

V. Mechanisms of tolerance and sensitization in the intestine and other organs of the body

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Summary

The intestinal tract is the major immunological organ of the human body.

The gut associated lymphoid tissues (GALT) play an important role in the suppression of adverse reactions to foods and in the induction of "Oral Tolerance".

Immunologically mediated clinical reactions to foods exist outside the gut environment and can affect virtually all organ systems.

Hypothesis

-It is postulated that food allergic diseases are caused by a breakdown of Oral Tolerance or by a failure of its induction.

Major research aims

-A better understanding of the immunological processes which govern the induction and maintenance of immunological suppression of adverse reactions to food and other proteins (Oral Tolerance).

Introduction

Considering the extent, variety and amounts of foods, viruses, bacteria and other of foreign material to which the intestinal tract and the gut associated lymphoid system (GALT) are exposed, it is surprising that only a small, although sizeable, proportion of the population (4-5% in Europe and North America) suffers from food allergies or food intolerances (1). One of the major tasks of the highly efficient local mucosal immune systems is the protection of mucosal surfaces and the prevention of adverse, immunological mediated, reaction which can affect the gut but also other organs of the body. The induction of "Oral Tolerance" after food intake, an antigen specific systemic down regulation of potentially adverse immune responses, forms an integral part of the

immunoregulatory capacity of the GALT (2-6).

Working definition of food allergy and intolerance

Adverse clinical reactions attributable to foods still tend to divide medical practitioners into two groups: those who ascribe a wide range of clinical complaints to foods and those who virtually deny that symptoms related to foods exist outside the gut.

The term *Food Allergy* (hypersensitivity) includes all immunological mediated adverse reactions to foods (IgE-mediated and those caused by other antibody and cell-mediated immune response). *Food Intolerance* reactions are by definition non-immunologically mediated reactions to foods.

Possible mechanisms are:

- Mucosal (e.g. lactase-deficiency in lactose intolerance) enzyme deficiencies or
- Systemic (e.g. phenylalanin-hydroxylase deficiency in phenylketonuria or fructose-1-phosphatase deficiency in fructose intolerance) enzyme deficiencies.
- Direct, toxin or chemically mediated intolerances as well as not yet defined immunologically mediated clinical symptoms may be included under the above heading.

Food Aversion is found in individuals with suspected - often self-diagnosed by affected individuals or parents - but as yet unconfirmed reactions to foods. In some individuals, food aversions may later be reclassified as belonging to one of the above categories (1).

The mucosal barrier

The mucosal lining of the gut as part of the GALT in combination with a host of non-specific factors (e.g. peristalsis, digestion, degradation, mucus) provides a highly effective barrier (Table 1) (7, 8).

Gut lumen

- Foreign proteins (e.g. foods)
- Bacterial/viral colonization/toxins
- Digestion, Secretions, Peristalsis

Mucosal surface

(non-specific and immunological factors)

- Barrier function

- Antigen:

- Binding, Processing (?), Uptake
 - Presentation to GALT
 - Interaction with intraepithelial lymphocytes

- Local immune defenses

- Secretory IgA, IgM
 - Cell-mediated immunity
 - Other Ig isotypes

- Lamina propria and GALT

- Antigen presentation
 - Induction of oral tolerance:
 - Suppression of humoral immunity
 - Suppression of cell-mediated immunity
 - Generation of memory

Table 1. Factors controlling antigen handling and immune regulation at mucosal sites

Any injury to the intestinal mucosal surface can compromise its functions and may lead to increased antigen absorption into the underlying lymphoid tissues and blood stream. This together with immunomodulatory effects of coexistent viral infections may lead to an increased rate of sensitizations. More evidence however is needed to evaluate whether this is a major contributing factor to sensitizations in individuals with a family history of atopy.

Minimal antigen access to the GALT and the bloodstream is a common occurrence and approximately 10^{-4} to 10^{-7} of the ingested food can be detected in the circulation at some time after intake (9).

A major defense factor is provided by the dimeric secretory IgA (IgA1 and IgA2) which is secreted by plasma cells within the gut, joint with a J chain and transported to the mucosal surface via the poly-Ig receptor. Secretory IgA within the gut consists in the majority of the IgA2 (>60%) subclass which seems to be less susceptible to enzymatic degradation than IgA1. In individuals with IgA-deficiency, IgM can provide similar functions and can use the same transport mechanism (10).

Anatomical aspects of the GALT

Epithelium

The epithelial cells, tightly connected at the apical parts via desmosomes (tight junctions) form a major part of the mucosal barrier. Lymphocytes (intraepithelial lymphocytes) mostly of the suppressor phenotype (CD8) are dispersed in between the epithelium, above the basal membrane, and are particularly close to the antigens which cross the epithelium (11, 12).

