Intestinal Immunology and Food Allergy,
edited by Alain L. de Weck and Hugh A. Sampson,
Nestlé Nutrition Workshop Series, Vol. 34. Nestec Ltd.,
Vevey/Raven Press, Ltd., New York © 1995.

Uptake and Transport of Macromolecules by the Intestine

Ian R. Sanderson and W. Allan Walker

Mucosal Immunology Laboratory of the Combined Program in Pediatric Gastroenterology and Nutrition, Massachusetts General Hospital, The Children's Hospital, Harvard Medical School, Charlestown, Massachusetts 02129, USA

INTRODUCTION

The intestine is routinely exposed to a limitless variety of macromolecules derived from many sources, including resident bacteria, ingested food, invading viruses, etc. Because of their size, macromolecules can act as antigens—some of which are harmful to the host while others pose no threat. The intestine must deal with this diversity. To do this, a unique immune system exists at mucosal surfaces, which is distinct from the systemic immune system. An important mechanism by which the intestine surveys antigens in the intestinal lumen is by allowing small quantities to cross the epithelium and interact with the mucosal and systemic immune systems. However, excessive or inappropriate exposure of antigens to the intestinal immune system may lead to gastrointestinal disease (1,2). There has been a considerable amount of work on the physiologic and pathologic consequences of macromolecular transport in recent years. For example, the uptake of intestinal growth factors necessary for normal growth and development of the gut and other organ systems has been studied extensively (3–5). In addition, important progress has been made on the mechanisms of macromolecular transport (6,7).

Two important intestinal diseases of childhood underscore the notion that it is the molecular structure of macromolecules that is critical to the pathogenesis of some immunologically mediated gastrointestinal diseases. In celiac disease, the appearance of an enteropathy sensitive to gluten is dependent on the integrity of the grain protein. Partial digestion by papain of any of the gluten-related family of macromolecules will abrogate its activity. The second disease entity, cow's milk sensitive enteropathy, responds to a diet in which casein or whey milk protein has been hydrolyzed commercially. Thus, the pathogenic nature of these macromolecules resides within their antigenic structure.

An understanding of the physiology of macromolecular transport is therefore crucial to an appreciation of its contribution to the pathogenesis of gastrointestinal disease. For example, it is possible that a limited exposure to antigens (which constitutes a normal mechanism of surveying the contents of the intestinal lumen) may at times lead to damage of the barriers to transport, allowing chronic immune and inflammatory responses to develop, as may occur in inflammatory bowel disease and allergic gastroenteropathy. Thus we must consider how uptake is limited so that immune reactions do not work adversely in the host. Furthermore, oral tolerance (the phenomenon whereby prior exposure to an antigen by the enteric route induces a specific immunological unresponsiveness on subsequent systemic exposure to the same antigen) may depend, in part, on the pathway(s) of antigen uptake and the manner in which luminal antigens are handled by the gut. For example, if a luminal antigen is taken up inappropriately, the result may provide the basis for autoimmune states (8).

Some mechanisms of macromolecular uptake are specific and constitute a physiologic process by which the molecules can perform their beneficial function. Other mechanisms are nonspecific and may constitute a potentially damaging result that could cause disease.

PHYSIOLOGIC TRANSPORT

While the majority of macromolecules necessary for normal body functions are synthesized *de novo,* there are some essential macromolecules that are taken up by the intestinal epithelium from the lumen of the bowel. Physiologic transfer of macromolecules is particularly important during infancy and childhood when organ development is incomplete. Specific transepithelial mechanisms have evolved to facilitate the uptake of a number of proteins, including growth factors and immunoglobulins. Growth factors are present in breast milk including nerve growth factor (NGF), epidermal growth factor (EGF), and transforming growth factor-α (TGF-α) (9). Some factors are important in the growth and differentiation of the intestine and must therefore interact with enterocytes directly, whereas other factors are involved in the development of organ systems outside the intestine, in which case they must not only be taken up by the intestine but must also be transported into the circulation. In the former, the intestine is the target organ, whereas in the latter it acts as a conduit. This is achieved in many cases by binding of luminal factors to specific receptors that can shuttle them across the intestine without intracellular hydrolysis (Fig. 1). Receptors are also involved in the transport of immunoglobulins across the intestine. Since endogenous immunoglobulin G (IgG) concentrations are below protective levels in young infants, protection depends on passive transfer of maternal antibodies. In many animals, this is achieved by receptor-mediated transport across the intestine (10,11).

Surveillance of antigens in the gastrointestinal tract involves their uptake by the intestinal mucosa. Lymphoid elements juxtaposed to the intestinal epithelial surface and in specialized aggregates or follicles (Peyer's patches) constitute the mucosal

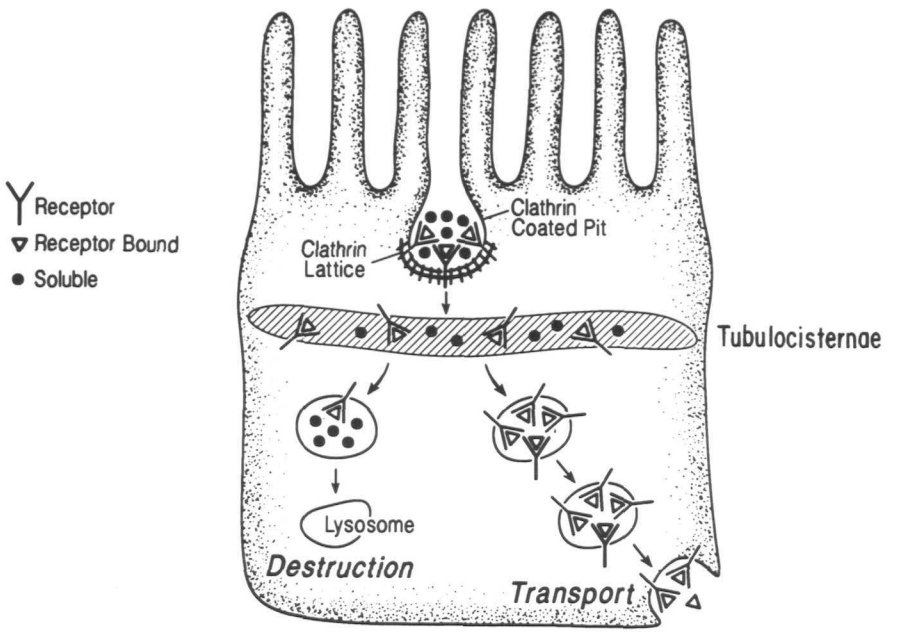

FIG. 1. Macromolecular endocytosis in enterocytes. Plasma membrane between microvilli invaginates to form vesicles. Clathrin, a protein that forms a membrane lattice, controls the curvature of the membrane. Macromolecules can enter the vesicle bound to surrounding membrane by their own receptors or by nonspecific attraction; they can also enter free in solution. After entry, they move to the tubulocisternae, where they are sorted and pass either to vesicles that travel toward the lysosome or to vesicles that transverse the cell to the basolateral pole. Membrane-bound molecules are more likely than those in solution to traverse the cell. (Reproduced with permission from Sanderson IR, Walker WA. *Gastroenterology* 1993; 104: 622–39.)

