# BASOPHIL ACTIVATION TEST FOR ALLERGY TESTING (BASOTEST)

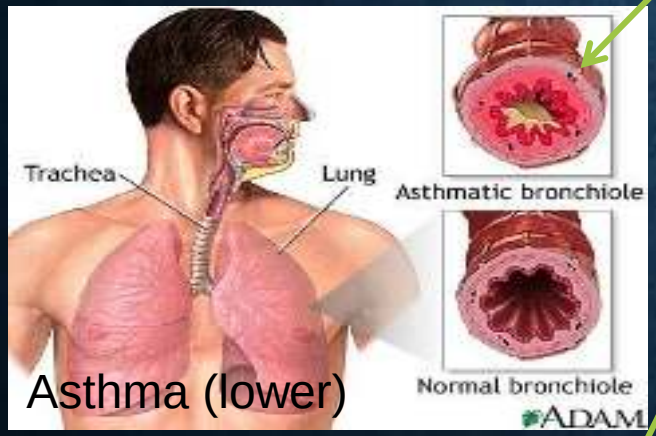
Maha Abdullah

# DEFINITION

- Allergy: a hypersensitive response of the immune system upon exposure to an allergen
- IgE-mediated hypersensitivity OR Hypersensitivity type I OR Immediate hypersensitivity
- An allergen is a usually harmless substance capable of triggering a response in the immune system and results in an allergic reaction.
- Atopy: genetic tendency to develop allergic diseases

# Allergies - 1 of 3 Malaysians is currently suffering from some form of allergy (MSAI, website)

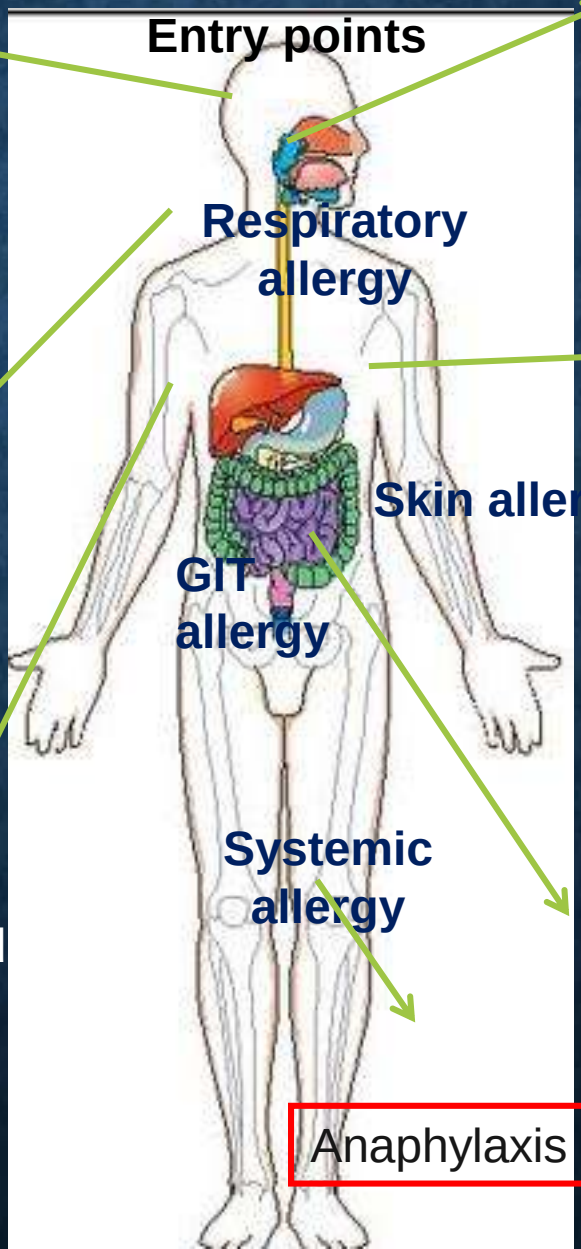
Rhinitis (upper)
Itchy eye, runny nose, sneezing

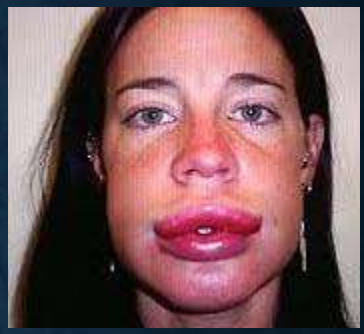



Asthma (lower)

Shortness of breath, wheezing and chest tightness

Food allergy: cramps, vomitting, diarrhea, urticaria



Angioedema

Hives/urticaria

Atopic dermatitis

# PREVALENCE OF ALLERGIES

| | American Academy of Allergy Asthma & Immunology | | AllergyUK | European Academy of Allergy and Clinical Immunol | Wang et al (2015) |
|---|---|---|---|---|---|
| | | worldwide | | | |
| **Allergic rhinitis** | 7-10% (paed)<br>7.8% (>18 yo) | 10-30% | 20% | 10-20% | 3.9-20% (paed)<br>8.7-34% (adult) |
| **Asthma** | 7.3-8.2% | | | 1-18% (paed) | 9.4% (<18 yo)<br>8.2% (>18 yo) |
| **Atopic dermatitis /eczema** | | | | 20% (paed)<br>2-10% adults | 10-20% (paed)<br>1-3% (adult) |
| **Skin allergies/ urticaria** | 10-17% (paed) | >20% | | | 20% |
| **Drug allergy** | | 10% (20% fatalities) | | 7% | 10% (penicillin) |
| **Food allergy** | 8% (paed) | | 6% (vs 12% food intolerance) | | 4-8% (paed)<br>5% (adult) |
| **Allergic** | | | | 25% | 40% (west) |

