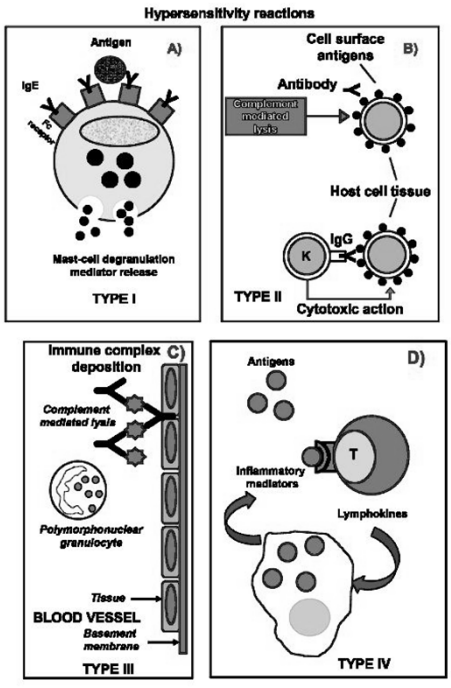
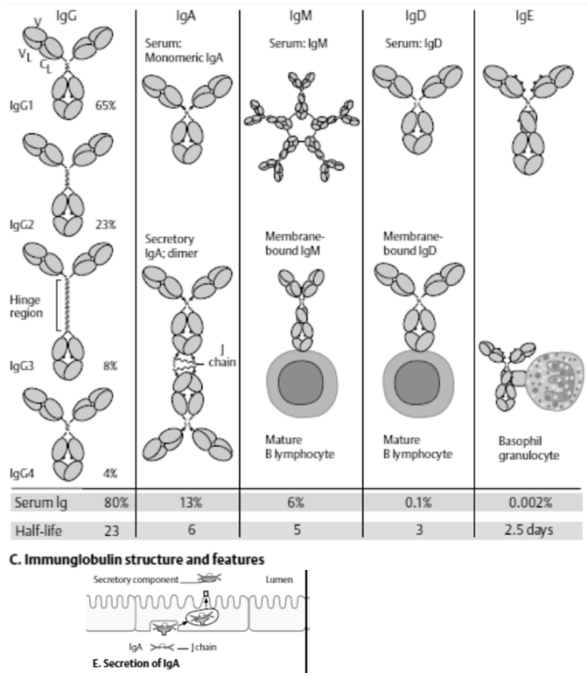
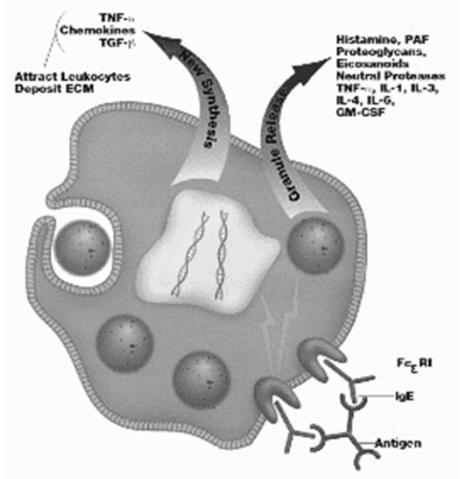
Stati patologici determinati dal sistema immunitario:

Ipersensibilità	(risposta esagerata)
Autoimmunità	(risposta rediretta)
Immunodeficienza	(risposta non sufficiente)

