skin in the region of the angle of the jaw. The bruit, thrill, and tumor subsided coincident with the passage of the transvascular electrode into the arteries feeding this malformation from the left external carotid system; the arteries were then obliterated with successive radio-frequency lesions as the catheter was withdrawn (Fig. 3). Postoperative angiography has demonstrated the lesion to be almost completely obliterated, and the patient is cosmetically much improved and able to resume a normal school life. The bruit that was so annoying to her is absent.

A second case of a congenital arteriovenous malformation involving the left side of the face, palate, lip, and jaw in an 8-year-old girl has been treated in similar fashion. No bruit was present, but this lesion was pulsatile and disfiguring and was hazardous to the patient because the teeth and tonsils were almost embedded in a mass of anomalous vessels. This lesion had also been judged to be surgically nonresectable. Partial obliteration of this lesion was obtained by radio-frequency transvascular coagulation without significant cosmetic improvement but with reduction of the arterial components of the malformation. Postoperative angiography confirmed the obliteration of the major feeding vessels from the left external carotid artery but also demonstrated partial filling of the malformation by other vessels from the opposite carotid system. However, the lesion has been so reduced in size that surgical resection can be attempted.

In both of these cases, the catheter was introduced into the external carotid artery and guided to the lesion site under local anesthesia supplemented by intravenous ketamine hydrochloride (*6*). It is well known that total obliteration of these lesions is necessary to prevent recurrence. Thus, although these cases support the feasibility of the use of transvascular radio-frequency thrombosis in humans, they must await long-term evaluation.

Transvascular radio-frequency thrombosis of cranial blood vessels has theoretical potential advantages: it may be used in the awake patient, and no retraction or destruction of the brain is required. There are also potential disadvantages, however: the risk of spasm or occlusion of the parent blood vessel, and extension of the thrombus or embolization from the catheter's tip. The limited results to date are encouraging in two cases of arteriovenous malformations that could not be treated by any other means. This method is currently being explored in the treatment of intracranial arteriovenous malformations and aneurysms.

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Vibriolytic Antibody-Forming Cells: A New Application of the Pfeiffer Phenomenon

Abstract. *Complement-mediated immune lysis was first described by Pfeiffer after observing an immune reaction against* Vibrio cholerae. *Application of this reaction in agar gel, with viable vibro organisms, gave results which were unique compared to the red blood cell or enterobacterial systems. Among these was the lack of a detectable "background," a lengthened latent period and differentiable results among the three major cell wall antigens characteristic of this bacterial group.*

Lysis of *Vibrio cholerae* in vivo by specific antibody and guinea pig complement was first described by Pfeiffer (*1*). The application of this phenomenon of lysis in the presence of complement has been used in both diagnostic serology and basic immunologic research. Standard techniques have been developed for the detection and enumeration of individual lymphoid cells secreting antibody specific for target red blood cells (*2*). These techniques have also been adopted for the detection of antibodies to antigens such as serum proteins, chemical haptens, or bacterial extracts absorbed onto erythrocytes (*3*). The same technique has been modified so that living bacterial cells may be used directly as the indicator target cells (*4*).

Serologic tests based on lysis have been used as sensitive indicators of serum antibody to *V. cholerae* (*5*). However, the nature or quantity of cells involved in formation of such antibody have not been studied (*6*). The plaque procedure described here permits direct detection and enumeration of cells producing specific vibriolytic antibody in vitro and determination of the rise and fall of the cellular immune response to the various cell surface antigens of *V. cholerae*. The three major surface antigens of *V. cholerae* are found separated on the two strains, Ogawa and Inaba. These share a major common antigen (A), but can be distinguished, with absorbed serum, by a type-specific antigen—B in the case of Ogawa and C with Inaba (*7*).

Adult NIH albino A mice, each weighing approximately 25 to 30 g, were immunized by intraperitoneal injection of vaccine of heat-killed *V. cholerae* of either the Ogawa (antigens A and B) or Inaba (antigens A and C) strains. The bacteria were cultured on brain heart infusion agar for 18 hours and harvested by washing with saline. The vaccine was standardized to approximately 5×10^9 organisms per milliliter. The organisms were then killed by heat (60°C for 1 hour).

We developed a direct plaque assay for vibriocidal antibody to enumerate individual plaque-forming units (PFU). Immunized mice were killed, and their spleens were removed immediately. Dispersed cell suspensions were prepared, and 0.1-ml portions, adjusted to contain 1 to 2×10^7 viable nucleated cells, were added to tubes containing 2.0 ml of 0.7 percent melted Bacto agar (Difco), maintained at 48° to 50°C in a water bath. To each tube, 1 mg of DEAE-dextran (*8*), molecular weight 2×10^6, was added as an inhibitor of the anticomplementary activity of the agar (*2*, *3*). A suspension (0.1 ml) of an

