

Antigen handling by the gut

The epithelial surface of the gastrointestinal tract represents an extensive surface area exposed to a hostile intraluminal environment containing a variety of antigenic macromolecules. These intraluminal substances—such as food antigens, microorganisms, and toxins—can potentially penetrate the mucosal barrier and gain access to the interstitial space or be taken up into the systemic circulation. Penetration of the mucosal barrier may in turn result in clinical disease manifested by infection, allergy, or autoimmunity. In order to combat the potential for antigen penetration across the mucosal barrier, man has created an elaborate system of immunological and nonimmunological defences within the intestinal lumen or on the intestinal surface (glycocalyx) which act to maintain the epithelium as a barrier to uptake of macromolecules. Of particular importance are recent studies which have begun to demonstrate the role of the glycocalyx as an active metabolic compartment, both for the control of bacterial adherence to the epithelial surface,¹ and for interference with the attachment and uptake of antigens and enterotoxins.²

Intestinal permeability

Despite the notion that the gut is an impenetrable barrier to the uptake of intraluminal antigens, experimental and clinical evidence suggests that the mucosal barrier to antigenic material may be incomplete, allowing for absorption of macromolecules, not in sufficient quantities to be of nutritional importance, but in quantities that may be antigenic or biologically active.³ The concept of intestinal permeability to intraluminal antigens stems primarily from studies in newborn babies.

In combined morphological and physiological experiments, the small intestinal epithelial cell can be demonstrated to engulf macromolecular antigens by an endocytotic process indistinguishable from the pinocytosis process described in human macrophages. The initial event in this process is an interaction between large molecules within the intestinal lumen and components of the microvillous membrane of intestinal absorptive cells (adsorption). When a sufficient concentration of molecules comes in contact with the cell membrane, invagination occurs and small vesicles are formed. After invagination, antigens migrate within membrane-bound vesicles (phagosomes) to the supranuclear region of the cell

where vesicles coalesce with lysosomes to form large vacuoles (phagolysosomes). Within these structures intracellular digestion occurs. However, small quantities of ingested molecules escape breakdown and migrate to the basal surface of the cell to be deposited in the interstitial space by a reversal of pinocytotic process. Under normal circumstances, macrophages and plasma cells present in the lamina propria of the small intestine and interact with antigens as a secondary line of defence against the penetration of antigen into the circulation. However, when excessive quantities of antigen traverse the intestinal epithelial cell or when secondary defences are lacking or diminished, as in secretory IgA, antigens may diffuse into the interstitial space and enter the systemic circulation. The penetration of foreign proteins into the circulation may in turn evoke allergic or toxigenic reactions manifested as disease states. Several clinical studies suggest that macromolecules can cross the mucosal barrier under normal physiological conditions in man.⁴ As the pinocytotic process of antigen absorption probably represents the residual of a primitive absorptive mechanism in the alimentary canal, the capacity to absorb large molecules may be more extensive in the immature small intestine than in the mature and more highly developed intestine. In fact, this observation is supported by evidence suggesting that premature and newborn infants can absorb greater quantities of ingested food antigens than older infants or adults.⁵ Rothberg⁶ for example, has measured bovine serum albumin (BSA) in the serum of premature infants fed quantities of this protein normally present in their daily milk. In contrast, circulating BSA could not be detected in serum samples from older children fed equivalent quantities of protein. He and others^{7,8} have also reported a larger percentage of infants with serum samples containing antibodies to food antigens, suggesting that food proteins are absorbed intact into the circulation of infants in sufficient quantities to evoke a systemic immune response. The implication of these studies is that the neonatal intestine may absorb antigenic quantities of ingested protein more readily than the more mature adult intestine. To support this hypothesis, Lev and Orlic⁹ in recent morphological studies with fetal monkeys, and Moxey and Trier¹⁰ with human fetuses, have shown excessive uptake of large molecules by intestinal epithelial cells. They also described

morphological features of epithelial cells suggesting structural immaturity. This same immaturity of gastrointestinal function and structure may persist beyond fetal life into the newborn period, at a time when the small intestine is exposed to increased quantities of both bacterial and food antigens.

In a recent study,¹¹ we have shown that levels of milk and soy serum agglutinins are considerably higher in infants fed milk and soy formulae within the first 3 months than in those introduced to these protein antigens after 3 months of age. This study suggests that the intestinal tract is more permeable to antigens during the first 3 months. It is, therefore, important that more studies be done to define clearly intestinal permeability to specific antigens during the perinatal period.

