ALLERGY DIAGNOSIS

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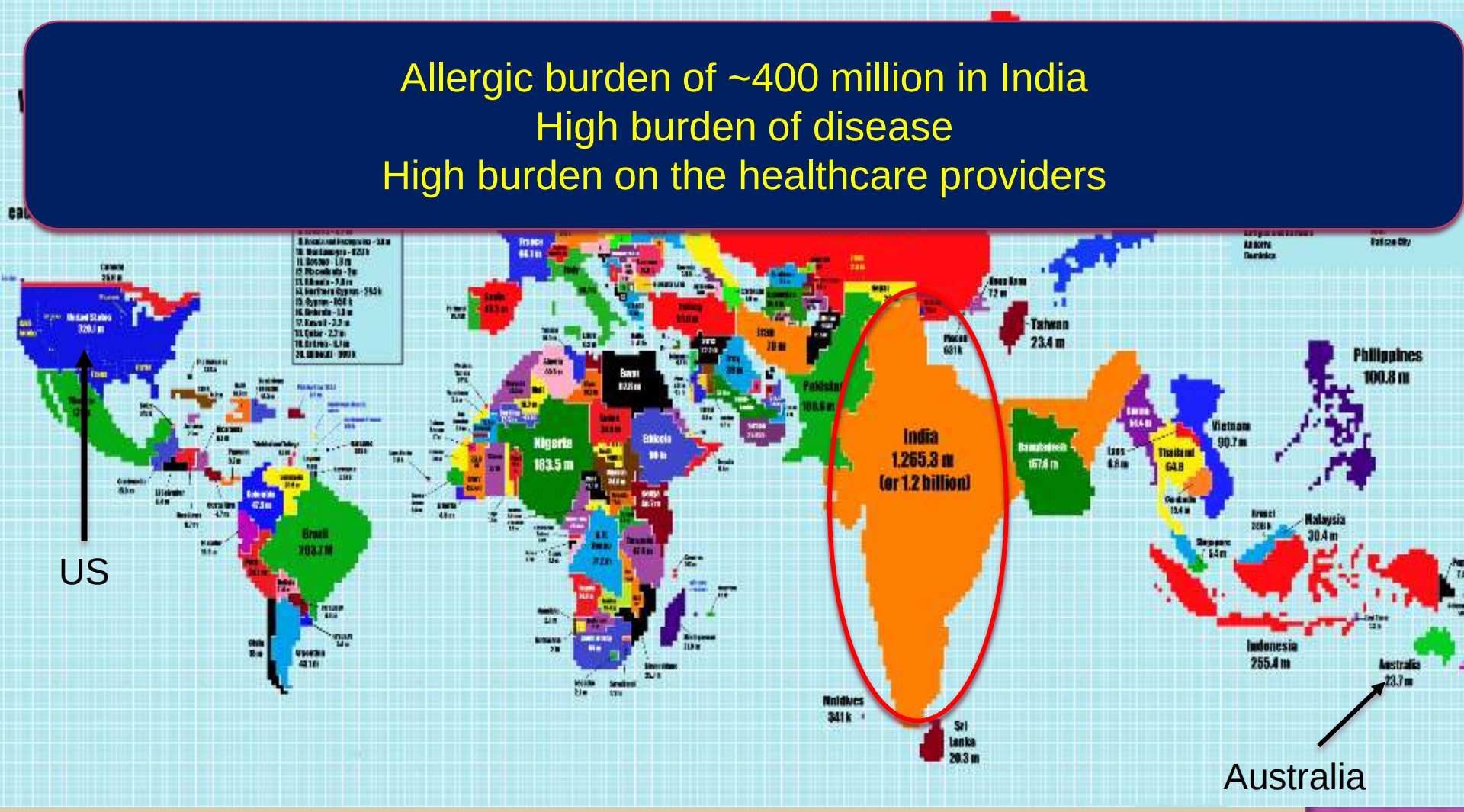
~20-30% Of The Indian Population Suffers From At Least One Of The Allergic Diseases[1]

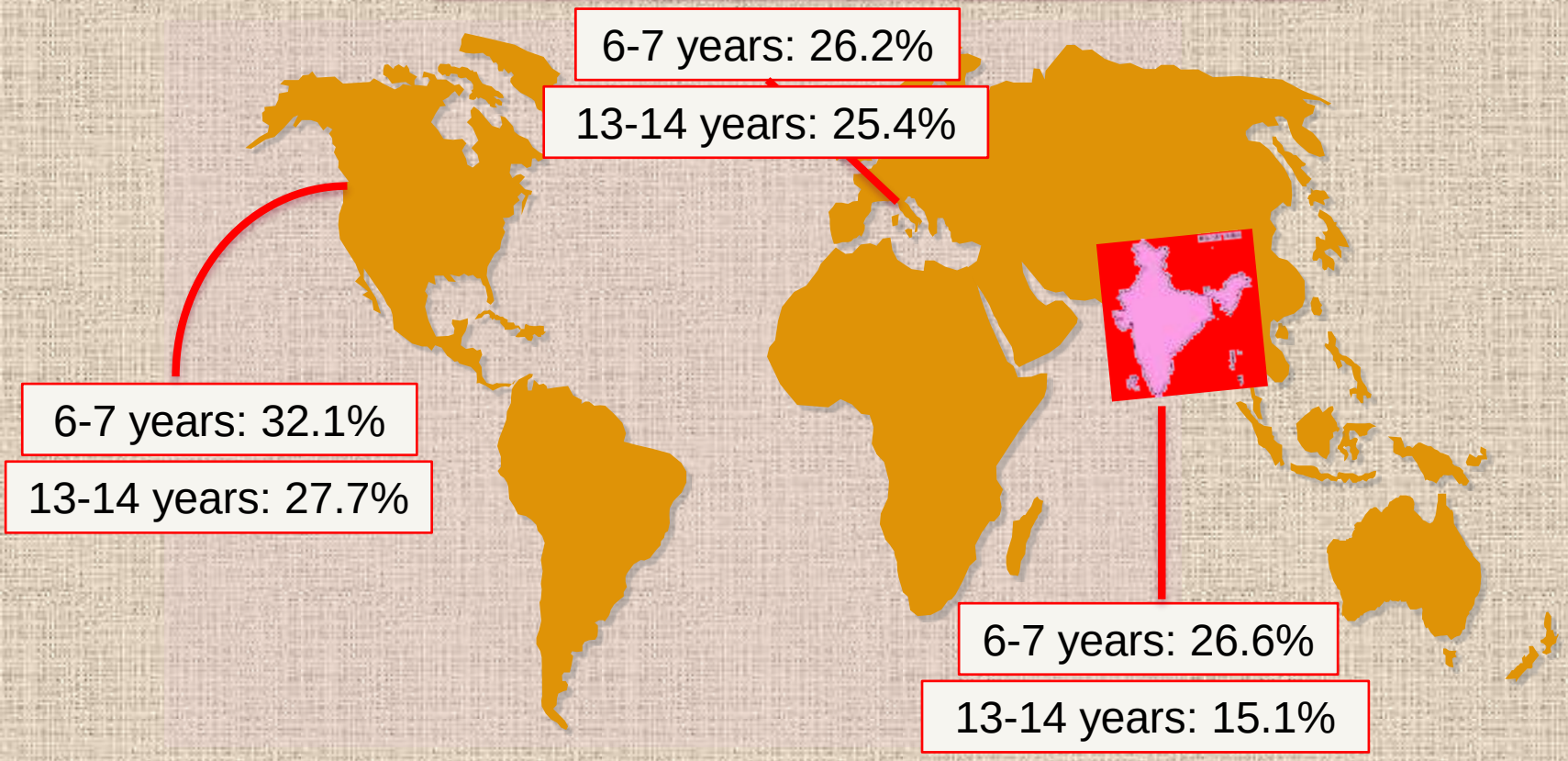
Allergic burden of ~400 million in India
High burden of disease
High burden on the healthcare providers



BURDEN OF ALLERGY

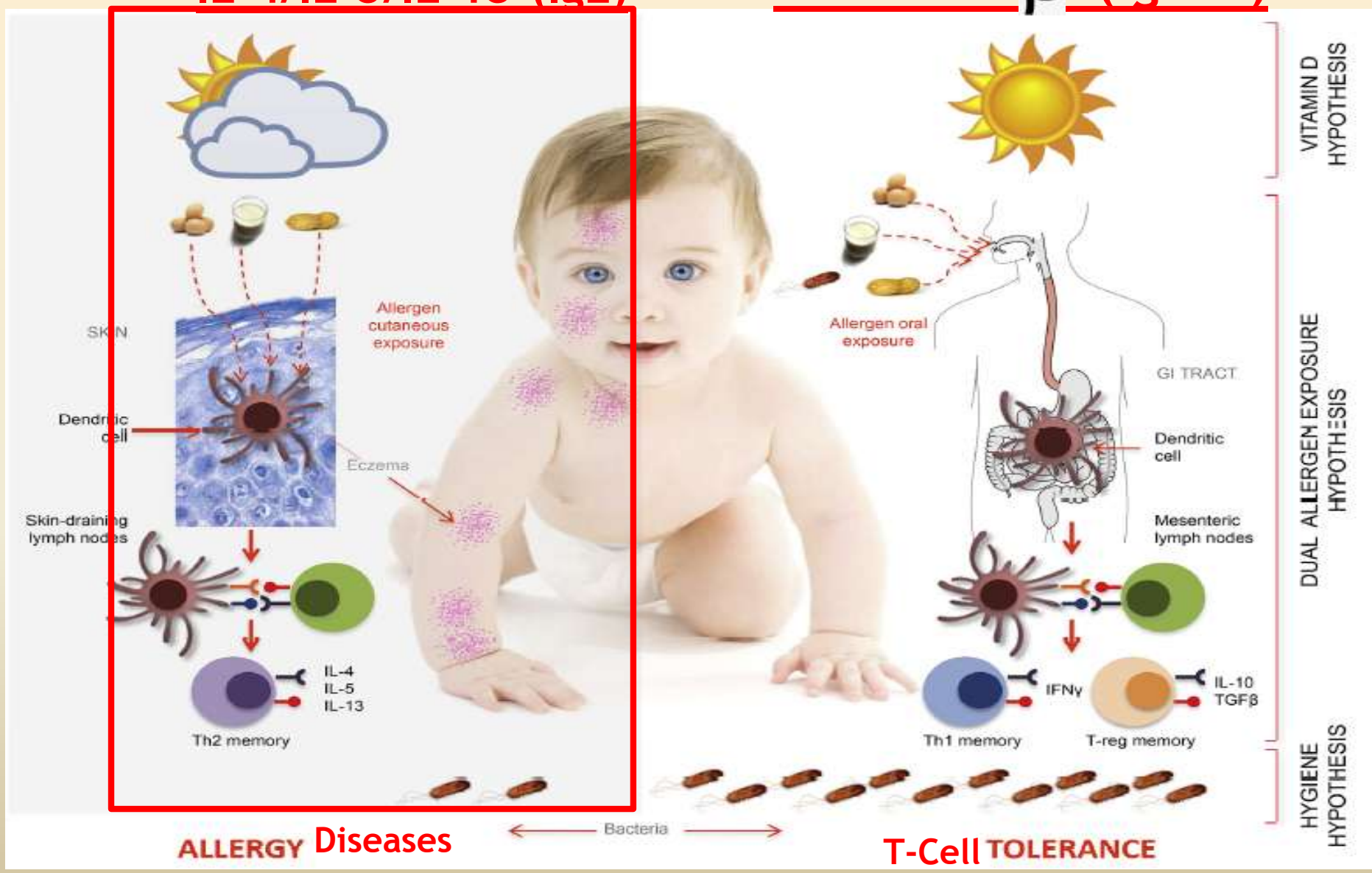
Children with Rhinoconjunctivitis having concomitant Asthma
ISAAC Phase Three

Similar prevalence was observed globally



•Mallol et al. Allergol Immunopathol (Madr). 2013;**41(2)**:73---85

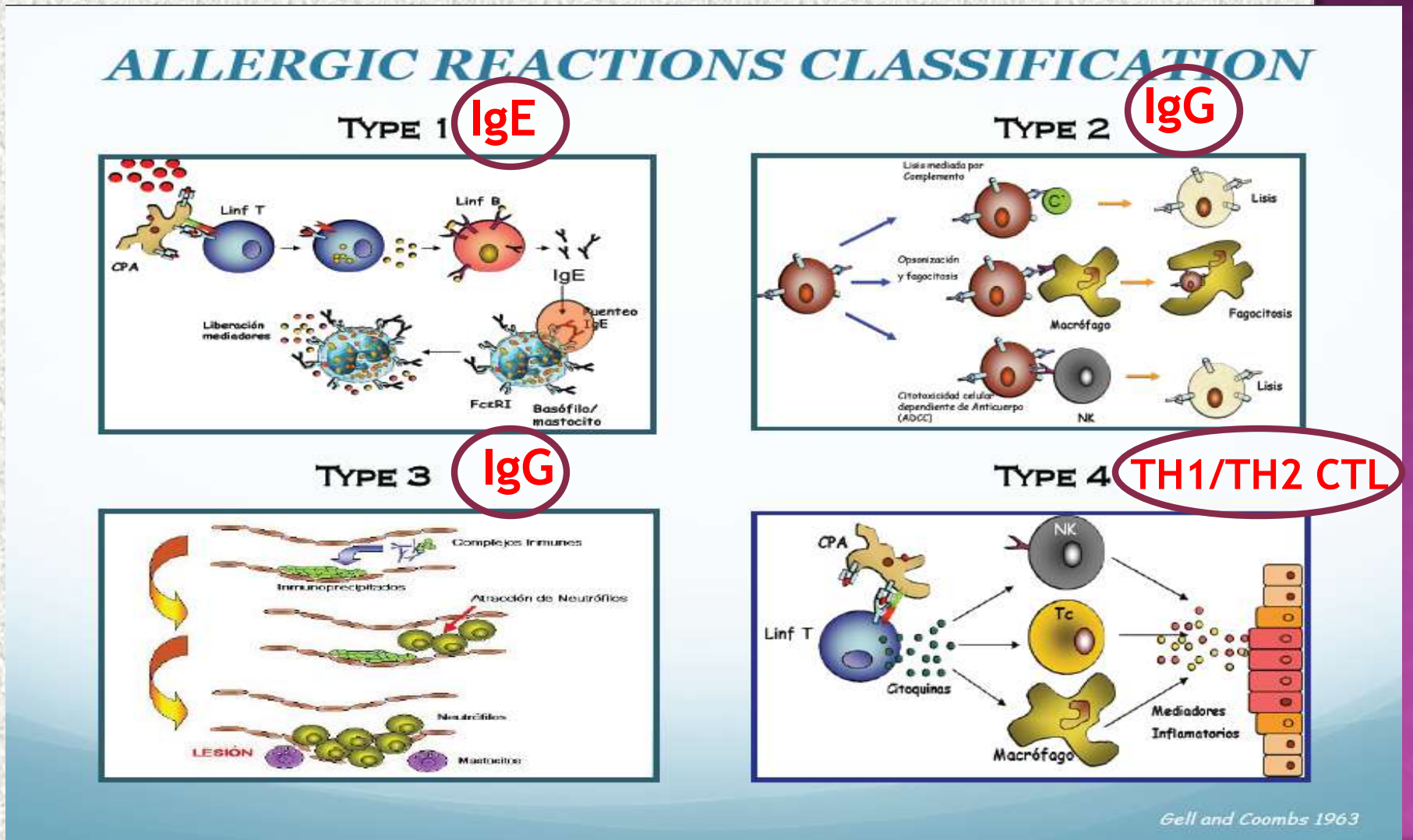
DEVELOPMENT OF ALLERGY VS TOLERANCE



1. Antigens – exogenous/ endogenous

2 Recognition

3. Specificity



Gell-Coombs Classification

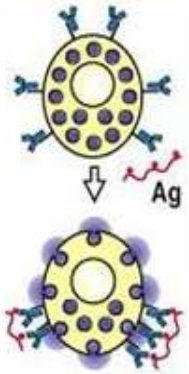
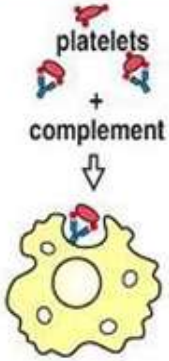
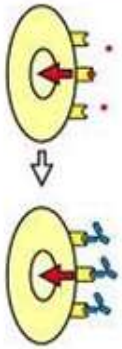
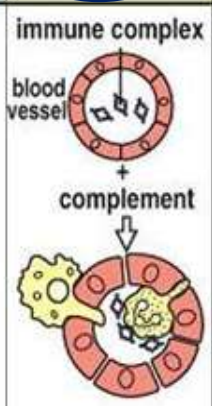
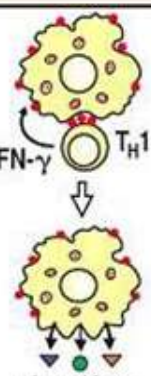
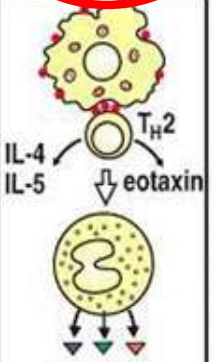
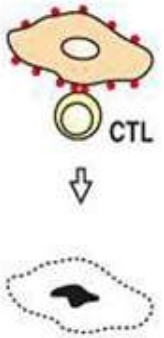
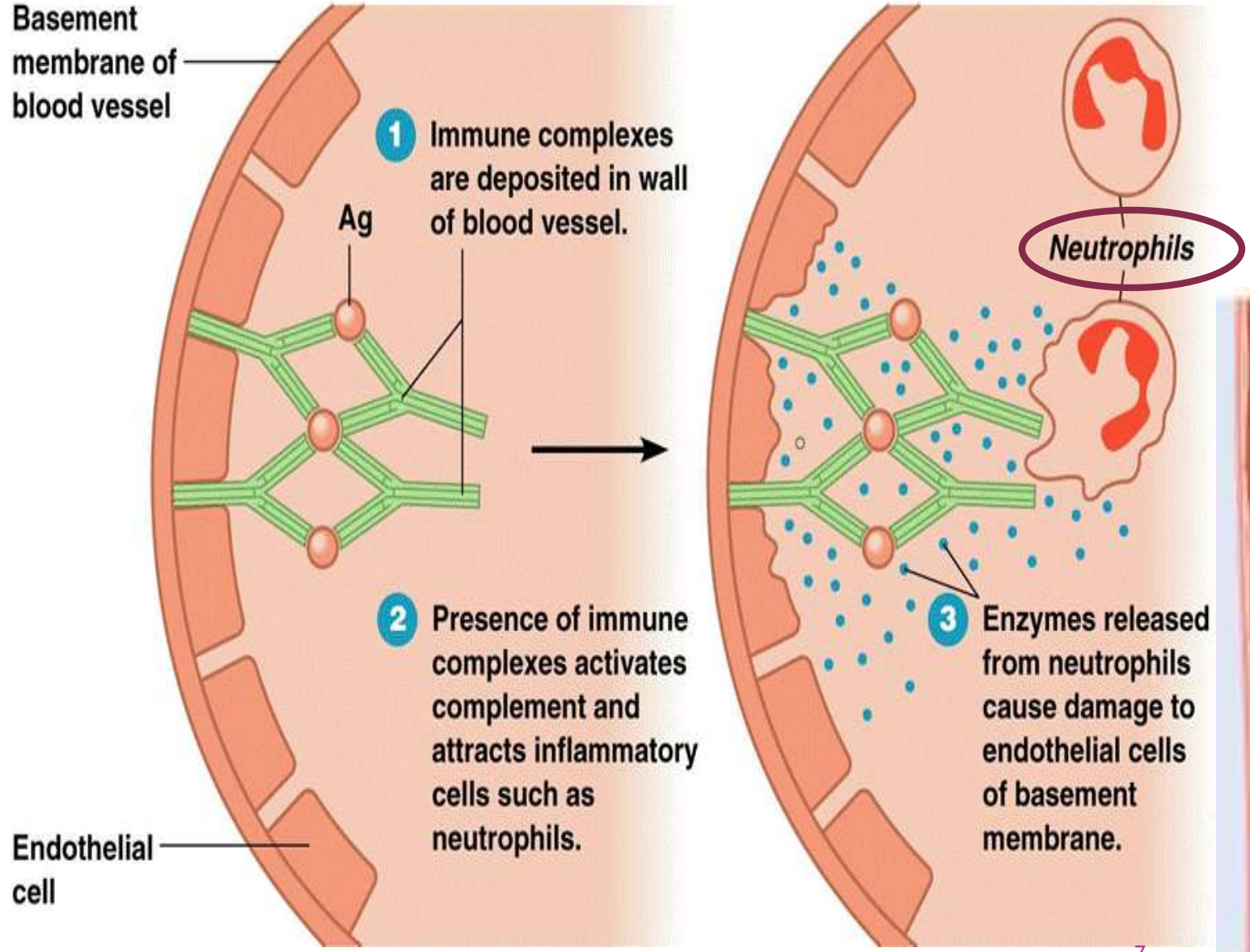
	Type I	Type II		Type III	Type IV		
Immune reactant	IgE	IgG		IgG	T_H1 cells	T_H2 cells	CTL
Antigen	Soluble antigen	Cell- or matrix-associated antigen	Cell-surface receptor	Soluble antigen	Soluble antigen	Soluble antigen	Cell-associated antigen
Effector mechanism	Mast-cell activation	Complement, FcR^+ cells (phagocytes, NK cells)	Antibody alters signaling	Complement, Phagocytes	Macrophage activation	IgE production, Eosinophil activation, Mastocytosis	Cytotoxicity
							
Example of hypersensitivity reaction	Allergic rhinitis, asthma, systemic anaphylaxis	Some drug allergies (eg, penicillin)	Chronic urticaria (antibody against FCεR1α)	Serum sickness, Arthus reaction	Contact dermatitis, tuberculin reaction	Chronic asthma, chronic allergic rhinitis	Contact dermatitis

Figure 12-2 Immunobiology, 6/e. (© Garland Science 2005)

HYPERSENSITIVITY TYPE 3 (IgG)- Excess of Ag/Ab

The Basic Pathology Of Serum Sickness



Allergy Diagnosis

GELL- Coombs

< 1hrs.

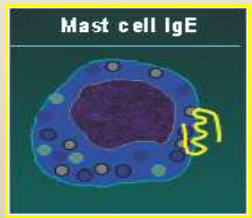
IL-4

Type I

Th2 (IL-4 /13)
Respiratory , Food,
Insect & Drugs allergy

Cell- bound IgE

. SPT
.IDST
. PPT

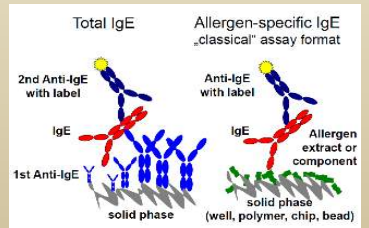


IgE
Soluble antigen
Mast-cell activation

Free IgE

Specific IgE
(immunocap- allergen)

Specific IgE ISAC
(component RD)



IL-5

> 1hrs.

Type IV

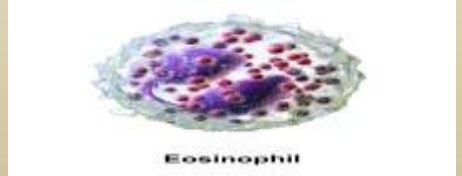
T_H2 cells
Soluble antigen
IgE production, Eosinophil activation, Mastocytosis

Delayed Respiratory & Food, Insect & Drugs Allergy

*Delayed IDST reaction
Atopy Patch TEST

BAT (CD63) assay
CAST(FLOW & ELISA)

Eosnophils



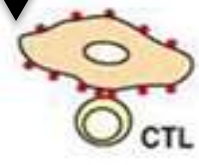
CTL
Cell-associated antigen
Cytotoxicity

*Contact dermatitis
*Drug allergy (T-cells)

Patch test
(T-cells)

LTT &
ELISPOT

Tissue damage
& BHR

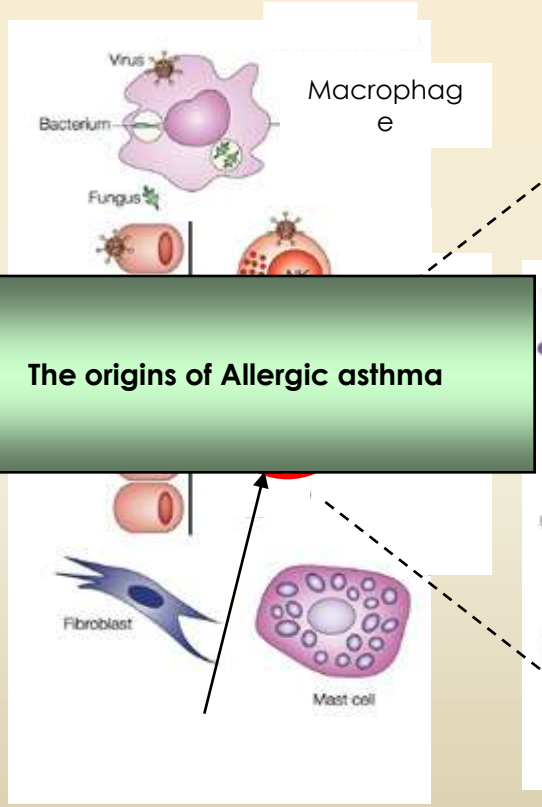


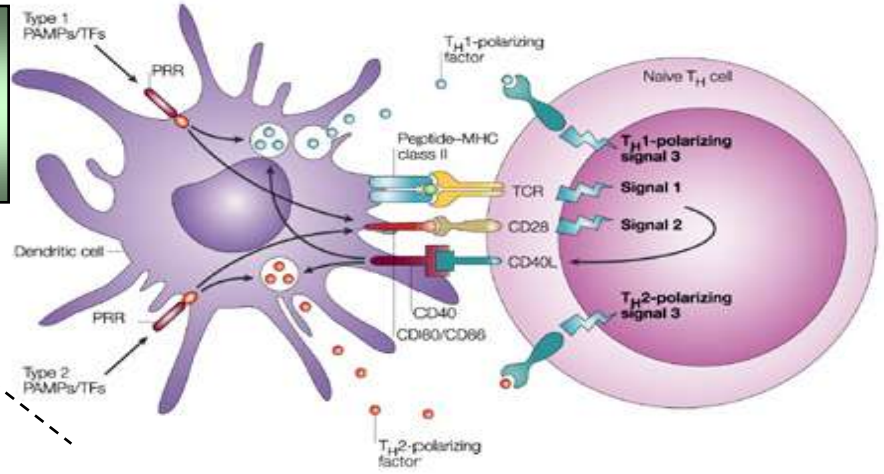
Dendritic cells link innate and adaptive immunity

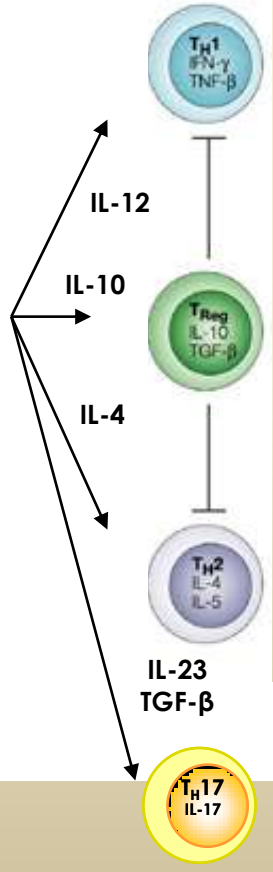
Innate Immunity
Recognition of pathogen associated molecular patterns (PAMPs) via a fixed repertoire of pathogen recognition receptors (PRRs) including TLRs`

Adaptive Immunity
- Specific activation and proliferation of B and T cells.
- Flexibility to respond to any antigen via versatile recombination of antigen receptors.
- Development of immunological memory.

