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Allergy

• Allergic diseases, such as asthma, hay fever and eczema are well-known chronic disease in many societies, with every third child having some type of allergy.
• In the 19th century, allergy was rarer, → suggestions that something may happen early in life to determine whether or not a child will become allergic.
• By looking at the pathogenetic mechanisms involved in allergic diseases, perhaps an explanation and cure for the increasing incidence of allergies may be found.
• In order to understand allergy, one must understand the different immune reactions to allergens, which we shall discuss today.

What is…

• Allergic disease – hypersensitivity reactions caused by the immune system reacting erroneously to external material to which it normally shouldn’t react.
• Allergen – special type of (otherwise harmless) Ags that elicit allergic reactions in sensitised individuals. Usually enter via skin or mucosal membrane.
• Atopy – the genetic propensity to develop an IgE Ab response to common allergens. Asthma is one of the most common clinical manifestations of this.
• Atopic allergens – IgE-inducing allergens.
Allergic reactions:

Allergies and symptoms which can occur:
- Atopic dermatitis (eczema)
- Urticaria (hives)
- Anaphylaxis (and shock)
- Asthma
- Hayfever
- Contact dermatitis
- Allergic conjunctivitis and rhinitis
- Goodpasture syndrome
- Food Allergy
- Insect Allergy
- Drug Allergy
- Serum sickness
- Graves Disease
- Arthus reaction
- Systemic lupus erythematosus (SLE)
- Delayed and contact hypersensitivity
- Allergic alveolitis
- Haemolytic Anaemia
- Ocular cicatrical pemphigoid
- Rhesus D incompatibility
- Myasthenia Gravis

And more…

What happens in allergy?

- Firstly, allergens gain access via:
  - skin
  - mucosal membrane
  - injection (stings, bites, drugs) or
  - endogenous production (autoallergens or allergens produced by invading parasites).

- Sensitisation to the allergen occurs usually after repeated exposure or in young children before complete tolerance has occurred.
Sensitization

Environmental Adjuvants
- Maternal smoking
- Pollution
- ? Infections
- ? Immunizations

Allergen exposure
- In utero
- Breast milk
- Environment (inhaled/ingested)

Genetic Predisposition

Atopic Disease
- food allergy
- atopic dermatitis
- respiratory allergic disease

Immune Response
("Atopic" cytokine profile)

1° Prevention
2° Prevention

IgE
Gell and Coombs Classification System 1963

- Attempt to define hypersensitivity:
  - **Type 1** – Immediate and late hypersensitivity caused by IgE Ab.
  - **Type 2** – Ab-mediated cytotoxic.
  - **Type 3** – Immune complex mediated.
  - **Type 4** – T Cell-mediated.

Now clear that many mechanisms that cause hypersensitivity subdivide and cross these boundaries.

Immediate and Delayed Allergy

- There is distinction between immediate and delayed patterns of allergic reactivity which loosely correspond to IgE-mediated allergy and non-IgE mediated responses.

- **Immediate allergy** - pattern is easily recognized because it involves quick and dramatic symptoms.
  Hay fever is the most common type 1 allergy that can be diagnosed by allergy skin tests and by IgE antibody tests.
  Hay fever is a reaction to airborne plant pollens in the nose. Allergy tests are positive, antihistamines help and allergy shots can sometimes reduce the reactivity over time.

- **Delayed allergy** – patterns are not so obvious and generally go unrecognized. Allergy skin tests do not show this problem. Symptom onset is delayed after exposure to the trigger foods. Allergic reactions to drugs such as penicillin and to foods are usually delayed hypersensitivity.
• The **immediate** model tends to dominate allergy literature.
• This is because it is simple: the same responses are expected from a sensitized individual with repeated challenges.
• Unrealistic model since no one reacts in the same way.
• Many **delayed** chronic diseases are either degenerative or inflammatory & many are recognized to be immune-mediated or hypersensitivity diseases.
• The delayed patterns of allergy can be the cause of chronic and disabling hypersensitivity disease.
• Hypersensitivity diseases which involve humoral and cell-mediated immunity:
  • Asthma, allergy, rheumatic diseases, autoimmune diseases, MS, diabetes, thyroiditis, psoriasis.

