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 that are damaging rather than helpful to the host.
- Why?

Hypersensitivity

- Gell and Coombs clasification
  - 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

Type of hypersensitivity	Pathologic immune mechanisms	Mechanisms of tissue injury and disease
Immediate hypersensitivity: Type I	IgE antibody	Mast cells and their mediators (vasoactive amines, lipid mediators, cytokines)
Antibody mediated: Type II	IgM, IgG antibodies against cell surface or extracellular matrix antigens	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
Immune complex mediated: Type III	Immune complexes of circulating antigens and IgM or IgG antibodies	Complement-and Fc receptor-mediated recruitment and activation of leukocytes
T cell mediated: Type IV	1. $CD4^+$ T cells (delayed-type hypersensitivity) 2. $CD8^+$ CTLs (T cell-mediated cytolysis)	1. Macrophage activation, cytokine-mediated inflammation 2. Direct target cell killing, cytokine-mediated inflammation

History

In 1906 C.Pirquet and B.Schick observed unwanted reactivity in chlildren 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 vs. anaphylaxis vs. atopy

- **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).

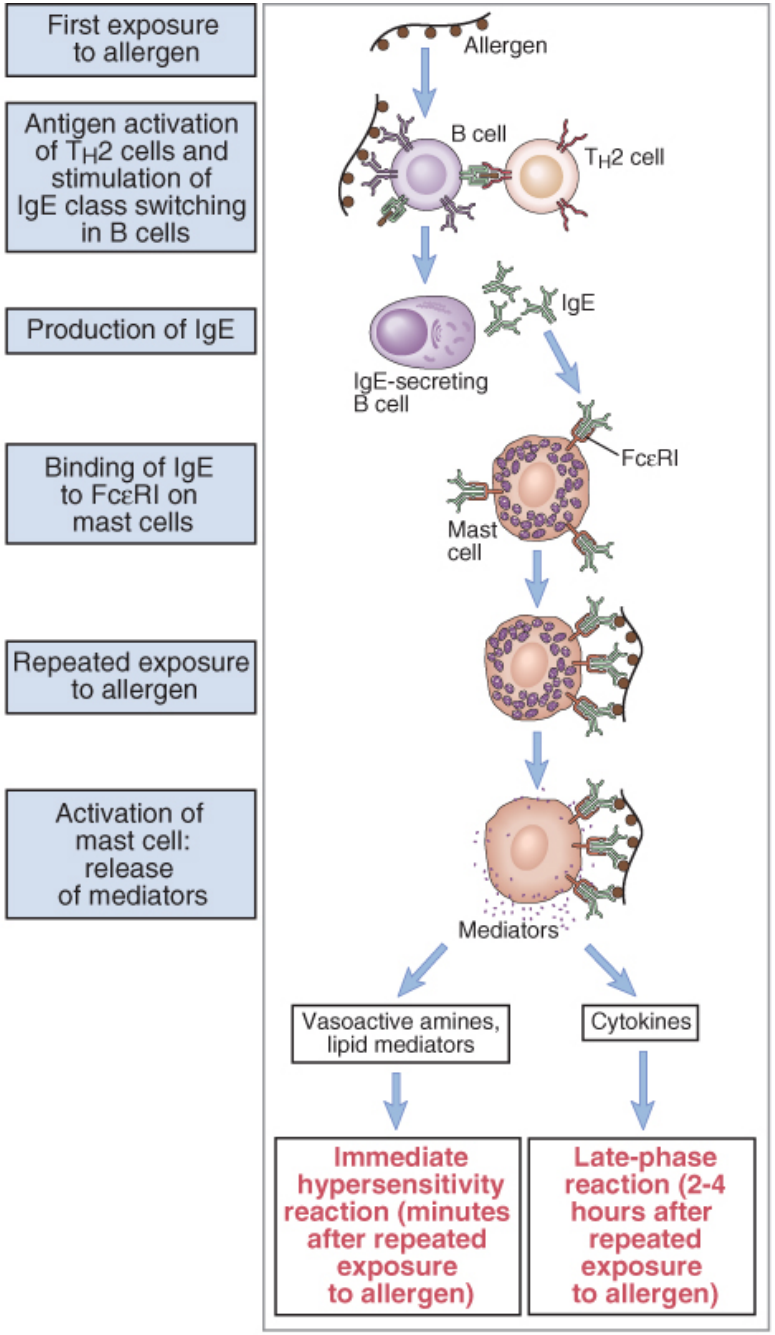
- **Atopy**

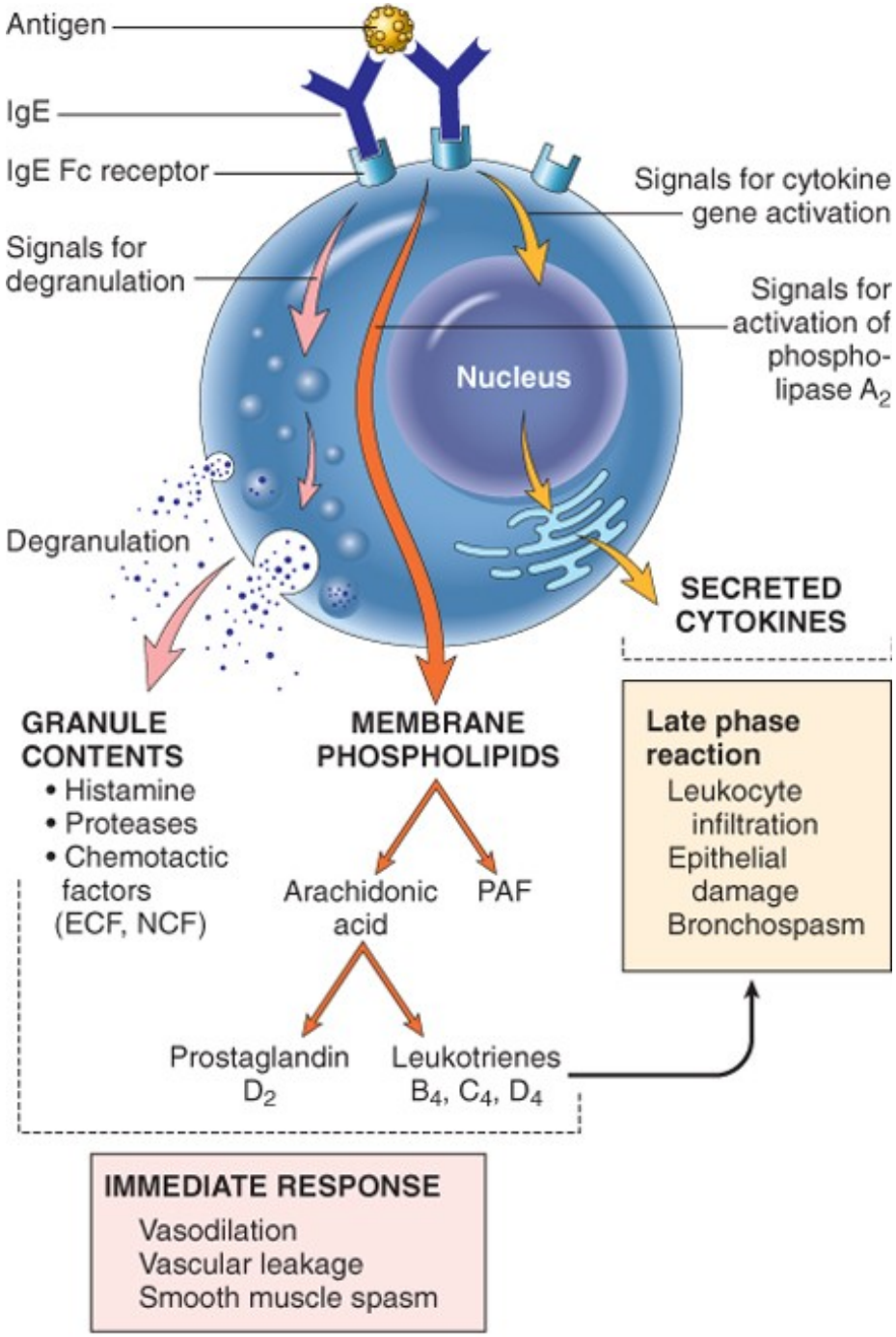
Atopy (Greek *ατοπία - placelessness*) or **atopic syndrome** is an allergic hypersensitivity affecting parts of the body not in direct contact with the allergen. It may involve eczema (atopic dermatitis), allergic conjunctivitis, allergic rhinitis and asthma. There appears to be a strong hereditary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

