Chapter 2: Type I allergy: Mechanisms in effector phase and Anaphylaxis

Eva Untersmayr-Elsenhuber
Department of Pathophysiology and Allergy Research
Medical University Vienna
Topics of lecture

- Clinical presentation of allergic reactions
- Key players in allergic inflammation
  - IgE
  - Allergen
  - Mast cells
  - Eosinophils, inflammatory cells
- Mechanisms of anaphylactic reactions
Clinical presentation of allergic reactions

Allergy against inhalative proteins

Å Respiratory symptoms
Wheezing, rhinitis
Conjunctivitis
Asthma  Mild → severe
In all age groups
  Gibson PG et al. Lancet 2010

Å Cutaneous and gastrointestinal symptoms
Eczema
Oral allergy syndrom
  Katelaris CH. Curr Opin Allergy Clin Immunol. 2010

http://www.raksar.com/shop/r/raksar/i mg-lib/con_20051229134550_iJPG
Clinical presentation of allergic reactions

Allergy against food compounds

Å Gastrointestinal symptoms
  Abdominal pain
  Nausea, vomiting
  Diarrhea

Å Respiratory and cutaneous symptoms
  Rhinitis, conjunctivitis
  Asthma
  Eczema, urticaria, angioedema

Å Generalized anaphylactic symptoms

Sicherer SH, Sampson HA. JACI 2010
Clinical presentation of allergic reactions

Allergy against drugs

Å Respiratory symptoms
  Wheezing, rhinitis
  Conjunctivitis
  Asthma

Å Cutaneous symptoms
  Eczema
  Urticaria
  Angioedema

Å Generalized anaphylactic symptoms
Time course of allergic reactions

Immediate reaction
Onset within seconds due to preformed or rapidly synthesized mediators → vascular permeability → contraction of smooth muscle

Late phase reaction
Induced synthesis and release of mediators → recruiting of eosinophils and Th2 lymphocytes → second phase of smooth muscle contraction, sustained edema, airway hyperreactivity

Time course of allergic reactions

Key players in immediate reactions
IgE bound via FcεRI to mast cells and basophils
→ mediator release

Time course of allergic reactions

Key players in late phase reaction
Network of inflammatory cells: eosinophils (50% of infiltrate), allergen-specific T-cells, mast cells, basophils, Th1 cells

Laché M et al. Nat Rev Immunol 2006
**Chronic allergic inflammation**

Due to repetitive or persistent allergen exposure, innate immune cells (eosinophils, basophils, neutrophils, and monocyte/macrophages) and adaptive immune cells (Th2-cells, other T-cells, B-cells) take up residence in tissue.

Chapter 2: Type I allergy: Mechanisms in the effector phase and Anaphylaxis

Eva Untersmayr-Elsenhuber
Department of Pathophysiology and Allergy Research
IgE antibodies

Mean serum concentration:
- IgE: 0.02-0.5 mg/ml
- IgG: 8-16 mg/ml

Percentage of total Ig:
- IgE: 0.002 %
- IgG: 80 %

Serum half life time: 2 days

Peak IgE levels occure 4-6 weeks after peak of pollen season

Total IgE >1000ng/mL → major diagnostic criteria for allergic bronchopulmonary aspergillosis
Diseases with elevated IgE levels

- Atopic diseases
- Parasitic infections (eg. Strongyloidiasis, ascariasis, schistosomiasis)
- Nonparasitic infections (eg. EBV, CMV, HIV, M. tuberculosis)
- Inflammatory disease (eg. Kimura disease, Churg-Stauss vasculitis, Kawasaki disease)
- Malignancies (eg. Hodgkin lymphoma, IgE myeloma)
- Cutaneous diseases (eg. Bullous pemphigoid)
- Cystic fibrosis
- Nephrotic syndrome
- Primary immunodeficiency diseases (eg. Hyper-IgE syndrome, Wiskott-Aldrich syndrome, Omenn syndrome, immune dysregulation, X-linked inheritance, atypical DiGeorge syndrome)
Immunoglobulin class switch

Ig switch: - transcription through upstream constant switch region
- DNA cleavage of ssDNA at transcription site
- DNA repair: recombine VDJ domain + new C domain

2 signals for IgE switch
1) IL4 or IL13 via STAT6: activates transcription at S\(\delta\)
2) CD40L (T-cells) and CD40 (B-cells): activates DNA switch recombination
Sensitisation to allergens

Dendritic cell sampling
Entrance through disrupted epithelia
Protease activity → cleaving of epithelial tight junctions

