selected topics in clinical Immunology
Allergology

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Overview Part 1

- Definitions
- IgE mediated allergies, mechanism
- Allergen overview (pollen, venom, other)
- Cross reactivity
- Allergic diseases (Hay fever, asthma, anaphylaxis, food allergy, hympenoptera venom allergy)
Definition of Allergy

1. Immune mediated reaction

2. Non replicating (harmless?) substances

3. Clinical symptoms

4. In contrast to infections: symptoms are caused almost exclusively by the immune reaction, not by the „bug“ (virus, bacteria, etc.)
Allergens

Examples of allergens:
- Pollen
- House dust mite
- Hymenoptera venom
- Food
- Drugs

Some allergens appear to have structural features able to activate the innate immunity (enzymes like papain, Der p2, some pollen grains)
Immune response to “non replicating” substances (“allergens”)

• No immune response - Ignorance
• (right) immune response - Tolerance
• (false ?) immune response - Allergy
Classification according to mechanisms

IgE mediated immune reactions:
e.g. rhinitis & conjunctivitis, asthma, urticaria, anaphylaxis

IgG mediated reactions:
vasculitis, immune hemolytic anemia, thrombocytopenia and granulocytopenia, Arthus reaction

T-cell reactions:
contact dermatitis, drug allergies, atopic dermatitis, asthma
Definitions

• **Atopy**: genetic determined readiness to react by IgE formation to substances taken up via aerogen or gastro-intestinal routes

• **Sensitization**: immune reaction to a foreign substance (proven in skin tests, serology, cellular tests...)

• **Allergy**: immune reaction to a non replicating substance (protein, chemical, drug, metal,...), which leads to clinical symptoms
**Sensitization Phase**

- Allergen
- Epithelial barrier
- DC
- T cell (naiv)
- Clonal expansion
- TH2
- TH2
- TH2
- IL-4, IL-5, IL-13
- B cell
- Ig Switch
- Production of allergen-specific IgE
- Plasma cell
- Re-exposure to the allergen

**Effector Phase**

- late phase
  - TH2
  - TH17
- early phase
  - TNF-α
  - Histamin
  - Prostaglandin
- Mast cell
- Basophil
- Tissue cells

**lymph node**
Airway immune response

Air pollutants

Bacteria

Allergen

Antigen

Virus

DC modulating factors

TSLP

PGE2

TGF-β

IL-10

GM-CSF

Inflammatory cytokines

Recruitment by chemokines

Lymph node

Treg cells

Naïve Th cells

Th1 cells

Th2 cells

Th17 cells

Airway immune response

J. van Tongeren et al. Allergy 2008: 63: 1124–1135
Sensitization Phase

- Allergen
- DC (naïve T cell)
- Lymph node
- Clonal expansion
  - TH2
  - TH2
  - TH2
- B cell
  - Ig Switch
  - Production of allergen-specific IgE
- Plasma cell
  - Re-exposure to the allergen

Effector Phase

- TH2
- TH17
- TH1
- Mast cell
- Basophil
  - TNF-α
  - Histamin
  - Prostaglandin
  - Late phase
  - Early phase
  - Tissue cells

Re-exposure to the allergen
Sensitization Phase

- Allergen
- DC
- T cell (naiv)
- B cell
- Plasma cell
- Production of allergen-specific IgE
- Ig Switch
- IL-4, IL-5, IL-13
- clonal expansion

Effector Phase

- late phase
  - TH17
  - mast cell
- early phase
  - TNF-α
  - histamin
  - prostaglandin
- basophil
- tissue cells
- Re-exposure to the allergen
- lymph node
Source: http://www.biolegend.com/category_costimulatory_molecules_human
Immunoglobulin: recognizes intact antigens in soluble form (surface epitopes)

T cell receptor (TCR): recognize fragments of antigens that have been processed by a separate antigen presenting cell and then bound to an MHC class I or class II molecule
The immunoglobulin light chain; \( \text{V}_L \), variable region of the immunoglobulin heavy chain; \( \text{CH} \) complex of one complex. In humans, but not in mice, \( \text{Fc} \) IgE signals through CD79, which has an immunoreceptor tyrosine-based activation co-stimulatory signals, also triggers B cell proliferation and differentiation. Membrane IgE mediates antigen uptake and presentation by B cells and, in combination with switching to IgE; and a secreted form that is produced by plasma cells. Membrane IgE receptor form that is expressed on the surface of B cells that have undergone class switching.

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<tr>
<th>Membrane IgE</th>
<th>Secreted IgE</th>
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<td>V(_L)</td>
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<td>( \text{CH} ) complex</td>
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<td>CD79 Immunoglobulin domain</td>
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<td>M1 M2 ITAM</td>
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<td>IgE B cell</td>
<td>IgE plasma cell</td>
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<td>Fc(\epsilon)RI</td>
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<td>Mast cells and basophils</td>
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<td>Immunoglobulin domain</td>
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<td>IgE B cell</td>
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<th>Membrane IgE</th>
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<td>Fc(\epsilon)RI (humans)</td>
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<td>Dendritic cells and macrophages</td>
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<td>Immunoglobulin domain</td>
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<td>( \alpha ) ( \beta ) ITAM</td>
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<td>IgE B cell</td>
<td>IgE plasma cell</td>
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<th>Membrane IgE</th>
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<td>Fc(\epsilon)RII</td>
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<td>B cells</td>
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<td>C-type lectin domain</td>
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<td>Leucine zipper</td>
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Lawren C. Wu and Ali A. Zarrin et al. Nature Immunology Review 2014
## IgE: very low amount in serum; cell affine (Fc-IgE-RI)

<table>
<thead>
<tr>
<th>Receptor</th>
<th>FcγRI (CD64)</th>
<th>FcγRII-A (CD32)</th>
<th>FcγRII-B2 (CD32)</th>
<th>FcγRII-B3 (CD32)</th>
<th>FcγRII (CD16)</th>
<th>FcεRI</th>
<th>FcαRI (CD89)</th>
<th>FcαRI/μR</th>
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<tr>
<td>Structure</td>
<td>α 72 kDa</td>
<td>α 40 kDa</td>
<td>γ-like domain</td>
<td>ITIM</td>
<td>α 50-70 kDa</td>
<td>α 45kDa</td>
<td>α 55-75kDa</td>
<td>α 70kDa</td>
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<tr>
<td>Binding</td>
<td>IgG1 10^8 M^-1</td>
<td>IgG1 2x10^6 M^-1</td>
<td>IgG1 2x10^6 M^-1</td>
<td>IgG1 2x10^6 M^-1</td>
<td>IgG1 5x10^5 M^-1</td>
<td>IgG1 IgG3</td>
<td>IgA1, IgA2 10^7 M^-1</td>
<td>IgA, IgM 3x10^8 M^-1</td>
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<td>Order of affinity</td>
<td>1) IgG1=IgG3 2) IgG4 3) IgG2</td>
<td>1) IgG1=IgG3 2) IgG4 3) IgG2</td>
<td>1) IgG1=IgG3 2) IgG4 3) IgG2</td>
<td>1) IgG1=IgG3 2) IgG4 3) IgG2</td>
<td>1) IgG1=IgG3 2) IgG4 3) IgG2</td>
<td>IgE 10^10 M^-1</td>
<td>IgA1=IgA2</td>
<td>IgA, IgM</td>
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<tr>
<td>Cell type</td>
<td>Macrophages Neutrophils Eosinophils Dendritic cells</td>
<td>Macrophages Neutrophils Eosinophils Platelets Langerhans' cells</td>
<td>Macrophages Neutrophils Eosinophils</td>
<td>B cells</td>
<td>NK cells Eosinophils Macrophages Neutrophils Mast cells</td>
<td>Mast cells Eosinophils Macrophages Neutrophils Basophils</td>
<td>Macrophages Neutrophils Eosinophils</td>
<td>Macrophages B cells</td>
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<tr>
<td>Effect of ligation</td>
<td>Uptake Stimulation Activation of respiratory burst Induction of killing</td>
<td>Uptake Granule release (eosinophils)</td>
<td>Uptake Inhibition of stimulation</td>
<td>No uptake Inhibition of stimulation</td>
<td>Induction of killing (NK cells)</td>
<td>Secretion of granules</td>
<td>Uptake Induction of killing</td>
<td>Uptake</td>
</tr>
</tbody>
</table>
Sensitization Phase

