Antigen Recognition and Processing by the Intestinal Mucosa: Immune Consequences of Eating

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The gastrointestinal tract is uniquely adapted to facilitate the digestion and absorption of essential nutrients, the diffusion of water, and the prevention of penetration by potential pathogens. These diverse functions are accomplished by intraluminal enzymes and antibodies as well as by the presence of a functional barrier at the intestinal epithelial surface. When pathogens or antigenic macromolecules penetrate the epithelial barrier, a mucosal immune response may ensue. The protective mechanisms by which the mucosal immune system responds may also lead to intestinal injury and systemic disease. In this chapter, we shall briefly discuss aspects of intestinal antigen processing that are both protective and potentially pathogenic in intestinal disease.

THE SPECIALIZED NATURE OF THE MUCOSAL IMMUNE SYSTEM

The gut-associated lymphoid tissue (GALT) is composed of organized lymphoid structures within the intestinal mucosa (Peyer’s patches), mesenteric lymph nodes, and lymphocytes diffusely scattered in the epithelium and lamina propria. The intraepithelial lymphocytes are situated at the basolateral aspect of the intestinal epithelial cells and are adjacent to the basement membrane (Fig. 1). Phenotypically, these lymphocytes are predominantly CD45RO⁺,CD8⁺ T cells, which indicates that they have been activated by previous exposure to antigen and recognize antigen in association with major histocompatibility complex (MHC) class I or MHC class I-like molecules (1,2). While lymphocytes bearing the γδ T-cell receptor (TCR) are preferentially found in the human mucosal epithelium, they are a relatively small population compared to the intraepithelial lymphocytes expressing the αβ T-cell receptor (3). The intraepithelial lymphocytes also have an oligoclonal T-cell receptor repertoire of variable-region gene segment usage compared to peripheral blood lymphocytes, suggesting that intraepithelial lymphocytes recognize a limited array of antigens (4–7). Recent evidence indicates that the oligoclonality of intraepithelial lymphocytes
FIG. 1. Schematic representation of the small intestinal mucosa. The absorptive cells are polarized, columnar epithelial cells anchored to a basement membrane and to each other by a junctional complex at the apical surface. Intraepithelial lymphocytes are T cells located on or above the basement membrane and the basolateral surface of the epithelial cells. The interstitial space between the basement membrane and the underlying muscularis mucosa contains a large population of immunoglobulin-containing plasma cells and T lymphocytes.

represents a large expansion of a relatively small number of dominant clones that populate the entire length of the intestinal mucosa and are probably determined by environmental factors such as diet or colonization with gut flora (8,9). In contrast to the intraepithelial lymphocytes, lymphocytes that predominate in the lamina propria are of B-cell origin. Approximately 60% of the T cells are CD4+, which recognize antigen in association with MHC class II molecules. Oligoclonal expansion of lamina propria lymphocytes also occurs with overlapping but distinct clones compared to the intraepithelial lymphocytes (7). The anatomic and phenotypic compartmentalization of mucosal lymphocytes suggest that there are functional differences in these mucosal lymphocyte populations.

Scattered within the domed epithelium overlying Peyer's patches are specialized epithelial cells (M cells) that facilitate the uptake and processing of luminal antigens for presentation to the underlying macrophages and T lymphocytes. Most of the antigenic uptake by M cells is by receptor-mediated endocytosis. After antigenic stimulation, both T and B cells in the Peyer's patches migrate to mesenteric lymph nodes, proliferate, and enter the systemic circulation through the thoracic duct. From the systemic circulation, these antigen-specific activated lymphocytes preferentially home to the intestinal mucosa at sites distant to the original exposure to antigen
The preferential homing of these lymphocytes to mucosal sites is accomplished by the expression of tissue-specific adhesion molecules on their cell surface which recognize ligands on specialized endothelial cells. After migrating from the vascular space, additional adhesion molecules facilitate the anchoring of lymphocytes to adjacent cells or cellular matrix proteins. Thus, mature lymphocytes are poised to respond to reexposure of antigen by antibody secretion from plasma cells or a cell-mediated response by T cells.

