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New Model for Analysis of Mucosal Immunity: Intestinal Secretion of Specific Monoclonal Immunoglobulin A from Hybridoma Tumors Protects against *Vibrio cholerae* Infection

LOUIS WINNER III,[1,2] JULIE MACK,[1,2] RICHARD WELTZIN,[1,2] JOHN J. MEKALANOS,[3] JEAN-PIERRE KRAEHENBUHL,[4] AND MARIAN R. NEUTRA[1,2]*

GI Cell Biology Laboratory, Children's Hospital,[1] and Harvard Digestive Diseases Center[2] and Department of Microbiology and Molecular Genetics,[3] Harvard Medical School, Boston, Massachusetts 02115, and Institute of Biochemistry, University of Lausanne, and The Swiss Institute for Experimental Cancer Research, 1066 Epalinges, Switzerland[4]

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Secretory immunoglobulin A (sIgA) plays a role in defense against *Vibrio cholerae* and other microorganisms that infect mucosal surfaces, but it is not established whether sIgA alone can prevent disease. We report here a strategy for identifying the antigen specificities of monoclonal sIgA antibodies that are capable of providing such protection. IgA hybridomas were generated from Peyer's patch lymphocytes after oral immunization with *V. cholerae* Ogawa 395. A clone was selected that produced dimeric monoclonal IgA antibodies directed against an Ogawa-specific lipopolysaccharide carbohydrate antigen exposed on the bacterial surface. Hybridoma cells were used to produce subcutaneous "backpack" tumors in syngeneic mice, resulting in secretion of monoclonal sIgA onto mucosal surfaces. Neonatal mice bearing anti-lipopolysaccharide hybridoma backpack tumors were specifically protected against oral challenge with 100 50% lethal doses of virulent Ogawa 395 organisms. Thus, the IgA hybridoma backpack tumor method identifies protective epitopes in the mucosal system and demonstrates that a single monoclonal sIgA can be sufficient to protect against intestinal disease.

Mucosal pathogens account for a significant amount of morbidity and mortality worldwide. Although it is known that secretory immunoglobulin A (sIgA) antibodies are involved in protective mucosal immunity, it is not known whether sIgA alone can protect against disease. If protective IgAs could be identified and produced in large quantities, they could potentially be used for passive protection or therapeutic intervention on mucosal surfaces. We undertook this study to determine whether a single monoclonal sIgA, secreted physiologically onto mucosal surfaces via the normal epithelial transport mechanism, is sufficient to confer protection against a mucosal pathogen. We chose *Vibrio cholerae* in neonatal mice as a model system because the pathogenesis of disease in this system is comparable to that of cholera in humans. To produce toxin and diarrheal disease, the organisms must avoid nonspecific host defenses (such as entrapment in mucus and clearance by peristalsis), gain access to the small intestinal mucosal surface, multiply, and form adherent colonies (6, 14).

Mucosal colonization by *V. cholerae* evokes a mucosal immune response in the host, including secretion of IgA antibodies that play a role in limiting the duration of primary disease and preventing colonization and disease on subsequent oral challenge (3, 18). Mucosal immunity to cholera in rabbits and humans involves polyclonal sIgA antibodies directed against both toxin and bacterial cell components, including the outer membrane lipopolysaccharide (LPS) (8–11, 18). The contributions of individual sIgAs to protection have not been tested, however, because of the lack of suitable monoclonal IgA antibodies and the lack of an experimental model in which the protective actions of individual IgA antibodies could be assessed.

* Corresponding author.

We previously demonstrated that the specificity of mucosal immune responses to enteric viruses can be analyzed by oral or mucosal immunization with intact viruses, followed by generation of hybridomas from Peyer's patch cells and characterization of the monoclonal IgA antibodies produced in hybridoma cell culture (25). In the present study, we used the same approach to generate monoclonal IgA directed against *V. cholerae* and to identify *V. cholerae* surface antigens that serve as efficient mucosal immunogens. With the IgA hybridomas, we devised a method that provides continuous delivery of specific monoclonal sIgA into the intestine in vivo. This approach can be used to identify *V. cholerae* epitopes that are protective in the mucosal system and to identify monoclonal IgA antibodies capable of protecting against colonization and disease.