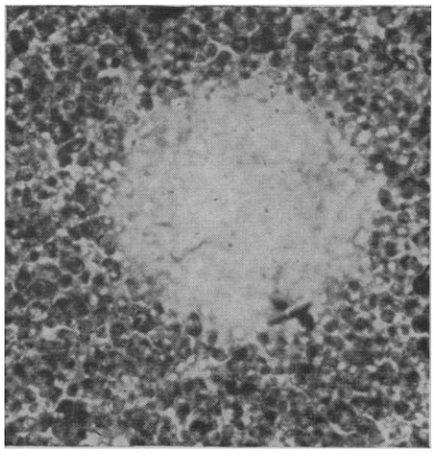

Fig. 1. Appearance of a typical discrete zone of vibriolysis in agar plate containing viable organisms and dispersed spleen cell suspensions from mice immunized with *V. cholerae*. Zone of "no growth" indicates antibody released from individual lymphoid cell (× 25).

overnight culture of the appropriate *V. cholerae* strain, containing 2×10^8 organisms, was then added to each tube. The contents were well mixed and quickly poured onto the surface of a petri plate (100 mm in diameter) containing a base layer of 15 to 20 ml of 1.4 percent brain heart infusion agar. When the upper layer had solidified, the plates were incubated for 90 minutes at 37°C. They were then treated with complement (5 ml of a 1 : 15 dilution of sterile guinea pig serum) and further incubated for 2 hours at 37°C. The complement was washed off the plates with Hanks solution, and the plates were incubated again at 37°C for 4 to 6 hours until, except for discrete zones of no growth, a confluent "lawn" of vibrio appeared. These zones, representing the antibody-forming cells releasing vibrolysins, appeared as sharp, round holes approximately 0.5 to 2 mm in diameter in the bacterial lawn (Fig. 1).

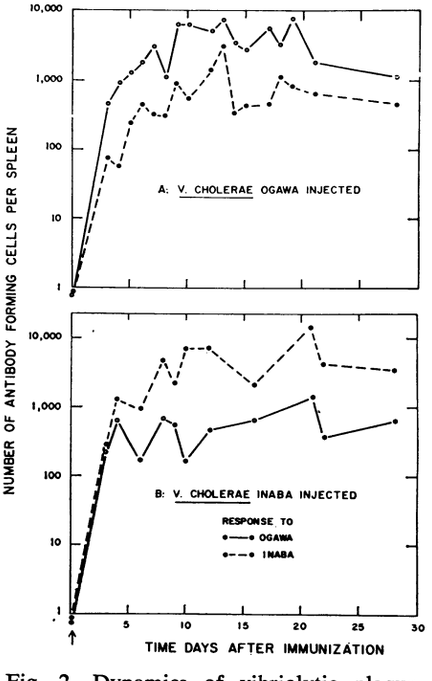

Fig. 2. Dynamics of vibriolytic plaque-forming cells in mice immunized with *V. cholerae* vaccine, strain Ogawa (Fig. 2A), or Inaba (2B). Each spleen cell suspension was tested for antibody-forming cells against both strains. Each point represents average response of 10 to 15 mice.

The number of plaques was markedly reduced when goat antibody to mouse globulin was incorporated into the mixtures before they were poured onto the base layer. The use of this type of control indicates that immune globulins are secreted by the spleen cells and induced in plaque formation (*2*, *3*). Plaques could also be suppressed by incorporation of either the immunizing antigen (whole cell vaccine) or lipopolysaccharide (*9*) from these cells into the agar gel.

The increase in number of vibrolytic plaques found in agar plates containing lymphoid cells from immunized mice was directly related to the time after inoculation of the vaccine (Fig. 2). The number of background plaques was not subtracted from the values at day zero as there actually was no detectable "background" for these organisms since spleen cells from normal, nonimmunized animals formed no detectable plaques, even when the entire organ was tested. After immunization with 10^9 *V. cholerae* Ogawa cells, few PFU appeared before days 2 to 3. Several hundred PFU appeared about the 3rd day after immunization, and this number increased greatly during the next 7 to 9 days (Fig. 2A). The peak number of PFU in these mice, generally between 10,000 to 15,000 per spleen, occurred on the 10th to 12th day after immunization. There was a persistent decline during the following 5 to 10 days, so that by 3 weeks after immunization there were 3000 or fewer PFU per spleen.

Unlike serologic results, the antibody plaque response showed relative specificity for the vaccine strain used for immunization. The maximum plaque response occurred when spleen cell suspensions from animals immunized with Ogawa cells were plated against the homologous organisms (Fig. 2A). However, if the same spleen cells were plated against Inaba cells as the target, a much lower response was noted. Presumably the response against the Ogawa strain is a combined reaction against both the A antigen, common to the Ogawa and Inaba strains, and the B antigen which is type-specific. However, except for the first few days of the response, over 90 percent of the plaque-forming cells were specific for the strain used as the immunogen. Thus, in the reverse situation, in which a suspension of *V. cholerae* Inaba was used as the immunogen, similar results were observed (Fig. 2B).

The advantages of this plaque technique with vibrio antigens for the quantitation of antibody-secreting lymphoid cells are (i) the absence of a detectable lytic "background" of antibody-forming cells to vibrio in nonimmune animals (which contrasts with the relatively large number of "natural" plaque-forming cells to red blood cells and other bacteria); (ii) relatively long latent period and delayed peak response, permitting a detailed investigation of the early immune reaction; (iii) a marked type specificity of the response, involving both common and strain-specific antigens; and (iv) the feasibility of investigating directly the immune response to bacterial antigens other than those from the Enterobacteriaceae.

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