Molecules, mediators and mechanisms of human allergic reactions

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Learning Objectives

• What is allergy?

• IgE and how it binds to its high affinity receptor FcεRI

• Activation of cells bearing FcεRI and its immediate and delayed clinical and inflammatory consequences including those involving eosinophils

• Examples of allergic diseases

• Treatment of allergic diseases: from mechanistic to real world
  – Approved agents
  – Those undergoing testing
Examples of Allergic Diseases

- Atopic Dermatitis/Eczema
- Food Allergy
- Allergic Asthma
- Allergic Rhinitis
- Anaphylaxis
Allergic Diseases: Why are they important? High prevalence

- According to recent data from Asthma and Allergy Foundation of America, allergic disease affects 50 million Americans.
  - Adults: allergic disease is the 5th-leading chronic disease: allergic rhinitis affects 10-30% of adults
  - Children: allergic disease is the 3rd-leading chronic disease: allergic rhinitis affects up to 40% of children
- Prevalence peaks in early ages
  - In 80% of cases, symptoms develop before age 20
  - 40% of cases prior to age 6
  - 20% of cases prior to age 3
## Food Allergy Prevalence Rates

<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>0.4%</td>
<td>0.4%</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>6.0%</td>
<td>3.7%</td>
</tr>
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</table>
The Rising Prevalence of Food Allergy

- Evidence that peanut allergy has doubled in the last 5–10 years
- Data less clear for other foods but trends appear similar
- Similar increases have occurred in other allergic diseases, although the timing may not be the same
- Also evidence that food allergy may be more persistent than it previously was
Allergic reactions

• Also known as type I hypersensitivity reactions

• Requires prior exposure and sensitization (IgE)

• Atopy: familial disposition towards allergy
  • “He comes from an atopic family”

• Allergic sensitization
  • Production of IgE and arming of FcεRI-bearing cells but does not imply clinical disease
IgE versus IgG

Does not cross placenta

Crosses placenta

IgE: The Culprit in Allergy
What Makes an Antigen IgE-Promoting?

- Protein, not lipid; rarely carbohydrate
- Mucosal exposure
- Low concentration but must be multivalent
- Stable, water soluble
- Many have protease activity
  - Grass pollens
- Some resemble helminthic parasite antigens
  - Filarial tropomyosin is similar to house dust mite, shellfish and cockroach proteins
Role of ILC2 cells in asthma

Sequence of Events Leading to IgE Production and Cellular Sensitization

1) Antigen presented

2) Type 2 response (IL-4, IL-5 and IL-13) from T cells, ILC2 cells and others

3) IL-4, IL-13 -> IgE production
   -> type 2 chemokines (eotaxins)

4) IL-5 -> eosinophil production

4) IgE loads mast cells and basophils via FcɛRI
Binding of IgE to the high-affinity receptor (FcεRI, αβγ2)

IgE

Allergen-binding site

Cε1

VH

VL

Cε2

Cε3

Cε4

FcεRI

α2

α1

extracellular

intracellular

High-affinity IgE Fc receptor (FcεRI)

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 19-5
Allergic Rhinitis

- Affects 10 to 30% of adults, 20-40% of children
- The vast majority of asthmatics have rhinitis
- Large financial burden to society
- Quality of life issues
  - Nasal
  - Systemic
  - Psychological
- Types of allergic rhinitis
  - Seasonal: present for less than 4 months/year
  - Perennial: present for more than 9 months/year
Asthma

- Reversible airway obstruction (includes one or all of the following symptoms):
  - Cough
  - Shortness of breath
  - Chest tightness
  - Wheezing
- Factors that influence the incidence of asthma (common triggers):
  - Allergen exposure
  - Air pollution
  - Tobacco smoke
  - Viral Infections
Asthma Phenotypes vs Endotypes

Phenotype:
- Eosinophilic asthma
- Exacerbation-prone asthma
- Exercise-induced asthma
- Fixed Airflow obstruction
- Poorly steroid-responsive asthma

Endotype:
- Allergic asthma; AERD; ABPA
- Above ± premenstrual asthma ± viral-exacerbated asthma
- Elite athlete’s asthma
- Non-eosinophilic (neutrophilic) asthma
- Airflow obstruction caused by obesity

Why Have IgE Responses? Designed to Fight Helminths!