Lamina propria

The lamina propria including the villi contains a large number of cells which are involved in local and systemic immunity: these include mast cells, eosinophils, T-lymphocytes (mostly of the CD4 helper/memory phenotype), accessory cells, natural killer cells and other cell types.

-The Peyer's Patch (PP) of the small intestine is the major organized lymphoid tissue which lies between the muscularis mucosae and the epithelium. It is covered with a specialized epithelium which contains M or microfold cells which are thought to play an important role in antigen (particulate, helminth) presentation to the underlying lymphoid tissue. The number of human Peyer's Patches varies with age and is highest in childhood (approx. 150-200/small intestine). These contain large numbers of B-cells in particular areas (interfollicular) but they are also found with T-lymphocytes in the dome area (Fig 1, p.20).

Lymphocyte recirculation and homing

The extent of migration of lymphocytes through the intestine and other mucosal sites is dependent on lymphoid drainage, the mucosal origin and activation pattern but also on a great number of additional factors, all of which are not clearly understood. In principle, activated cells of the GALT leave the Peyer's Patch via efferent lymphatics to the draining mesenteric lymph nodes which drain via the thoracic duct into the subclavian vein (13). After having reached the circulation cells derived from the intestinal tract have the propensity to return to the induction site but may also reach other mucosal sites and tissues such as the mammary gland respiratory and urogenital tract (Fig 2, p.21). A recently emerging large family of lymphocyte and endothelial surface molecules (Homing receptors and addressins) are involved in the migration and distribution of these cells (14-16).