Recent studies have demonstrated that a specialised epithelial cell may exist within the gut to facilitate the access of antigens to intestinal lymphoid tissue. This process may be important in the IgA-producing plasma cell cycle. These clusters of specialised epithelial cells, known as membranous epithelial cells (M-cells), have been recognised overlying gut-associated lymphoid tissue (GALT) in the ileum of several species, including man, and may represent an important pathway for the direct access of intestinal antigens to lymphoid tissue.¹² Morphological features of M-cells, including a paucity of microvilli, a poorly developed glycocalyx, and an absence of lysosomal organelles, support the view that these cells are especially adapted for antigen transport. Histochemical studies have demonstrated a preferential uptake of horseradish peroxidase into M-cells after exposure of the gut to small quantities of that antigen. After exposure to larger amounts of the enzyme, uptake of horseradish peroxidase was noted, not only in M-cells, but in all epithelial cells, suggesting that the mode of antigen access to GALT may be dependent on the concentration of antigens in the intestinal lumen. At physiological or lower levels of luminal antigen, the specialised uptake pathway is preferred; at increased antigen levels, a more generalised uptake of antigen takes place. After uptake into M-cells, horseradish peroxidase is rapidly released into the interstitial space and processed by lymphoid cells circulating through Peyer's patches. This mechanism of antigen handling by the gut appears to represent an important specialised access route for intestinal antigens to reach lymphoid tissues and thereby stimulate the local immune system.

Intestinal antibodies

An important, if not the most important, component of host defence at the epithelial surface is the

presence of intestinal antibodies. There is now substantial evidence that the main function of intestinal antibodies is the process of immune exclusion at the mucosal surface. William and Gibbons¹³ examined the adherence properties of oral pathogens, such as *Streptococcus viridans*, to epithelial cell surfaces before and after exposure of these organisms to specific secretory IgA antibodies. They observed a definite decrease in adhesion of these bacteria to the cell membrane after exposure to secretory antibodies. They concluded that secretory IgA antibodies block specific binding sites on the bacterial cell wall and thereby interfere with bacterial adherence to epithelial surfaces. A decrease in adherence results in decreased colonisation as well as enhanced clearance of the bacteria by oral secretions. Additional studies have shown interference in the attachment of *Vibrio cholerae* to intestinal mucosa by secretory IgA intestinal antibodies.¹⁴ The presence or absence of intestinal antibodies capable of interfering with specific bacterial adherence may also be important in determining the nature of indigenous gut bacterial flora.

Intestinal antibodies can also protect against the effects of toxic bacterial by-products (for example, enterotoxins). Secretory antitoxins complexing with cholera toxin can prevent toxin binding to receptors on intestinal microvillus membranes and thereby interfere with the activation of adenylate cyclase, a necessary step in the active secretion associated with toxigenic diarrhoea. In like manner, intestinal antibodies interfere with the uptake of nonviable antigens introduced directly into the gastrointestinal tract.¹⁵ We have previously reported that intestinal antigens become rapidly associated with antibodies present in the glycocalyx. Antigen-antibody complex formation at that site appears to prevent migration of antigen to the cellular membrane surface and thereby to interfere with pinocytosis by enterocytes. Other investigators have demonstrated that IgA myeloma antibodies injected into the respiratory tract of laboratory animals interfere with the uptake of human serum albumin by respiratory epithelium.¹⁶

Other defence mechanisms

A number of nonimmunological factors, present either within the intestinal lumen or on the intestinal mucous surface, exist and are of importance as an adjunct to more classical host defence mechanisms of the gut. These factors include peristaltic movement, goblet cell release of mucus, pancreatic enzymes, indigenous flora, and hepatic reticulo-endothelial filtration. We have reported¹⁷ that

pancreatic enzymes adsorbed to the surface of the small intestine facilitate the breakdown of antigen-antibody complexes at that site. More recently, we have shown that antigen-antibody complex formation within the intestinal lumen may trigger the release of goblet-cell mucus which in turn can interfere with further antigen penetration across the mucosal barrier.¹⁸ These nonspecific defences may work synergistically with immunological defences in the control of proliferation of micro-organisms present in the gastrointestinal tract, by decreasing adherence of organisms to the gut surface; these factors may also be important in limiting the available antigen mass that may otherwise overwhelm local immunological defence mechanisms and penetrate the mucosal barrier or enter the systemic circulation. Taken individually these factors contribute very little to the overall protection of epithelial surfaces. However, when all nonspecific defences are operational their combined contribution provides important additional protection.