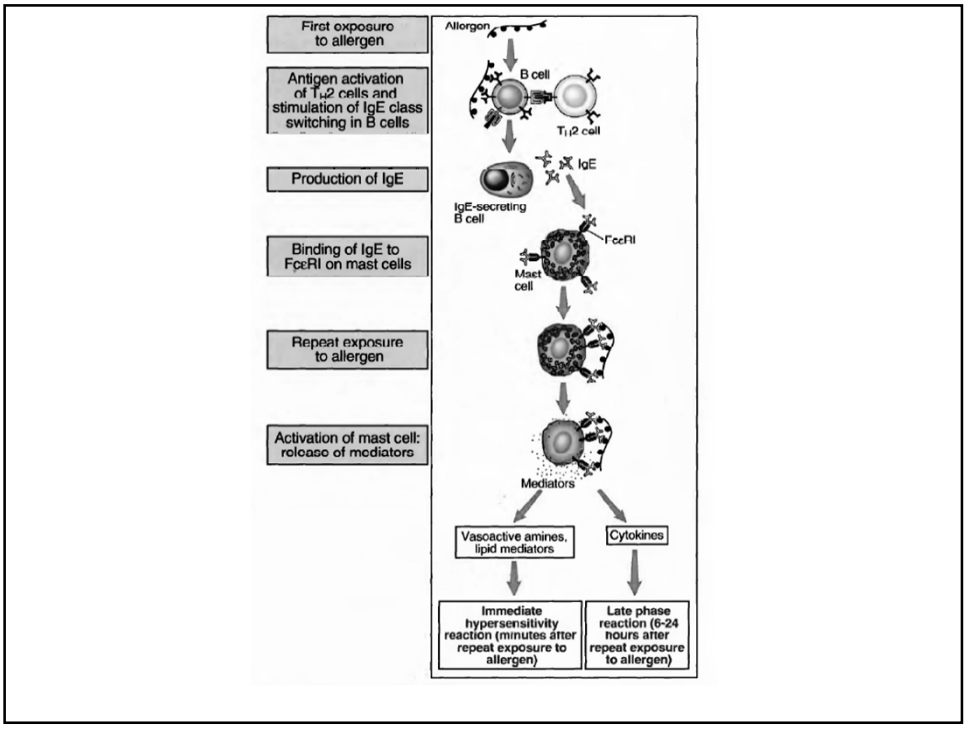
Ipersensibilità

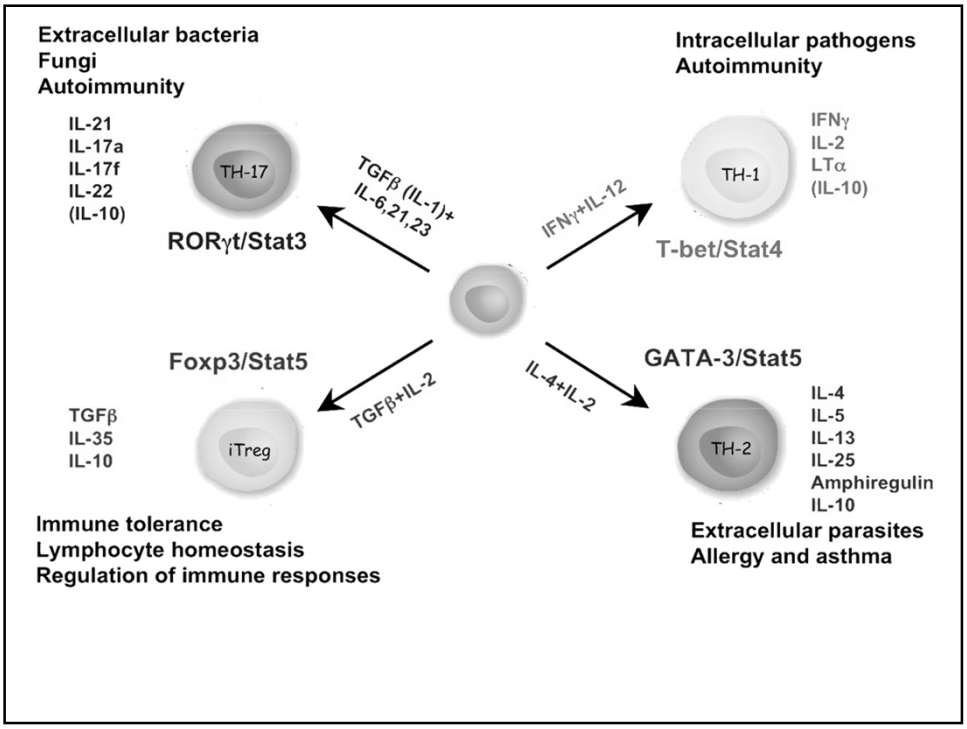






Le IgE sono presenti nel siero in modesta concentrazione, sono formate da due catene pesanti chiamate ε, 20000 dalton più pesanti delle catene γ delle IgG e di conseguenza presentano un ulteriore dominio. Le IgE sono responsabili delle allergie pertanto possono trovarsi negli individui allergici in elevata concentrazione. L'ulteriore dominio permette il legame alla superficie delle mastcellule, ciò comporta una reazione che porta alla liberazione di sostanze farmacologicamente attive come istamina e serotonina causa di dilatazione capillare, alterazione della permeabilità e costrizione bronchiale


First exposure to allergen
Antigen activation of TH2 cells and stimulation of IgE class switching in B cells
Production of IgE
Binding of IgE to FcεRI on mast cells
Repeat exposure to allergen
Activation of mast cell: release of mediators
Allergen
B cell
TH2 cell
IgE
IgE-secreting B cell
FcεRI
Mast cell
Mediators
Vasoactive amines, lipid mediators
Cytokines
Immediate hypersensitivity reaction (minutes after repeat exposure to allergen)
Late phase reaction (6-24 hours after repeat exposure to allergen)


Extracellular bacteria
Fungi
Autoimmunity
IL-21
IL-17a
IL-17f
IL-22
(IL-10)
TH-17
RORγt/Stat3
TGFβ (IL-1)+ IL-6,21,23
IFNγ+IL-12
Intracellular pathogens
Autoimmunity
IFNγ
IL-2
LTα
(IL-10)
TH-1
T-bet/Stat4
Foxp3/Stat5
TGFβ
IL-35
IL-10
iTreg
TGFβ+IL-2
IL-4+IL-2
GATA-3/Stat5
IL-4
IL-5
IL-13
IL-25
Amphiregulin
IL-10
TH-2
Immune tolerance
Lymphocyte homeostasis
Regulation of immune responses
Extracellular parasites
Allergy and asthma

SINTOMI DI REAZIONI ALLERGICHE AGLI ALIMENTI

Respiratori

Naso che cola o congestione nasale
Starnuti
Asma (difficoltà a respirare)
Tosse
Respiro affannoso-sibilante

Cutanei

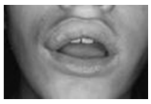
Gonfiore di labbra, bocca, lingua, faccia e/o gola (angioedema)
Orticaria
Eruzioni cutanee o rossori
Prurito
Eczema

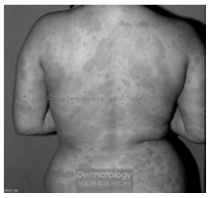
Gastrointestinali

Crampi addominali
Diarrea
Nausea
Vomito
Coliche
Gonfiore

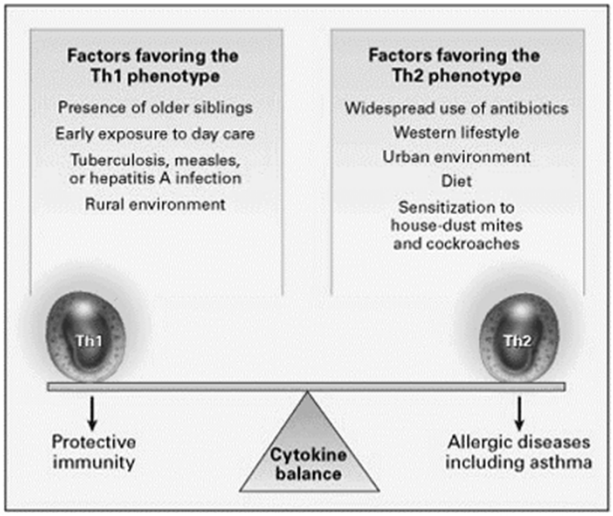
Sistemici

Shock anafilattico (grave shock generalizzato)





Fattori influenzanti il fenotipo linfocitario Th1 e Th2 (ipotesi dell'eccessivo igiene come favorente le patologie allergiche)



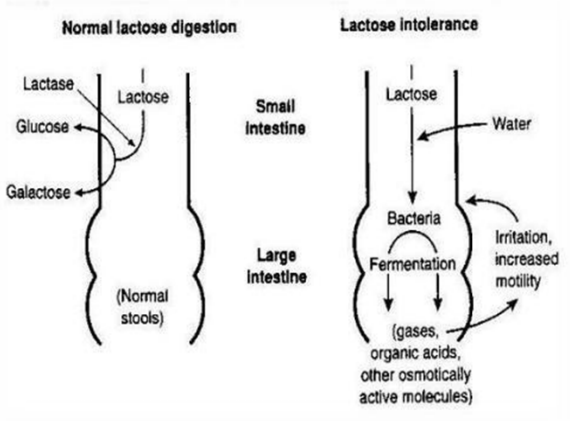
Qual è la differenza tra allergie e intolleranze alimentari?

La reazione negativa al cibo è spesso erroneamente definita allergia alimentare. In molti casi è provocata da altre cause come un'intossicazione alimentare di tipo microbico, un'avversione psicologica al cibo o un'intolleranza ad un determinato ingrediente di un alimento.

L'allergia alimentare è una forma specifica di intolleranza ad alimenti o a componenti alimentari che attiva il sistema immunitario. Un allergene (proteina presente nell'alimento a rischio che nella maggioranza delle persone è del tutto innocua) innesca una catena di reazioni del sistema immunitario tra cui la produzione di anticorpi. Gli anticorpi determinano il rilascio di sostanze chimiche organiche, come l'istamina, che provocano vari sintomi: prurito, naso che cola, tosse o affanno. Le allergie agli alimenti o ai componenti alimentari sono spesso ereditarie e vengono in genere diagnosticate nei primi anni di vita.

L'intolleranza alimentare coinvolge il metabolismo ma non il sistema immunitario. Un tipico esempio è l'intolleranza al lattosio: le persone che ne sono affette hanno una carenza di lattasi, l'enzima digestivo che scompone lo zucchero del latte.