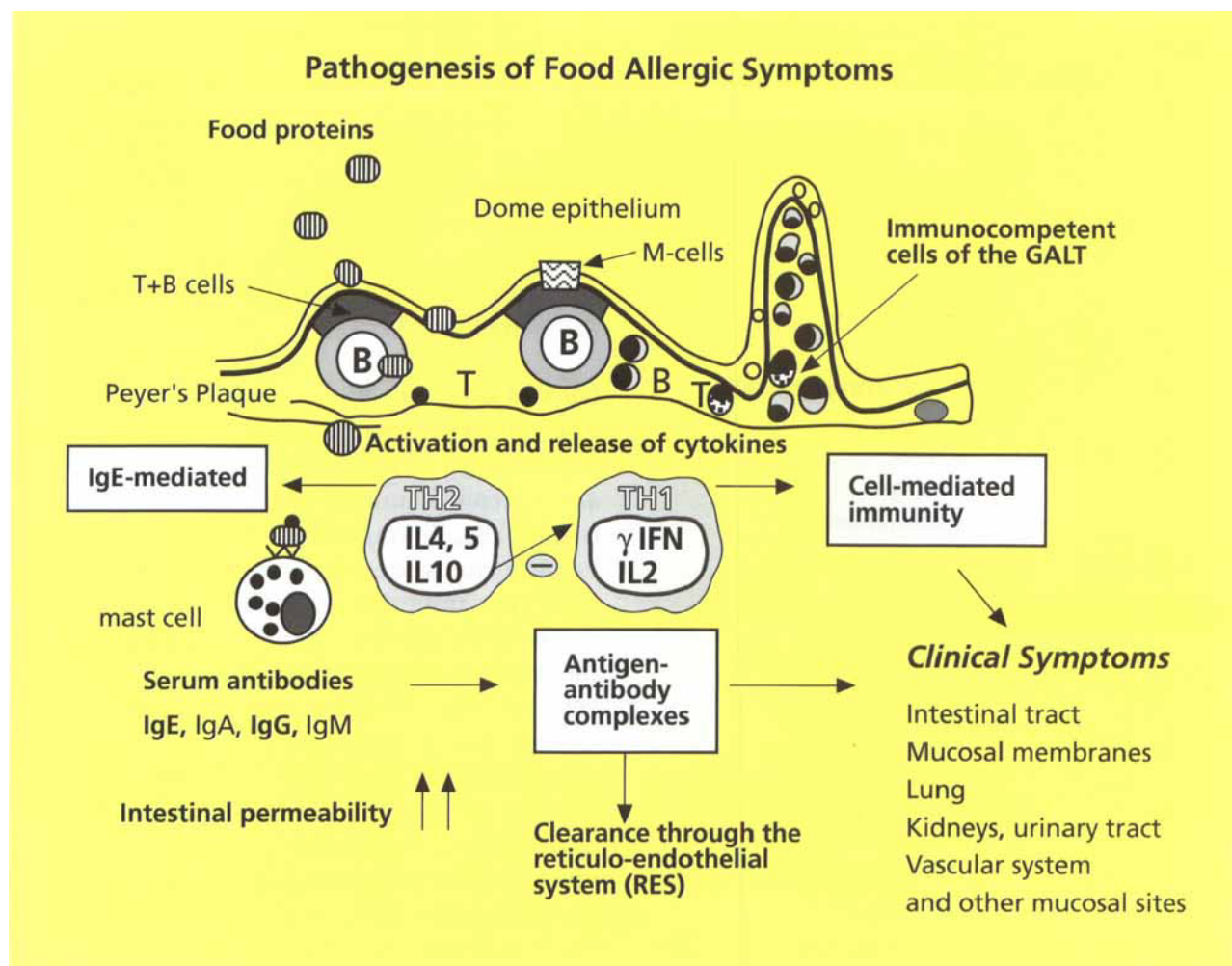


Fig 1. Pathogenesis of Food Allergic Symptoms (hypothesis)

Food proteins reach the immunocompetent cells of the GALT in small quantities undigested or after processing and presentation by antigen presenting cells. A poorly understood process of local antigen processing and presentation through epithelial cells, B-cells, follicular dendritic cells and dendritic cells generally leads to a suppression of potential adverse IgE- and cell-mediated immune responses without affecting protective local secretory immunity (IgA/IgM). Under pathological conditions, cell-mediated immune responses play a major role in causing mucosal damage. This cellular immune response can be locally modified by a particular cytokine release pattern and preferential activation of T-lymphocytes (e.g. Th2 cells) that favour IgE production or trigger other inflammatory amplification loops that add to alterations of mucosal morphology and function. Secondary phenomena such as an increased intestinal permeability and increased formation of circulating antigen-antibody immune complexes can lead to clinical symptoms that can affect virtually all organ systems. IL10 may be able to down-regulate TH1 responses.

Functional aspects of the GALT

A central role of the GALT is to prevent adverse immunological reactions to potential hazardous antigens and at the same time initiate active defense mechanism without damage to the body's tissues.

Oral tolerance

The ingestion of food antigens generally leads to the induction of oral (peripheral) tolerance (17). This tolerance is defined as a specific immunologic hyporesponsiveness after prior mucosal (enteral) exposure to that antigen. This state of tolerance includes suppression of IgE and cell-mediated

immunity but does not prevent the production of IgG antibodies to foods which is a normal occurrence, especially in young infants and children. It does however prevent tissue-damaging, mostly cellular immune responses. The exact regulation of this central immunological tolerance mechanism remains to be fully understood although evidence suggests that 3 basic mechanisms are involved in its regulation (17-21)(Fig 2).

- T-lymphocyte mediated suppression
- anergy (silencing), and possibly,
- clonal deletion