immune system. A normal immune response depends on B cell recognition of antigen; thus intraluminal and enterocyte destruction of antigenic structure will reduce its antigenicity. The immune response also depends on T cell recognition of peptides that are processed from antigen. Both types of lymphocytes are located adjacent to the intestinal epithelium. The immune responses of the normal mucosa are varied. The mucosal immune system is capable of producing secretory IgA (S-IgA) specific to antigens in the lumen. It is also able to inhibit responsiveness by the systemic immune system (tolerance) to orally ingested antigens. Oral tolerance has been shown in a range of animal species (12,13). It is possible that this phenomenon plays a role in preventing food allergy and autoimmune states (14–16). Many diseases are related to the ingestion of food antigens. Some are quick reactions usually mediated by an IgE response such as urticaria, vomiting, and, most severely, systemic anaphylaxis (17). Food-sensitive enteropathies tend to be slow to develop and are related to cell-mediated immune responses (17). Such responses are normally prevented by means of oral tolerance, although the mechanism of this response is still incompletely understood. It is clear, however, that antigen must cross the epithelium in order for the

mucosal immune system to function normally in limiting infectious disease and preventing food allergy. Intact antigen is capable of crossing the epithelium through specialized epithelial cells [microfold cells (M cells); follicular epithelial cells], which have characteristics that make them effective in transporting macromolecules. Whether this is the only physiologic pathway of macromolecular entry that does not involve specific membrane receptors is not yet clear. It is possible, however, that some antigens are absorbed by enterocytes not associated with lymphoid follicles and presented to T cells, leading to alterations in the immune response of the intestine. Although there is no *in vivo* evidence yet that enterocytes can present antigen to T cells, experiments with epithelial cells isolated from the intestine (18–20) indicate that T cells do recognize antigen that has been taken up and processed by enterocytes.

Receptor-Mediated Uptake of Growth Factors

The growth and differentiation of the small intestine depend on exposure to an array of growth factors that have complementary actions on intestinal epithelial cells. Some factors are synthesized by enterocytes themselves (autocrine), some are delivered from the circulation, and some enter the epithelium directly from the gastrointestinal tract. Epidermal growth factor (EGF) (3,5), found in human milk and saliva, can cross the intestinal epithelium. EGF is a peptide consisting of 53 amino acids that has trophic effects on both adult and neonatal intestine.

Macromolecules are transferred by a mechanism that is altogether different from those that transport nutrients such as glucose and amino acids. Nutrient molecules enter the intestinal cell cytoplasm at the apical membrane and exit via the basolateral membrane. Growth factor macromolecules, on the other hand, transverse the cell (Fig. 1) in membrane-bound compartments that invaginate from the apical membrane (endocytosis). The first step in this process is attachment to receptors on the apical surface of enterocytes. Studies of EGF binding to microvillous membranes and isolated enterocytes show that intestinal cells have receptors that are specific for EGF. Other growth factors may use the EGF receptor (i.e., TGF-α) or use their own receptor (i.e., IGF-1).

The mechanisms involved in the transit of membrane-bound ligands from the apical to the basolateral surface of the enterocyte are still poorly understood (21). In electron microscopic studies, the apical membrane of absorptive cells can be seen invaginating to form endosomes (Fig. 1). Further transit into the cell may occur by the movement of separated vesicles.

Antibody Uptake

The newborn makes very little immunoglobulin and most circulating antibody is IgG-derived passively from the mother. For the most part, in humans IgG is transferred by the placenta during late gestation, whereas in many animals the transfer occurs from maternal milk through the proximal small intestine. The transfer of IgG

across the gut is mediated by receptors that bind to the Fc portion of the immunoglobulin molecule (22).

Fc receptors are able to cross the epithelial cells. This transcytosis occurs in both directions. Receptors are carried in membranes that traffic from lumen to serosa and return by other membrane transport mechanisms. Some membrane proteins contain specific amino acid sequences that direct the protein within epithelial cells, that is, the polymeric IgA receptor (23) that transports the IgA from the basolateral membrane to the apical membrane. However, the amino acid sequences that determine the movement of the apical IgG Fc receptor, have not yet been elucidated. Transfer of maternal IgG in the neonatal rodent falls markedly at 21 days of age, that is, at weaning (a phenomenon known as closure). This phenomenon is now known to be due to the decrease in the expression of the Fc receptor gene, and it is likely that factors in breast milk may affect Fc receptor gene expression.

Nonreceptor Transport

Cells Specialized for Macromolecular Transport, Membranous Epithelial Cells (M Cells)

The generation of secretory immune responses by the intestinal mucosa depends on transfer of antigens across the epithelium. Any loss of the molecular structure of the antibody recognition sites, the epitopes, on antigens during transport would render them unrecognizable by B cells. The passage of intact macromolecules across the gut is at variance with the role of the gut as a macromolecular barrier. In order for macromolecules to cross the gut in a controlled manner, specialized epithelial cells have evolved that overlay lymphoid follicles (Fig. 2). These M cells have few microvilli on their surface and correspondingly little of the glycocalyx that typifies enterocytes. There is also less mucus covering the cell surface. In addition, lysosomal enzymic activity within the cell is reduced. Thus, these components of normal barrier function are less well developed in M cells than in other epithelial cells. Furthermore, there is a deep invagination of their basal membrane into which cells of the immune system can intrude. This invagination is separated by only a narrow band of cytoplasm from the apical membrane. Thus lymphocytes and macrophages can position themselves close to the intestinal lumen.

Amerongen and colleagues (24) have reviewed the microorganisms and other macromolecules that are known to be transported by M cells (Table 1). M cells also transport luminal antigen from the gut and therefore represent the primary physiologic route for nonreceptor transport of macromolecules. This has been shown by electron microscopy using antigens including ferritin and horseradish peroxidase. Soluble macromolecules are incorporated into membrane-bound compartments, transferred across the cell, and extruded from the serosal surface into the interstitium containing lymphoid cells.

