one or more days after administration of treatment materials. Challenge was achieved by intraperitoneal inoculation of 500 to 1,000 $LD_{50}$ in a 5% gastric mucin suspension. Vaccines were prepared by heating known numbers of bacterial cells at 60 C for 30 min. Attempts to culture viable vibrios from the vaccines were unsuccessful.

Mice were immunized with one or two subcutaneous doses of vaccine given on either day 1 or days 1 and 14. Sera obtained from these dosage regimens were designated immune mouse sera and hyperimmune mouse sera (HIMS), respectively. Treatment materials were harvested 14 days after the last immunization and consisted of spleen, peritoneal cells, or sera. Appropriate treatment materials were obtained from normal mice. Donors were bled by decapitation, spleens were removed aseptically, and a cell suspension was prepared in Hanks balanced salt solution (HBSS). Peritoneal cells were obtained by rinsing an unstimulated peritoneal cavity with 2 ml of HBSS. All cell suspensions were washed three times with cold HBSS prior to further treatment. Treatment of cells with sera from normal or immune donors was achieved by combining equal volumes of sera and cells at 25 C for 45 to 75 min. The cells then were washed with a total of 50,000 cell volumes of cold HBSS. Nucleated cell concentrations were de-

termined with a hemocytometer. All treatment materials were administered in 0.1 ml of HBSS via the intraperitoneal route. No viable bacterial contamination was detected upon culturing of these materials. Vibriocidal titers were determined by using a modification of the method of Benenson et al. (1).

## RESULTS

The ability of cell transfer to protect recipient mice was examined first. Eight groups of strain A/JAX mice were challenged 1 day after intraperitoneal (ip) administration of the test materials shown in Table 1. This table shows that immune peritoneal exudate cells (IPEC) or HIMS fully protected mice against *V. cholerae* challenge. Spleen cells from either normal or immune donors failed to protect. Normal peritoneal exudate cells (NPEC) exposed in vitro to normal mouse serum (NMS) also failed to protect; however, exposure of NPEC to HIMS followed by thorough washing was highly protective.

The second experiment was designed to examine the duration of protection induced by transfer of IPEC and the possible ability of immune spleen cells (ISC) to adoptively immunize recipients. The results in Table 2 show that IPEC protects recipient A/JAX mice challenged 1 or 14 days after cell transfer, therefore inducing long-lasting protection. ISC again failed to protect recipients challenged 1 day after cell transfer; however, ISC did adoptively immunize mice as was evidenced by complete survival when challenge was 14 days after cell transfer. A limited sampling of pooled sera obtained 18 days after mice were treated with IPEC revealed a $\log_2$ vibriocidal titer of 4.7.

The third experiment was designed to examine the duration of protection conferred by NPEC treated in vitro with HIMS. CFW mice were challenged 1 or 4 days after ip injection of NPEC exposed to HIMS. The protection observed was found to be of short duration. The survival rate decreased by 50% 2 to 3 days after cell transfer. The ability of NPEC or normal spleen cells (NSC) to serve as carriers for sera of different origin was next studied. NPEC exposed to HIMS again was capable of inducing a high degree of resistance to CFW mice when challenge was 1 day posttransfer. Decreased protection was observed when immune mouse serum (IMS) was used in place of HIMS (Table 3). Transfer of NSC exposed to either IMS or HIMS to mice resulted in moderate protection when challenge was 1 day later. Administration of NPEC or NSC exposed to immune dog serum having a high vibriolytic titer failed to protect mice against challenge. This same dog serum administered ip was protective for mice.

Hyperimmune dog serum with a high vibriocidal titer (16,700 vibriocidal units/ml) was compared with hyperimmune mouse serum (512 vibriocidal units/ml) for ability to protect CFW mice against ip challenge with *V. cholerae* 1 day after administration of sera. The sera were diluted and administered ip (0.1 ml per dose). It can be seen from Fig. 1 that equal volumes of each serum gave approximately equal protection; however, the hyperimmune mouse serum was more than 60 times more efficient ($ED_{50}$ = 0.12 vibriocidal units) than the hyperimmune dog serum ($ED_{50}$ = 7.9 vibriocidal units).

## DISCUSSION

Peritoneal exudate cells or spleen cells taken from animals immunized with killed whole-cell vaccine of *V. cholerae* Ogawa 41 were capable of adoptively immunizing recipient mice. These recipients, challenged 14 days posttransfer of immune cells, were protected against ip challenge with the homologous serotype. Examination of a pooled serum sample obtained 18 days after transfer of peritoneal exudate cells

TABLE 1. *Transfer of protection to Vibrio cholerae Ogawa 41 in mice challenged 1 day after treatment*

| Material | In vitro exposure | Survived/tested |
|---|---|---|
| Immune peritoneal exudate cells ($7.5 \times 10^6$) | | 12/12 |
| Normal peritoneal exudate cells ($3 \times 10^6$) | Hyperimmune mouse serum | 13/14 |
| Normal peritoneal exudate cells ($3 \times 10^6$) | Normal mouse serum | 0/14 |
| Immune spleen cells ($1.6 \times 10^6$) | | 0/13 |
| Normal spleen cells ($6.5 \times 10^7$) | | 0/13 |
| Hyperimmune mouse sera (20 $\mu$liters) | | 13/13 |
| Normal mouse sera (100 $\mu$liters) | | 0/13 |
| Hanks balanced salt solution (100 $\mu$liters) | | 0/14 |