**ANTIGEN UPTAKE MAY OCCUR AT MUCOSAL SITES OTHER THAN PEYER'S PATCHES**

The primary function of the intestinal villi is absorption of essential nutrients and water. However, antigens may also gain access to the internal environment of the mucosa by several routes. Macromolecules may enter the lamina propria at the extrusion zone at the tip of the villus where the epithelial cells are shed into the intestinal lumen. Nonselective uptake of antigen by the absorptive epithelial cells occurs by fluid phase endocytosis and is transported in smooth vesicles. Selective uptake occurs by receptor-mediated binding or adherence to glycoproteins, usually at the base of microvilli in clathrin-coated pits. The endocytosed coated vesicles are transported intracellularly and fuse with lysosomes. After degradation, the macromolecules are transported to the basolateral surface of the enterocyte and released into the intercellular space (below the tight junctions which are located near the apical surface). Antigens may also pass from the intestinal lumen through the junctional complex between epithelial cells directly into the intercellular space. The permeability of the junctional complex is greatly affected by cytokines such as Interferon (INFγ) and interleukin (IL)-4, which are produced by mucosal lymphocytes (12).

Intestinal epithelial cells constitutively express MHC class I and class II molecules, which are required antigen presenting elements for T-cell antigen recognition. *In vitro* studies have shown that intestinal epithelial cells are capable of presenting antigen to T cells, resulting in the preferential proliferation of a suppressor CD8+ T-cell population which can be blocked by antibodies against CD8 or MHC class II (13–15). These original experiments were done using T cells isolated from peripheral blood and thus may not reflect accurately events occurring in the intestinal mucosa. More recently, these studies have been extended to demonstrate that lamina propria lymphocytes proliferate in response to allogeneic intestinal epithelial cells but their proliferation is not blocked by antibodies against MHC class I or class II (16). These results suggest that mucosal lymphocytes may respond to a distinct restriction element present on intestinal epithelial cells.

CD1 molecules are a family of non-MHC-encoded, nonpolymorphic, β2-microglobulin-associated glycoproteins present on most antigen-presenting cells. CD1d is one of the protein products of the CD1 gene locus that has been identified on human intestinal epithelial cells (17). The potential significance of this finding was enhanced
when a cultured human intraepithelial lymphocyte line showed CD1-specific cytotoxicity of CD1a-d-transfected target cells (5). Although a clone of the intraepithelial lymphocyte line was specific for CD1c, which is not present on epithelial cells, the fact that this cell line responded to CD1d-transfected target cells suggests that CD1d may be an intestinal epithelial cell ligand recognized by intraepithelial lymphocytes in vivo.

The structural similarity of CD1 molecules to antigen presenting molecules encoded by the MHC genes has led to speculation that the CD1 family of proteins may also be capable of presenting antigen to lymphocytes. This concept was supported by the development of an αβ TCR+ CD4+, CD8+ T-cell line (DN1) that is reactive with *Mycobacterium tuberculosis* when processed by antigen presenting cells, and the T-cell response is restricted by CD1b (18,19). The antigen recognized by the DN1 T cells has now been shown to be mycolic acid, a lipid found in mycobacteria (20). These findings show that T cells can respond to nonprotein antigens and that CD1b functions as an antigen-presenting molecule. Since intraepithelial lymphocytes are oligoclonal and, therefore, have a limited repertoire of antigen recognition, and since the CD1 molecules are nonpolymorphic, it may be that intraepithelial lymphocytes respond to a limited array of microbial antigens presented by CD1d on intestinal epithelial cells.

**CELIAC DISEASE RESULTS FROM ORAL INGESTION OF A SPECIFIC DIETARY PROTEIN**

Celiac disease (gluten-sensitive enteropathy) is characterized by small intestinal mucosal injury resulting in malabsorption and weight loss. In children, a decrease in linear growth may occur as well. Histologically, there is subtotal villous atrophy, crypt hyperplasia, an increase in the number of intraepithelial lymphocytes, and a mixed lymphocyte infiltrate of the lamina propria. Although the intestinal epithelial cells are often abnormal, with a cuboidal appearance, mucosal ulceration does not occur. The mucosal injury results in susceptible individuals following the ingestion of a group of gliadin proteins present in wheat and, to a lesser extent, related proteins in other grains such as rye, barley, and possibly oats. Complete recovery of the mucosal injury occurs after these grains are eliminated from the diet. Gluten does not appear to be directly toxic to intestinal epithelial cells but initiates immune-mediated injury. Some evidence suggests that this may be an autoimmune disease because of its association with specific MHC HLA antigens, the presence of circulating autoantibodies, and other autoimmune diseases such as type 1 diabetes mellitus.