MATERIALS AND METHODS

Adherence and transport of *V. cholerae* in Peyer's patches of BALB/c mice. *V. cholerae* (Ogawa 395) was grown under conditions that favor expression of pili as previously described (21). Organisms in growth phase were pelleted, suspended in phosphate-buffered saline (PBS), and injected into ligated loops of distal ileum of anesthetized BALB/c mice. Each loop included a Peyer's patch. After 4 h, the loop was removed and the lumen was flushed, first with PBS and then with a fixative solution consisting of 2% freshly depolymerized formaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4. Peyer's patch tissue was excised, immersed in fixative solution for 4 h, and further processed for electron microscopy (EM) as previously described (16). EM sections were stained with uranyl acetate and lead citrate and photographed with a JEOL 100 CX electron microscope.

Production and screening of anti-*V. cholerae* IgA hybrid-

omas. Three BALB/c mice were orally immunized on day zero by gastric intubation of 10^9 live, virulent *V. cholerae* Ogawa 395 organisms in 0.2 M sodium bicarbonate. Immunization was repeated on days 14 and 21; on day 26, mice were sacrificed by cervical dislocation and seven or eight Peyer's patches were excised from each mouse. Peyer's patch lymphocytes were isolated, pooled, and fused with P3X63/Ag8U.1 mouse myeloma cells as previously described (25). Fusion products were seeded in 96-well plates with a feeder layer of thymocytes freshly isolated from adult DBA/2 mice.

Culture supernatants were screened by enzyme-linked immunosorbent assay (ELISA) using whole Ogawa 395 organisms that had been coated on plates in carbonate-bicarbonate buffer, pH 9.6, and positive wells were detected by using anti-mouse IgG-IgA-IgM coupled to peroxidase (Zymed Laboratories, South San Francisco, Calif.). Positive supernatants were then rescreened by using anti-mouse IgA coupled to peroxidase (Zymed). Specific IgA-producing hybridomas were expanded and cloned three or four times by limiting dilution.

Analysis of IgA antigen specificity. Whole-cell *V. cholerae* extracts were separated on 10% reducing gels and transferred to nitrocellulose. Nitrocellulose blots were blocked with 5% nonfat dry milk and incubated with hybridoma culture supernatant followed by a goat anti-mouse IgA coupled to alkaline phosphatase (Hyclone Laboratories, Logan, Utah). To destroy carbohydrate epitopes of antigens on Western blots, nitrocellulose strips were treated with periodic acid at pH 4.5 prior to application of IgA antibodies (27); control strips were incubated in pH 4.5 buffer without periodic acid.

To analyze the molecular forms of monoclonal IgA antibodies produced in hybridoma cell culture, IgA was immunoprecipitated by incubation of fresh hybridoma supernatant with intact vibrios overnight at 4°C. Prior to use in immunoprecipitation, vibrios were fixed for 30 min in 2% formaldehyde in PBS, and free aldehydes were quenched with 50 mM ammonium chloride for 30 min at 4°C. *V. cholerae*-antibody aggregates were rinsed three times, suspended in sample buffer without 2-mercaptoethanol, and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 3 to 10% gradient gels under nonreducing conditions. After electrotransfer as described above, nitrocellulose blots were incubated with rabbit anti-mouse IgA (Zymed) followed by goat anti-rabbit IgG coupled to alkaline phosphatase (Hyclone).

Selection of IgA antibodies that recognize surface epitopes on intact *V. cholerae*. The abilities of monoclonal IgA antibodies to bind to surface-exposed epitopes on intact *V. cholerae* organisms were tested by agglutination assay, indirect immunofluorescence, and EM immunogold labeling. Stationary cultures of *V. cholerae* Ogawa 395, grown under conditions that favor piliation, were washed, resuspended in PBS, and incubated with undiluted culture supernatant for 4 h at 23°C. Agglutination was monitored by phase-contrast microscopy. To visualize surface-bound IgA, *V. cholerae* were washed twice in PBS containing 0.5% bovine serum albumin (BSA), incubated in hybridoma culture supernatant, washed with PBS-BSA, and mixed with goat anti-mouse IgA-IgG-IgM antibody coupled to rhodamine (Organon Teknika-Cappel, Malvern, Pa.). For immunogold labeling, intact *V. cholerae* organisms were briefly fixed in 2% paraformaldehyde in PBS, dispersed onto Formvar-coated nickel grids, and treated either with monoclonal anti-*V. cholerae* IgA or with a control anti-reovirus monoclonal IgA, followed by rabbit anti-mouse IgA coupled to 10 nm colloidal gold.

