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Review Article

Immunopathogenesis of cow's milk allergy

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Cow's milk allergy has become a prominent clinical problem in pediatrics in recent years. The reasons for this seem to be a greatly increased use of milk formula, even at very early ages, together with improvements in health conditions, which permit us to spent time on the less serious allergic manifestations. In the history of medicine, we knew that Hippocrates himself described typical atopic manifestation of cow's milk; however, systematic investigations, have been conducted for the most part only since 1950.[1]

It has not yet been possible to arrive at an unanimously accepted terminology for milk allergy, mainly because the clinical entity as such is poorly defined and the pathologic mechanisms behind the clinical symptoms are as yet not clear. The expression "intolerance" is often used when it is not known how adverse reaction to milk is mediated. When the mechanism is immunological, use of the term "hypersensitivity" is justified, and when it is IgE-mediated the term 'allergy" is correct, sometimes "atopy" is also used interchangeably.

The prevalence of cow's milk allergy vary from a part of the world to another, in average up to 8% of children less than 3 years of age and approximately 2% of the adult population experience cow's milk-induced allergic disorders.[2] Prevention of food allergy using hypoallergenic milk formula in the first trimester of life is very important, because once IgE response to cow's milk protein is initiated, it progresses throughout the infant life and sensitzation to other food allergens may develop.

Given the public's increasing awareness of cow's milk allergy and their frequent misperception that various illness is caused by cow's milk-induced allergic reactions, the physician must retain some skepticism throughout the evaluation and rely on objective measures to arrive at the final diagnosis. Over diagnosis of cow's milk allergy has led to malnutrition, eating disorders, and psychosocial problems, as well as family disruption, whereas under diagnosis leaves the patient suffering unnecessarily and may result in growth failure and permanent physical impairments. The following discussion provides an immunological basis of cow's milk allergy in an attempt to improve our understanding in clinical manifestations, diagnosis and management of the disease.

Symptoms of cow's milk allergy

The primary target organs for cow's milk allergy are skin, gastrointestinal tract, and the respiratory system. Both common acute reactions (hives and anaphylaxis) and common diseases: asthma, atopic dermatitis and gastrointestinal disorder may be caused or exacerbated by milk ingestion.[3] Common manifestations of milk allergy which may be IgE mediated or non IgE mediated are listed in **Table 1**.

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TABLE 1. COMMON MANIFESTATION OF COW'S MILK ALLERGY.

Target Organ	IgE-mediated disorder	Non IgE-mediated disorder
Skin	Urticaria and angioedema Atopic Dermatitis	Atopic Dermatitis Dermatitis Herpetiformis
Gastrointestinal	Oral Allergy Syndrome Gastrointestinal anaphylaxis Allergic eosinophilic gastroenteritis	Proctocolitis Enterocolitis Allergic osinophilic gastroenteritis Enteropathy syndrome Celiac Disease
Respiratory Tract	Asthma Allergic Rhinitis	Heiner Syndrome
Multisystem	Anaphylaxis Exercise-induced anaphylaxis	

The clinical manifestations of cow's milk allergy are not limited only on the list mentioned above, which are familiar.[4] There are some unusual manifestations of cow's milk allergy (**Table 2**). Since the term "unusual" is relative, perhaps some of what is mentioned here as unusual may be considered usual by others.

TABLE 2. UNUSUAL CLINICAL MANIFESTATIONS

Target organ	Unusual Clinical manifestation
Skin	Vasculitis
	Fixed Skin Eruption
ENT	Chronic Serous Otitis Media
Respiratory	Chronic Pulmonary disease (Heiner Syndrome)
	Hypersensitivity pneumonitis
Gastrointestinal	Constipation
	Gastroesophageal reflux
Multi system	Irritability/Sleeplessness in infants
	Arthropathy
	Nephropathy
	Thrombocytopenia

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Cow's milk allergens

Although many different cow's milk protein are included, a relatively small number accounts for the vast majority of cow's milk-induced allergic reaction. However, the allergenic fraction of food is generally comprised of heat stable, water-soluble glycoproteins ranging in size from 10 to 70 KD. As depicted in **Table 3**, the allergenic proteins in many foods have been identified, isolated, sequenced, and cloned.[5-8] Casein accounts for about 80% of the total protein content in cow's milk, whereas whey protein comprises the rest. Casein consists of 4 protein fractions, as1-casein, as2-casein, b-casein, k-casein, comprising 32%, 10%, 28%, and 10% of the total cow's milk protein, respectively.