A genetic basis for celiac disease has long been suspected because of an increased prevalence among first-degree relatives and an association with the MHC class I antigen HLA-B8 and the MHC class II antigens HLA-DR3 and DR7. However, non-MHC genes must also be a factor because there is a higher concurrence rate of disease among monozygotic twins than among HLA-identical siblings. A potential functional relevance of the association between celiac disease and HLA-DQ2 has
recently been shown. Knut et al. propagated αβ⁺ TCR, CD4⁺, CD8⁻ T-cell lines as well as a panel of T-cell clones that were reactive with a peptic-tryptic digest of gluten, and the response was restricted to antigen presentation by the HLA-DQ(α1*0501, β1*0201) heterodimer (21). Because not all individuals who express this HLA-DQ heterodimer develop celiac disease, its presence does not ensure the development of disease. However, it is now clear that a T-cell-mediated immune response to dietary gluten can occur in the intestinal mucosa and the resulting activation of T cells and subsequent release of cytokines may have a role in the pathogenesis of celiac disease.

POSSIBLE CORRELATION BETWEEN EARLY INTRODUCTION OF DIETARY PROTEINS AND DEVELOPMENT OF AUTOIMMUNE DISEASES

Postnatally, the intestinal mucosa undergoes several maturational changes including glycoconjugate composition on epithelial cell surface proteins and increased capacity to exclude the absorption of intact macromolecules. Clinical studies on human infants have shown measurable quantities of bovine serum albumin (BSA) in the serum of premature infants fed the protein but not in older children fed an equivalent amount of the protein (22). Since the uptake of antigenically active macromolecules can reach the circulation, a systemic immune response may ensue. Thus, it was of particular interest when it was first suggested that the absence of breast-feeding or the early introduction of cow’s milk formula were potential predisposing factors in the development of insulin-dependent diabetes mellitus (IDDM) in genetically susceptible individuals (23). A subsequent meta-analysis of 20 selected reports indicated a modest but statistically significant association between the early introduction of cow’s milk protein and the development of IDDM in childhood (24). Continuing efforts to define the nature of this association have often focused on the presence of circulating antibodies against BSA or a specific 17-amino-acid BSA peptide that have been reported to be present at a higher frequency among patients with IDDM, first-degree relatives of IDDM patients, and individuals with other autoimmune diseases. The implication would be that oral tolerance has not been induced or has been broken, thus predisposing susceptible children to IDDM.

Although antibodies against insulin are often identified in patients with IDDM, the islet cell injury is likely to be a cell-mediated immune response. The chronic lymphocytic infiltrate in the pancreas of patients with IDDM, as well as in animal models, is primarily T cells. Adoptive transfer experiments of islet-reactive T-cell clones in nonobese diabetic (NOD) mice induce the onset of diabetes (25), and blocking antibodies against the T-cell costimulatory molecule CD28 and one of its ligands, B7-2, prevent the development of diabetes (26). Since IDDM is most likely to be a T-cell-mediated disease, the presence of anti-BSA antibodies in patients with IDDM may be an epiphenomenon and unrelated to the pathogenesis of disease. In fact, T cells isolated from the peripheral blood of patients with IDDM (new onset and established) do not respond to BSA or to the 17-amino-acid peptide of BSA in a cell-mediated proliferation assay, but some T cells may respond when insulin is used as
a control antigen (27). The lack of a cell-mediated response to BSA suggests that BSA antigens are not pathogenic in the development of IDDM. However, the presence of anti-BSA antibodies may reflect a defect in the induction of oral tolerance in susceptible individuals, thus predisposing them to autoimmune diseases.

Another consequence of an immune-mediated response to dietary proteins is allergic colitis in infants (reviewed in 28). The suspected antigens are from cow’s milk or soy proteins in proprietary formulas or may be present in human breast milk, presumably derived from the maternal diet. Evidence of rectal bleeding is usually observed in the first 3 months of life and resolves after removal of the inciting protein from the diet. The need for exclusion of particular proteins from the diet is only temporary, since children are usually tolerant of the protein by 1–2 years of age.

The immune response elicited by exposure to infant formula proteins may be an IgE-mediated immediate hypersensitivity (type I), an IgG immune complex reaction (type III), or a cell-mediated delayed hypersensitivity response (type IV). The type of immune response elicited is variable and probably depends on the nature of antigen presentation to the effector cells and the cytokines subsequently released. The most significant histologic feature of allergic colitis is a predominate eosinophilic infiltration of the lamina propria as well as eosinophils within the epithelium. Upon activation, usually by crosslinking cell surface immunoglobulins, eosinophils degranulate, releasing potent mediators of inflammation that affect recruitment of additional inflammatory cells, smooth muscle contraction, and intestinal permeability. The factors that affect susceptibility of infants to the development of allergic colitis remain unknown, but research has focused on intestinal macromolecular uptake and mechanisms of oral tolerance.