Production of IgA hybridoma tumors in adult BALB/c mice. Hybridoma cells producing anti-*V. cholerae* LPS IgA (clone 2D6), anti-reovirus sigma 3 IgA (clone RB3), anti-reovirus mu-1c IgA (clone RB8), or anti-*V. cholerae* IgG (clone Sh3, Medical Biotechnology Center, University of Maryland), were grown in RPMI 1640 medium supplemented with 1% Nutridoma SP (Boehringer Mannheim Corp., Indianapolis, Ind.), harvested, and washed twice in PBS. Cells (1×10^6 to 2×10^6) in 0.5 ml of PBS were injected subcutaneously into the upper backs of adult BALB/c mice. After 1 to 3 weeks of tumor growth, serum samples were taken and intestinal contents were collected by flushing the isolated small intestine with 3 ml of PBS. IgA or IgG levels were quantitated by ELISA using *V. cholerae* as the immobilized antigen and a reference sample of hybridoma culture supernatant as the standard. Antibody in the reference culture supernatant was quantitated by sandwich ELISA using purified IgA or IgG as standards (26). SDS-PAGE and Western blot (immunoblot) were performed as described above.

Production of IgA hybridoma tumors and *V. cholerae* challenge in neonatal BALB/c mice. On day 1 of mouse life, 10^6 hybridoma cells in 0.1 ml of PBS were injected subcutaneously and neonates were returned to their mothers. On day 6 to 8, 10^7 *V. cholerae* organisms, growing in L broth under conditions that favor piliation, were washed twice to remove secreted toxin, suspended in 0.1 ml of L broth including blue marker dye, and administered via gastric intubation. Neonates were maintained in a 30°C incubator for 40 h or until death. Mice were judged moribund if they met all of the following criteria: gray-blue skin coloring, markedly reduced skin turgor, and poor response to stimuli.

RESULTS

***V. cholerae* adheres selectively to M cells in BALB/c mice.** A strong mucosal immune response to *V. cholerae* may depend in part upon efficient transepithelial transport of organisms by M cells of Peyer's patch mucosa, a phenomenon previously demonstrated in rabbits (17). We therefore tested whether M cells of BALB/c mice efficiently bind and transport *V. cholerae*, since such transport would be expected to lead to localized accumulation of antigen-sensitized B lymphocytes in the intestinal mucosa. Within 4 h after intraluminal injection of organisms into ligated loops, vibrios were clustered on M cell luminal surfaces, closely associated with the M cell plasma membrane (Fig. 1). Vibrios were also observed within M cell vesicles and under the Peyer's patch epithelium (not shown). Thus, mouse M cells efficiently deliver *V. cholerae* to the underlying mucosal lymphoid tissue of Peyer's patches.

IgA hybridomas generated after mucosal immunization with *V. cholerae* show anti-LPS specificity. Of the hybridomas derived from Peyer's patch lymphocytes of three mice, 48 produced anti-*V. cholerae* antibodies, and of these, 20 were of the IgA class. After cloning, two of these antibodies recognized a broad 33- to 38-kDa band on Western blots of whole-cell extracts of *V. cholerae* Ogawa 395 proteins separated by SDS-PAGE. This band corresponds in mobility and other properties to the LPS of *V. cholerae* (see below). One anti-LPS monoclonal antibody, IgA 2D6, was selected for further study (Fig. 2A). Staining by IgA 2D6 of the 33- to 38-kDa LPS band on Western blot was abolished by prior oxidation of the blot with periodic acid at pH 4.5, suggesting that the epitope is carbohydrate in nature (27). The epitope is