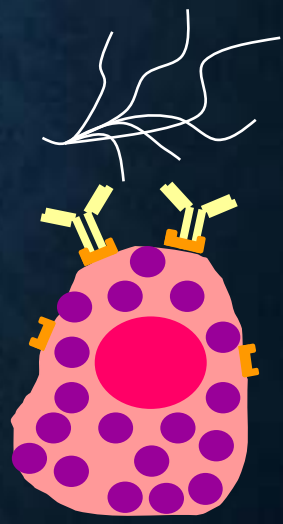
# MAJOR COMPONENTS OF AN ALLERGIC REACTION

IgE-mediated hypersensitivity

1) Mast cells and basophils ($Fc_eRI$ )

2) IgE

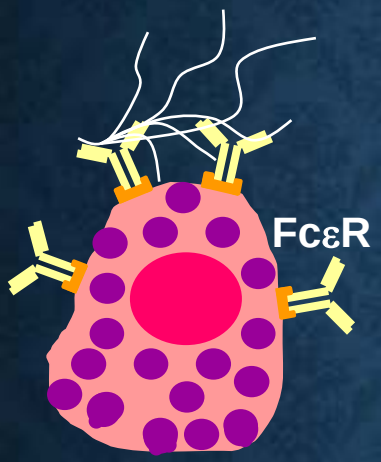
3) Allergens



- ***Rhinitis, asthma, anaphylaxis***

# Type I Hypersensitivity (phases)

First exposure




IgE

Sensitization
(50% of population)

Re-exposure

Activation
(20% of population)