- Allergen
- T cell (naiv)
- DC
- clonal expansion
- IL-4, IL-5, IL-13
- B cell
- Ig Switch
- Production of allergen-specific IgE
- Plasma cell
- Re-exposure to the allergen

Effector Phase

- late phase
  - TH2
  - TH17
  - TH1
- early phase
  - TNF-α
  - histamin
  - prostaglandin
  - mast cell
  - basophil
  - tissue cells
  - lymph node
Cross linking of 2 Fc-IgE-RI
Is required for mast cell activation

Mediator release
**Sensitization Phase**

- Allergen
- Epithelial barrier
- DC
- T cell (naive)
- Clonal expansion
- TH2
- TH2
- TH2
- TH2
- IL-4, IL-5, IL-13
- B cell
- Ig Switch
- Production of allergen-specific IgE
- Plasma cell
- Re-exposure to the allergen

**Effector Phase**

- Lymph node
- Mast cell
- Basophil
- Histamin
- Prostaglandin
- TNF-α
- Late phase
- TH2
- TH17
- TH1
- Early phase
- Tissue cells
- TNF-α
- Histamin
- Prostaglandin
FIG 2. A, Suppression of TH2 cell-mediated features of allergic inflammation by Treg cells. Treg cells use multiple suppressor factors to regulate undesired activity of effector TH2 cells. IL-10 and TGF-$\beta$ suppress IgE production and induce IgG4 and IgA, respectively. Both cytokines directly suppress allergic inflammation induced by effector cells such as mast cells, basophils, and eosinophils. In addition, TH2 cells are suppressed by Treg cells and can therefore no longer provide cytokines such as IL-3, IL-4, IL-5, IL-9, and IL-13. These cytokines are required for the differentiation, survival, and activity of mast cells, basophils, eosinophils, and mucus-producing cells, as well as for the tissue homing of TH2 cells and eosinophils (red line, suppression; black line, stimulation).

B, Suppression of TH1 cell-mediated features of allergic inflammation by Treg cells. The TH1 cytokine, IFN-$\gamma$, in combination with TNF-$\alpha$ and/or FasL, induces apoptosis of smooth muscle cells, keratinocytes, and bronchial epithelial cells as essential tissue injury events in atopic dermatitis and asthma (red line, suppression; black line, stimulation).

FasL, Fas-ligand; IP-10, IFN-$\gamma$–inducible protein 10; mig, monokine induced by IFN-$\gamma$; iTac, IFN-$\gamma$–inducible chemoattractant.

Akdis et al. JACI 2007
The immune system is highly specific and needs danger signals to become activated.

How can a harmless/innocuous substance like a pollen potentially induce an IgE mediated immune reaction?
Allergens = 2% of proteins

Biochemical functions of allergens

TABLE III. The 15 GO terms associated with the highest number of allergens in AllFam

<table>
<thead>
<tr>
<th>GO term</th>
<th>Allergens</th>
</tr>
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<tbody>
<tr>
<td><strong>Molecular function</strong></td>
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<tr>
<td>Hydrolase activity (GO:0016787)</td>
<td>119</td>
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<tr>
<td>Peptidase activity (GO:0008233)</td>
<td>56</td>
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<tr>
<td>Metal ion binding (GO:0046872)</td>
<td>73</td>
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<tr>
<td>Calcium ion binding (GO:0005509)</td>
<td>56</td>
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<tr>
<td>Nutrient reservoir activity (GO:0045735)</td>
<td>55</td>
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<tr>
<td>Lipid binding (GO:0008289)</td>
<td>53</td>
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<tr>
<td>Actin binding (GO:0003779)</td>
<td>48</td>
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<tr>
<td><strong>Biologic process</strong></td>
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<tr>
<td>Transport (GO:0006810)</td>
<td>105</td>
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<tr>
<td>Metabolic process (GO:0008152)</td>
<td>45</td>
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<tr>
<td>Proteolysis (GO:0006508)</td>
<td>58</td>
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<tr>
<td>Carbohydrate metabolic process (GO:0005975)</td>
<td>45</td>
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<tr>
<td>Cytoskeleton organization and biogenesis (GO:0007010)</td>
<td>45</td>
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<tr>
<td><strong>Cellular component</strong></td>
<td></td>
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<tr>
<td>Extracellular region (GO:0005576)</td>
<td>109</td>
</tr>
<tr>
<td>Cytoplasm (GO:0005737)</td>
<td>76</td>
</tr>
<tr>
<td>Cytoskeleton (GO:0005856)</td>
<td>45</td>
</tr>
</tbody>
</table>
Ability of „innocuous“ proteins to activate immune system

1. House dust mite allergen Der p1: cysteine protease cleaves tight junction protein occludin → Increased epithelial permeability and facilitating its entry into the tissue

2. House dust mite allergen Der p2: structural and functional homology with MD-2, LPS-binding component of TLR 4 signaling complex → facilitates signaling through direct interactions with the TLR4 complex

3. Pollen-associated lipid mediators (PALMs): When pollen grains are hydrated on the respiratory epithelia, they release allergens and eicosanoid lipids → so-called pollen-associated lipid mediators (PALMs) → act as stimulators of DC
PAR2. PARs (1, 2, 3, 4) are a family of proteolytically activated mediated through activation of the protease-activated receptor 2 an open question. Actually gain functional access to lymphocytes. It should be noted that whether intact proteases such as Der p 1 suggest that disruption of the balance between proteases and Antitrypsin can inhibit this effect of Der p 1 on CD23 cleavage, IgE synthesis, thereby amplifying the allergic response. Potentially disrupt the negative feedback signal and enhance tive regulator of IgE synthesis, Der p 1 cleavage of CD23 could form of the IgE receptor is thought to exert its effect as a negative immune responses as well, through cleavage of molecules such as CD25 and CD23. Specifically, Der p 1 has been shown to be able to cleave the low-affinity receptor for IgE, CD23, from the surface of human B cells, releasing the soluble form of the receptor. 30 (IgE, CD23, from the surface of human B cells, releasing the soluble form of the receptor. 30) IgE, CD23, from the surface of human B cells, releasing the soluble form of the receptor. 30

The biological effects of some allergenic proteases may also be allergic sensitization through PAR2 activation. The generality of this pathway, as well as its molecular identification, remains to be defined. The overall effect may be to shift the balance of immune responses from a tolerogenic response to one favoring a Th2 pattern of response. Allergenic proteases such as Der p 1 have shown to Der p 9, along with German cockroach extract, have been shown to exhibit increased expression of PAR2 on respiratory epithelial cells. Several house dust mite allergens (Der p1, Der p 3, Der p 2) have been shown to be able to cleave and activate PAR2 (as Asp). As thymic stromal lymphopoietin promotes DC polarization of naive T cells to a Th2 phenotype, these results underscore a potential role for PAR2 in allergic sensitization.