The origins of Allergic asthma







Immature dendritic cell
- **Excellent antigen capture and processing**
- **Poor T cell stimulator**

Mature dendritic cell
- Poor antigen capture and uptake.
- Up regulation of MHCII and co-stimulatory molecules **CD40, CD80, CD86.**

Balance in healthy individuals

T-cell

Th1-derived Auto-Immune diseases

Allergic diseases is an Th2-related disease

- Eliminates micro organisms and tumors
- Auto-immune disease
- Transplant rejection

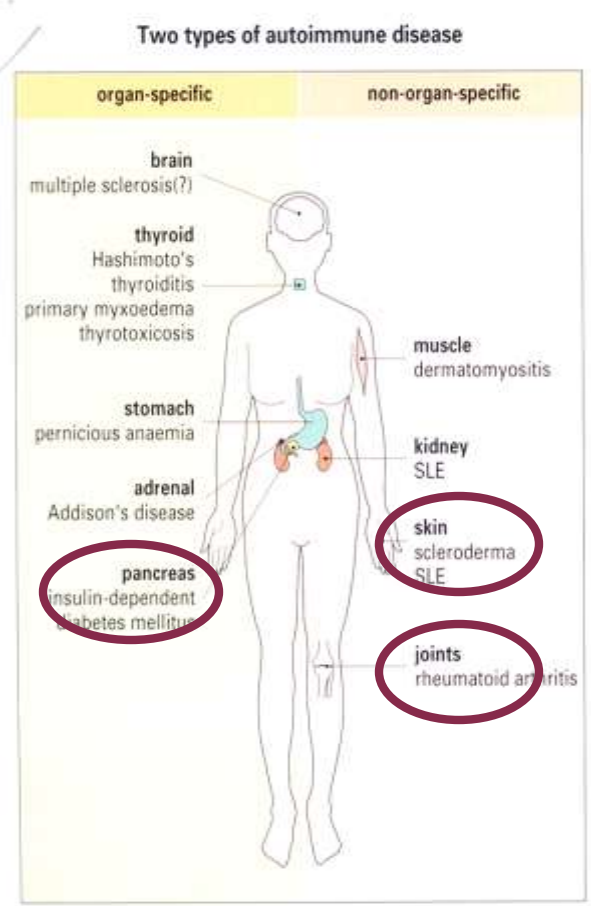
- Eliminates parasite
- Allergic diseases

Th1
IFN-γ, IL-2

Th2
IL-4, 5, 13

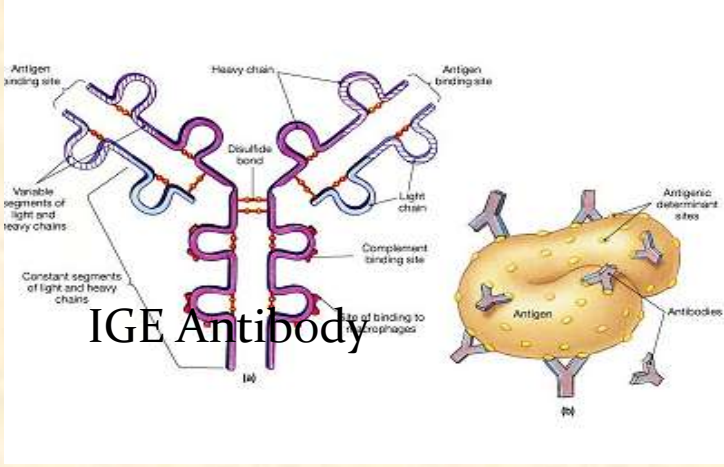
Tc
CD8+

Th
CD4+



ALLERGY - DEFINITION

- *GENETICALLY CONTROLLED*
-
-



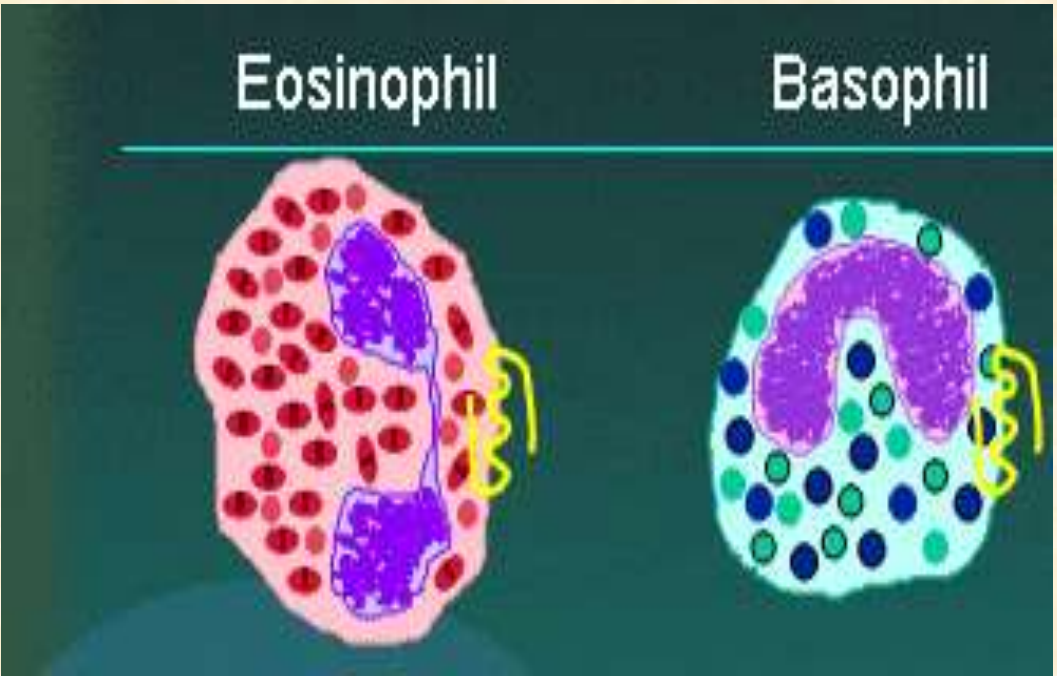
IGE Antibody

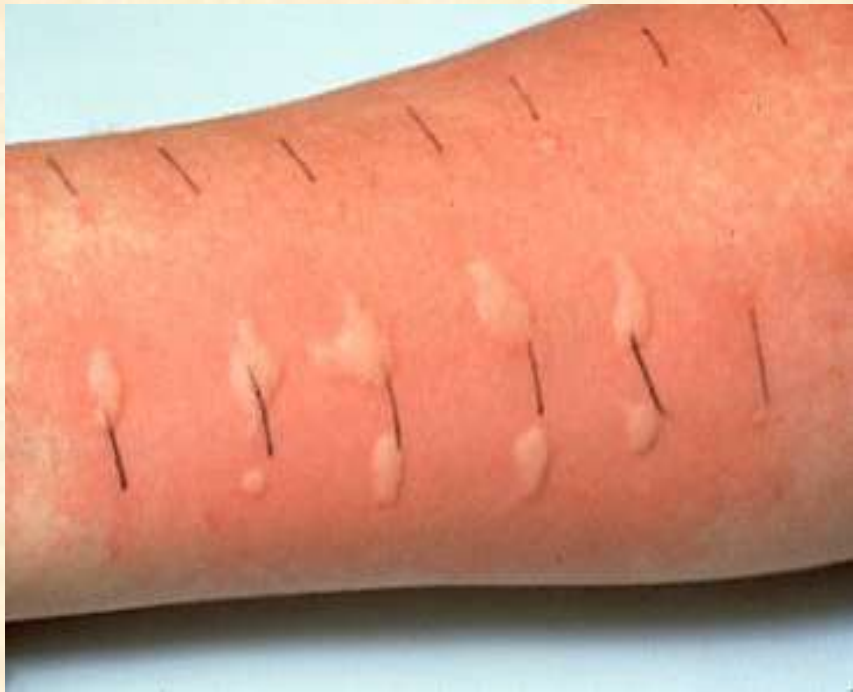
TH-2 Cytokines-

(A)IL-4 → IgE inflammation → Mast Cells Dgranulation

(1) Sensitization phase (2)effecter phase

(B)IL-5/13 → Eosinophil inflammation → ch. remodeling

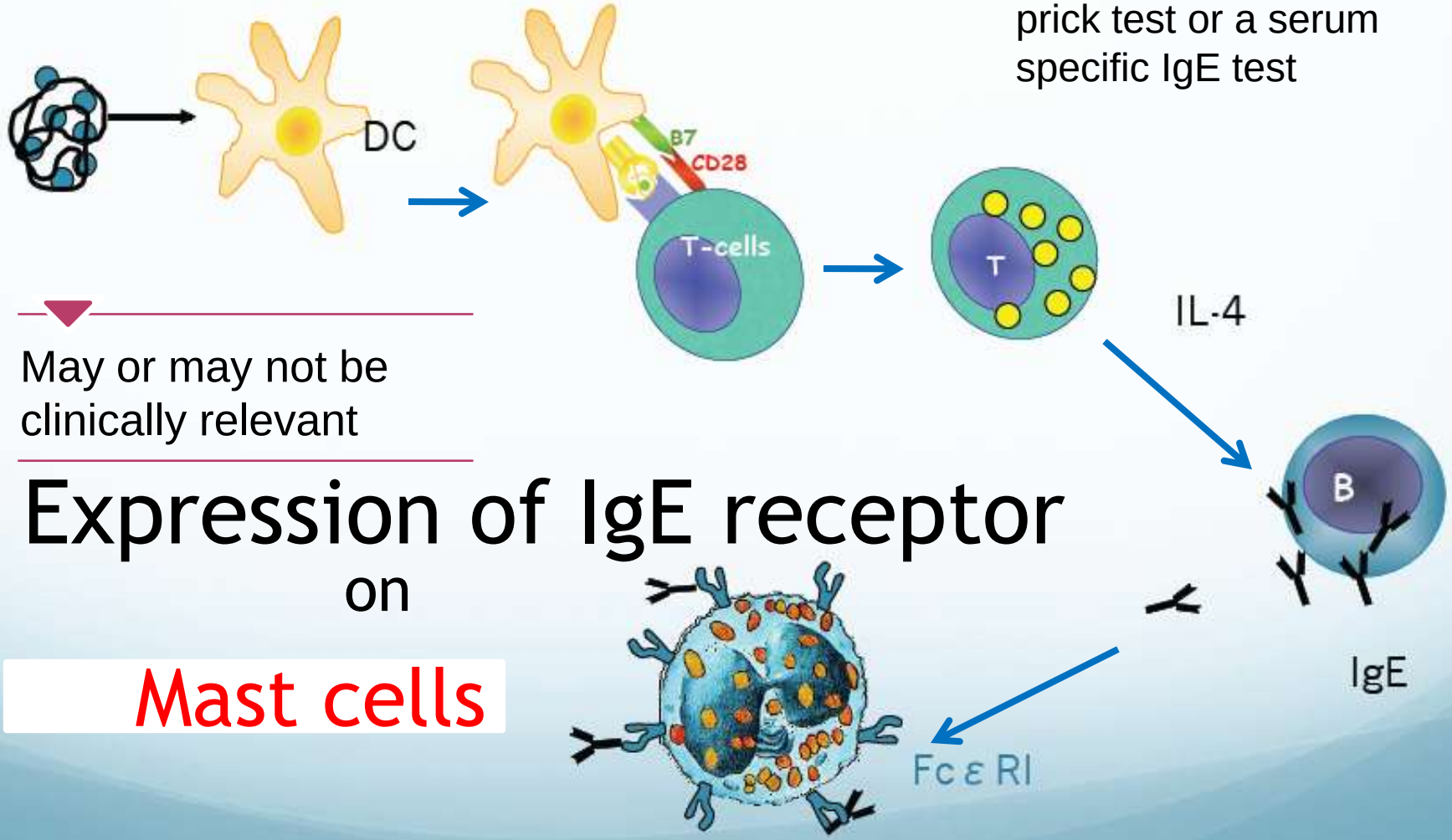




Mechanism of IgE mediated reactions

1st allergen contact (sensitisation)

A positive result on skin prick test or a serum specific IgE test



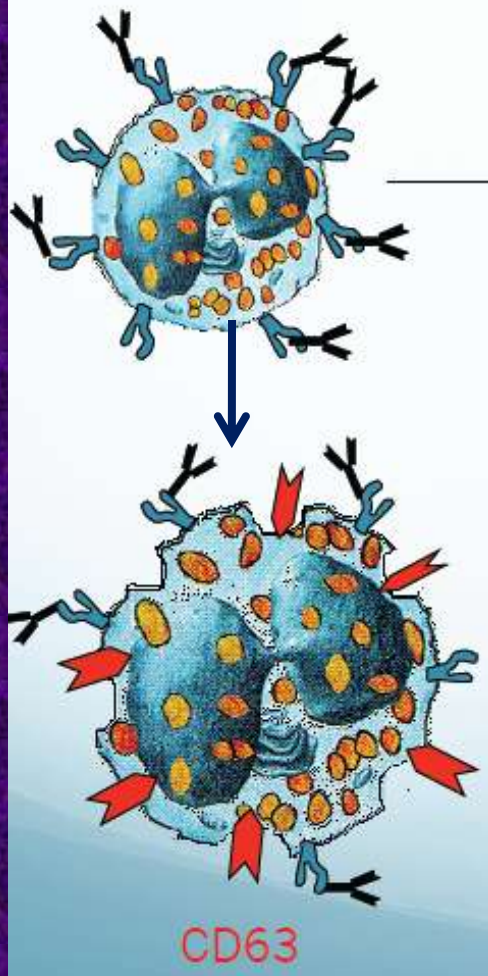
May or may not be clinically relevant

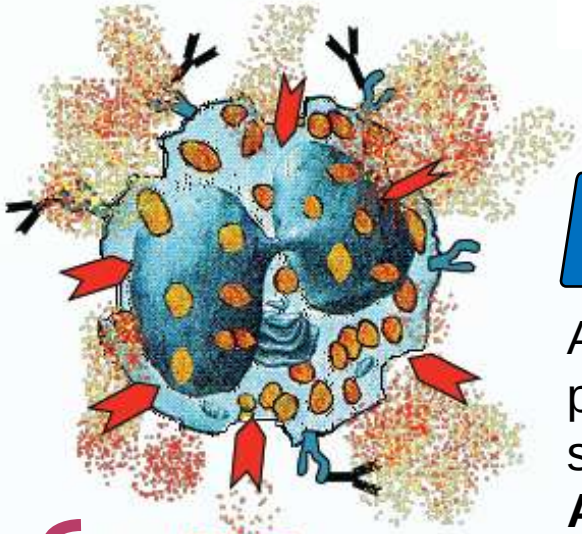
Expression of IgE receptor on

Mast cells

Mechanism of IgE mediated reactions:

2nd allergen contact **Effecter Phase**





Degranulation of Activated basophil & Release of mediators

Allergy

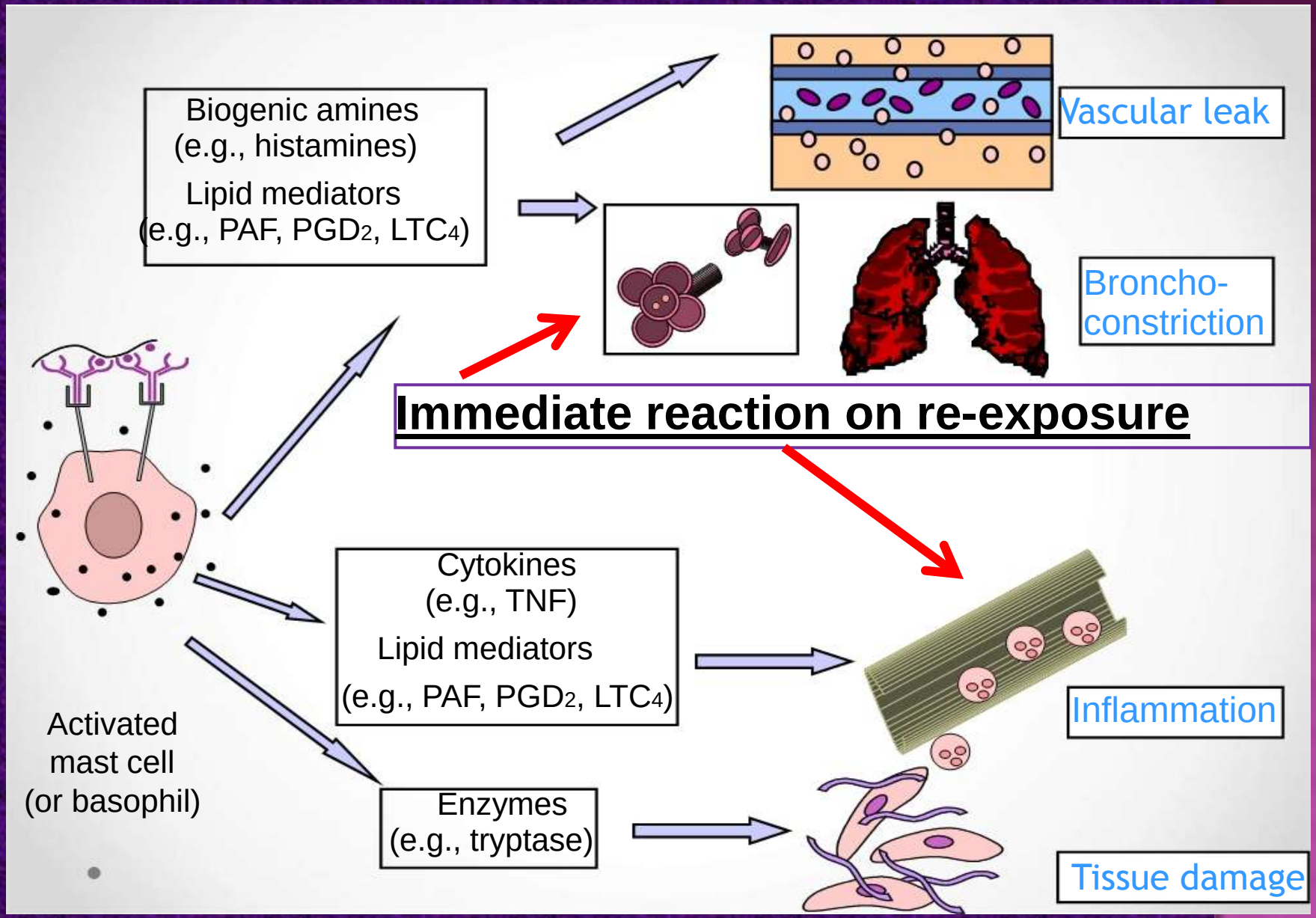
A positive result on skin prick test or a serum specific IgE test

AND

Correlating clinical history

Clinically relevant

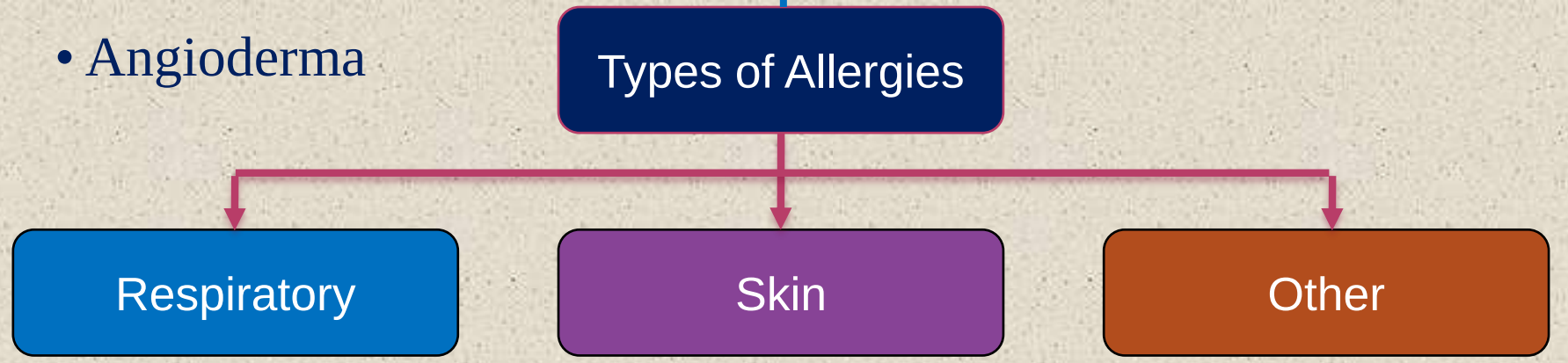
Clinical Allergy – symptoms



ALLERGIC DISORDERS

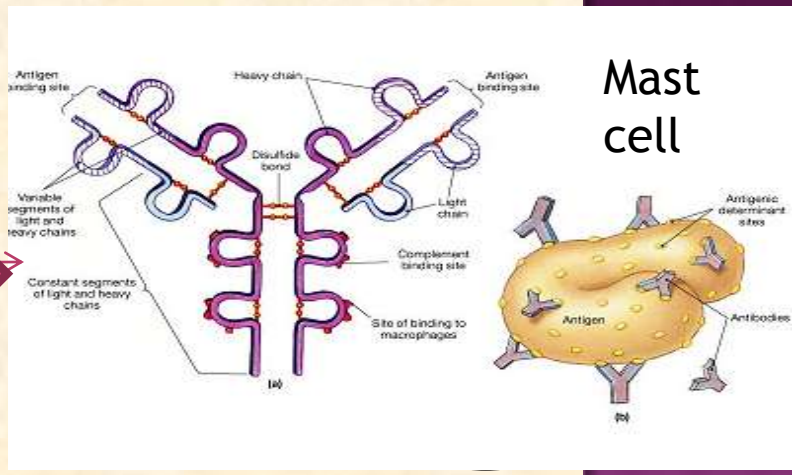
- Rhino conjunctivitis
- Rhino-sinusitis
- Rhino otitis
- Allergic BR-asthma
- Occupational-allergy
- Urticaria
- Atopic dermatitis
- Angioderma

- Food allergy
- Drug allergies
- Insect allergy
- Oral allergy syndrome
- Latex allergy
- **Anaphylaxis**



Spectrum of Allergen Sources

IgE Antibodies



Mast cell

Birth:TH_2

Allergen Exposure

TH_1
No allergies

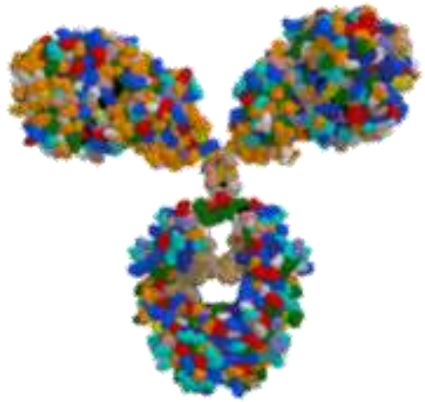
Still TH_2
Allergies

Source: Busse WW, Lemanske RF. N Engl J Med 2001.

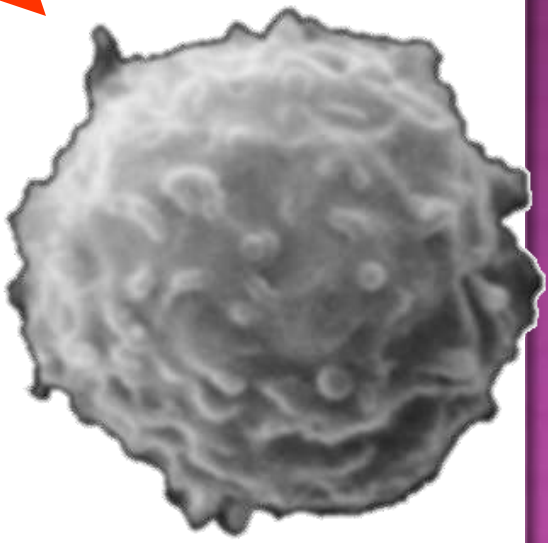
1st Exposure Of Antigen

A. IGE FORMATION

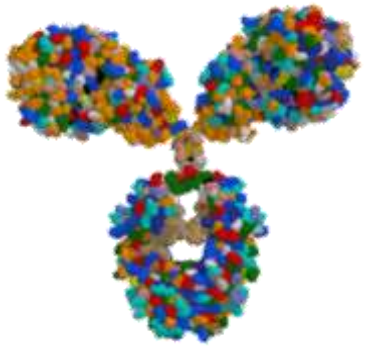
1. Antigen meets B cell



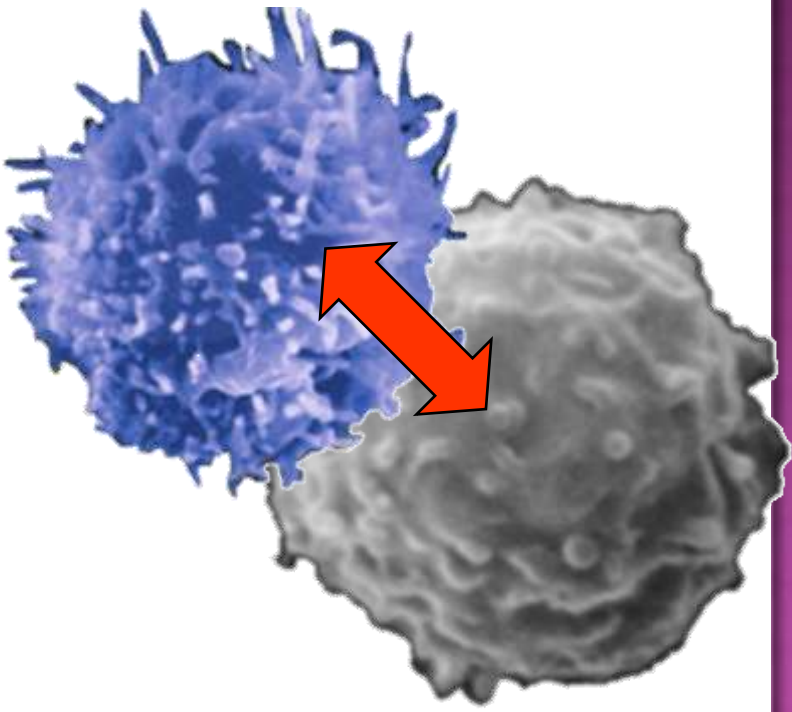
Ag



A. IGE FORMATION

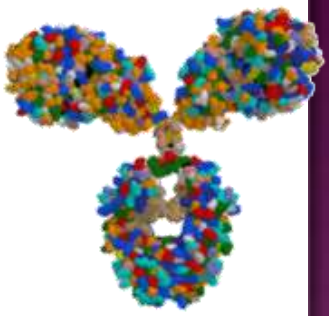


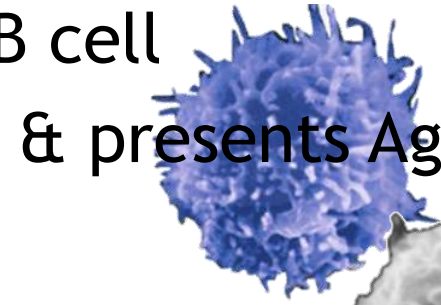
1. Antigen meets B cell
2. B cell packages & presents Ag to helper T cell

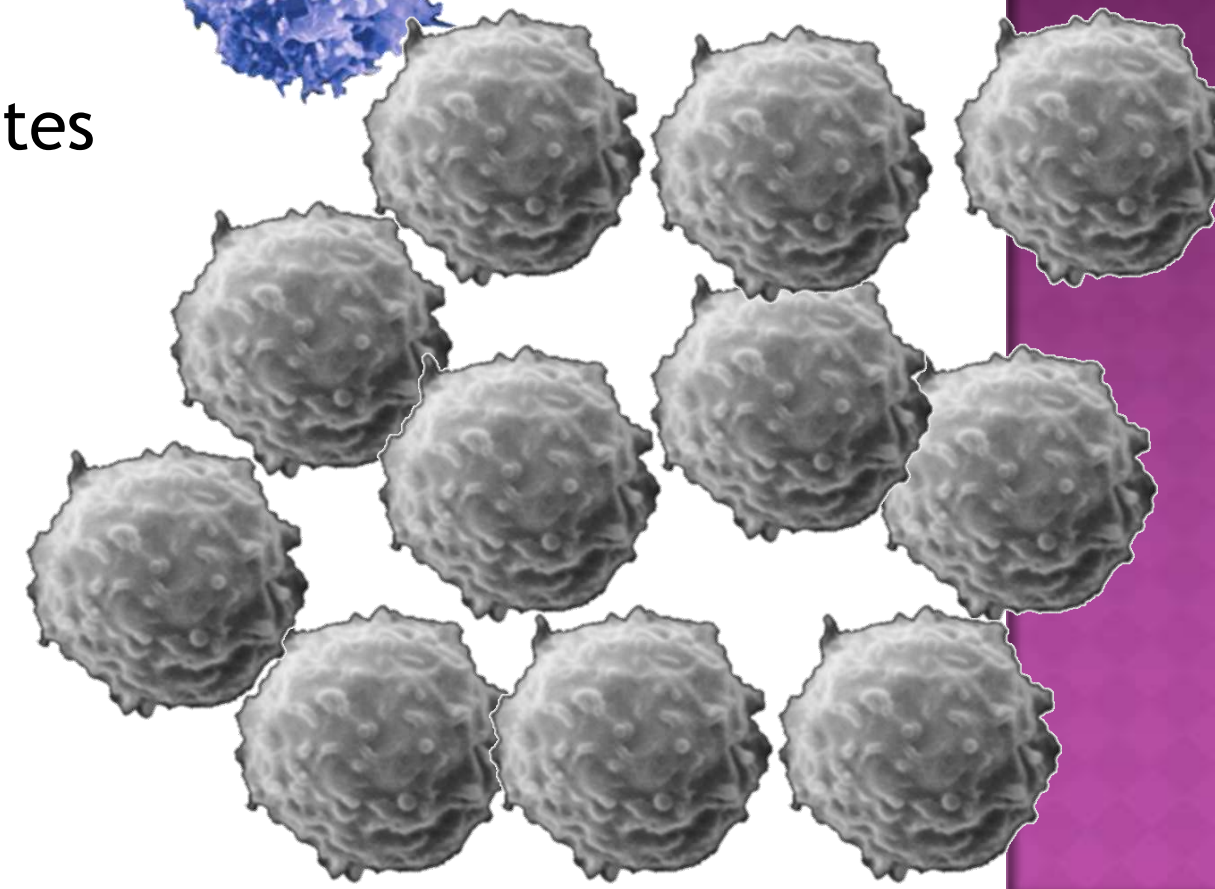


A. IGE FORMATION

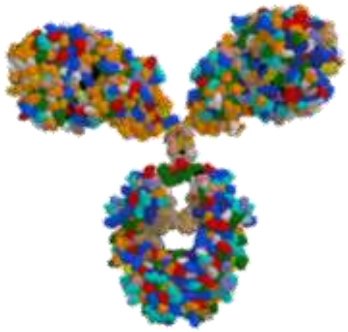
1. Antigen meets B cell
2. B cell packages & presents Ag to helper T cell
3. B cell proliferates