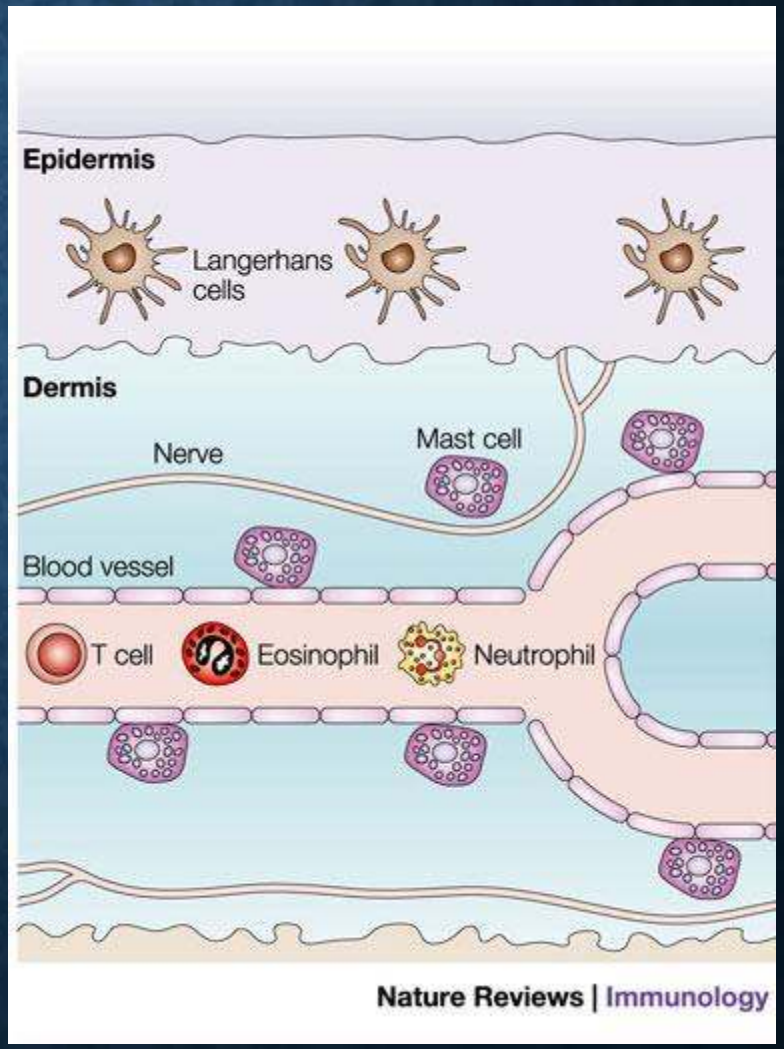
Late phase reaction

Allergic symptoms

# SKIN

Mast cells

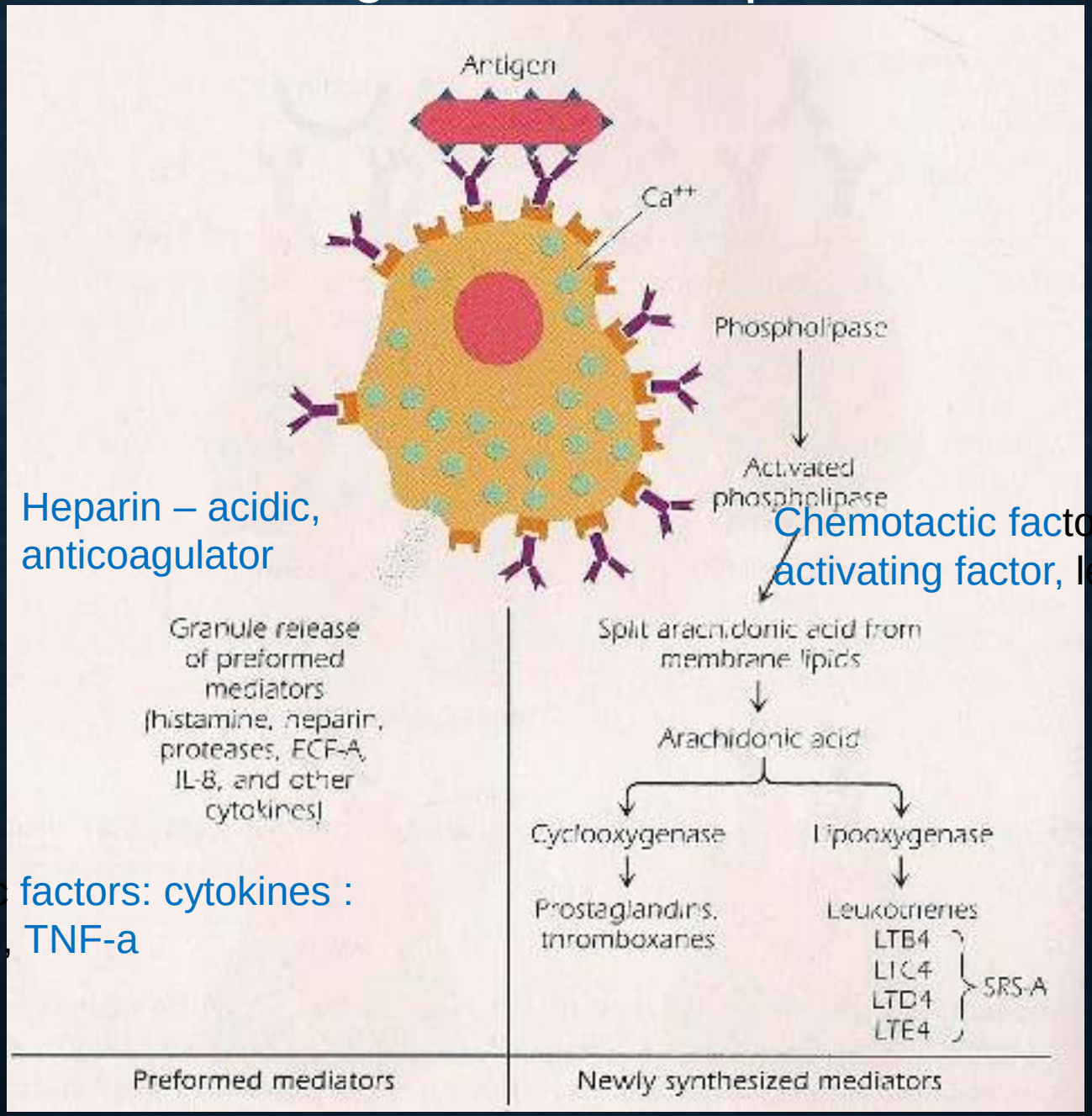
Skin tests
immediate hypersensitivity
Purified allergens –
percutaneously or
intradermally




Marshall JS (2004). Mast-cell responses to pathogens. *Nature Rev Immunol;* **4**, 787-799