Gastrointestinal allergy

As a result of the pathological transport of antigens across the small intestine, toxic quantities of bacterial breakdown products, proteolytic and hydrolytic enzymes, and ingested antigens may traverse the mucosal barrier and predispose to gastrointestinal diseases. The gastrointestinal diseases possibly associated with antigen absorption are gastrointestinal allergy, inflammatory bowel disease, coeliac disease, toxigenic diarrhoea, chronic hepatitis, necrotising enterocolitis, and autoimmune diseases. Since the evidence cited to support the hypothesis that intestinal permeability to antigens is involved in the pathogenesis of human disease is largely indirect, the reader should realise that these comments are highly speculative and remain to be proved by more direct evidence.⁹ Probably the most striking association between antigen handling and clinical disease is shown with gastrointestinal allergy. Several clinical allergy symptoms have been described which appear to relate specifically to the ingestion of specific foods (particularly cows' milk). These conditions may be localised to the gastrointestinal tract and present with diarrhoea, gastrointestinal bleeding, or protein-losing enteropathy, or they may be represented by systemic manifestations of allergy ranging in severity from exanthema to anaphylaxis. The clinical expression of allergy may relate (a) to the transport of antigens into the lamina propria alone (local allergic reaction), or (b) into both the lamina propria and systemic circulation (systemic allergic response). Factors

which determine the nature of the allergic response are not entirely understood, but they undoubtedly relate to the degree of sensitivity of the allergic patient and/or the concentration of allergen ingested. Although the mechanism(s) of gastrointestinal allergy is at present obscure, it would appear that the intestinal transport of allergens is a necessary initial step in the process. In fact, it has been suggested that during the neonatal period when increased antigen permeability exists, susceptible infants may become sensitised to specific ingested protein. With re-exposure at a time when much less macromolecular absorption is occurring, minute quantities of allergen may be absorbed and result in allergic symptoms.

Although the pathogenesis of inflammatory bowel disease, which includes chronic ulcerative colitis and Crohn's enterocolitis, remains unclear, one of the current hypotheses is that bacterial antigens taken up from the intestine evoke a local hypersensitivity reaction which in turn causes local intestinal inflammation, mucosal ulceration, and granulomatous reaction. Shorter *et al.* suggest that a deranged permeability of the gut mucosa allows for the passage of macromolecules and nonenteropathic Gram-negative bacteria into the gut wall which contains aggregates of lymphoid tissue previously sensitised to these same antigens.¹⁹ This penetration of antigens triggers a hypersensitivity reaction in the intestinal wall which ultimately results in the pathological condition of inflammatory bowel disease.

The pathogenesis of coeliac disease is not at present fully understood. However, some evidence suggests that a local immunological response within the small intestine may account for the pathological changes of epithelial cell destruction and submucosal inflammation characteristic of this disease.²⁰ It is possible that the intestinal uptake of gluten (a major component of wheat protein) or its polypeptide breakdown product (gliadin), could account for the hypersensitivity response noted in susceptible individuals. It remains to be established whether the experimental evidence supports the contention that a primary immunological response to increased gluten uptake accounts for the disease state, or whether a generalised increase in the absorption of all intestinal antigens secondary to mucosal barrier damage accounts for the immunopathology present in the small intestine.

Bacterial toxins

An increased interest in the mechanism of action of bacterial exotoxins on intestinal mucosa has recently developed because of the number of reports of toxins causing a variety of diarrhoeal disease

states.²¹ From experiments on the mechanism of action of cholera toxin, we now know that toxigenic organisms do not penetrate the intestinal mucosa but instead release a protein (enterotoxin) which attaches to the intestinal epithelial cell surface and stimulates an increased secretion of fluids from the serosal to the mucosal surface. Some studies suggest that the toxin may either be taken up into the cell or in some way penetrates the intestinal mucosal barrier before exerting a biological effect.

Hepatic disorders

In addition to those clinical conditions described above, a number of other diseases may be associated with a pathological absorption of macromolecular antigens owing to an altered mucosal barrier. These conditions, although primarily associated with a selective deficiency in IgA, are thought to be due to an enhanced entry of macromolecules after interaction with the epithelial surface of the gut. An increased incidence of certain liver diseases is associated with selective IgA deficiency.²² It is suggested that hepatotoxic substances or breakdown products of intestinal flora (enzymes, endotoxins, etc.) within the gut lumen can be taken up directly into the portal circulation in concentrations that exceed the protective capacity of the reticuloendothelial system, and thus can result in direct damage to hepatocytes. Furthermore, the chronic active hepatitis associated with inflammatory bowel disease may be caused by excessive uptake of hepatotoxins across areas of denuded intestinal mucosa. This concept is based on the observation that sometimes when inflamed bowel is removed the liver condition improves, suggesting a decreased uptake of toxins.

From the foregoing, it is apparent to the reader that more research is needed before we can understand how the gastrointestinal tract handles antigens. For example, recent evidence suggests that intraepithelial lymphocytes are increased in number in those conditions associated with enhanced antigen permeability.²³ What role do these cells play in that process? How can we selectively turn on secretory IgA and not IgE or IgG antibodies? What methods are available to protect passively and actively the gut from antigenic uptake? Better understanding of antigen handling by the gut may lead us to deal with these problems more appropriately.