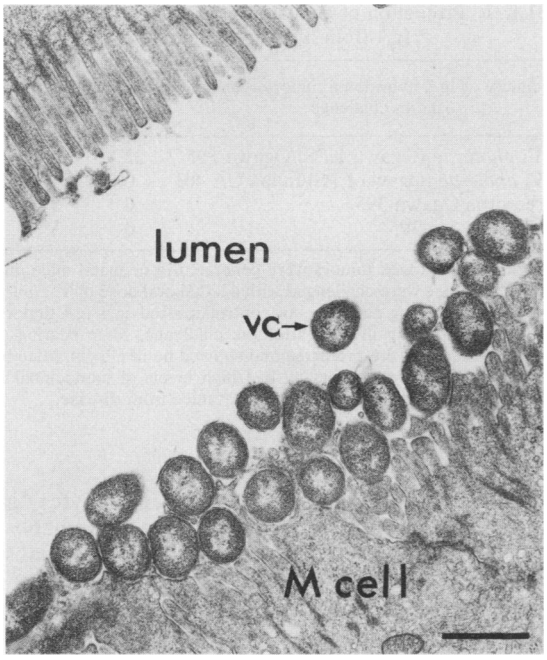

FIG. 1. Uptake of *V. cholerae* into mucosa-associated lymphoid tissue. *V. cholerae* selectively binds to M cells in Peyer's patch epithelia of BALB/c mice. *V. cholerae* Ogawa 395 was injected into ligated loops of mouse ileum which included Peyer's patches, and tissue was harvested 4 h later. Vibrios (vc) adhered preferentially to M cells and were internalized and transported across the epithelium, as previously reported for rabbits (8). Bar, 1 μm.

Ogawa serotype specific, since monoclonal antibody 2D6 did not recognize LPS on Western blots of *V. cholerae* CA401, a virulent strain of the Inaba serotype (data not shown). These data suggest that IgA 2D6 is directed against a carbohydrate epitope associated with Ogawa-specific O-side chain structures on the LPS, rather than against core oligosaccharides.

Cultured 2D6 hybridoma cells produced IgA antibodies in normal dimeric and polymeric forms that would be recognized by epithelial poly-Ig receptors. This was demonstrated by Western blot analysis of antibodies extracted from hybridoma culture supernatant using intact *V. cholerae* organisms as an immunoabsorbent (Fig. 2B).

IgA 2D6 recognizes an externally exposed LPS antigenic site on Ogawa 395. IgA 2D6 aggregated live Ogawa 395 organisms in vitro as shown by slide agglutination and labeled the surfaces of live organisms by indirect immunofluorescence (data not shown), suggesting that the antibody recognizes an externally exposed epitope. The ability of IgA 2D6 to react with the surfaces of intact organisms was confirmed by immunogold staining of briefly fixed organisms on Formvar-coated EM grids. Purified 2D6 bound specifically to the entire surface of the bacterium including the flagellar sheath (Fig. 2C), a labeling pattern identical to that seen in previous studies characterizing the cell surface reactivity of serotype-specific anti-LPS monoclonal IgG antibodies (4). The ability of IgA 2D6 to bind to the intact *V. cholerae* surface and to cross-link these organisms in vitro suggested that it could be effective in vivo in agglutinating organisms in the lumen of the intestine, facilitating their entrapment in mucus, and thus preventing colonization.

Monoclonal IgA from hybridoma backpack tumors is secreted into the intestinal lumen in vivo. To obtain continuous monoclonal IgA delivery in vivo, hybridoma cells were injected subcutaneously onto the upper backs of adult BALB/c mice. This resulted in hybridoma "backpack"

