Allergy testing

Dr Katherine Nicholls
Clinical Immunologist and Immunopathologist
The Royal Melbourne Hospital
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Outline

- Mechanisms and classification of allergy
- Process of sensitization
- Diagnosis of allergy
- Identification of trigger
- Forms of allergy testing
- Clinical indications for testing

Classification

- Gell and Coombs still holds true, but has been resolved further in recent years
- I-IV based on pathophysiology, clinical phenotype and rapidity of symptom onset

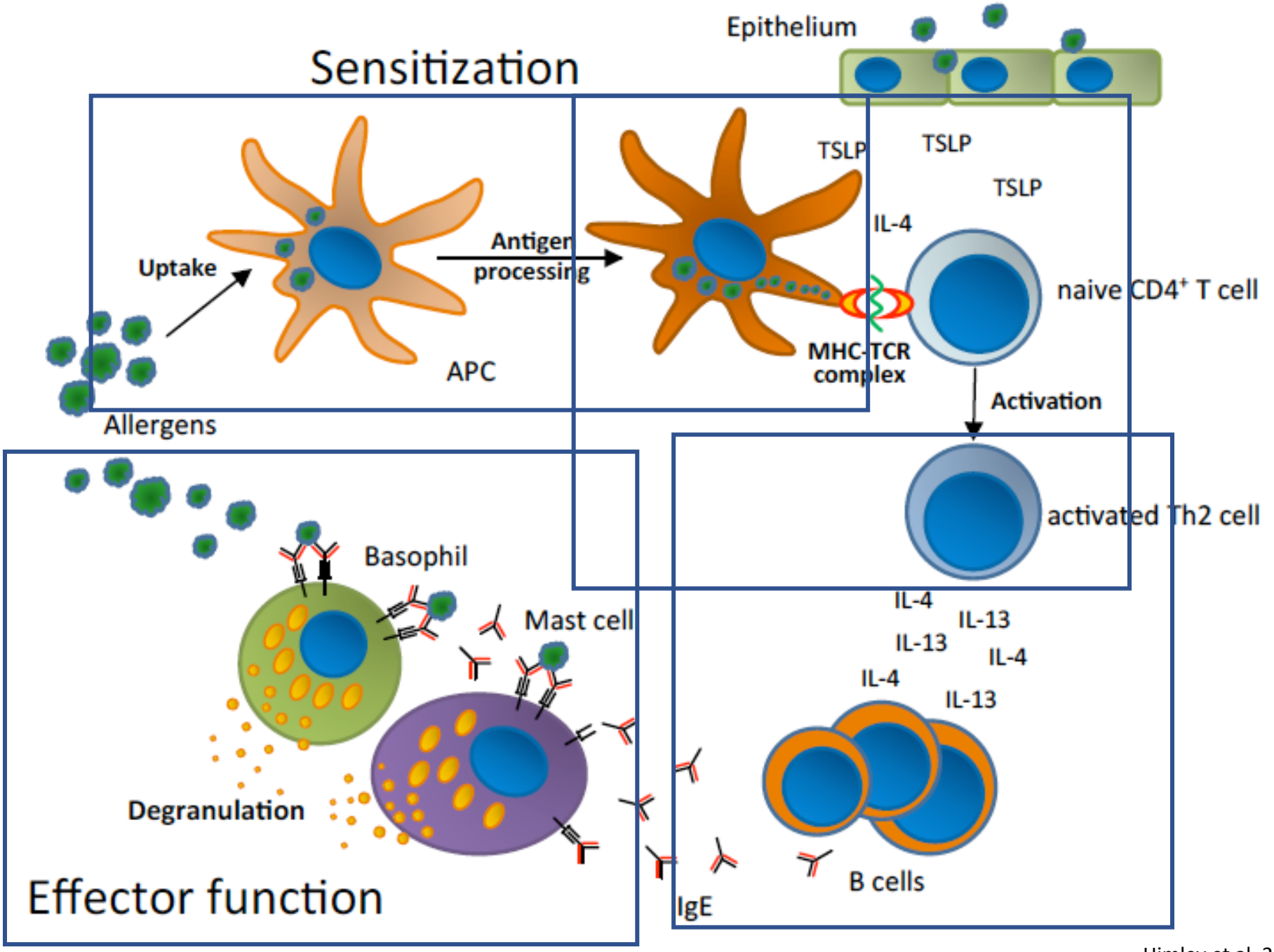
Allergy Classification

Gell–Coombs classification	Mechanism	Timing	Typical clinical features
I	'Immediate' or IgE-mediated mast cell degranulation	Minutes to hours	Anaphylaxis Urticaria, angioedema
II	Complement-dependent cytolysis (IgG/IgM)	Variable	Hemolytic anaemia Thrombocytopenia Interstitial nephritis
III	Immune complex damage	1 to 3 weeks after exposure	Serum sickness Drug fever Some cutaneous eruptions Vasculitis
IV	'Delayed' or cellular hypersensitivity	2 to 14 days or longer	Contact dermatitis Morbilliform eruptions SJS/TEN DRESS AGEP