INDUCTION OF ORAL TOLERANCE IS MEDIATED BY IMMUNOLOGICAL EVENTS IN THE INTESTINAL MUCOSA

One of the distinguishing features of the mucosal immune system compared to the rest of the peripheral immune system is that, overall, it has a suppressor function. Because of the vast amount of antigenic material to which the intestinal mucosa is exposed, mechanisms exist which make the mucosal immune system “tolerant” of repeated exposure to some antigens. Induction of oral tolerance produces a state of systemic immunologic unresponsiveness by means of active suppression or clonal anergy (reviewed in 29). Experimental animal models have suggested that the dose of the orally fed antigen appears to be important in determining the mechanism of tolerance. Low dose of antigen leads to the generation of antigen-specific regulatory cells which, upon antigenic restimulation, secrete transforming growth factor β (TGFβ), IL-4, and IL-10. This cytokine profile is characteristic of Th2 cells, predominantly generated in the mucosa, and has a general downregulatory immunologic effect. High doses of oral antigen lead to anergy of the Th1 cells. The unresponsiveness of anergic cells can be overcome by exposure to exogenous IL-2. The role of costimulatory molecules in the activation of T cells is now becoming apparent. T cells exposed to antigen presented by an MHC molecule may become activated if a costimulatory
signal (such as the CD28/B7 receptor interaction) is also present. However, T cells recognizing antigen in context with MHC molecules, but in the absence of the second signal, do not respond and become anergic to subsequent antigen exposure (30).

Since oral tolerance is induced primarily by T cells secreting suppressive cytokines, especially TGFβ, the effect may be a nonspecific suppression of responses to antigens other than the initiating fed antigen. This "bystander suppression" has been the basis for studies investigating the induction of oral tolerance in the presence of autoimmune disease. Oral administration of myelin basic protein (MBP) or specific peptides of MBP suppress experimental autoimmune encephalomyelitis (EAE) in rodents. This effect is mediated by antigen-specific CD8+ cells secreting TGFβ or by clonal anergy apparently depending on the dose of the fed antigen (29,31,32). Recently, the effect of feeding antigen to induce tolerance has been examined in humans. Husby et al. subcutaneously immunized volunteers with keyhole limpet hemocyanin (KLH) following oral ingestion of KLH (33). The antigen-specific T-cell proliferation and delayed skin test responses were significantly less in the group fed antigen before immunization than in the group which did not receive oral antigen. However, the serum anti-KLH antibodies and anti-KLH IgA in mucosal secretions were higher in the KLH-fed group, indicating that antigen feeding before immunization induced T-cell tolerance but B-cell priming. Thus, oral administration of specific antigen may induce tolerance and suppress human autoimmune diseases that are T-cell-mediated. Indeed, clinical trials investigating the effectiveness of feeding antigen-specific peptides to suppress multiple sclerosis, rheumatoid arthritis, and uveitis are ongoing (29).

REFERENCES

3. Trejosiewicz LK, Smart CJ, Oakes DJ, Howdle PD, Malizia G, Campana D, Boylston AW. Expression of T-cell receptors TcR1 (gamma/delta) and TcR2 (alpha/beta) in the human intestinal mucosa. Immunology 1989; 68: 7–12.
7. Blumberg RS, Yockey CE, Gross GG, Ebert EC, Balk SP. Human intestinal intraepithelial lymphocytes are derived from a limited number of T cell clones that utilize multiple Vβ T cell receptor genes. J Immunol 1993; 150: 5144–53.


DISCUSSION

*Dr. Whitehead:* Could you comment on the very widespread problems in developing countries? The issue of stunting in children was raised earlier and there is speculation that the grossly abnormal gut structures that are found in the Third World could be a major component.
Dr. Kleinman: The cycle that has been described in infected and malnourished youngsters is now fairly well characterized. One of the first consequences of severe malnutrition is a decrease in cell-mediated immunity. So in that respect, a very important part of the intestine that defends against the outside world is deficient, so that sets the youngsters up for repeated cycles of infection following exposure to contaminated water, contaminated food, contaminated milk, and so on. Their inability to defend themselves adequately results in increasingly frequent infections, they become more malnourished, and ultimately if that cycle is not broken, they die. So one of the most important things that we can do is to try to interrupt that cycle at various points; probably the most effective thing to do is to encourage breast-feeding since that provides passive immunity for young infants, as well as a clean source of food.