High affinity IgE receptor FcεRI

Tetrameric FcεRI

Antigen dependent effects

Mediator release

Antigen independent effects

Signalling

mast cells, basophils

According to: Kraft S, Kinet JP. Nature Immunol 2007
Trimeric Fc\(_{RI\alpha 2}\)

monocytes, macrophages, dendritic cells, Langerhans cells, eosinophils

According to: Kraft S, Kinet JP. Nature Immunol 2007
**Fc\(_\epsilon\)RI and IgE interaction**

1. Fc\(_\epsilon\)RI chain with 2 asymmetric IgE interaction sites:
   - 4 solvent-exposed tryptophans \(\rightarrow\) large hydrophobic surface
   - \(\gamma\)-\(\gamma\) loop in the receptor D2 domain

\(\rightarrow\) ligand:receptor = 1:1
\(\rightarrow\) high affinity \((10^{10} \text{ M}^{-1})\) due to low dissociation rate, each binding site has lower intrinsic affinity

Wan T et al. Nature Immunol 2002

Metzger H. Immunol Rev 1992
Low affinity IgE receptor FcεRII/CD23

Glycoprotein, 45 kDa, single chain homology to C-type (Ca\(^{2+}\)-dependent) lectins → IgE and CD21 bind to lectin domain

Mossalayi MD et al. EMBO J 1992

lower affinity for IgE (10\(^7\) M\(^{-1}\))

B-cells, activated T-cells, monocytes, macrophages, eosinophils, Langerhans cells, intestinal epithelial cells, platelets
CD23 regulates IgE synthesis

**Up-regulation of IgE synthesis**

**Co-crosslinking of mLgE and CD21 by trimeric sCD23**

- Hibbert RG et al. JEM 2005

**IgE binding to mCD23 protects mCD23 against proteolysis and prevents formation of sCD23**

**Down-regulation of IgE synthesis**

- Co-crosslinking of mCD23 and mLgE by allergen-IgE complex
  - Inhibition of B-cell proliferation and IgE production

- Induction of B-cell apoptosis → B-cell population regulation
  - Hibbert RG et al. JEM 2005
Chapter 2: Type I allergy: Mechanisms in the effector phase and Anaphylaxis

Eva Untersmayr-Elsenhuber
Department of Pathophysiology and Allergy Research
### Characteristics of allergens

<table>
<thead>
<tr>
<th>Pollen allergens</th>
<th>Food allergens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoproteins</td>
<td>Stable to head, acid, proteases</td>
</tr>
<tr>
<td>low dose $\rightarrow$ activation of IL4-producing CD4-T-cells</td>
<td>some are enzyme inhibitors</td>
</tr>
<tr>
<td>low molecular weight ($10-70$ kDa) $\rightarrow$ diffusion through epithelia facilitated solubility</td>
<td>abundance in food</td>
</tr>
<tr>
<td>Stability</td>
<td></td>
</tr>
<tr>
<td>enzymatically active, protease</td>
<td></td>
</tr>
</tbody>
</table>

**Pollen allergens**

[Image of pollen allergens](http://www.umass.edu/tei/TEI/images/UMassEnv/pollen.gif)

**Food allergens**

IgE – allergen interaction

IgE epitopes on allergens are mainly conformational

\[ \text{Xia L et al. Mol Immunol 2010} \]
\[ \text{Padavattan S et al. J Immunol 2009} \]
\[ \text{Pedraza-Escalona M et al. Mol Immunol 2009} \]

IgE to conformational rather than linear epitopes correlate with tolerance development in milk and egg allergic children

\[ \text{Järvinen KM et al. Allergy 2007} \]
\[ \text{Vila L et al. Clin Exp Allergy 2001} \]

Repeated epitope presentation on allergen surface required for crosslinking

\[ \text{→ allergens are multimers} \]

High binding affinity and slow dissociation rates of IgE to the allergen

\[ \text{IgE: } 10^{-10} \text{ bis } 10^{-11} \text{ M} \]
\[ \text{IgG: } 10^{-7} \text{ bis } 10^{-8} \text{ M} \]

\[ \text{→ IgE affinity to allergen among the highest biologically relevant binding strength} \]

\[ \text{Kim KE et al. Mol Immunol 1996} \]
\[ \text{Pierson L et al. J Immunol Meth 1998} \]
\[ \text{Jackola DR et al. Mol Immunol 2002} \]
\[ \text{Hantusch B et al. Immunol Lett 2005} \]
\[ \text{Foote J et al. PNAS USA 1995} \]
Chapter 2: Type I allergy: Mechanisms in the effector phase and Anaphylaxis

Eva Untersmayr-Elsenhuber
Department of Pathophysiology and Allergy Research
Morphology of mast cell