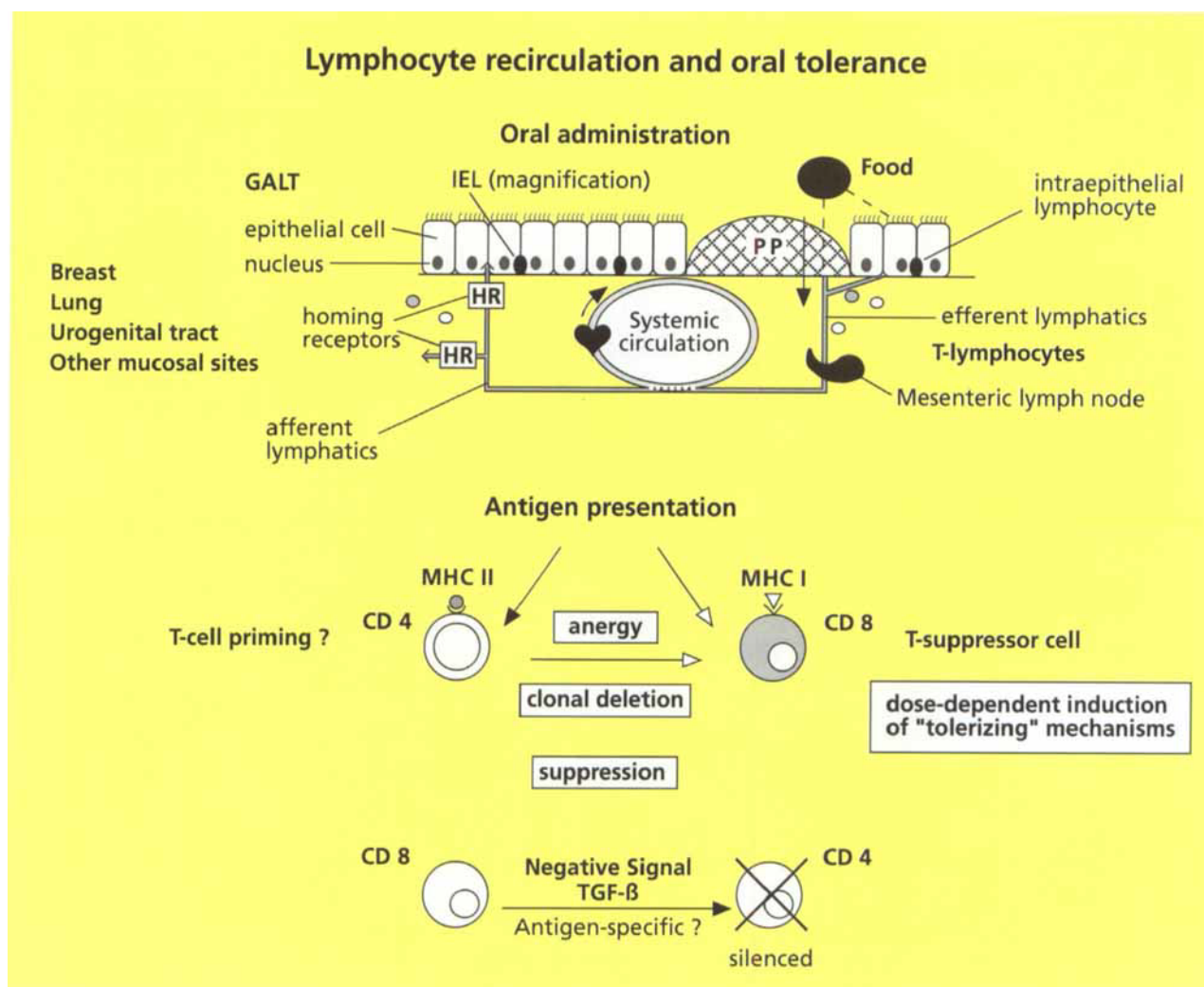


Fig 2. Lymphocyte recirculation and oral tolerance

After passage through the mucosa and processing by the GALT (including enterocytes) antigen is presented in connection with major histocompatibility complex class I and/or class II molecules. Presentation in association with class I antigens may lead to activation of specific CD8+ T suppressor cells. Presentation in association with class II antigens will activate CD4+ T cells and lead to memory induction. A regulatory interaction between CD4+ and CD8+ T-cells in this context is conceivable. Activated CD8+ T cells can also provide a negative signal to (auto) reactive cells in affected target organs. A negative signal could be provided by TGF- β . In the absence of a costimulatory signal (e.g. via CD28-CD80 (B-7 family)) T-cell anergy (tolerance) could be induced. Other regulatory pathways are likely to exist and the role of the CD8+ regulatory cell needs further clarification. Other "fail-safe" mechanisms may include immunological help provided by CD4+ cells. Their exact role in tolerance induction remains to be established. The amount of antigen presented to the GALT may be able to select between mechanisms such as anergy and suppression. Under experimental conditions, smaller antigen doses are more likely to induce suppression.

A number of confounding variables needs to be considered when analyzing the effects of food antigen administration:

- genetic background
- age at first antigen exposure
- nature of antigen (e.g. soluble, particulate, replicating)
- dose of antigen and frequency of administration
- immunological status (e.g. virus infection, gastroenteropathy) of the host
- antigen transmission via breast milk and previous dietary exposure of the mother

Immunological mechanisms involved in food allergic diseases in the gut and other sites of the human body.

A breakdown of this important regulatory mechanism may lead to the induction of food allergic disease (Figs 1, 2). For the sake of clarity, immune responses (possibly) active in food allergic diseases are separated into four groups (Type I-IV, Gell and Coombs classification) although it is evident that adverse clinical reactions can and are likely to be triggered by more than one immune mechanism (4, 17, 22-26).

Amplification of ongoing immune response through secretion of inflammatory mediators