Lots of TH$_2$ cells
Lots of IgE
Lots of eosinophils
Eosinophils 101

- Identified by Paul Ehrlich in 1879 and named based on the staining: ‘eosin (acid stain) loving’
Eosinophils 101

- Granules contain cationic proteins:
  - major basic protein (core)
  - eosinophil cationic protein
  - eosinophil-derived neurotoxin
  - eosinophil peroxidase

- Contain and release cytokines (interleukins, growth factors) and lipid mediators (leukotrienes)

- Mediate parasite defense, allergic responses, tissue inflammation, immune modulation
Eosinophil-derived mediators

<table>
<thead>
<tr>
<th>GRANULE PROTEINS</th>
<th>OXIDATIVE PRODUCTS</th>
<th>ENZYMES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major basic protein (MBP)</td>
<td>Superoxide radical anion (OH⁻)</td>
<td>Collagenase</td>
</tr>
<tr>
<td>MBP homolog (MBP2)</td>
<td>Hydrogen peroxide (H₂O₂)</td>
<td>Metalloproteinase-9</td>
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<tr>
<td>Eosinophil cationic protein (ECP)</td>
<td>Hypohalous acids</td>
<td>Indoleamine 2,3-dioxygenase (IDO)</td>
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<tr>
<td>Eosinophil-derived neurotoxin (EDN)</td>
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<tr>
<td>Eosinophil peroxidase (EPX)</td>
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<tr>
<td>Charcot-Leyden crystal (CLC) protein</td>
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<tr>
<td>Secretory phospholipase A₂ (sPLA₂)</td>
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<tr>
<td>Bactericidal/permeability-inducing protein (BPI)</td>
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<td>Acid phosphatase</td>
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<tr>
<td>Arylsulfatase</td>
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<tr>
<td>β-Glucuronidase</td>
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<table>
<thead>
<tr>
<th>LIPID MEDIATORS</th>
<th>CYTOKINES*</th>
<th>CHEMOKINES</th>
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</thead>
<tbody>
<tr>
<td>Leukotriene B₄ (negligible)</td>
<td>IL-1α</td>
<td>CXCL8 (IL-8)</td>
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<tr>
<td>Leukotriene C₄</td>
<td>IL-2</td>
<td>CCL2 (MCP-1)</td>
</tr>
<tr>
<td>5-HETE</td>
<td>IL-3</td>
<td>CCL3 (MIP-1α)</td>
</tr>
<tr>
<td>5,15- and 8,15-diHETE</td>
<td>IL-4</td>
<td>CCL5 (RANTES)</td>
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<td>5-oxo-15-hydroxy-6,8,11,13-ETE</td>
<td>IL-5</td>
<td>CCL7 (MCP-3)</td>
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<td>Platelet-activating factor (PAF)</td>
<td>IL-6</td>
<td>CCL11 (eotaxin)</td>
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<td>Prostaglandin E₁ and E₂</td>
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<td>CCL13 (ECP-4)</td>
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<td>Thromboxane B₂</td>
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<td>IL-16</td>
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<td>Leukemia inhibitory factor (LIF)</td>
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<td>Interferon-γ (IFN-γ)</td>
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<td>Tumor necrosis factor–α (TNF-α)</td>
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</table>

| GROWTH FACTORS                                    | Nerve growth factor (NGF)           |                                 |
|---------------------------------------------------|------------------------------------|                                 |
|                                                   | Platelet-derived growth factor (PDGF)|                                 |
|                                                   | Stem cell factor (SCF)              |                                 |
|                                                   | Transforming growth factor (TGF-α, TGF-β)|                             |
The eosinophil surface phenotype

<table>
<thead>
<tr>
<th>Chemokine, complement and other chemotactic factor receptors</th>
<th>Adhesion molecules</th>
<th>Apoptosis, signaling and others</th>
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<tr>
<td>CD35 CCR1</td>
<td>CD11a CD44</td>
<td>CD9 CD134 EMR1</td>
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<tr>
<td>CD88 CCR2</td>
<td>CD11b CD49d</td>
<td>CD12 CD137 Glucocorticoid receptor</td>
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<td>C3aR CCR3</td>
<td>CD11c CD49f</td>
<td>CD17 CD139 Siglec-8</td>
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<td>CD15s CD147</td>
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<td>CysLT₁ CXCR1</td>
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<td>CysLT₂ CXCR2</td>
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<td>fMLPR CXCR3</td>
<td>αδ integrin</td>
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<td>CD86* CD300f</td>
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<td>Leukemia IL-33</td>
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<td>Inhibitory factor Stem cell</td>
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<td></td>
<td></td>
<td>PAR-2</td>
</tr>
</tbody>
</table>

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Bochner, Imm Allergy Clin NA 2015
Why do we have eosinophils?