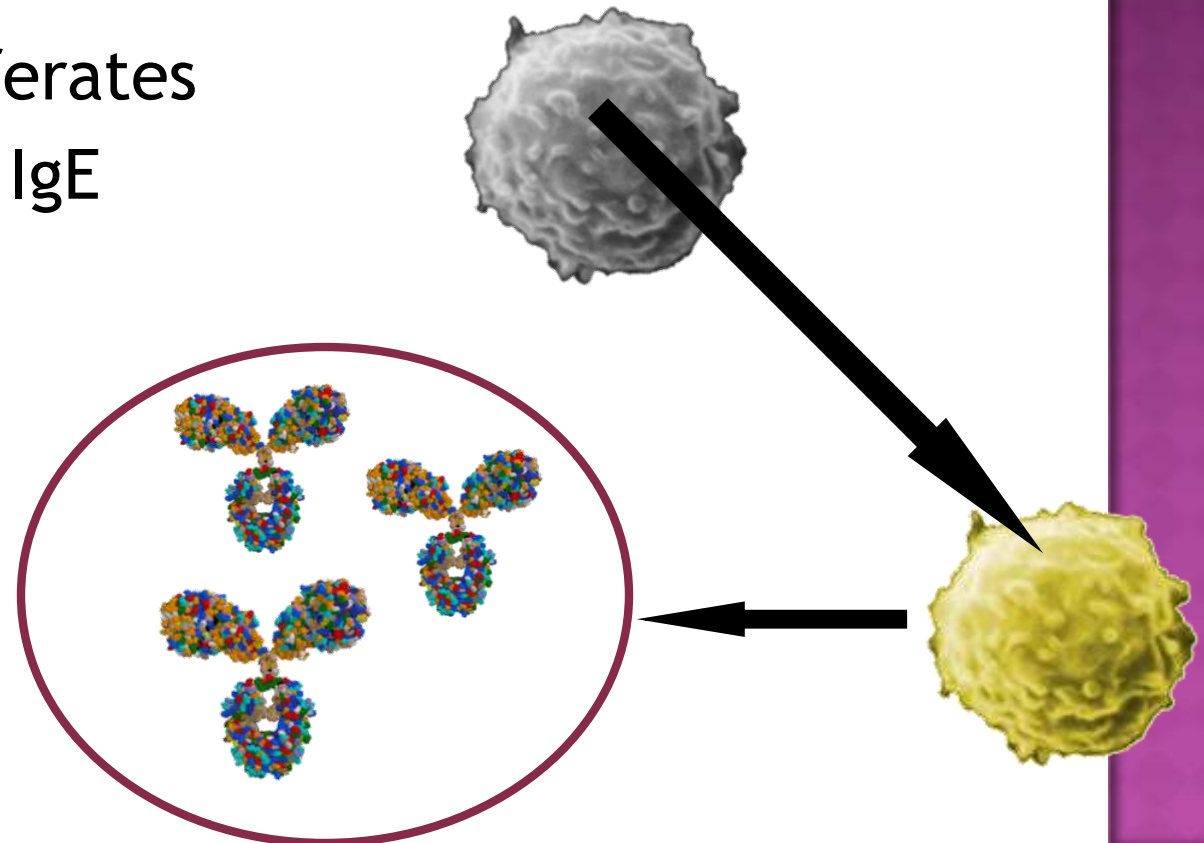




A. IGE FORMATION

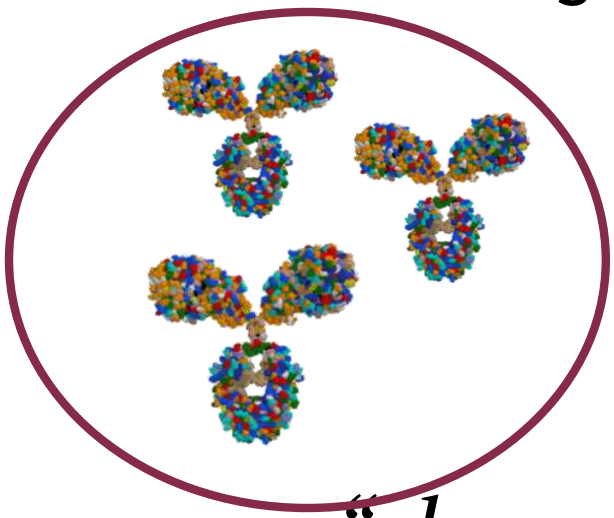


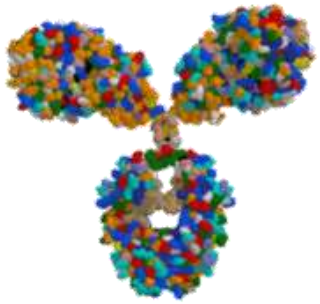
1. Antigen meets B cell
2. B cell packages & presents Ag to helper T cell
3. B cell proliferates
4. A *switch* to IgE



A. IGE PRODUCTION

1. Antigen meets B cell
2. B cell packages & presents Ag to helper T cell
3. B cell proliferates
4. A switch to IgE





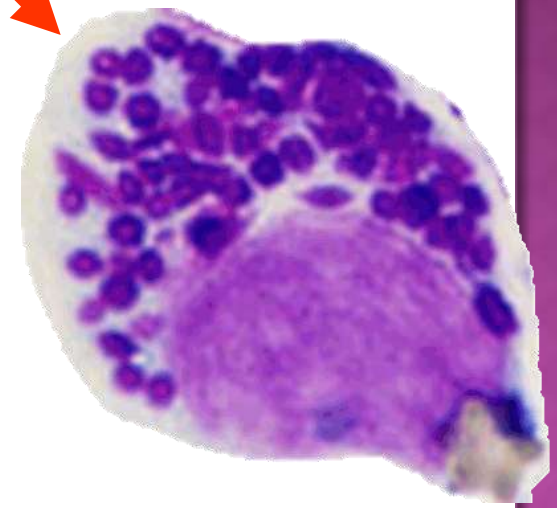
CD40L

IL4

"class switch recombination"

B. BASOPHIL & MAST CELL

IgE binds to the mast cell



HOW?

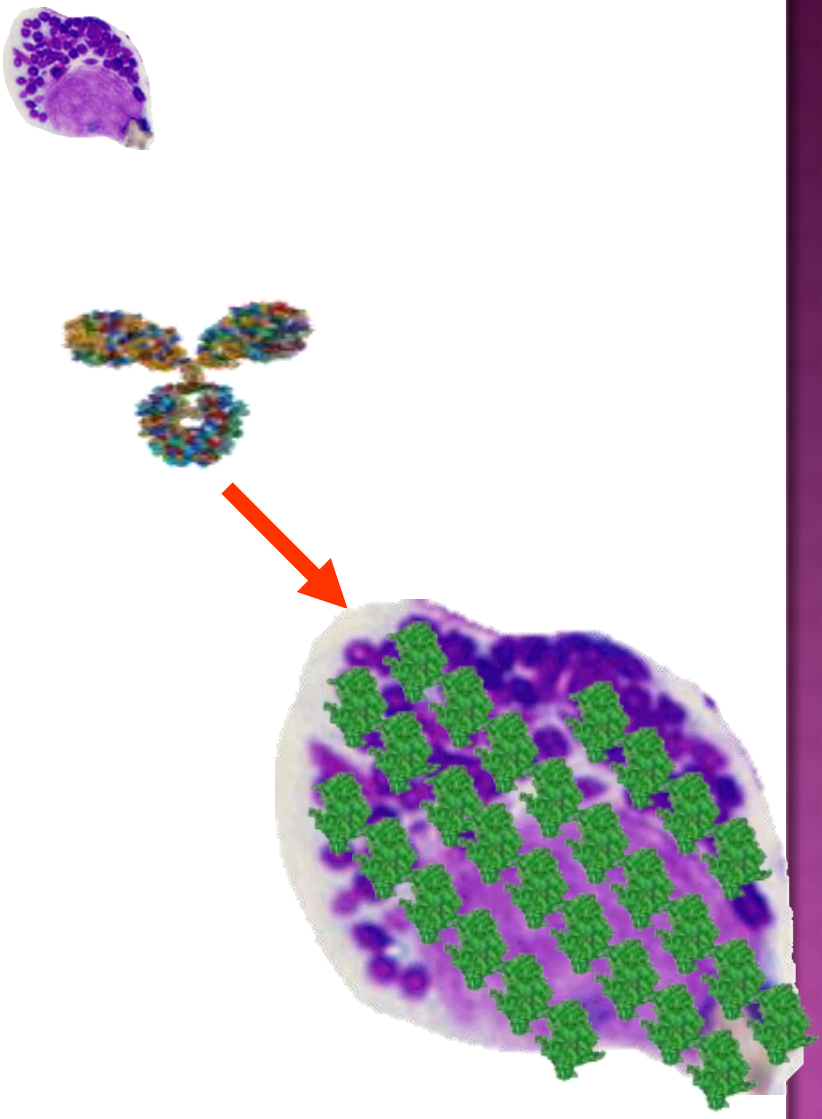
immunoCAp
specific IgE

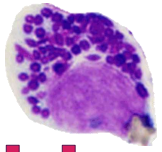
B. MAST CELL

IgE binds to the mast cell

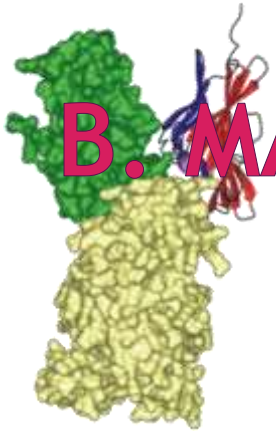
HOW?

FcεRI



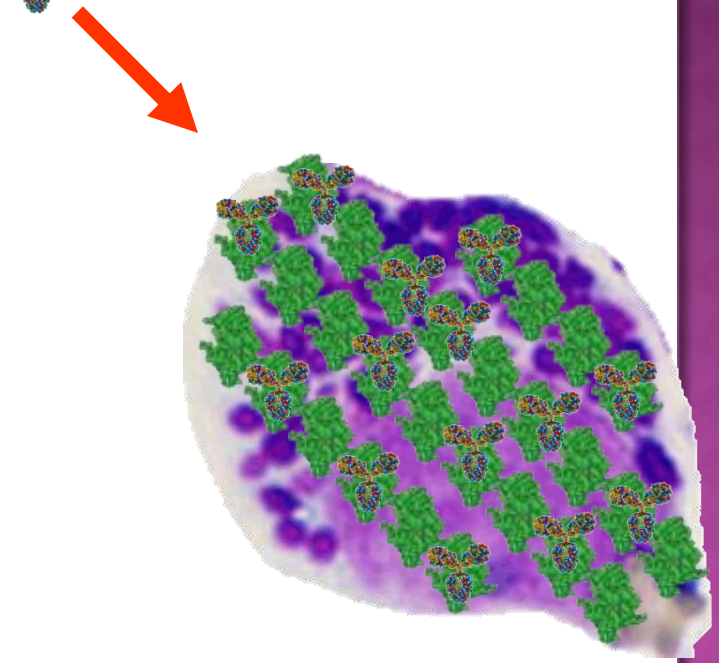


Sensitization phase

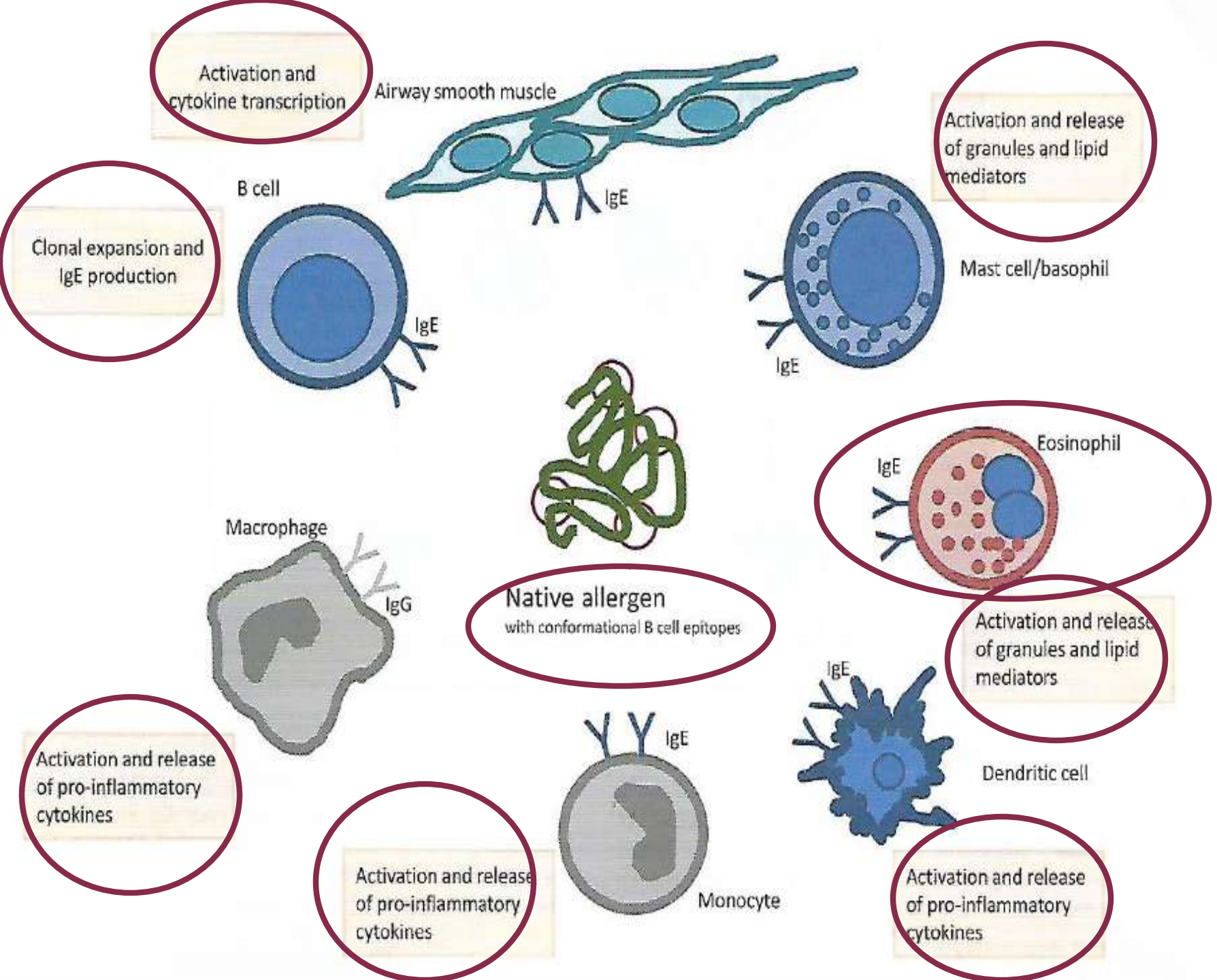


B. MAST CELL

FcεRI



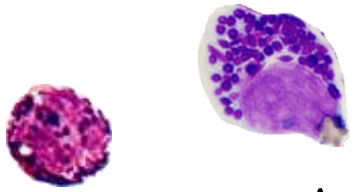
- *High* affinity
- 200 000/cell
- 10% occupancy works fully!


Activation and cytokine transcription
Airway smooth muscle
IgE
Activation and release of granules and lipid mediators
Clonal expansion and IgE production
B cell
IgE
Mast cell/basophil
IgE
Eosinophil
IgE
Macrophage
IgG
Native allergen
with conformational B cell epitopes
Activation and release of granules and lipid mediators
IgE
Activation and release of pro-inflammatory cytokines
IgE
Dendritic cell
Activation and release of pro-inflammatory cytokines
Monocyte
Activation and release of pro-inflammatory cytokines

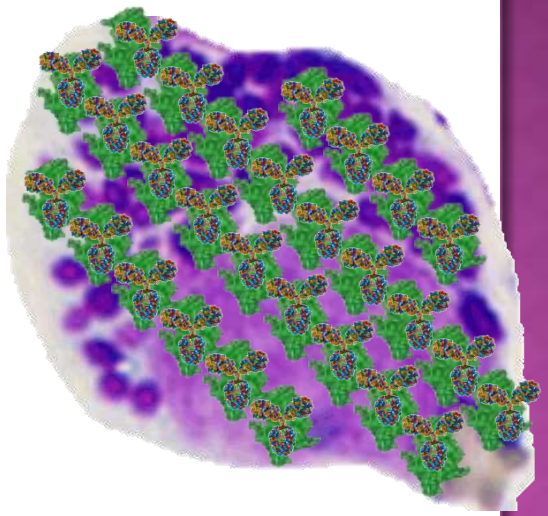
2nd Exposure Of Antigen

Effector phase

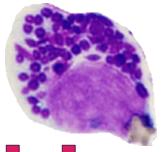
B. MAST CELL



Ag

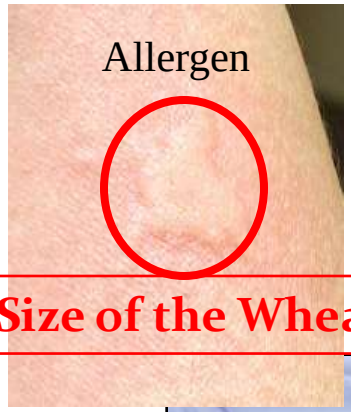


Degranulation

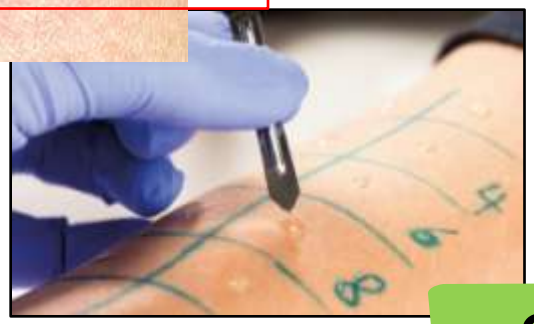


B. MAST CELL

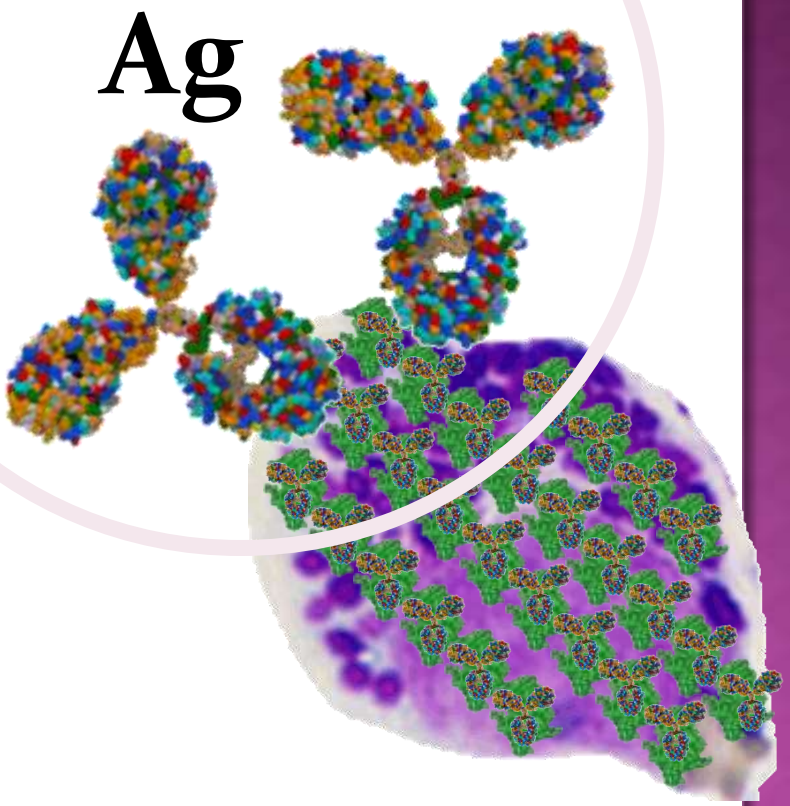
Degranulation



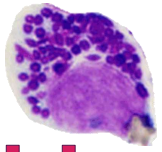
Size of the Wheal



- SPT

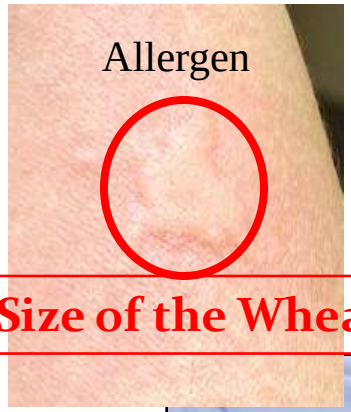


Allergy skin test (SPT/IDST)

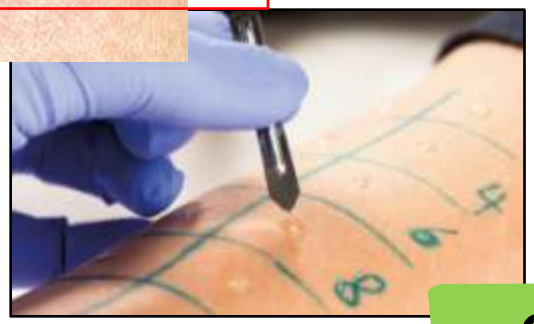


B. MAST CELL

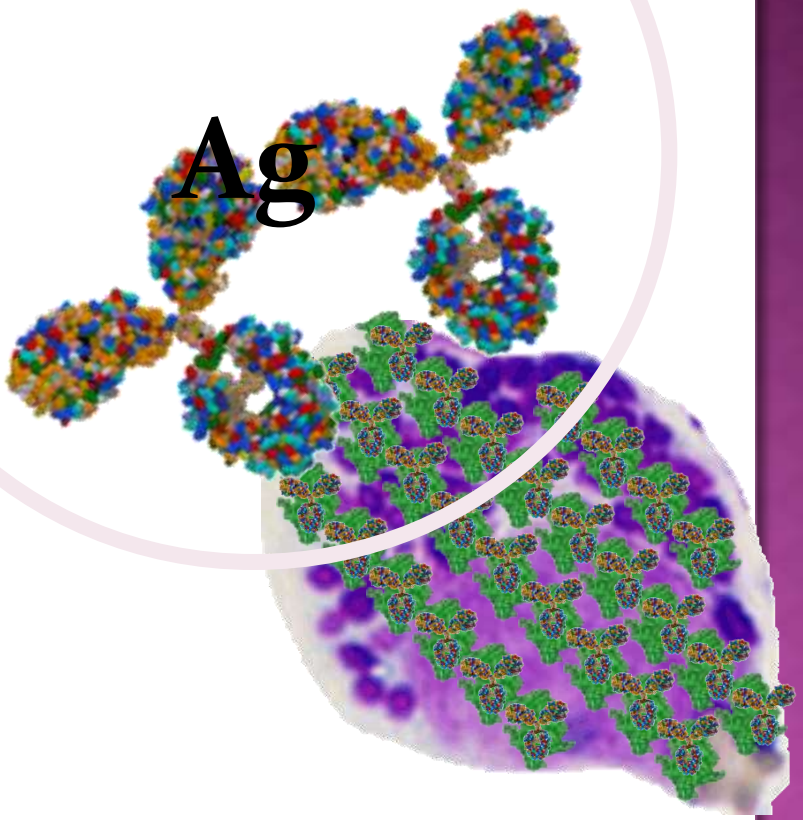
Degranulation

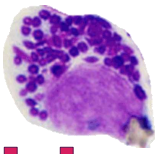


Size of the Wheal



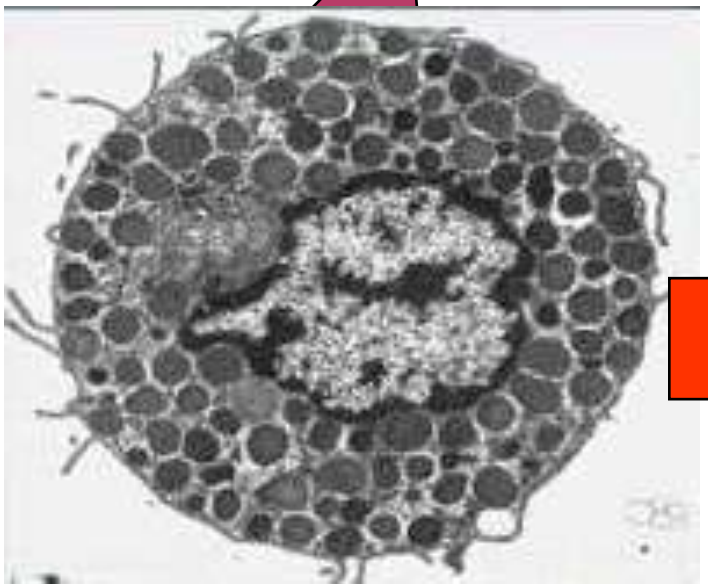
- SPT



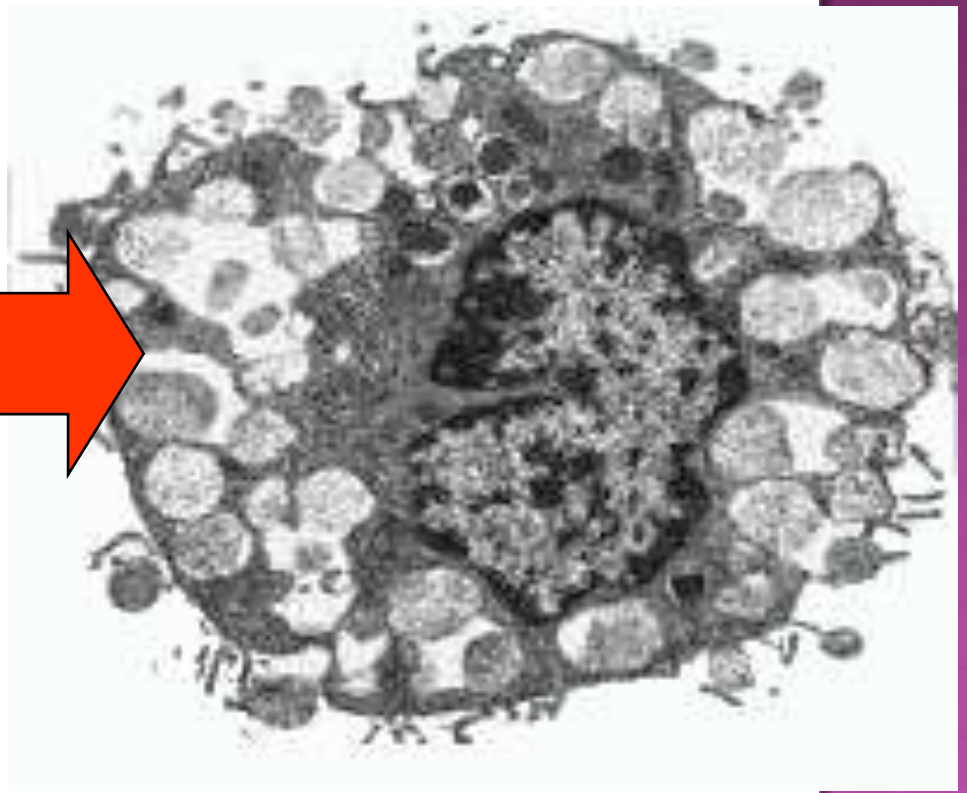


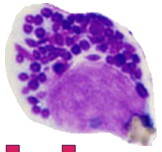
B. MAST CELL

PI3 kinase



Degranulation





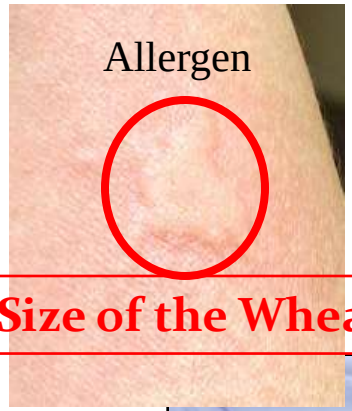
B. MAST CELL

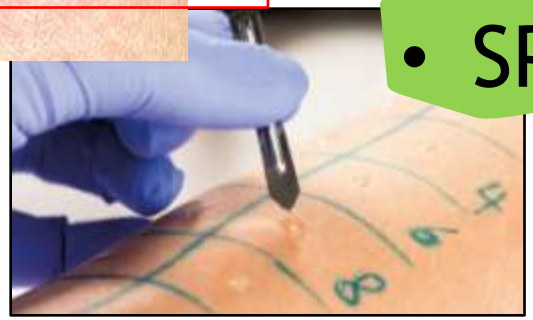
Degranulation

- *Serotonin*
- *Pg, leukotrienes*
- *Proteases*
- *Chemokines*
- *Cytokines*

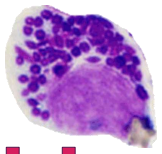
Histamine

(H1, H2, H3)