# Mechanism of damage: Activation phase

Mast cell granules

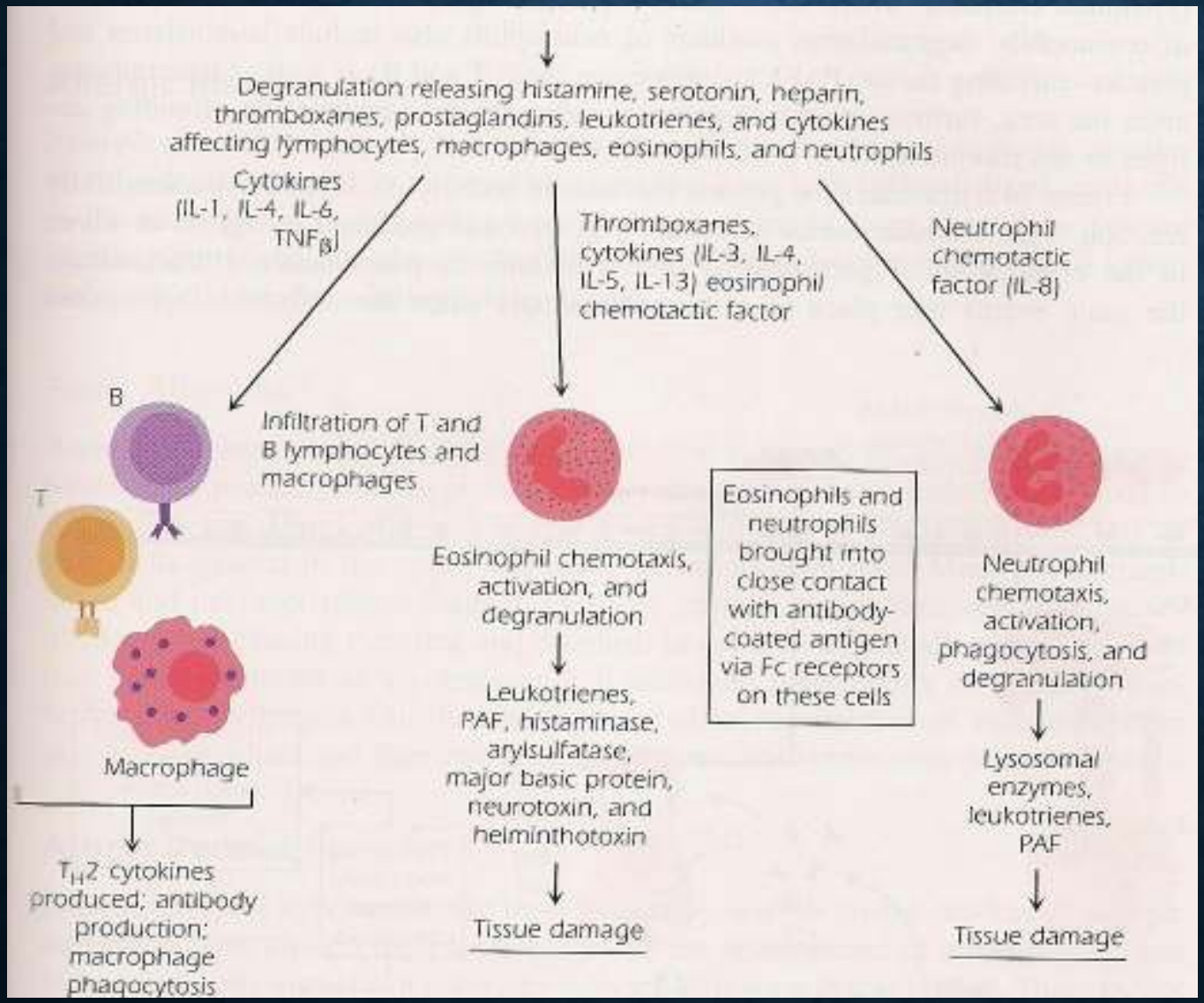



Heparin – acidic, anticoagulator

Chemotactic factors: platelet activating factor, leukotrienes

*Chemotactic factors: cytokines : GM-CSF, IL5, TNF-a

# Mechanism of damage: Late-phase reaction




## Activation phase

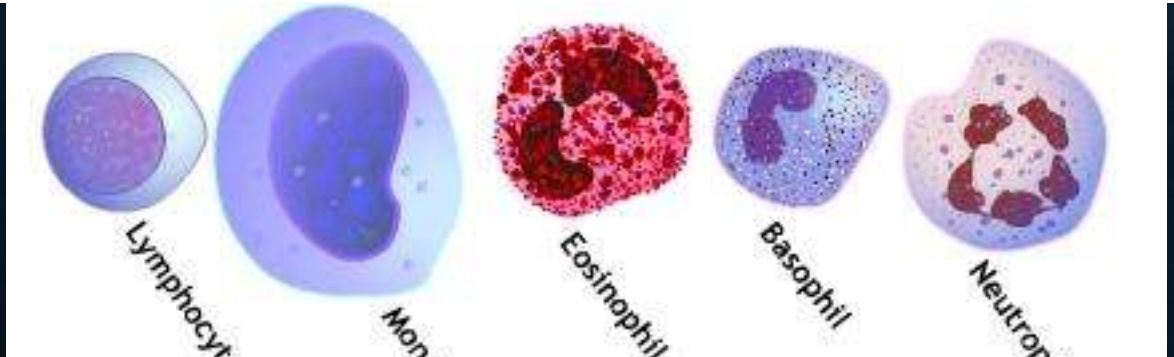
Occurs within 10-15 min

## Late-phase reaction

Occurs within 4-8 hr; persist several days

# Differential white blood cells count (normal adult)

| | Absolute Values | Percentage. |
|---|---|---|
| Neutrophil | 2.0-7.0 x$10^9$/L | 40-75 % |
| Lymphocytes | 1.0-3.0 x$10^9$/L | 20-45 % |
| Monocytes | 0.2-1.0 x$10^9$/L | 2-10 % |
| Eosinophil | 0.02-0.5 x$10^9$/L | 1-6 % |
| Basophile | 0.02-0.1 x$10^9$/L | 0-2 % |



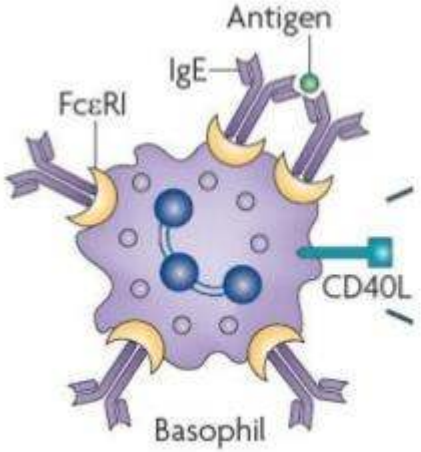
# Fc receptors and transporting Fc receptors