TABLE 2. *Transfer of protection to Vibrio cholerae Ogawa 41 in mice challenged 1 or 14 days after treatment*

| Material | Interval (days) | Survived/tested |
|---|---|---|
| Immune peritoneal exudate cells ($1 \times 10^6$) | 1 | 13/16 |
| Immune spleen cells ($1 \times 10^6$) | 1 | 1/17 |
| Immune peritoneal exudate cells ($1 \times 10^6$) | 14 | 10/10 |
| Immune spleen cells ($1 \times 10^6$) | 14 | 10/10 |
| Hyperimmune mouse sera (2 μliters) | 1 | 3/5 |
| Hyperimmune mouse sera (2 μliters) | 14 | 0/10 |
| Hanks balanced salt solution (100 μliters) | 14 | 0/10 |

TABLE 3. *Transfer of protection to Vibrio cholerae Ogawa 41 in mice challenged 1 day after treatment: effect of exposure of $10^6$ cells to mouse and dog sera*

| Material | In vitro exposure | Survived/tested |
|---|---|---|
| Normal peritoneal exudate cells | Immune mouse sera | 3/9 |
| Normal peritoneal exudate cells | Hyperimmune mouse sera | 7/9 |
| Normal peritoneal exudate cells | Hyperimmune dog sera | 0/8 |
| Normal spleen cells | Immune mouse sera | 5/9 |
| Normal spleen cells | Hyperimmune mouse sera | 3/9 |
| Normal spleen cells | Hyperimmune dog sera | 0/8 |
| Normal peritoneal exudate cells | | 0/8 |
| Normal spleen cells | | 0/8 |
| Hyperimmune dog sera (100 μliters) | | 5/7 |

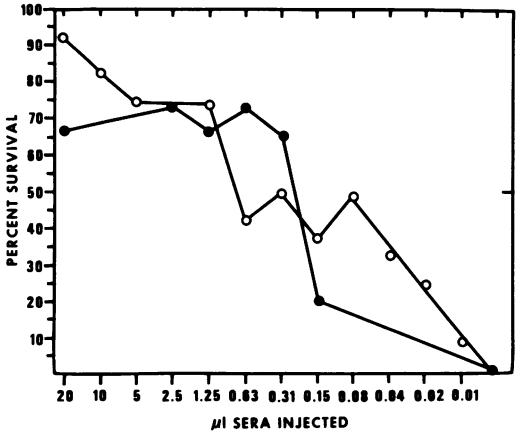


FIG. 1. *Effect of passive administration of dog* (○) *or mouse* (●) *hyperimmune sera on survival of mice challenged with Vibrio cholerae Ogawa 41. Dog serum contained 16,700 vibriocidal units/ml. Mouse serum contained 512 vibriocidal units/ml.*

showed the presence of high titers of vibriocidal antibody. The failure of ISC to protect 1 day after transfer is further evidence of adoptive immunity for protection was observed 14 days after transfer. The protection observed with IPEC was similar to that recently reported by Lukasewycz and Berry (Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 102, 1972). They showed that IPEC from CFW mice could transfer immunity to recipient mice. In their study, challenge was with either Ogawa or Inaba serotypes at 14 days after cell transfer.

The observation that IPEC will protect even at 1 day after transfer (Tables 1 and 2) can probably be explained by our studies with normal cells exposed to immune serum. NPEC or spleen cells exposed in vitro to HIMS, followed by thorough washing, are capable of protecting mice from vibrio challenge 1 day after ip transfer (Tables 1 and 3). The protection observed was short-lived with a half-life of 2 to 3 days. The phenomenon apparently was species specific in that hyperimmune dog serum with a high vibriocidal titer failed to adhere to NSC or peritoneal exudate cells (Table 3). This same dog serum was capable of protecting mice when administered intraperitoneally prior to challenge; however, it was approximately 60 times less efficient than mouse serum (Fig. 1).

It was recently reported that mouse macrophages cultivated in vitro are susceptible to *V. cholerae* (4). The vibrios, which grow intracellularly as well as on the surface of the macrophages, ultimately destroyed the host cells. The vibrios failed to grow in cell cultures obtained from immune mice, provided immune

mouse serum was present. Serum complement was not needed for protection in this system. Unfortunately it was not possible to determine the role of adherence of immune elements in serum to the macrophages in this in vitro system, because the combination of normal cells and immune serum was not attempted. It is of interest that immune rabbit serum added to immune mouse macrophages did not allow for protection in vitro. This may be analogous to our finding that immune elements in dog serum also failed to adhere to NMS or peritoneal cells.