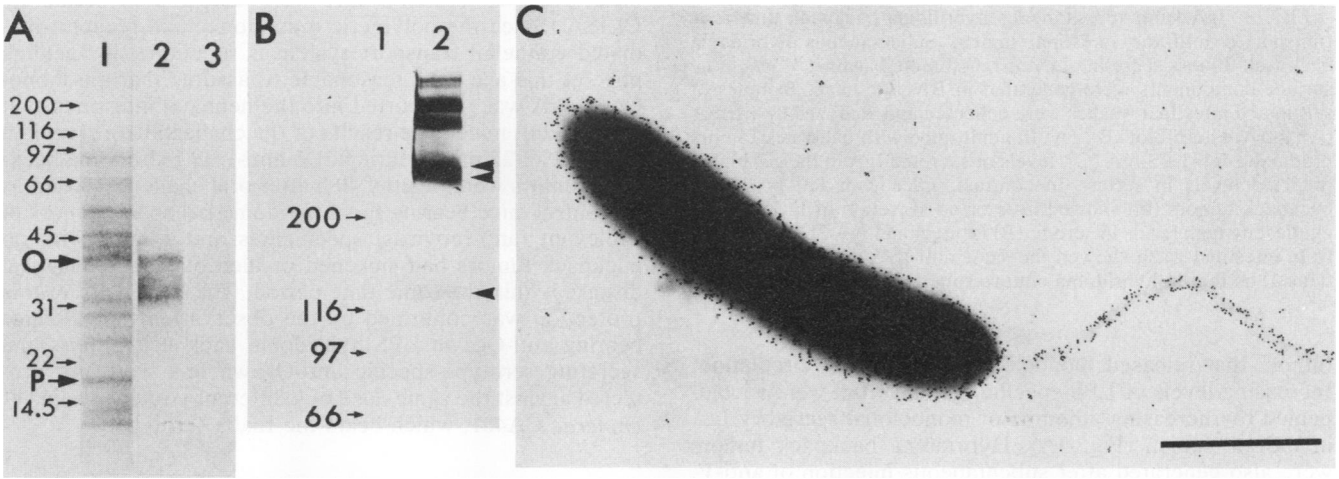

FIG. 2. Characterization of anti-*V. cholerae* IgA monoclonal antibody directed against Ogawa-specific LPS. (A) Antigen specificity of anti-*V. cholerae* monoclonal IgA 2D6 was determined by Western blot of whole-cell extracts of Ogawa 395 vibrios lysed in SDS sample buffer. Lane 1, stained with amido black, shows the major bacterial proteins including the 38-kDa outer membrane protein Omp-U (O) and toxin-coregulated pilin (P). Lane 2 shows that IgA 2D6 recognized a series of broad 33- to 38-kDa bands corresponding in mobility to outer membrane LPS. Lane 3, stained with control anti-reovirus monoclonal IgA, shows that irrelevant IgA antibodies did not bind to *V. cholerae* proteins. (B) Monoclonal antibodies were immunoprecipitated from culture supernatant by using intact *V. cholerae* organisms, demonstrating that the epitope is exterior and that the monoclonal IgA is secreted from 2D6 hybridoma cells as dimers and higher polymers. Lane 1 shows that no detectable IgA was precipitated by *V. cholerae* organisms from control anti-reovirus hybridoma supernatant. Lane 2 shows that IgA monomers (◀), dimers (◀), and higher polymers were extracted from anti-*V. cholerae* 2D6 hybridoma supernatant by intact organisms. (C) Immunogold labeling of intact *V. cholerae* with monoclonal IgA 2D6 confirms that the LPS epitope is exposed on the bacterial surface. Bar, 1 μm.