Other studies have shown that overexpression of PAR2 in mice and enhanced Th2 cytokine production, something dependent to Der p 1 show markedly reduced Th1 cytokine production to be able to cleave the -chain of the IL-2 receptor (CD25) on T cells exposed directly on the protease activity or Der p 1. Cleavage of CD25 might also, of course, alter regulatory function, as IL-2 stimulation on the protease activity or Der p 1. Cleavage of CD25 might also, of course, alter regulatory function, as IL-2 stimulation on the protease activity or Der p 1. Cleavage of CD25 might also, of course, alter regulatory function, as IL-2 stimulation on the protease activity or Der p 1. Cleavage of CD25 might also, of course, alter regulatory function, as IL-2 stimulation on the protease activity or Der p 1. Cleavage of CD25 might also, of course, alter regulatory function, as IL-2 stimulation on the protease activity or Der p 1. Cleavage of CD25 might also, of course, alter regulatory function, as IL-2 stimulation on the protease activity or Der p 1. Cleavage of CD25 might also, of course, alter regulatory function, as IL-2 stimulation on the protease activity or Der p 1. Cleavage of CD25 might also, of course, alter regulatory function, as IL-2 stimulation on the protease activity or Der p 1. Cleavage of CD25 might also, of course, alter regulatory function, as IL-2 stimulation.
IgE-mediated allergic diseases overview

- Pollen allergy
- House dust mite allergy
- Food allergy
- Medication allergy
- Hymenoptera allergies
Sensitization Routes

Surfaces: Antigen presenting cells
Major part of allergens will rest in the upper respiratory System (pollen grain: 15-40 µm size)

Allergens might also be carried by small carrier proteins (2-10 µm) → might reach lung
Hay fever: symptoms of immediate reaction

Eyes: Conjunctivitis
Nose: Rhinitis
Lungs: Asthma
Skin: Urticaria
50 % of asthma patients have atopy; Symptoms: dyspnea and/or cough
Allergic asthma: allergic asthma is triggered by inhaling allergens; mostly seasonal asthma
Symptoms of IgE mediated allergies

**Acute**
- vascular phase of inflammation with dilatation of vessels, permeability ↑, constriction of smooth muscle and itching

**Chronic**
- cellular phase of inflammation with infiltration of cells (*Eosinophilia*, Th2-lymphocyte, macrophages,..) and destruction of tissue as consequence of inflammation
Symptoms of the IgE mediated (immediate) - reaction

**acute**

- Red eyes
- Swelling
- pruritus
- sneezing
- Runny nose

(Histamine, PAF, LT, PG)

- Vascular dilatation
- Increased permeability
- Sensory nerves
- Sensory nerves
- Hyper secretion

H₁
Symptoms of IgE induced late reaction

chronic

Swelling
Infiltration by inflammatory cells
Damage to epithelia
Thickening of basal membrane, restructuring of lung tissue
Mucus production

(LT, cytokine, PG, PAF, ECP, EPO, EDN,... IL-13, TNFα)
blocked nose
bronchial hyper reactivity
reduced lung function
Allergens overview

Overview of allergens important for IgE mediated allergic reactions

Diagnostic possibilities
Most frequent sensitizations of aero allergens

Number of allergens to be tested to assess allergenic sensitization in epidemiologic studies: results of the European Community Respiratory Health Survey I

P.-J. Bousquet et al. 2007 Clinical and Experimental Allergy, 37, 780–787

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Prevalence alone (%)</th>
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<tbody>
<tr>
<td><em>Dermatophagoïdes pteronyssinus</em></td>
<td>20.9</td>
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<tr>
<td>+Grass</td>
<td>16.6</td>
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<tr>
<td>+Cat</td>
<td>10.2</td>
</tr>
<tr>
<td>+Birch</td>
<td>8.2</td>
</tr>
<tr>
<td>+Alternaria</td>
<td>4.4</td>
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<tr>
<td>+Olive</td>
<td>3.7</td>
</tr>
<tr>
<td>+Cladosporium</td>
<td>2.3</td>
</tr>
<tr>
<td>+Parietaria</td>
<td>1.5</td>
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<tr>
<td>+Ragweed</td>
<td>1.3</td>
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</tbody>
</table>

nine allergens, 35.6% of the 11 355 subjects were sensitized
Allergy diagnosis

Allergy = sensitization + clinical symptoms

Skin prick test

Serum IgE
Basophil activation Test
Symptom assessment

Symptom calendar

Peak flow calendar
Conjunctival provocation tests (CPT, allergen solution)

No standardization of CPT; no grading of ocular reactions

Digital image analysis possesses the potential of being an objective evaluation method compared to the wide-spread subjective

Dogan et al. Int Arch Allergy Immunol 2014;163:59–68
Pollen
Allergenic pollen in Europe

Grass pollen: 10,000 species, most important airborne grass pollens:

Timothy (Phleum pratensae, Lieschgrass)

Major cause of pollinosis
Lowest dose for symptom induction: 10-50 grains / m³
(Davies R et al Clin Allergy 1973)

Tree pollen:
1. Fagales (Betulacae (birch and alder); Corylaceae (hazel); cross reactivity
2. Oleaceae (Olive, Mediterraneam region), Fraxinus (Ash)
3. Cupressaceae
Weed pollen:
Urticaceae (Brennessel, Parietaria (Glaskraut))

Compositae (Asteraceae): 20,000 species
Ragweed (Ambrosia) and mugwort (Artemisia)

Spores:
Alternaria, aspergillus, caldoproium, penicillium
# Flowering time of plants

<table>
<thead>
<tr>
<th>Type of pollen</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
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<tr>
<td>Hazel (Corylus)</td>
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<td>Yew (Taxus)</td>
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<td>Elm (Ulmus)</td>
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<td>Willow (Salix)</td>
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<td>Poplar (Populus)</td>
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<td>Birch (Betula)</td>
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<td>Ash (Fraxinus)</td>
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<td>Oil seed rape (B.napus)</td>
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<td>Pine (Pinus)</td>
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<td>Nettle (Urtica)</td>
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<td>Dock (Rumex)</td>
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<tr>
<td>Mugwort (Artemisia)</td>
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The main period of pollen release: 
- **Green**: Main period of pollen release
- **Blue**: Peak periods
The allergy is **not** directed to pollen, but to proteins within pollen!

Pollen = carrier (grain) + allergen (surface) + lipids
Betula verrucosa 1
Bet v 1
**Major and Minor allergens**

Major allergen: >50% of patients sensitive to the allergen

Minor allergen: <50% of patients sensitive to the allergen
Major and Minor grass pollen allergens

Heterogeneity of molecular sensitization profiles in grass pollen allergy – implications for immunotherapy?
U. Darsow et al. Clinical & Experimental Allergy, 2014 (44) 778–786
Food allergy

1. Toxic

2. Nontoxic
   A) Immune mediated
      - IgE mediated
      - Non-IgE mediated

   B) Non immune mediated (food intolerance)
      - enzymatic (e.g. lactase deficiency)
      - pharmacological (abnormal reactivity to substances e.g. amines)
      - undefined (e.g. food additive intolerance)
Prevalence

- 6-8% of children have food allergy during the first 3 years of life
- Up to 85% outgrow the food allergy in the first 5-10 years of life
- Adults: USA 4% have food allergy (real allergy?)

Symptoms

- Gastrointestinal tract, skin, airways, anaphylaxis
- Co-factors: alcohol, exercise, medications
2 Groups of food allergy

- Food sensitization develops as a consequence of sensitization to airborne allergens
  - Mostly adults, cross reactivity

- Food sensitization occurs by gastrointestinal tract (often stable proteins)
  - Mostly in children “real food allergy”
Cross reactivity

Cross reaction: provoked in a patient by various offending allergen sources containing common allergen structures.