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**Key Players:**

Mast cells and basophils are the principle effector cells of IgE mediated reactions.

Mast cells release:
  • Histamine
  • Tryptase
  • PGD2
  • LTC4
  • PAF
  • Bradykinin
  • Cytokines

Cytokines are released at many stages.

- Interaction of APC with Th cell
  - GM-CSF, IL3-6, 9-10 &13
- Induction of eosinophil proliferation IL5
- B cell differentiation IL4-6
- Eosinophils secrete IL1 which favours Th2 cell proliferation and IL3 – mast cell growth factor.
- Mast cells release IL4-6 &13, also TNFα and GM-CSF which cause IgE production.
Key Players: IgE

- **IgE**: the antibody which produces typical allergy or immediate hypersensitivity reactions such as: hay fever, asthma, hives & anaphylaxis.
- Its normal function is anti-parasite defence.
- Found throughout body, although predominantly in association with mucosal tissues.
- Attaches to mast cells and acts as a receptor to Ag.
- Not found in breast milk, and only in very low amounts in other secretions such as saliva.

Other Igs involved in allergy:

- **IgA**: circulating and secreted on all defended body surfaces, the 1st defence against invaders.
- If deficient, ↑ gut permeability to Ags and delayed patterns of food allergy and diseases that are related to type 3 & 4 - the ‘autoimmune diseases’ ie celiac disease.
- **IgG**: is the major circulating Ab which enters tissues freely, and participates in diverse immune events.
- represent a large vocabulary of antigen recognition molecules.
- IgG (and IgM) activate complement.
- **IgM**: the multivalent Ab, capable of capturing and binding Ags to form large insoluble complexes which are readily cleared from the blood.
- IgM levels may be elevated in patients with delayed patterns of food allergy, and probably manifest a protective defence response.
First Contact to a novel Allergen

- Antigen Presenting Cells, whether they be macrophages, B cells or dendritic cells internalize the allergen by phagocytosis or endocytosis and the re-express a part of the allergen together with an class II MHC molecule on their membrane.

- The physical complex that forms between the allergen epitope (degraded allergen peptide sequence) and class I or class II MHC molecule depends on the route the allergen takes to enter. (Class II = exogenous, class I = endogenous)

- Most allergens enter exogeneously (by phagocytosis or endocytosis after administration via routes described earlier such as mucosal membranes and injection). The antigen presenting cells process the allergen into peptide fragments (epitopes) within the endosomal processing pathway. These epitopes then bind to the cleft within the class II MHC molecule and this complex is exported to the cell surface.

- T cells displaying CD4 (a membrane bound glycoprotein recognising class II MHC bound allergen epitopes) function as T helper cells. These T cells are MHC class II restricted.

- Whether APC is B cell or macrophage depends on the concentration of the allergen.
  - Low concentration = B cell
  - High concentration = Macrophage

- Depends on cytokine profile of T cells.
3D structure and recognition of the antigen (allergen) by different APC is essential in the development of a distinct T cell cytokine profile.

Introduction of T cell to APC

- The macrophage presents the processed allergen associated with class II MHC molecules to the T cells.
- This interaction, together with interleukin-1 (IL-1) induces a series of membrane changes in the Th cell leading to its activation.
- Activation of the Th cell induces the secretion of IL-2.
- The allergen-specific Th cells then differentiate into phenotypes that secrete other cytokines and now play a role in the activation of B lymphocytes.
Effective antigen presentation and subsequent T-cell activation requires interactions between multiple cell surface molecules. The initial interaction between T-cells and APC’s is via low affinity interactions between lymphocyte function-associated antigen (LFA-1) on the T-cell and intracellular adhesion molecules (ICAMs) on the APC. The interaction of CD2 with LFA-3 facilitates communication between the two cells.