- SPT



B. MAST CELL

Target organs

ANAPHYLAXIS

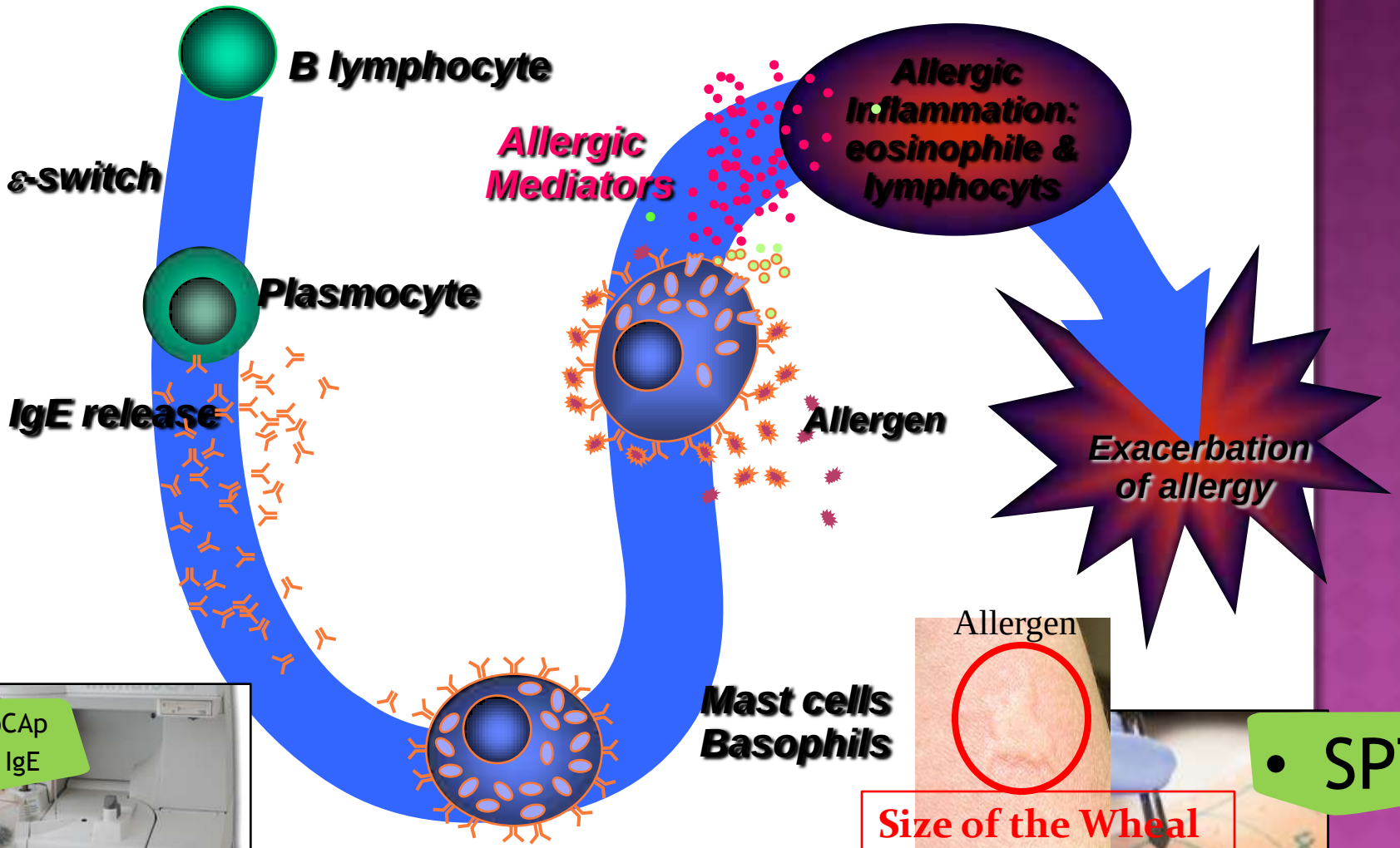
- Lungs
- Heart
- Vasculature
- Cutaneous

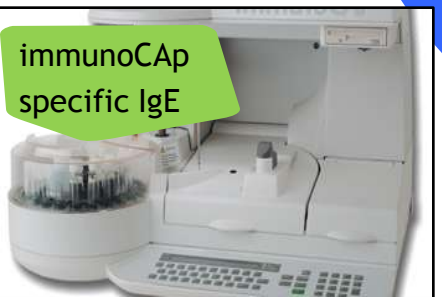
Cardiovascular collapse

Urticaria

Bronchospasm

ALLERGIC CASCADE



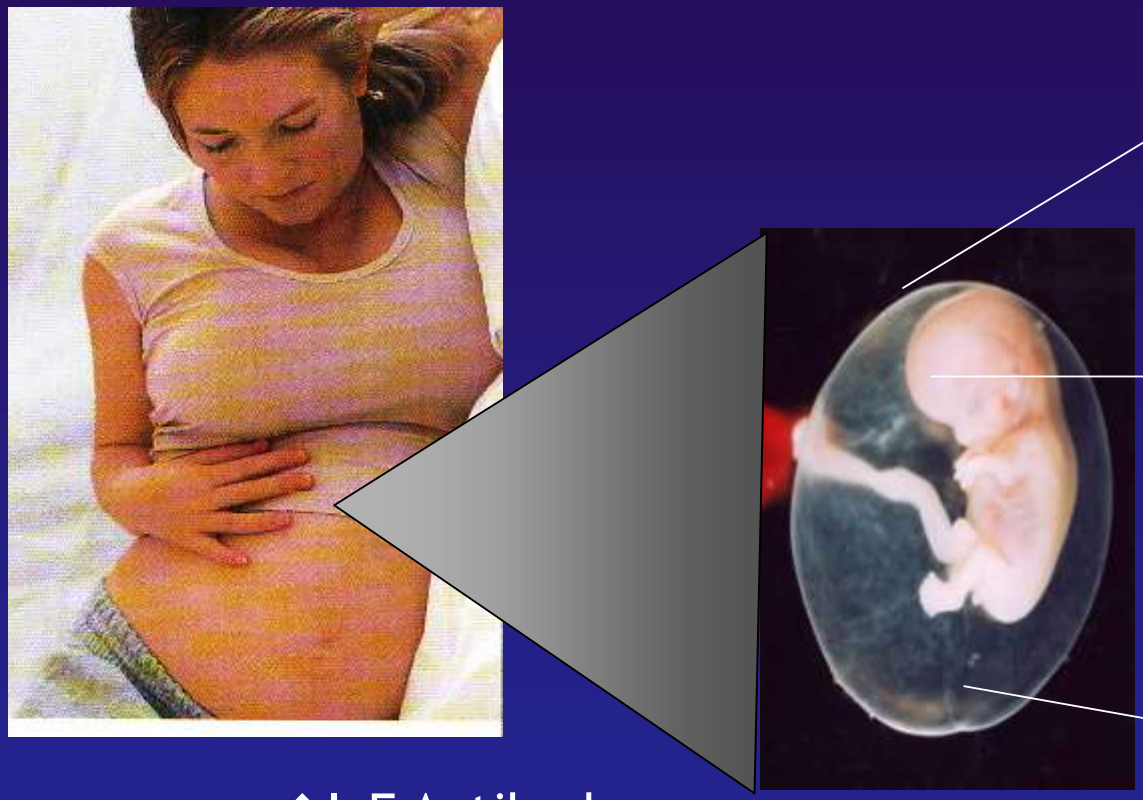


Size of the Wheal

- SPT

Why is there an Increase in Allergic Diseases?

Mother, fetus and infant interaction In the development of allergy



Allergen, IgE, Th2 promoters

Fetal Swallowing

IgE facilitated allergen uptake and sensitization

- IgE Antibody
- Does not Cross the placenta
- Produced by Fetus from 6 weeks onwards

A 'Designer' Allergic Infant

atopic parents

C/section

1st born

male

atopic siblings

mum smokes

bottle fed

early weaning ?

little contact with other young children

warm high humidity home

WHY IS THERE AN INCREASE IN ALLERGIC DISEASES?

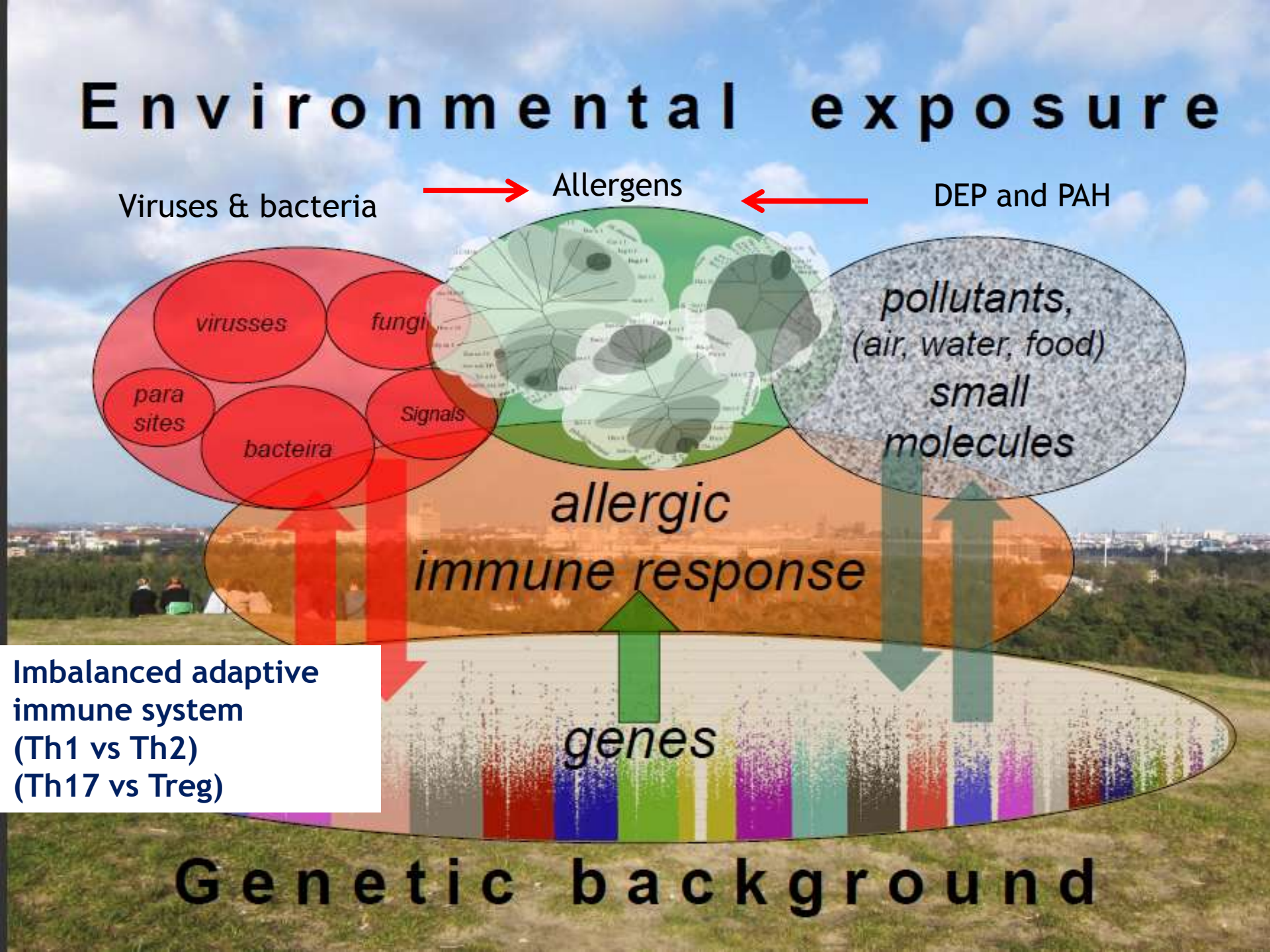
Risk factors for allergy

Genes

Environment ⟷ Lifestyle

- Hygiene hypothesis
- Air pollution
- Allergen exposure
- Indoor climate

- Diet (e.g. antioxidants)
- Vitamin D (decreased sunlight)
- Obesity and overweight
- Physical inactivity
- Alcohol

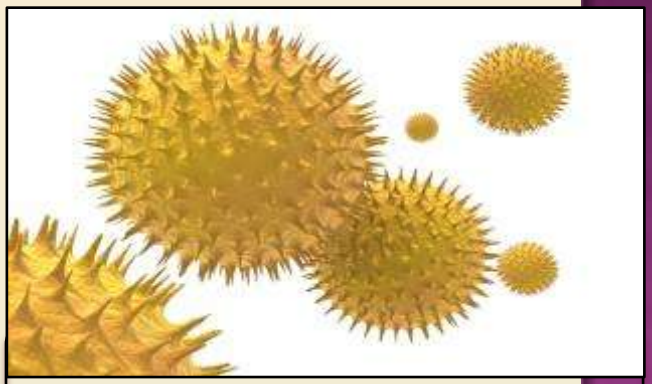


Imbalanced adaptive immune system
(Th1 vs Th2)
(Th17 vs Treg)

Respiratory Allergens
Aero-allergens Are The Most Common

- Triggers include:

Dust Mites



Pollen

Cockroach

Animal Dander

Mold

What makes Food Allergic ?

Food allergy in adults

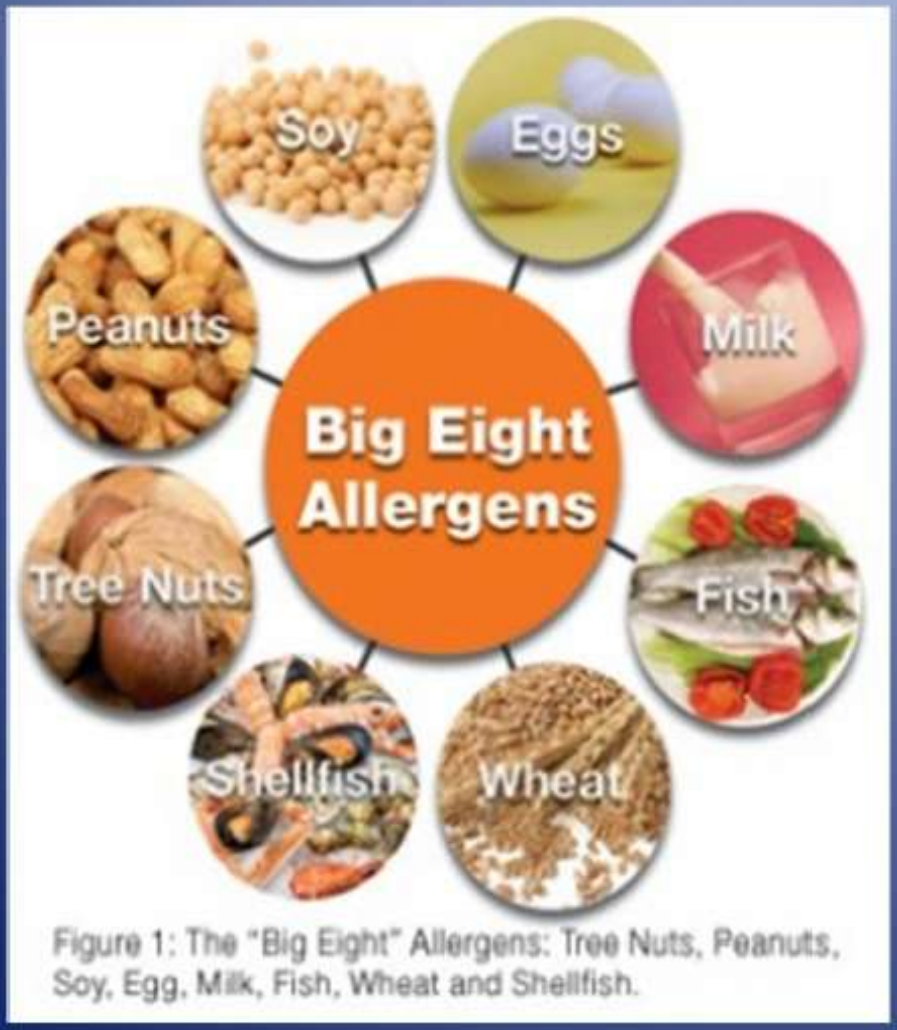


Figure 1: The "Big Eight" Allergens: Tree Nuts, Peanuts, Soy, Egg, Milk, Fish, Wheat and Shellfish.

- *Fish*
- *Shellfish*
- *Nuts/seeds*
- *Peanuts*
- *Sesame*

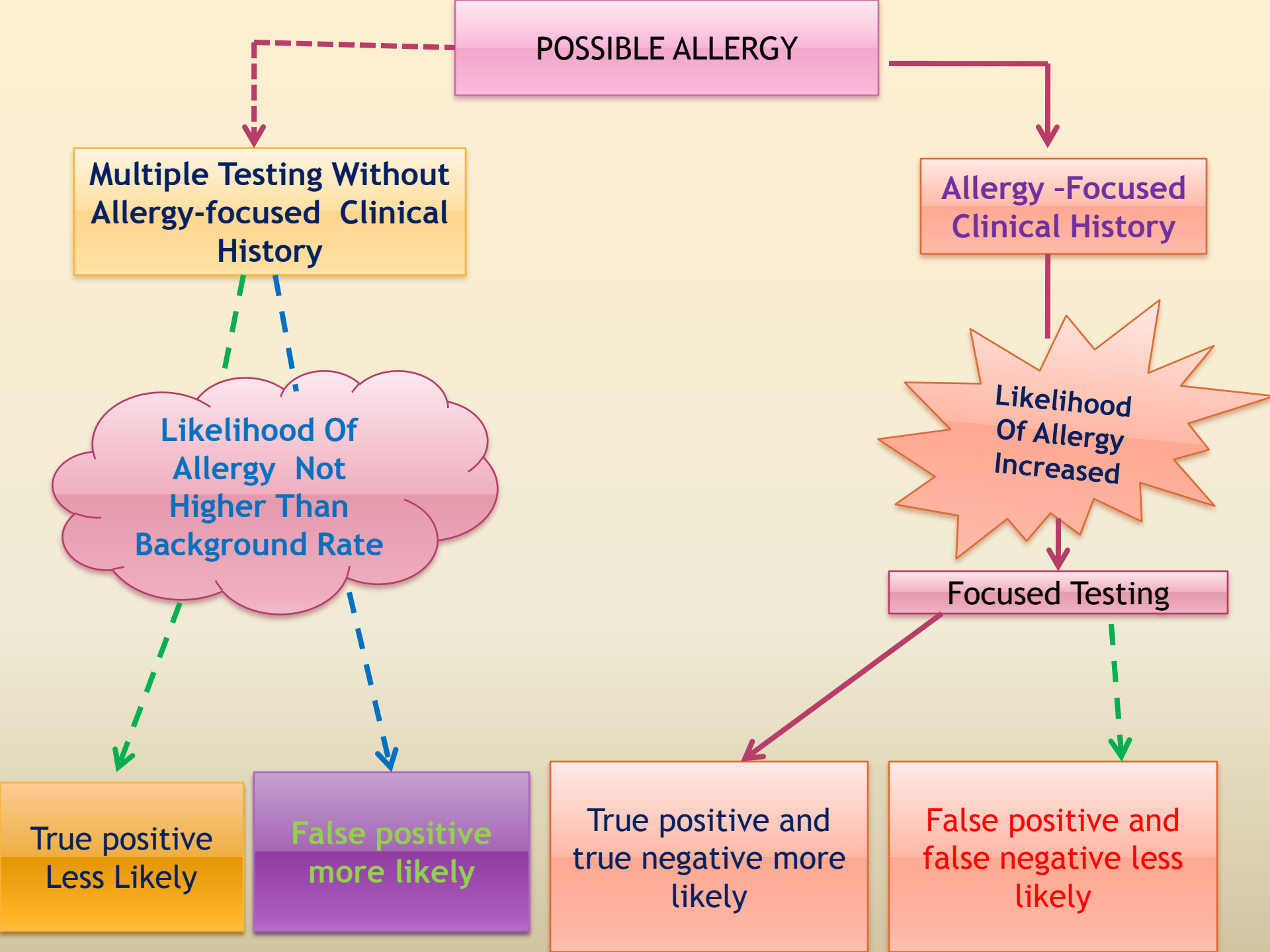
ALLERGY DIAGNOSTICS

- **The diagnosis of an allergy is based on the correlation between**

History

Sensitization tests

Provocation tests/CRD


POSSIBLE ALLERGY
Multiple Testing Without Allergy-focused Clinical History
Allergy -Focused Clinical History
Likelihood Of Allergy Not Higher Than Background Rate
Likelihood Of Allergy Increased
Focused Testing
True positive Less Likely
False positive more likely
True positive and true negative more likely
False positive and false negative less likely

ALLERGIC DISEASES IN CHILDHOOD

Skin allergy

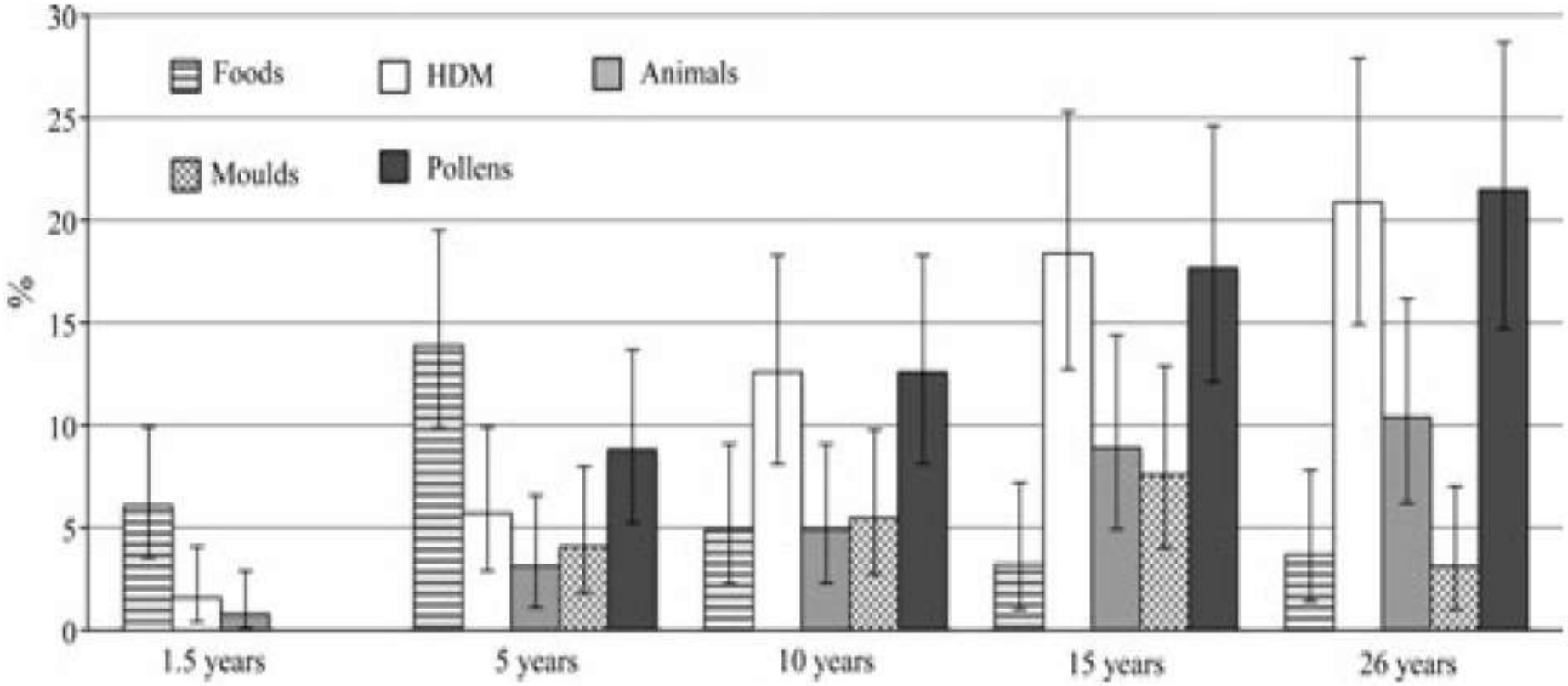
Respiratory allergy

Table 1. Allergic diseases in childhood

Age	Diagnosis	Prevalence (%)	IgE sensitization (%)
Early childhood	Food allergy	7–8	40–60
School age	Food allergy	1–2	60–70
Childhood	Atopic dermatitis	15–20	33–40
Early childhood	Recurrent wheeze/asthma	21–34	30–60
School age	Asthma	7–10	70–90
Childhood	Rhinitis and conjunctivitis	10–15	60–80

Eigenmann PA. Allergy 2005: 60 (Suppl. 79): 6–9

SENSITIZATIONS ACCORDING TO AGE



- Food sensitization reduces with increasing age
- Aeroallergen sensitizations increase with increasing age

Nissen SP et al. Pediatr Allergy Immunol 2013: 24: 549–555.

Age, Common Allergies & Common Allergens

< 5 years

Common Allergies:
- Eczema
- Food

Common Allergens:
- Food
- HDM

5-10 years

Common Allergies:
- Asthma & Rhinitis
- Food
- Eczema

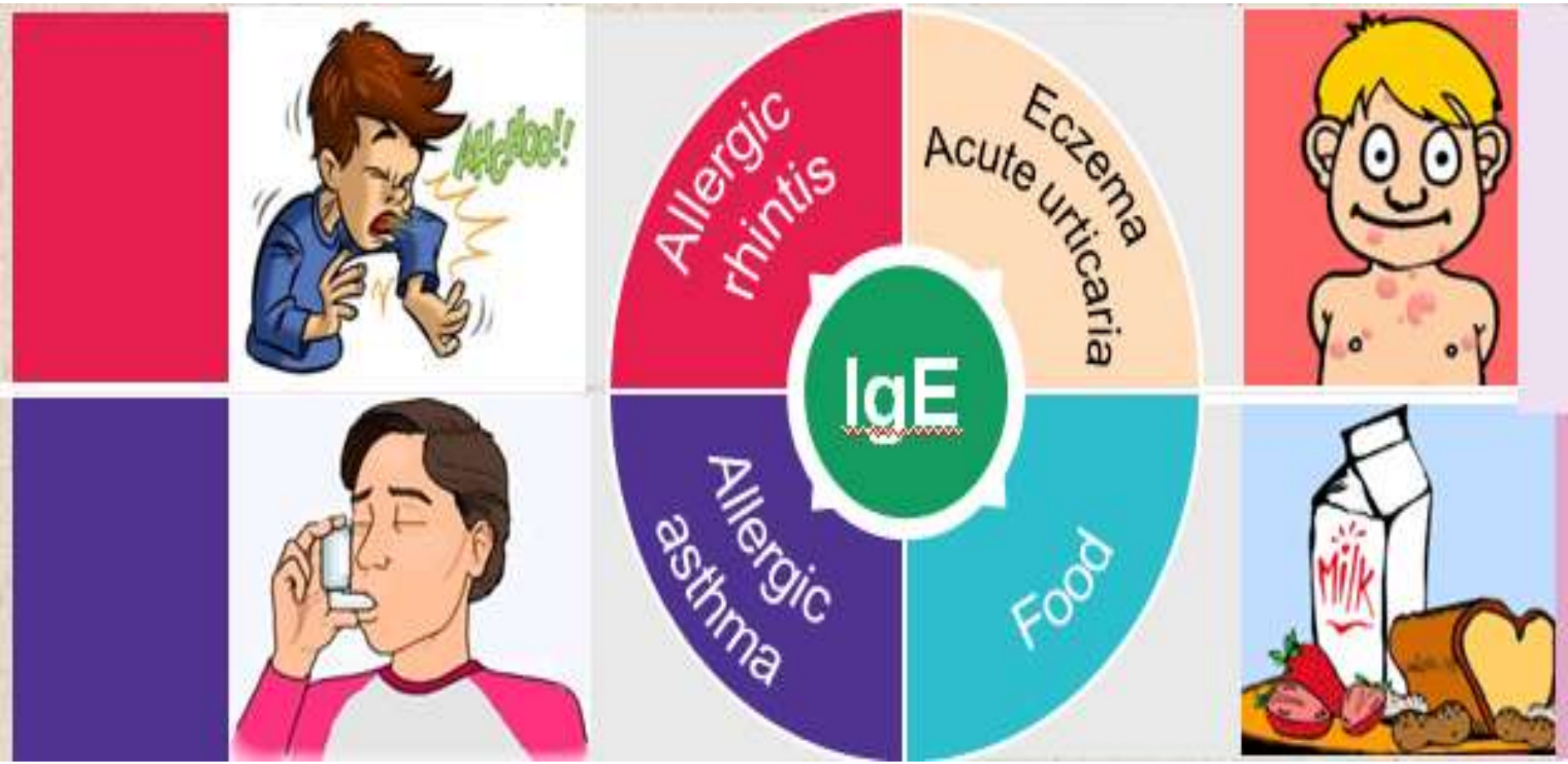
Common Allergens:
- Food
- HDM
- Pollen

>10 years

Common Allergies:
- Asthma & Rhinitis

Common Allergens:
- Aeroallergens (all aero-allergy)
- Food

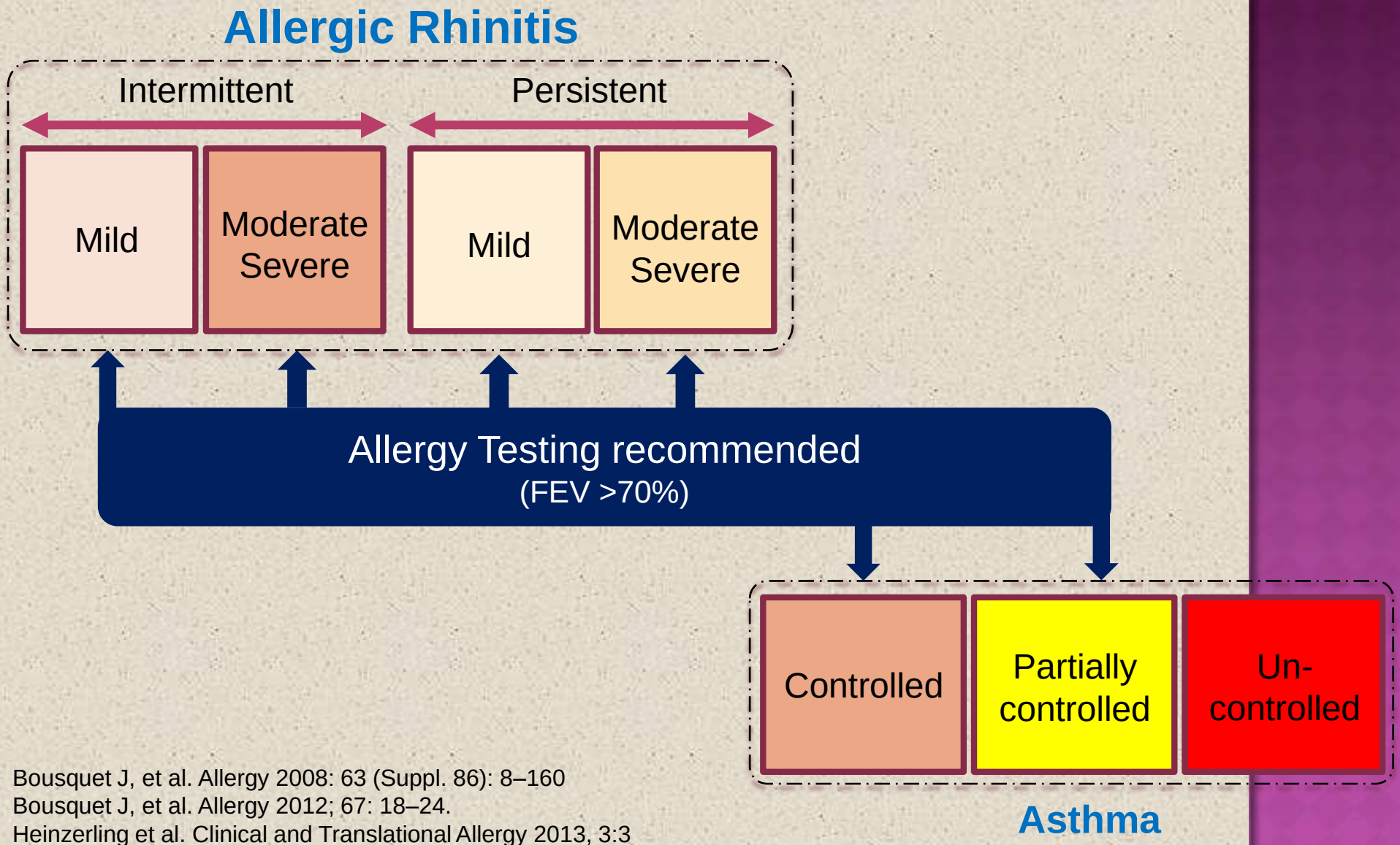
Common IgE mediated allergic disease



-Drug allergy
-Insect allergy
-Letex allergy

-Food allergy
-Anaphylaxis
-Angioedema

Indications For Allergy Diagnostic Tests In Allergic Rhinitis & Asthma



Bousquet J, et al. Allergy 2008: 63 (Suppl. 86): 8–160
Bousquet J, et al. Allergy 2012; 67: 18–24.
Heinzerling et al. Clinical and Translational Allergy 2013, 3:3

WHOM TO TEST? RHINITIS

Recurrent

Rhinitis

Seasonal rhinitis/conjunctivitis should be tested in treatment-resistant cases

Perennial rhinitis/conjunctivitis should be tested in all cases

Eigenmann et al. Pediatric Allergy and Immunology 2013; 24:195–209

WHOM TO TEST? ALLERGIC ASTHMA

Asthma

All children with recurrent (>3 times) wheeze not triggered by upper airway infections, chronic wheeze or possible asthma diagnosis should be tested for IgE sensitizations.