An important but as yet unanswered question is whether specific receptors exist

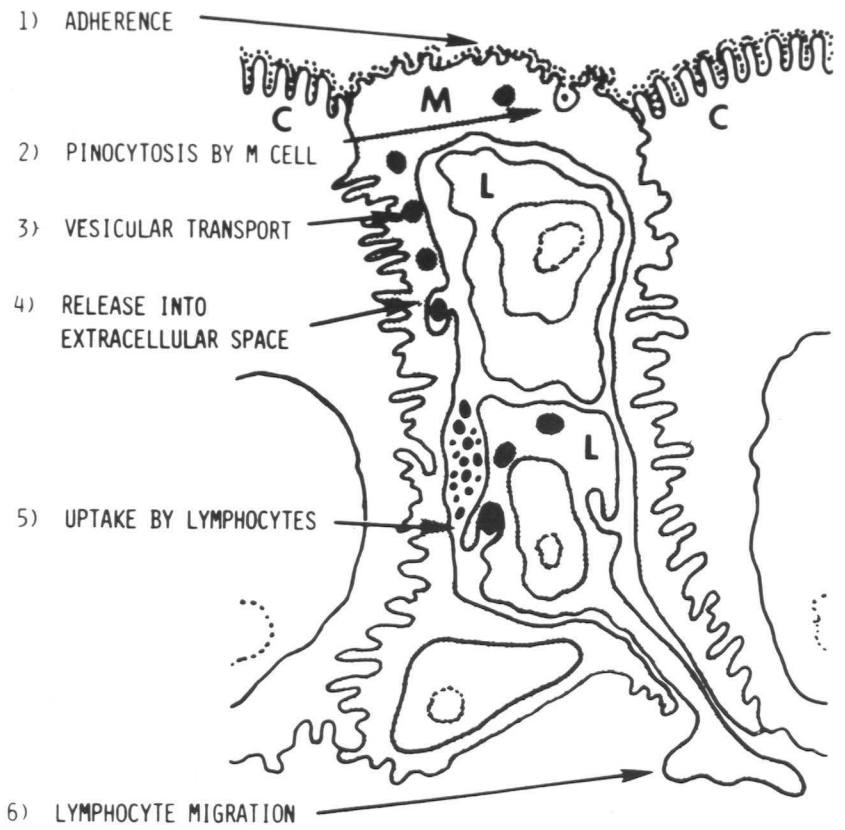

FIG. 2. M cell after adherence and endocytosis: macromolecules only need travel a short distance from the apical surface to the basal pole, where they are released close to immune cells that have migrated into the "pocket" at the basal surface of the M cells. (Reproduced with permission from Owen RL. *Gastroenterology* 1977; 72: 440–51.)

on the surface of M cells, which aid the transport of macromolecules across the epithelium. Some infectious agents, including reovirus (25) and *Escherichia coli* (strain RDEC-1) (26), bind selectively to M cells. The question of receptors is made more complex by the fact that different agents are taken up in different ways by M cells. For example, poliovirus (27) is taken into clathrin-coated pits by endocytosis, whereas reovirus is taken up in vesicles that do not contain clathrin (25).

Enterocytes as Antigen-Presenting Cells

Intact antigens or antigen fragments traverse the M cell and may encounter immunoglobulin in solution or on B cell surfaces as part of an immune response. Effective

TABLE 1. *Microorganisms and nonliving particles adherent to M cell apical membranes*

Bacteria	Viruses	Protozoa	Nonliving particles
Vibrio cholerae *Salmonella typhi* *Yersinia enterocolitica* Bacille Calmette–Guérin (BCG) *Campylobacter jejuni* *Shigella flexneri* RDEC-1 strain of *Escherichia coli*	Reovirus Poliovirus Human immunodeficiency virus-1 (HIV-1)	Cryptosporidium	Carbon particles Latex beads Copolymer microspheres Hydroxyapatite

From Amerongen MH, Weltzin RW, Mack JA, *et al. Ann N Y Acad Sci* 1992; 664: 18–26.

immune responses to antigenic proteins also require the help of T lymphocytes. Stimulation of T cells in turn depends on exogenous antigen being presented by antigen-presenting cells (APCs). The APCs must internalize, digest, and link a small fragment of the antigen to a surface glycoprotein—the major histocompatibility complex (MHC) class II or HLA-D in humans—that interacts with a T cell receptor. Various cells of the immune system can act as APCs, including B cells, macrophages, and dendritic cells. The ability of these cells to present exogenous antigen depends on the expression of MHC class II on their surface (28). Cytotoxic T lymphocytes also express the T cell receptor. These cells are part of the effector immune response. Their activation depends on MHC class I molecules (which most cell types express) but may also at times depend on MHC class II molecules.

MHC class II molecules are also present in the epithelia of normal small intestine, particularly on villous cells, in both humans and rodents. *In vitro* studies (18,19) have shown that isolated enterocytes from rat and human small intestine can present antigens to appropriately primed T cells. This raises the possibility that in the intestine MHC class II molecules might present peptides from cellular membrane compartments to cells of the immune system that are localized below the epithelium. In support of this concept, MHC class II molecules have been detected in adult rat jejunal villi in association with intracellular organelles (29). Class II molecules were never detected in microvillous brush border or vesicles at the base of microvilli. However, organelles below the terminal web and throughout the apical cytoplasm were stained specifically. Basolateral membranes clearly showed MHC class II molecules. These molecules are therefore in an ideal position for binding with polypeptides that may have been taken up and processed within the epithelial cell (Fig. 3). Antigen presentation by enterocytes may result in different immunologic responses from antigen presentation by lamina propria cells following transport by a different pathway. Antigen presentation by enterocytes *in vitro* results in CD8 stimulation rather than the CD4+ proliferation (19) produced by other APCs.