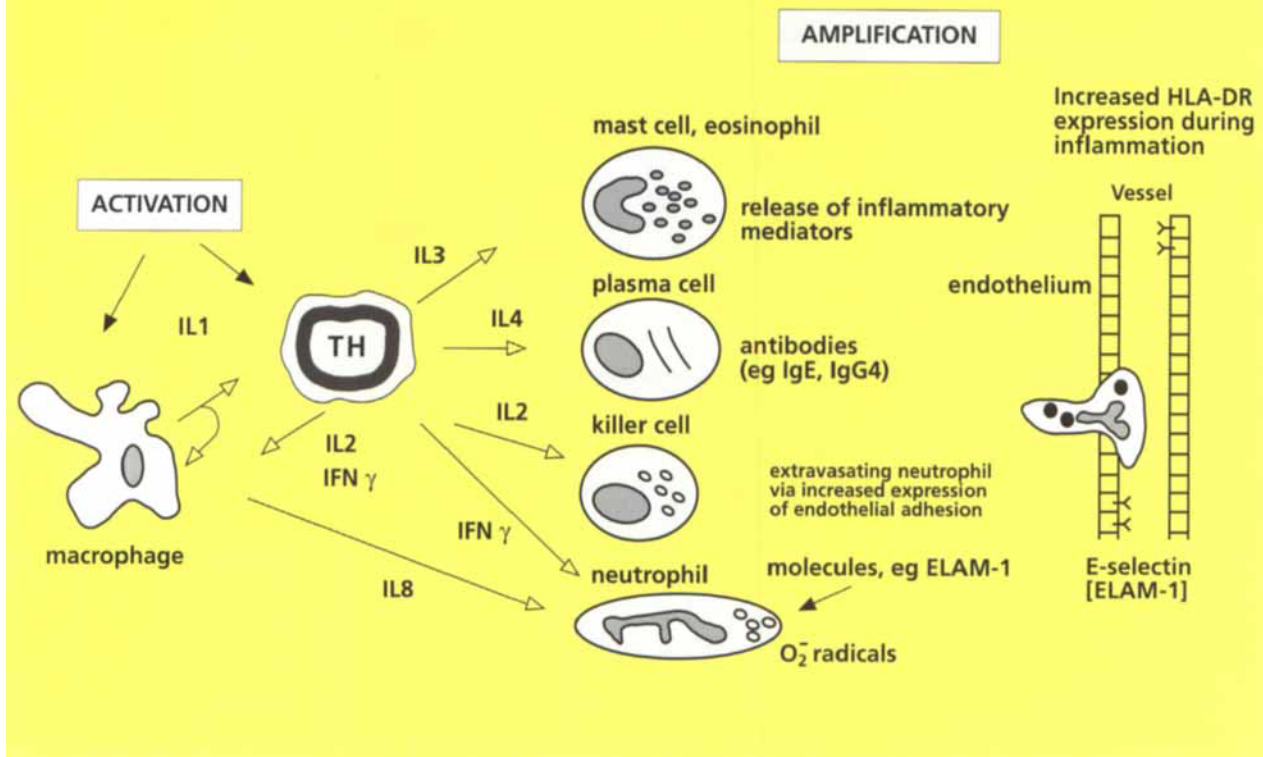


Fig 3

Amplification through inflammatory cytokines

After activation of T-lymphocytes and macrophages or both a range of regulatory and inflammatory cytokines are released. Depending on the cytokine and activated cells, a variety of immune responses can be triggered by T-cells with preferential secretion of γ -interferon and interleukin 2 (IL2) (Th1 cells) and by T-cells secretion of mainly IL4, IL6, IL10 and IL13. Other inflammatory cells can be recruited and can amplify the inflammatory process. Stimulation of neutrophil-endothelial interactions and subsequent extravasation and migration to inflamed sites can add to tissue damage.

Immediate hypersensitivity reaction (Type I) Intestinal anaphylaxis, IgE-mediated

The acute onset of IgE-mediated clinical symptoms of intestinal anaphylaxis (diarrhea, vomiting) after eating a triggering food is familiar to most medical practitioners (and parents). During a mucosal anaphylactic reaction, proteins can cross the intestinal and vascular barrier in two ways. They can “leak” into the gut or, under certain circumstances, pass from the gut lumen into the vascular system. Altered mucosal integrity and function or both can be assessed with appropriate *in vivo* absorption tests.

Macromolecular protein absorption does not necessarily correlate with sugar permeability and both have to be assessed separately. The release of mast cell or eosinophil-specific enzymes can be a marker of intestinal anaphylaxis under experi-

mental conditions. The usefulness of measuring the release of inflammatory mediators as a means of diagnosis in the individual patient remains to be shown. Preformed or newly generated mediators of inflammation and cytokines released or produced from mast cells or other cells can amplify the pathogenic process (Fig 3). In rodents, the regulation of IgE antibody production seems to be under a delicate cytokine control network whereby IL4 (secreted by Th2 cells) augments IgE production and interferon- γ (secreted also by Th1 cells) has a suppressive activity (18, 27, 28).

Recent studies have provided substantial evidence that lymphocytes within the lamina propria differ from lymphocytes in other tissues and in the circulation. Mucosal lymphocytes are phenotypically distinct and show evidence of activation and recent memory induction. On activation

lamina propria lymphocytes have a high level of expression of mRNA for the production of IL2, IL4, IL5 and γ IFN. The high level of expression of IL4 and IL5 is similar to mouse Th2 clones and there is increasing evidence at present that normal human lymphocytes exist with a similar functional pattern to murine Th1 or Th2 clones. Activation or imbalance or both of these delicate local cytokine pathways could finally trigger mechanisms that lead to mucosal damage.

The effect of so-called "mast-cell stabilizing" drugs in the prevention of presumably IgE-mediated clinical symptoms has been unsatisfactory despite the obvious involvement of mucosal mast cells. Reports about the usefulness of orally administered cromoglycate for treatment of food allergies have been conflicting (29, 30).