Lactose Intolerance



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No single major allergen has been identified in cow's milk according to either challenge tests or laboratory procedures. Indeed, clinical challenge tests demonstrate that most CMA patients react to several protein fractions of cow's milk and each allergenic protein may have several epitopes, which are widely spread along the molecules. The cow milk proteins prevalently implicated in allergic responses in children are the whey proteins α-Lactalbumin (α-La)(Bos d 4) and β-Lactoglobulin (β-Lg) (Bos d 5), in addition to the casein (CN) fraction (Bos d 8). In adults, the predominant allergen is CN, whereas sensitization to whey proteins is rare.

Cow's Milk Proteins (100%)	Protein	Allergen Name	Allergenicity	Total Protein %	MW (kDa)	pI	Amino Acid Residues	Calcium sensitivity	Phosphate groups
Caseins (80%)	α_{s1}-Casein	Bos d 8	Major	32	26.6	4.9 - 5.0	199	+++	8-9
	α_{s2}-Casein	"	"	10	25.2	5.2 - 5.4	207	++++	10-13
	β-Casein	"	"	28	24,0	5.1 - 5.4		++	4-5
	γ1-Casein	"	"	Traces	20,5	5.5	181	+	1
	γ2-Casein	"	"	Traces	11,9	6.4	104		
	γ3-Casein	"	"	Traces	11.5	5.8	102		
	κ-Casein	"	"	10	19	5.4 - 5.6	169		
Whey proteins (20%)	α-Lactalbumin	Bos d 4	Major	5	14.2	4.8	123		
	β-Lactoglobulin	Bos d 5	Major	10	18.3	5.3	162		
	Immunoglobulins	Bos d 7	–	3	150	–	–	–	1-3
	BSA	Bos d 6	–	1	66.3	4.9 - 5.1	582		
	Lactoferrin	–	–	Traces	80	8.7	703		

- **Arachidi**
- **Cereali che contengono glutine**
- **Crostacei**
- **Bisolfito (usato come antiossidante e conservante, per es. nella frutta secca, vino e patate conservate)**
- **Latte**
- **Lupino (un tipo di legume appartenente alla famiglia delle Febacee)**
- **Molluschi**
- **Noci**
- **Pesce**
- **Sedano**
- **Semi di sedano**
- **Senape**
- **Soia**
- **Uova**

I più comuni allergeni alimentari

Tutti gli alimenti possono potenzialmente causare allergie, tuttavia, in Europa sono **14** gli allergeni che presentano i maggiori rischi allergici e che sono perciò soggetti a etichettatura legislativa.

Valori di soglia

Del 3-4% di adulti e del 5-8% di bambini che soffrono di allergie alimentari, esiste un alto grado di variabilità su come molti allergeni debbano essere presenti in un alimento per scatenare una reazione allergica. La minima concentrazione di allergene in grado di scatenare una reazione allergica viene definita **soglia**. A causa delle notevoli differenze nei valori soglia tra gli individui, attualmente è molto difficile identificare un valore universalmente valido **per stabilire la massima concentrazione di allergene presente in un alimento che, se ingerito, non causi una reazione avversa**. Un importante traguardo della ricerca per trovare una soluzione a questo problema è sviluppare la capacità di prevedere la gravità delle reazioni negli individui.

Legislazione della Unione Europea (UE)

Attualmente, non esiste una cura per l'allergia alimentare, se non evitare di ingerire cibo contenente gli allergeni. Per assicurare il corretto livello di informazione, la Commissione Europea (CE) ha stabilito che i maggiori 14 potenziali allergeni (vedi Tabella) debbano essere chiaramente indicati sull'etichetta di tutti i cibi preconfezionati, quando essi o qualunque ingrediente fatto da essi vengano usati a qualsiasi livello (eccetto per il bisolfito che è esente da dichiarazioni quando in concentrazioni minori di 10mg/kg).

Table 1
Major food allergens

Food allergen family	Food source	Allergen examples
Animal food protein families		
Caseins	Mammalian milk	αs1, αs2, β, κ-casein — cows' milk
EF-hand proteins (mainly parvalbumin)	Fish	Gad c 1 — cod
Tropomyosin	Crustaceans and mollusks	Pen a 1 — shrimp
Plant food protein families		
Bet v 1 superfamily	Fruits, vegetables, soy	Gly m 4 — soy; Mal d 1 — apple
Cupin superfamily		
7S globulin	Peanut, tree nuts, legumes, seeds	Ara h 1 — peanut; β-conglycinin — soy
11S globulin	Peanut, tree nuts, legumes	Ara h 3 — peanut; glycinin — soy
Cysteine protease C1 family	Soy, kiwi	Gly m 1 — soy
Profilins	Fruits, vegetables, legumes	Ara h 5 — peanut Api g 4 — celery
Prolamin superfamily		
Prolamins	Cereals	α- and γ-gliadin — wheat
Nonspecific lipid-transfer proteins	Fruits and vegetables	Mal d 3 — apple Pru p 3 — peach Cor a 8 — hazelnut
α-Amylase/trypsin inhibitors	Barley and rice	Hor v 1 — barley
2S albumins	Peanut, tree nuts, seeds	Ara h 2 — peanut

In Switzerland, hazelnut allergy is the most common food allergy in adults and is due to cross-reacting IgE to a birch pollen pathogenesis-related protein

In Israel, where sesame is incorporated prominently in the diet, sesame seed is the most common cause of anaphylaxis in young children, and peanut allergy is rare

In Singapore, edible bird's nest soup is commonly implicated in pediatric food allergy, while in Japan, buckwheat (grano saraceno) is an important cause of food allergy in school-age children

Table 8 International food allergen labeling requirements [119–121]

Country/block	USA	European Union	Australia–New Zealand	Canada	Japan
Cow's milk	Yes	Yes	Yes	Yes	Yes
Hen's egg	Yes	Yes	Yes	Yes	Yes
Wheat	Yes	Yes	Yes	Yes	Yes
Soy	Yes	Yes	Yes	Yes	No
Peanut	Yes	Yes	Yes	Yes	Yes
Tree nuts	Yes	Yes	Yes	Yes	No
Fish	Yes	Yes	Yes	Yes	No
Crustaceans	Yes	Yes	Yes	Yes	No
Molluscs	No	No	Yes	Yes	No
Sesame seed	No	Yes	Yes	Yes	No
Mustard seed	No	Yes	No	No	No
Celery	No	Yes	No	No	No
Buckwheat	No	No	No	No	Yes

The gastrointestinal mucosal immune system must **constantly** analyze antigenic information and respond appropriately to **pathogens**, **commensals**, and **food antigens**. These responses require a **complex immunoregulatory network**, of which the hallmark is the **induction of oral tolerance**. Both **host-** and **antigen-specific** properties, as well as **dietary** and other **environmental factors**, are important in determining the proper adaptive immune response.