Dr. Guesry: Martin Esteban has shown that of babies allergic to cow's milk, eggs, or fish at 9 months of age, only 20% will remain allergic to cow's milk, 40% to eggs, and 60% to fish 3 or 4 years later. How do you explain the development of tolerance for milk protein in 80% of the population of the babies who have developed allergy to it?

Dr. Kleinman: At present this question is unfortunately unanswerable. We don't understand the process involved in tolerance. What is even more interesting is that if you do skin tests in these youngsters at the age of 2 or 3 or 4, you can demonstrate that IgE antibody is being produced and yet there is no clinical response to the ingestion of the antigen orally. You have raised one of the remaining puzzles in mucosal immunology.

Dr. Lentze: Breast milk contains a variety of food proteins according to the mother's diet and it has always been speculated that this is a way immune tolerance can be achieved by the baby. Could you speculate on whether this is a good concept?

Dr. Kleinman: Probably the best data on the ability to suppress the IgE immune response are those from Bob Zeiger. He showed that if you exclude all of the most common antigens from the maternal diet during infancy and the third trimester of pregnancy, then the infant will not develop food allergy during the first few years of life, but you won't prevent other types of allergic disease, either during that time or later (1). We don't have any information that breast-feeding per se tolerizes the immune system. In fact, the information is more directed toward demonstrating that breast-feeding may prime the immune system. Breast-fed infants, for example, develop peak levels of IgA in their secretions more quickly than non-breast-fed infants, and that is independent of the IgA that is present in the breast milk itself, so something in the breast milk is stimulating the infant to begin to produce IgA on its mucosal surfaces earlier than the formula-fed infants. You can also demonstrate transfer of tuberculin sensitivity to breast-feeding infants. So, all of the evidence that I know of shows that the immune system of the infant is, if anything, primed by breast milk rather than tolerized; we don't know much more than that.

Dr. Schofer: Transfer of maternal immune cells to the infant via the breast milk has to be considered as a haplo-identical lymphoid graft to the child, so it would be very surprising if these cells really functioned as immune cells. They should either excite a host-versus-graft reaction or, if there were a clonal expansion, they should stimulate graft-versus-host disease or some sort of reaction at least. So is anything known about the function and fate of these cells? Do they stay in the lumen of the gastrointestinal tract and get excreted after a while, or what happens to them?

Dr. Kleinman: I don't believe there are any studies to show that these cells are functional in the gut over time. There is some evidence in animals of very early graft-versus-host reactions, but obviously that does not happen regularly or infants would not be able to thrive on their own mother's milk. However, in carefully manipulated animal experiments, you can demonstrate that type of reaction.

Dr. Schofer: You mentioned experiments on mice or rats, where they were fed antigens within the first few days after birth. However, as shown in the field of marrow and organ
transplantation, newborn rodents have a very immature immune system. Are these results really relevant to the newborn infant?

Dr. Kleinman: These animals receive immunity in a way that is quite similar to humans, i.e., there is transplacental transfer of antibody in the same way as in the human. Obviously the situations are not identical, so you can’t extrapolate all the conclusions from the animal studies to humans. But there is clearly an increased transfer of antigens from the premature infant’s intestine into the circulation, and there may be a priming of the immune response in the human infant as well, although that is much less certain. Whether you can exploit that model for transplantation purposes is now under active investigation. Investigators in a number of centers are looking at transplantation antigens to see whether you can depress cellular responses to those HLA antigens after an organ has been transplanted. Some of this work is quite encouraging in animal studies.

Dr. Gruskin: What is your view on the reintroduction of milk products to infants who have had acute gastroenteritis. Did you wait, or did you do it early?

Dr. Kleinman: Opinion is divided on that topic. In the developed countries, postenteritis enteropathy, which is another way of saying allergic enteropathy following a viral gastroenteritis, is quite a rare phenomenon. I have seen only a few cases in 20 years. My feeling is that in developed countries, there is really no role for a hypoallergenic diet following the usual case of acute gastroenteritis. Almost 100% of these youngsters do very well when returned either to the breast or to standard infant formula afterward.

Dr. Lentze: In European countries, 30–40% of children are already receiving hypoallergenic formula as a preventive measure. If these children get gastroenteritis, what should they be fed then? Our current view is that you should not give a cow’s milk formula to such children if they are already receiving a hypoallergenic formula, but there are no hard data about this. The question has not been resolved.