**TABLE 12–3 Fc Receptors**

| FcR | Affinity for Immunoglobulin | Cell Distribution | Function |
|---|---|---|---|
| FcγRI (CD64) | High ($K_d < 10^{-9}$ M); binds IgG1 and IgG3, can bind monomeric IgG | Macrophages, neutrophils; also eosinophils | Phagocytosis; activation of phagocytes |
| FcγRIIA (CD32) | Low ($K_d > 10^{-7}$ M) | Macrophages, neutrophils; eosinophils, platelets | Phagocytosis; cell activation (inefficient) |
| FcγRIIB (CD32) | Low ($K_d > 10^{-7}$ M) | B lymphocytes | Feedback inhibition of B cells |
| FcγRIIC (CD32) | Low ($K_d > 10^{-7}$ M) | Macrophages, neutrophils, NK cells | Phagocytosis, cell activation |
| FcγRIIIA (CD16) | Low ($K_d > 10^{-6}$ M) | NK cells | Antibody-dependent cell-mediated cytotoxicity |
| FcγRIIIB (CD16) | Low ($K_d > 10^{-6}$ M); GPI-linked protein | Neutrophils | Phagocytosis (inefficient) |
| FcεRI | High ($K_d > 10^{-10}$ M); binds monomeric IgE | Mast cells, basophils, eosinophils | Cell activation (degranulation) |
| FcεRII (CD23) | Low ($K_d > 10^{-7}$ M) | B lymphocytes, eosinophils, Langerhans cells | Unknown |
| FcαR (CD89) | Low ($K_d > 10^{-6}$ M) | Neutrophils, eosinophils, monocytes | Cell activation? |
| FcRn | „neonatal" Fc receptor | Placenta, endothelial and epithelial cells, etc. | IgG transfer, IgG salvage |
| pIgR | poli Ig receptor | Epithelial cells | IgA, IgM transfer |

# BASOPHIL

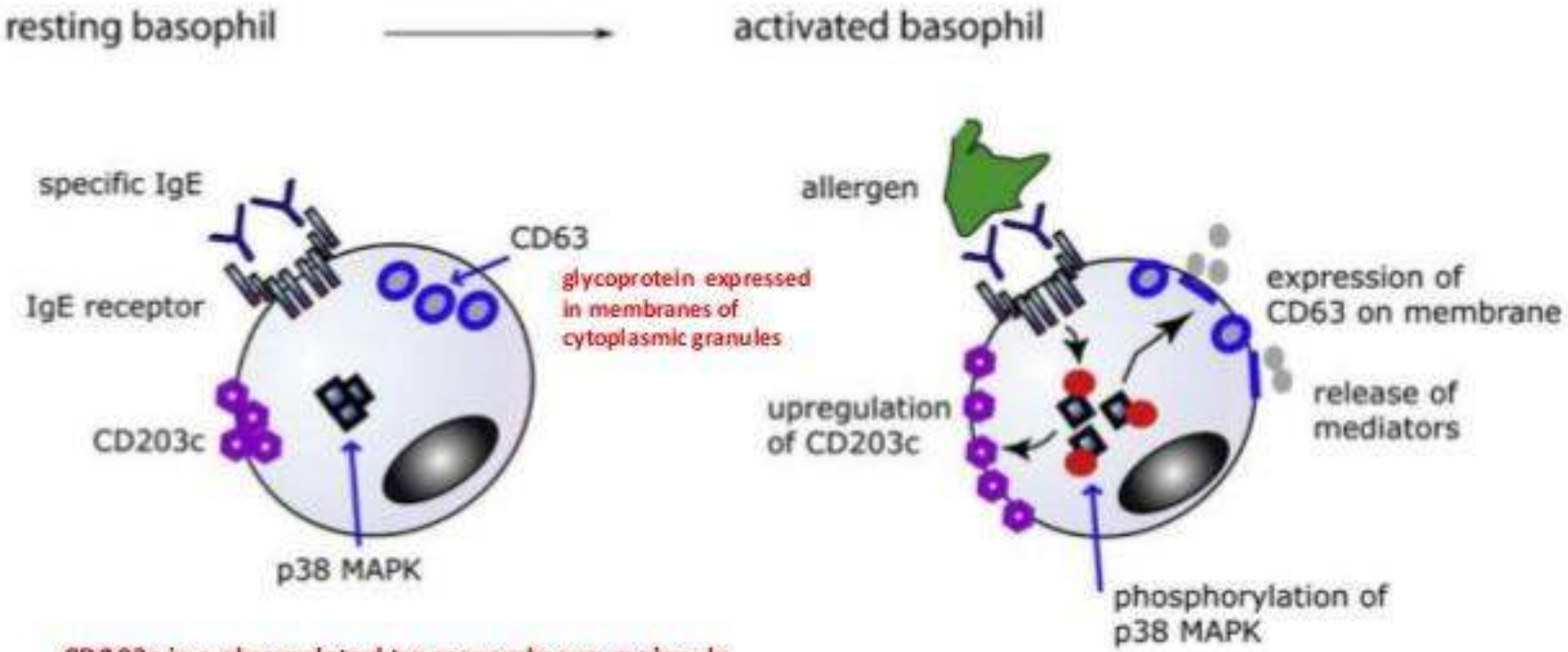
- Proliferative capacity –none
- Survival in circulation (days) ~3-7




Human basophils may express between 5,000 and 1 million FcεRI sites per cell

Estimated 200 IgE-receptor cross-links needed to initiate mediator release from basophils

# Basophil activation markers: CD63 & CD203c




CD203c is a glycosylated transmembrane molecule

Basophils express low level of CD203c, which is increased with basophil activation and may correspond to piecemeal degranulation

Cromheecke JL. et. al. Curr Allergy Asthma Rep 2014;14:408
Hausmann OV. et. al. Immunol Allergy Clin N Am 2009;29:555–66

# BAT

- BASOTEST allows the quantitative determination of human basophil degranulation.
- The FC method correlates well with histamine release assay.
- BASOTEST allows the diagnosis of immediate-type HS.
- The success of immunotherapies can be monitored.