Homology between proteins (pollen (Bet v1) and fruit associated Mal d1)

Structures of Pru a1 and Bet v1
Determination of cross-reactivity

Fig. 5 Western blot result of IgE binding to different immobilized nuts along with their pistachio nut inhibited extracts, probed using three individual patients' sera (a, b, and c). Negative control (N); A1 (Almond); A2 (immobilized almond inhibited with pistachio); Pe1 (Peanut); Pe2 (immobilized peanut inhibited with pistachio); C1 (Cashew); C2 (immobilized cashew inhibited with pistachio); Pi (Pistachio); Lo (low molecular weight marker (Amersham) (MW).

Fig. 6 Competitive inhibition ELISA of IgE binding to immobilized raw pistachio nut extract by increasing concentrations of pistachio, cashew, almond, and peanut extracts using patients' pooled sera.

Inhibition (%)

Pistachio  Cashew  Almond  Peanut  BSA

Inhibitor conc. [μg mL⁻¹]

0.01  0.1  1  10  100  1000

Noorbahsh et al. Allergology International 2011
Oral allergy syndrome

Sensitization to heat/pepsine labile plant-derived proteins in patients with pollen allergy

Cross reactivity between homologous plant derived proteins and pollen proteins

Bet v 1 → nuts, apple, kiwi

heated normally well tolerated

Allergen cross reactivity seems to be due to IgE antibodies that recognize structurally similar epitopes on different proteins that are phylogenetically closely related or present evolutionarily conserved structures
Profilin, a plant panallergen

Profilin are found in all eukaryotic cells; = actin binding protein (actin cytoskeleton)

Highly conserved proteins, sequence comparison among several Plants: identity of 75%

Detectable in many plants: pollen, fruits, vegetables, latex

was first identified in birch (bet v2)
Lipid transfer proteins

Role in defence against fungi and bacteria

Heat stable, begin to unfold above 95°, protein refold on cooling

More severe allergic reactions

Nut allergy (hazelnut, peanut, walnut)
2 Groups of food allergy

Food sensitization develops as a consequence of sensitization to airborne allergens

Mostly adults, cross reactivity

Food sensitization occurs by gastrointestinal tract (often stable proteins)

Mostly in children “real food allergy”
Allergy to stable food proteins

Most frequently involved foods: egg, milk, peanut, nuts, fish

Children may outgrow their allergy

Milk allergy: 50% have already lost their milk allergy during first year 85% by age of 3 years

Patients with nut, peanut or fish will mostly remain clinically reactive
Diagnosis of food allergy

No single *in vivo* or *in vitro* test is able to provide a reliable prediction of the clinical reactivity of a patient.

Sensitization (IgE, positive skin prick test…) does not predict clinical allergy upon food exposure.

High sensitivity, negative predictive accuracy (>90%) BUT lower specificity and positive predictive accuracy (50-85%).

Provocation (false negative, missing co-factors)
predictive decision points previously established proved highly efficient in diagnosing symptomatic food hypersensitivity. Because these 95% predictive decision points were generated from a population of children with atopic dermatitis, a patient group that typically has the most elevated IgE levels, patients without atopic dermatitis (who typically have much lower IgE antibody levels than patients with atopic dermatitis) would be quite likely to experience a positive food challenge result if their food-specific IgE levels exceeded these 95% decision points. The generally lower levels of IgE antibodies in patients without atopic dermatitis at least partially compensates for the lower prevalence of food allergy in the patients without atopic dermatitis compared with the population with atopic dermatitis. However, it must be appreciated that predictive values are significantly affected by disease prevalence within the population under investigation.

An alternative approach that minimizes the influence of disease prevalence when establishing decision points among different populations is to establish the diagnostic decision points from specificity values (eg, 95% or 90%). With such an approach, one accepts that 5% or 10% of nondiseased individuals may be erroneously labeled as clinically reactive (ie, allergic). Applying diagnostic decision points on the basis of 90% specificity values in our retrospective study (Table IV), the positive predictive values for the 3 major food allergens (ie, egg, milk, and peanut) were 95% or greater in the prospective study. As noted with the 95% predictive diagnostic decision points generated in the retrospective study, the 90% specificity diagnostic decision points have poor sensitivity, and therefore physicians cannot assume that a patient with a food-specific IgE value less than the diagnostic decision point will not have a clinical reaction to that food. Only a physician-supervised food challenge can clearly establish clinical reactivity.

Because the Pharmacia CAP System FEIA uses standardized allergens and is calibrated against the World Health Organization IgE standard, the predictive values that are reported in this study should be useful for the diagnosis of food allergy throughout the world. Table V presents the diagnostic decision points (ie, the food-specific IgE antibody levels recommended for identifying patients with >95% probability of reacting to a food challenge). For example, a physician would not need to perform an oral milk challenge on a child without atopic dermatitis if the milk-specific IgE level was 15 kUA/L or

![Image](https://example.com/image.png)

**FIG 1.** Probability of reacting to a food at a given IgE value.

Hymenoptera venom allergy

- the prevalence of an allergy systemic reaction after a Hymenoptera sting is estimated between 1 and 7% in Europe

- bee sting 50 µg (30-140 µg) of venom proteins, after a wasp sting 3–5 µg of venom proteins are introduced to the body of the victim

- Toxic reaction > 50-100 stings (in adults); > 10 stings (in children)
### Allergens present in hymenoptera venom

<table>
<thead>
<tr>
<th>Identified allergens of bee venom</th>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Allergen</strong></td>
<td>MW kDa</td>
</tr>
<tr>
<td>Phospholipase A2 Api m1</td>
<td>16</td>
</tr>
<tr>
<td>Hyaluronidase Api m2</td>
<td>45</td>
</tr>
<tr>
<td>Acid phosphatase Api m3</td>
<td>49</td>
</tr>
<tr>
<td>Melittin Api m4</td>
<td>2,8</td>
</tr>
<tr>
<td>Dipeptidyl peptidase Api m5</td>
<td>102</td>
</tr>
<tr>
<td>Protease inhibitor Api m6</td>
<td>8</td>
</tr>
<tr>
<td>CUB serine protease Api m7</td>
<td>39</td>
</tr>
<tr>
<td>Carboxyl esterase Api m8</td>
<td>70</td>
</tr>
<tr>
<td>Carboxypeptidase Api m9</td>
<td>60</td>
</tr>
<tr>
<td>Icarapin Api m10</td>
<td>55</td>
</tr>
<tr>
<td>MRJP 8 Api m11</td>
<td>65 60</td>
</tr>
<tr>
<td>Vitellogenin Api m12</td>
<td>200</td>
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<table>
<thead>
<tr>
<th>Identified allergens of wasp venom (Vespula spp.)</th>
<th>Table 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Allergen</strong></td>
<td>MW kDa</td>
</tr>
<tr>
<td>Phospholipase A1 Ves v1</td>
<td>34</td>
</tr>
<tr>
<td>Hyaluronidase Ves v2</td>
<td>38</td>
</tr>
<tr>
<td>Dipeptidyl peptidase</td>
<td>102</td>
</tr>
</tbody>
</table>

**MW, molecular weight; CCD, cross-reactive carbohydrate determinants; + = present; - = not present**

**Major allergen:** > 50 % of patients with wasp venom allergy have specific IgE antibodies,

**Minor allergen:** < 50 % of patients with wasp venom allergy have specific IgE antibodies

---

12-15% of dry weight of bee venom

>50% of bee venom allergic patients are sensitized to PLA2

---

A. Helbling, U. Müller Update on hymenoptera venom allergy with special aspects of diagnostics and therapy Allergo J 2013
Cross-reacting carbohydrate determinants

