

Revisiting the “hygiene hypothesis” in gastrointestinal allergy

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The “hygiene hypothesis” in its original form hypothesized that infection in early childhood acquired through unhygienic contact with siblings or the mother may prevent the development of allergic disease. Several recent epidemiologic surveys showing an inverse relationship between the frequency of infectious disease and the incidence of allergic diseases lend support to this hypothesis. Allergen sensitization of the immune system can occur early *in utero* against a background of neonatal commitment to a Th2 immune response involving the production of Th2 cytokines (eg, interleukin (IL)-4, IL-5, IL-13) that are the principal mediators of allergic inflammation. Continued allergen exposure is associated with predominantly CD4⁺ Th2 cell proliferation but does not exclude a minor Th1 allergen-specific subpopulation that can be further expanded nonspecifically and polyclonally by microbial superantigens or as bystanders, by interferon-gamma (IFN- γ) and IL-2 released from Th1 effectors responding to antigens associated with infectious pathogens. Th1 cytokines can also subvert allergen-specific Th2 lymphocytes to become allergen-specific Th1 cells—a process reminiscent of the increased tendency of the maturing immune system of growing adults to mount a Th1 response to some environmental and dietary antigens. Unlike Th2 cytokines, IFN- γ and IL-2 inhibit B-cell production of IgE and, hence, delimit the capacity of mast cells to degranulate and release allergenic mediators. The ability of infectious agents through their danger signals to initiate a Th1 response that deviates the Th2 allergenic bias is the basis of the hygiene hypothesis. *Curr Opin Gastroenterol* 2002, 18:705–710 © 2002 Lippincott Williams & Wilkins, Inc.

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Abbreviations

CMP	cow's milk protein
FPIES	food protein-induced enterocolitis syndrome
IL	interleukin
TGF-β	transforming growth factor-beta
Th1	T helper 1 lymphocytes
Th2	T helper 2 lymphocytes
TNF-α	tumor necrosis factor-alpha
Tr1	T regulatory 1 (lymphocytes)

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Nature of the immune response in food allergies

Role of IgE

Food allergy and intolerance are adverse reactions to food represented by various clinical signs or symptoms that can occur within minutes to hours following ingestion of the offending ingredient [1]. These reactions can be more generalized, with involvement of the skin, nose, eyes, and gastrointestinal tract and are accompanied by symptoms including rhinitis, asthma, urticaria, dermatitis, and diarrhea. In more severe cases, anaphylactic reactions, including shock and laryngeal edema, and cardiovascular symptoms, including hypotension, cardiac dysrhythmias, and death, can occur [2]. Other gastrointestinal tract forms of food allergy include food protein-induced enterocolitis or gastroenteropathy, celiac disease, and some cases of eosinophilic gastroenteritis. Almost all of these adverse food reactions are believed to be IgE mediated [3]. For this reason, it is technically feasible to identify the allergenic protein or glycoprotein by electrophoretically separating the food component into multiple antigenic peptides based on charge and size in a gel matrix. These are then transferred to a nitrocellulose membrane and immunoblotted with sera from patients with adverse reactions to food. Probing the membrane with an enzyme-conjugated antibody against IgE will then identify the location of the allergen [4] whose identity can then be characterized by *N*-terminal sequencing. Not all food allergens are caused by ingested proteins and it has been shown that long-term supplementation of food reductone in the diet (eg, 2,5-dimethyl-4-hydroxy-3[2H]-furanone; 2%, w/w), detected in many foodstuffs including soy sauce, and hydroxyhydroquinone (1,2,4-benzenetriol; 1.2%, w/w), detected in coffee, can predispose experimental animals toward both type IV and I allergic responses [5]. As a rule, the incidence of IgE-mediated food allergies is more common in newborn and young children and only rarely in young adults (<1%) [6•].

Th2 lymphocytes and IgE production

Based on Mosman's classification [7], helper T cells capable of producing IL-4, IL-5, IL-6, and IL-13 cytokines (known otherwise as T helper 2 lymphocytes (Th2) with a CD4⁺ phenotype) provide help for B cells to secrete noncomplement fixing IgG4 and IgE antibodies. IL-4 and IL-13 are important switch factors for B cells to produce IgE, whereas IL-5 is a potent growth and differentiation factor for eosinophils. Degranulation of eosino-

phils and mast cells by IgE-mediated cross-linking of receptors cascades a series of events leading to contraction of airway smooth muscle and production of mucus, all classical symptoms of chronic allergic inflammation.