- TEST KIT
- Positive control: Chemotactic peptide N-formyl-Met-Leu-Phe (fMLP)
- Allergens  (Mite mix)
- Anti-IgE-PE
- Anti-CD63-FITC (recognizes gp53 expressed on activated basophils

# ALLERGY SECTION

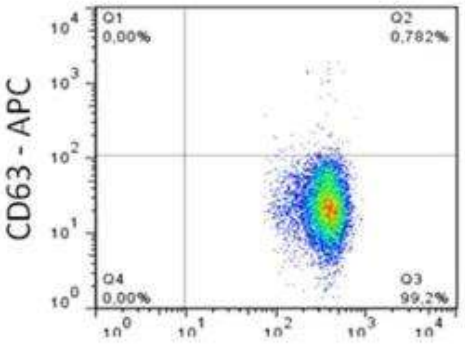
Counts
- Percentage
- Absolute counts
- Levels

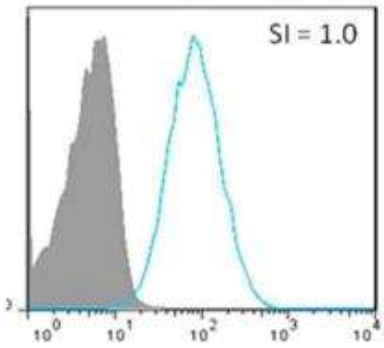
- Total IgE
  (Allergies, parasitic infections, WAS, Churg-Strauss, Hyper-IgE, some forms of immunodeficiencies)
- Specific IgE
  (aeroallergen, food, drugs etc.)
- Anaphylaxis (Tryptase)

- Functional assay

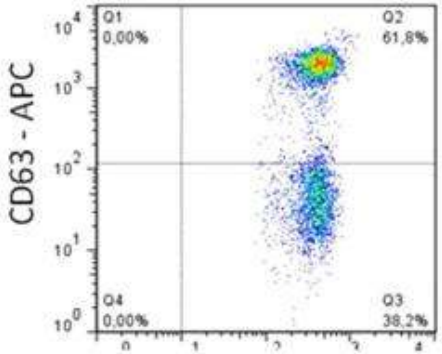
- Skin prick test
- Oral food challenge
- Basophil activation test