1) BREAKING TOLERANCE: THE INDUCTIVE PHASE OF FOOD ALLERGY

- Physico-chemical characteristics of potential allergenes
- Increased intestinal permeability
- Antigen dose and timing of exposure
- Nutritional/dietary factors
- Genetic predisposition
- Cutaneous/airway sensitization to food allergens (?)
- Intestinal microbiome

2) EFFECTOR MECHANISMS OF FOOD ALLERGY

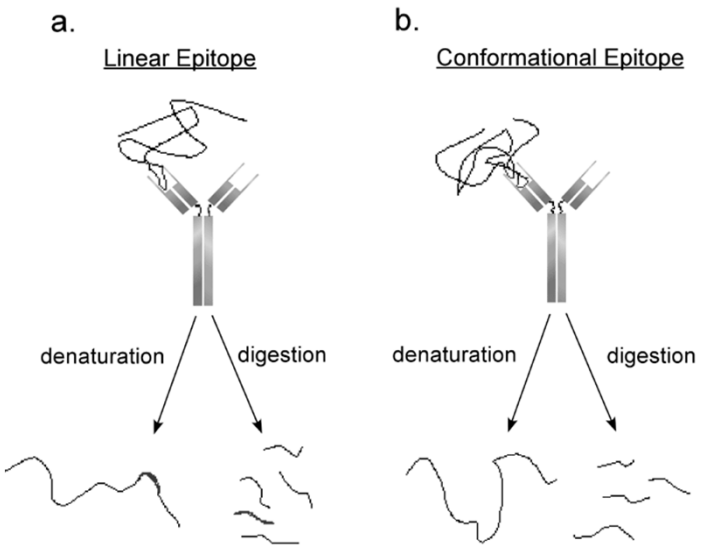
- Antigen uptake
- Local manifestations of food allergy
- Systemic manifestations of food allergy
- Mechanisms of systemic anaphylaxis

1) BREAKING TOLERANCE: THE INDUCTIVE PHASE OF FOOD ALLERGY

•Physico-chemical characteristics of potential allergenes

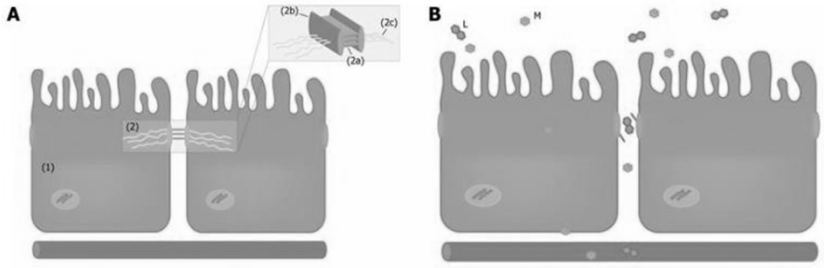
TABLE I. Biochemical factors that promote allergenicity

Molecular weight <70 kd
Glycosylation
Resistance to thermal or chemical denaturation
Abundance in food source
Linear epitopes
Solubility in water



1) BREAKING TOLERANCE: THE INDUCTIVE PHASE OF FOOD ALLERGY

- Increased intestinal permeability



Intestinal permeability was assessed in infants with food allergy by calculating the urinary ratio after ingestion of freely diffusible mannitol (M) and normally unabsorbed lactulose (L). **Infants with food allergy** were noted to have a **lower ratio (M/L)**, indicating **increased intestinal permeability**, when compared with normal healthy young children.

1) BREAKING TOLERANCE: THE INDUCTIVE PHASE OF FOOD ALLERGY

- Antigen dose and timing of exposure

In murine models **high-dose** exposure to antigen **in early life**, even a single isolated dose, can produce **lymphocyte anergy**, whereas **low-dose exposure**, especially when repeated, induces **Treg cell development**.

Although oral tolerance has been shown to occur across a range of doses, **frequent or continuous exposure to relatively low doses** typically **results in robust oral tolerance** induction.

Emerging evidence in human disease suggests that exposure to the proper dose of antigen during this critical period in early life is important for the shaping of the appropriate immune response to foods. Several epidemiologic studies have implicated **delayed introduction** in the **increased prevalence of peanut allergy**. Similarly, there is evidence that **delayed introduction of cereals** is associated with a **higher risk of wheat allergy**.

1) BREAKING TOLERANCE: THE INDUCTIVE PHASE OF FOOD ALLERGY

- Antigen dose and timing of exposure

Two recent studies suggest that the role of **timing** of allergen exposure may **vary for different foods**. Early **egg exposure, by 4 to 6 months of age**, appeared to be **protective** for egg allergy; in contrast, **introduction of milk** in the **first 2 weeks** of life was **protective**, while introduction between **4 and 6 months of age** was associated with the **highest risk of developing milk allergy**. While these questionnaire-based studies are subject to recall bias and/or reverse causation, they point out that studies on one food allergen may not be applicable to other foods.

Differences may also be due to **variations in the form of foods being introduced** (i.e., natural egg vs. baked egg) or the **quantity of exposure** at each age period.

1) BREAKING TOLERANCE: THE INDUCTIVE PHASE OF FOOD ALLERGY

- Antigen dose and timing of exposure

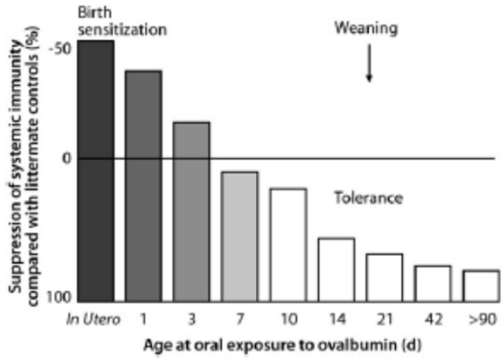


FIG 1. Immunity but not tolerance occurs after allergen exposure in early life. Neonatal mouse pups were exposed to ovalbumin through intra-amniotic injection 24 to 36 hours before birth or fed ovalbumin (1 mg/g body weight) or saline at days 1, 3, 7, 14, or 42. When rechallenged, animals exposed to ovalbumin before the seventh day of life did not have tolerance but instead robust humoral and cell-mediated immune responses, which persisted up to 14 weeks. Although it had long been known that tolerance was the default response to oral antigen administration in adult mice, these experiments demonstrated that oral exposure in early life could result in active immunologic priming rather than suppression.

1) BREAKING TOLERANCE: THE INDUCTIVE PHASE OF FOOD ALLERGY

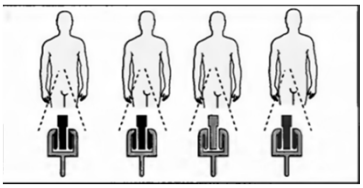
- Nutritional/dietary factors

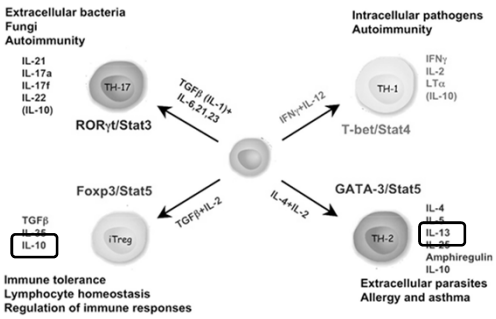
- **Breast-feeding** (?)
- **Vitamin D** (?) increases T-reg
- **ω-6 LC-PUFAs** (long-chain polyunsaturated fatty acids) may lead to the production of PGE2, which can inhibit the production of Th1 cytokines and promote synthesis of Th2 cytokines. In comparison, **ω-3 LC-PUFAs** may inhibit PGE2 synthesis.
- **Vitamin C** **vitamin E** (?)