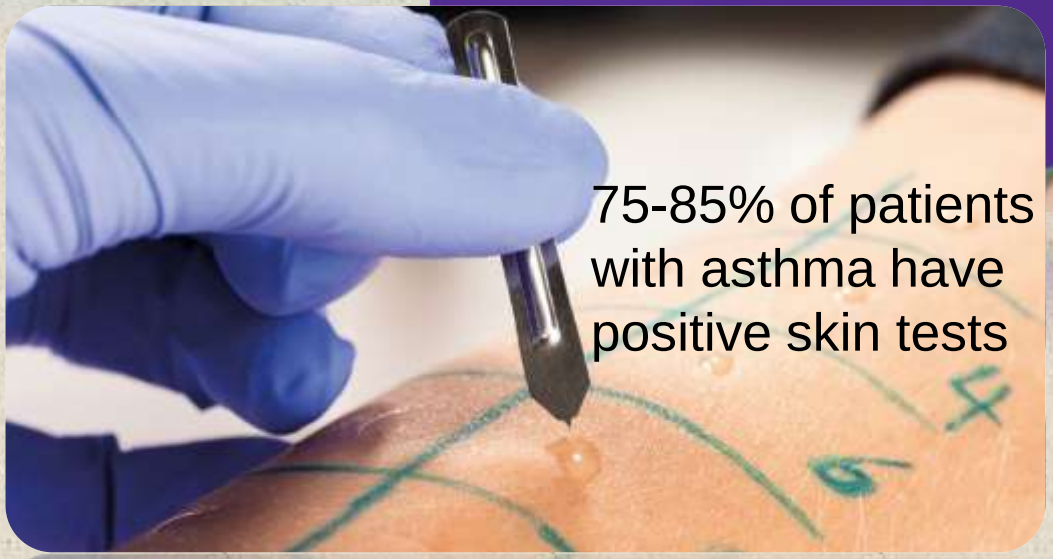
Testing becomes increasingly desirable with increasing age, positive family history, as well as the presence of additional allergic symptoms

Eigenmann et al. Pediatric Allergy and Immunology 2013; 24:195–209

WHY ALLERGIES ASTHMA?

Allergens trigger asthma attacks in 60-90% of children

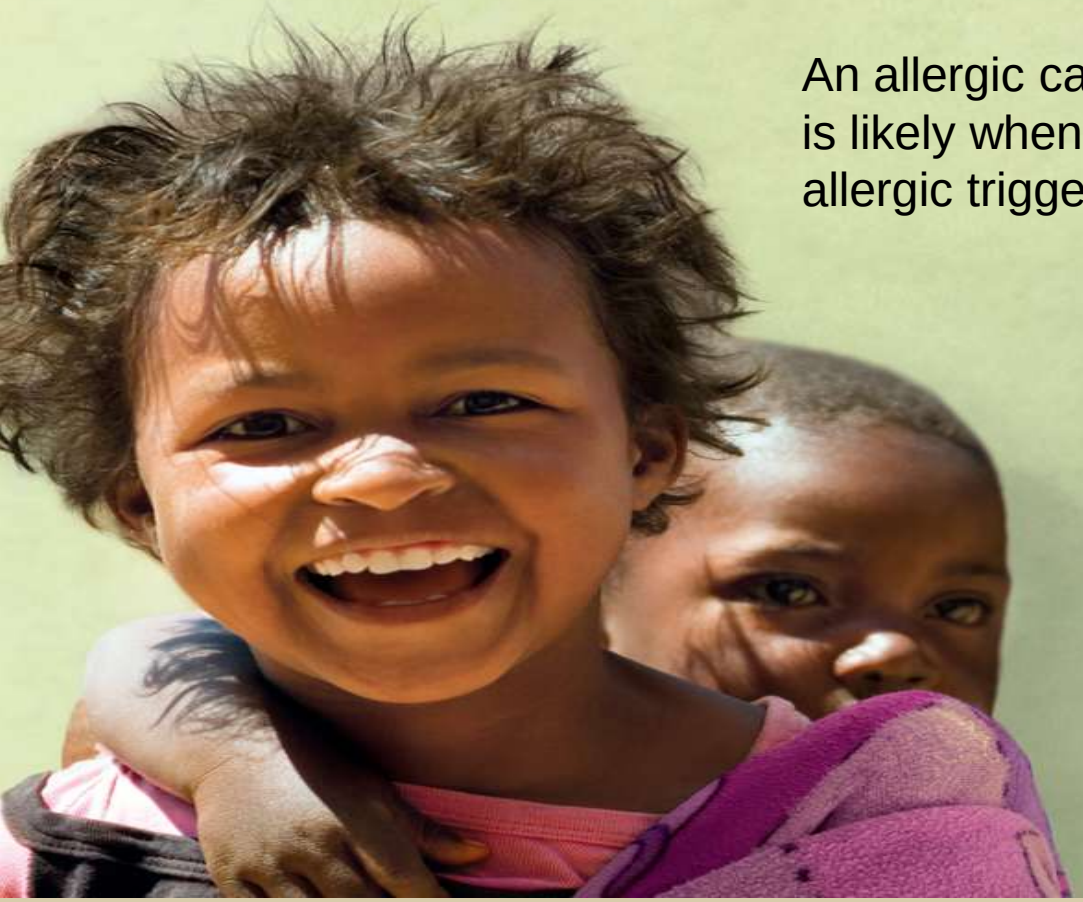
Allergens trigger asthma attacks in 50% of adults



75-85% of patients with asthma have positive skin tests

Do et al. Allergy 2015

WHOM TO TEST? URTICARIA & ECZEMA



Acute Urticaria

An allergic cause for acute urticaria or angioedema is likely when they occur within 2 h of a potential allergic trigger and the symptoms last for <24 h

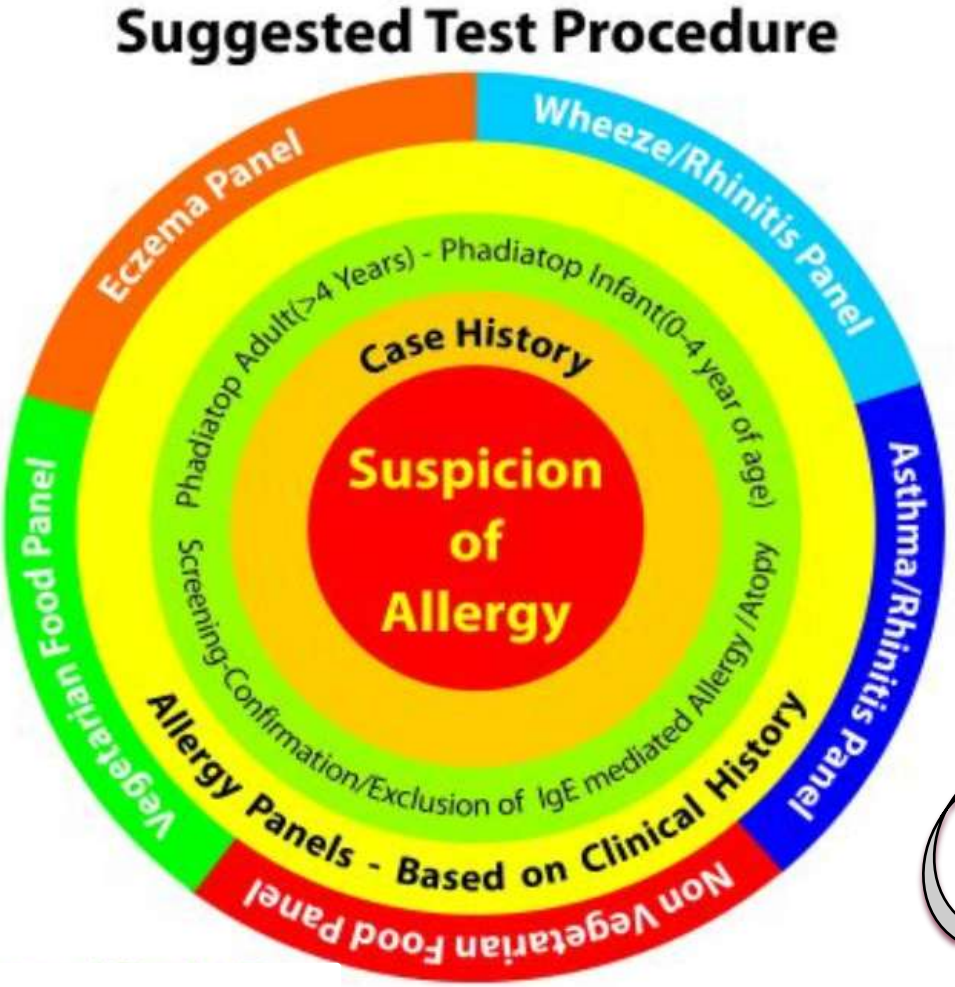
Atopic Eczema

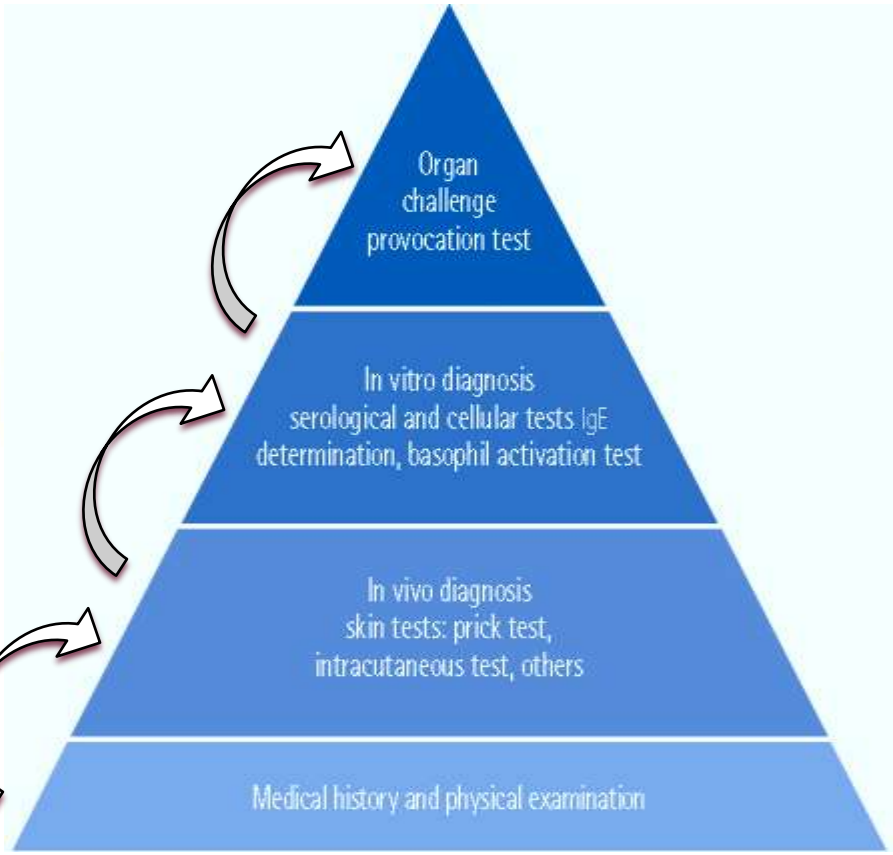
Allergy diagnosis will aim to identify potential allergic triggers for eczema flares in infants and children at high risk, for example, infants and children with moderate to severe, persistent eczema

Eigenmann et al. Pediatric Allergy and Immunology 2013; 24:195–209

DIAGNOSTIC PYRAMID

"weather Prick/puncture tests or specific IgE are the preferred techniques for diagnosing IgE-mediated hypersensitivity"





Sohrab S, et al. Allergo J 2013;22(2):128-34.
Bernstein IL, et al. Ann Allergy Asthma Immunol. 2008 Mar;100(3 Suppl 3):S1-148.

Historical Context of Diagnosis Methods of Human Allergic Disease

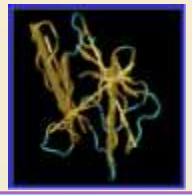
Provocation testing

Characterisation of IgE

RAST*

First allergens cloned

Diagnostic recombinant allergen panels



Handbook of Molecular Allergology (EAACI)

1880 — 1967 — 1988-91 — 1995-1999 — 2015

In-vivo **testing with Allergen Extracts**

In-vitro **testing with allergen extracts**

Molecular Allergen-specific IgE

Research → Application

Allergy Diagnosis

(IgE- Mediated Allergic Diseases

Non - IgE- Mediated Allergic Diseases

- **Test for Immediate Hypersensitivity**
- Skin Prick Tests
- Intradermal Skin Tests
  - Prick-Prick Test
- Serum Specific- IgE (Free IgE)
  - Immuno-CAP (RAST)

Cell bound (IgE)

Test for Delayed Hypersensitivity

- Patch Tests
  - Contact Dermatitis
  - Delayed food reactions
  - Drug reactions

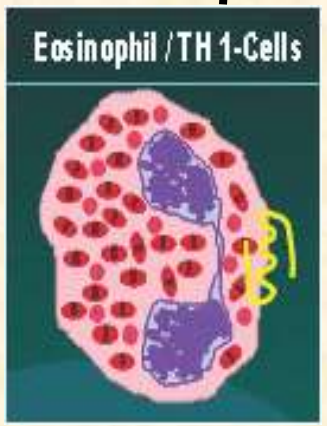
Provocation Tests

- Oral Challenge
- DBPCFC
- Nasal & Bronchial Challenges

Component Resolved Diagnosis (CRD)
BASOPHIL Activation Test (BAT)

Cell bound (IgE)

Th2 (IL-4 /5/13)
(IgE- Mediated Allergic Diseases



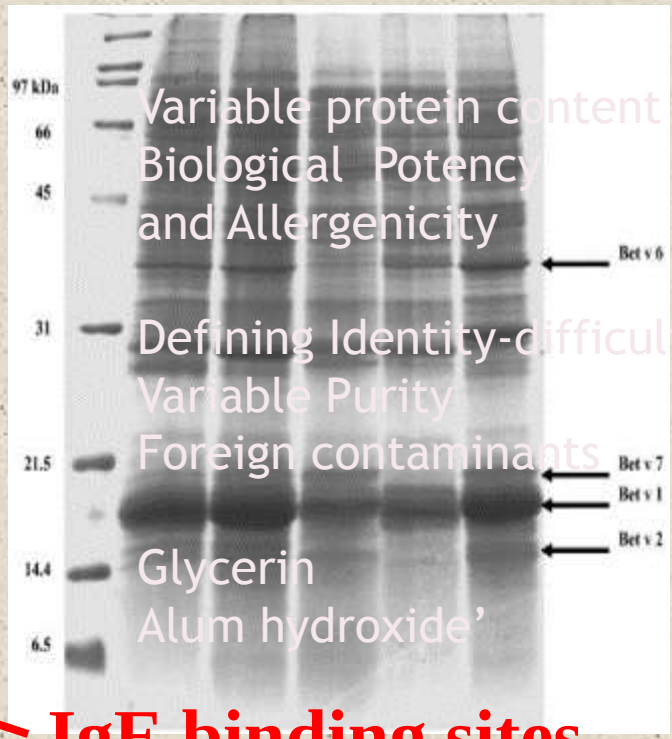
Th1 (CTL)
(Non - IgE- Mediated Allergic Diseases

Crude Extract Preparation (*Betula verrucosa*)

Collect Pollen

Isolate Pollen

Perform Aqueous Extraction Defat and Analyze

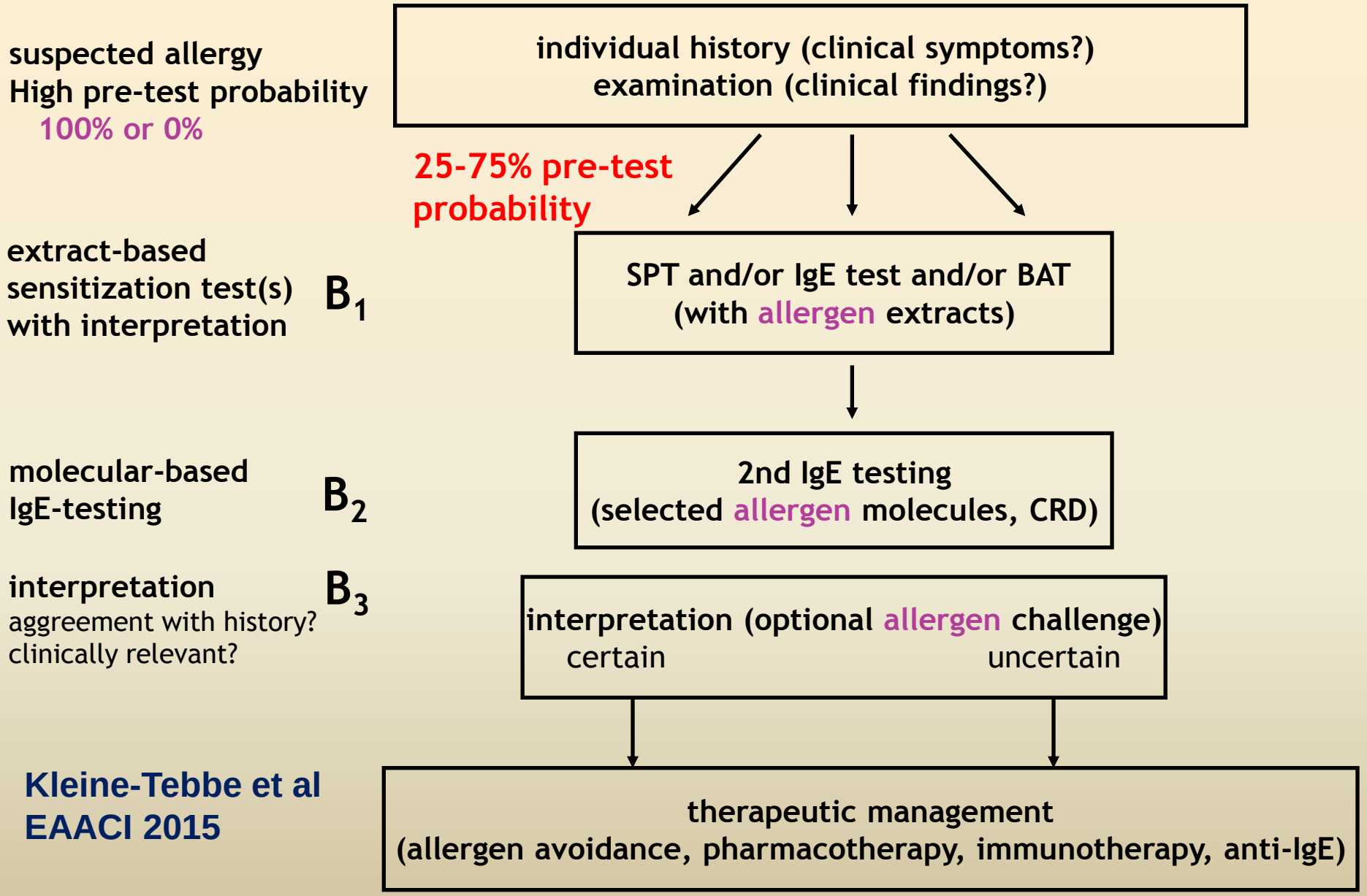


cDNA clone Isolated from pollen expression library Breiteneder et al. EMBO J, 1989

Bet v 1 17 kDa

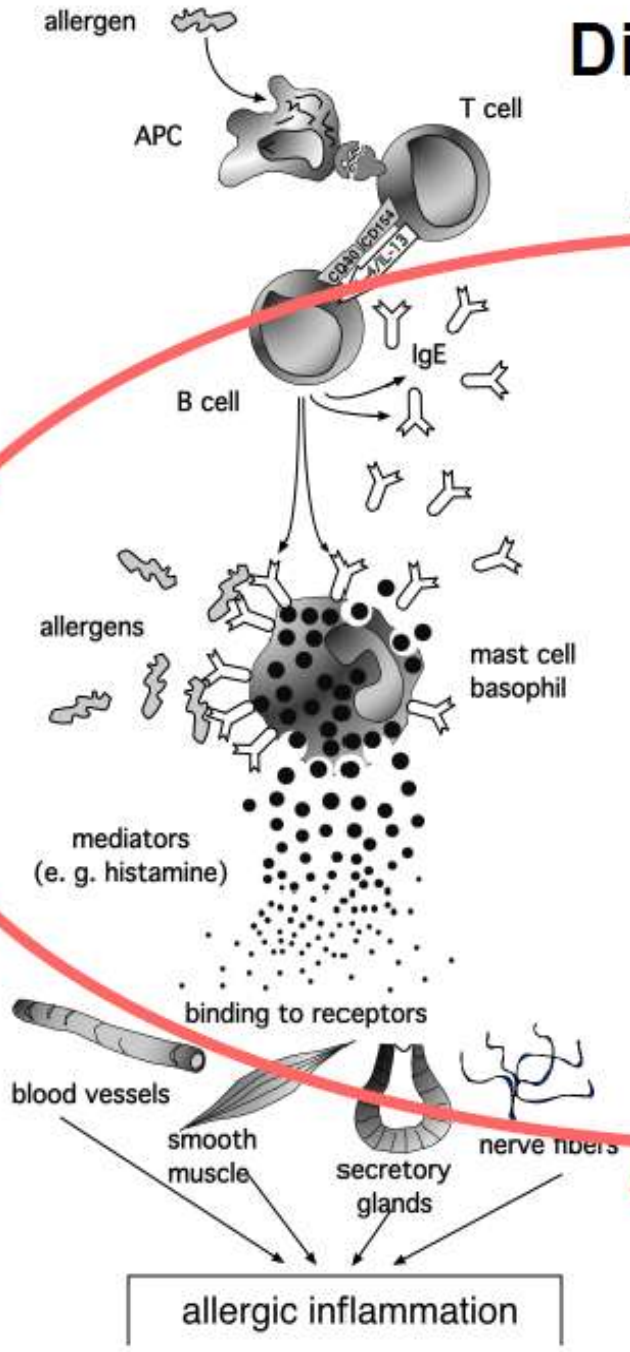
IgE binding sites

Diagnostic Algorithm for Human Allergic Disease



Kleine-Tebbe et al
EAACI 2015

Diagnostic repertoire to work up allergic diseases



T cell function

lymphocyte stimulation test

assays for IgE

allergenspecific IgE
total IgE

cellular
ex vivo tests

histamine release
leukotriene production
basophil activation tests
(BAT: CD63, CD203c)

in vivo
challenge tests

skin, nose, bronchi

directly

indicate IgE
(=sensitization)

indirectly

"Sensitization Tests"

DIAGNOSTIC TESTS: MULTITUDE OF TESTS

In-vivo	*In-vitro*
Skin prick test	Blood test - total IgE lower diagnostic and clinical value
Intradermal test anaphylaxis/lower sensitivity and specificity	Blood test - serum specific IgE
Patch test only for contact dermatitis	What do we choose?
Challenge test only for research or uncertain diagnosis	

Bousquet J, et al. Allergy 2012; 67: 18–24. Bernstein IL, et al. Ann Allergy Asthma Immunol. 2008 Mar;100(3 Suppl 3):S1-148. ASCIA Skin Prick Test Manual. 2013.

SPT Is The Gold Standard For Allergy Diagnosis

Skin Prick Test (SPT) Is The First Choice in respiratory & food allergy

World Allergy Organisation 2011

Skin tests are the most accurate diagnostic tool for demonstrating that a specific allergen has induced an IgE Ab response and are regarded as the **gold standard** for detection of IgE Abs

Global Allergy and Asthma European Network (GA2LEN); Allergic Rhinitis and its Impact on Asthma (ARIA)
EAACI Position Paper 2012

Skin tests represent the first diagnostic method in patients with a suggestive clinical history of allergic rhinitis (conjunctivitis) and/or asthma

American Academy of Allergy, Asthma and Immunology (AAAAI) &
American College of Allergy, Asthma and Immunology (ACAAI) - 2008

Prick/puncture tests are the preferred techniques for IgE-mediated hypersensitivity

Bousquet J, et al. Allergy 2012; 67: 18–24.
Bernstein IL, et al. Ann Allergy Asthma Immunol. 2008 Mar;100(3 Suppl 3):S1-148.
WAO. White Book of Allergy. http://www.worldallergy.org/

GINA GUIDELINES 2016

SPT

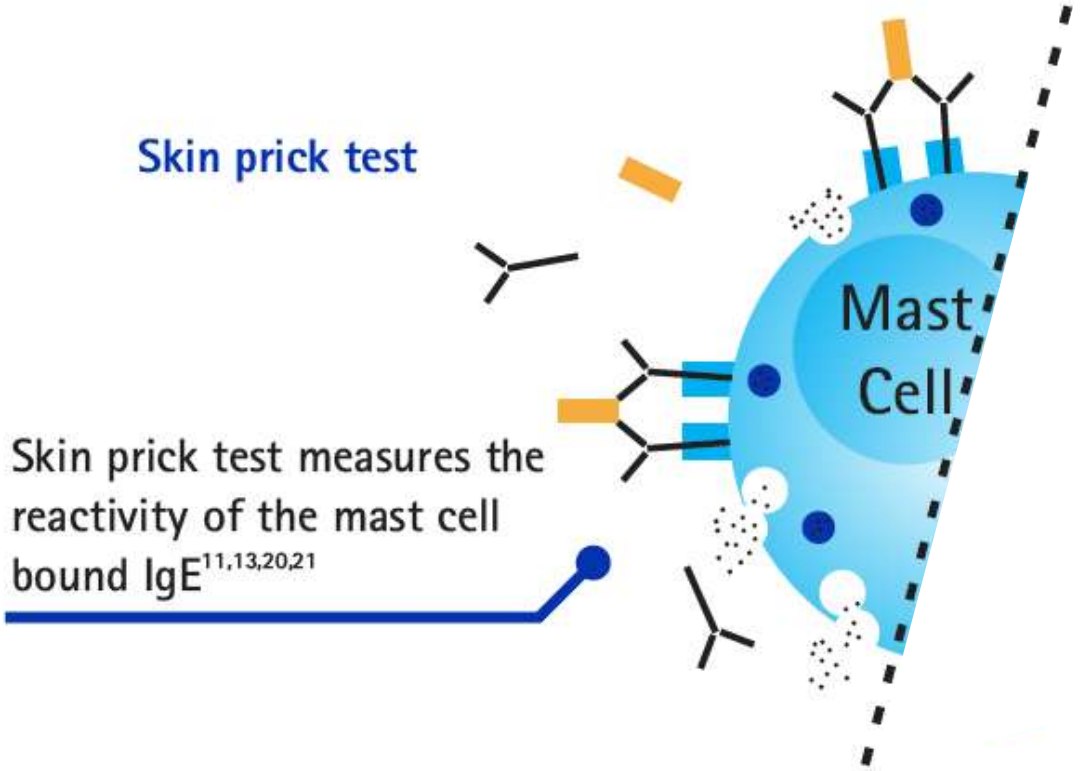
“Skin prick testing with common environmental allergens is simple and rapid to perform and, when performed by an experienced tester with standardized extracts, is inexpensive and has a high sensitivity.”