Th1 lymphocytes and cellular immunity

A second category of helper T cells, known as helper 1 lymphocytes (Th1), is able to produce interferon-gamma (IFN- γ), IL-2, and IL-12. These T cells are mainly involved in providing protective immunity toward intracellular microorganisms because of their ability to provoke an inflammatory response. This occurs by activation of B cells to produce complement-fixing and opsonizing antibodies that activate the microbial effector functions of macrophages associated with inflammatory tissue damage.

Role of Th2 cells in allergy

T-cell activation by specific food proteins appears to be the etiologic basis of a number of gastrointestinal disorders, including allergic eosinophilic gastroenteritis and food protein-induced enteropathy associated with milk hypersensitivity. Such involvement can be studied by examining the cytokine profiles of milk-specific lymphocytes isolated from the duodenal lamina propria from children with milk-induced gastrointestinal disease [8••]. Duodenal biopsy specimens were obtained from 10 patients with allergic eosinophilic gastroenteritis, food protein-induced enteropathy, or both, and also from 12 control subjects. Tissues were mechanically minced and cultured with either mitogens (*ie*, polyclonal T-cell expansion) or milk proteins (*ie*, milk-specific T-cell expansion). By using flow cytometry, expanded T cells were phenotyped with anti-CD4, anti-CD8. Th1 and Th2 profiles were obtained by intracellular staining with monoclonal antibodies against IFN- γ and anti-IL-4 and anti-IL-5, respectively. The milk specificity of the lines was evaluated by lymphocyte proliferation assay using milk proteins. In addition, the release of Th1, Th2, and Th3 cytokines was determined after restimulation. In patients and control subjects, polyclonal expansion of mucosal lymphocytes resulted in predominantly Th1 cells. Milk-specific mucosal T-cell lines could be established in 60% of the patients but in none of the control subjects. In contrast to the polyclonal expansion of T cells, the milk-specific expansion of mucosal T cells showed a clear Th2 cytokine profile. On restimulation with milk protein, these cells showed a high proliferative response and released Th2 cytokines, predominately IL-13, but failed to release Th3 cytokines important in the development of oral tolerance. The release of Th2 cytokines after stimulation of milk-specific mucosal T cells is in agreement with a pathogenic role in inflammatory changes seen associated with milk-induced gastrointestinal disorders. The finding that polyclonal T-cell expansion resulted in Th1 lymphocytes in contrast to allergen cow milk protein-expanded lymphocytes

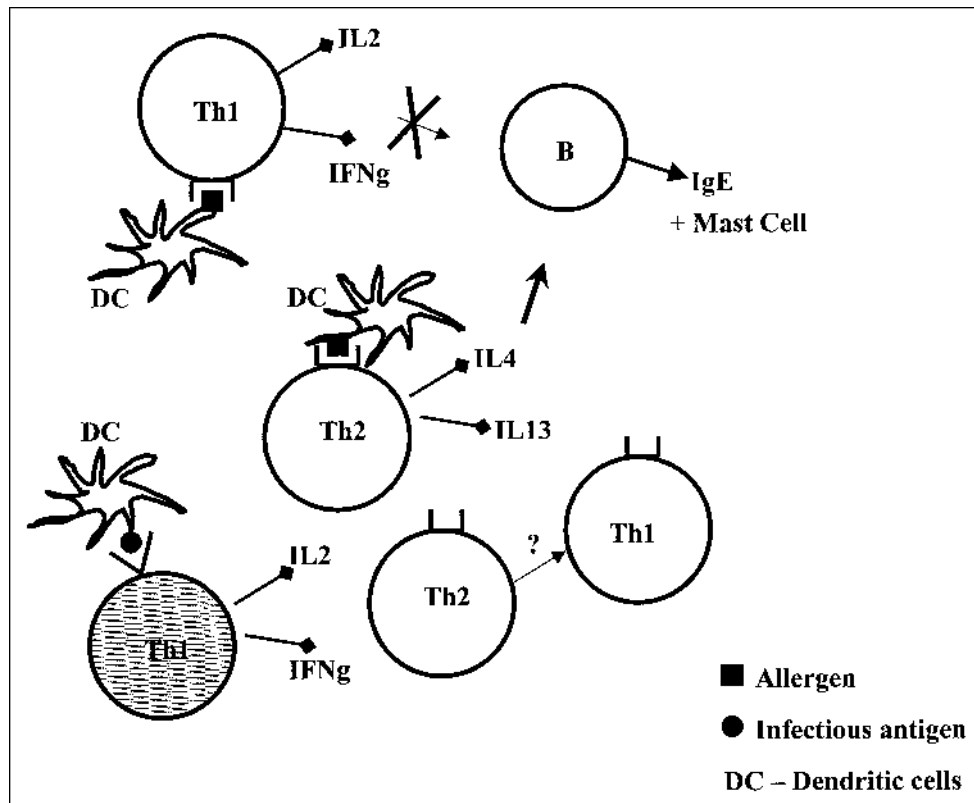
with a Th2 profile is an important observation and highlights a little known fact that Th1 and Th2 responses are not mutually exclusive. Also, it is likely that the Th2 bias manifested in clinical allergies can be sustained by the continued presence of the allergen and its presentation by antigen presenting cells (Fig. 1) in the mucosa.