1) BREAKING TOLERANCE: THE INDUCTIVE PHASE OF FOOD ALLERGY

- Genetic predisposition

To date, more than 10 genes have been linked to FS and/or FA in at least one single study. These genes include the HLA class II gene family (**HLA-DRB1, HLA-DQB1, HLA-DPB1**), CD14, forkhead box P3 (FOXP3) STAT6, SPINK5, **IL10**, **IL13**, NLRP3, and **FLG** genes





1) BREAKING TOLERANCE: THE INDUCTIVE PHASE OF FOOD ALLERGY

- Cutaneous/airway sensitization to food allergens (?)

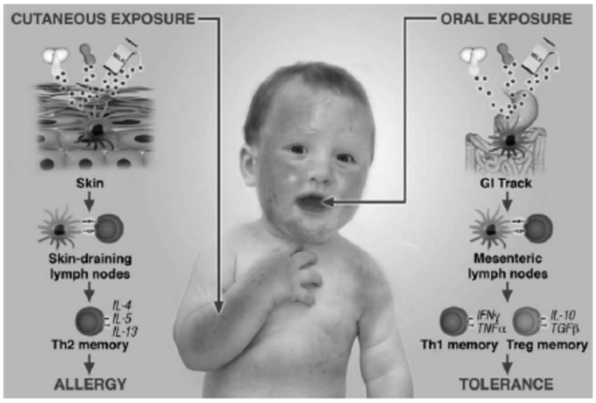


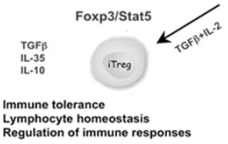
FIG 1. Dual-allergen exposure hypothesis for the pathogenesis of FA. Allergic sensitization results from cutaneous exposure, and tolerance occurs as a result of oral exposure to food. *GI*, Gastrointestinal. Reprinted with permission from Lack.[1]

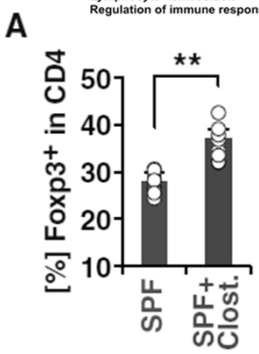
1) BREAKING TOLERANCE: THE INDUCTIVE PHASE OF FOOD ALLERGY

- Cutaneous/airway sensitization to food allergens (?)

1) BREAKING TOLERANCE: THE INDUCTIVE PHASE OF FOOD ALLERGY

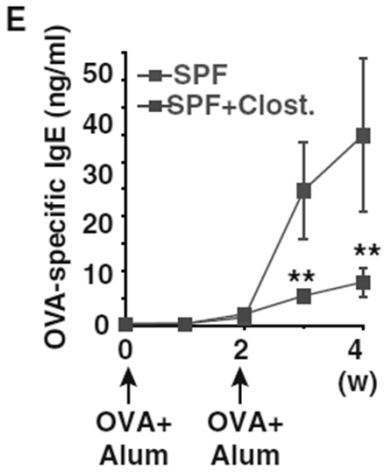
- Intestinal microbiome

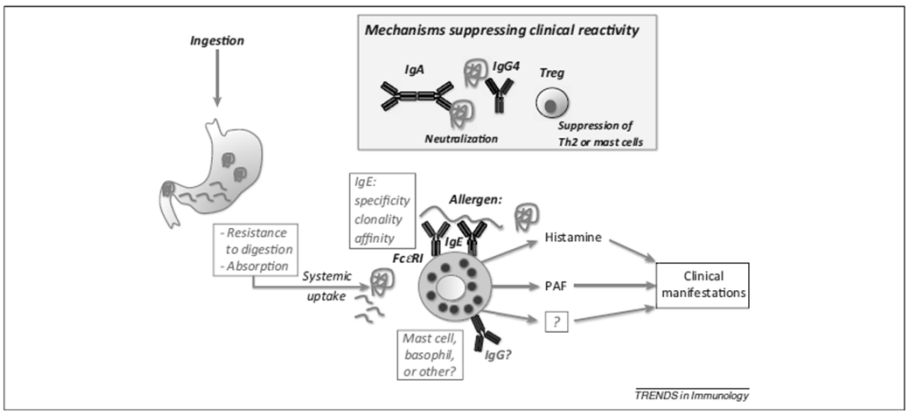


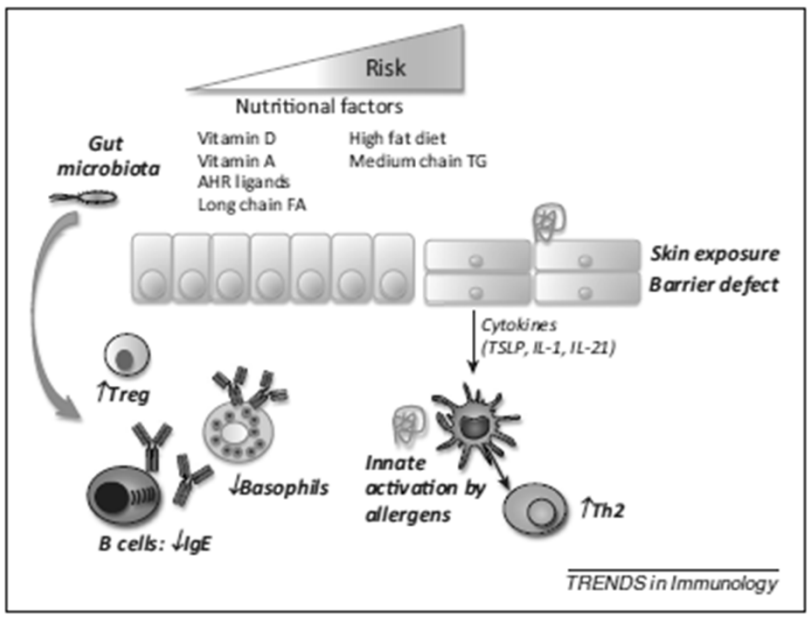


1) BREAKING TOLERANCE: THE INDUCTIVE PHASE OF FOOD ALLERGY

- Intestinal microbiome




Ingestion
Mechanisms suppressing clinical reactivity
IgA
IgG4
Treg
Neutralization
Suppression of Th2 or mast cells
IgE: specificity clonality affinity
Allergen:
IgE
FcεRI
- Resistance to digestion
- Absorption
Systemic uptake
Histamine
PAF
?
Clinical manifestations
Mast cell, basophil, or other?
IgG?
TRENDS in Immunology