Antibody-dependent cytotoxicity (ADCC) (Type II)

Antibody-dependent cytotoxicity is characterized by the effects of natural killer (NK) cells and T-lymphocytes which possess a high affinity immunoglobulin receptor (Fc γ RIII, CD16) which is used for target cell recognition (in association with a specific antibody binding to the cell). These immunological mechanisms are not restricted by the recognition pathway via the histocompatibility complex HLA-DR (Class II antigens). Large granular lymphocytes (LGL) which can be identified in the lamina propria and gut epithelium display several of these activities *in vitro*. ADCC activity has also been reported *in vitro* in infants with cow's milk-protein induced gastroenteropathy without other clinical responses to milk. The role of ADCC in clinical food allergic reactions remains to be established (31).

Immune complex (Antigen-antibody, Arthus type) disease (Type III)

Production of anti-food antibodies in children is a physiological response and not generally correlated with disease. Increased levels of food-specific antibodies in serum or secretions, however, are more frequently found in groups of patients with clinical symptoms of food sensitivity. Immune complex (IC) deposition in tissues and their histological demonstration can be a sign of an underlying immunologically mediated disease (32). It would still be premature to suggest that the IC identified on intestinal sections of patients with inflammatory bowel disease or with food-sensitive

enteropathies represent the immunological cause for the mucosal damage, although there is increasing evidence for a putative role of local immune complement activation based on immunohistochemical evidence in patients with (non food-related) ulcerative colitis. The contribution of other pathogenetic processes e.g. local vasculitic processes in the generation of mucosal injury needs to be considered but its contribution to the disease process remains unclear.

Delayed type hypersensitivity reaction (Type IV)

Cell-mediated delayed type hypersensitivity reactions represent a major immunological pathway responsible for mucosal damage (3, 25, 33). Effects on intestinal morphology have been investigated in detail during immunologically mediated processes (e.g. graft versus host reaction or rejection of transplanted bowel). The small intestinal mucosa is the major target organ but changes are seen throughout the whole length of the intestinal tract. The striking similarities of these histological changes with intestinal abnormalities observed in food protein-induced gastroenteropathies (celiac disease, cow's milk-induced enteropathy) have led to the suggestion that a T-lymphocyte mediated process also plays an important role in the pathophysiology of villus atrophy in humans.

A first sign of this mucosal immune response is an increase of lymphocytes within the intestinal epithelium. IEL infiltration is frequently followed by an increase in crypt cell turnover and an increase of the crypt depth which ultimately can progress to villus atrophy (23, 33).

The role of "enteropathic" cytokines

Convincing experimental evidence that direct cytotoxicity mechanisms play an important role in damaging the mucosa is lacking and it is tempting to speculate that immunologically mediated damage is triggered by systemic or more likely local release of mediators (cytokines). These "enteropathic" cytokines may modulate the function (?) of intraepithelial lymphocytes and could also affect lamina propria fibroblasts and intestinal crypt stem cells (34-37). Alterations of the cytokine activity of intestinal mucosa of patients with inflammatory bowel diseases have been reported. The local release of cytokines from various sources is likely to provide an important inflammatory

amplification loop in adverse reactions to foods not only in the intestinal tract but also in other organs of the body.