Tissue based inflammatory cells
Respond to signals of innate and adaptive immunity with immediate and delayed release of inflammatory mediators

20 μm diameter
Ovoid or irregularly elongated cells with ovoid nucleus
Abundant metachromatic cytoplasmatic granules (metachromatic staining due to sulfated proteoglycans)

cKIT (CD117) and FcεRI positive
Other cell-surface receptors depending on location and activation:
FcεRIIa (in resting state)
FcεRI (CD64) (in presence of IFN-γ)
β2-adrenergic receptor, adenosine receptor A2B, prostaglandin E2 receptor, C3a, C5a-receptor, IL-3R, IL-4R, IL-5R, IL-9R, IL-10R, GM-CSFR, IFN-γR, CCR3, CCR5, CXCR2, CXCR4, nerve growth factor R, TLRs,
Mast cell maturation and tissue distribution

Arise from CD34+ progenitors:
- Hematopoietic Stem cell
- Mast cell progenitor

cKIT (CD 117) + SCF Ligand

Phenotype of mature mast cells depends on growth factor milieu:
- Mucosal mast cell: Tryptase pos.
  - In respiratory and GI mucosa → increased with inflammation
- Connective tissue mast cell: Tryptase, mast cell-specific chymase pos.
  - In connective tissue (dermis, submucosa of GI tract, heart, conjunctivae, perivascular)
  - Small bowel of end-stage immunodeficiencies
Mechanisms of mast cell activation

- Antigen-crosslinked surface-bound IgE
- Divalent Ab against IgE Fc region
- Anti-idiotypic Ab
- Anti-Fc receptor Ab
- Covalent cross-linked IgE
- Lectins
- Complement (C3a, C5a) through C3aR, C5aR (CD88)
- Nerve growth factor
- IgG by FcγRI
- TLR ligands (eg. TLR3 dsDNA → IFN-γ production in mast cells)
Mast cell mediators

**Granule-ass. preformed mediators**
- Histamine
- Neutral proteases (tryptase in MC\textsubscript{T}, tryptase, chymase, cathepsin G, carboxypeptidase in MC\textsubscript{TC})
- Proteoglycans (heparin, chondroitin sulfates) neg. charged → complexes with histamine

**Newly formed mediators**
- Lipoxygenase pathway products: SRS-A (LTC\textsubscript{4}, LTD\textsubscript{4}), leukotrienes (LTB\textsubscript{4})
- Cyclo-oxygenase products: prostaglandines and thromboxanes
- PAF
**Effector function of mediators**

**Chemo-attractants**
- NCF
- ECF-A
- LTB₄

**Activators**
- Histamine
- PAF
- Tryptase
- Kininogenase

**Spasmogens**
- Histamine
- PGD₂
- LTC₄
- LTD₄

**Attractants of**
- Neutrophils, eosinophils, monocytes, basophils

**Vasodil., Vascular permeability**

**Microthrombi**

**Proteolyt. enzyme, activates C3**

**Kinins → Vasodil. → edema**

**Brachial smooth muscle contraction**

**Mucosal edema**

**Mucus secretion**

Preformed

Newly formed
Clinical aspects of mast cell activation

Allergen dosage and entrance route are decisive for clinical reaction.

<table>
<thead>
<tr>
<th>MC&lt;sub&gt;TC&lt;/sub&gt;</th>
<th>MC&lt;sub&gt;T&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>intravenous high dose</td>
<td>inhalation low dose</td>
</tr>
<tr>
<td>subcutaneous low dose</td>
<td>uptake with food</td>
</tr>
</tbody>
</table>

Mast cell activation

- Anaphylaxis
- Wheal and flare reaction
- Allerg. rhinitis
- Asthma
- Nausea, pain, vomiting diarreha

Allergen (Allerg. rhinitis)
Morphology of basophils

Share many features with mast cells:
- FcεRI
- secretion of Th2 cytokines
- metachromic staining
- release of histamine after activation

But distinct lineage

- 5-8 μm diameter, segmented condensed nucleus, little proliferative capacity
- Rapid and potent expression of IL-4 and IL-13
- Express Cytokine receptors (IL-3R, IL-5R, GM-CSF receptor)
  - Chemokine receptors (CD11c, CD11c, CD35 and CD88)
  - Ig receptors (FcεRI, FcγRIIb)
  - TLR
Basophil development and activation

- CD34+ progenitors
- Differentiate and mature in bone marrow
- Circulate in periphery, <1% peripheral leukocytes
- Differentiation driven by IL3
- Express integrins and chemokine receptors → able to infiltrate inflammed tissue (skin in AD, airway of respiratory allergies)