The perceived Th2 bias of T cells in regional biopsies of the gastrointestinal tract is also reflected by a similar bias in circulating lymphocytes [9]. Cow's milk protein (CMP)-specific T-cell clones were established from blood of infants with cow's milk allergy (CMA) and atopic dermatitis, from atopic controls with atopic dermatitis but without CMA, and from nonatopic controls. T-cell clones were also established from infants with CMA after they had spontaneously developed tolerance to cow's milk. T-cell clones were analyzed for flow cytometry for phenotypic expression of markers for T-cell activation—CD25, CD26, and CD30. Cow's milk protein-specific T cells from infants with CMA expressed much higher levels of CD25 and CD30 than CMP-specific T cells from infants without CMA. Expression of CD26 was much lower than in normal controls. After development of tolerance for cow's milk, expression of CD25 and CD30 was decreased, whereas the expression of CD26 was increased to normal levels. It was concluded that antigen-specific T cells from patients with food allergy display an increased expression of cell-surface markers of activation compared with cells of patients without food allergy. This suggests an intrinsically stronger food-specific T-cell response in patients with food allergy, and points to the key role of food-specific T cells in the pathogenesis of food allergy.

Th3 cytokines in food allergy

Tumor necrosis factor-alpha (TNF- α) secreted by activated T cells is known to increase intestinal permeability, whereas transforming growth factor-beta (TGF- β) has the ability to protect the epithelial barrier. To investigate a role for these cytokines in the pathogenesis of food protein-induced enterocolitis syndrome (FPIES), Chung *et al.* [10] determined the expression of TGF- β 1, its receptors, and TNF- α on the mucosa of small intestine of 28 infants diagnosed with FPIES by clinical criteria and challenge test results. On the basis of immunologic staining, TGF- β 1 expression was generally depressed in patients. Expression of type 1 TGF- β receptor was significantly lower in the patients who had villous atrophy compared with those patients who did not ($P < 0.001$) and negatively correlated with the severity of atrophy ($r = -0.59$, $P < 0.001$). Expression of type 2 TGF- β receptor showed no significant difference between the patients with or without villous atrophy. The immunoreactivity for both TGF- β receptors on lamina propria cells was slight or negative. TNF- α expression was detected on both epithelial and lamina propria cells and was significantly greater in the patients who had

Figure 1. T helper 2 lymphocytes (Th2) immune deviation by cytokines produced by the T helper 1 (Th1) lymphocytes responding to allergen or infectious antigen presented by dendritic cells



villous atrophy compared with patients who did not ($P < 0.01$). Villous atrophy arising from FPIES may be caused by reduced levels of TGF- β 1, a cytokine with an important role countering the activity of T-cell cytokines. The significantly lower expression of type 1 TGF- β receptor compared with type 2 receptor suggests the differential contribution of each receptor to the diverse biologic activities of TGF- β in the intestinal epithelium.

The CD4⁺ T cells that specifically secrete TGF- β represent another class of T regulatory lymphocytes referred to as Th3 that are capable preventing T cell-mediated diseases [11].