Asparagine – second AS serine or threonine

Van Ree Int Arch Allergy Immunol 2002;129:189–197

Hemmer et al. JACI 2001
Local reactions after hymenoptera stings

Large local reaction=
Swelling exceeding 10 cm

Unclear mechanism
(T cells?)
Classification of hymenoptera allergic reactions

Classification according to Mueller
Anaphylaxis

= potentially life threatening situation; rapid onset

Massive mediator release

different organs are involved (skin, respiratory, cardiovascular system)

most frequent cause in Switzerland: hymenoptera venom allergy, drug allergy, food allergy
Products of mast cell activation

- Histamine
- Proteases: tryptase, carboxypeptidase, chymase, cathepsin G, elastase, plasminogen activator, renin, matrix metalloprotease 9
- Cytokines: IL-4, IL-5, IL-6, IL-8, IL-13, GM-CSF, TNF-α, fibroblast growth factor, stem cell factor
- Lipid mediators: prostaglandin D₃, leukotriene C₄, platelet activating factor
- Other Enzymes: β-hexosaminidase, β-glucuronidase, arylsulphatase

Products of basophil activation

- Histamine
- Proteases: tryptase, elastase, cathepsin G
- Cytokines
- Proteoglycans: heparin, chondroitin sulphate
- Basic proteins: basogranulin, 2D7 antigen, eosinophil major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin
- Lipid mediators: leukotriene C₄, platelet activating factor
- Other Enzymes: β-hexosaminidase, β-glucuronidase, eosinophil peroxidase

Late phase reaction in 20% (delay 2-8 hours) !!!
Is anaphylaxis always IgE mediated???

No, Anaphylaxis may involve other mechanisms than IgE, e.g.:

- Complement activation
- IgG and IgM immune complexes
- Non-immunologic mechanisms
- Pseudo allergies (radio contrast media)
- Toxic effects of insect venom
- Non-steroidal anti-inflammatory drugs
Symptoms of anaphylaxis

1. Skin (e.g. urticaria, angioedema)       90%
2. Respiratory system (e.g. asthma)       50%
3. Gastrointestinal symptoms (e.g. vomiting) 30%
4. Circulation (e.g. shock)              10%
Important facts about hymenoptera venom allergy

Prevalence of hymenoptera venom sensitization: 9.3-28.7%

Prevalence of hymenoptera venom allergy: about 3%

1 sting: first month 30% of adults are sensitized; becomes negative in 30% of sensitized patients after 2 years

During sensitization no symptoms

Sensitization does not predict a future reaction

Risk of systemic reaction increases if the first sting is preceded by a second sting during 2 month
Beekeepers

Systemic reactions in 45% of beekeepers with <15 bee sting / year

No/less systemic reactions in Beekeepers with > 200 bee sting / year

Why???
PART 2

Therapy principles

Allergen-specific immunotherapy

Non-IgE mediated allergies
  - T-cell mediated allergies
  - IgG mediated allergies

Hygiene hypothesis

Drug allergy
Therapy principles

Inhibition of inflammatory mediators released during effector phase:

- anti histamines
- mast cell stabilizers
- leukotriene antagonists
- anti IL-4, IL-5
- anti IgE

Glucocorticoids

No causal therapy

Allergen-specific Immunotherapy = alters course of disease
Action of antihistamines

A. Active state of the histamine H1-receptor is in equilibrium with the active conformation, and shifts the equilibrium toward the inactive state.15,18

B. The agonist, histamine, has preferential affinity for the active state, stabilizes the receptor in this conformation, and shifts the equilibrium toward the active state.

C. Inverse agonist (antihistamine), has preferential affinity for the inactive state, stabilizes the receptor in this conformation, and shifts the equilibrium toward the active state.

Reverse receptor agonist

Simon et al
J ALLERGY CLIN IMMUNOL
DECEMBER 2011

Table E2

<table>
<thead>
<tr>
<th>Medication</th>
<th>Dosage</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levocetirizine</td>
<td>5 mg</td>
<td>Excellent</td>
</tr>
<tr>
<td>Desloratadine</td>
<td>5 mg</td>
<td>Good</td>
</tr>
<tr>
<td>Loratadine</td>
<td>10 mg</td>
<td>Moderate</td>
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<tr>
<td>Fexofenadine</td>
<td>180 mg</td>
<td>Poor</td>
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Table E3

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<thead>
<tr>
<th>Condition</th>
<th>Recommended Medications</th>
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<tbody>
<tr>
<td>Allergic rhinitis</td>
<td>Levocetirizine, Desloratadine, Fexofenadine</td>
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<tr>
<td>Allergic conjunctivitis</td>
<td>Levocetirizine, Desloratadine, Fexofenadine</td>
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<tr>
<td>Chronic urticaria</td>
<td>Levocetirizine, Desloratadine, Fexofenadine</td>
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Table E4

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<thead>
<tr>
<th>Medication</th>
<th>Efficacy</th>
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<tbody>
<tr>
<td>Azelastine</td>
<td>Excellent</td>
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<tr>
<td>Ketotifen</td>
<td>Good</td>
</tr>
<tr>
<td>Pseudoephedrine</td>
<td>Moderate</td>
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<td>Decongestants</td>
<td>Poor</td>
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Table E5

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<tr>
<th>Medication</th>
<th>Route</th>
<th>Efficacy</th>
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</thead>
<tbody>
<tr>
<td>Azelastine</td>
<td>Nasal</td>
<td>Excellent</td>
</tr>
<tr>
<td>Ketotifen</td>
<td>Oral</td>
<td>Good</td>
</tr>
<tr>
<td>Pseudoephedrine</td>
<td>Oral</td>
<td>Moderate</td>
</tr>
<tr>
<td>Decongestants</td>
<td>Oral</td>
<td>Poor</td>
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of the 40 patients became symptom free on 10 or 20 mg of levocetirizine without experiencing adverse effects at the higher doses. Additionally, in objective tests, such as in patients with acquired cold urticaria, high-dose desloratadine (20 mg) or rupatadine (20 mg) decrease wheal volume and improve cold provocation thresholds significantly compared with standard doses. Treatment guidelines for chronic urticaria now recommend second-generation H1-antihistamines as the medications of choice, starting with standard doses and increasing the doses up to 4-fold as needed to provide relief. This approach has not yet been validated in children.

For patients with severe chronic urticaria refractory to non-sedating H1-antihistamines, it can be helpful to add an H2-antihistamine, montelukast, omalizumab, cyclosporine, or dapsone and treat exacerbations with a glucocorticoid for 3 to 7 days. Small randomized controlled trials support the use of H1-antihistamines to prevent and relieve itching and flushing in patients with mastocytosis, prevent and relieve itchy large local allergic reactions to mosquito bites, and reduce adverse reactions and modulate allergen-specific immune responses during stinging insect venom immunotherapy.

Diseases in which H1-antihistamines are not medications of first choice

Largely on the basis of tradition, H1-antihistamines remain widely used in many diseases in which the evidence base for their efficacy and safety is weak and generally not supported by randomized controlled trials that meet current standards (Fig 4).

Atopic dermatitis.