TABLE 3. MAJOR COW'S MILK ALLERGENS THAT HAVE BEEN ISOLATED AND CHARACTERIZED.

Cow's milk	
Caseins	αs1-casein, as2-casein
	β-casein
	κ-casein
Whey	β-lactoglobulin
	α-lactalbumin

In cow's milk, different caseins form complexes and ordered aggregates, i.e. micelles. These globular complexes are composed of a peripheral hydrophilic layer and a hydrophobic core. In the core, caseins are assembled by means of intermolecular interactions between the colloidal calcium phosphate and the phosphoserine groups of the as1, as2, and the b-casein, whereas the C terminal polar fragment of the k-casein and the polar domains of the other caseins are exposed at the periphery.[9] Whey fraction contains essentially globular protein, a-lactalbumin and b-lactoglobulin, containing 4 and 2 disulfide bridges and comprising 5% and 10% of total cow's milk protein, respectively.

The relative allergenicity of each cow's milk protein remains unclear, although data from recent stud-

ies have emphasized the importance of the caseins as major milk allergens, and significant reactivity to the whey protein (a-lactalbumin and b-lactoglobulin) was also noted. Some studies have recently mapped the major IgE and IgG binding epitopes on as1-casein, as2-casein, b-casein, k-casein, a-lactalbumin and b-lactoglobulin.[10, 11]

Development of cow's milk allergy

Within hours of birth, a newborn's gastrointestinal tract and gut-associated lymphoid tissue are confronted with foreign proteins in the form of bacteria and food antigens. This immature system must process ingested food into a form that can be absorbed and used for energy and cell growth, mount rapid and potent responses against various pathogens (development of immunity) and remain unresponsive to enormous quantities of foreign nutrient antigen (development of tolerance). A number of immunologic and non-immunologic mechanisms (**Table 4**) operate to prevent foreign antigens from penetrating the gut barrier.[12]

However, immaturity of this mechanism in infants reduces the efficiency of the infant mucosal barrier. For example, basal acid output is relatively low during the first month of life, intestinal proteolytic activity does not reach mature levels until approximately 2 years of age, and intestinal microvillus membranes are immature, resulting in altered antigen binding and transport through mucosal epithelial cells. In addition, the newborn lacks IgA and IgM in exocrine secretions and salivary s-IgA concentration, which are absent at birth and remain low during the early months of life.[13] The relatively low concentrations of s-IgA in the young infant's intestine and the relatively large quantities of ingested proteins place a tremendous burden on the immature gut-associated lymphoid tissue. Not surprising, the early introduction of numerous food antigens has been shown to stimulate excessive production of IgE antibodies.[14] The development of an IgE-mediated response to an allergen (generally a glycoprotein) is the result of a series of molecular and cellular interactions involving antigen-presenting cells (APC's), T cells and B cells. APCs present small peptide fragments (T-cell epitope) in conjunction with MHC class II molecules to T cells, which in turn will bind to the peptide-MHC complex. This interactive "first signal" leads to T-cell proliferation, cytokine generation initiation of "second" signal, which promotes an IgE response (T_{h2}-like activation). These cells and their products, in turn, interact with B-cells bearing appropriate antigen-specific receptors, leading to isotype switching and the generation of antigen-specific IgE. At all stages, a number of specific cytokines are secreted, which modulate the cell interactions. The antigen-specific IgE then binds to the surface receptors of mast cells, basophil, macrophage and other APCs, arming the immune system for an allergic reaction with the next encounter of the specific antigen.[15]

TABLE 4. COMPONENT OF THE GUT BARRIER.