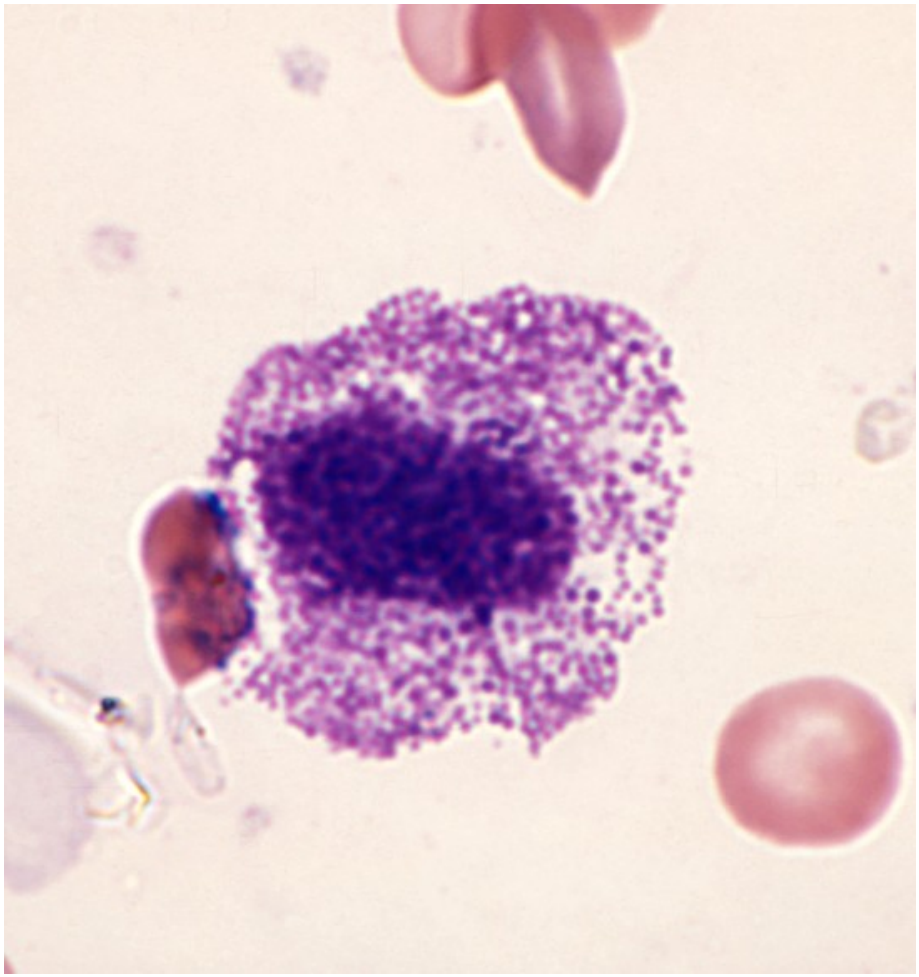
Immediate hypersensitivity

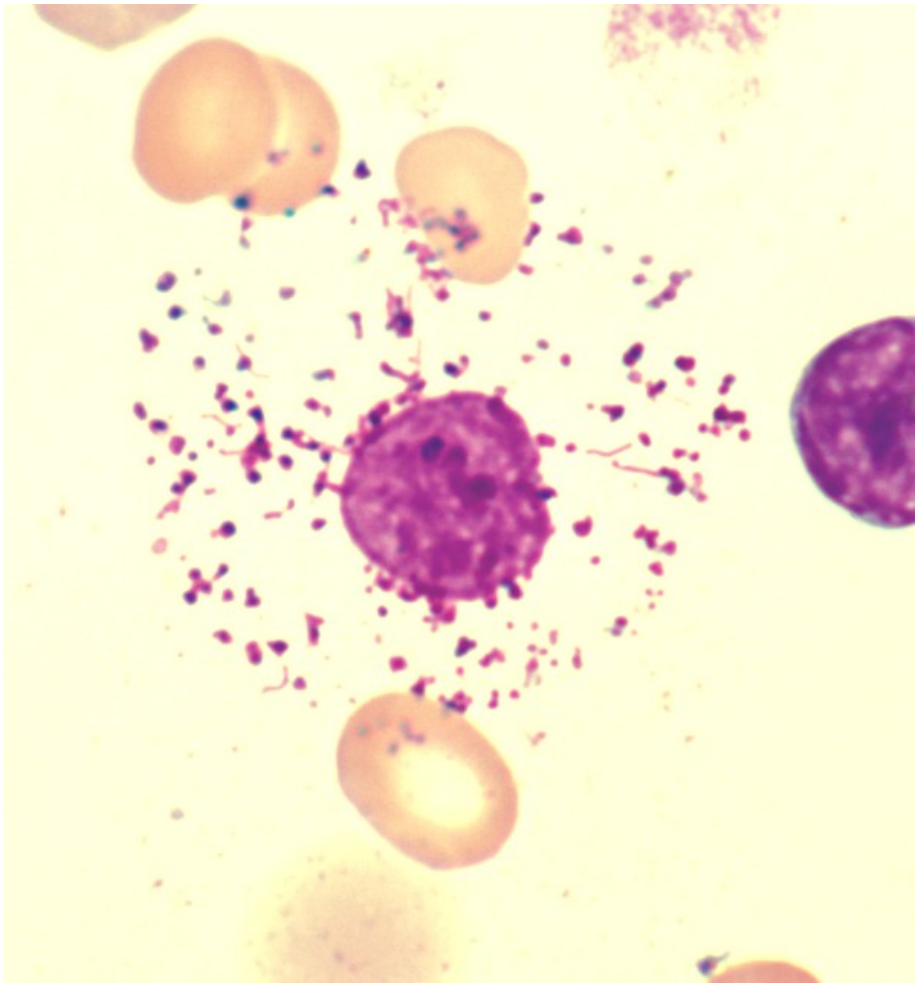
The typical sequence of events in immediate hypersensitivity consists of:

- 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




Antigen
IgE
IgE Fc receptor
Signals for degranulation
Signals for cytokine gene activation
Signals for activation of phospho-lipase A2
Nucleus
Degranulation
SECRETED CYTOKINES
GRANULE CONTENTS
• Histamine
• Proteases
• Chemotactic factors (ECF, NCF)
MEMBRANE PHOSPHOLIPIDS
Arachidonic acid
PAF
Prostaglandin D2
Leukotrienes B4, C4, D4
Late phase reaction
Leukocyte infiltration
Epithelial damage
Bronchospasm
IMMEDIATE RESPONSE
Vasodilation
Vascular leakage
Smooth muscle spasm





Immediate hypersensitivity

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

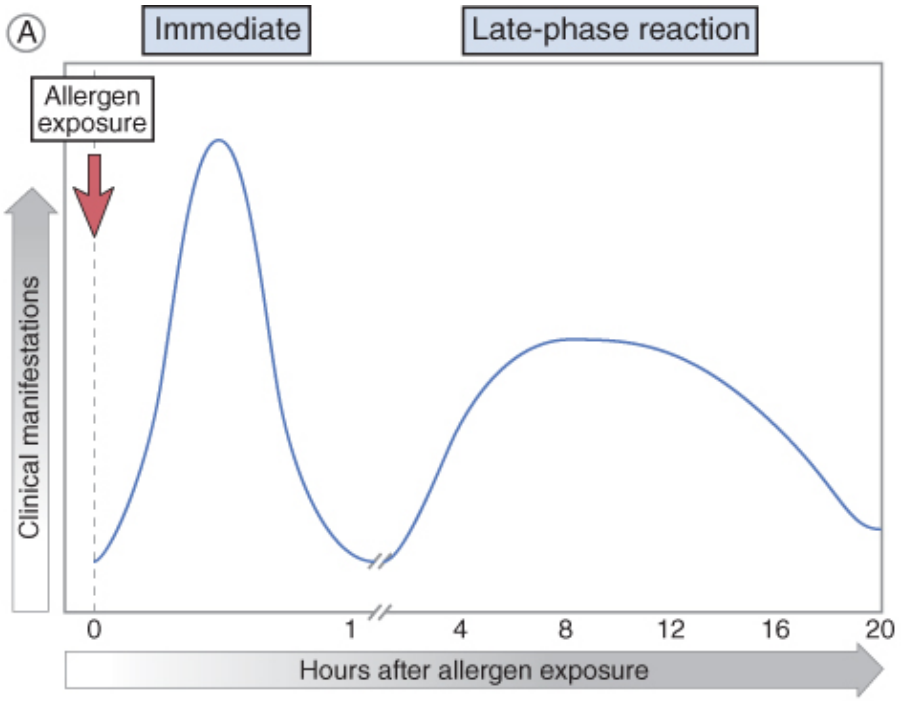
- vascular and smooth muscle reaction that develops rapidly after repeated exposure to the allergen (the immediate reaction)

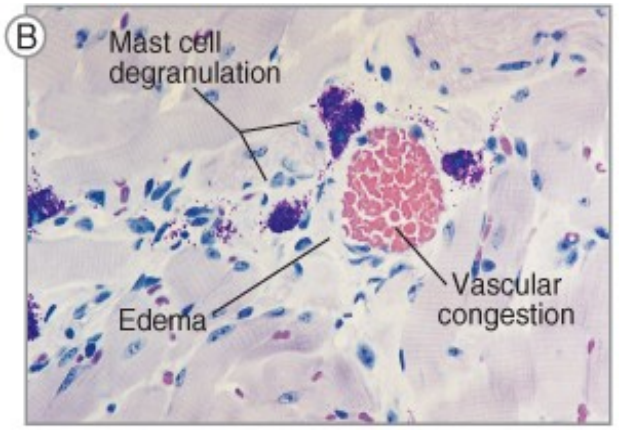
- and a delayed late-phase reaction consisting mainly of inflammation

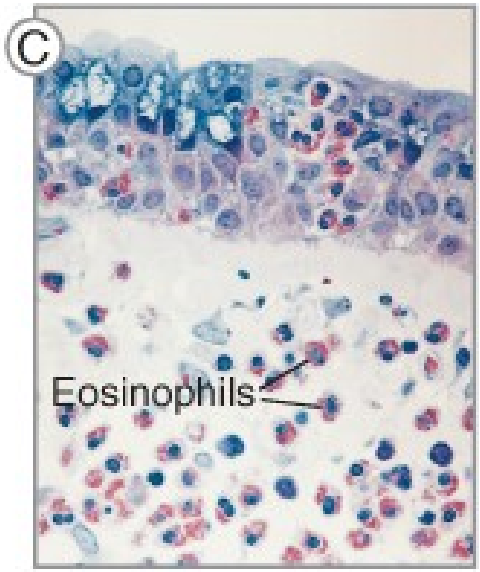
Immediate hypersensitivity reactions are manifested in different ways, including:

- skin and mucosal allergies,
- food allergies
- asthma
- systemic anaphylaxis

The immediate and late-phase reactions

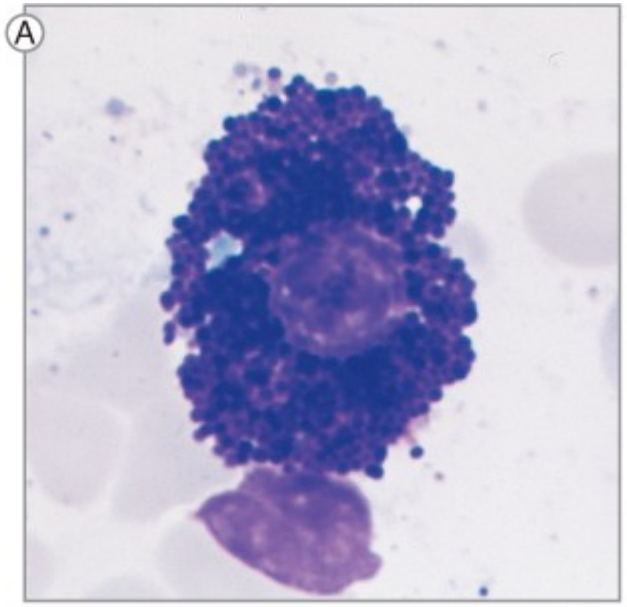


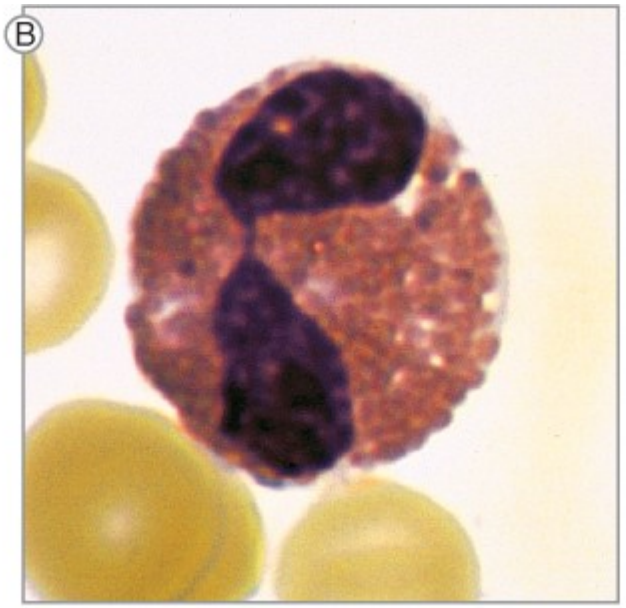




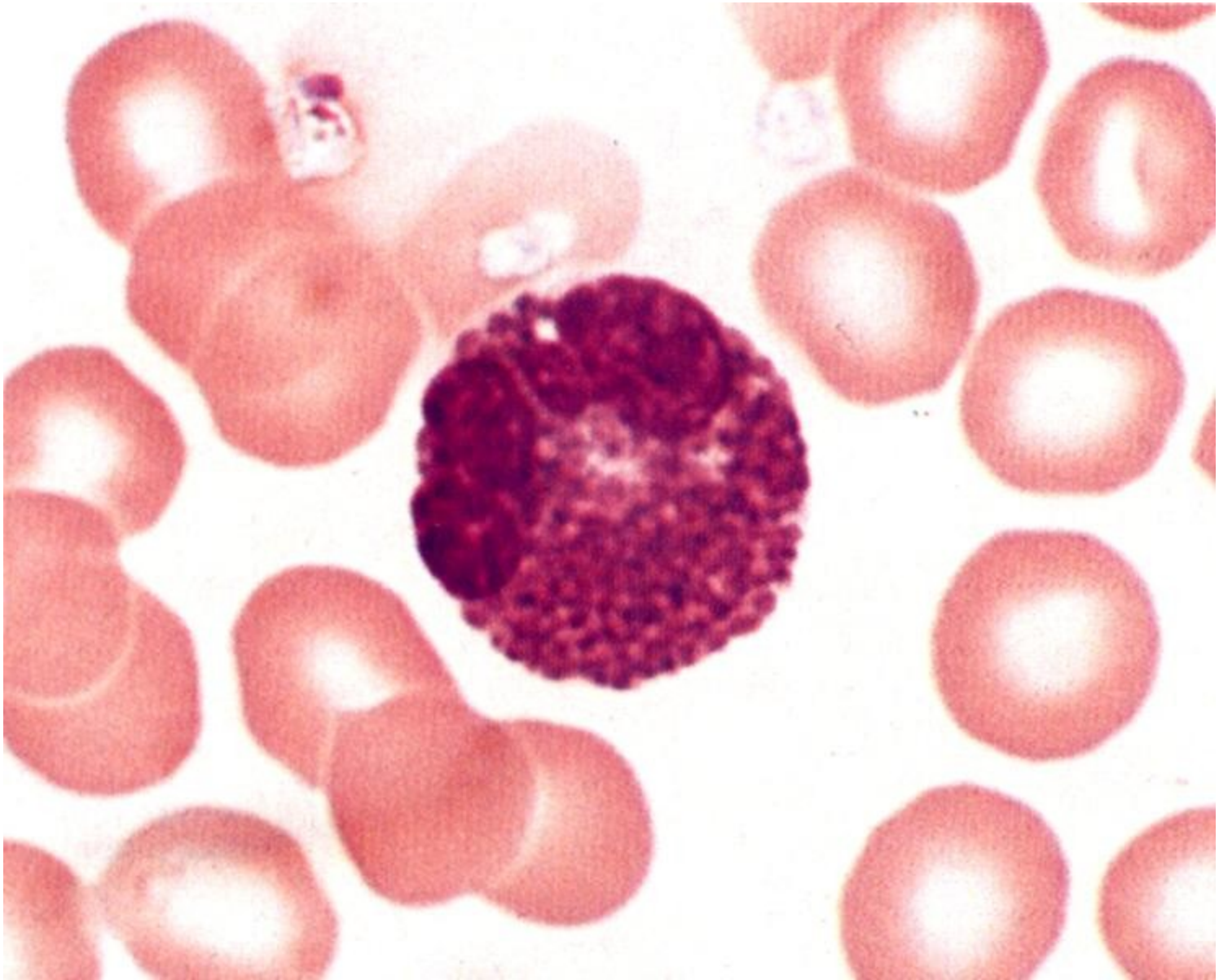
The immediate vascular and smooth muscle reaction to allergen develops within minutes after challenge