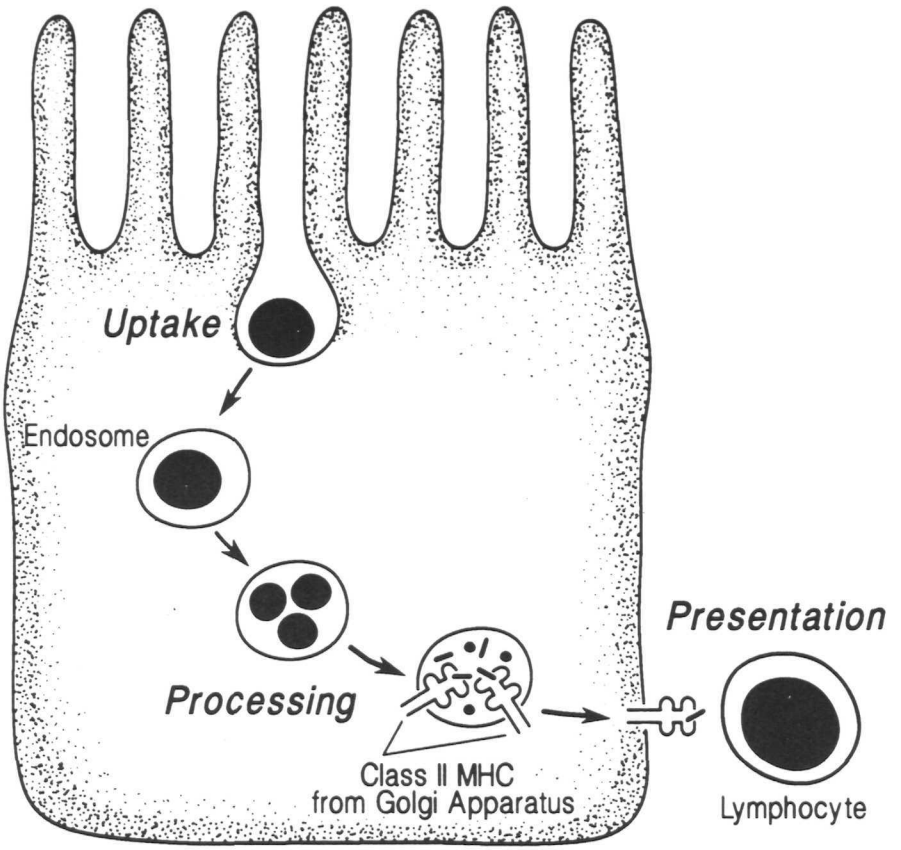

FIG. 3. Model of antigen presentation by enterocyte. Macromolecules can enter membrane-bound organelle of the enterocyte. Instead of binding to the surrounding membrane or being destroyed in lysosomes, antigen is processed within the endosomal component into fragments that can bind to MHC class II molecules on the inner membrane of the components. From there they are presented on the basolateral surface of the cell. (Drawing prepared with the help of Dr. L. Mayer.) (Reproduced with permission from Sanderson IR, Walker WA. *Gastroenterology* 1993; 104: 622–39.)

PATHOLOGIC TRANSPORT

Controlled uptake of macromolecules from the intestine is important in delivering growth factors and immunoglobulins to the circulation. It also enables the mucosal immune system to sample antigen in the lumen. Thus, physiologic transport is dependent on specific mechanisms that control macromolecular entry. However, if macromolecules are taken up nonspecifically, these regulatory mechanisms could be circumvented. In this case, antigens could cross the epithelium in excessive amounts. Such transport may well set up immune reactions that are not limited to the local

immune response. These reactions, in the face of unrestricted antigen entry, may become widespread and thus ultimately cause disease in the gastrointestinal tract or other organ systems.

Nonspecific transfer can occur by two pathways. First, vesicular traffic moving across the cell will transport molecules that have adhered to receptors on the surface membrane. Second, junctions between cells, which normally act as a barrier, could loosen and become leaky. These nonspecific pathways become more permeable when the intestine receives an insult or during its developmental stages, thus making chronic gastrointestinal disease more likely at these times.

Mucosal Barrier to Antigens

Antigens only gain access to the surface of the intestine after passing a number of mechanisms that act as a barrier (Fig. 4). This barrier consists of some components that are under immunological control and others that are nonspecific. Breakdown in any of these components could result in an increase in the nonspecific passage of antigen into the intestine. Thus the integrity of these mechanisms is necessary to prevent disease caused by excessive uptake of antigens. Antigen absorption is limited by a number of nonimmunological factors that operate in the gastrointestinal tract. These include gastric acidity, proteolytic digestion, mucus secretion, and peristalsis (Fig. 4). These mechanisms have been reviewed extensively and will not be described in detail (1,30).

Intracellular Transport

Direct evidence of nonspecific macromolecular transport through vesicular compartments of enterocytes has been demonstrated in ultrastructural studies of horseradish peroxidase (HRP) in mature intestine (31). Macromolecules can be taken up by the enterocyte without the involvement of specific receptors. This can occur in two ways. Molecules can bind to the apical membrane in a nonspecific manner and then be taken up by endocytosis; conversely, molecules in solution close to the invaginating membrane will be engulfed by the developing vesicle (Fig. 1). Macromolecules are more adherent to the surface of immature cells than to mature cells (32,33) and may adhere preferentially because of their structure or charge. This means that when the contents of the lumen have easy access to immature enterocytes, macromolecules will adhere readily. Immature cells are found on the surface of the intestine in the young and when the lifespan of the enterocyte is reduced, as happens in many enteropathies (Table 2), including viral gastroenteritis in which villous enterocytes are preferentially destroyed and replaced by increased numbers of immature crypt enterocytes.

Various macromolecules, including bovine serum albumin and HRP, are transported more readily in young animals. The passage of these macromolecules falls markedly with age and this is considered another form of "closure." Closure there-