References

- ¹Walker, W. A. (1976). Host defense mechanisms in the gastrointestinal tract. *Pediatrics*, **57**, 901-916.
- ²Walker, W. A. (1975). Antigen absorption from the small intestine and gastrointestinal disease. *Pediatric Clinics of North America*, **22**, 731-746.
- ³Walker, W. A., and Isselbacher, K. J. (1974). Uptake and transport of macromolecules by the intestine. Possible role in clinical disorders. *Gastroenterology*, **67**, 531-550.
- ⁴Wilson, S. J., and Walzer, M. (1935). Absorption of undigested proteins in human beings. IV. Absorption of unaltered egg protein in infants and in children. *American Journal of Diseases of Children*, **50**, 49-54.
- ⁵Gruskay, F. L., and Cooke, R. E. (1955). The gastrointestinal absorption of unaltered protein in normal infants and in infants recovering from diarrhea. *Pediatrics*, **16**, 763-769.
- ⁶Rothberg, R. M. (1969). Immunoglobulin and specific antibody synthesis during the first weeks of life of premature infants. *Journal of Pediatrics*, **75**, 391-399.
- ⁷Katz, J., Spiro, H. M., and Herskovic, T. (1968). Milk precipitating substance in the stool in gastrointestinal milk sensitivity. *New England Journal of Medicine*, **278**, 1191-1194.
- ⁸Korenblat, R. E., Rothberg, R. M., Minden, P., and Farr, R. S. (1968). Immune response of human adults after oral and parenteral exposure to bovine serum albumin. *Journal of Allergy*, **41**, 226-235.
- ⁹Lev, R., and Orlic, D. (1973). Uptake of protein in swallowed amniotic fluid by monkey fetal intestine *in utero*. *Gastroenterology*, **65**, 60-68.
- ¹⁰Moxey, P. C., and Trier, J. S. (1975). Structural features of the mucosa of human fetal small intestine (abstract). *Gastroenterology*, **68**, 1002-1003.
- ¹¹Eastham, E. J., Lichauco, T., Grady, M. I., and Walker, W. A. (1978). Antigenicity of infant formulas: role of immature intestine on protein permeability. *Journal of Pediatrics* (in press).
- ¹²Owen, R. L. (1977). Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: an ultrastructural study. *Gastroenterology*, **72**, 440-451.
- ¹³William, R. C., and Gibbons, R. J. (1972). Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. *Science*, **177**, 697-699.
- ¹⁴Fubura, E. S., and Freter, R. (1973). Protection against enteric bacterial infections by secretory IgA antibodies. *Journal of Immunology*, **111**, 395-403.
- ¹⁵Walker, W. A., Isselbacher, K. J., and Bloch, K. J. (1972). Intestinal uptake of macromolecules: effect of oral immunization. *Science*, **177**, 608-610.
- ¹⁶Walker, W. A., and Isselbacher, K. J. (1977). Intestinal antibodies. *New England Journal of Medicine*, **297**, 767-773.
- ¹⁷Walker, W. A., Isselbacher, K. J., and Bloch, K. J. (1975). Intestinal uptake of macromolecules. IV. The effect of pancreatic duct ligation on breakdown of antigen and antigen-antibody complexes on the intestinal surface. *Gastroenterology*, **69**, 1223-1229.
- ¹⁸Walker, W. A., Wu, M., and Bloch, K. J. (1977). Stimulation by immune complexes of mucus release from goblet cells of the rat small intestine. *Science*, **197**, 370-372.
- ¹⁹Shorter, R. G., Huizenga, K. A., and Spencer, R. J. (1972). A working hypothesis for the etiology and pathogenesis of non-specific inflammatory bowel disease. *American Journal of Digestive Diseases*, **17**, 1024-1032.
- ²⁰Hobbs, J. R., Hepner, G. W., Douglas, A. P., Crabbé, P. A., and Johansson, S. G. O. (1969). Immunological mystery of coeliac disease. *Lancet*, **2**, 649-650.
- ²¹Grady, G. F., and Keusch, G. T. (1971). Pathogenesis of

- bacterial diarrhea. Part I. *New England Journal of Medicine*, **285**, 831–841.
- ²²Wilson, I. D., Onstad, G. R., Williams, R. C., Jr, and Carey, J. B., Jr (1968). Selective immunoglobulin A deficiency in two patients with alcoholic cirrhosis. *Gastroenterology*, **54**, 253–259.
- ²³Ferguson, A. (1977). Intraepithelial lymphocytes of the small intestine. *Gut*, **18**, 921–937.

W. ALLAN WALKER
*Harvard Medical School,
Pediatric Gastrointestinal and Nutrition Unit,
Massachusetts General Hospital,
Boston, Massachusetts 02114,
USA*