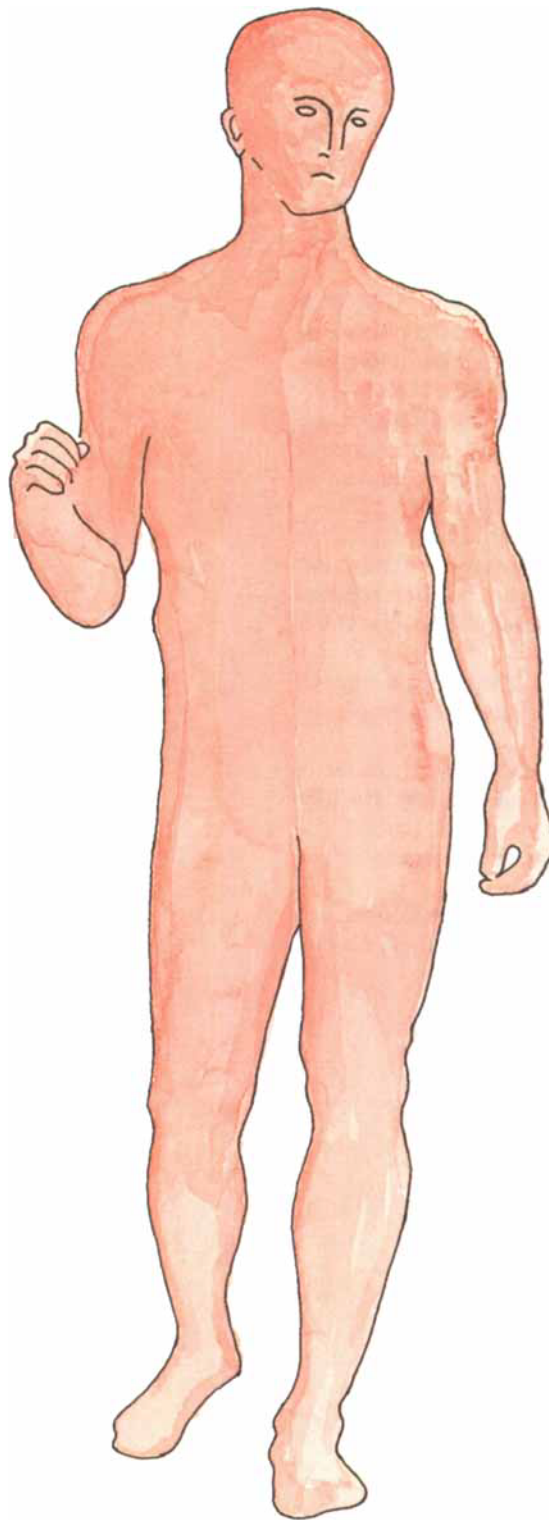
Clearly more work is needed to substantiate these hypotheses and to increase our understanding of the exciting field of gastrointestinal immunoregulation and its relationship to health and disease.

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References

1. Strobel S. Epidemiology of food sensitivity in childhood - with special reference to cow's milk allergy in infancy. In: Burr ML, ed. *Epidemiology of clinical allergy*. Basel: Karger, 1993:119-130. (Hanson LA, Shakib F, eds. *Monographs in allergy*; vol 31).
2. Strobel S. Dietary manipulation and induction of tolerance. *J Pediatr* 1992;121:S74-79.
3. Strobel S, Ferguson A. Modulation of intestinal and systemic immune responses to a fed protein antigen in mice. *Gut* 1986;27:829-37.
4. Mowat AM. The regulation of immune responses to dietary antigens. *Immunol Today* 1987;8:93-98.
5. Weiner HL, Friedman A, Miller A, et al. Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Ann Rev Immunol* 1994;12:809-838.
6. Wells H. Studies on the chemistry of anaphylaxis. III Experiments with isolated proteins, especially those of the hen's egg. *J Infect Dis* 1911;9:147-151.
7. Walker WA. Role of mucosal barrier in antigen handling by the gut. In: Brostoff J, Challacombe, ed. See pp. 209-222. *Food allergy and intolerance*. 1st ed. London: Bailliere Tindall, 1987:209-222.
8. Sanderson I, Walker W. Uptake and transport of macromolecules by the intestine: possible role in clinical disorders (an update). *Gastroenterology* 1993;104:622-639.
9. Husby S. Dietary antigens: uptake and humoral immunity in man. *Acta Pathol Microbiol et Immunologica Scand*. 1988;96 (Suppl 1), 1-40.
10. Brandtzaeg P, Bjerke K, Kett K, et al. Production and secretion of immunoglobulins in the gastrointestinal tract. *Ann Allergy* 1987;59.
11. MacDonald T. Antigen-dependent and antigen-independent development of mucosal immunity and hypersensitivity. In: Walker WA, Harmatz PR, Wershil BK, eds. *Immunophysiology of the gut*. San Diego: Academic Press, 1993:99-109. Ed. V. Moreno, Bristol Myers/Squibb/ Mead Johnson Nutrition Symposia.
12. Spencer J, MacDonald TT, Finn T, Isaacson PG. The development of gut associated lymphoid tissue in terminal ileum of fetal human intestine. *Clin Exp Immunol* 1986;64:536-543.
13. Gowans JL, Knight EJ. The route of recirculation of lymphocytes in the rat. *Proc Royal Soc London (Biol)* 1964;159:257-281.
14. Jalkanen S, Bargatze RF, de la Toyos J, Butcher EC. Lymphocyte recognition of high endothelium: antibodies to distinct epitopes of an 85-95 kD glycoprotein antigen differentially inhibit lymphocyte binding to lymph node, mucosal or synovial endothelial cells. *J Cell Biol*. 1987;105:903-990.
15. Picker LJ, Treer JR, Ferguson-Darnell B, Collins P, Buck D, Terstappen LWM. Control of lymphocyte homing in man: I Differential regulation of the peripheral lymph node homing receptor L-selection on T-cells during the virgin to memory transition. *J Immunol* 1993;150:1105-1121.
16. Ottaway CO. Lymphocyte migration to the gut mucosa. In: Heatley RV, ed. *Gastrointestinal and hepatic immunology*. Cambridge: Cambridge University Press, 1994:24-47. Eds. Oliveira DBG, Peters DK, Weetman AP. *Cambridge Review in Clinical Immunology*.
17. Strobel S. Food allergy-role of mucosal immune regulation and oral tolerance: facts, fiction, and hypotheses. In: Walker WA, Harmatz PR, Wershil BK, Eds. *Immunophysiology of the gut*. San Diego: Academic Press, 1993:336-357. Ed. V. Moreno. Bristol Myers/Squibb/ Mead Johnson Nutrition Symposia.
18. McMenamin C, Holt PG. The natural immune response to inhaled soluble protein antigens involves major histocompatibility complex (MHC) class I-restricted CD8+ T cell-mediated but MHC class II-restricted CD4+ T cell-dependent immune deviation resulting in selective suppression of immunoglobulin E production. *J Exp Med* 1993;178:889-99.
19. Melamed D, Friedman A. Direct evidence for anergy in T lymphocytes tolerized by oral administration of ovalbumin. *Eur J Immunol* 1993;23:935-42.
20. Gregerson DS, Obritsch WF, Donoso LA. Oral tolerance in experimental autoimmune uveoretinitis. Distinct mechanisms of resistance are induced by low dose vs high dose feeding protocols. *J Immunol* 1993;151:5751-61.
21. Strobel S, Mowat AM, Drummond HE, Pickering MG, Ferguson A. Immunological responses to fed protein antigens in mice. II. Oral tolerance of CMI is due to activation of cyclophosphamide-sensitive cells by gut-processed antigen. *Immunology* 1983;49:451-6.
22. Ferguson A. Models of immunologically-driven small intestinal damage. In: Marsh N, ed. *Immunopathology of the small intestine*. Chichester: John Wiley and Sons, 1987:225-252.
23. Strobel S. Immunologically mediated damage to the intestinal mucosa. *Acta Paediatr Scand*. 1990;356:S46-S57.
24. Strobel S. Food intolerance and allergy. In: Heatley RV, ed. *Gastrointestinal and hepatic immunology*. Cambridge: Cambridge University Press, 1994:147-168. Eds. Oliveira DBG, Peters DK, Weetman AP. *Cambridge Reviews in Clinical Immunology*.
25. MacDonald TT, Spencer J. Evidence that activated mucosal T cells play a role in the pathogenesis of enteropathy in human small intestine. *J Exp Med* 1988;167:1341-1349.

