Protection against Mucosal Infections by Secretory IgA

David F. Keren, M.D.
Biochemistry Section
Department of Pathology
The University of Michigan
Ann Arbor, Michigan

In the past decade, several model systems have been developed to study the details of the production of secretory IgA by the mucosal surface. Until recently, most attention in vaccine research has been directed toward establishing a vigorous systemic (predominantly IgG) humoral immune response to the infectious agent. With the recognition, however, that secretory IgA is overwhelmingly the predominant immunoglobulin along mucosal surfaces and likely responsible for host defense, much work has been directed at understanding initiation of the secretory IgA response and to establishing a secretory IgA memory response. Also, the mechanisms by which secretory IgA affects protection are being sought. Unlike IgG, secretory IgA does not opsonize for phagocytosis or efficiently activate complement for cell destruction by membrane damage. Rather, secretory IgA seems to afford protection by preventing attachment of microorganisms and/or their toxic product to the delicate surface epithelium that lines the mucosa.

Antigen Processing and Regulatory T Cells in the Mucosal Immune System

Specialized surface epithelial cells which overlie lymphoid follicles, Peyer's patches, and the appendix have been identified that take up both luminal macromolecules and microorganisms for antigen processing. These M cells can take up macromolecules such as horseradish peroxidase within a few minutes of their application to the intestinal lumen (12). Larger structures, such as reovirus or *Shigella flexneri,* are taken up more slowly, requiring 1 to 1.5 hr before ingestion can be reliably demonstrated (16). A potential problem with antigen processing in the gut, however, is that when invasive microorganisms such as *Shigella flexneri* or enteropathogenic viruses are taken up by M cells, the pathogens may use the M cells as sites for replication and further invasion. Indeed, in a recent editorial, Sneller and Strober suggested that this may be the route of invasion by the AIDS virus (15). The rectal mucosa has abundant isolated lymphoid follicles which contain such M cells. Preliminary work from our own laboratory, however, suggests that preexisting secretory IgA will prevent this M cell uptake by live, invasive *Shigella*. Therefore, with appropriate vaccination, it may be possible to control uptake of undesirable microorganisms and toxic molecules by the gastrointestinal tract lymphoid tissue.

Following uptake of antigen, the material is brought to the underlying lymphoid tissues that are enriched in precursor B lymphocytes for an IgA response. At this stage of development, most B lymphocytes in the gut-associated lymphoid tissues (GALT) express surface IgM and/or IgD. Immunoregulatory T lymphocytes are present in GALT that influence the development of IgA precursor B lymphocytes. These regulatory cells include switch T cells as described by Kawanishi and associates (6). Under the influence of lipopolysaccharide, clones of these switch T cells alter the surface immunoglobulin expression of Peyer's patch B lymphocytes from IgM to IgA. Several other laboratories have described helper T cells for the IgA response. These cells encourage differentiation of B lymphocytes which already bear surface IgA, toward becoming mature plasma cells (14). In addition to regulatory T cells, it is clear that the B lymphocytes within GALT have an inherent genetic propensity for becoming IgA synthesizing and secreting plasma cells (2).

Oral antigen administration also sets immunosuppressive events in motion. Following mucosal stimulation with particulate or soluble antigens, suppressor T cells that inhibit systemic IgG, IgM, IgE, and cell-mediated immune responses have been described. These suppressor T cells have been identified within GALT for the first 2 or 3 days following mucosal stimulation with antigen. Thereafter, they leave GALT and can be found in the spleen and other systemic sites (10). Teleologically, there would be an advantage in preventing the potentially damaging systemic IgG response to the many harmless antigens present along the gastrointestinal tract. If such vigorous systemic immunity resulted in all luminal antigens, it would set the stage for Arthus-type reactions to trivial antigen stimulation along the delicate intestinal epithelium.

Lymphocyte Trafficking in the Gut

Following their stimulation in GALT, antigen-specific IgA precursor B lymphocytes travel to the mesenteric lymph nodes, thoracic duct, mature further in the spleen, and eventually migrate back to mucosal surfaces (1). In addition to the location where initial antigenic stimulation occurred, these antigen-specific IgA B lymphocytes (and preplasma cells) populate other mucosal sites around the body: mammary glands, lacrimal glands, salivary glands, and bronchial mucosal. This may serve to protect a variety of mucosal surfaces against antigens present in the environment. To a certain extent, the presence of antigen at one particular surface can enhance the number of IgA-secreting plasma cells that select that site (13).