Genesis of the "hygiene" hypothesis

Compared with eastern Europe, accumulating epidemiologic evidence over the last decade have increasingly shown a distinctive trend in the West toward a higher incidence of atopic disorders [12••]—the phenomenon of the East-West Gradient (Table 1). The International Study of Asthma and Allergies in Childhood (ISAAC) with supporting surveys from the European Community Respiratory Health Survey (ECRHS) have noted up to a 20 times variation in the prevalence of self-reported symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema [13]. An important factor in this trend appears to be crowding and sibship size, where the number of siblings is inversely related to the prevalence of

Table 1. Prevalence of atopic diseases in eastern and western Europe

Condition	West (% children) West Germany	East (% children) East Germany	Significance
Hay fever*	8.6	2.7	$P < 0.001$
Asthma*	9.3	7.2	$P < 0.5$
Airway hyperresponsiveness*	8.3	5.5	$P < 0.001$
Skin prick test*	36.7	18.2	$P < 0.001$
	Sweden	Estonia/Poland	
Atopic sensitization [†]	30.3	10.9/13.6	$P < 0.001$

*Surveyed children aged 9–11 y. [†]Surveyed children aged 10–12 y. Data from [12••].

self-reported inhalant allergy, atopic eczema, skin test reactivity, and the presence of specific IgE antibodies in the serum in children, adolescents, and adults [14]. A large Australian survey also found, for youngest children, having two siblings rather than one was associated with a significantly lower odds ratio for asthma and hay fever. In addition, a short interbirth interval to the next older sibling was associated with a lower rate of hay fever [15]. Strachan [16] first noted, more than a decade ago, that birth order is important in the incidence of allergies, which led to the hygiene hypothesis: “infection in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally from a mother infected by contact with her older children” may prevent the development of allergic disease.

Three important concepts are implicit in the hygiene hypothesis. First, epidemiologic associations asserting an inverse relationship between infection and allergy apply primarily to neonates and children in whom an already known and proven developmental bias exists toward a Th2 immune response. Second, exposure and infection in these individuals to bacteria or viruses via the respiratory or gastrointestinal tract can elicit a Th1 response. Third, the elicitation of a Th1 response by infectious agents can diminish the capacity of the Th2 mucosal immune response to allergens even under conditions in which no Th1 engagement in an ongoing and active infection exists. In short, the consequence of the hygiene hypothesis is that manifestation of the atopic phenotype may be suppressed by reversing the Th2 bias toward a Th1 response.

Inherent bias toward a Th2 mucosal immune response

Although environmental and genetic factors predispose atopic individuals toward an allergic phenotype, it is not clear exactly how and when allergen reactive T cells are triggered after birth. One school of thought is that exposure of neonates to allergens during the window of sensitization at infancy commits T cells toward an allergen-reactive repertoire of Th2 memory cells (Fig. 1) that can be further expanded on exposure in adult life. In contrast, nonatopic individuals have the capacity to switch the Th2 bias toward a Th1 response against environmental and dietary allergens. An interesting approach used by Holt's group has shown that the “sensitization window” may actually occur *in utero* and is expressed at birth. Cord blood derived mononuclear cells were collected from newborn infants and expanded *in vitro* by lymphoproliferation in the presence of a number of well-known allergens, including dust mite and rye grass extracts. Expanded cord blood derived mononuclear cells produced cytokines comprising IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, all characteristic of a Th2 bias and in keeping with the need for the fetomaternal interface to be skewed away from possible allogeneic rejection

mechanisms facilitated by a Th1 response [17]. Indeed, more recent work from the same laboratory [18] has shown that CpG and non-CpG sites at the IFN- γ promoter of cord blood derived CD4⁺/CD45RO⁺ T-cell subset are highly methylated and produce, on stimulation, significantly less IFN- γ than a similar adult subset. This difference in methylation of the IFN- γ promoter was not present between neonatal and adult CD8⁺ and natural killer cells, thereby providing further evidence of an evolutionary safeguard against errant IFN- γ gene expression and the need for a Th2 bias.

Infection and the Th1 response

Experiments have not yet been carried out to demonstrate that animals challenged with nonlethal doses of enteric or respiratory pathogens in early life are more resilient to experimentally induced allergies. However, most pathogens and, especially, virulent intracellular microorganisms, carry danger signals that are capable of engaging TOLL receptors of the innate immune system [19••] to activate a Th1 response. This phenomenon has been experimentally demonstrated in mice by oral feeding of certain *Lactobacillus* strains [20]; by using heat-killed *Listeria monocytogenes* as an adjuvant [21], or by oral delivery of a lyophilized extract consisting of eight common respiratory pathogens [22••] (*Haemophilus influenza*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus viridans*, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Staphylococcus aureus* and *Moraxella catarrhalis*). Th1 polarization and inhibition of allergy has also been successfully achieved by chronic mucosal exposure to recombinant *Lactobacillus plantarum* expressing a recombinant and immunodominant T-cell epitope (Der p 1) of house dust mites [23••]. Intradermal injection of killed *Mycobacterium vaccae* was associated with an improvement in atopic dermatitis in children with moderate to severe disease [24•]. From accumulating evidence in the literature, it appears vaccination with bacteria components can provide a practical therapy for atopic disorders [25••].