Dr. Kleinman: I think it is not one that will be resolved. In the USA of course this is not the practice. The percentage of infants there who are fed on a hypoallergenic formula is probably in the range of 5% or so, and 95% are getting a fairly standard formula with intact protein in it. On average in the USA, the infant between birth and 3 years of age has about three episodes of diarrhea a year, so it is not an insignificant problem, but all of these children are being fed on a complex complete formula and yet we don’t have any higher prevalence of postgastroenteritis enteropathy than I see reported from European countries. So that is the reason why I take the position that it is not necessary either to treat before or to treat afterwards. I think the data are now also quite clear on the prevention of allergy in high-risk children. We would restrict that treatment to those youngsters who have somewhere between a 25% and a 70% risk of allergy, based on family history—one parent, one parent/one sibling, or both parents—and in those cases, my own practice is to give the parents the choice. If it is a breast-fed infant, then I offer the mother the options of restricting milk, egg, wheat, soy, nuts, and fish from her diet, and doing that for a period of 6 months to a year, which is an extremely restrictive diet, or of using a hypoallergenic formula together with restrictions on the introduction of solid foods into the infant’s diet. Most parents decide to wait and see whether their youngster will actually develop allergic symptoms or not before starting on that type of hypoallergenic regime, and I think that is quite reasonable. This accounts for the low usage of hypoallergenic formulas.

Dr. Ballabriga: Some years ago, we collaborated with Dr. Hilpert in Switzerland to study the possible beneficial role of milk immunoglobulins in the prevention of E. coli gastroenteritis in infants. Cows were hyperimmunized during the last 6–8 weeks of gestation against different serotypes of enteropathogenic E. coli. Five days after calving, colostral whey proteins with
predominating IgG1 were isolated. The use of such a specific bovine milk immunoglobulin would be a method of providing passive local protection of the newborn against enteric infections. Passive hemagglutination and immunoelectrophoresis revealed clear evidence for a considerable resistance of bovine milk immunoglobulin against proteolytic degradation. In various clinical trials, the therapeutic efficiency of specific anti-*E. coli* milk immunoglobulin in infant *E. coli* gastroenteritis was studied. These experiments were stopped later for several reasons, but I would like to have your opinion on this.

**Dr. Kleinman:** This concept has now been carried several steps further by genetic engineering such that you can breed animals so that these antibodies are in their immune repertoire and are reproduced in large quantities in the milk. It is an active area for the infant formula manufacturers, to determine whether or not they are going to add these immunoglobulins to infant formulas. Individuals with AIDS have benefited from oral bovine immunoglobulin both in the protection against, and in the decrease in severity of, intestinal infections. Overall, I don't know what impact these immunoglobulins are going to have on public health in the developed nations, where the prevalence of these kinds of infectious illness is relatively low, but there may be benefits for developing nations. But this technique involves IgG antibodies, which are less effective than IgA in protecting against these diseases because they survive transit down the intestinal pathway less well. Overall, I don't know of any studies in large numbers of infants which show that this is an effective strategy for providing passive immunization but I know it is under active consideration.

**Dr. Agostoni:** I remember that 10 years ago, there were some studies linking atherosclerosis to the development of antibodies in human milk. What is your opinion of this?

**Dr. Kleinman:** Apart from the Barker studies—and, like Dr. Rey, I am skeptical about these—I don't think there is much to support a role for human milk feeding in protection against later heart disease, either immunologically or by affecting the regulation of cholesterol metabolism. However, some interesting experiments have been done that involve immunizing adult individuals against cholesterol. The individual is induced to produce antibodies against cholesterol or various metabolites of cholesterol. It can be shown, at least in the animal studies, that there is a significant decrease in cholesterol levels and in a few studies, a decrease in occlusions by vascular lesions. This process appears to enhance the clearance pathway of cholesterol from the blood.

**Dr. Sarles:** From a clinical point of view, it seems that adolescence is a critical period for respiratory allergy. Do we have any evidence that this could be the same for intestinal allergy?

**Dr. Kleinman:** No, the highest prevalence for intestinal allergy is between birth and 3 months of age. The peak prevalence is around 3 months of age, perhaps 3–5 months of age. It is very unusual to see those specific intestinal allergic syndromes beyond that age with the exception of eosinophilic gastroenteritis, which you do see during adolescence, but most of those cases do not respond to allergen avoidance or allergen withdrawal. Adolescence is one of the peak periods for inflammatory bowel disease. Whether there is any relationship between inflammatory bowel disease and dietary antigens remains an open question.

**REFERENCES**