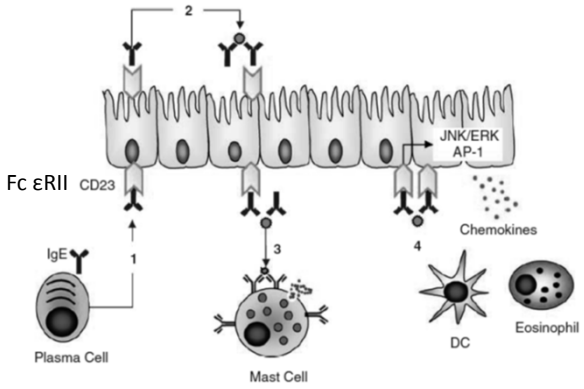

Risk
Nutritional factors
Gut microbiota
Vitamin D
Vitamin A
AHR ligands
Long chain FA
High fat diet
Medium chain TG
Skin exposure
Barrier defect
Cytokines (TSLP, IL-1, IL-21)
↑Treg
↓Basophils
B cells: ↓IgE
Innate activation by allergens
↑Th2
TRENDS in Immunology

2) EFFECTOR MECHANISMS OF FOOD ALLERGY

- Antigen uptake

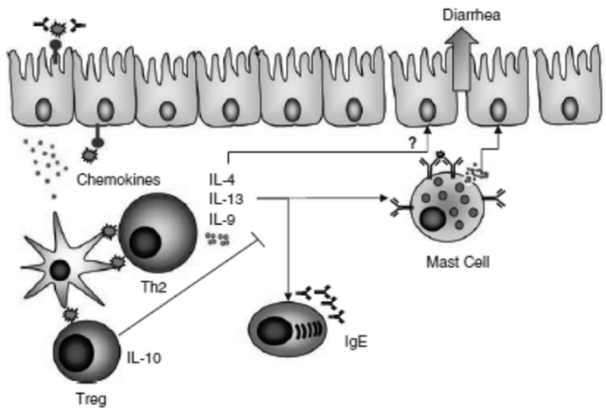
Ingestion of food allergens by food-allergic individuals leads to symptoms that can occur very rapidly after ingestion. It has not been possible to pinpoint the site of antigen absorption: it is possible that some uptake of antigen is occurring in the mouth across buccal or sublingual mucosa. The small intestine, in particular the jejunum, is thought to be the site of greatest absorption in the gastrointestinal tract.

- IgE-mediated antigen sampling across the intestinal epithelium



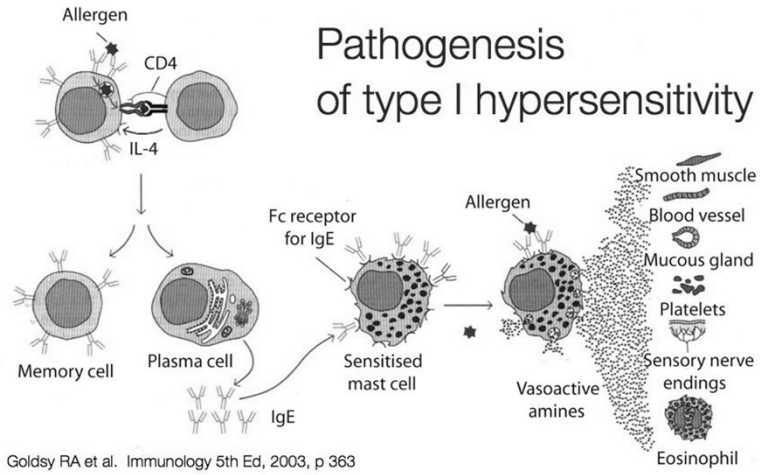
2) EFFECTOR MECHANISMS OF FOOD ALLERGY

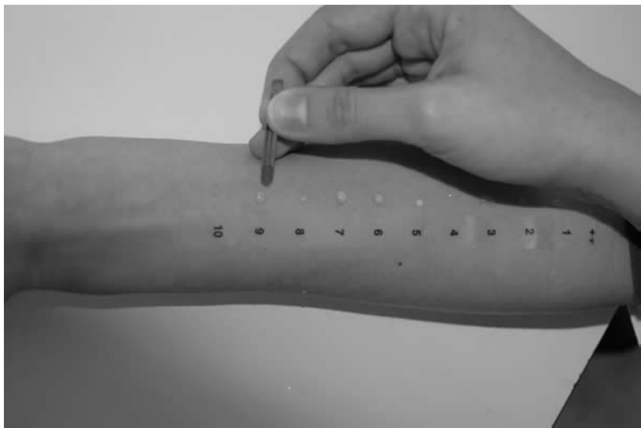
- Gastrointestinal manifestations of food allergy

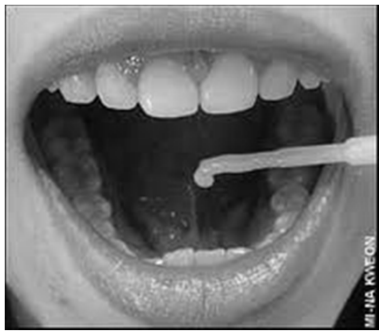


2) EFFECTOR MECHANISMS OF FOOD ALLERGY

- Systemic manifestations of food allergy
- Mechanisms of systemic anaphylaxis






MI-NA KWEON


Sublingual
Drops

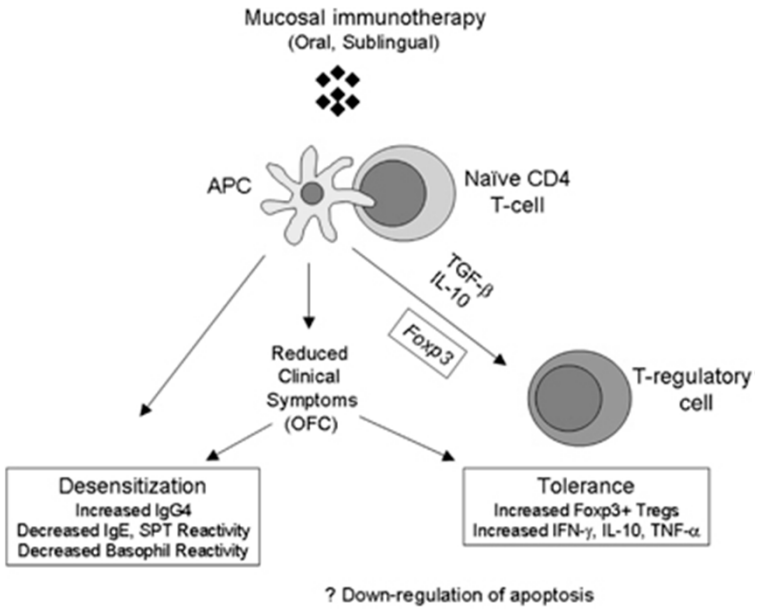

Mucosal immunotherapy
(Oral, Sublingual)
APC
Naïve CD4
T-cell
TGF-β
IL-10
Foxp3
T-regulatory
cell
Reduced
Clinical
Symptoms
(OFC)
Desensitization
Increased IgG4
Decreased IgE, SPT Reactivity
Decreased Basophil Reactivity
Tolerance
Increased Foxp3+ Tregs
Increased IFN-γ, IL-10, TNF-α
? Down-regulation of apoptosis