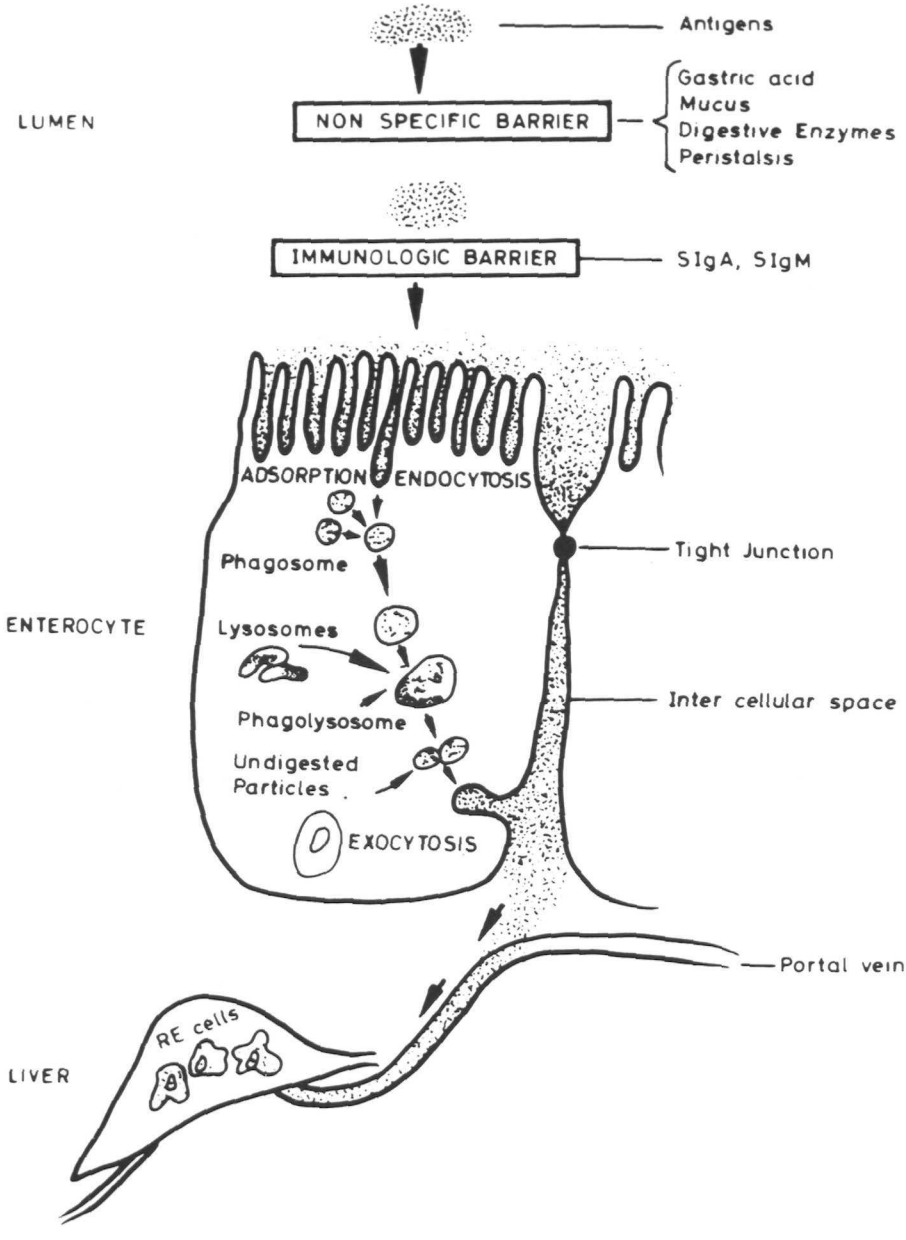

FIG. 4. Barriers to macromolecular absorption. Antigen entry is prevented by nonspecific and immunologic mechanisms in the gastrointestinal tract, as well as by the physical structure of the epithelium itself. (Reproduced with permission from Iyngkaran N, Yadav M. In: Marsh MN, ed. *Immunopathology of the small intestine.* Chichester: Wiley 1987; 415–49.)

TABLE 2. *Enteropathies where the ratio of immature to mature cells increases on the intestinal surface*

Celiac disease	Autoimmune enteropathy
Post-enteritis enteropathy	Radiation enteritis
Allergic enteropathy	

fore encompasses more than one mechanism. It was originally applied to the cessation of passage of immunoglobulins across the intestine, which is now known to be due to reduction in expression of the Fc receptor gene, but the term, closure also includes cessation of enhanced nonspecific transfer. A similar, but more subtle, decrease in the transport of antigens is seen in the human newborn (34,35). Formula-fed preterm neonates have higher serum concentrations of β-lactoglobulin than term neonates.

Paracellular Transport

Transepithelial transport can occur by the paracellular route as well as through cells. There has been a significant change in our perception of the importance of this route in recent years. It has been appreciated that water, sodium, potassium, and chloride can pass between cells, but there is now evidence that large solutes can, under certain circumstances, penetrate at this site. The rate-limiting barrier to diffusion is the tight junction, which in healthy intestine prevents passage of large macromolecules such as HRP. The structure of the tight junction (36) in freeze–fracture preparations consists of strands that pass between cells. The composition of these strands is not known, but they are likely to be proteins of high tensile strength. It is the number of strands that determines the ionic resistance of an epithelial monolayer. Pappenheimer and colleagues (37–39) have calculated that the rate of uptake from the lumen of molecules smaller than 5500 daltons was proportional to the rate of fluid absorption—a concept known as solvent drag. This gives an effective pore size of 5 nm at the tight junction (36). Sodium-dependent solute, such as glucose and amino acids, induces expansion of intercellular spaces associated with condensation of microfilaments of the actinomycin ring associated with the tight junction. While these observations have enormous importance on the physiology of absorption of nutrients, their impact on our understanding of macromolecular transport has yet to be fully assessed. The calculated pore radius of the open tight junction (5 nm) is similar to that of small macromolecules: glucose–sodium transport will in fact allow the passage of polypeptides 11 amino acids long (MP-1) (40), but larger immunogenic proteins may not pass through this route under physiologic conditions. HRP, for example, does not pass the tight junctions (40) even when they have been rendered permeable to MP-1.

On the other hand, *pathologic* insults to the intestine may open these pores sufficiently to allow passage of antigens. The permeability of the gut to macromolecules in disease models needs to be reexamined using Pappenheimer's methodology. Macromolecular markers of different sizes, charge and hydrophilicity have all been used

TABLE 3. *Insults that increase macromolecular permeability of the intestine*

Intestinal disorders	Systemic insults	Drugs
Gastrointestinal food allergy	Excessive radiation	Nonsteroidal anti-inflammatory drugs
Celiac disease	Extensive burns	
Acute gastroenteritis	Septicemic shock	
Chronic intestinal infections	Hypovolemic shock	
Inflammatory bowel disease	Malnutrition	
Surgery		

independently *in vivo* in both animals and humans (see below), but the physical characteristics of these molecules have not been used to predict pore size in disease.

There is no doubt that uptake of antigens is increased in a number of diseases of the small intestine (Table 3).

CONCLUSION

There remain many questions to be answered in the study of macromolecule transport. Macromolecules may penetrate the mucosal barrier by different pathways, but we do not know the relevant importance of those pathways in immune surveillance or in the initiation of gastrointestinal disease. In some circumstances, macromolecule passage increases immune activity (as in the initiation of allergic disease); in other circumstances, oral antigens suppress immune reactions that are already underway. Do these different responses to oral antigen represent different pathways of transport?

Finally, we need to determine whether antigen uptake is always essential for immune surveillance. Can luminal macromolecules be recognized without their penetrating the epithelium? If MHC class II molecules on the epithelium present processed luminal peptides on their surfaces without releasing them, then this is indeed a possibility. Can antigens be recognized within the lumen of the intestine? There is no evidence for this yet, but two observations suggest that uptake may not always be essential. First, lymphocytes can enter the lumen of the intestine from Peyer's patches (41) and could conceivably function as part of the afferent arm of the immune response. Second, the apical surfaces of mucosal epithelial cells express a number of molecules belonging to the immunoglobulin superfamily. At present, their function is unknown, but they have been identified because they form the attachments for invading viruses. One member of the immunoglobulin superfamily (ICAM-1) allows rhinovirus attachment in nasal epithelium; a poliovirus receptor in intestinal epithelium is also a member of the immunoglobulin superfamily. Since the primary function of these molecules on mucosal surface cannot be to serve as a conduit for viral infection, it is tempting to suggest that they and other similar molecules might play a role in the immunosurveillance of macromolecules in the gastrointestinal tract. If macromolecules are recognized within the gastrointestinal tract, is this information

correlated with information garnered from the previous penetration of similar macromolecules? At present, we can only speculate on questions such as these, but future research in this area will bring fascinating insights.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Institutes of Health (PO1-DK33506, RO1-DK37521, RO1-HD12437). Dr. Sanderson is the recipient of a Fulbright Scholarship and a grant from the Crohn's and Colitis Foundation of America.