Histamine acts as a pruritogen through H4-receptors in patients with atopic dermatitis, and other mediators, including IL-31 and other cytokines, play an important role. No high-quality randomized controlled trials of H1-antihistamines confirm their efficacy in patients with atopic dermatitis. Despite the absence of such trials, first-generation H1-antihistamines are...
Action of glucocorticoids

Inflammatory cells
- Number ↓ (apoptosis)
  - Eosinophils
- Cytokine ↓
  - Th2 cells
- Number ↓ Mediator ↓ Cytokine ↓ FcεRI ↓
  - Mast cells
- IgE ↓
  - B cells
- Number ↓
  - Dendritic cells
- Mediator ↓
  - Basophils

Constitutive cells
- Cytokine ↓
  - Epithelial cells
  - Adhesion molecule ↓
- Adhesion molecule ↓ Permeability ↓
- Endothelial cells
  - Proliferation ↓
  - Adhesion molecule ↓
  - Chemokine ↓
- Fibroblasts
  - Mucin secretion ↓
- Glands
  - Goblet cells

Glucocorticosteroid
- Number ↑
  - Regulatory T cells (Treg)
  - Regulatory cytokines ↑
  - (IL-10, TGF-β)

M. Okano  Clinical and Experimental Immunology, 158: 164–173 2009
Fig. 1. Genomic mechanisms of GC action: the ligand-activated GC receptor translocates into the nucleus where it induces or inhibits gene transcription. (I) Induced synthesis of anti-inflammatory proteins through binding of the ligand-activated glucocorticoid receptor to positive GREs; (II) suppressed transcription of inflammatory genes via negative GREs; (III) “transrepression” through direct or indirect interaction with transcription factors; (IV) competition for nuclear coactivators between the GC/cGCR complex and transcription factors. Abbreviations: cGCR, cytosolic glucocorticoid receptor; GRE, glucocorticoid response elements; nGRE, negative GRE; AP-1, activator protein 1 (Buttgereit et al., 2004).

Fig. 2. Summary of genomic and non-genomic mechanisms of GC action: lipophilic glucocorticoids pass easily through the plasma membrane and bind to the cGCR, which mediate genomic (I) and non-genomic effects (II). Non-genomic effects are also suggested to be mediated via membrane bound GCR (III) and by interactions with cellular membranes (IV) (Buttgereit et al., 2004).
Side effects of glucocorticoid treatment:

e.g. metabolic effects

Osteoporosis
Hypertension
Hyperglycaemia
Cushing syndrome …

Less side effects with topical glucocorticoids (e.g. asthma spray)
Anti IgE therapy (omalizumab)

Binding of omalizumab to the cε3 domain of IgE.

Adapted from Francés et al. 2014 Actas Dermosifiliogr. 2014;105:45-52. - Vol. 105 Num.01
Immunotherapy, a causal therapy of allergic diseases???
Short term effect: Tachyphylaxis

Injection time point 0 min 30 min 60 min 1.5 h 2.5 h 3.5 h

Dose of wasp venom 0.1 µg 1 µg 10 µg 20 µg 30 µg 50 µg

- temporarily decreased release of mediators from effector cells
- transient increase of intracellular cyclic AMP

Mechanisms of SIT

A

- Early desensitization: decrease in mast cell and basophil activity for degranulation and systemic anaphylaxis
- T cell tolerance induction of T_{Reg} cells suppression of Th2-Th1 cells
- Late desensitization: decrease in tissue mast cells and eosinophils and release of their mediators

B

- Type I skin test reactivity
- Specific IgE
- Specific IgG4

Burks et al. J
Allergy Clin Immunol 2013;131:1288-96.
Suppression of TH2 cells and IgE producing B-cells by Treg-cells:

Allergen-specific immunotherapy → Treg → TGF-β, IL-10 → TH2 → B cell

Ig Switch

IgE↓ IgG4↑

Inhibition of inflammatory mediators released during effector phase:
- basophils
- antihistamines
- mast cell stabilizers
- leukotrien antagonists
- anti-IL-4, IL-5
- anti-IgE

Treg: Regulatory T cells
B cell: B lymphocyte
TH2: Type 2 helper T cell
IL-10: Interleukin-10
TGF-β: Transforming growth factor-β
Side effects of SCIT

Severity of side effects: Mild symptoms to life-threatening anaphylaxis and even death

Table 2. Systemic Reactions (SRs) per 10,000 Injection Visitsa between July 2008 and 2010

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Any type of SR</td>
<td>10.2</td>
<td>9.7</td>
</tr>
<tr>
<td>Grade 1 (mild SRs)</td>
<td>7.6</td>
<td>6.7</td>
</tr>
<tr>
<td>Grade 2 (moderate SRs)</td>
<td>2.3</td>
<td>2.7</td>
</tr>
<tr>
<td>Grade 3 (severe SRs)</td>
<td>0.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Immediate and delayed-onset systemic reactions after subcutaneous immunotherapy injections: ACAAI/AAAAI surveillance study of subcutaneous immunotherapy–year 2


SLIT has a better safety profile than SCIT (home administration possible)
Which pollen extracts are used for immunotherapy?

Evidence for efficacy is found for the following allergens relevant in Europe:

- Birch
- Alder
- Hazel
- Olive
- Ash
- Grass
- Cypress
- Parietaria
- Ambrosia
- Dermatophagoides pteronyssinus and farinae
- Cat
Extracts for SCIT

- SIT is performed using natural allergen extracts
- Most commercial extracts are standardized for major allergen content, but amounts of minor allergenic components can be very low or variable!!!
Recombinant allergens for SCIT?

Specific subcutaneous immunotherapy with recombinant grass pollen allergens: first randomized dose-ranging safety study

L. Klimek¹, P. Schendzielorz¹,², R. Pinol¹ and O. Pfaar¹
¹Center for Rhinology and Allergology, Wiesbaden, Germany and ²Department of Otorhinolaryngology, University Hospital of Ruprecht-Karls University, Mannheim, Germany

- Recombinant Phleum allergens (Phl p 1, 2, 5a, 5b, 6)
- No severe side effects (50 patients)
- Increases of allergen-specific IgG4 and IgG1 levels
- Improved tolerance in conjunctival provocation test
Adjuvants

- Aim: less side effects, enhanced immune response

- Aluminium hydroxide-adsorbed allergens

- Aluminium salt: enhancer of immune response

- Possible mechanisms:
  1. depot effect, slow antigen release -> prolonged presentation to antigen presenting cells (HogenEsch et al. Front Immunol 2013)


  3. stimulation of prostaglandin E2 production → regulation of Th2 responses (Kuroda et al. 2011 Immunity)
**Allergoid vaccines**

Allergoid vaccines = allergen extracts modified chemically e.g. by glutaraldehyde or formaldehyde

- destruction of IgE epitopes; no impact on sequential T cell epitopes

- claim of allergoids: decreased allergenicity, thereby allowing faster and higher up-dosing

- with preserved or even improved immunogenicity


Hygiene hypothesis
Prevalence of allergic diseases

- East Germany: before the fall of the wall lower asthma incidence compared to West Germany, now no difference any more

- Prevalence of atopic diseases has doubled or tripled during the last 3 decades

- West – East gradient in Europe
Prevalence of Allergic diseases
Prevalence of Autoimmune diseases
Prevalence of Infectious diseases
According to this theory, the immune system at birth is immature (TH2-like pattern of cytokine production persists), leading to an increased risk of asthma and other atopic diseases. LPS, lipopolysaccharide.