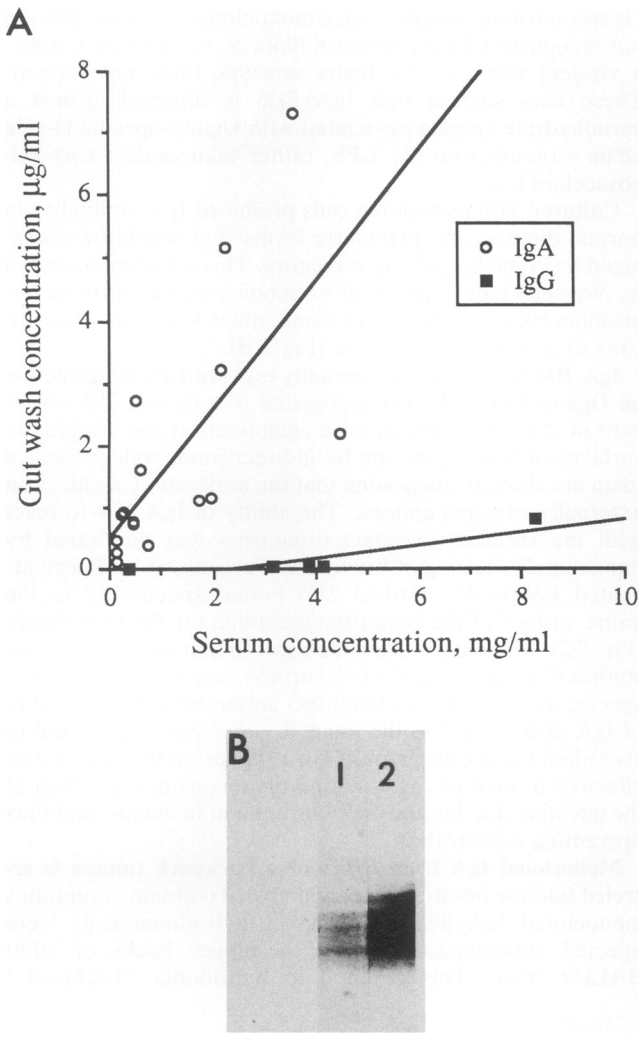

FIG. 3. IgA from subcutaneous hybridoma backpack tumors is transported into the intestinal lumen. Subcutaneous hybridoma backpack tumors secreting IgA or IgG directed against *V. cholerae* surface components were generated in BALB/c mice. Samples of serum and intestinal washes were collected and analyzed by ELISA (A) and Western blot (B). (A) In adult mice with backpack tumors producing anti-LPS IgA (○), levels of secreted IgA in the gut lumen reflected levels in serum. In contrast, mice with IgG-producing backpack tumors (■) showed little or no secretion of IgG into the gut despite high levels in serum. (B) Monoclonal IgA 2D6 recovered from intestinal wash showed the same anti-LPS antigen specificity (lane 1) as that in hybridoma culture supernatant (lane 2).

tumors that released monoclonal IgA into the circulation. Increasing levels of LPS-specific IgA in serum were accompanied by increasing amounts of monoclonal secretory IgA in the gut lumen (Fig. 3A). Hybridoma backpack tumors were also generated after subcutaneous injection of anti-*V. cholerae* IgG hybridoma cells. Although subcutaneous IgG-producing tumors resulted in very high levels of IgG in serum, monoclonal IgG was not transported into the intestine (Fig. 3A). These data suggest that tumor-derived dimeric or polymeric monoclonal IgA antibodies were specifically transported into the gut lumen via the normal receptor-mediated epithelial transport mechanism that also provides for addition of secretory component (SC). The methods used here did not distinguish between IgA transported by hepatocytes into bile and IgA transported by enterocytes directly into the intestinal lumen; both pathways presumably contributed to the monoclonal IgA recovered in intestinal secretions. Secretory IgA recovered from the intestines of adult mice bearing 2D6 hybridoma tumors recognized the Ogawa LPS band on Western blot (Fig. 3B), confirming that antigen-binding capacity of the monoclonal antibodies was unaffected by circulation and secretion in vivo.

TABLE 1. Protection of neonatal mice by monoclonal anti-LPS IgA from hybridoma tumors[a]

Specificity of IgA hybridoma tumor/organism used in challenge	No. of animals[b]:		
	Well	Moribund	Dead
Anti-*V. cholerae* (Ogawa LPS)/Ogawa 395	22	0	0
Anti-*V. cholerae* (Ogawa LPS)/Inaba CA 401	0	5	6
Anti-reovirus/Ogawa 395	0	5	15
No tumor/Ogawa 395	0	5	2

[a] Hybridoma backpack tumors were generated in neonatal mice on day 1 after birth, and mice were challenged with a lethal oral dose of *V. cholerae* on day 6 to 8. Mice bearing no tumors or control anti-reovirus IgA tumors were either dead or morbidly ill 40 h after the challenge. Mice bearing anti-*V. cholerae* LPS tumors were protected and showed no ill effects. Mice bearing anti-LPS or anti-reovirus tumors all had high levels of monoclonal IgA in serum. Thus, irrelevant IgAs did not protect mice from disease.
[b] At 40 h.