The total time for the lymphocyte recirculation following oral antigen stimulation is 4 to 6 days. In studies wherein the Peyer's patches are directly injected with antigen, an IgA specific response to the antigen can be seen within 2 to 3 days. This implies that the initial antigen processing event, from intestinal lumen through M cell, to antigen presenting cell, requires approximately 2 days.

Secretory IgA against Enteropathogens

As secretory IgA lies at the portal of entry for enteropathogens, several model systems have been developed to determine the optimal means of producing a vigorous mucosal immune response to these agents. Further, recent

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studies have indicated that the presence of secretory IgA to enteropathogens and/or their toxic products can inhibit the pathologic events.

To follow the kinetics of the IgA response in intestinal secretions, our laboratory developed a chronically isolated, ileal loop model in rabbits. With proper daily care, these chronically isolated, ileal loops can be maintained for at least one month in 90% of the rabbits. Using this model system, we have followed the mucosal immune response to *S. flexneri* and other enteropathogens. Following oral immunization with live or killed *S. flexneri*, a weak, primary IgA response is detected in secretions by 6–8 days following oral stimulation. The response is stronger when a live vaccine is used than when the killed oral vaccine is given. The kinetics of the development of the response are the same for both, however. This model system was used to document the existence in intestinal secretions of a secretory IgA memory response after appropriate priming. For a mucosal memory response, animals were primed with 3 weekly oral doses of live *S. flexneri*, then left for 60 days, and rechallenged orally with the same strain of *Shigella*. A vigorous secretory IgA response could be detected in intestinal secretions within two days following rechallenge (7). In addition to accelerated kinetics, the IgA activity attained was significantly stronger than that achieved by the primary stimulation. Interestingly, heat-killed *Shigella* given orally were unable to prime for a mucosal memory response regardless of the dosage given.

Since it was possible that the invasive capabilities of the *Shigella* used in our initial studies were responsible for the IgA memory response obtained in the intestinal secretions, we used 2 noninvasive strains of *Shigella* in subsequent experiments. One strain, *S. flexneri* 2457-0, was noninvasive by the Sereny test; however, it contained a 140 megadalton virulence plasmid, which is found in pathogenic strains and thought to be associated with invasion. The second strain, M4243A$_1$, has been cured of this virulence plasmid and shows no invasive capabilities by Sereny test or rabbit ileal loop model. Both strains were able to prime rabbits for a secretory IgA memory response that was as strong as had been seen previously with the virulent strains (8). This indicates that even noninvasive, live bacteria are processed by the mucosal immune system to prime the animal for a mucosal memory response. Such findings offer considerable promise for future mucosal vaccine research. Whether the secretory IgA produced against these bacteria is protective has not been determined. In similar studies in our laboratory using cholera toxin and Shiga's toxin, however, the secretory IgA produced was able to interfere with the pathologic effects of those molecules.

The same kinetics of the secretory IgA response have been found by several groups using different model systems. For instance, Clements and colleagues gave heat-labile *Escherichia coli* enterotoxin B subunit (nontoxic) orally to BALB/c mice. They collected the intestinal contents at time of sacrifice and were able to determine that the purified IgA anti-B subunit was capable of neutralizing the biologic activities of the *E. coli* toxin in vitro. Further, they were able to determine that either oral or intraperitoneal dose of antigen could boost, or hyperstimulate the mucosal IgA response (3). Although they were only able to obtain one datum point per mouse, the use of inbred mice for these studies is a considerable advantage over the rabbit model for understanding the immunogenetics involved in the response. The parallel to the rabbit system is, however, striking. For instance, in our most recent studies, we have found that a combination of parenteral and oral doses of antigen will give a boost to the primary IgA response to *Shigella*.