• Eosinophils go back to metazoan species
  • All five classes of vertebrates have eosinophils or similar cells, so they are ≈400 million years old

• Eosinophil granule protein genes and their cousins extend well beyond fish

• Main role is in host defense against parasitic infections, especially those caused by certain worms, so they have a conserved role in innate immunity
EOSINOPHIL MORPHOLOGY MAMMALS

Nicole I. Stacy, DVM, DrMedVet, DACVP (Clinical)
Department of Large Animal Clinical Sciences - Aquatic Animal Health Program
College of Veterinary Medicine, University of Florida
Allergic Diseases: when IgE is made against airborne, food or other antigens

• Instead of IgE being used as a sentinel system for detecting migrating parasites,

• Imagine the consequences of this happening in the eyes, nose or lung exposed to an airborne allergen in an IgE-sensitized individual

• Imagine the consequences of this happening throughout the body following ingestion of an allergic food, drug or after a sting in an IgE-sensitized individual
Common Aeroallergens and Exposure

- **Seasonal/Outdoor**
  - Trees - Spring
  - Grasses - Summer
  - Weeds - Fall

- **Perennial/Indoor**
  - Molds
  - Pets
  - Cockroaches
  - Dust mites
Allergen Exposure
Mast cell vs. Basophil Mediator Profiles: Preformed versus Newly Generated (Human)

<table>
<thead>
<tr>
<th>Mediator</th>
<th>MC</th>
<th>Baso</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Tryptase</td>
<td>+++</td>
<td>+/-</td>
</tr>
<tr>
<td>LTs</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>PG’s</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>IL-4</td>
<td>+/-</td>
<td>+++</td>
</tr>
<tr>
<td>IL-13</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Other cytokines</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

Dvorak AM, J. Histochem Cytochem 53:1043, 2005

Courtesy J Schroeder, Ph.D.
Clinical Effects of Mediators

- Nerve activation
  - Pruritus
  - Sneezing

- Vasodilation & extravasation
  - Tissue swelling
  - Hypotension

- Gland secretion
  - Rhinorrhea
  - Phlegm production

- Smooth muscle contraction
  - Bronchoconstriction
  - Diarrhea
Symptoms of Allergic Diseases: Allergic rhinoconjunctivitis (“rose” or “hay” fever)

- Sneezing
- Runny nose
- Nasal congestion
- Itchy eyes
- Seasonal, perennial or both
Allergen-induced Release of Histamine following Experimental Nasal Allergen Challenge

*\( p<0.05 \) vs. Pre
Biophysiologic Effects of Histamine

- Nerve activation
- Vasodilation & edema
- Gland secretion
- Smooth muscle contraction
Biophysiologic Effects of Histamine via Receptors

Histamine

- H1 receptor
- H2 receptor
- H3 and H4 receptors
Receptors for Cysteinyl Leukotrienes

- LTC4
  - CysLT1 receptor
  - CysLT2 receptor
Biophysiologic Effects of Cysteinyl Leukotrienes

Cys LTs

- Smooth muscle contraction
- Vasodilation & edema
- Gland secretion
Diagnosis and management of allergic diseases

- History and Physical exam
- Detecting relevant IgE
- Allergen avoidance
- Pharmacologic agents
- Immunotherapy
Immediate hypersensitivity reaction in the skin ("wheat and flare")

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 19-3
Detection of Specific IgE-Mediated Sensitivity

- Skin Testing: wheal and flare responses
- Laboratory measurement of serum specific IgE levels
Laboratory measurement of allergen-specific serum IgE levels

The allergen of interest, covalently coupled to the solid phase, reacts with the specific IgE in the patient’s serum sample.

After washing away non-specific IgE, enzyme-labeled antibodies against IgE are added to form a complex.
After incubation, unbound enzyme-labeled anti-IgE is washed away and the bound complex is then incubated with a developing agent.