The T-cell receptor recognises antigenic peptides presented via MHC molecules to provide the first signal for activation. The second signal, required for proliferation and differentiation, comes via co-stimulatory molecules B7-1 or B7-2 on the APC. These bind to the CD28 molecule on the T-cell.

**B cell Role**

- Activation of B cells from the bone marrow occurs through cross linkage of the allergen epitope with the B cells membrane receptor and growth factors secreted by macrophages and Th cells.
- The B cell processes the allergen and presents it in association with MHC class II molecules on the cell surface. This complex is recognised by the now specific Th cell.
- As the B cell is an APC, it facilitates the presentation of the epitope-MHC class II complex to the specific Th cells inducing the secretion of cytokines including IL-2, IL-4, IL-6 and IFN-γ, resulting in B cell differentiation and division in association with IL-1 from the macrophage (as previously described).
- This period of division and differentiation results in two populations of B cells:
  1. Antibody secreting plasma cells (in allergy, primarily IgE)
  2. Memory B cells

These processes are SENSITIZATION. Subsequent exposure to an allergen will now produce a faster, specific response.
Mechanism of IgE Action

- IgE is secreted from the plasma cells

- IgE binds to IgE receptors (FcεRI and FcεRII). FcεRI is a high affinity receptor and found on mast cells and basophils. The FcεRII receptor is low affinity and found on B cells, macrophages and eosinophils.

- The receptor interacts with the Fc portion of IgE

- FcεRI receptors are specific for the CH2/CH2 domain of IgE. The low affinity FcεRII are specific for the CH3/CH3 domain. This receptor is also known as CD23.

- FcεRI
  This consists of 4 polypeptide chains, α, β and 2 disulphide linked γ chains. The α chain interacts with high affinity to the CH2/CH2 domain of IgE. The β chain spans the plasma membrane (4 times) linking the α and γ chains.

FcεRI

FcεRI is a tetrameric holoreceptor
The allergen binds to IgE that is bound by FcεRI to mast cells (or basophils). The allergen binds by bridging two membrane bound molecules of IgE as shown.

Before contact with the allergen, the binding of IgE to FcεRI has no effect on the target cell. Only once the allergen has bound forming the cross-linked IgE receptor complex does degranulation begin.

Within 15 seconds of this cross-linkage, methylation of various membrane phospholipids occurs and phosphatidylcholine (PC) facilitates formation of Ca²⁺ channels in the mast cell membrane.

The Ca²⁺ channels open due to methylation of membrane phospholipids and there is an influx of Ca²⁺ peaking at 2 minutes. Degranulation of mast cells does NOT occur in the absence of Ca²⁺.

Ca²⁺ then activates the enzyme phospholipase A2 promoting the breakdown of PC to lysophosphatidylcholine and arachidonic acid.

Arachidonic acid is converted into 2 different classes of potent mediators:
1) Prostaglandins
2) Leukotrienes

Simultaneously, with phospholipid methylation and Ca²⁺ influx, there is a transient rise in membrane bound adenylate cyclase activity, peaking 15 seconds after cross-linkage. The cAMP effects are exerted through activation of cAMP-dependent protein kinases which phosphorylate the granule membrane proteins changing the granules permeability to water and Ca²⁺.

This causes swelling of the granules facilitating fusion of the granules to the plasma membrane. The increase in Ca²⁺ and cAMP followed by the subsequent decrease in both and microtubular assembly causes the fusion of granules to the membrane and the release of mediators from the granule such as histamine.
Anaphylaxis

• Life-threatening
• Sudden release of mast-cell and basophil derived mediators into the circulation
• Food and medicines – most anaphylaxis

Mast cell  Basophil

Anaphylaxis

• Symptoms: urticaria, angioedema, bronchospasm, laryngeal edema, dizziness, unconsciousness, hyperperistalsis, hypotension, cardiac arrhythmias
• Also: nausea, vomiting, headache
• Signs within 5 to 30 min (very rarely hours)
• Recurrent (biphasic) anaphylaxis – occurs 8-10h after the initial attack
• Persistent anaphylaxis – can last for up to 32h