Our data are consistent with the operation of two modes of protection, one involving a cell-adherent or cytophilic antibody and the other not involving a cell-adherent factor. The means by which the cell-adherent antibody functions in vivo can only be speculated on at this time. Tizard's recent review (12) of macrophage-bound immunoglobulins points up the lack of progress in the elucidation of their biological function. Our findings may be similar to that reported for mouse typhoid by Rowley et al. (11). In their studies, protection was transferred passively by IPEC and was attributed to the presence of a cell-bound antibody. The results suggest that cytophilic antibody is not the only mechanism of protection because immune dog serum not capable of adhering to peritoneal cells can protect mice. Antibodies are reported to play a role in protection of mice challenged with *V. cholerae* via the ip or oral routes. A number of investigators have reported passive protection to ip challenge (2, 6, 7, 9). Newborn mice are protected against ip or oral challenge by immune milk (3, 9, 14). In a system similar to our infection model in mice, Pike and Chandler have demonstrated that rabbit anti-*V. cholerae* immunoglobulin M was twice as effective as immunoglobulin G when both globulins were administered ip (8). In any event, protection may only in part be due to a vibriocidal effect. We have observed protection of mice with no detectable vibriocidal titer. It is of interest that Chiacumpa and Rowley have shown that 5- to 6-day-old mice inoculated orally with organisms exposed in vitro to hyperimmune rabbit serum did not succumb to infection (3). The mechanism of this protection is still not clear for it is difficult to ascribe this protection to a vibriocidal or opsonization effect.

It should be pointed out that these initial experiments still leave many questions unanswered. Further work should be carried out with respect to the identification of the class of antibodies and cells involved. In addition, it would be important to determine the duration of the serological and protective responses after induction of adoptive immunity. Only limited evidence is available on the occurrence of cytophilic antibody in the infective processes (13). Our data, which strongly suggest the participation of a cytophilic antibody in the *V. cholerae* mouse system, may serve as a useful model in studying this group of immunoglobulins whose biological functions are presently only hinted at.

## ACKNOWLEDGMENTS

We thank Ron Schoengold and Pincus Stern for their excellent technical assistance throughout this study.

## LITERATURE CITED

1. Benenson, A. S., A. Saad, and W. H. Mosley. 1968. Serological studies in cholera. II. The vibriocidal antibody response of cholera patients determined by a microtechnique. Bull. W. H. O. **38**:277-285.
2. Burrows, W., A. N. Mather, M. E. Elliott, and D. Havens. 1947. Studies on immunity to Asiatic cholera. III. The mouse protection test. J. Infect. Dis. **81**:157-174.
3. Chiacumpa, W., and D. Rowley. 1972. Experimental cholera in infant mice: protective effects of antibody. J. Infect. Dis. **125**:480-485.
4. Dodin, A., J. Wiart, and J. Gallut. 1971. Utilisation des macrophages de souris pour apprécier l'immunité anti-cholérique post-vaccinale. C. R. Acad. Sci. **272**: 1189-1191.
5. Feeley, J. C., and M. Pittman. 1962. A mouse protection test for assay of cholera vaccine, p. 92-94. *In* SEATO Conference on Cholera, Dacca, East Pakistan, 1960. Post Publishing Co., Bangkok.
6. Griffits, J. J. 1944. Mouse protective antibodies in human serums following injections with cholera vaccine. Pub. Health Rep. **59**:1374-1384.
7. Panse, M. V., and N. K. Dutta. 1964. Cholera vaccines and placental transmission of antibodies. J. Immunol. **93**:243-245.
8. Pike, R. M., and C. H. Chandler. 1971. Passive protection of mice against intraperitoneally injected *Vibrio cholerae* by γG and γM antibody. Infect. Immunity **4**:589-592.
9. Pitkin, D., and P. Actor. 1972. Immunity to *Vibrio cholerae* in the mouse. I. Passive protection of newborn mice. Infect. Immunity **5**:428-432.
10. Pittman, M., and J. C. Feeley. 1963. Protective activity of cholera vaccines against El Tor cholera vibrios. Bull. W. H. O. **28**:379-383.
11. Rowley, D., K. J. Turner, and C. R. Jenkin. 1964. The basis for immunity to mouse typhoid. 3. Cell-bound antibody. Aust. J. Exp. Biol. Med. Sci. **42**:237-248.
12. Tizard, I. R. 1971. Macrophage-cytophilic antibodies and the functions of macrophage-bound immunoglobulins. Bacteriol. Rev. **35**:365-378.
13. Tizard, I. R., and M. A. Soltys. 1971. Macrophage cytophilic antibodies to *Trypanosoma brucei* in rabbits. Trans. Roy. Soc. Trop. Med. **65**:407-408.
14. Ujiiye, A., and K. Kobari. 1970. Protective effect on infections with *Vibrio cholerae* in suckling mice caused by the passive immunization with milk of immune mothers. J. Infect. Dis. **121** (suppl.): 50-55.
15. Watanabe, Y., and W. F. Verwey. 1965. The preparation and properties of a purified mouse-protective lipopolysaccharide from the Ogawa serotype of the El Tor variety of *Vibrio cholerae*, p. 253-259. *In* Proceedings of the Cholera Research Symposium. Public Health Service publication no. 1328, Washington, D.C.