Adapted from Middleton's Allergy

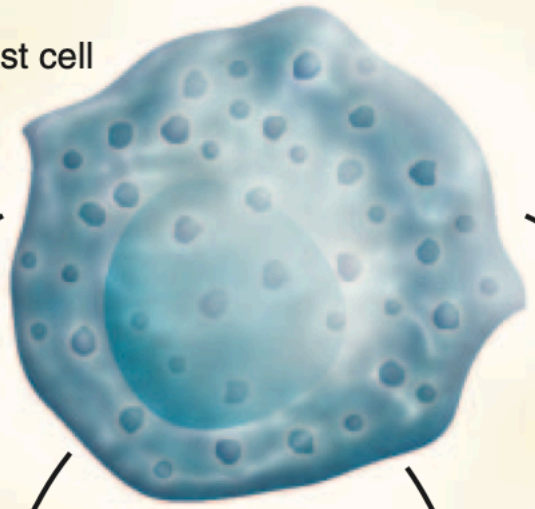
Immediate hypersensitivity

- Typical clinical features:
  - Angioedema
  - Urticaria
  - Asthma/bronchospasm
  - Allergic rhinoconjunctivitis
  - Nausea, vomiting, diarrhoea
  - Hypotension
  - Anaphylaxis
- Typically <1-2hrs after exposure to trigger
  - IV drugs, venoms typically within minutes
  - EXCEPT a-gal allergy (3-6hrs after consuming red meat or meat products)



Himley et al. 2016

MEDIATOR RELEASE AND PHYSIOLOGICAL REACTIONS OF MAST CELL DEGRANULATION



Growth factors
- Stem cell factor
- Granulocyte macrophage
- Colony stimulating factor
- Gonadotrophin releasing hormone
- Fibroblast growth factor
- Vascular endothelial growth factor
- Nerve growth factor

Proteases
- Tryptase
- Chymase
- Carboxypeptidase
- Histamine
- Proteoglycan (heparin)

Action
- Tissue remodeling
- Cellular recruitment
- Vascular permeability
- Acute allergic disease

Leukotrienes
- LeukotrieneC4
- LeukotrieneB4
- Platelet activating factor
- Prostaglandin D2
- Prostaglandin E2

Action
- Bronchoconstriction
- Vascular permeability
- Eosinophil recuitment
- Neutrophil recruitment

Cytokines and chemokines
- Interferon γ
- Tumour necrosis factor
- Macrophage inhibitory factor
- Transforming growth factor
- Interleukin 1, 3-6, 9, 10, 13, 16
- CCL 2-5
- CXCL ligand 8-11