26. MacDonald TT, Spenser J. Cell-mediated immune injury in the intestine. *Gastroenterol Clin North Am* 1992;21:367-86.
27. Kay AB. Origin of type 2 helper T cells. *N Engl J Med*. 1994;330:567-568.
28. Mosman TR, Coffman RL. Th1 and Th2 cells: different patterns of cytokine excretion lead to different functional properties. *Annu Rev Immunol* 1989;7:145-173.
29. Fälth-Magnusson K, Kjellman MI, Magnusson KE, Sundqvist T. Intestinal permeability in healthy and allergic children before and after sodium-cromoglycate treatment assessed with different-sized polyethyleneglycols (PEG 400 and PEG 1000). *Clin Allergy* 1984;14:277-86.
30. Ventura A, Rinaldi S, Florean P, Agosti E. Intestinal permeability, atopic eczema and oral disodium cromoglycate. *Pediatr Med Chir* 1991;13:169-72.
31. Saalman R, Carlsson B, Fällström SP, Hanson LÅ, Ahlstedt S. Antibody dependent cell-mediated cytotoxicity to β -lactoglobulin coated cells with sera from children with cow's milk protein allergy. *Clin Exp Immunol* 1991;85:446-452.
32. Halstensen TS, Mollnes TE, Garred P, Fausa O, Brandtzaeg P. Epithelial deposition of immunoglobulin G and activated complement (C3b and terminal complement complex) in ulcerative colitis. *Gastroenterology* 1990;98:1264-1271.
33. Mowat AM, Borland A, Parrott DMV. The delayed type hypersensitivity reaction in the small intestine. VII. Induction of the intestine phase of the murine graft versus host reaction by Lyt 2-T cells activated by I-A alloantigens. *Transplantation* 1986;41:192-198.
34. Beagley K, Elson C. Cells and cytokines in mucosal immunity and inflammation. *Gastroenterol Clin North Am* 1992;21:347-366.
35. Fiocchi C. Production of inflammatory cytokines in inflammatory bowel disease. *Immunol Res* 1991;10:239-246.
36. Kagnoff MF. Immunology of the intestinal tract. *Gastroenterology* 1993;105:1275-80.
37. Sartor RB. Cytokines in intestinal inflammation: pathophysiological and clinical considerations. *Gastroenterology* 1994;106:533-539.



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