Neonatal mice bearing anti-LPS IgA hybridoma tumors are protected from a lethal oral dose of *V. cholerae*. Adult mice are resistant to infection with *V. cholerae* and do not readily develop diarrheal disease. Neonatal mice, however, are highly susceptible; the oral 50% lethal dose (LD_{50}) for unprotected mice up to 9 days of age is 10^5 organisms, and an oral challenge of 100 LD_{50} (10^7 organisms) results uniformly in moribund symptoms or death within 36 h (21). To test protection in neonates, hybridoma cells were injected 1 day after birth, and the animals were challenged by intragastric intubation of 10^7 *V. cholerae* on days 6 to 8. Although nodular tumors were not apparent at the time of challenge, high levels of anti-LPS IgA were detected in serum by ELISA. Since the polymeric immunoglobulin receptor-mediated epithelial transport system is functional in suckling mice at this age, it is reasonable to assume that the monoclonal IgA was transported into the neonatal intestines as it was in adult mice. The results of the challenge are shown in Table 1: all 22 mice bearing 2D6 anti-LPS hybridoma backpack tumors were healthy 40 h after oral challenge, whereas 20 control mice bearing IgA hybridoma backpack tumors of irrelevant (anti-reovirus) specificities and 7 mice with no backpack tumors had sickened or died of severe diarrheal disease within the same time period. The specificity of this protection was confirmed by the observation that 11 mice bearing anti-Ogawa LPS hybridoma backpack tumors and secreting serotype-specific anti-Ogawa IgA were not protected against the same dose of a different virulent strain, *V. cholerae* CA401, which bears the Inaba serotype.

DISCUSSION

By generating anti-*V. cholerae* IgA hybridomas from Peyer's patch lymphocytes after mucosal immunization with *V. cholerae* Ogawa 395, we confirmed that surface LPS is an effective mucosal immunogen. Monoclonal anti-LPS IgA antibodies, released from subcutaneous hybridoma tumors and secreted into the intestine, were sufficient to protect suckling mice against a lethal oral dose of *V. cholerae*.

Protective immunity in survivors of cholera includes poly-

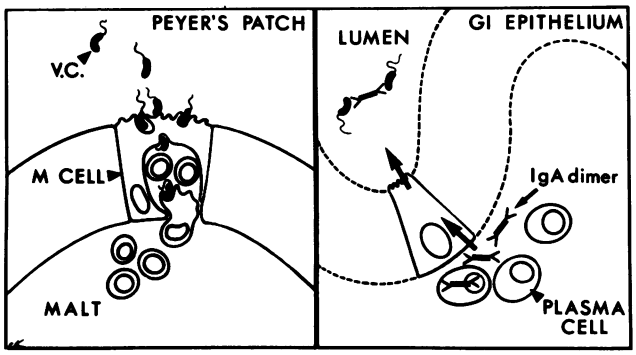

FIG. 4. Diagram summarizing proposed mechanism of normal secretory immune protection. After M cell transport of *V. cholerae* (V.C.) into mucosa-associated lymphoid tissue (MALT) of Peyer's patch, specific antigen-sensitized lymphoblasts leave MALT, proliferate, circulate, and home back to mucosal tissues to become IgA-secreting plasma cells. Dimeric IgA is bound by poly-Ig receptors on basolateral membranes of intestinal epithelial cells; the receptor-ligand complex is transepithelially transported and released into the intestinal lumen as sIgA. It is thought that sIgA protects the mucosa by cross-linking microorganisms and preventing epithelial colonization, a phenomenon called immune exclusion (13, 23).