- “Measurement of sIgE is no more reliable than skin tests and is more expensive, but may be preferred for uncooperative patients, those with widespread skin disease, or if the history suggests a risk of anaphylaxis.”

Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2016. Available from: www.ginasthma.org

Skin Prick Testing Has Better Sensitivity Than IgE Testing

The Sensitivity Of Blood Specific IgE Allergy Testing Is 25 To 30% Lower Then That Of Skin Testing



Disagreement between skin prick test and specific IgE in young children

A.-M. M. Schoos, B. L. K. Chawes, N. V. Følsgaard, N. Samandari, K. Bønnelykke & H. Bisgaard

SPT and sIgE (ImmunoCAP) levels were assessed simultaneously for 16 common inhalant and food allergens at **age ½, 1½, 4, and 6 years** in 389 children from the Copenhagen Prospective Study on Asthma in Childhood$_{2000}$ (COPSAC2000) at-risk birth cohort

	Differences Between Positive Predictive Value of Skin Prick Test & ImmunoCAP (Fovouring SPT)
Inhalant allergens	**15.6%**
Food allergens	**54.5%**

SPT results were better co-related to symptoms than ImmunoCAP for:
- inhalant allergen but not food allergens

SPT[1]	Serum sIgE
SPT is highly specific and sensitive • Specificity: 70 to 95% • Sensitivity: 80 to 97%	• Specificity[2]: 30-95% • Sensitivity[3]: average 60-90% • False positive is more than 50%

Positive Rate Of SPT & Immunocap-IgE- Healthy Controls

	Normal Control (n = 40)	
	SPT	ImmunoCAP
Dermatophagoides pteronyssinus	0 (0%)	2 (5%)
Dermatophagoides farinae	0 (0%)	5 (12.5%)
Cat	0 (0%)	0 (0%)
Dog	0 (0%)	0 (0%)
Cockroach	0 (0%)	5 (12.5%)
Birch	0 (0%)	2 (5%)
Oak	0 (0%)	2 (5%)
Ragweed	0 (0%)	3 (7.5%)
Mugwort	0 (0%)	2 (5%)
Alternaria	0 (0%)	0 (0%)

SPT = skin-prick test.

Allergy serum assays are also known to give occasional **false positive results** because of nonspecific binding of antibodies used in the assays.

Cho JH, et al. Am J Rhinol Allergy 2014;28:388–391

CLINICAL DIAGNOSTIC SENSITIVITIES OF TESTS FOR ALLERGY DIAGNOSIS

Allergen	Serum IgE Assay	Prick /puncture Skin Test	Intradermal Skin Test
Respiratory Allergy	Acceptable	Acceptable	Usually not needed (false positives)
Food Allergy	Acceptable	Acceptable	Not needed (false positives)
Latex Allergy	Inadequate	Performed	Not needed
Venom and Drug Allergy	Complementary to ID Skin Test	Not sufficient	Preferred

Skin Prick Test Vs Serum Specific Ige Testing (Immunocap): Favouring Skin Prick Test

	Skin Prick Test	Serum sIgE test
Quick results	✓	✗
Recommended as diagnostic test of first choice by various authorities	✓	✗
Better sensitivity and specificity	✓	✗
No interference from high total IgE*	✓	✗
Clear demonstration to patients of their allergies	✓	✗
Resembles physiological reaction to allergen**	✓	✗
Clinical Correlation#	Excellent	Good
Cost#	Low	High
Selection of antigens#	Excellent	Good
Require areas of normal skin for testing	✗	✓
Patients unable to stop medications such as antihistamines	✗	✓
Patients having dermatographism	✗	✓
Pregnant women	✗	✓

IN VITRO TEST VERSUS IN VIVO TEST

- ***Age:*** Infants (<3 months) and elderly patients (>50 or 65 years old) have reduced reactivity of the skin **(in vitro > in vivo)**
- ***Color of the skin:*** The measurement of skin response may be difficult in patients with darkly pigmented skin. **(in vitro > in vivo)**
- ***Condition of the skin:*** Severe dermatographism can make the interpretation difficult. **(in vitro > in vivo)**
- ***Skin disorders:*** Performance of the test is difficult in case of active skin disorders (atopic dermatitis, egzema, acute urticaria) **(in vitro > in vivo)**
- ***Drugs :*** If the patient is not able to stop these drugs, skin test response will be effected: Antihistamines, tricyclic antidepressants and phenothiazines **(in vitro > in vivo)**

IN VITRO TEST VERSUS IN VIVO TEST-2

- ***Drugs (cont.) :*** Long term (>1 week) systemic steroid therapy suppress the cutaneous mast cell response. **(in vitro > in vivo)**
- ***The conditions in which adrenalin injection is contrindicated or ineffective*** **(in vitro > in vivo)**
  - The patients who must use β bloker
  - Pregnancy
  - Patients with high risk cardiac diseases
- ***Asthmatic patients with low FEV1*** (even after appropriate medical thearapy) **(in vitro > in vivo)**
- ***Pathological Conditions :*** There is reduced skin test reactivity in patients with cancer, chronic renal failure, regular hemodialysis treatment, spinal cord injuries, diabetic neuropathy **(in vitro > in vivo)**

COMPARISON OF IN VIVO TEST TO IN VITRO TEST

The studies performed with earlier generation in vitro tests demonstrated that;
skin tests are more sensitive than invitro tests.

BUT

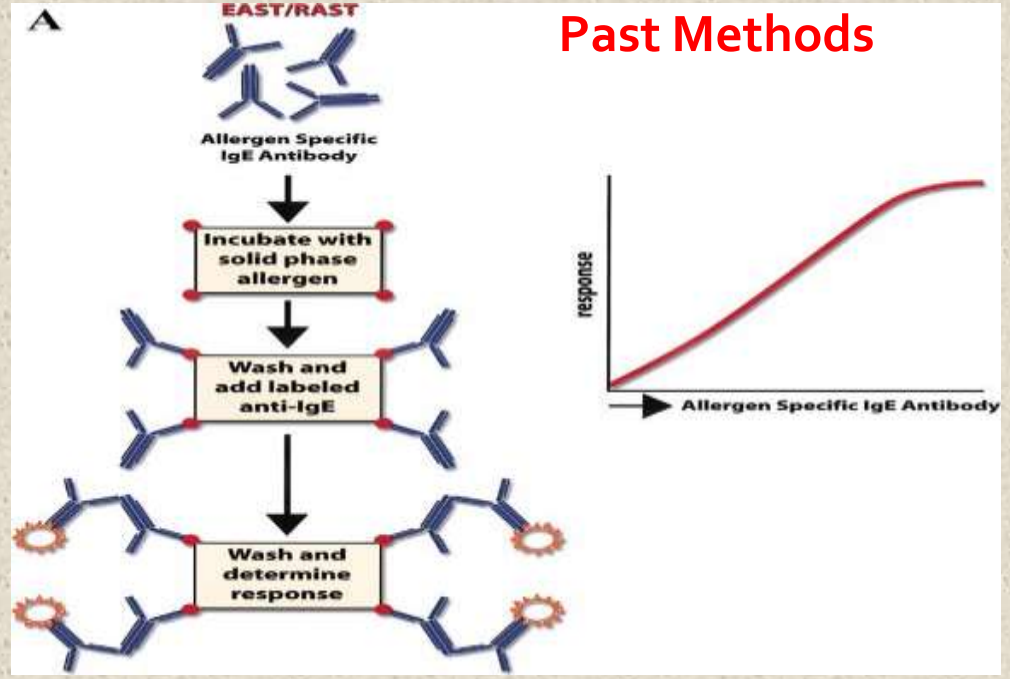
2nd and 3rd generation assays provide quantitative results.

Third generation IgE Ab assays **produce higher test sensitivity and specificity.**

The newer quantitative allergen specific IgE antibody assays use the most advanced assay calibration methods

PAST METHODS

Common Assay Design For Measurement Of Allergen Specific IgE Antibody In Serum



Specific IgE antibody assay technology

Step 1:
Separate allergen-specific IgE from other antibodies present with a solid phase allergosorbent

Step 2:
A buffer wash separates bound IgE from unbound antibodies

Step 3:
Bound IgE is detected with a labeled anti-human IgE reagent

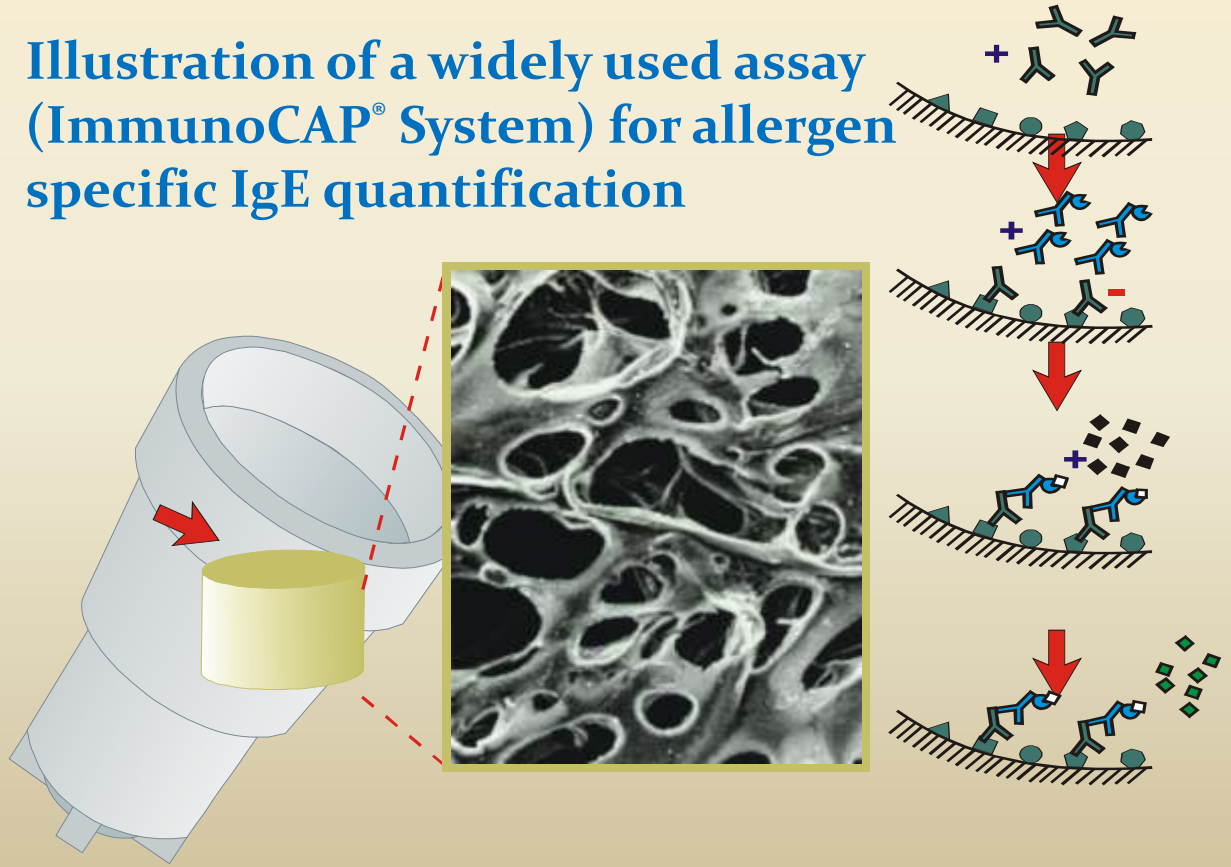
Past Methods

RAST® =Radioallergosorbent test, (formally Pharmacia, currently Phadia)
FAST = Allergenics/Biowhittaker, fluorescent allergosorbent test
MAST = Hitachi: thread pipette
EAST = Sanofi Dignostics Pasteur
Magic Lite = ALK/Corning
Matrix = Abbott
Alastat, Diagnostic Products Corp. (DPC, biotin-allergen)
Avida Centaur (Bayer Diagnostics)

Current Methods

1- Hycor-Stratagene: Hy-Tec System Siemens:
2- Immulite System (formally Diagnostic Products Corporation)
3- Phadia: ImmunoCAP System® (formally Pharmacia Diagnostics)

Illustration of a widely used assay (ImmunoCAP® System) for allergen specific IgE quantification



Patient IgE

Allergen coupled to ImmunoCAP

Conjugate; Enzyme Anti-IgE

Patient IgE ab bound to ImmunoCAP allergen

Fluorogenic substrate

Conjugate bound to patient IgE

Conjugate enzyme reacts with substrate forming a fluorescent product

WHAT ARE THE DISADVANTAGES OF IN VITRO TEST

- Delayed result when compared with skin testing.
- Insufficient sensitivity in some commercial assay methods.
- Not always consist of all allergens individually.
- Cost

Levels of allergen specific IgE measured by different commercial assays are not equivalent.

Because each assay varies :

- ✓ **in the composition of allergen reagents,**
- ✓ **methods of measurement and**
- ✓ **standardization**

Poor Agreement Of IgE Antibody Laboratory Results

Results:

Poor agreement among the 3 systems for soy and peanut

- **Using a cutoff of 0.35 kUa/L showed some differences in the ability to detect sIgE sensitization with Turbo RAST most variable**

- **Studies suggest various assays measure different populations of IgE antibody.**

- **Currently, it is not known which of the major assays provides the most accurate evaluation of allergen s-IgE in patients' serum.**

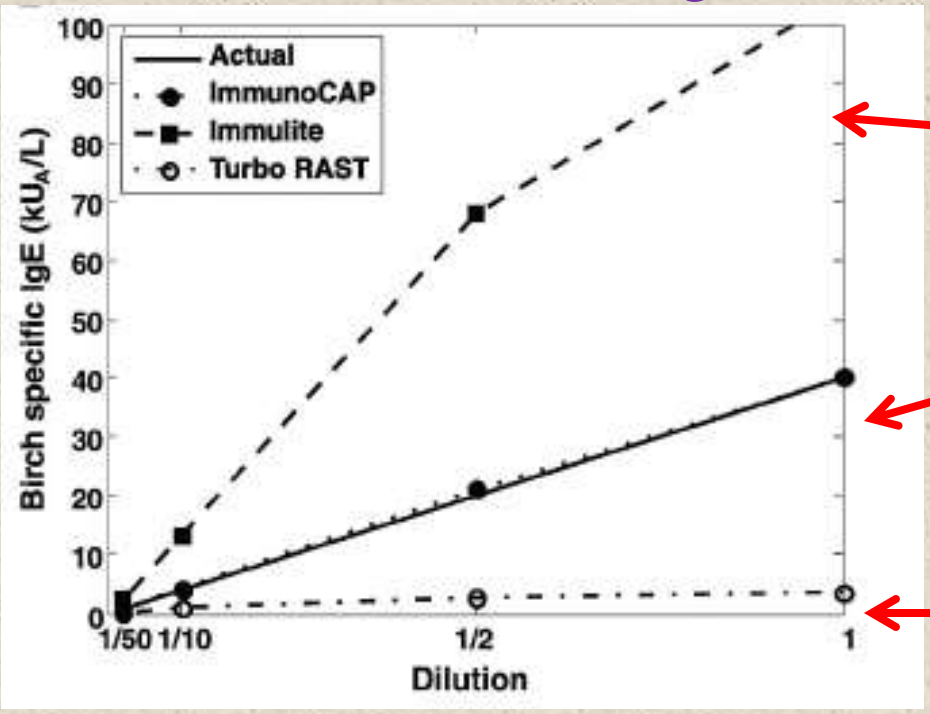
Table 1. Dichotomous Comparison of Assay Performances With Peanut and Soy Antibodies in Serum

Assays	Peanut antibodies		Soy antibodies	
	Positive	Negative	Positive	Negative
Cutoff of 0.35 kU/L				
ImmunoCAP	50	10	17	3
Immulite	47	10	16	3
Turbo RAST	39	9	12	3
Cutoff of 0.10 kU/L				
ImmunoCAP	55	5	17	3
Immulite	54	4	17	3
Turbo RAST	48	5	17	3

Abbreviation: RAST, radioallergosorbent test.

Interassay Variability Of IgE Antibody Laboratory Results

- **Results:** Chimeric antibodies: Widely disparate results amongst the 3 assays
  - Immunlite considerably overestimated sIgE
  - Turbo RAST underestimated s-IgE



Immunlite

ImmunoCAP

Tubo RAST

Wood et al.,Annals Allergy, Asthma & Immunol 2007; 99:34-41

RESPIRATORY{INHALANT} ALLERGY

- Using standardized and good quality allergens or extracts in both tests, the percentage concurrence between in vitro allergen-specific IgE tests and skin prick-puncture tests ranges : 85 % 95 % (depending on the evaluated allergens)

Allergy Skin Testing & Specifec IgE Report

	TESTING REPORT	Skin test	Sp IgE
1	D. Farines	+++	26.5 kU A/l
2	D. Pteronyssinus :	++++	34.4 k U/A/l
3	Prosopis Juli	+++	
4			
5			
6	Grass mix panel		4.57 kua/l

SPT ≥8 mm = "diagnostic" of peanut allergy

SPT 3 to 7 mm = IMMUNOLOGICAL GREY ZONE

SPT <3 mm = very unlikely to be peanut allergy

SPT Is Superior To IgE CAPRAST For The Diagnosis Of Infantile Food Allergy

- Study: Infants with suspected egg and milk allergy with negative specific-IgE at the time of first visit
- Results:
  - Egg: 72/89 (80%) suspected-HE allergies with negative IgE CAPRAST, were diagnosed as HE allergy by the elimination and provocation tests . 39 had positive egg SPT
  - Milk: 42/125 (33%) suspected-CM allergy infants with negative IgE were diagnosed as CM allergy, and 21 (50%) had positive milk SPT
- Authors' Conclusions: "SPT seemed to be more useful than EW- or CM- IgE CAPRAST for the diagnosis of HE or CM allergies in early infantile period."

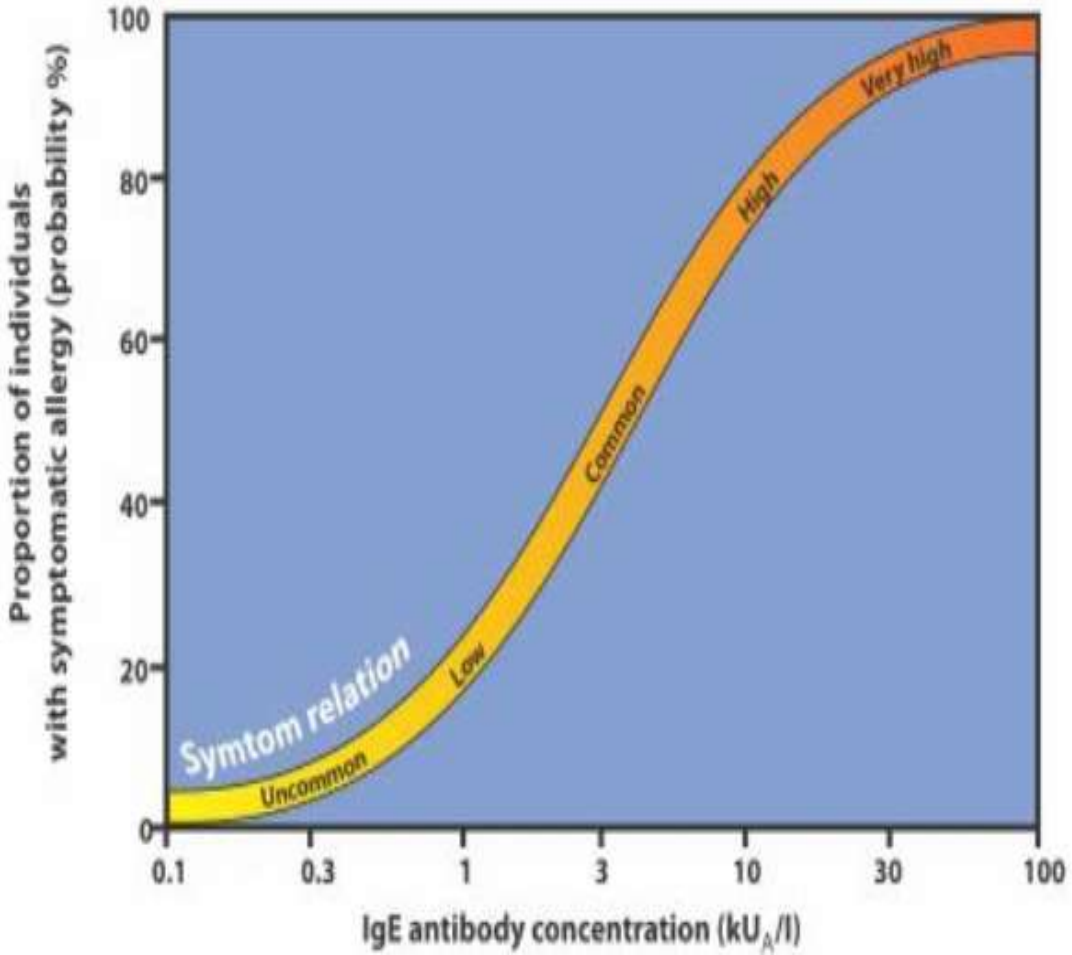
Ebisawa M et al, J Allergy Clin Immunol 2009;123(2):S23.

Normal values

ImmunoCAP Allergen : < 0.1 kU_A/l

ImmunoCAP Phadiatop/Allergen mix : < 0.35 KU_A/l

The higher the level of Specific IgE antibodies, i.e. sensitization, the higher the risk for symptomatic allergy



Factors to consider for a final diagnosis :

- Age
- Degree of atopy
- Allergen load
- Type of sensitizing allergens
- Previous symptoms
- Other triggering factors

No severity or intensity of Disease by allergens (SPT/specific-IgE)

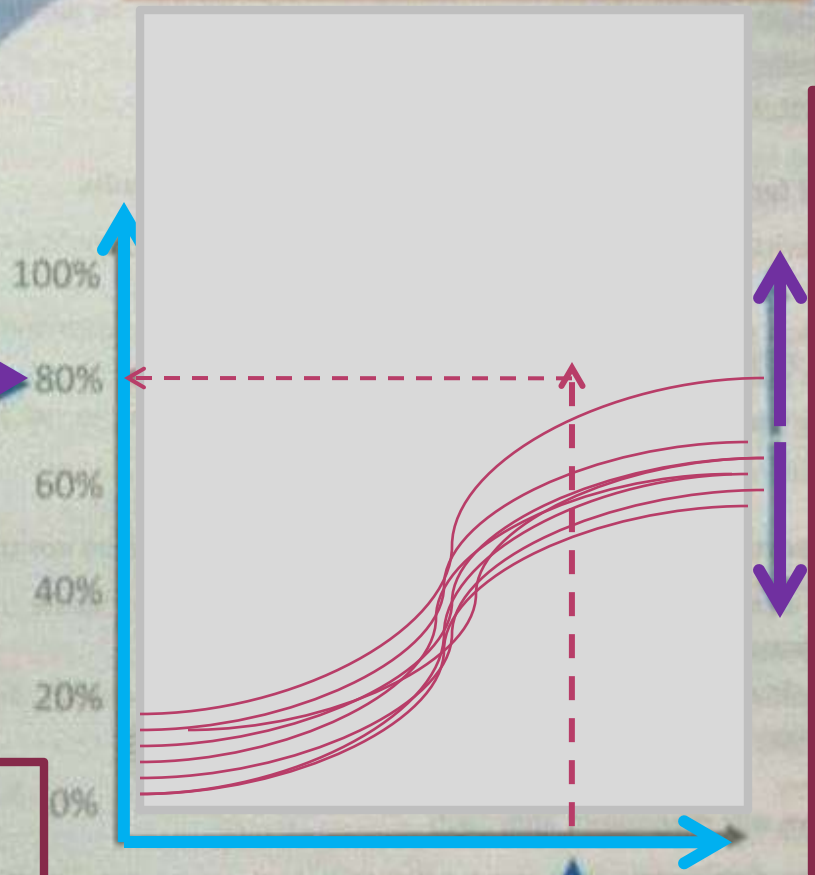
95% PREDICTIVE DECISION LEVELS

Allergen	Decision Pt (kUA/L)	PPV	Sens.	Spec.
Egg	7	98%	61%	98%
(≤ 2 yrs of age)+	2	95%		
Milk	15	95%	57%	94%
(≤ 1yr of age)++	5	95%		
Peanut	14	100%	57%	100%
Soy	30	73%	44%	94%
Wheat	26	74%	61%	92%
Tree nuts+++	15	95%	----	----

+ Boyano MT, et al. *Clin Exp Allergy* 2001; 31:1464-9.
++ Garcia-Ara C, et al. *JACI* 2001; 107:185-90.
+++ Clark AT, Ewan P. Clin Exp Allergy 2003; 33:1041-45.

Sampson *JACI* 2001; 107:891-96.

Integration Of Patient -Specific Factors And IgE- Sensitization Test Result



❹Probability of clinical allergy

Integration of a specific IgE sensitization test result and specific patient factors

❸Patient-specific factors

- Internal: gender, age, presenting features of possible allergic reaction, coexisting diseases
- External: cofactors such as stress, viral infection

Each factor may modulate up or down, possibly in an additive fashion, the relationship between IgE result and probability of clinical allergy; impact may not be linear.