Figure 3

Microbial exposure boosts Th1 response
Microbial exposure alters Th2 response

Response starts in utero

Protective effect of the farm environment

Protective effect by parasite infection

Gern et al. Nature Reviews 2002
Western life style

- Low microbial load
- Th2
- Allergy

Traditional life style

- High microbial load
- Th1 / Treg
- Tolerance, no allergy
**Probiotics**

= non pathogenic microorganisms that exert a positive influence on host health and physiology

Important for shaping the immune system

Human gut: $10^{14}$ bacteria

Placebo controlled trial in Finland:
Lactobacillus intake for mothers for 2-4 weeks before delivery and postnatally could decrease the incidence of atopic dermatitis

German population: same study no protective effects

→ Role of probiotics in immune allergic diseases not clear
„Allergic“ diseases
- non IgE -

- Exogen allergic alveolitis (IgE/IgG/T-cells)
- contact dermatitis (T-cells)
- Various, frequent forms of drug allergies (mostly T-cells)
- „Pseudo - allergies“ (?)
- Some forms of asthma bronchiale
- Some forms of atopic dermatitis (z.T. T cells)
- Some forms of eosinophilic esophagitis
- chronic urticaria (no antigen/allergen)
Drug allergy
Introduction

The adverse events seen after contrast media (CM) administration may be divided into three different types: (i) allergic and nonallergic hypersensitivity reactions as defined by the European Academy of Allergy and Clinical Immunology (1), (ii) toxic reactions (2) and (iii) events unrelated to CM exposure (Fig. 1). Hypersensitivity reactions are either immediate reactions, which occur within 1 h after CM administration, or nonimmediate reactions, which become apparent more than 1 h after CM exposure (3). About 70% of the immediate symptoms are reported to start within the first 5 min of CM administration (4).

This paper summarizes current state of the art regarding pathomechanisms, diagnosis and prevention of CM-induced hypersensitivity reactions and outlines open fields for further research in this area.

Prevalence of contrast medium reactions

Three large observational studies conducted in the mid-1980s indicated that mild adverse reactions of the immediate type occur in 3.8–12.7% of patients receiving intravenous injections of high-osmolar, ionic CM and in 0.7–3.1% of patients receiving low-osmolar nonionic CM (4–6). Severe immediate reactions have been reported to occur with a frequency of 0.1–0.4% for ionic CM and with a frequency of 0.02–0.04% for nonionic CM (4–7). Although the adverse reactions observed with the nonionic CM are usually less severe than the reactions induced by the ionic CM, the death rates for the two types of products are not significantly different. The mortality rate has been estimated to be in the range of 1 in 100,000 examinations (7). Severe and fatal reactions represent a serious problem in regard to the more than 70 million applications of CM per year worldwide (8).

The frequency of nonimmediate adverse reactions was recently reviewed by Webb et al. (9). In the 10 studies cited in this paper, the frequency ranged from 0.5 to 23%. This large variation may be due to the difficulty in verifying whether symptoms occurring hours or days after CM exposure are in fact caused by the CM. When radiological examinations with use of CM were compared with examinations without CM, most nonimmediate symptoms except skin reactions were found to be unrelated to the CM administration (10, 11). Thus, various types of exanthema seem to account for the majority of the CM-induced nonimmediate hypersensitivity reactions. Such eruptions have been reported to affect some 1–3% of CM-exposed patients (3, 11–13).

Organ toxicity

T-cell-mediated

Nonallergic or IgE-mediated?

Pharmacological effect

Immediate reaction ≤1 h

Nonimmediate reaction 1 h–7 days

Hypersensitivity

Organ toxicity

Allergic symptoms

Exanthematous skin eruption

Unspecified symptoms

Unrelated event

Pharmacological toxicity

Adverse event

Unrelated event
T cells react with the drug in two different ways. 'Immunologically', namely by 'recognizing' a hapten-modified peptide with their T cell receptor (TCR) in the frame of MHC molecules. This corresponds to a classical immune response and is highly relevant for contact dermatitis, and is due to haptens covalently bound to proteins and peptides. Alternatively, T cells may be stimulated 'pharmacologically' – the drug itself binds either directly to certain regions of the TCR, which results, together with MHC interaction, in a stimulation of this T cell with the particular T cell receptor, or the drug may modify MHC peptide complexes directly, making them immunogenic for T cells.

<table>
<thead>
<tr>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
<th>Type IVa</th>
<th>Type IVb</th>
<th>Type IVc</th>
<th>Type IVd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune reactant</td>
<td>IgE</td>
<td>IgG</td>
<td>IgG</td>
<td>IFNγ, TNFa (TH1 cells)</td>
<td>IL-5, IL-4/IL-13 (TH2 cells)</td>
<td>Perforin/granzymeB (CTL)</td>
</tr>
<tr>
<td>Antigen</td>
<td>Soluble antigen</td>
<td>Cell- or matrix-associated antigen</td>
<td>Soluble antigen</td>
<td>Antigen presented by cells or direct T cell stimulation</td>
<td>Antigen presented by cells or direct T cell stimulation</td>
<td>Cell-associated antigen or direct T cell stimulation</td>
</tr>
<tr>
<td>Effector</td>
<td>Mast cell activation (phagocytes, NK cells)</td>
<td>FcR+ cells complement</td>
<td>Macrophage activation</td>
<td>Eosinophils</td>
<td>T cells</td>
<td>Neutrophils</td>
</tr>
<tr>
<td>Example of hypersensitivity reaction</td>
<td>Allergic rhinitis, asthma, systemic anaphylaxis</td>
<td>Hemolytic anemia, thrombocytopenia (e.g. penicillin)</td>
<td>Serum sickness, Arthus reaction</td>
<td>Tuberculiner reaction, contact dermatitis (with IVc)</td>
<td>Chronic asthma, chronic allergic rhinitis</td>
<td>Contact dermatitis Maculopapular and bullous exanthema hepatitis</td>
</tr>
</tbody>
</table>
Chemical structure of drug molecule

- No protein drugs, small molecules 1-2 kDa, haptens
- Protein drugs, large molecules >20 kDa
Haptens

Small molecules alone are not immunogenic!
Haptens = reactive proteins binding to a larger protein → hapten-carrier complex
→ resistant to intracellular processing
→ danger signal (activation of innate immunity e.g. DC’s)
→ forms neoantigenic determinants able to induce both a T-cell and B-cell immune response.

\[
\text{hapten} \quad + \quad \text{carrier protein} \quad = \quad \text{hapten-carrier complex}
\]

Not immunogenic  Not immunogenic  Immunogenic
Prohapten – metabolism is required

sulfamethoxazole hydroxylamine

sulfamethoxazole nitroso sulfamethoxazole

sulfamethoxazole protein conjugate

HYPERSENSITIVITY

Cribb & Spielberg, 1992
Gill et al., 1997
IgE mediated drug allergies (immediate reactions)

<table>
<thead>
<tr>
<th>Anaphylactic IgE-mediated reactions</th>
<th>Antibiotics</th>
<th>Chemotherapy drugs</th>
<th>Monoclonal antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flushing, pruritus, urticaria, angioedema, laryngeal edema, rhinorrhea, conjunctivitis, shortness of breath, wheezing, bronchospasm, nausea, vomiting, diarrhea, hypotension</td>
<td><strong>Beta-lactams</strong>&lt;br&gt; Penicillins, cephalosporins, amino-penicillins&lt;br&gt; <strong>Fluroquinolones</strong>&lt;br&gt; Ciprofloxacin, levofloxacin</td>
<td><strong>Platins</strong>&lt;br&gt; Carboplatin, cisplatin, oxaliplatin</td>
<td><strong>Rituximab, trastuzumab</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sepsis</strong>&lt;br&gt; Meningitis&lt;br&gt; Pneumonia&lt;br&gt; Pyelonephritis</td>
<td></td>
<td>Primary and recurrent metastatic cancers (breast, ovarian, colon)</td>
<td>Chronic inflammatory diseases, cancers (leukemias, breast, ovarian )</td>
</tr>
</tbody>
</table>

Desensitization often possible!!!
### Non-IgE mediated drug allergies (immediate reactions)

**Table 3: Desensitization to aspirin in a patient with asthma**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>90</td>
<td>81</td>
</tr>
<tr>
<td>180</td>
<td>162</td>
</tr>
<tr>
<td>240</td>
<td>325</td>
</tr>
<tr>
<td>330</td>
<td>650</td>
</tr>
</tbody>
</table>

Aspirin to be continued at 650 mg orally, twice a day. Adapted from White et al. [30].