REFERENCES

1. Sanderson IR, Walker WA. Uptake and transport of macromolecules by the intestine: possible role in clinical disorders (an update). *Gastroenterology* 1993; 104: 622–39.
2. Juvonen P, Jakobsson I, Lindberg T. Macromolecular absorption and cow's milk allergy. *Arch Dis Child* 1991; 66: 300–3.
3. Weaver LT, Walker WA. Epidermal growth factor and the developing human gut. *Gastroenterology* 1988; 94: 845–7.
4. Koldovsky O, Thornburg W. Hormones in milk. *J Pediatr Gastroenterol Nutr* 1987; 6: 172–96.
5. Carpenter G, Wahl MI. The epidermal growth factor family. In: Sporn MB, Roberts AB, eds. *Peptide growth factors and their receptors, I.* New York: Springer-Verlag, 1991; 69–171.
6. Mostov KE, Simister NE. Transcytosis. *Cell* 1985; 42: 389–90.
7. Neutra MR, Wilson JM, Weltzin RA, Kraehenbuhl JP. Membrane domains and macromolecular transport in intestinal epithelial cells. *Am Rev Respir Dis* 1988; 138: S10–16.
8. Targan SR, Kagnoff MF, Brogan MD, Shananhan F. Immunologic mechanisms in intestinal disease. *Ann Intern Med* 1987; 106: 853–70.
9. Koldovsky O, Britton J, Davis D, *et al.* The developing gastrointestinal tract and milk-borne epidermal growth factor. In: Mestecky J, *et al.*, eds. *Immunology of milk and the neonate.* New York: Plenum Press, 1991; 99–106.
10. Rodewald R, Kraehenbuhl JP. Receptor-mediated transport of IgG. *J Cell Biol* 1984; 99: 159–64s.
11. Simister NE, Rees AR. Isolation and characterization of an Fc receptor from neonatal rat small intestine. *Eur J Immunol* 1985; 15: 733–8.
12. Peng J-H, Turner MW, Strobel S. The generation of a tolerogen after the ingestion of ovalbumin is time-dependent and unrelated to serum levels of immunoreactive antigen. *Clin Exp Immunol* 1990; 81: 510–15.
13. Strobel S. Immunologically mediated damage to the intestinal mucosa. *Acta Paediatr Scand Suppl* 1990; 365: 46–57.
14. Higgins PR, Weiner HL. Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein and its fragments. *J Immunol* 1988; 140: 440–5.
15. Miller A, Lider O, Weiner HL. Antigen-driven bystander suppression after oral administration of antigen. *J Exp Med* 1991; 174: 791–8.
16. Nagler-Anderson C, Bober LA, Robinson ME, Siskind GW, Thorbeck GJ. Suppression of type II collagen-induced arthritis by intragastric administration of soluble type II collagen. *Proc Natl Acad Sci USA* 1986; 83: 7443–6.
17. Walker-Smith JA. Dietary protein intolerance. In: Walker-Smith JA, ed. *Diseases of the small intestine in childhood.* London: Butterworth, 1988; 144–84.
18. Bland PW, Warren LG. Antigen presentation by epithelial cells of the rat small intestine. I. Kinetics, antigen specificity and blocking by anti-Ia antisera. *Immunology* 1986; 58: 1–8.
19. Mayer L, Shlien R. Evidence for function of Ia molecules on gut epithelial cells in man. *J Exp Med* 1987; 166: 1471–83.
20. Kaiserlian D, Vidal K, Revillard J-P. Murine enterocytes can present soluble antigen to a specific class II-restricted $CD4^+$ T cells. *Eur J Immunol* 1989; 19: 1513–6.

21. Simons K, Wandinger-Ness A. Polarized sorting in epithelia. *Cell* 1990; 62: 207–10.
22. Simister N, Mostov KE. An Fc receptor structurally related to MHC class I antigens. *Nature* 1989; 333: 184–7.
23. Casanova JE, Apodaca G, Mostov KE. An autonomous signal for basolateral sorting in the cytoplasmic domain of the polymeric immunoglobulin receptor. *Cell* 1991; 66: 65–75.
24. Amerongen MH, Weltzin RW, Mack JA, *et al.* M-cell mediated antigen transport and monoclonal IgA antibodies for mucosal immune protection. *Ann N Y Acad Sci* 1992; 664: 18–26.
25. Wolf JL, Rubin DH, Finberg R, *et al.* Intestinal M cells: a pathway for entry of reovirus into the host. *Science* 1981; 212: 471–2.
26. Inman LR, Cantey JR. Specific adherence of *Escherichia coli* (strain RDEC-1) to membranous (M) cells of the Peyer's patch in *Escherichia coli* diarrhea in the rabbit. *J Clin Invest* 1983; 71: 1–8.
27. Sicinski P, Rowinski J, Wasrchol JB, *et al.* Poliovirus type 1 enters the human host through intestinal M cells. *Gastroenterology* 1990; 98: 56–8.
28. Unanue ER. Antigen presenting function of the macrophage. *Annu Rev Immunol* 1984; 2: 395–428.
29. Mayrhofer G, Spargo LDJ. Distribution of class II major histocompatibility antigens in enterocytes of the rat jejunum and their association with organelles of the endocytic pathway. *Immunology* 1990; 70: 11–9.
30. Walker WA. Antigen handling by the small intestine. *Clin Gastroenterol* 1986; 15: 1–20.
31. Cornell R, Walker WA, Isselbacher KJ. Small intestinal absorption of horseradish peroxidase. A cytochemical study. *Lab Invest* 1971; 25: 42–8.
32. Pang KY, Bresson JL, Walker WA. Development of the gastrointestinal mucosal barrier: evidence for structural differences in microvillus membranes from newborn and adult rabbits. *Biochim Biophys Acta* 1983; 727: 201–8.
33. Stern M, Pang KY, Walker WA. Food proteins and gut mucosal barrier. II. Differential interaction of cow's milk proteins with the mucous coat and the surface membrane of adult and immature rat jejunum. *Pediatr Res* 1984; 18: 1252–7.
34. Roberton DM, Paganelli R, Dinwiddie R, Levinsky RJ. Milk antigen absorption in the preterm and term neonate. *Arch Dis Child* 1982; 57: 369–72.
35. Axelsson I, Jakobsson I, Lindberg T, Poleberger S, Benediktsson B, Raiha ON. Macromolecular absorption in preterm and term infants. *Acta Paediatr Scand* 1989; 78: 532–7.
36. Madara JL. Pathobiology of the intestinal epithelial barrier. *Am J Pathol* 1990; 137: 1273–81.
37. Pappenheimer JR, Reiss KZ. Contribution of solvent drag through intercellular junctions to absorption of nutrient by the small intestine of the rat. *J Membr Biol* 1987; 100: 123–36.
38. Pappenheimer JR. Physiological regulation of transepithelial impedance in the intestinal mucosal of rat and hamster. *J Membr Biol* 1987; 100: 137–48.
39. Madara JL, Pappenheimer JR. Structural basis for physiological regulation of paracellular pathways in intestinal epithelia. *J Membr Biol* 1987; 100: 149–64.
40. Atisook K, Madara JL. An oligopeptide permeates intestinal tight junctions at glucose-elicited dilatations. Implications for oligopeptide absorption. *Gastroenterology* 1991; 100: 719–24.
41. Owen RL, Bhalla DK, Appel RT. Migration of Peyer's patch lymphocytes through intact M cells into the intestinal lumen in mice, rats, and rabbits (Abstract). *J Cell Biol* 1982; 95: 332a.
42. Owen RL. Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: an ultrastructural study. *Gastroenterolgy* 1977; 72: 440–51.
43. Jyngkaran N, Yadav M. In: Marsh MN, ed. *Immunopathology of the small intestine.* Chichester: Wiley 1987; 415–49.