clonal serum IgG antibodies and sIgA antibodies directed against bacterial outer membrane proteins, LPS, and cholera toxin (11, 18), but the relative contributions of each of these factors to protection have not been established (10). The results of this study demonstrate for the first time that specific sIgA alone can protect in the absence of other nonspecific and specific immunologic defense mechanisms and that a monoclonal sIgA antibody directed to a single LPS carbohydrate epitope can be sufficient to protect against disease. These results are thus consistent with studies involving orally immunized human volunteers and convalescent patients that suggest a role for polyclonal anti-LPS IgA antibodies in conferring protection against cholera (8, 11). Although we have shown that a monospecific sIgA is protective in an intact animal model, the exact mechanism of protection remains to be determined. Anti-LPS sIgA may prevent disease by agglutinating vibrios and entrapping them in mucus, by inhibiting their mobility, by preventing their adherence to the epithelium (13, 23), by enhancing the antibacterial effects of lactoferrin (1) and lactoperoxidase (22), and perhaps by "arming" of luminal lymphocytes leading to antibody-dependent cellular cytotoxicity (19, 20) directed specifically at vibrios. The hybridoma backpack tumor model described here offers the unique opportunity to dissect the spectrum of anti-bacterium protective mechanisms displayed by individual sIgA antibodies.

The strong mucosal immune response observed after *V. cholerae* infection in rabbits and humans is thought to be initiated by adherence of vibrios to M cells of the lymphoid follicle-associated epithelium of Peyer's patches (17). Transepithelial transport of *V. cholerae* organisms by M cells to the mucosa-associated lymphoid tissue results in production of specific antigen-sensitized IgA lymphoblasts; these go on to proliferate, migrate, and populate mucosal tissues throughout the intestines and other mucosal surfaces as IgA-producing plasma cells (13, 23) (Fig. 4). Dimeric and polymeric IgA antibodies produced by IgA plasma cells in the lamina propria specifically bind to polymeric immunoglobulin receptors on basolateral membranes of epithelial cells. Receptor-IgA complexes are endocytosed and transported to luminal cell surfaces where the receptors are proteolytically cleaved, releasing dimeric IgA coupled with the extracellular receptor domain known as SC (15). Association with SC is thought to protect secretory IgA from bacterial and pancreatic proteases in the intestinal lumen (2, 24).

To test the protective effects of monoclonal IgA antibodies, we sought a mode of administration of antibody in vivo that would mimic as closely as possible the normal secretion of IgA onto mucosal surfaces. For this, we took advantage of the fact that hybridoma cells can form immunoglobulin-secreting tumors in vivo (5) and that circulating IgA dimers readily exit the fenestrated subepithelial capillaries of the liver and the intestinal mucosa, bind to epithelial polymeric immunoglobulin receptors on hepatocytes and enterocytes, and are efficiently transported into bile and mucosal secretions as sIgA (7, 25). The model described here provides for the first time an experimental system that closely mimics normal IgA production and secretion. In a previous study, monoclonal anti-Sendai virus IgA antibodies, applied directly to the nasal mucosa, provided protection against respiratory infection after nasal challenge with virus (12). Such an approach may not reliably identify all protective IgA antibodies in the intestine, however, since monoclonal IgA from culture supernatant lacks SC and may be degraded in protease-rich environments. Furthermore, oral or nasal administration does not provide a physiologic, uniform distribution of IgA on mucosal surfaces. In contrast, monoclonal IgA in mice with backpack tumors is transported by normal epithelial cells, complexed with endogenous SC, and released continuously into secretions.

In this study, mucosal immunization with whole bacterial cells was used to identify immunogenic epitopes and to define a single epitope that elicits protective secretory antibodies in the intestine. This strategy will allow us to define other protective monoclonal IgA antibodies against other *V. cholerae* surface structures known to be associated with virulence (14, 21). Since specific receptor-mediated IgA transport is conducted by epithelial cells and glands of the digestive, respiratory, and genital tracts as well as mammary and lacrimal glands, monoclonal IgA from hybridoma backpack tumors in mice could be used to assess protection at these mucosal surfaces. Thus, the approach documented here can potentially be used to define effective mucosal immunogens and to identify protection sIgA antibodies against the wild variety of bacterial and viral pathogens that infect or invade mucosal surfaces. With protective sIgA antibodies thus identified, we are currently conducting experiments to determine whether monoclonal IgA can be used prophylactically to provide passive mucosal protection or therapeutically to arrest the disease process once mucosal colonization is established.

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