Table 3
Treatments for food allergy that are currently under investigation

	Mechanism	Effects	Concerns
Allergen-specific therapies			
OIT	Gradual exposure to allergens to induce desensitization or tolerance	Improved clinical tolerance; clinical trials for egg, milk, and peanut currently underway	Unclear whether the effects are desensitization or induction of tolerance; side effects are common and unpredictable
SLIT	Gradual exposure to allergens to induce desensitization or tolerance	Improved clinical tolerance	Unclear whether the effects are desensitization or induction of tolerance; side effects are common
Recombinant vaccines	Mutate IgE-binding sites; proteins stimulate T cells to proliferate, but have greatly reduced IgE-binding capacity	Protection against peanut anaphylaxis in mice; clinical trials currently underway	Improved safety profile compared with conventional IT; requires identification of IgE-binding sites for each allergen
Peptide immunotherapy	Peptide fragments contain T cell epitopes, but are not of sufficient length to cross-link IgE and therefore cannot trigger mast cell or basophil activation	Protection against peanut anaphylaxis in mice	Improved safety profile compared with conventional IT; requires identification of T cell epitopes for each allergen
ISS-conjugated protein immunotherapy	ISS bound to proteins can act as adjuvants to promote switching to a Th1 response	Protection against peanut sensitization in mice	Concern for excessive Th1 stimulation and potential for autoimmunity
Plasmid DNA immunotherapy	Allergen gene immunization to promote endogenous allergen production resulting in possible induction of tolerance	Less severe and delayed peanut-induced anaphylaxis in a murine model	Serious concerns regarding safety in view of strain-dependent effects in mice
Allergen-nonspecific therapies			
Anti-IgE	Decreases circulating free IgE, inhibits the early- and late-phase allergic response, suppresses inflammation and provides improved control for allergic diseases	Provides an improved threshold against peanut-induced reactions in 80% of treated patients	May be useful in combination with immunotherapy
Chinese herbal medicine	Inhibit Th2 immune response	Long-term protection from peanut anaphylaxis in a murine model. Also effective in murine model of multiple food allergies.	Oral, generally safe and well tolerated; phase I study completed
Cytokine/ anti-cytokine	Block proallergic cytokines	Anti–IL-5 causes reduction in tissue eosinophils, but does not induce resolution of histologic or clinical features of eosinophilic esophagitis (EoE).	Concerns for systemic side effects
TLR-9	Induction of Th1-type immune responses	Protect from peanut anaphylaxis in a murine model	Concern for excessive Th1 stimulation and potential for autoimmunity

IT, immunotherapy; ISS, immunostimulatory sequence.

Animal models for assessment of allergenicity

It is a commonly held belief that in order to be of utility an animal model must reflect all aspects of the clinical situation, including sensitization and challenge **using the oral route, production of clinically relevant symptoms on challenge, identification of similar IgE epitopes to those observed in human sera, the induction of IgE antibody, selectivity of responses for known allergens, lack of requirement for adjuvant and reproducibility of results within and between laboratories.** Another commonly held opinion is that it will **not be possible to develop useful animal models** due to the wide variation among different animal strains and species with respect to immune responsiveness to particular proteins.

A **complete recapitulation** of the human experience should **not be the goal** of animal model development in the context of safety assessment needs for novel proteins – or indeed for any other toxicological application. Rather, the objective is to provide a model that will provide useful and reliable information that when used in tandem with other relevant data will allow sound judgements to be made about the nature of likely hazards. For an animal model to be truly of value in this context there is a need to understand performance characteristics and to acknowledge limitations, particularly with respect to reliability under different circumstances.

Dearman et al. 2009

Animal models for assessment of allergenicity

Currently, several animal models of food allergy are used for these purposes, including **mouse, rat, swine and dog**. Food allergy is a complex disease, with genetic predisposition, environmental factors and exposure conditions all contributing to inter-individual differences in susceptibility. It is therefore very unlikely that a single method using experimental animals will be developed that is capable of predicting accurately all aspects of the likely prevalence, persistence and severity of food allergy among human populations exposed to a novel allergen in the diet.

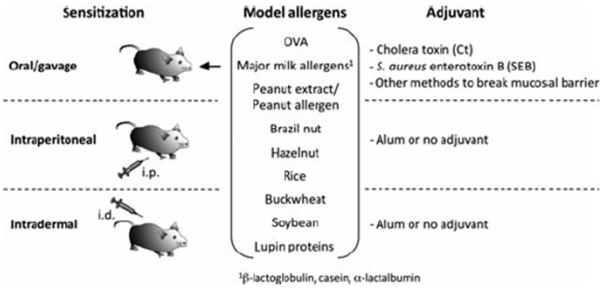


Figure 1 Food allergy models in the mouse. Many different mouse models for food allergy are in use. The biggest differences are the use of model allergens and the sensitization strategy prior to oral challenge [reviewed in (67–69)]. For oral sensitization, addition of an adjuvant (or other method to manipulate the intestinal epithelium) is needed in most cases to break tolerance in the gut. Cholera toxin (Ct) is most commonly used. However, *Staphylococcus aureus* enterotoxin B (SEB) has been shown to be effective as adjuvant and may be clinically much more relevant for human food allergy (70). Alternatively, mice are systemically sensitized (i.p. or i.d.) prior to oral challenge, resulting in anaphylactic reactions. Systemic sensitization models in the presence or absence of adjuvant (most commonly alum) are established.

Murine experimental models.
The species **most commonly favoured** with respect to animal model development is the **mouse**. This is largely driven by the availability of **various immunological and molecular reagents**, including **transgenic animals** in which particular genes of interest have been over expressed or deleted. It is generally accepted that for many aspects of immune regulation similar mechanisms are shared between man and mouse. Thus, mouse models have been used extensively for the characterization of the cellular and molecular mechanisms of various types of IgE-mediated allergic disease, including asthma to proteins or to protein detergent enzymes. In addition, a major advantage for studies involving IgE antibody responses are the availability of inbred and congenic high IgE responder mouse strains, such as the **high IgE responder BALB/c strain**. As such, this strain is analogous with the susceptible (atopic) human phenotype that has a propensity to develop IgE-mediated disease, facilitating the identification of potentially allergenic proteins. However, caution must be exercised with the interpretation of a negative IgE antibody response to a particular protein. It has been known for many years that the major histocompatibility complex (MHC) class II haplotype (H-2) among strains of mice can play important roles in the immune recognition of proteins and the development of antibody responses

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Article

Sequential class switching is required for the generation of high affinity IgE antibodies

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IgE antibodies with high affinity for their antigens can be stably cross-linked at low concentrations by trace amounts of antigen, whereas IgE antibodies with low affinity bind their antigens weakly. In this study, we find that there are two distinct pathways to generate high and low affinity IgE. High affinity IgE is generated through sequential class switching ($\mu \rightarrow \gamma \rightarrow \varepsilon$) in which an intermediary IgG phase is necessary for the affinity maturation of the IgE response, where the IgE inherits somatic hypermutations and high affinity from the IgG1 phase. In contrast, low affinity IgE is generated through direct class switching ($\mu \rightarrow \varepsilon$) and is much less mutated. Mice deficient in IgG1 production cannot produce high affinity IgE, even after repeated immunizations. We demonstrate that a small amount of high affinity IgE can cause anaphylaxis and is pathogenic. Low affinity IgE competes with high affinity IgE for binding to $Fc\varepsilon$ receptors and prevents anaphylaxis and is thus beneficial.