**Table 4: Desensitization to aspirin in a patient with aspirin-related urticaria-angioedema**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>20</td>
<td>0.3</td>
</tr>
<tr>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>60</td>
<td>3</td>
</tr>
<tr>
<td>80</td>
<td>10</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td>120</td>
<td>40</td>
</tr>
<tr>
<td>140</td>
<td>81</td>
</tr>
<tr>
<td>160</td>
<td>162</td>
</tr>
</tbody>
</table>

Aspirin to be continued at 162 mg once per day. Adapted from Wong et al. [5].

### Anaphylactoid

**Direct mast cell/basophil, complement, and leukotriene metabolism reactions**
- Flushing, pruritus, urticaria, angioedema, throat tightness, shortness of breath, nausea, vomiting, diarrhea, hypotension, hypertension, back and/or abdominal pain

### Aspirin/NSAIDs

**Cardiac protection, asthma w/ nasal polyposis, chronic inflammatory diseases (RA, Crohn's)**

### Vancomycin

**MRSA**

### Chemotherapy drugs

**Taxenes**
- Paclitaxel, docetaxel

**Primary and recurrent metastatic cancers (breast, ovarian, colon)**

### Pseudo-allergic reactions radio contrast media: direct membrane effects related to the osmolarity of contrast media solution
IgG mediated drug hypersensitivity (Typ II)

Haemolytic anaemia

Drug binds on Cell surface

Alters antigen on red blood cell
Hapten

Recognition By antibodies

Cell lysis

Culprit drugs: e.g. penicillins, cephalosporins
Symptoms: cytopenia (anemia, thrombocytopenia etc.)
DIRECT COOMB’S TEST

Patient Sample + anti-Hu IgG Coomb’s Reagent = Agglutination

IgG mediated typ II hemolysis → normally positive
IgG mediated drug hypersensitivity (Typ III)

Immune complexes may deposit preferentially in joints because the synovial endothelium is fenestrated → is more accessible to proteins and protein complexes → triggers an inflammatory response.

Clinic: vasculitis, skin, serum sickness, lungs, joints, fever
T cell mediated drug hypersensitivity
(Type IV reaction)
Symptoms of T cell mediated drug allergy

• Makulo-papular Exanthem
• bullous Exanthem
• Acute generalized exanthematous Pustulosis (AGEP)
• Stevens-Johnson Syndrome (SJS) toxic-epidermal Necrolysis (TEN)
• DRESS, Hepatitis, interstitial Nephritis, Pneumonitis
Sequence of T cell mediated drug reaction

1       2       3       4       5       6       7        8       9       10  days

few precursor cells → multiplication Reaching a certain number
symptoms (depending on kind of immune reaction)

Clonal expansion
Secretion of pro-Inflammatory cytokines
Tissue damage
Pharmacological interaction with immune receptors

- p-i concept

- Chemical inert drug, unable to bind covalently to proteins

- Binding to immune receptors

- Drug-protein interaction $\rightarrow$ activation and expansion of antigen-specific cells

- Initiation of a specific immune response
**p-i concept:**

a) the drug binds first to the TCR (by non covalent bonds; not restricted to a HLA-allele)

or

b) the drug binds first to the HLA molecule, and the HLA-peptide-drug complex is then recognized by the TCR (HLA-class I restricted, CD8)
Hapten

Immune reaction

p-i (TCR)

Pharmacological interaction

p-i (HLA)
Are pi reactions predictable???
MHC alleles

Allele = number of alternative forms of the same gene or same genetic locus.
## Gene complexity at the MHC locus in man

<table>
<thead>
<tr>
<th>Class I</th>
<th>Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>gene</td>
<td>alleles</td>
</tr>
<tr>
<td>HLA-A</td>
<td>1,519</td>
</tr>
<tr>
<td>HLA-B</td>
<td>2,069</td>
</tr>
<tr>
<td>HLA-C</td>
<td>1,016</td>
</tr>
<tr>
<td>HLA-E</td>
<td>10</td>
</tr>
<tr>
<td>HLA-F</td>
<td>22</td>
</tr>
<tr>
<td>HLA-G</td>
<td>46</td>
</tr>
</tbody>
</table>

Data from the European Bioinformatics Institute (EBI) server ([http://www.ebi.ac.uk/imgt/hla/stats.html](http://www.ebi.ac.uk/imgt/hla/stats.html))
## HLA-alleles and drug hypersensitivity

<table>
<thead>
<tr>
<th>DRUG</th>
<th>HLA Allele</th>
<th>HLA Carriage Rate</th>
<th>Prevalence of diagnosis</th>
<th>Negative Predictive Value</th>
<th>Positive Predictive Value</th>
<th>NNT to prevent “1”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abacavir</td>
<td>B*5701</td>
<td>6-8% Caucasian &lt;1% African/Asian 2.5% African American</td>
<td>8% (includes 3% true HSR and 2-7% false positive diagnosis)</td>
<td>100% for patch test confirmed</td>
<td>55%</td>
<td>13</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>B*5801</td>
<td>9-11% Han Chinese 1-6% Caucasian</td>
<td>1/250-1/1000</td>
<td>100% in Han Chinese</td>
<td>3%</td>
<td>250</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>B*1502</td>
<td>10-15% Han Chinese &lt;0.1% Caucasian</td>
<td>&lt;1-6/1000</td>
<td>100% in Han Chinese</td>
<td>3%</td>
<td>1000</td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>B*5701</td>
<td>As for abacavir</td>
<td>8.5/100,000</td>
<td>99.99%</td>
<td>0.12%</td>
<td>13819</td>
</tr>
</tbody>
</table>
HLA - A*02:01, A*31:01
- B*10:02; B*57:01
- C*02:01; C*06:01
- DR B1*01:01; 04:02
- DR B5*01:01
- DP*04:04; 08:01
- DQ*01:05; 05:01

HLA - A*02:01, A*32:01
- B*15:02; B*58:01
- C*02:01; C*06:01
- DR B1*01:01; 04:02
- DR B5*01:01
- DP*03:04; 07:01
- DQ*02:04; 03:02
HLA - A*02:01, A*31:01
- B*10:02; B*57:01
- C*02:01; C*06:01
- DR* B1 01:01; 04:02
- DR* B5 01:01
- DP* 04:04; 08:01
- DQ* 01:05; 05:01

Risk for Carbamazepine hypersensitivity

Risk for Abacavir hypersensitivity

HLA - A*02:01, A*32:01
- B*15:02; B*58:01
- C*0201; C*06:01
- DR* B1-01:01; 04:02
- DR* B5-01:01
- DP* 03:04; 07:01
- DQ* 02:04; 03:02

Risk for Allopurinol hypersensitivity

Risk for Carbamazepine hypersensitivity
T cell mediated allergy - conclusion

**HAPTON**
- Chemical (hapten)
- MHC-APC directed
- (processing and metabolism)
- Very heterogeneous and often combined immune responses (Ig, T-cells.....)

**P-I CONCEPT**
- Structural (fitting into TCR)
- TCR – T-cell directed
- No processing/no metabolism
- T-cell reactions of different types (exanthema, DRESS, AGEP, TEN.....)
Summary

Allergy → specific immune reaction to harmless foreign substances with clinical symptoms

Innate and adaptive immune system is involved

Allergens: proteins or haptens, often activate immune system

Antibody mediated (IgE, IgG)

T cell mediated immune reactions

pharmacological interaction (pi concept)

Allergen-specific immunotherapy is the only causal therapy for allergic diseases