❶Clinical history

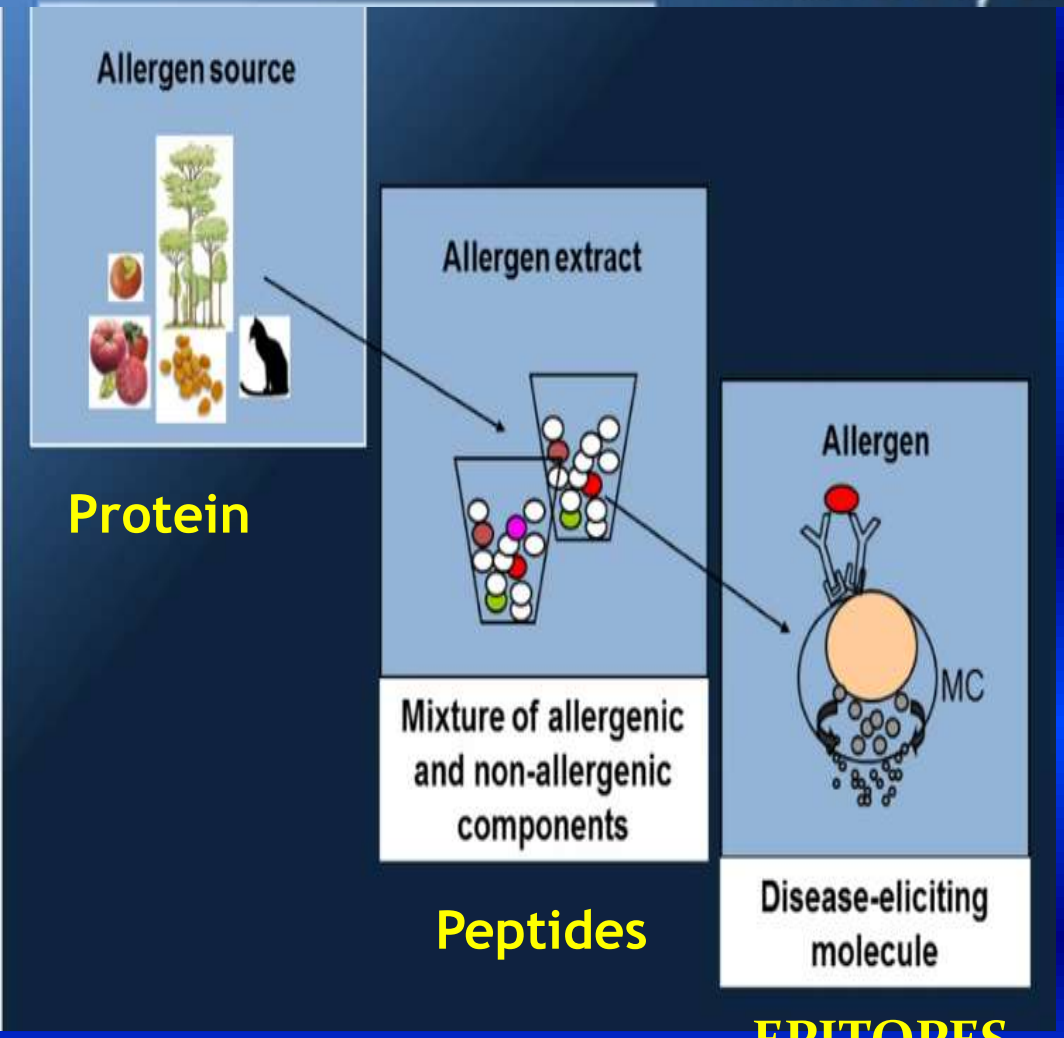
for example any previous reaction

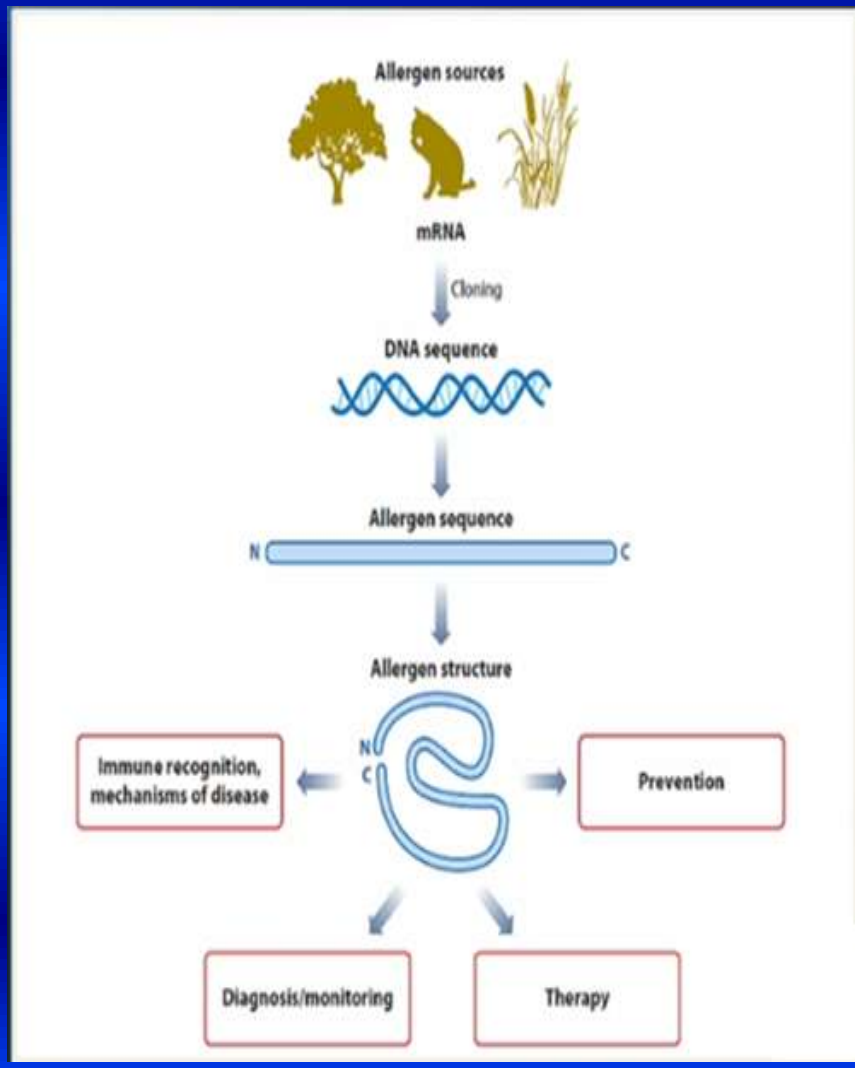
History informs decision about best IgE sensitization test

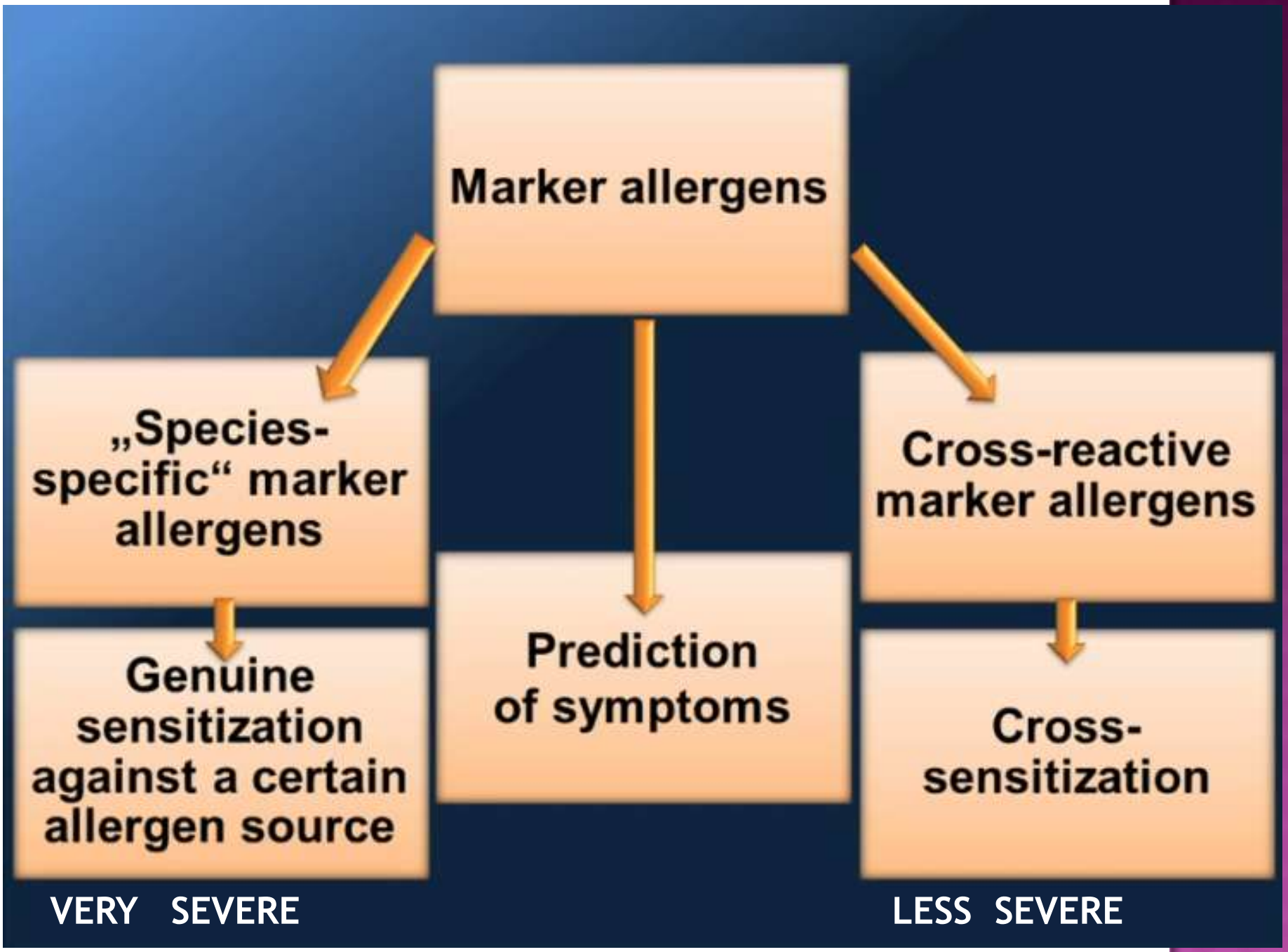
❷IgE sensitization test

eg skin prick test, serum-specific IgE

Definition: Allergen source, allergen extract, allergen



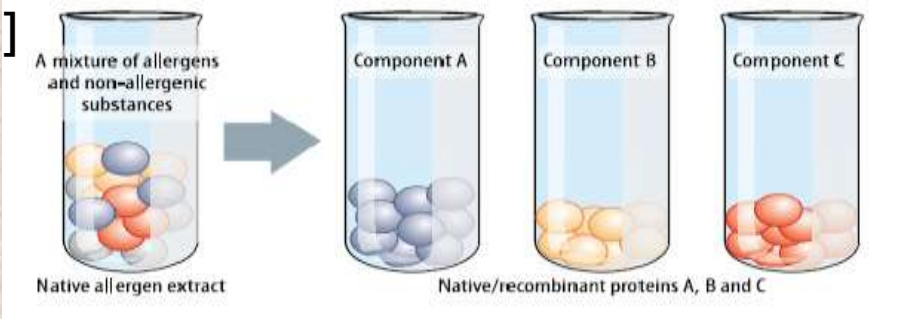



Marker allergens
„Species-specific“ marker allergens
Cross-reactive marker allergens
Genuine sensitization against a certain allergen source
Prediction of symptoms
Cross-sensitization
VERY SEVERE
LESS SEVERE

General reasons why molecular allergy (MA) is applied to diagnostic methods (IgE testing)

Role Of Component-resolved Diagnostics (CRD)

A science that make it feasible to quantify IgE antibodies to specific allergen proteins on a molecular allergologic]



Whole Allergen Components,(Individual Allergenic Epitopes Molecules)

CRD have been introduced in order to increase the probability of

1- True Food/aero-allergens & insects Allergy diagnosis

2- Identify patients at high risk of reactions

3- Identify patients more prone to persistent disease

"Rule of thumb": How to use MA

Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, Ollert M et al. Pediatr Allergy Immunol 2016;27(Suppl.23):1-250 (free access)

- Suspected allergen from family with **broad cross-reactivity?** i.e.
  - PR-10,
  - nsLTP
  - profilin
  - polcalcin
  - albumin
  - parvalbumin
  - tropomyosin

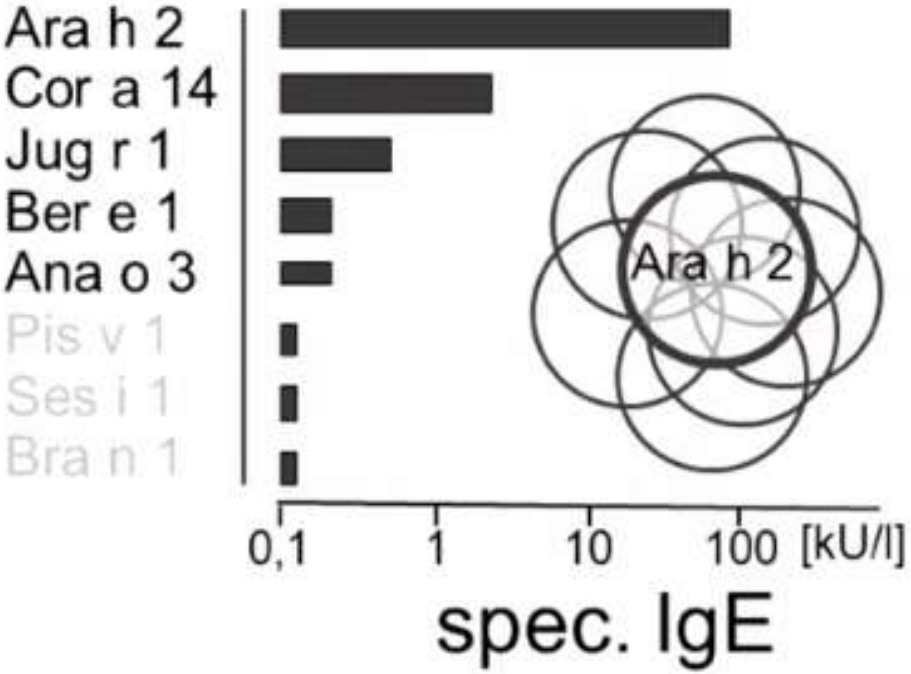
- Test specific IgE only to **one representative** member, i.e.
  - i.e. Bet v 1
  - Pru p 3
  - Phl p 12 or Bet v 2
  - Phl p 7 or Bet v 4
  - Fel d 2
  - Gad c 1
  - Pen a 1

"Rule of thumb": How to use IgE tests in MA

Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, Ollert M et al. Pediatr Allergy Immunol 2016;27(Suppl.23):1-250 (free access)

- Allergen from family with **limited cross-reactivity?** (seed storage proteins, lipocalins)

2S albumins



- test suspicious member(s) and related ones*
- hierarchy indicates primary sensitizer
- *if negative, cross-reactivity is unlikely

Vegetable Origin

Rule of Thumb

A. Profilin and PR10 proteins

- Highly cross reactive (PR 10 especially to Birch)
- Often associated with less serve reactions e.g. OAS

B. nsLTP's and Storage Proteins **(highly reactive)**

- Associated with more severe reactions
- More heat/digestive enzyme resistant and therefore can be more often associated with OAS and well as digestive problems

Peanut allergens:

Is it low risk allergy Or risk for severe reaction?

Seed storage proteins

- Ara h 2/6 — 2S-albumin
- Ara h 1 — 7S-globulin (vicilin)
- Ara h 3 — 11S-globulin (legumin)

oleosins: Ara h 10/11, Ara h 14/15

defensins: Ara h 12, Ara h 13

nsLTP: Ara h 9, Ara h 16/17

PR-10: Ara h 8

profilin: Ara h 5

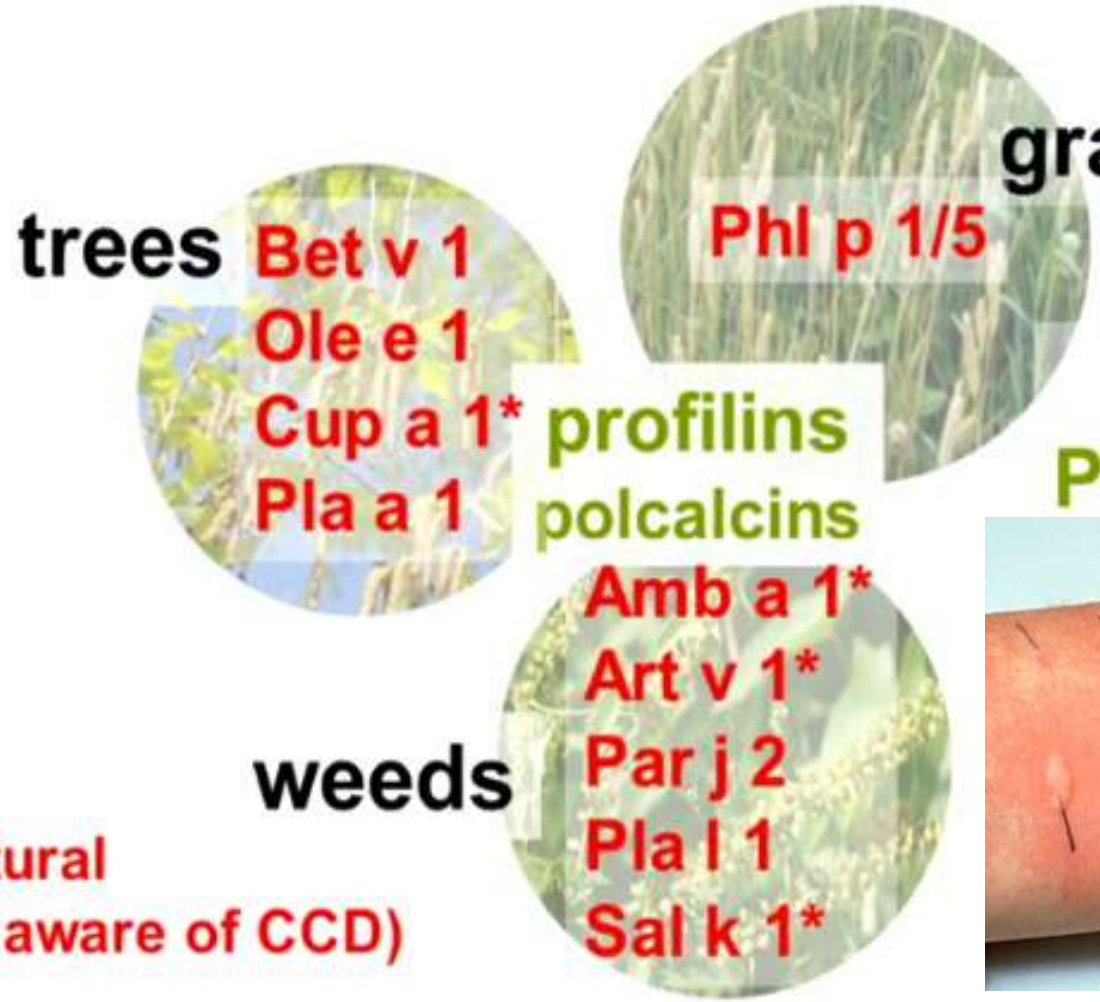
Pru p 3

Ara h 5	Ara h 8	Ara h 9	Ara h 10, Ara h 11	Ara h 1, Ara h 2/6, Ara h 3
CCD	profilins	PR-10	nsLTP	oleosins	storage protein

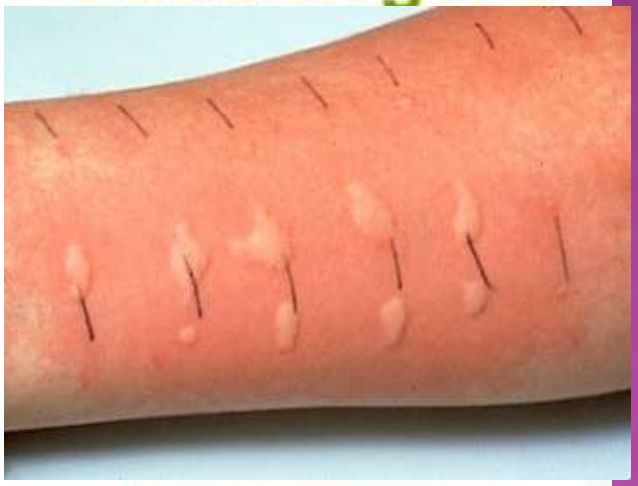
labile proteins, low amount → **Low risk**

stabile proteins, large amounts → **High risk**

Multiple sensitizations due to crossreactivity or true co-sensitization?

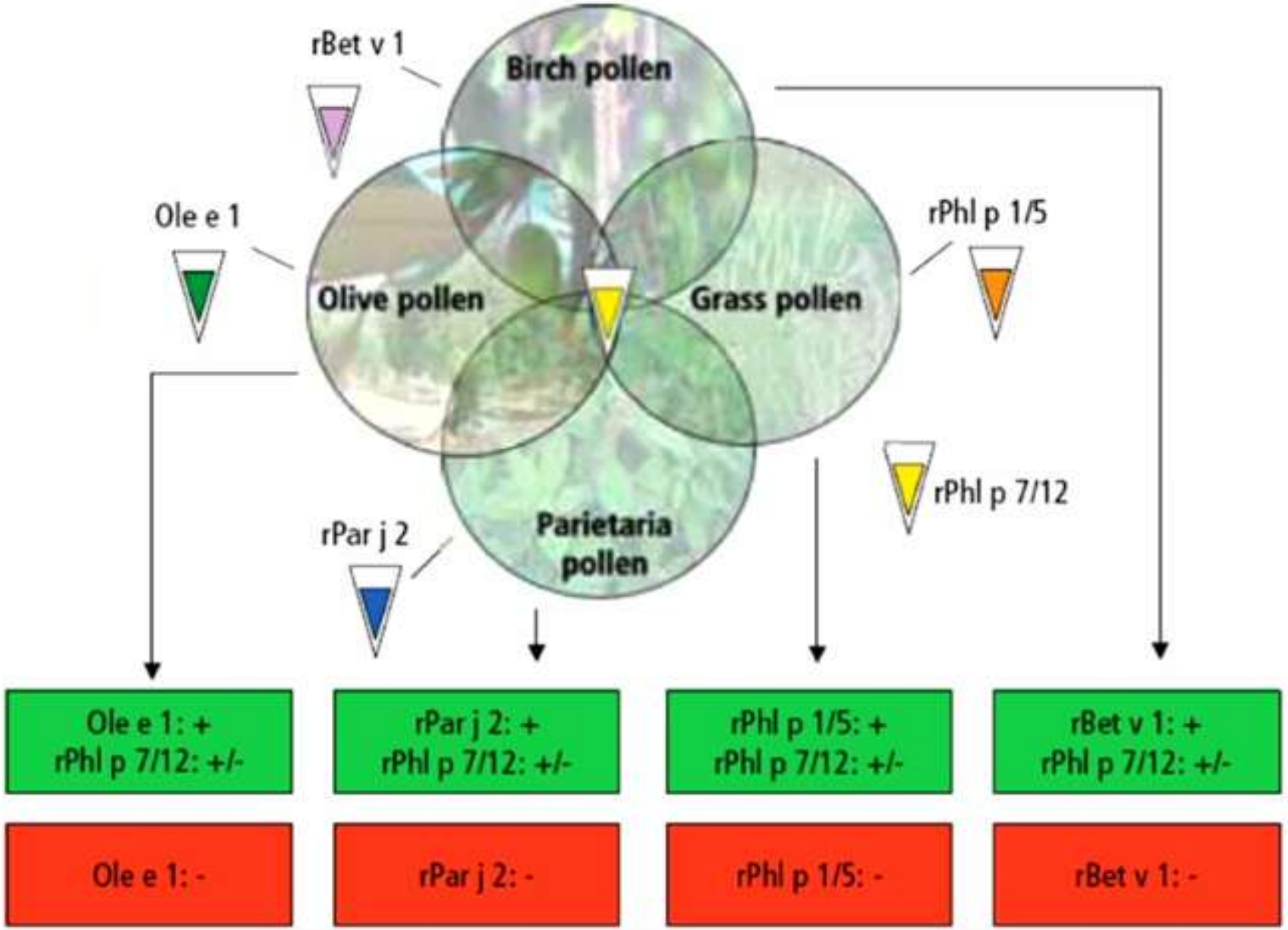


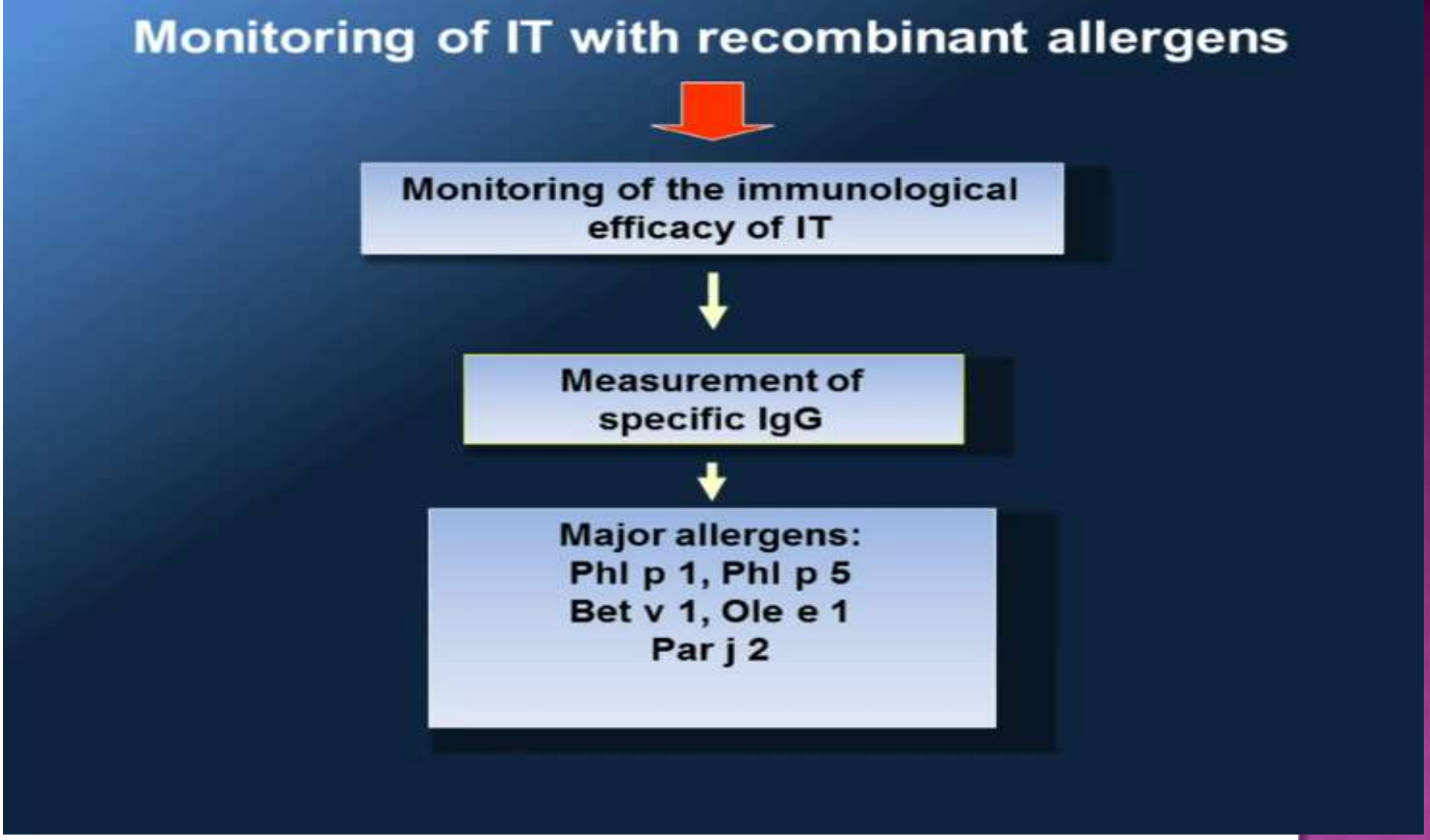
Pollen-Panallergens



Diagnosing components before AIT?