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Extrathymically generated regulatory T cells control mucosal T_H2 inflammation

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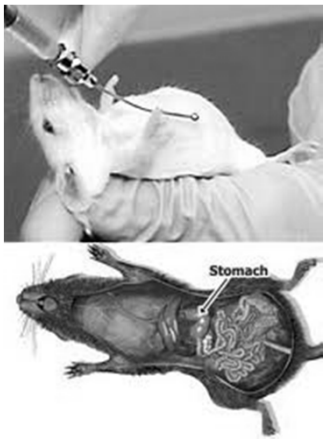
A balance between pro- and anti-inflammatory mechanisms at mucosal interfaces, which are sites of constitutive exposure to microbes and non-microbial foreign substances, allows for efficient protection against pathogens yet prevents adverse inflammatory responses associated with allergy, asthma and intestinal inflammation[1]. Regulatory T (T_{reg}) cells prevent systemic and tissue-specific autoimmunity and inflammatory lesions at mucosal interfaces. These cells are generated in the thymus (tT_{reg} cells) and in the periphery (induced (i)T_{reg} cells), and their dual origin implies a division of labour between tT_{reg} and iT_{reg} cells in immune homeostasis. Here we show that a highly selective blockage in differentiation of iT_{reg} cells in mice did not lead to unprovoked multi-organ autoimmunity, exacerbation of induced tissue-specific autoimmune pathology, or increased pro-inflammatory responses of T helper 1 (T_H1) and T_H17 cells. However, mice deficient in iT_{reg} cells spontaneously developed pronounced T_H2-type pathologies at mucosal sites—in the gastrointestinal tract and lungs—with hallmarks of allergic inflammation and asthma. Furthermore, iT_{reg}-cell deficiency altered gut microbial communities. These results suggest that whereas T_{reg} cells generated in the thymus appear sufficient for control of systemic and tissue-specific autoimmunity, extrathymic differentiation of T_{reg} cells affects commensal microbiota composition and serves a distinct, essential function in restraint of allergic-type inflammation at mucosal interfaces.

Rat experimental models.
Other rodent species, particularly the Brown Norway (BN) rat, a strain that has been characterized as mounting strong IgE antibody responses, have been the experimental model of choice for many investigators. One of the attractions of this approach is that due to the size of the species, it is possible to monitor within individual animals the kinetics of specific serum antibody (IgE and IgG) responses. In addition, oral challenge-induced responses in previously sensitized animals may be studied as a function of changes in gut permeability, respiratory functions and blood pressure. The approach employing BN rats that has attracted most interest is one in which the test protein is delivered by daily gavage over a period of some weeks in the absence of adjuvant.



Dog experimental models.
A less commonly used experimental species for protein allergenicity studies is the dog. The dog is one of the few species in which **atopic allergies develop naturally**, and canine IgEmediated food hypersensitivity is a commonly presenting complaint in veterinary surgeries. There are several general additional advantages to the use of this large animal model: the **gut anatomy and physiology and nutritional requirements are similar to humans**, it is possible to perform repeated endoscopic analysis of the gastrointestinal tract, high IgE responder animals can be identified and the large size of primary and secondary immune organs and blood volume facilitates certain analyses, including some longitudinal analyses .
However, these advantages lend themselves more readily to mechanistic studies than to the development of more routine testing strategies for safety assessment. In addition there are limited strains available and greater interanimal variation than in rodent strains, there is a **lack of commercially available immunological reagents** and such animals are expensive to maintain, often leading to studies with smaller power.

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Evaluation of a Spontaneous Canine Model of Immunoglobulin E-Mediated Food Hypersensitivity: Dynamic Changes in Serum and Fecal Allergen-Specific Immunoglobulin E Values Relative to Dietary Change

Hilary A. Jackson, BVM&S, DVD' and Bruce Hammerberg, DVM, PhD

The purpose of the pilot study reported here was to evaluate serum and fecal total and allergen-specific immunoglobulin E (IgE) responses to dietary change in five Maltese x beagle dogs with suspected food hypersensitivity, compared with those of five clinically normal dogs. Clinical arameters (pruritus, otitis, and diarrhea) improved in the Maltese x beagle dogs during feeding of a novel diet, and signs were exacerbated by oral allergen provocation.
Relative concentrations of serum and fecal wheat-, corn-, and milk-specific IgE were determined by use of an ELISA. The onset of clinical signs of disease was accompanied by an increase in serum allergen-specific IgE concentrations. In contrast, changes in clinical signs of disease or allergen-specific IgE values were not seen in the control group undergoing the same regimen. Total serum IgE concentration was measured by use of the ELISA, and comparison with known quantities of a monoclonal IgE allowed absolute values to be reported. Values were high in the Maltese x beagle colony (7 to 34 µg/ml), compared with those in the control dogs (0.7 to 6 µg/ml). Total serum and total fecal IgE concentrations did not change in either group during the study. Although allergen-specific IgE was detected in the feces of both groups, significant interassay variability made interpretation of the results difficult. The authors concluded that these Maltese x beagle dogs satisfied the currently recognized clinical criteria for the diagnosis of canine food hypersensitivity. Furthermore, the clinical and serologic responses seen in these dogs in response to oral allergen provocation suggest that this may be a useful model for the study of spontaneous food hypersensitivity

Swine experimental models.
The final less common model that has been proposed utilizes another large animal species, the neonatal pig. The same general advantages and disadvantages apply to this experimental system as those identified for the dog. The pig has been used rather more extensively in studies that examine the development of mucosal immunity, as the pig closely resembles the human in this respect . Intraperitoneal injection in the presence of cholera toxin (CT) adjuvant is the method of immunization that has been utilized and responses to peanut proteins and the HEW allergen ovomucoid only have been determined.

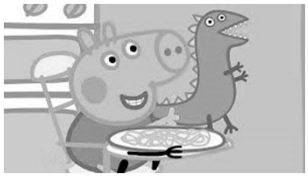

Table 1. Advantages/disadvantages of nonrodent animal food allergy models.

Advantages	Disadvantages
Confirmed clinical/immunologic of natural food allergy	Limited species/strains
Anatomy/physiology/nutritional requirements similar to those of humans	Knockout strains not available
Immunopathogenic/mechanistic/therapeutic intervention strategies similar to those for humans	Lack of complete array of immunologic reagents
Repeated endoscopic analysis of gastrointestinal tract	Large size and smaller experimental animal numbers/group
Large size/numbers of primary and secondary immune organs/cells	Expensive to maintain colonies
Smaller concentration of sensitizing antigen/allergen per gram of body weight	