Activation via
- IgE crosslinking
- C3a, C5a,
- TLR2 and TLR4 → IL-4, IL-13 secretion and potentiation of IgE activation
- IL-33 through ST2 receptor
Role in health and disease

Mediators: preformed: Histamine, less heparin, low tryptase levels
newly synthesized: LTC4, LTD4, LTE4, no PGD2 production

cytokines: IL-4, IL-13, GM-CSF → source of early IL-4 for Th2
cell differentiation and amplification of IgE synthesis

Sullivan BM et al. Immunity 2009

Â Physiological function remains unknown (host defense against parasites?)
Â Innate immunity (TLR2 expression)
Â Predominant source of IL-4 in allergen and helminth parasite activated
PBMCs
Â In late-phase allergic responses, found in increased numbers in lungs of
asthma patients dying of asthma

Karasuyama H et al. Nat Rev Immunol 2009
Min B. Nat Immunol 2008
Sullivan BM et al. Immunity 2009
### Features of mast cells and basophils

<table>
<thead>
<tr>
<th>Origin</th>
<th>Mast cells</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maturation</td>
<td>Hematopoietic stem cell</td>
<td>Hematopoietic stem cell</td>
</tr>
<tr>
<td>Lifespan</td>
<td>Connective tissue</td>
<td>Bone marrow</td>
</tr>
<tr>
<td>Location</td>
<td>Months</td>
<td>Days</td>
</tr>
<tr>
<td>Size</td>
<td>Tissue</td>
<td>Intravascular circulation</td>
</tr>
<tr>
<td>Nuclear size</td>
<td>6-12 μm</td>
<td>5-8 μm</td>
</tr>
<tr>
<td>Granules</td>
<td>Oval or round</td>
<td>Segmented</td>
</tr>
<tr>
<td>Peptido-glycans</td>
<td>Smaller and more</td>
<td>Larger and fewer</td>
</tr>
<tr>
<td>Tryptase content</td>
<td>Heparin and chondroitin sulfates</td>
<td>Predom. chondroitin sulfates</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
</tr>
</tbody>
</table>

*Stone KD et al. JACI 2010*
Chapter 2: Type I allergy: Mechanisms in the effector phase and Anaphylaxis

Eva Untersmayr-Elsenhuber
Department of Pathophysiology and Allergy Research
Morphology of eosinophils

Blood and tissue eosinophilia hallmarks for
Å helminth infection
Å allergy and asthma,
Å eosinophilic gastrointestinal disorders
Å other rare disorders (eg. Hypereosinophilia Churg-Strauss syndrom)

Bilobed nucleus, highly condensed chromatin
Stained with acidic dye eosin

2 major types of granules:
**Specific granules** with electron-dense core, contain cationic proteins:
- major basic protein MBP,
- eosinophil peroxidase EPO,
- eosinophil cationic protein ECP,
- eosinophil-derived neurotoxin EDN

**Primary granules** similar to those found in other granulocytes
enriched in Charcot-Lyden crystal protein
Cell surface receptors

- IgG receptor (FcγRII/CD32)
- IgA receptor (FcαRI/CD89)
- Complement receptors (CR1, CR3, CD88 for C3a and C5a)
- Cytokine receptors (IL-3R, IL-5R; receptors for GM-CSF, IL-1α, IL-2, IL-4, IFN-γ, TNF-α)
- Chemokine receptors (CCR1, CCR3)
- Adhesion molecules (very late antigen4, Εβ7, siglec-8)
- Leukotrien receptors (CysLT1R, CysLT2R, LTB4 receptor)
- PG receptors (PGD2 type 2 receptor)
- PAF receptor
- TLR7/8

FcεRII expression is minimal, does not activate eosinophils, unclear functional significance
Development and activation

IL-5, IL-3, GM-CSF → eosinophil development from CD34+ progenitor cells

Release from bone marrow into circulation after IL-5 and CCL11 (eotaxin-1, via CCR3) stimulation

IL-4, IL-13 promote eosinophil trafficking to mucosal tissue by eotaxin (CCL11, CCL26) upregulation

Eosinophil chemotactic factors:
PAF, LTD$_2$, C5a, CCL5 (RANTES)
Effector function of eosinophils

Release of proinflammatory mediators:
Granule-stored cationic proteins, newly synthesized eicosanoids and cytokines

**MBP** 50% of granule protein mass
Acitivity against parasites (helminths, schistosomula)
In asthma: serum and BALF MBP correlates with bronchial hyperresponsiveness