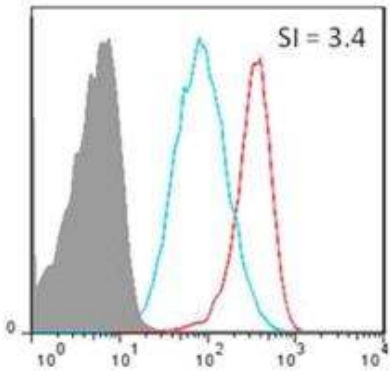
No stimulation



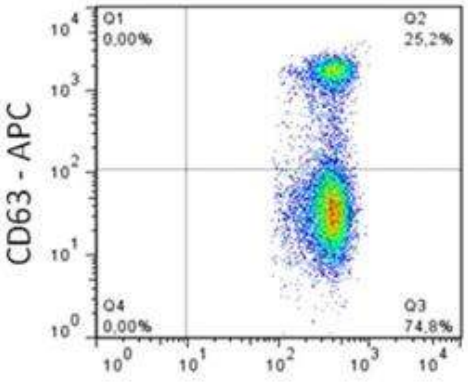



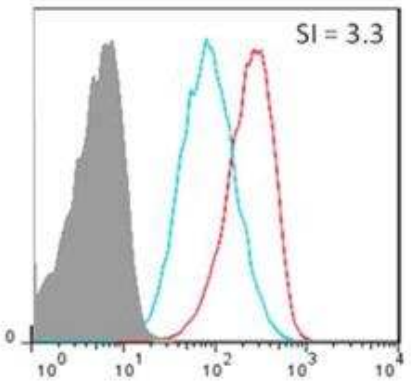

Peanut extract
10 ng/ml




Anti-IgE
1µg/ml



## Table: Studies assessing the utility of BAT to diagnose food allergy

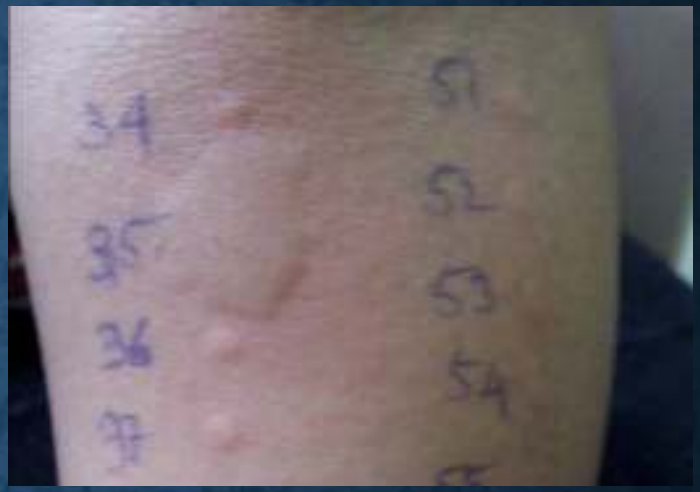
| Food | Author year | N | Cut-offs | Sensitivity | Specificity |
|---|---|---|---|---|---|
| Peanut | Santos 2014 [15] * | N = 104 | ≥4.78 % CD63+ | 97.6 % | 96.0 % |
| | | Validation population N = 65 | | 83.3 % | 100 % |
| | Glaumann 2012 [12] | N = 38 | ND | 92 % | 77 % |
| | Javaloyes 2012 [16] | N = 26 | ND | 92 % | 95 % |
| | Ocmant 2009 [17] | N = 75 | ≥9.1 % CD63+ | 87 % | 94 % |
| Hazelnut | Brandström 2015 [28] | N = 40 | CD-sens > 1.7 | 100 % | 97 % |
| Egg | Ocmant 2009 [17] | N = 67 | ≥5 % CD63+ | 77 % | 100 % |
| Cow's milk | Sato 2010 [19] | N = 50 | SI CD203c ≥ 1.9 | 89 % | 83 % |
| Wheat | Tokuda 2009 [22] | N = 58 | ≥14.4 % CD203c+ | 85 % | 77 % |
| Apple (PFS) | Ebo 2005 [34] | N = 61 | Vs sensit. ≥17 % CD63+<br>Vs NA ≥10 % | Vs sensit. = 88 %<br>Vs NA = 100 % | Vs sensit. = 75 %<br>Vs NA = 100 % |
| Hazelnut (PFS) | Erdmann 2003 [33] | N = 30 | ≥6.7 % CD63+ | 85 % | 80 % |
| Celery (PFS) | | | ≥6.3 % CD63+ | 85 % | 80 % |
| Carrot (PFS) | | | ≥8.9 % CD63+ | 85 % | 85 % |

*Allowed a reduction in the number of OFC required by 66 %.