Valenta R et al. J Investig Allergol Clin Immunol 2007;17:88-92

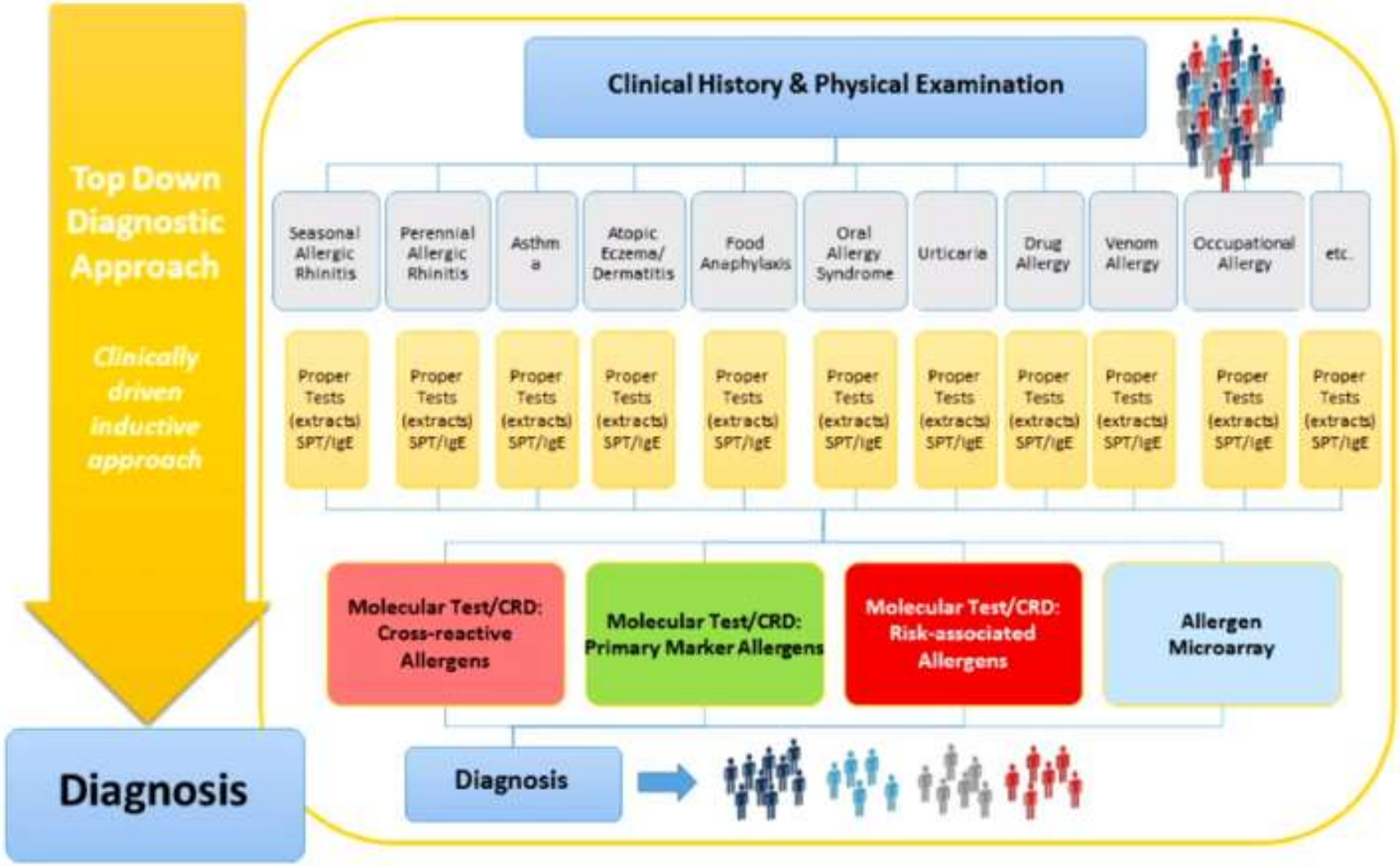




Allergen molecule-based tests for accurate prescription of immunotherapy

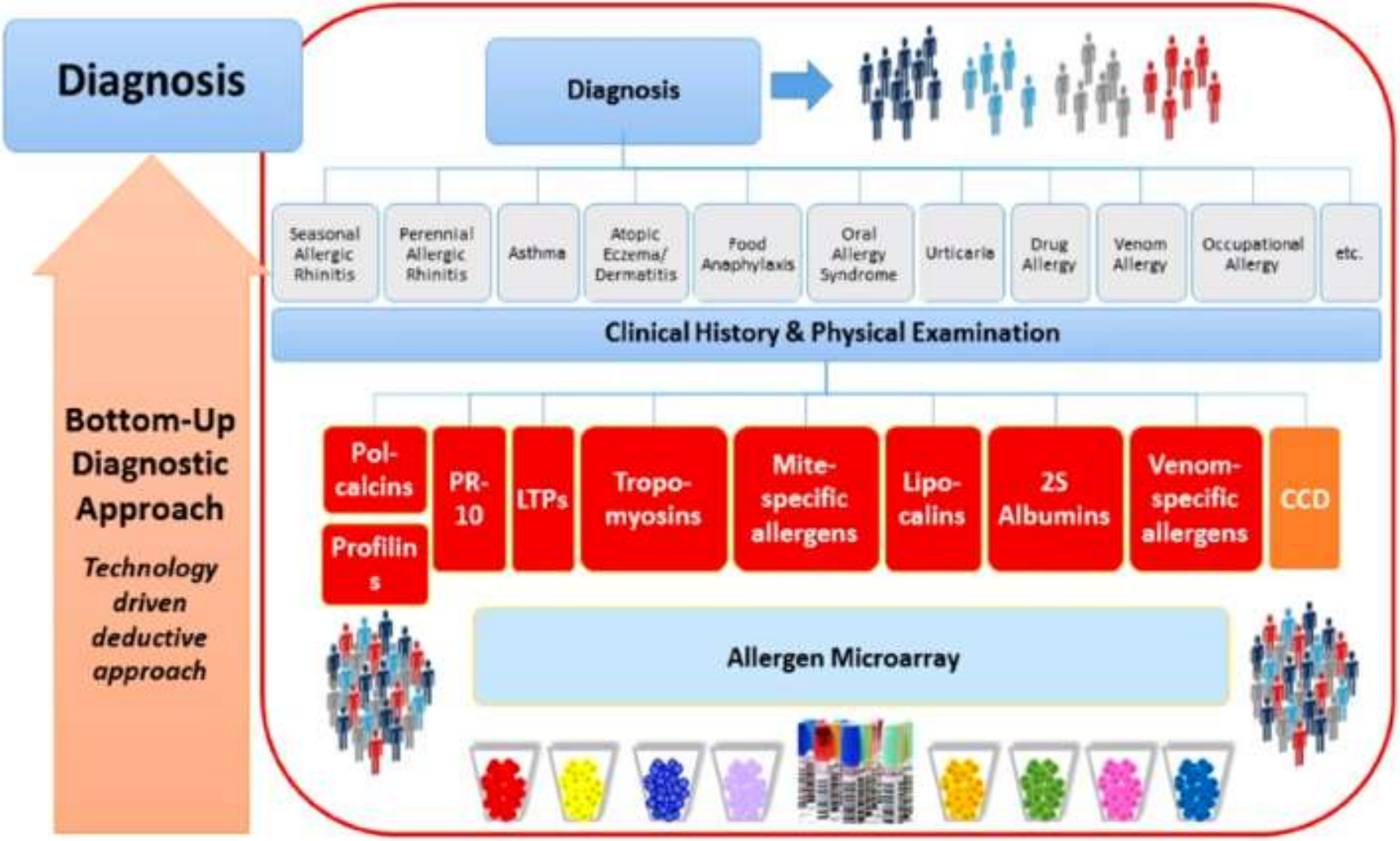
Top-down diagnostic approach

Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, Ollert M et al. Pediatr Allergy Immunol 2016;27(Suppl.23):1-250 (free access)

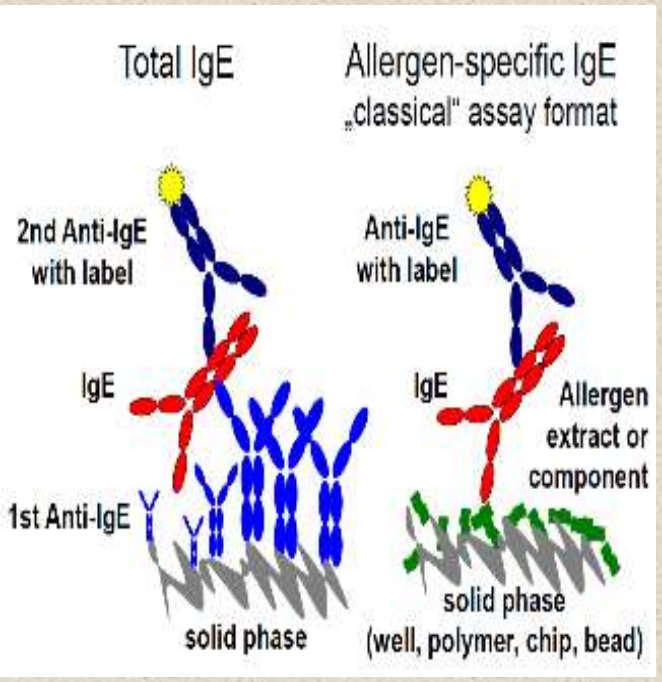


Bottom-up diagnostic approach

Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, Valenta R, Hilger C, Hofmaier S, Ollert M et al. Pediatr Allergy Immunol 2016;27(Suppl.23):1-250 (free access)



IgE Antibody Single-Plex Autoanalyzers



Basic reagents and chemistries are similar

*** Allergen on solid phase binds antibody**
*** Buffer wash to remove unbound protein**
*** Enzyme anti-IgE detects bound IgE**

***All assays report in similar units with comparable analytical sensitivities of 0.1 kUa/L**

•All assays principally use allergen extracts;
•Increasing number of molecular allergens available

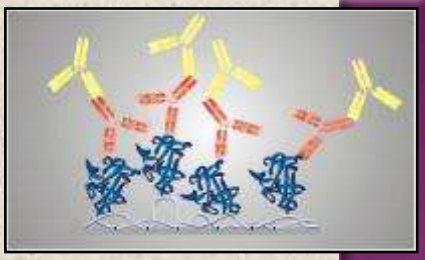
CHIP (ISAC®-112) TECHNOLOGY (CRD)

Collect serum → **Pipette sample** → **Add Anti-IgE-Fluoro Conjugate**

40 µl

Static Planar array

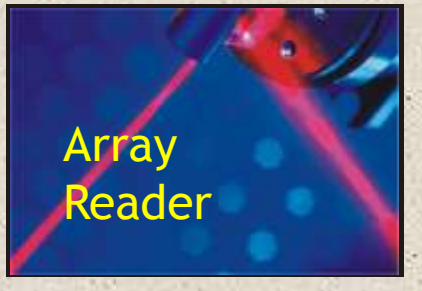
Wash



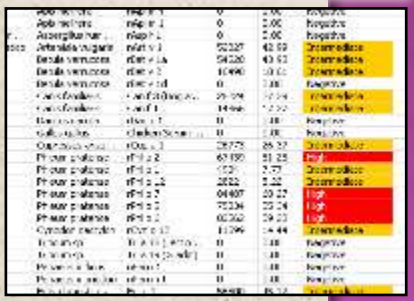
Duration: 3 hours

Scan biochip → **Analyse images** → **Report: ?Too much Information?**

Wash



Semi Quant-itative



Duration: 10 min.

EU-MeDALL Allergen Chip (super array: 170 allergens): IgG and IgE analyses

Lupinek C et al Methods 2014:66 106-19

Single- or multiplex assays? *"choose wisely* or *precision medicine"*?

Singleplexing

- targeted sensitization test
- great assay sensitivity (0.1 kU_A/l)
- interpretation with spec./total IgE ratio
- ultimate exclusion of allergen-spec. IgE
- economically superior

Multiplexing

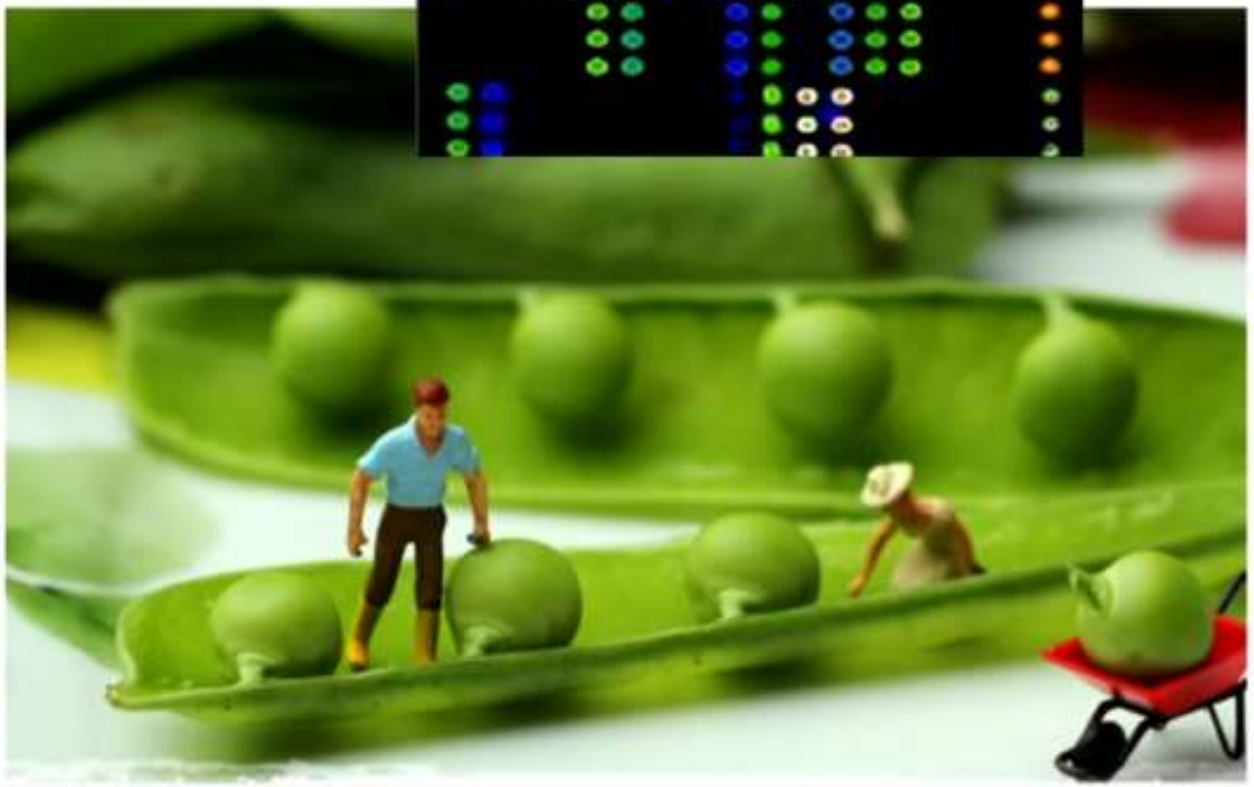
- broad sprectrum sensitization test(s) (>100, >200?)
- broad screening
- semiquantitative
- sensitization profiles sometimes redundant
- limited (analytical) sensitivity (<1 kU_A/l?)

What is your personality type?

Hunter? or Collector?

singleplex IgE,
i.e. ImmunoCAP

multiplex IgE,
i.e.ISAC2
FABER...



Total Serum IgE Levels - Allergic Diseases

Patients with allergic asthma may have increased total serum IgE concentrations, but this is not an allergy-specific finding:-

- **60% of "allergic" asthmatics have increased IgE**
- **40% of "allergic" rhinitis patients have increased IgE**

Measurement of total serum IgE may be of value in patients with:

- **Gastrointestinal symptoms/eosinophilic esophagitis**
- **Suspected occupational allergy with unclear genesis**
- **Anaphylaxis**
- **Allergic Bronchopulmonary Aspergillosis (ABPA)**
- **Allergic Fungal Sinusitis**

Total serum IgE may be measured to determine the dosage of omalizumab

doi: 10.1111/j.1365-2222.2006.02525.x

Clinical and Experimental Allergy, 36, 1019–1027

ORIGINAL PAPER

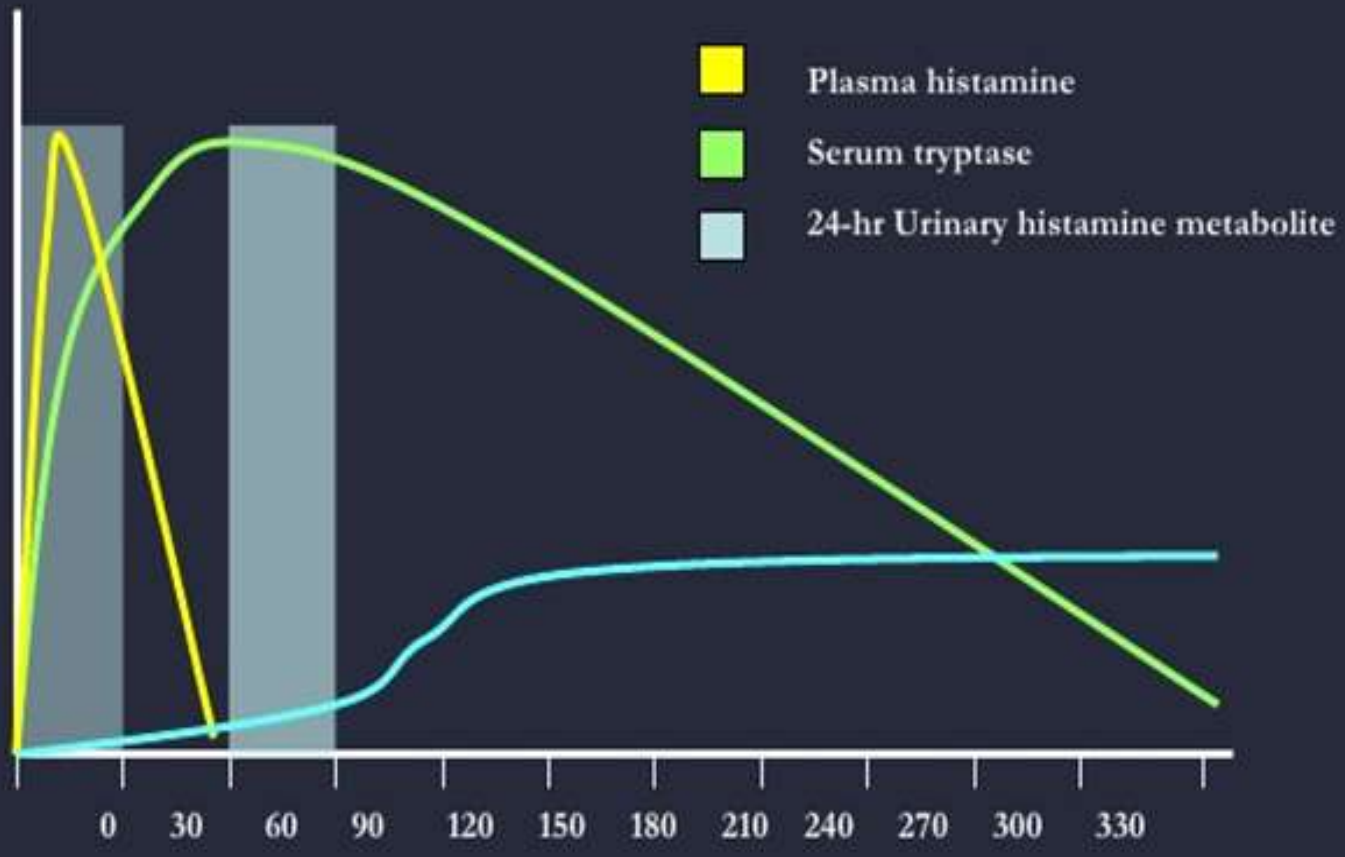
Total serum immunoglobulin E levels in a case–control study in asthmatic/allergic patients, their family members, and healthy subjects from India

S. Sharma*, P. C. Kathuria†, C. K. Gupta‡, K. Nordling§, B. Ghosh* and A. B. Singh*

*Institute of Genomics & Integrative Biology, Delhi, India †National Allergy Centre, Delhi, India ‡Anupam Apartments, Vasundhara Enclave, Delhi, India and §Division Medisinsk Service Sykehuset Innlandet, Gjovik, Norway

Conclusion:- **The IgE level in Indian allergic patients is significantly related to atopy ,but due to wide overlap of IgE levels in patients and healthy subjects , its diagnostics significance in Indian population seems to be limited**

Laboratory tests in the diagnosis of anaphylaxis



DRUG ALLERGY

Suspected IgE Mediated

sIgE BAT

Skin Prick/Intradermal Test

Suspected T Cell Mediated

LTT

Late Reading Intradermal Test/Patch Test

Drug Provocation Test

For High Risk Patients
Or
Severe Reaction
Or
For Drugs Where Skin Test Are Not Available
It Might Be Advisable To Perform In Vitro Tests Before In Vivo Test

Basophile Activation Tests

- Based on flow cytometry and measuring activation markers (CD63 and CD203c)
  - For inject able drugs and mimics in-vivo response

Sensitivity

40 – 60 % depending on the drug

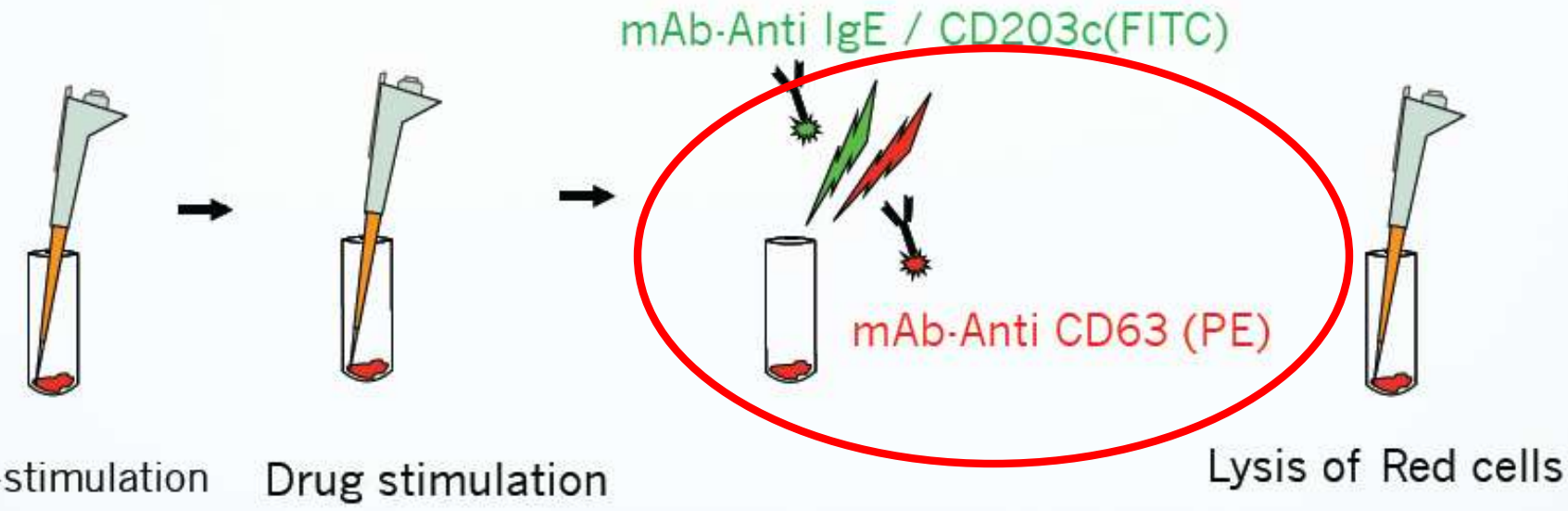
Specificity

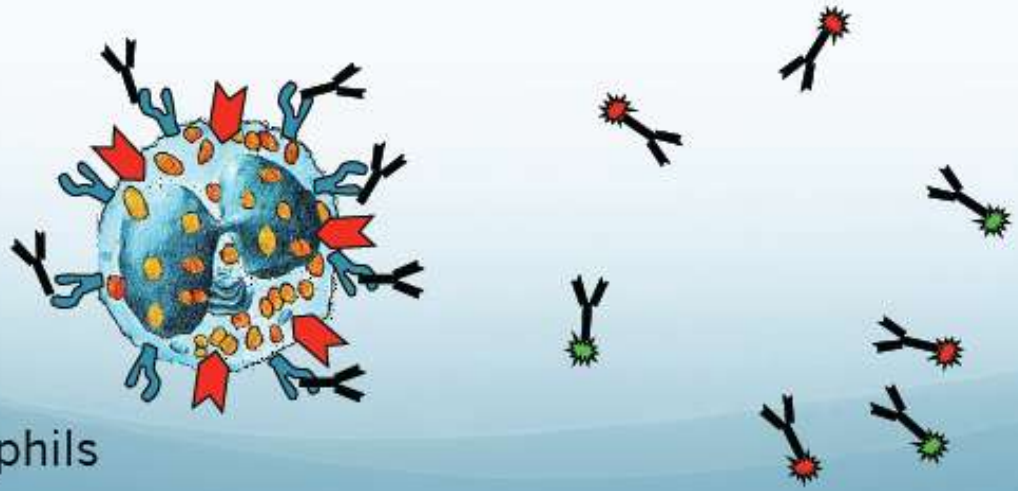
85 – 100%

Drug	Sensitivity
Penicillin	22-55%
Clavulanic acid	53%
Rocuronium	92%
NMBA	64-85%
Fluoroquinolones	36-71%

- At present no standardised approach and variations between laboratories

BAT protocol for flow cytometry

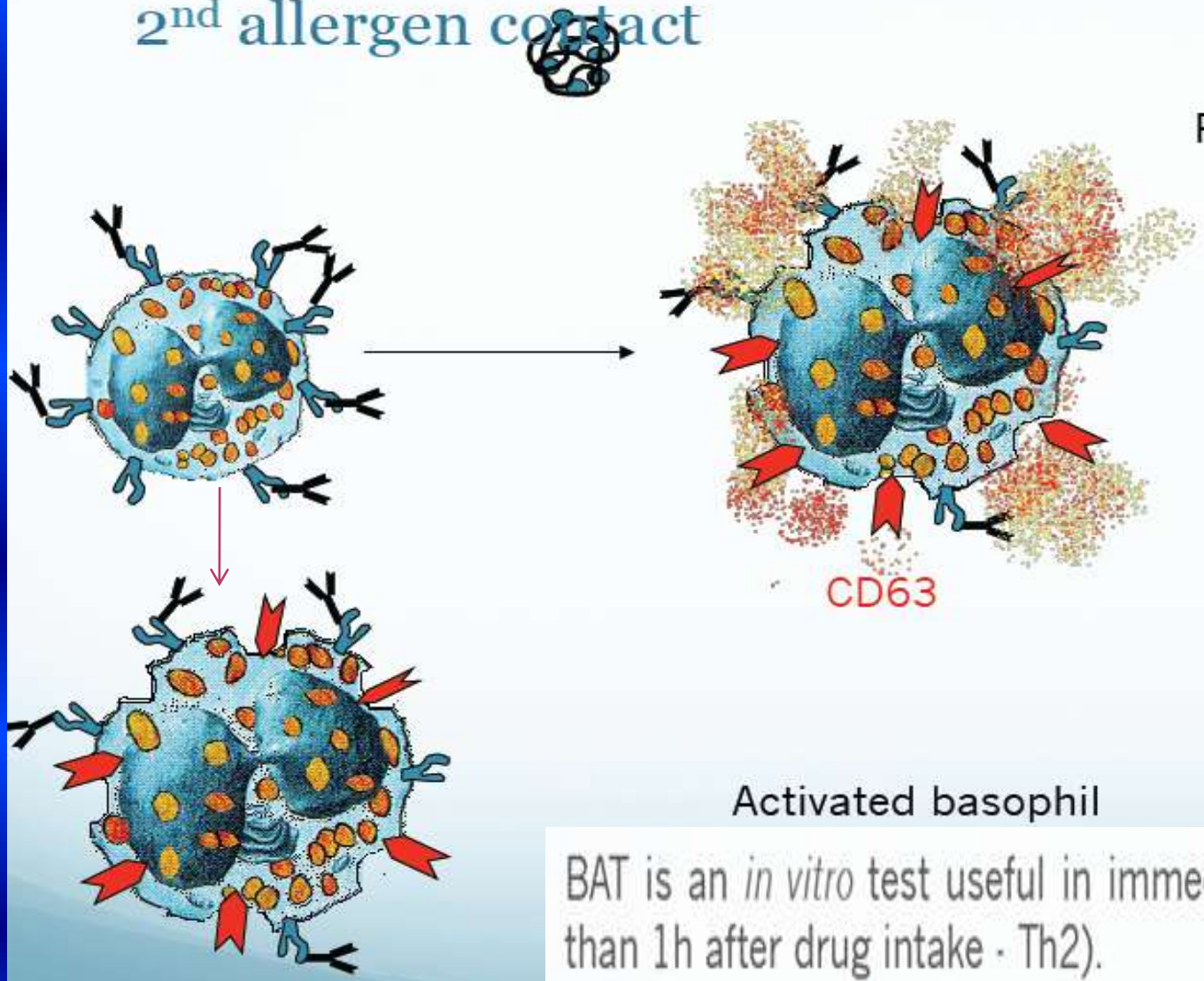




Drug allergy-IgE

Mechanism of IgE mediated reactions:

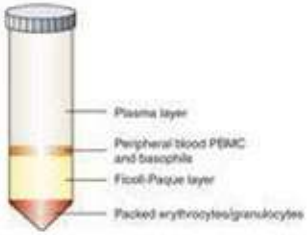
2nd allergen contact

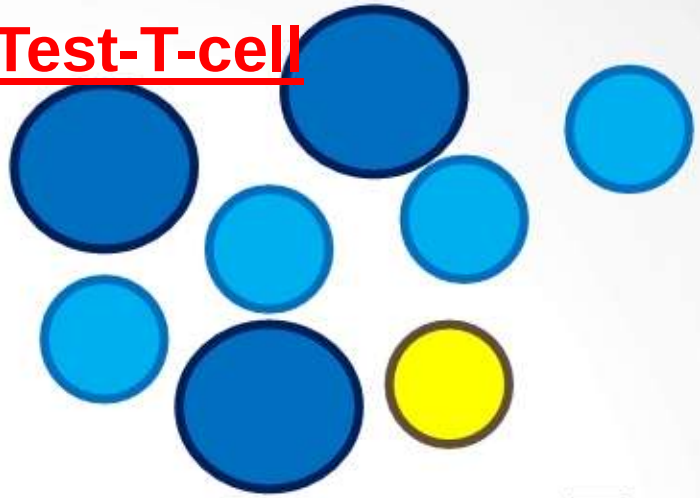


Activated basophil

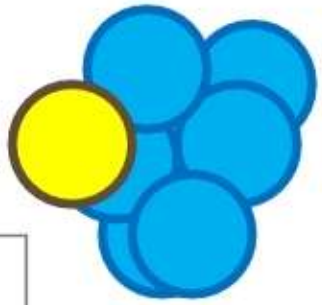
BAT is an *in vitro* test useful in immediate responses (less than 1h after drug intake · Th2).

Lymphocyte Transformation Test-T-cell

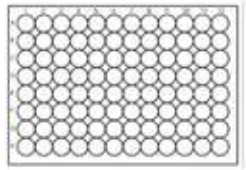


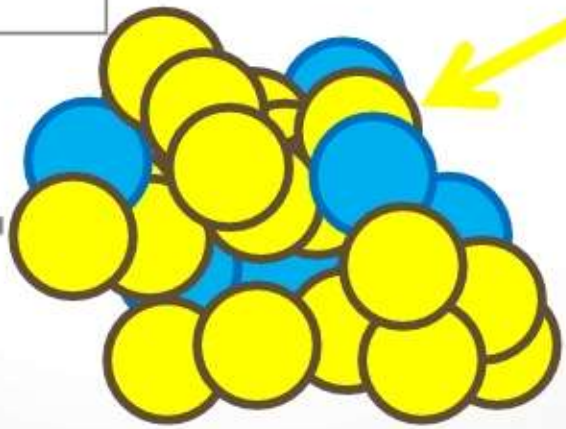


Drug allergy non-IgE



Radioactive THY





Diagnostic Interpretation

- Pos. allergen-specific IgE indicates an allergic sensitization/cross reaction
- Being clinically relevant ***ONLY*** in case of corresponding symptoms
- Neg. allergen-specific IgE rules out an allergic sensitization/cross reaction convincingly, but only *IF*
  - Total IgE is high enough,
  - Allergen intact, fully represented &
  - Analytical sensitivity has been optimized
- *The Allergist determines the clinical relevance of an allergic senitization/cross reaction, NOT the test*

Thanks

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ORGANIZED BY

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