**EDN, ECP** RNAse activity
Toxicity to parasites and ssRNA viruses

**EPO** 25% of granule protein mass
Toxic to microorganisms and host cells

Role of eosinophils in airway remodelling, airway hyperreactivity, and mucus production
The allergic effector unit

Soluble mediators modulate the reciprocal interaction between mast cells and eosinophils

GM-CSF, IL-3, IL-5, TNF-\(\alpha\), Heparin, LTC\(_4\), LTD\(_4\), LTE\(_4\), Chymase, PGD\(_2\), LTB\(_4\), IL-2, Tryptase, Histamine, PAF

SCF, NGF, MBP, Eotaxin, EDN, LTC\(_4\), LTD\(_4\), LTE\(_4\), ECP, EPO

The allergic effector unit

Physical interactions between mast cells and eosinophils

Receptor-ligand couples

Mast cell

Eosinophil

TRAIL \text{ï} TRAIL ligand
CD300a \text{ï} CD300a
CD48 \text{ï} CD244
ICAM-1 \text{ï} LFA-1

Early phase of allergy

- Allergen-specific IgE cross-linking → FcεRI aggregation → mast cell activation
- Mediator release
- Bronchoconstriction
- Vasodilatation
- Increased vascular permeability, increased mucus production

Transition to late phase: leukocyte recruitment

Late phase of allergy

Action of innate and adaptive immune cells and secretion of inflammatory mediators from tissue-resident cells

Degradation of type III collagen, injury of epithelial cells

Bronchoconstriction

Chronic allergic inflammation

- Prolonged allergen exposure
- Increased mucus production, thickening of subepithelial tissue
- Formation of EMTU (epithelial-mesenchymal tropic unit)
- Smooth muscle hyperplasia
- Severe narrowing of airway lumen
Tissue remodelling in asthma

Normal small bronchus

Severe asthma
Mucus fills airway lumen
Numerous goblet cells
Thickened lamina reticularis
Many eos and MC in submucosa
More bronchial smooth muscle

Anaphylaxis

= Serious allergic reaction with rapid onset, which can cause death

Anaphylaxis is unpredictable, can occur in anyone, anywhere at any time
Underrecognized by patients and underdiagnosed by MDs

Incidence: doubled from 21/100.000 person/year (1980s)
to 50/100.000 person/year (1990s)
with 70/100.000 person/year younger than 19 years

Triggers: Food (peanut, treenut, shellfish, fish, milk, eggs)
Medication (β-lactam antibiotics)
Insect stings/bites
Natural latex rubber
IgG-Ag complexes
Complement and coagulation system activation
exercise, cold air or water, medication

Simons FER. JACI 2009
Chapter 2: Type I allergy: Mechanisms in the effector phase and Anaphylaxis

Eva Untersmayr-Elsenhuber
Department of Pathophysiology and Allergy Research

Mechanisms

- **Gastrointestinal tract**
  - Increased fluid secretion, increased peristalsis
  - Expulsion of gastrointestinal tract contents (diarrhea, vomiting)

- **Airways**
  - Decreased diameter, increased mucus secretion
  - Congestion and blockage of airways (wheezing, coughing, phlegm)
  - Swelling and mucus secretion in nasal passages

- **Blood vessels**
  - Increased blood flow, increased permeability
  - Increased fluid in tissues causing increased flow of lymph to lymph nodes, increased effector response in tissues

http://www.bio.davidson.edu/courses/immunology/Students/spring2006/Witcher/figure%2012-11.jpg
Patient-specific risk factors

Severe concomitant allergic rhinitis and eczema
Severe asthma
Chronic obstructive pulmonary disease
Other respiratory diseases
Cardiovascular diseases
Mastocytosis and clonal mast cell disorders
Diseases impeding prompt recognition (CNS, psychiatric disorders,..)

Increased risk of severe, life-threatening or fatal anaphylaxis

Diagnosis of anaphylactic event: histamine in serum and 24h urine tryptase

Confirmation of anaphylactic trigger: History, skin tests, allergen-specific IgE Challenge tests

Simons FER. JACI 2009
Therapy of anaphylaxis

Preventive measures:
Å Avoidance of specific triggers
Å Immunomodulation (oral desensitization, sc. anti-IgE, TCM medication, immunotherapy with insect venom)

Emergency preparedness:
Å Self-injectable epinephrine

Emergency treatment:
Å Stop allergen exposure
Å O₂ and volume substitution
Å Epinephrine gavage
Å Antihistamines
Å Corticosteroids
Å β₂-Symptatomimetica

Simons FER. JACI 2006
Srivastava KD et al. JACI 2009
Patient training for EpiPen use

The correct use can save lives!
Thank you for your attention!