DISCUSSION

Dr. El Gamal: Your talk again confirms the danger of increasing the incidence of atopy with the early administration of food, before the age of 6 months, both in preterm babies with immature gut and in normal babies where there is a positive family history of atopy. Atopy is a dominantly transmitted disorder related to a gene on chromosome 11. Do you think that the excessive antigen uptake related to the M cells determines this genetic predisposition?

Dr. Walker: I don't think we know enough about M cells to say this. There is controversy

as to whether or not soluble or particulate antigens, particularly microorganisms, bind preferentially to M cells as opposed to just having easier access in the absence of a mucous coat.

Dr. Chapoy: It is believed that at about 3 months of age the phenomenon of endocytosis is switched off or shut down. Is this due to hormonal changes or to other mechanisms?

Dr. Walker: There are those who would disagree with the statement that the endocytotic process shuts down at 3 months of age. They feel that it tends to shut down much earlier in the human, perhaps in late gestation, and that endocytosis is probably more active in premature than in full-term infants. We can assume that 3 months postpartum is the time when endocytosis changes, without much evidence other than what I presented to you, and that factors in breast milk can facilitate closure. However, we still have a lot of work to do. To answer these questions, we must develop more comprehensive cell and molecular techniques to study maturation events in a cell culture system with a normal human cell line, because it is very difficult for us to biopsy normal children during the first few months of life to enable us to look specifically at endocytosis.

Dr. Chapoy: Would you advise using steroids to mature the gut to prevent uptake of antigen?

Dr. Walker: It is difficult to apply basic observations to clinical applications. Most of the clinical questions have not yet been investigated by full trials. I believe that steroids are likely to be helpful in necrotizing enterocolitis, for example, because there is good clinical evidence to support this statement. I don't know of any good clinical evidence to show that either prenatal or postnatal steroids would affect allergen expression other than by turning off allergic responses by anti-inflammatory means.

Dr. Chandra: Is the increased uptake of antigens in low birthweight infants a function of gestational age, of or weight? And, more specifically, would you see the same phenomenon in small-for-gestational age low birthweight babies as you see in preterm infants?

Dr. Walker: It is a combination. If you have a preterm infant who is also small-for-gestational age, you are likely to get a greater uptake of antigen because malnutrition and lack of enteric stimulation will also contribute to the immaturity of the intestine.

Dr. Brandtzaeg: You placed a lot of emphasis on the M cells but you did not distinguish between soluble and particulate antigens. How much do you actually know about uptake of soluble antigens by the M cells? I think we know very little.

Dr. Walker: Generally, what we know about M cells has to do with microorganisms. Very little is known about specific molecules and we don't know if the M cell route is the preferred one. I spoke about M cells because half of my assignment was to discuss M cell transport. If this appeared to result in overemphasis, it was unintentional. I don't know the specific role of M cells in soluble antigen-induced intestinal allergies.

Dr. Brandtzaeg: My second question relates to the Fc receptor, which you referred to on the M cells. Marian Neutra's group in Boston has evidence (1) that this Fc receptor is not specific for any particular type or class of immunoglobulin but is a general receptor. Do you have any information on attempts to clone and identify this receptor?

Dr. Walker: I think Dr. Neutra showed that both Fab and Fc immunoglobulin fragments can bind to the surface of the M cell. One point to be emphasized is that the M cell is different from the enterocyte in that it seems not to express poly-A receptor and it therefore does not transmit IgA. Except for a recent article by Jerry Trier (2), I don't know of anyone who has been able to show class II (HLA) molecules on the M cell.

Dr. Husby: Marian Neutra also developed a beautiful model showing the importance of IgA antibodies in the regulation of the uptake. Do you have any further comments on whether

that regulation is nonspecific, for example, due to coating by bile and secretions, or is it specifically related to the M cell?

Dr. Walker: Dr. Neutra has done a lot of studies. You may be referring to some of her earlier work where she was not doing physiologic experiments because she was using monomeric and not dimeric IgA. This is a nonphysiologic situation. However, she was able to show in that study that IgA antibodies did affect antigen uptake. I do not know of any specific receptor on the M cell allowing antibodies either to attach or to be taken up, other than what we just discussed.

Dr. Schmitz: I would like to discuss the data you presented about the effect of food on triggering HLA on the enterocyte. It seems that if mice have been fed a laboratory diet, this triggering is expressed to a much greater degree than if they are suckled. For how long is it possible to modulate this expression of HLA molecules in the mouse life span and do you know of a similar phenomenon in human babies?

Dr. Walker: The mice were followed on the elemental diet for 40 days, which is more than twice the natural weaning period, and there was no expression up to that point (3). So it appears that an elemental diet may stimulate the intestine in a different way from a complex diet with respect to atopic factors. I don't know of any studies that have been done in humans.

Dr. Schmitz: We have observed in some patients with very early weaning, who were put onto intravenous feeding, that HLA expression was very low compared to what would be expected in a normal baby.

Dr. Walker: They probably had very little enteric stimulus and that may be an important factor.

Dr. Sampson: I have always been impressed with how rapidly a food allergic reaction can develop. I have also been intrigued by Lauser's studies looking at large populations of normal patients who were passively sensitized. In those studies, Lauser clearly showed that in normal individuals with sufficient antigen absorption a passively sensitized site could be activated in as little as 20 minutes. How effective is the barrier? It seems as though there is rapid penetration of small amounts of antigen across this barrier in normal individuals as well as in allergic individuals, certainly enough to activate an immune response.