TABLE 1. Approximate incidence of anaphylaxis: Overall and with selected agents

| Overall | Approximately 154 annual fatal episodes per 1,000,000 hospitalised subjects; occur internationally.6
| The estimated risk of anaphylaxis per person in the United States is 1 in 79.3,4
| US projection on the basis of data from Olmsted County, Minn
| For population of 280 million and mortality rate of 1%; there will be an estimated 84,000 anaphylaxis cases and 840 fatalities annually.5,8

Selected Agents
Foods
An estimated 150 fatalities from food-induced anaphylaxis occur each year in the United States5; peanuts and tree nuts accounted for 30 (94%) of 32 fatal cases voluntarily reported to a national registry for food anaphylaxis.3

Antibiotics
β-Lactam antibiotics are alleged to cause 400 to 800 fatal anaphylactic episodes per year.6

Allergen vaccines
Fatalities from allergen immunotherapy occur approximately 1 per 2,000,000 injections.9 Fatal reactions to skin testing are rare.

Venoms
Insect stings probably cause at least 50 US fatalities annually; the true incidence of sting anaphylaxis and death is unknown.10

Idiopathic anaphylaxis
Estimated prevalence of 34,000 individuals in the United States.11

J Allergy Clin Immunol 2004;113:805-19
Mechanism

- Degranulation of mast cells and basophils
- Pre-formed substances: histamine, tryptase, chymase, heparin, histamine releasing factor, and other cytokines
- Newly generated lipid derived mediators – PGD2, leukotrienes LTB4, LTC4, LTD4, LTE4

Histamine

- H1- pruritus, rhinorrhea, tachycardia and bronchospasm
- H1 – stimulates endothelial cells to convert L-arginine to NO (vasodilator)
- H1 and H2 – headache, flushing, hypotension
- Serum histamine – severity of cardiopulmonary reactions, GI signs
- H3 – no known implications for human subjects and anaphylaxis

Photo of skin mast cells at 100X using an oil immersion lens and an olympus digital camera. The cells are stained with Tol Blue, and might appear slightly degranulated as they were activated using an artificial antigen during the course of an experiment. (wikipedia.org)
Tryptase

- Concentrated selectively in the granules of mast cells
- Plasma levels correlate with the severity of anaphylaxis
- Post-mortem measurement of serum tryptase within 15h of death
- β-tryptase stored in mast cells, α-tryptase secreted constitutively

Treatment

- Intramuscular injection of epinephrine into the thigh – more effective than injection into the arm or subcutaneous administration
Hypersensitivity

- Peanut Allergy (1% of the population)
- *Arachis hypogaea*
- Major cause of food-induced anaphylaxis
- Persists through to adulthood (21.5% resolution with increasing age)
- Need for safe and specific therapy

**TABLE I. Prevalence of food allergies in the United States**

<table>
<thead>
<tr>
<th>Food</th>
<th>Young children</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>2.5%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Egg</td>
<td>1.3%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Peanut</td>
<td>0.8%</td>
<td>0.6%</td>
</tr>
<tr>
<td>Tree nuts</td>
<td>0.2%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Fish</td>
<td>0.1%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Shellfish</td>
<td>0.1%</td>
<td>2.0%</td>
</tr>
<tr>
<td>Overall</td>
<td>6%</td>
<td>3.7%</td>
</tr>
</tbody>
</table>

*J Allergy Clin Immunol 2004;113:305-19*

Peanut Allergy – clinical features

- Develops early in life
- Associated with other atopic diseases
- Sensitization – consumption of peanut containing products
- Symptoms within minutes to hours
- Manifestation: oral pruritus, nausea, vomiting, urticaria, angioedema, bronchospasm
- Severe cases – anaphylaxis
### TABLE II. Food hypersensitivity disorders