Physiologic barriers	Block penetration of ingested antigens:
	Epithelial cells
	Glycocalyx
	Intestinal microvillus membrane structure
	Tight junctions joining adjacent enterocytes
	Intestinal peristalsis
	Break down ingested antigens:
	Salivary amylases and mastication
	Gastric acid and pepsins
	Pancreatic enzymes
	Intestinal enzymes
	Intestinal epithelial cell lysozyme activity
Immunologic barriers	Block penetration of ingested antigens:
	Antigen-specific s-IgA in gut lumen
	Clear antigens penetrating gastrointestinal barrier:
	Serum antigen-specific IgA and IgG
	Reticuloendothelial system

Even in the mature gut, about 2% of ingested food antigens are absorbed and transported throughout the body in an "immunologically" intact form.[16] Increased stomach acidity and the presence of other food in the gut decrease antigen absorption, whereas decreased stomach acidity (e.g., antacids) and ingestion of alcohol increase absorption.[17] The immunologically recognizable proteins that gain access to the circulation do not normally cause adverse reactions because tolerance develops in most individuals, but in the sensitised host they can provoke a variety of hypersensitivity responses. Although more common in the developing gut-associated lymphoid tissue of young children, it is clear that both cellular and IgE-mediated hypersensitivity responses to foods can develop at any age. Recent studies suggest that intestinal cells (IECs) play a central regulatory role in determining the rate and pattern of uptake of ingested antigens. Studies in sensitised rats indicate that intestinal antigen transport proceeds in 2 phases. In the first phase of antigen uptake, Trans epithelial transport occurs through endosomes, is antigen specific and mast cell independent, and occurs 10 times faster in sensitised rats compared with nonsensitized controls.[18] In the second phase Para cellular transport predominates, is mast cell dependent, is not antigen specific and is markedly increased by antigen challenge in sensitised rats compared with mast cell-deficient sensitised rats or nonsensitized controls. These studies clearly demonstrate that the rate and amount of antigen absorbed during IgE-mediated reactions in the gastrointestinal tract is markedly increased. They also suggest that both antigen-specific pathways most likely involve cytokines. Consistent with this concept IECs express receptors for a number of different cytokines (IL-1, IL-2, IL-6, IL-10, IL-12, IL-15, GM_CSF and IFN-g), and IECs have been shown to be functionally altered by exposure to these cytokines.

IgE antibody in relation to cow's milk allergy

Adverse reactions to foods are dose dependent. The amount of the offending food component absorbed depends on the amount ingested and the efficiency of the gut-barrier. The greater risk of developing food allergy in infancy as compared to later in life may be related to several factors. The artificially fed baby is massively exposed to food antigens by a large intake of proteins in relation to body weight. Intact antigens are absorbed in considerable amounts and reach antibody-forming cells in the intestinal mucosa and the gut-associated lymphoid organs, which probably explains why most infants develop food antibodies of the IgG, IgA and IgM types. In atopic children, who are prone to develop antibodies of IgE class, an early antigenic exposure can cause IgE production and subsequent sensitisation of mast cells in the gastrointestinal and respiratory tracts as well as the skin. IgE production starts early in the primary immune response to food in predisposed infants and seems to continue even when the allergen is avoided by an elimination diet. Food IgE-antibody concentrations seem to increase to individual peak levels and thereafter decline. Extremely atopic infants may also be sensitized through breast-milk by foods their mothers have eaten. This explains why some infants already react to their first intake of cow's milk formula. Infants with onset of allergic to one food, e.g., cow's milk, are also at high risk of developing allergy to other foods. IgE levels have been suggested as being under the same genetically control, as the predisposition to atopic disease.[1] Family history of atopic disease is common in children with atopic symptoms but will, in cases with food sensitivity, provide little support for a reaginic pathogenesis of the symptoms. However elevated serum IgE levels in early infancy, in addition to a family history of atopy, indicate an increased risk of developing atopic disease and in such cases food sensitivity might have a reaginic basis. Maternal IgE does not cross the placental barrier and detectable IgE in cord serum has been shown to be of fetal origin, indicating intra-uterine sensitization.[2]

Cow's milk antibodies belonging to immunoglobulin other than IgE.