Dr. Walker: You are right, it is an extremely rapid response. There is a spectrum of reaction to antigenic stimulus. The normal individuals to whom you refer, are likely to be those with a high degree of anaphylaxis, probably IgE mediated. In such circumstances, antigen may be absorbed from the stomach rather than the small intestine. In allergic patients, it is a different situation. Here, we have small quantities of antigen rapidly stimulating cells and the barrier function is immaterial. In other types of allergic reaction, which are more delayed and may not be IgE-mediated, the barrier function may indeed be important.

Dr. Sampson: But Lauser showed equally rapid penetration of allergens when he passively sensitized either normal children or normal adults.

Dr. Walker: I was trying to say that if you have an extraordinary allergic response—if the individual is highly atopic—sufficient antigen gets across in minute quantities to trigger the response and the barrier really has very little effect on that.

Dr. Bock: How much antigen may be absorbed in the stomach? How much mediator can the mast cells in the stomach release, and how much of that mediator can get absorbed compared to the amount of allergen that has to be absorbed? Do you think that all the allergen is going straight through the gastric mucosa?

Dr. Walker: All I can do is speculate. Because the response occurs so quickly, the antigen may be absorbed across the buccal mucosa or anywhere along the gastrointestinal tract. However, if you look at gastric emptying, it is unlikely that there is enough time for allergens

to get into the small intestine in these individuals, so absorption has to take place somewhere before that. The stomach is a potential site because there is ever increasing evidence that the stomach may be an extremely active immunologic organ system. Also, the buccal mucosa consists of stratified epithelium, which is not geared for absorption, so I doubt whether antigen would normally be taken up from it unless there was some underlying disruption of the surface.

Dr. Bock: Has anyone looked at the absorption of macromolecules through inflamed mucosa?

Dr. Walker: Any inflammation tends to disrupt the mucosal surface and can open up spaces between cells and cause migration of cells across the denuded area. This is likely to increase the possibility of absorption, but I don't know if anyone has specifically looked at that.

Dr. Duchateau: You have shown important data about the intestinal uptake of β-lactoglobulin and bovine serum albumin. β-Lactoglobulin is a very special protein that belongs to a protein family termed lipocalins. The corresponding member of this protein family in humans is retinol binding protein, and it has already been demonstrated that bovine β-lactoglobulin can increase the transport or uptake of vitamin A via different routes in the intestinal mucosa in various animal models. I think it is likely that β-lactoglobulin may cross the intestinal mucosa through a specific receptor system, which is normally used by other molecules from breast milk. Thus this protein of bovine origin finds its way into the body by an abnormal route and this enables it to become a good antigen.

Dr. Guesry: From what you have observed in immature animals and humans, would you recommend that when a premature baby's own mother's breast milk is not available the requisite infant formula should be made of hydrolyzed protein or of intact protein?

Dr. Walker: In a general sense hydrolyzed protein is not necessarily appropriate. What I was showing you was only the pathophysiology of antigen handling by the neonate. I did not mean to infer that all neonates are potentially susceptible to allergic reactions. However, if you are dealing with infants who are susceptible, that is, with an allergic history or with increased IgE level in the cord blood, then I think it probably is appropriate.

Dr. de Weck: You have indicated that local anaphylactic reactions increase the uptake of antigens and this is definitely also true for a number of nonspecific inflammatory conditions. In the early 1980s, a number of people tried to use this phenomenon, as manifested by increased protein or marker uptake, for diagnostic purposes, for example, to monitor disease progress. These efforts never seem to have gone into routine diagnostic use. Do you have any suggestion about how they could be improved? Is there so much individual difference between people in this respect that such a test really cannot be used in a routine manner?

Dr. Walker: There are several things to take into account. One is that there has been a number of different molecules used to measure macromolecular transport—polyethylene glycol in various sizes, lactulose, and other substances have all been used. I don't think the molecules reflect what is happening with proteins. Another problem with uptake tests is that, though there may be a 10-fold or even a 100-fold increase in uptake, the substance is then diluted in a huge intravascular pool, and unless you have a highly sensitive assay, it is very hard to detect any increase in uptake. When we measure and manipulate things by opening the intestine—that is, injecting substances into a gut sac of an animal or removing cells and looking at transport—this is very different from what is going on *in vivo*.

Dr. Moneret-Vautrin: Could you give us further information about the nature of the receptors for food antigen on the epithelial surface? Are they specific for chemical structures of different families of proteins or do you think there are specific secretory IgA molecules that are linked to the surface?

Dr. Walker: I can only speculate. My personal view is that the phenomenon is nonspecific. Molecules with a positive ionic charge are attracted to the negatively charged surface of the intestine. I don't believe that this attraction is mediated by a specific receptor, nor do I think that it is modulated by IgA. From our own work I can say that the nature of terminal sugars, such as sialic acid, is an important determinant as to how ligands bind and how bacteria attach to the surface of the intestine. That same phenomenon may be true of how molecules, such as antigens, attach.

Dr. Olives: What are the factors that can delay or inhibit normal intestinal closure in humans?

Dr. Walker: Those factors in breast milk that are known to cause maturation of epithelium may positively cause premature closure to occur. Situations that affect the gut, for example, viral gastroenteritis, tend preferentially to destroy differentiated villous cells and produce compensatory crypt-type aplasia, but the end result is a transiently "immature" surface area. This tends to reopen the epithelial barrier because of endocytosis. Starvation can be a potential cause of alteration of maturation. Many of the factors that normally cause maturation in a full-term infant occur *in utero* and any insult to the intestine that affects the capacity of cells to turn over and differentiate can adversely affect closure.

Dr. Cézard: Most of the data presented today are related to the immature intestine. What information do we have about allergy occurring in older children or adults? Are different mechanisms involved?

Dr. Walker: It is very difficult to do human studies in this area because of ethical constraints. I think we can assume that a predisposition to allergy or a transient insult to the intestine will allow a greater transfer of antigens and this is likely to set off a reaction. Malnutrition has been shown to result in an increase in antigen uptake. In infants and children in developing countries who are malnourished and/or parasitically infected, causing an IgE response, the chronic diarrheal state that is so common in these conditions might be due to ongoing allergen bombardment. This needs to be studied in the human.

REFERENCES

1. Weltzin R, Lucia-Jandris P, Michetti P, *et al.* Binding and transepithelial transport of immunoglobulins by intestinal M cells: demonstration using monoclonal IgA antibodies against enteric viral proteins. *J Cell Biol* 1989; 108: 1673–85.
2. Allan CH, Mendrick DL, Trier JS. Rat intestinal M cells contain endosomal–lysosomal compartments and express class II major histocompatibility complex determinants. *Gastroenterology* 1993; 104(3): 698–708.
3. Sanderson IR, Ouellette AJ, Carter EA, Harmatz PR. Ontogeny of Ia messenger RNA in the mouse small intestinal epithelium is modulated by age of weaning and diet. *Gastroenterology* 1993; 105(4): 974–80.