<table>
<thead>
<tr>
<th>Type</th>
<th>Gastrointestinal</th>
<th>Cutaneous</th>
<th>Respiratory</th>
<th>Generalized</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IgE mediated</strong></td>
<td>Oral allergy syndrome, gastrointestinal anaphylaxis</td>
<td>Urticaria, angioedema, morbilliform rashes and flushing</td>
<td>Acute rhinoconjunctivitis, bronchospasm (wheezing)</td>
<td>Anaphylactic shock</td>
</tr>
<tr>
<td><strong>Mixed IgE and cell mediated</strong></td>
<td>Allergic eosinophilic esophagitis, allergic eosinophilic gastroenteritis</td>
<td>Atopic dermatitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cell mediated</strong></td>
<td>Food protein–induced enterocolitis, food protein–induced proctocolitis, food protein–induced enteropathy syndromes, celiac disease</td>
<td>Contact dermatitis, dermatitis herpetiformis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Food-induced pulmonary hemosiderosis (Heiner syndrome)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*J Allergy Clin Immunol 2004;113:805-19*

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**Molecular mechanism**

The major cellular interactions during the mucosal allergic immune response

*Expert Reviews in Molecular Medicine © 2007 Cambridge University Press*
Allergenic components of Peanuts

- Eight allergens Ara h 1 – Ara h 8
- Main – Ara h 1, Ara h 2, Ara h 3
- Ara h 4 – Ara h 7 less allergenic
- Ara h 8 distinct from others
- Most allergens are seed storage proteins (exception Ara h 5 and Ara h 8 – pollen associated food allergy)

Biochemical properties

- Ara h 1, Ara h 2, Ara h 6 - Resistant to digestive enzymes and thermal processing
- Ara h 2 - Sequence homology with trypsin inhibitors
- Increased activity following roasting
Molecular model of Ara h 1

Crossreactivity

**Plantae**
Kingdom

Tracheobionta
Subkingdom
Suprachlorophyta
Supergroup
Magnoliophyta
Division
Magnoliopsida
Class

Hamamelidaceae
(Subclass)
Rosoideae
(Subclass)
Dilleniidaceae
(Subclass)

Fagales
(Order)
Juglandales
(Order)
Fabales
(Order)
Rosales
(Order)
Sapindales
(Order)
Lecythidales
(Order)

Betulaceae
(Birch family)
Juglandaceae
(Walnut family)
Fabaceae
(Pea family)
Proteaceae
(Rose family)
Anacardiaceae
(Sumac family)
Lecythidaceae
(Brazil nut family)

Corylus
(Hazel)
Juglans
(Roasted)
Arachis
(Peanut)
Macadamia
(Macadamia nut)
Pterocarya
(Chesnut)
Anacardium
(Cashew nut)
Bertolonia
(Brazil nut)

**Taxonomic classification of peanut and tree nuts**

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Immunotherapeutic options

• Subcutaneous immunotherapy – fatal anaphylaxis
• Allergen derivatives as vaccines
• Allergen preparations that do not crosslink IgE antibodies bound to effector cells
• Three possible approaches
Research

• Ultimate goal – inducing tolerance to peanuts
• Sublingual immunotherapy (SLIT) with hypoallergenic preparations
• Transgenic plants - hypoallergenic peanuts
• So far no optimal strategy for the treatment and prevention of peanut allergy
What is asthma?

- Chronic inflammatory disorder of the airways causing recurrent episodes of symptoms due to widespread but variable obstruction to bronchial airflow and increased airway hyperresponsiveness.
- Many different inflammatory cells are activated in asthmatic airways to produce a variety of mediators which act on target cells of the airway to produce pathophysiological features typical of asthma.
- Symptoms:
  - wheezing - cough
  - breathlessness - chest tightness

• The airways narrow due to:
  - Muscles around the airways tightening
  - The airway lining becomes inflamed
  - Mucus production, blocking the airway
Airway Inflammation