Morphology of basophils and eosinophils

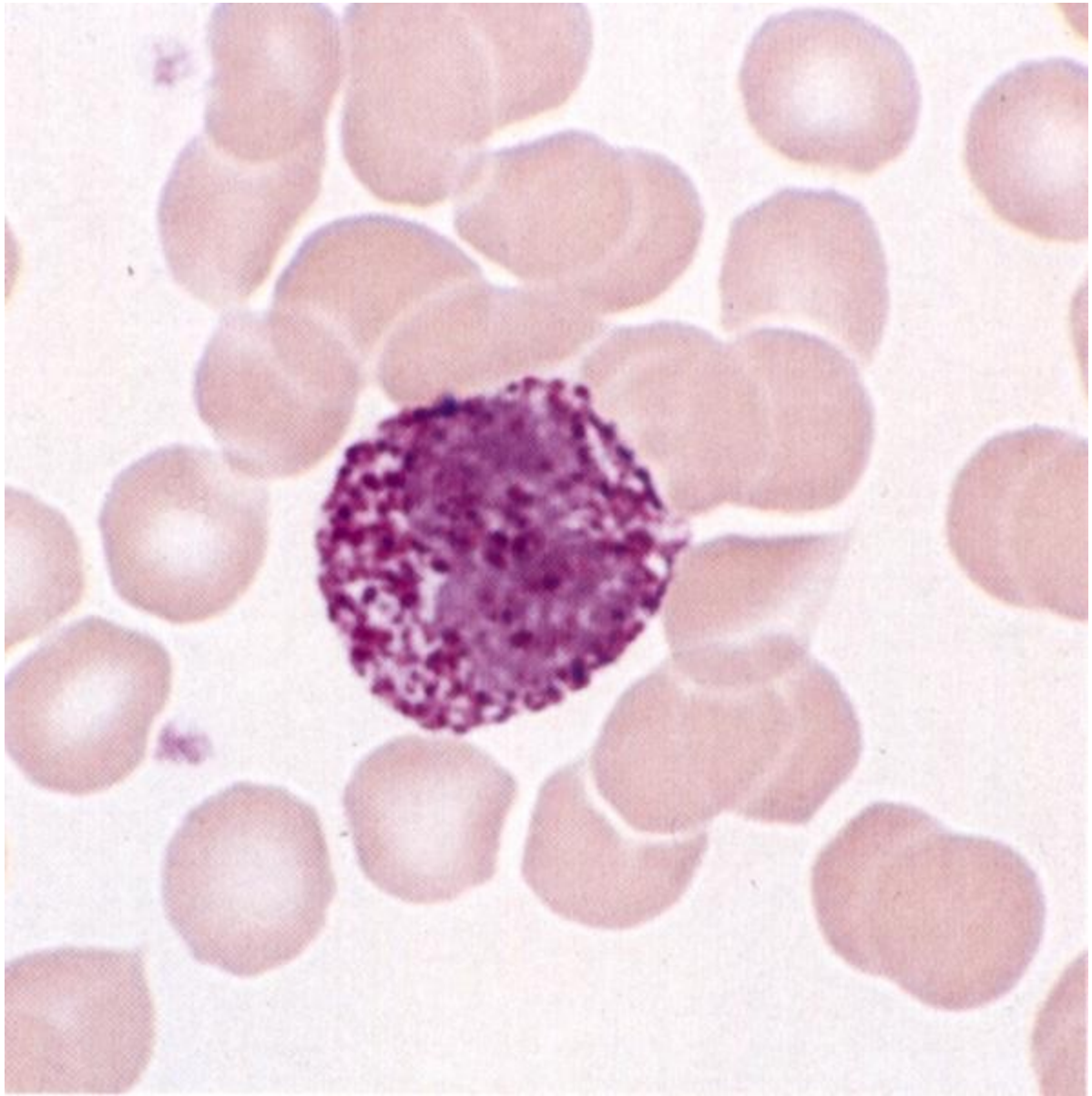




Eosinofily

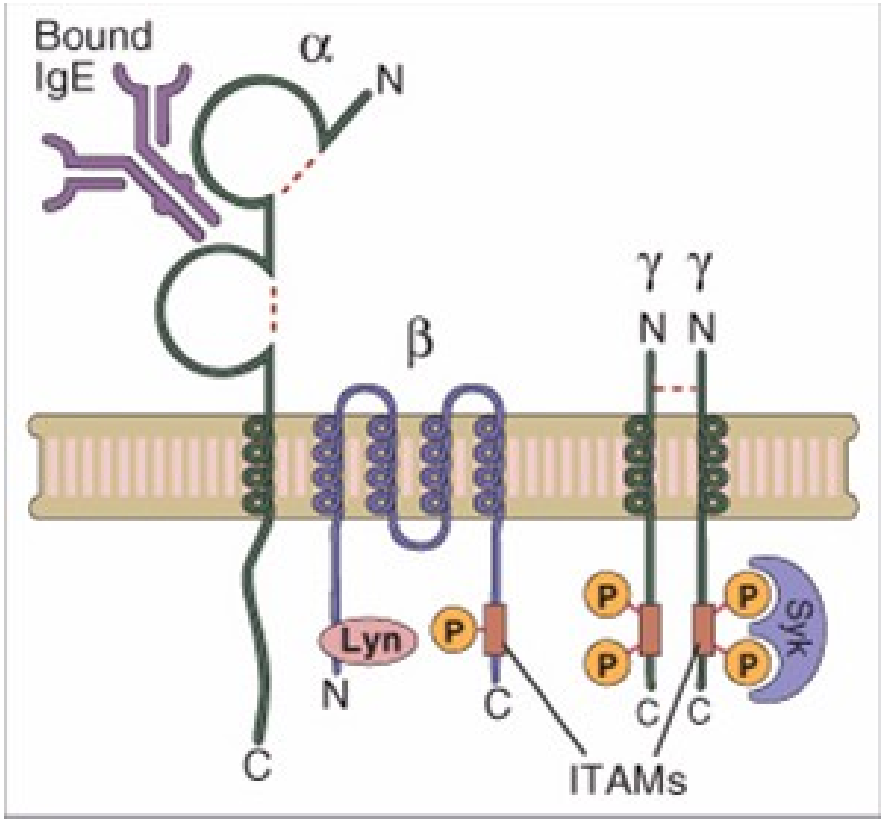


Basofily



Mast cell

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