It is obvious that not all of the adverse immunological reactions to foods can be explained by IgE-mediated mechanisms. Heat stable anaphylactoid IgG-antibodies have been found to occur in some milk-sensitive, but also in some cows milk-tolerant individuals. IgG and IgM-milk antibodies are able to form immune complexes in serum and in the intestinal

mucosa of some children with cow's milk sensitivity.[1] Such immune complexes might activate the complement system, thus giving rise to anaphylactic and chemotactic factors which can contribute to mast -cells degranulation and subsequent symptoms. High levels of precipitating and hemaglutinating antibodies to cow's milk have been shown to occur in such conditions as milk-induced malabsorption, protein-losing enteropathy, iron-deficiency anemia and gastrointestinal bleeding, pulmonary disease and sudden unexpected death in infancy. In addition to children with severe gastrointestinal disturbances, atopic children also seem to have food antibodies of IgG and IgM classes more often and in higher concentrations than the non-atopics, thus having the prerequisites for immune complex reactions. Although not proven, it seems likely that decreased complement levels reflect an activation of the complement system. The lack of correlation between such decrease and symptoms of clinical allergy indicates that formation of immune complexes and activation of complement might occur as a physiological phenomenon in childhood, not necessarily followed by symptoms.[2]

Induction of tolerance

The majority of children outgrow their cow's milk (cow's milk allergy become tolerant) by 3 to 4 years of age. However 15% of infants with IgE-mediated CMA retain their sensitivity into the second decade.[19] Recently, it was able to identify 6 IgE-binding sites on cow's milk protein that differentiate patients with persistent cow's milk allergy (CMA) and those with transient CMA. The presence of IgE antibodies against at least 1 of the 3 of these epitopes (AA123-132 on as1-casein, AA171-180 on as2-casein, 155-164 on k-casein) might be useful as a marker of persistent CMA.[20]

The dominant response in the gut associated lymphoid tissue is suppression or tolerance. The means by which the immune system is educated to sensitization to ingested food antigens is not well understood. Early studies suggested that M cells (specialized epithelial cells overlying the Payer's patches) were the major sites of immune antigen sampling in the intestine.[21] More recent studies, however, indicate that IECs may be central APCs used for generating immunosuppression in the gut.[22] These "non-professional" APC have been shown to express MHC Class II molecules, take up soluble proteins from the apical end, transport it basolaterally, and selectively activate CD8 suppressor cells.[23] The latter appears to be regulated by non classical class I molecules (CD1d) and other novel membrane molecules that interact with CD8.[24] It has been hypothesized that soluble antigens in the gut lumen are sampled and presented primarily by IECs, leading to suppression of the immune response, whereas M cells, leading to active immunity and generation of IgA, sample particulate antigens and intact bacteria, viruses, and parasites. The development of tolerance to food has little effect on B-cell function because antibody production against food protein is a universal phenomenon in both infants and adults, which is not generally associated with hypersensitivity to the antigen. Most low-level antibodies to foods in clinically tolerant individuals are of IgG class, with high-level IgE antibodies more likely to be an indicator of a pathologic process (e.g., cow's milk allergy).[25] Ingestion of dietary proteins normally activates CD8 suppressor cells, which reside in the gut-associated lymphoid tissue, and after prolonged ingestion of antigens in the spleen.[18] Initial activation of these cells depends on the nature, dose, and frequency of antigen exposure, the host age, and possibly LPS produced by host's intestinal flora. Refeeding dietary antigens generally promotes systemic unresponsiveness of delayed-type hypersensitivity. Several studies in human subjects have demonstrated increased lymphocyte proliferation or IL-2 production after food antigen stimulation in vitro in patients with food allergy, celiac disease and inflammatory bowel disease. However, in vitro T-cell responses are commonly found in normal individual as well.

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