Santos et al (2016). BMC Clinical and Translational

# SKIN PRICK TEST (IN VIVO METHOD)

Allergen and lancet



Wheal and flare

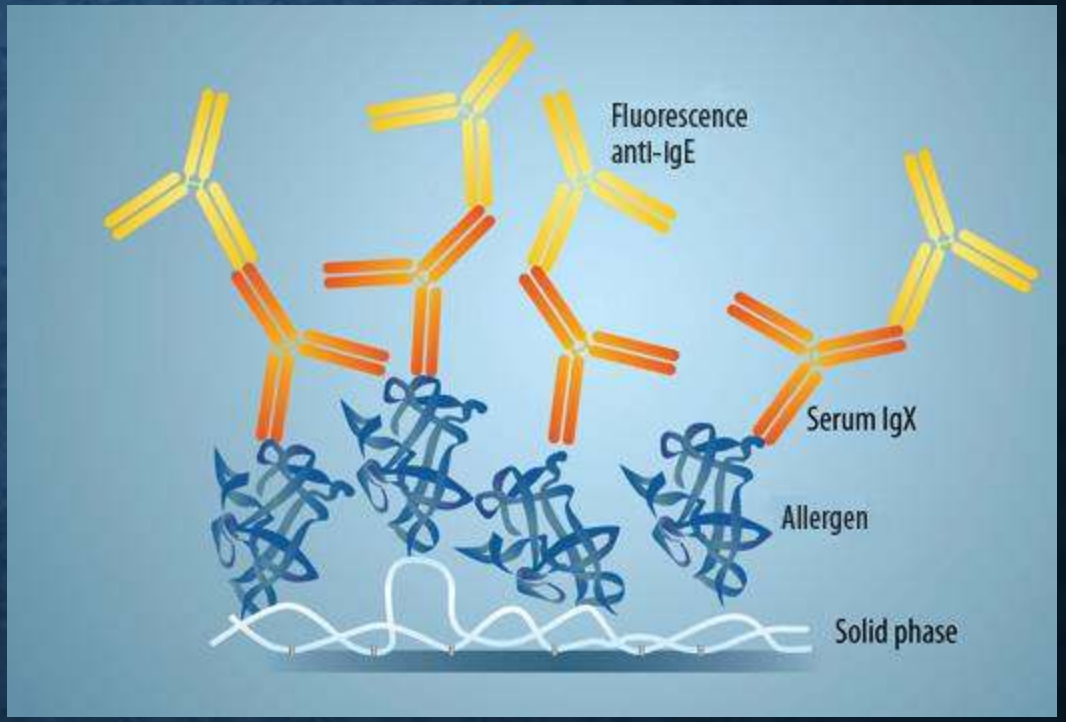
Marking , allergen drop and skin prick

Measuring flare

# ALLERGEN SPECIFIC IG-E ANTIBODY TEST (*IN VITRO* METHOD)

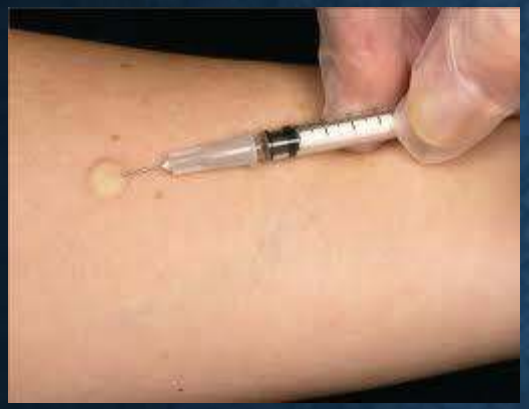

ImmunoCAP

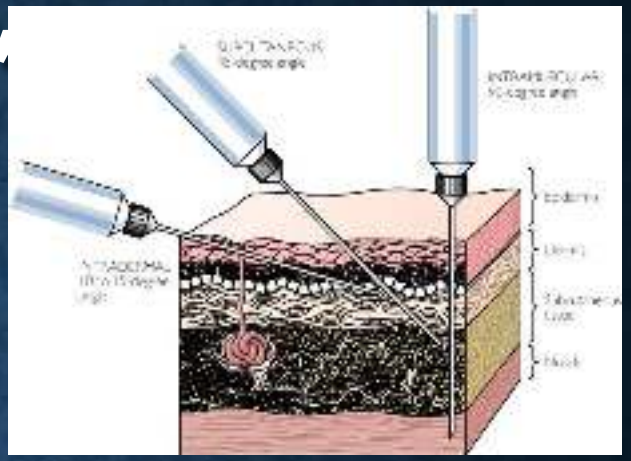
Principle of ELISA - FEIA




Allergen

# INTRADERMAL





The intradermal skin test involves:

- Injecting a small amount of allergen into the skin.
- Then the health care provider watches for a reaction at the site.


Intradermal tests are not used to test for food allergies because of high false-positive results and the danger of causing a severe allergic reaction.

Intradermal tests are much used to diagnose allergy and to test cellular immunity.

# SPT VS IDT

- Skin testing may be performed using either the prick/puncture (percutaneous) or intradermal (intracutaneous) technique.
- Intradermal testing is far more sensitive than prick/puncture testing, which means that it requires about 1000-fold less concentrated extracts than those used for prick/puncture testing to achieve a similar response.
- Although direct comparisons indicate that intradermal testing is more reproducible than percutaneous testing, there are many factors that favor the routine use of percutaneous allergy tests. These include economy of time, patient comfort and patient safety.
- Percutaneous testing allows the use of extract in 50% glycerin, which provides greater extract stability. Intradermal testing cannot use this diluent, as it may incite a false-positive irritant response.
- However, the most important consideration is that results of percutaneous testing correlate better with clinical allergy.
- The higher sensitivity of intradermal skin tests does not usually offer added benefit, since the results of skin prick tests performed with potent extracts are of sufficient sensitivity for use in clinical practice.

# PATCH TEST (TYPE IV HYPERSENSITIVITY)

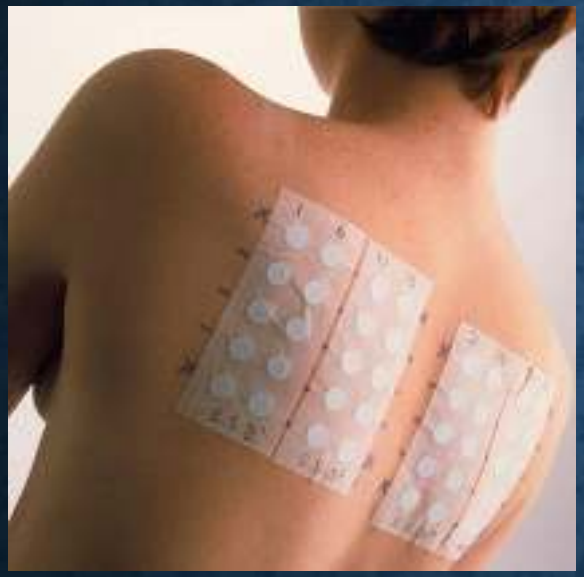
Patch testing is used to diagnose T cell allergy. Clinically these reactions manifest with an eczematous rash confined to the site of contact.

Strips of Patch test chambers applied to the strips.

*Some common Allergens (causing contact dermatitis) used in Patch Testing*

| Allergen | Sources |
|---|---|
| Nickel | Jewellery |
| Balsam of Peru | Perfumes, citrus fruits |
| Dichromate | Cement, leather, matches |
| Paraphenylenediamine | Hair dyes, clothing |
| Rubber chemicals | Shoes, clothing, gloves |
| Colophony | Sticking plasters |
| Benzocaine | Topical anesthetics |
| Neomycin | Topical medicaments |
| Parabens | Preservatives in cosmetics, creams |
| Epoxy resins | Glues |
| Formaldehyde | Clothing, cosmetics, paper |
| Wool alcohol | Lanolin, cosmetics, creams |

# PATCH TEST (CONTINUED)



Allergy test patches on back. Possible allergens are taped to the skin for 48 hours.
Area looked at after 72 - 96 hours.

The cell-mediated response appears 7 to 14 days after initial sensitisation and reactivates within 2 to 5 days of re-exposure.