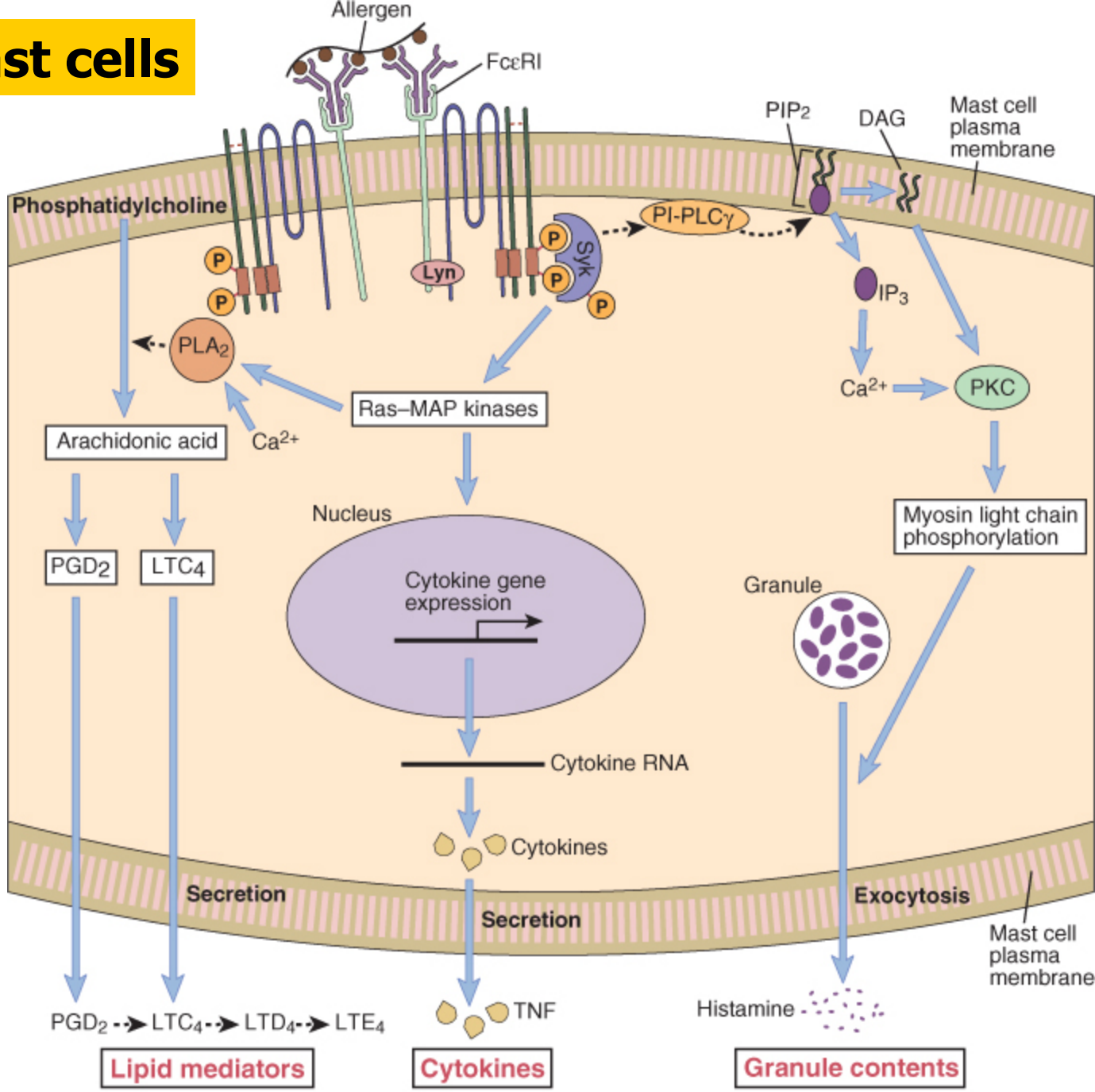


Mast cell activation

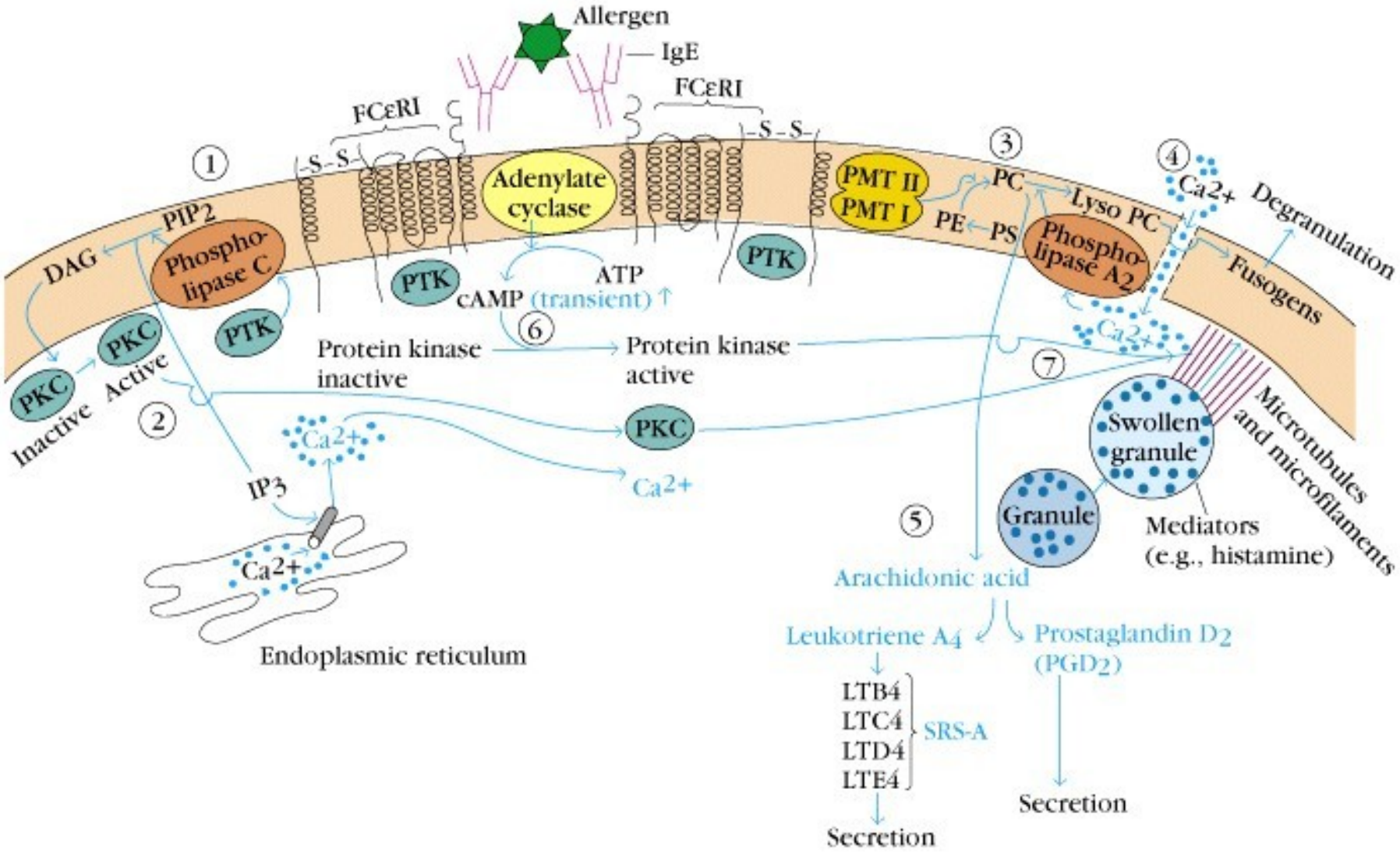
- Mast cells are activated by cross-linking of FcεRI molecules, which occurs by binding of multivalent antigens to the attached IgE molecules