Functional Significance of Secretory IgA Response to Enteropathogens

Although systemic humoral immunity, mainly IgG, affords protection via opsonization for phagocytosis, activation of complement, and, perhaps, antibody-dependent cell-mediated cytotoxicity, IgA does not seem to be proficient at these tasks. Since some enteropathogens, such as *S. flexneri*, often result in ulceration with elicitation of a polymorphonuclear leukocyte inflammatory response, it has been important to determine whether secretory IgA directed against these enteropathogens plays a role in opsonizing for phagocytosis. There has been some work that suggests that IgA may enhance phagocytosis. For instance, Edebo and associates coated *Salmonella typhimurim* with dinitrophenol (DNP). They reacted these DNP-coated bacteria with a mouse myeloma protein (MOPC 315), a monomeric IgA directed against DNP (4). Although this IgA was able to enhance the interaction of the bacteria with phagocytic cells, the IgA monoclonal was much less efficient at this process than was IgG. This is not the form of IgA that is usually present in intestinal secretions, however. Indeed, studies in which secretory IgA was used to study opsonization have found that it may inhibit the opsonization for phagocytosis (11).

The most likely protective role for secretory IgA was suggested by Fubara and Freter 15 years ago. They hypothesized that secretory IgA would interfere with colonization or attachment of microorganisms to the surface epithelium (5). More recent studies using parasites and small toxins to stimulate mucosal immunity support Freter's insightful suggestion. For instance, studies in which *Giardia* trophozoites were given via an intraintestinal immunization route, demonstrated that secretory IgA is readily produced to these parasites. Further, secretory IgA bound to these parasites and was able to increase the clearance of these microorganisms. Scanning electron microscopy detected IgA bound to the adhesive dishes of these parasites (9). Lastly, in recent studies using Shiga's bacillus toxin, our laboratory, working together with Brown, found that the IgA anti–Shiga toxin activity correlates significantly with the ability of intestinal secretions to neutralize the effects of Shiga's toxin on tissue culture cells.

The secretory IgA system provides an effective defense along mucosal surfaces to a wide variety of bacteria, virus, parasites, and toxins. When appropriately primed, the secretory IgA system will produce a strong memory

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response. The antibody elicited will prevent binding of bacteria, parasites, and toxic molecules to the epithelial cell surfaces. Considerable future work is needed to better understand the optimal means of immunization and to review the function of the secretory IgA molecules produced in dealing with enteric infections.

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Defensins

Robert I. Lehrer[1,4], M.D.
Tomas Ganz[1,3], M.D., Ph.D.
Michael E. Selsted[1,2], M.D., Ph.D.
Departments of Medicine[1] and Pathology[2]
The Will Rogers Institute Pulmonary Research Laboratory[3]
UCLA School of Medicine
Los Angeles, California
Department of Medicine[4]
Veterans Administration Medical Center
West Los Angeles, California

The ability of phagocytic leukocytes to kill ingested microorganisms arises from two general mechanisms. One of these depends on the phagocyte's ability to assemble and activate a multicomponent oxidase system that generates superoxide anion (O_2^-) by oxidizing its substrate, NADPH. The resultant superoxide anions are converted to other reactive oxygen derivatives (RODs), whose precise chemical nature is influenced by the biochemical composition of the phagocyte. In normal human neutrophils (PMN), these secondary RODs include H_2O_2, hydroxyl radicals, hypochlorous acid, and chloramines (18), all of which are considerably more microbicidal than superoxide itself. In addition to undergoing an oxidative attack by RODs, ingested microbes are exposed to antimicrobial constituents normally contained within the phagocyte's cytoplasmic granules.

This report will focus on defensins, a recently defined family of antimicrobial peptides whose members appear to be important effectors of ROD-independent antimicrobial activity by PMN and certain macrophages. The activity spectrum of human and rabbit defensins encompassess both gram-positive and gram-negative bacteria (3, 13), fungi (3, 14), certain viruses (1, 9), and even tumor cells (11). This review will describe both our recent studies with human PMN defensins and certain earlier studies that we performed on homologous peptides from rabbit leukocytes. Readers seeking information about other ROD-independent antimicrobial components of PMN, such as B/PI or cathepsin G, may consult other recent reviews (2, 17).

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