After stopping the reaction, the fluorescence of the eluate is measured. The higher the fluorescence, the more specific IgE is present in the sample.
The OFFICIAL discovery of anaphylaxis
Goal: Man-of-war prophylaxis

**Setting:** Princesse Alice II, between Toulon, France and the Cape Verde islands, off the West African Coast

**Hypothesis:** Immunization of dogs with low-dose Physalia toxin should confer resistance — similar to immunization.

**Early data:** Toxin dissolved in glycerol produced no ill effects upon first dose, but second dose caused the death of “a fine big dog by the name of Neptunus.”
Further developments

• Richet and Portier confirmed these results in more dogs and a variety of other species

• They confirmed the results with other toxins (actinia from sea anemones)

• Observed that second dose should be administered 14-23 days after first dose

• Symptoms included itching, vomiting, dyspnea, hypotension and death with time course <30-60min
Rather than providing protection, or *prophylaxis* against toxin, repeated low-dose administration caused anti-protection or *anaphylaxis*.

The Nobel Prize in Physiology or Medicine: 1913

Charles Richet
Definition and Diagnosis of Anaphylaxis

Anaphylaxis is highly likely when any one of the following three criteria is fulfilled:

1. Sudden onset of an illness (minutes to several hours), with involvement of the skin, mucosal tissue, or both (e.g. generalized hives, itching or flushing, swollen lips-tongue-uvula)

   AND AT LEAST ONE OF THE FOLLOWING:

   - Sudden respiratory symptoms and signs (e.g. shortness of breath, wheeze, cough, stridor, hypoxemia)
   - Sudden reduced BP or symptoms of end-organ dysfunction (e.g. hypotonia [collapse], incontinence)

2. Two or more of the following that occur suddenly after exposure to a likely allergen or other trigger* for that patient (minutes to several hours):

   - Sudden skin or mucosal symptoms and signs (e.g. generalized hives, itch-flush, swollen lips-tongue-uvula)
   - Sudden respiratory symptoms and signs (e.g. shortness of breath, wheeze, cough, stridor, hypoxemia)
   - Sudden reduced BP or symptoms of end-organ dysfunction (e.g. hypotonia [collapse], incontinence)
   - Sudden gastrointestinal symptoms (e.g. crampy abdominal pain, vomiting)

3. Reduced blood pressure (BP) after exposure to a known allergen** for that patient (minutes to several hours):

   - Infants and children: low systolic BP (age-specific) or greater than 30% decrease in systolic BP***
   - Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person’s baseline

Immunologic Causes of Anaphylaxis

Via IgE
- peanut
- tree nuts
- shellfish
- fish
- milk
- egg
- soybean
- peach
- sesame
- stinging insects
- β-lactam antibiotics*
- NSAIDs* **
- biologic agents*

Not via IgE
- Natural rubber latex
- Occupational allergens
- Seminal fluid
- Aeroallergens
- Radiocontrast media*
- Radiocontrast media*
- NSAIDs* **
- Dextrans (e.g. HMW*** iron or other source)
- Biologic agents* (e.g. some monoclonal antibodies)

*Trigger anaphylaxis by more than one mechanism  **NSAIDs, non-steroidal anti-inflammatory drugs  ***HMW, high molecular weight

# Non-Immunologic Causes of Anaphylaxis

## Nonimmunologic Mechanisms (Direct Mast Cell Activation)

<table>
<thead>
<tr>
<th>Physical factors (e.g. exercise, cold, heat, sunlight)</th>
<th>Ethanol</th>
<th>Medications* (e.g. opioids)</th>
</tr>
</thead>
</table>

## Idiopathic Anaphylaxis (No apparent trigger)

<table>
<thead>
<tr>
<th>Previously unrecognized allergen?</th>
<th>Mastocytosis/clonal mast cell disorder?</th>
</tr>
</thead>
</table>

*Trigger anaphylaxis by more than one mechanism  
**NSAIDs, non-steroidal anti-inflammatory drugs  
***HMW, high molecular weight

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Treatments for allergic diseases

• Identification and avoidance of triggers
• H1 antihistamines
• Topical steroids
• Epinephrine
• Leukotriene modifiers
• Bronchodilators, decongestants
• Allergy shots
• Biologics (anti-IgE, anti-IL-5)
Key concepts

- Can’t have allergies without IgE or FcεRI
- Mediators released during allergic reactions cause a characteristic pattern of signs and symptoms
- Treatments include identification of allergen triggers, avoidance, medications and immunotherapy