- 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



Mediators derived from **Mast Cells**

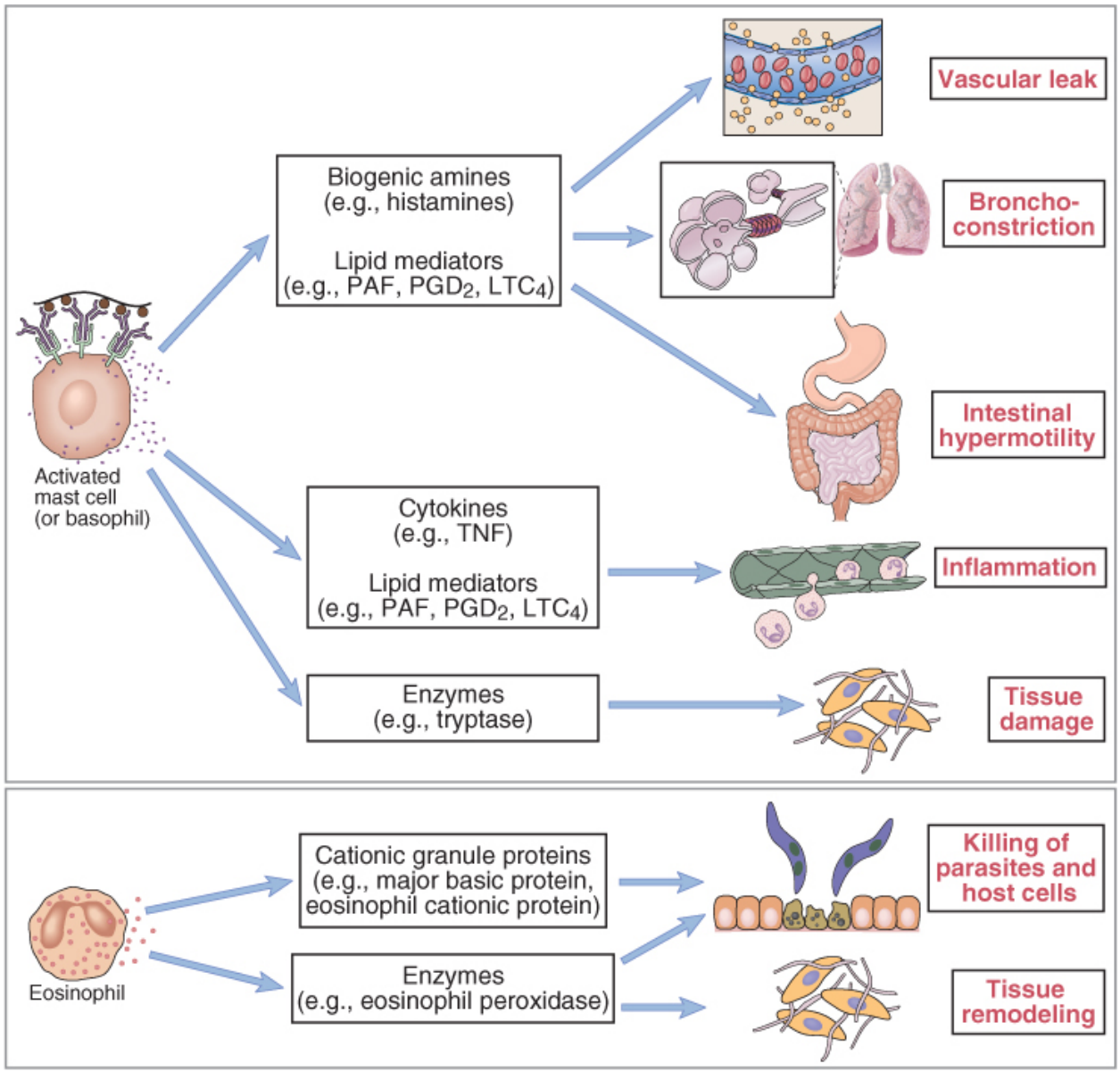
- The effector functions of mast cells are mediated by soluble molecules released from the cells on activation

- 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**

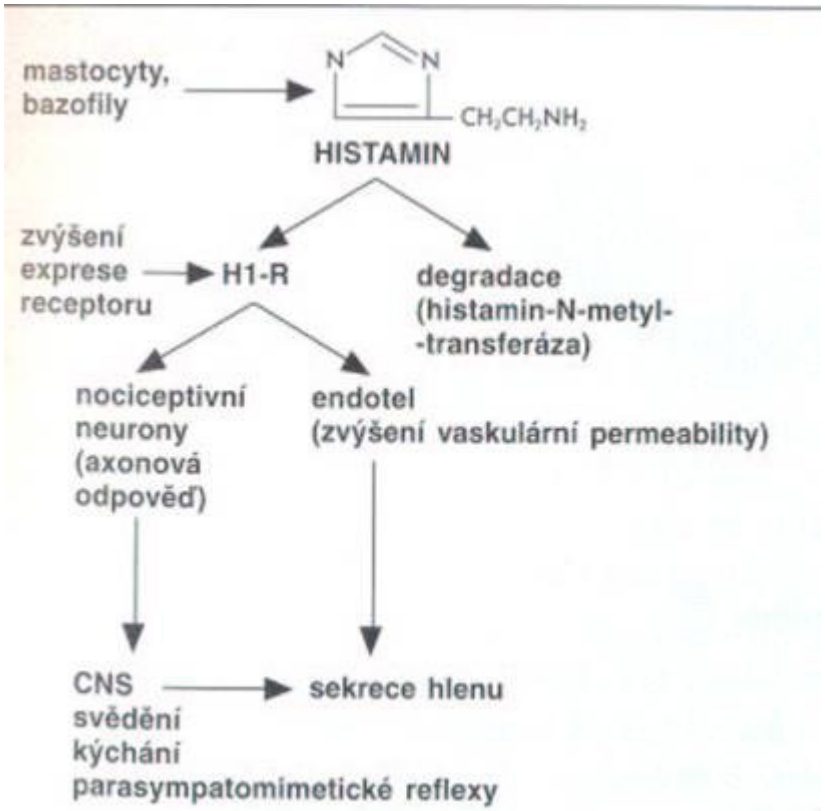
- Biogenic amines
  - histamine
- Granule proteins and proteoglycans (Enzymes)
  - Serine proteases
- Lipid mediators
  - Prostaglandins, leukotrienes
- Cytokines
  - TNF, IL

Biological effect of mediators



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

- **H_1- receptors**
  - Constriction of smooth muscle
  - Increased vascular leak
  - Irritation of sensitive nerves
  - Hypersecretion in salivary gland

- **H_2-receptors**
  - Stimulation of HCl secretion
  - Positive chronotropic and ionotropic effect
  - Anaphylaxis

- **H_3-receptors** (nerve cells).
  - Regulatory function – after activation – decrease of histamine and other mediators production in CNS
- **H_4-receptory** - eosinophils, bone marrow, lung
  - Regulation of immune system

