Experimental induced anaphylactic response in lab. animal

Part 1
Aim of practical

• Introduction to hypersensitive reactions
• Immediate hypersensitivity

• Counting of blood elements - eosinophils, WBC and platelets
Hypersensitivity

• Positive or negative?

• The term hypersensitivity is used to describe immune responses which are damaging rather than helpful to the host.

• Why?
Hypersensitivity

- Gell and Coombs classification
  - Type I - immediate hypersensitivity
  - Type II - is caused by specific antibody binding to cells or tissue antigens
  - Type III - is mediated by immune complexes
  - Type IV - is the only class of hypersensitive reactions to be triggered by antigen-specific T cells
<table>
<thead>
<tr>
<th>Type of hypersensitivity</th>
<th>Pathologic immune mechanisms</th>
<th>Mechanisms of tissue injury and disease</th>
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</thead>
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<tr>
<td>Immediate hypersensitivity: Type I</td>
<td>IgE antibody</td>
<td>Mast cells and their mediators (vasoactive amines, lipid mediators, cytokines)</td>
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<tr>
<td>Antibody mediated: Type II</td>
<td>IgM, IgG antibodies against cell surface or extracellular matrix antigens</td>
<td>Opsonization and phagocytosis of cells Complement-and Fc receptor-mediated recruitment and activation of leukocytes (neutrophils, macrophages) Abnormalities in cellular functions, e.g., hormone receptor signaling</td>
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<td>Immune complex mediated: Type III</td>
<td>Immune complexes of circulating antigens and IgM or IgG antibodies</td>
<td>Complement-and Fc receptor-mediated recruitment and activation of leukocytes</td>
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<td>T cell mediated: Type IV</td>
<td>1. CD4+ T cells (delayed-type hypersensitivity) 2. CD8+ CTLs (T cell-mediated cytolysis)</td>
<td>1. Macrophage activation, cytokine-mediated inflammation 2. Direct target cell killing, cytokine-mediated inflammation</td>
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In 1906 C.Pirquet and B.Schick observed unwanted reactivity in children after repeated application of diphteric serum – they called the reaction serum illness – term “allergy“.

In 1911 Ch.Richet and P.Portier studied influence of extract of sea animals (jelly-fish) in dogs. Rapid shock reaction which followed they termed as anaphylactic – unwanted (in contrast with prophylaxis).

1920 A.F.Coca atopy vs. genetically predisposition
**Allergy**
- **Allergy** is a disorder of the immune system that is often called **atopy**. Allergic reactions occur to environmental substances known as allergens; these reactions are acquired, predictable and rapid.

**Anaphylaxis**
**Anaphylaxis** is an acute systemic (multi-system) and severe Type I Hypersensitivity allergic reaction in humans and other mammals. The term comes from the Greek words ανα *ana* (against) and φύλαξις *phylaxis* (protection).

**Atopy**
**Atopy** (Greek ατοπία - *placelessness*) or **atopic syndrome** is an allergic hypersensitivity affecting parts of the body not in direct contact with the allergen. It may involve eczema (atopic dermatitis), allergic conjunctivitis, allergic rhinitis and asthma. There appears to be a strong hereditary component.
Immediate hypersensitivity

The typical sequence of events in immediate hypersensitivity consists of:

- exposure to an antigen
- activation of TH2 cells specific for the antigen,
- production of IgE antibody
- binding of the antibody to Fcε receptors of mast cells
- triggering of the mast cells by re-exposure to the antigen, resulting in the release of mediators from the mast cells and the subsequent pathologic reaction
First exposure to allergen

Antigen activation of T\(_{H2}\) cells and stimulation of IgE class switching in B cells

Production of IgE

Binding of IgE to FcεRI on mast cells

Repeated exposure to allergen

Activation of mast cell: release of mediators

Immediate hypersensitivity reaction (minutes after repeated exposure to allergen)

Late-phase reaction (2-4 hours after repeated exposure to allergen)

Immediate hypersensitivity

The clinical and pathologic manifestations of immediate hypersensitivity consist of the:

- vascular and smooth muscle reaction that develops rapidly after repeated exposure to the allergen (the immediate reaction)
- and a delayed late-phase reaction consisting mainly of inflammation

Immediate hypersensitivity reactions are manifested in different ways, including:

- skin and mucosal allergies,
- food allergies
- asthma
- systemic anaphylaxis
The immediate and late-phase reactions

The immediate vascular and smooth muscle reaction to allergen develops within minutes after challenge.
Morphology of basophils and eosinophils
Mast cell

- Respiratory and gastrointestinal system, derm
- Population of mast cells differ by type and amount of mediators
- Mast cells produce various cytokines: IL-1, IL-3, IL-4, IL-5, IL-6, GM-CSF, TGF-β, TNF-α
Mast cell activation

- Mast cells are activated by cross-linking of FcεRI molecules, which occurs by binding of multivalent antigens to the attached IgE molecules.