- Airway hyperresponsivness
- Bronchoconstriction
- Hypertrophy of airway smooth muscle
- Plug formation in airway lumen
  - mucous (increased numbers of goblet cells)
  - serum proteins
  - inflammatory cells
  - cellular debris
- Airway wall remodelling
  - collagen deposition in sub-basement membrane
  - tissue oedema
- Infiltration of bronchial wall with
  - eosinophils
  - T-lymphocytes
  - mast cells
  - macrophages
  - plasma cells

Development of Asthma

- May develop at any age due to:
  - genetic disorder
  - exposure to allergens
  - irritants
  - viruses
  - hygiene hypothesis
- Prevalence is increasing in industrialized countries. Why?
The Hygiene Hypothesis

- Improved hygiene in industrialized societies
  - Improved public health
  - Use of vaccines and antibiotics
- Decreased infections that normally stimulate the immune system to work against asthma.
- The following have a decreased risk of asthma:
  - bigger families
  - early placement in day-care
  - exposure to farm animals and products
  - exposure to bacterial endotoxins
  - limited use of antibiotics

TH1 vs. TH2

- Individuals developing asthma genetically may have weaker cell-mediated immune responses but increased humoral (IgE mediated) responses.
- Conditions in industrialized countries drive the development of Th2 responses.
- Pathogens suspected of limiting asthma development are those which induce the Th1 response.
**Inflammatory cells**

**Macrophages:**
- activated by allergen via IgE receptors
- produce large variety of cytokines
- can increase and decrease inflammation

**Eosinophils:**
“chronic eosinophilic bronchitis”
- release proteins and free radicals causing airway hyperresponsiveness
- inflamed airway signals increased eosinophil production.

**Neutrophils:**
- Found in airways of asthmatics, but don’t affect AHR, importance unknown

**T-lymphocytes:**
- coordinate inflammatory response through release of cytokines
- involved in recruitment and survival of eosinophils and mast cells in airways

**Epithelial Cells:**
- produce inflammatory mediators (endothelins, cytokines, chemokines)

**Inflammatory Mediators**

**Cysteinyl leukotrienes:** (LTC₄, LTD₄, LTE₄)
- bronchoconstriction of human airways
- increase AHR
- microvascular leakage
- mucus secretion
- chemoattract eosinophils

**Cytokines:**
- synthesised and released by inflammatory cells (macrophages, mast cells, eosinophils, lymphocytes) and structural cells (epithelial and endothelial).
- IL3 for survival of mast cells in tissues
- IL4 switches B-lymphocytes to produce IgE
- IL5 is important for differentiation and survival of eosinophils
- IL1, IL6 are released from cells and may amplify inflammatory response

**Endothelins:**
- vasoconstrictors and bronchoconstrictors induce airway smooth muscle proliferation and fibrosis

**Nitric Oxide:**
Effects of Inflammation

**Airway smooth muscle hypertrophy and hyperplasia**
- due to stimulation by growth factors from inflammatory cells.

**Vascular Response:**
- vasodilation
- increased microvascular leakage

**Airway mucus hypersecretion**
- contributes to mucus plugs which occlude asthmatic airways.
- Possibly due to inflammatory mediators acting on submucosal glands

Shedding of epithelium:
- due to proteases and free radicals.
- Epithelial lumps (Creola bodies) commonly found in sputum.
- Causes enhanced AHR by loss of barrier to allergens, loss of enzymes to degrade inflammatory mediators and exposure of sensory nerves.

**Subepithelial Fibrosis:**
- thickening of basement membrane due to collagen placement

Implications for Therapy

- Elimination of causative agents from the environment
- Eliminate underlying inflammation

**Pharmacotherapies:**
- Bronchodilators to relax airway smooth muscle
- Anti-inflammatory e.g. inhaled corticosteroids and leukotriene antagonists

**Enhancing Tolerance:**
- use of probiotics to improve/prevent atopic disease

**Oral allergen immunotherapy:**
- reduces symptoms of allergy

**Allergen immunotherapy:**
- allergen given subcutaneously in increasing amounts
- controls symptoms, reverses disease
- beneficial effects maintained for years after therapy finishes
  BUT needs >100 shots for completion