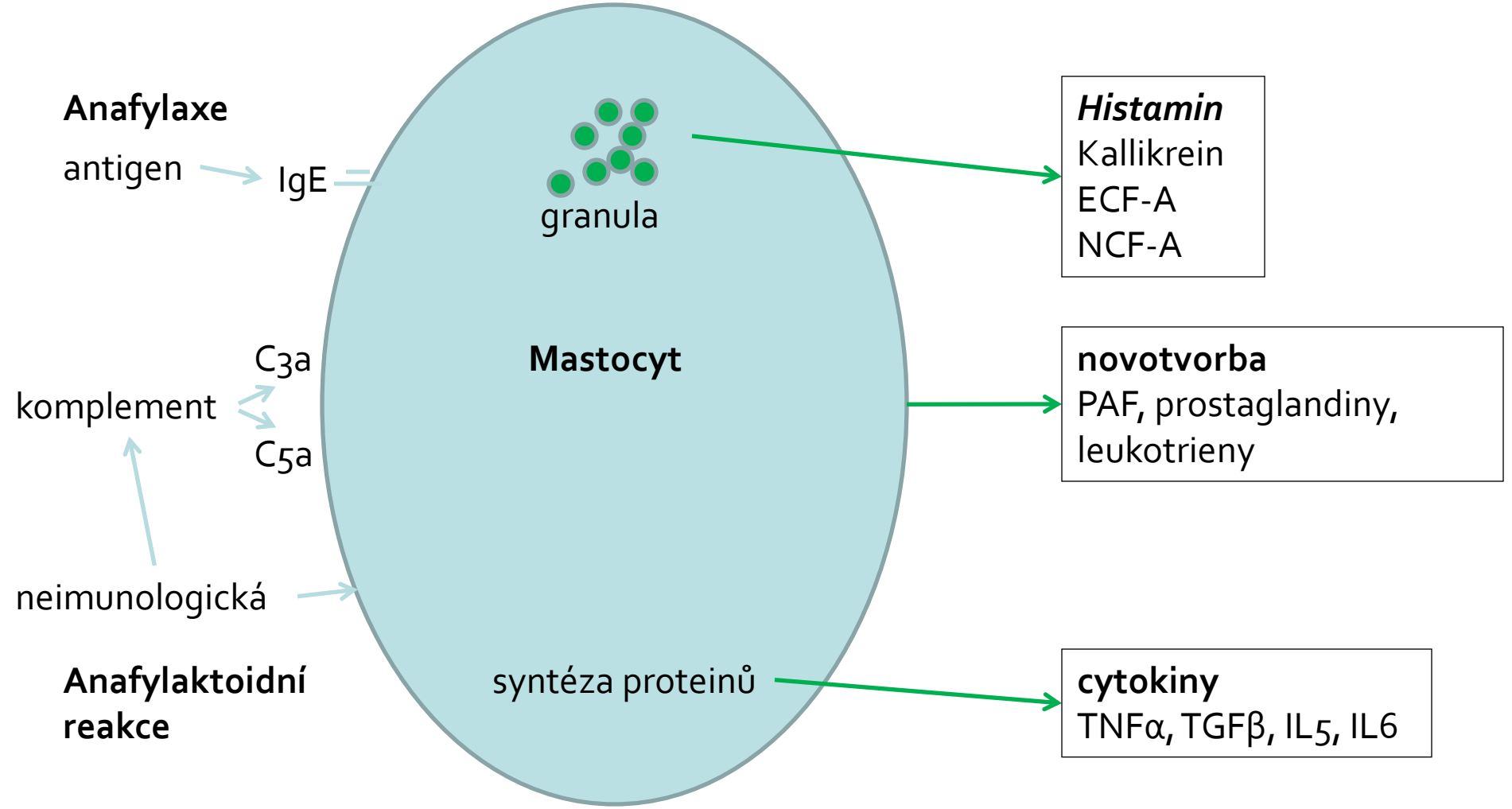
Hypersensitive reaction – type I

- **Systemic (anaphylactic reaction)**

  - generalized, endangering life, shock
  - anaphylactoid reaction

- **Localized reaction**
  - Asthma bronchiale
  - Nasal allergy
  - Atopic dermatitis
  - Food allergy

Úloha žírných buněk u anafylaxe

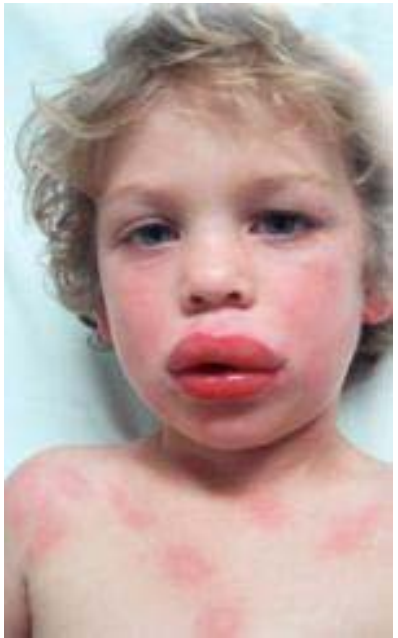


Clinical picture and manifestation

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

Symptoms depend on:

- Sesibilization level of patient
- Place of allergen entry
- Allergen type



Symptoms

Cardiovascular system:

Palpitation, tachycardia, hypotension, arrhytmia

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.

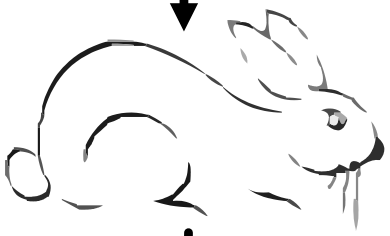
- Inhibition of leucotrien synthesis
-Inhibition of inflammatory cells infilration in place of allergy reaction
- Inhibition in cytokine production

Antihistaminics

Antihistaminics	Inhibition of H1 and H2 receptors in terminal cells
Theophylin	Prolongation of increasing level of cAMP in mast cells
Adrenaline	Stimulation of cAMP production due to binding to β-receptors in mast cells
Corticosteroids	Inhibition of leucotrien and cytokine synthesis

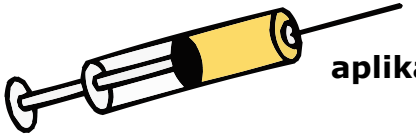
Practical part

opakovaná aplikace antigenu (koňské sérum) s.c. a 3 dny



za 3 týdno od začátku sensibilizace

(1) odběr krve z v. auricularis (2ml, +heparin) na hematologické vyšetření



aplikace 3ml koňského séra i.v.

(2) odběr krve z v. auricularis popř. intrakardiálně (2ml, +heparin) na hematologické vyšetření
(3) zhotovení nátěru periferní krve
(4) stanovení počtu krevních elementů (leukocyty, trombocyty, eozinofily)

VÝSLEDKY

(1) sledování průběhu anafylaktické reakce
(videozáznam)

(1) srovnání počtu krevních elementů
(před a po proběhlé anafylaxi)

(2) zhodnocení nátěru periferní krve
(ze vzorků před a po anaxylaxi, srovnání)