Role of microbes in subverting the Th2 response

As mentioned, although allergen-driven Th2 immune responses can occur predominantly, it does not necessarily exclude the induction of Th1 effector lymphocytes. It is this small population that may be inadvertently expanded by bacteria and viral infections. A likely mechanism could involve the activation of lymphocytes by polyclonal mitogens associated with microorganisms or even superantigens produced by pathogenic bacteria and viruses. Under these conditions, allergic Th2 responders would be bystanders that become subjected to modulation by Th1 cytokines produced by Th1 lymphocytes activated by infectious antigens (Fig. 1). Some of these allergen-reactive Th2 responders may even be modulated to become allergen-reactive Th1 lymphocytes, a scenario mimicked in the maturation process of the immune system as adults outgrow their childhood allergies.

Genetic limitations of the hygiene hypothesis

A limitation of the hygiene hypothesis is that it does not take into consideration genetic factors that are associated with allergic predisposition (*eg*, the relationship between polymorphism in the IL-4R α [Q576R] gene and atopy) [26]. More recently, Tamura *et al.* [27•] reported an association between allergic disease and an increased incidence of GT-dimer repeats in A1 and A3 alleles of the STAT-6 gene, which normally plays a crucial role in the production of Th2 cytokines. It may be that a subset of atopic individuals with genetic correlates of allergic predisposition may be resilient to microbial subversion of Th2 responses and perhaps even to immunotherapy.

Immunotherapy

The classical form of allergen immunotherapy involves intradermal or subcutaneous administration of increasing doses of allergen. This can reduce clinical symptoms and allergen-specific Th2 cytokine production when further challenged with allergen. Although the protocol is rarely used for food allergies, it appears to be beneficial for the treatment of rhinitis and insect venom allergy but is not as effective in allergic asthma. Occasionally, such therapy can induce severe systemic reactions when allergen-specific IgE cross-links with mast cells. Using a murine model of ovalbumin sensitized allergy, Janssen *et al.* [28] found that a single modified amino acid in the sequence ISQAVHAAHAEI-A-EAGR, was more efficient in skewing T lymphocytes toward the production of Th2 cytokines compared with the wild type ovalbumin allergenic epitope ISQAVHAAHAEINEAGR. In contrast, another modification in the same polypeptide (ISQAVHAAHAEIN-A-AGR) significantly altered the response toward Th1 and decreased airway eosinophilia and production of both ovalbumin-specific IL-4 and IL-5. The significance of this work is depicted in Figure 1 and shows how an allergen epitope-specific Th2 lymphocyte can be deviated into a similar allergen epitope-specific Th1 effector.

T regulatory cells and tolerance in allergy

Other mechanisms exist for suppressing the production of Th2 cytokines. CD4⁺ T cells stimulated to proliferate in the presence of IL-10 differentiate into T regulatory 1 (Tr1) lymphocytes that continue to produce high levels of IL-10 (but not IL-2 or IL-4) capable of suppressing IL-4-induced IgE synthesis [29•]. Passive transfer experiments have also shown that Tr1 cells and the IL-10 they produce can inhibit IL-5-mediated eosinophilia. An enigmatic problem in allergy is that mucosal exposure to environmental and food antigens should lead primarily to tolerance or immune suppression and not activation. Feeding ovalbumin to different mouse strains attenuated their allergic response to intraperitoneal sensitization with alum adjuvanted ovalbumin [30]. In this model, allergen-specific unresponsiveness was characterized by reduced airway eosinophilic inflammation, reduced air-

way hyperreactivity and mucus production, low levels of Th2 cytokines in bronchoalveolar lavage fluid, and decreased IgG1 and IgE antibodies against ovalbumin. Interestingly, the unresponsive state was not associated with increased levels of suppressive cytokines IL-10 and TGF- β or with immune deviation toward the Th1 pathway because of increased levels of IFN- γ and IL-12. Indeed, oral feeding with ovalbumin before priming exacerbated clinical asthma when mice were further challenged with the allergen. These findings suggest that still other immune mechanisms may exist within the population and that it would be simplistic to believe that any one specific treatment will effectively prevent allergies.

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