- Activation of mast cells results in three types of biologic response:
  - secretion of the preformed contents of their granules by a regulated process of exocytosis,
  - synthesis and secretion of lipid mediators,
  - and synthesis and secretion of cytokines.
Activation of mast cells
Mast cell degranulation

[Diagram showing the process of mast cell degranulation, including the roles of various molecules and enzymes such as Adenylate cyclase, Phospholipase C, Protein kinase C, and Ca²⁺.]
Mediators derived from Mast Cells

• The effector functions of mast cells are mediated by soluble molecules released from the cells on activation.

• These mediators may be divided into preformed mediators, which include biogenic amines and granule macromolecules, and newly synthesized mediators, which include lipid-derived mediators and cytokines.
Mediators derived from **Mast Cells**

- Biogenic amines
  - histamine
- Granule proteins and proteoglycans (Enzymes)
  - Serine proteases
- Lipid mediators
  - Prostaglandins, leukotrienes
- Cytokines
  - TNF, IL
Biological effect of mediators

- Biogenic amines (e.g., histamines)
- Lipid mediators (e.g., PAF, PGD₂, LTC₄)
- Cytokines (e.g., TNF)
- Lipid mediators (e.g., PAF, PGD₂, LTC₄)
- Enzymes (e.g., lysozyme)
- Eosinophil
- Cationic granule proteins (e.g., major basic protein, eosinophil cationic protein)
- Enzymes (e.g., eosinophil peroxidase)

Effects:
- Vascular leak
- Bronchoconstriction
- Intestinal hypermotility
- Inflammation
- Tissue damage
- Killing of parasites and host cells
- Tissue remodeling
Biological effect of histamine

- H1 receptor
  - bronchoconstriction
  - vascular leak
  - vasodilatation

- H2 receptor
  - secretion of HCl
  - release of histamine
  - regulation on immune response

- H3 receptor
Types of histamine receptors

- **H₁-receptors**
  - Constriction of smooth muscle
  - Increased vascular leak
  - Irritation of sensitive nerves
  - Hypersecretion in salivary gland

- **H₂-receptors**
  - Stimulation of HCl secretion
  - Positive chronotropic and ionotropic effect
  - Anaphylaxis

- **H₃-receptors** (nerve cells).
  - Regulatory function - after activation - decrease of histamine and other mediators production in CNS

- **H₄-receptory** - eosinophils, bone marrow, lung
  - Regulation of immune system
Hypersensitive reaction - type I

• **Systemic (anaphylactic reaction)**
  - generalized, endangering life, shock
  - anaphylactoid reaction

• **Localized reaction**
  - Asthma bronchiale
  - Nasal allergy
  - Atopic dermatitis
  - Food allergy
Clinical picture and manifestation

- Mucous membrane, derm
  - Erythema, exanthema, pruritus, edema
- Respiratory system
  - Acute rhinitis, nasal obstruction, sneezing, irritation to cough, breathing problems
- GIT
  - Vomitus, colic, diarrhoea

Symptoms depend on:
- Sesibilization level of patient
- Place of allergen entry
- Allergen type
Symptoms

**Cardiovascular system:**
Palpitation, tachycardia, hypotension, arrhythmia

**Urogenital system:**
Picture of renal colic

**General symptoms:**
Cognition disorders, spsms

**!!Reason of death!!**
Respiratory failure, cardiovascular failure
Treatment

**Adrenaline i.v.**

**Corticosteroids i.v.**
- Inhibition of leucotrien synthesis
- Inhibition of inflammatory cells infiltration in place of allergy reaction
- Inhibition in cytokine production

**Antihistaminics**
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<th>Inhibition of H1 and H2 receptors in terminal cells</th>
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<tr>
<td><strong>Theophylin</strong></td>
<td>Prolongation of increasing level of cAMP in mast cells</td>
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<tr>
<td><strong>Adrenaline</strong></td>
<td>Stimulation of cAMP production due to binding to β-receptors in mast cells</td>
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<tr>
<td><strong>Corticosteroids</strong></td>
<td>Inhibition of leucotrien and cytokine synthesis</td>
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Practical part

1. Repeated application of antigen (horse serum) s.c. and 3 days

2. Blood sampling from auricularis (2ml, +heparin) for hematological examination

3. Intravenous application of 3ml of horse serum

4. Blood sampling from auricularis (2ml, +heparin) for hematological examination and
5. Photographing the peripheral blood smear
6. Measurement of the number of blood elements (leukocytes, thrombocytes, eosinophils)

Results
1. Monitoring the course of the anaphylactic reaction (overview)
2. Comparison of the number of blood elements (before and after anaphylaxis)
3. Evaluation of the peripheral blood smear (from samples before and after anaphylaxis, comparison)