Action
- Pro and anti-inflammatory responses
- Immune regulation

Holdsworth and Summers 2008

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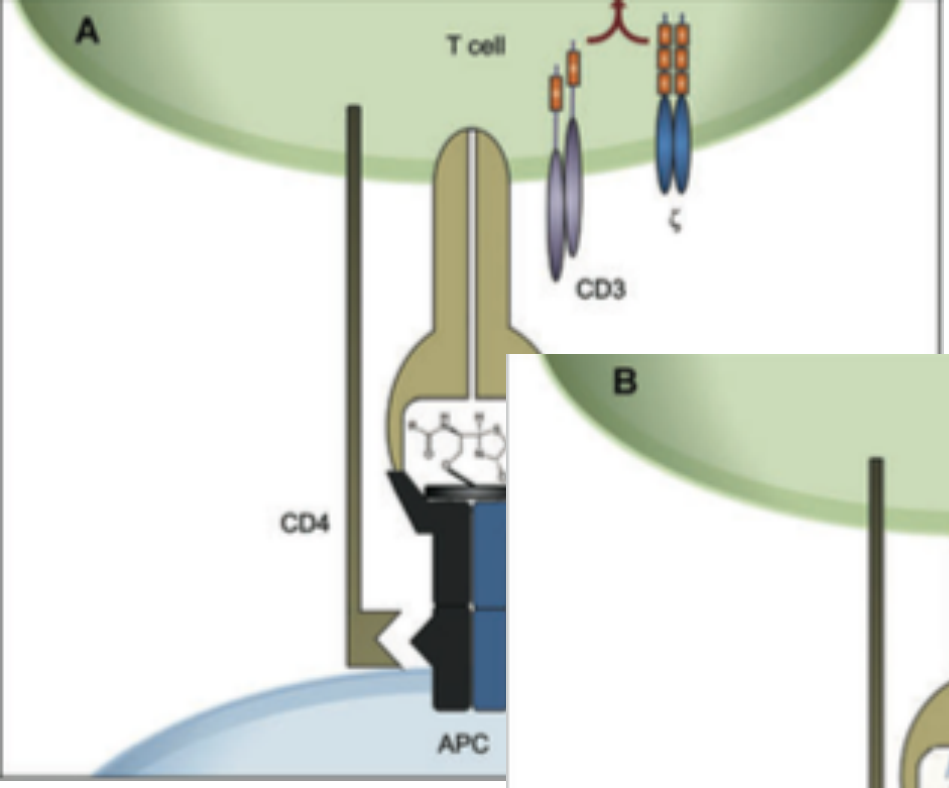
Non-Immediate hypersensitivity

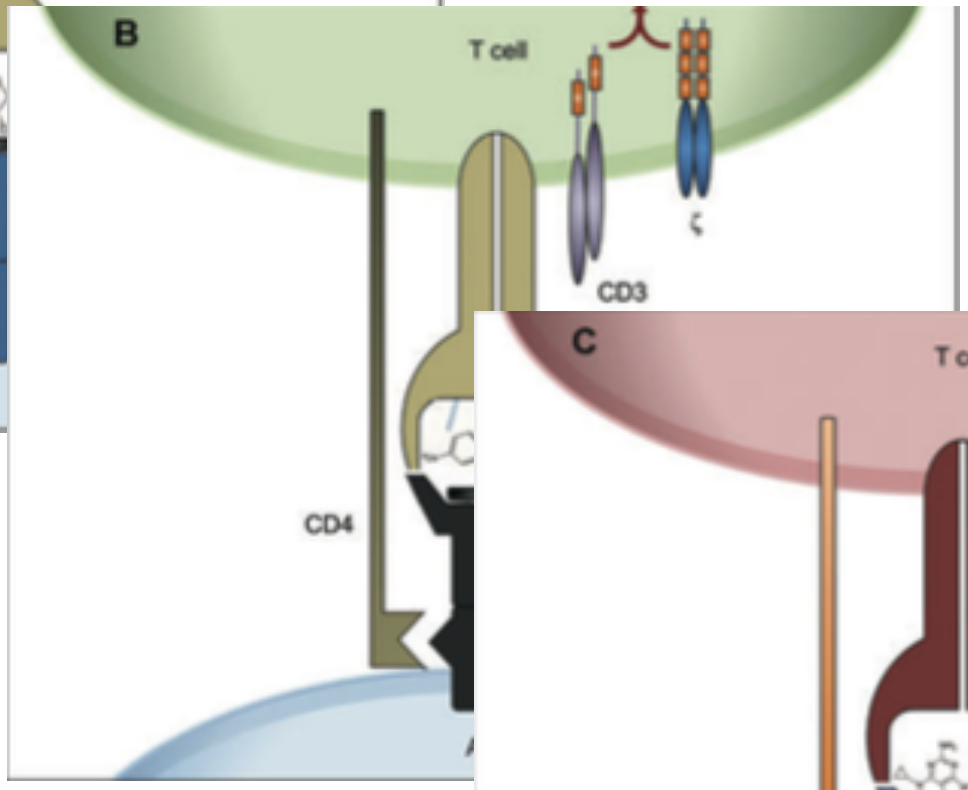
- Type IV – cellular hypersensitivity
- “Sensitisation” occurs down several different pathways:
  - Via typical DC/T-cell interaction for large peptides
  - Hapten, prohapten, P-I concept pathway for small otherwise non-immunogenic peptides

Hapten:

Small non-reactive molecules can covalently bind to extracellular, membrane or intracellular proteins Drug-protein complex can then be presented by HLA complex to T cells

Eg beta-lactams

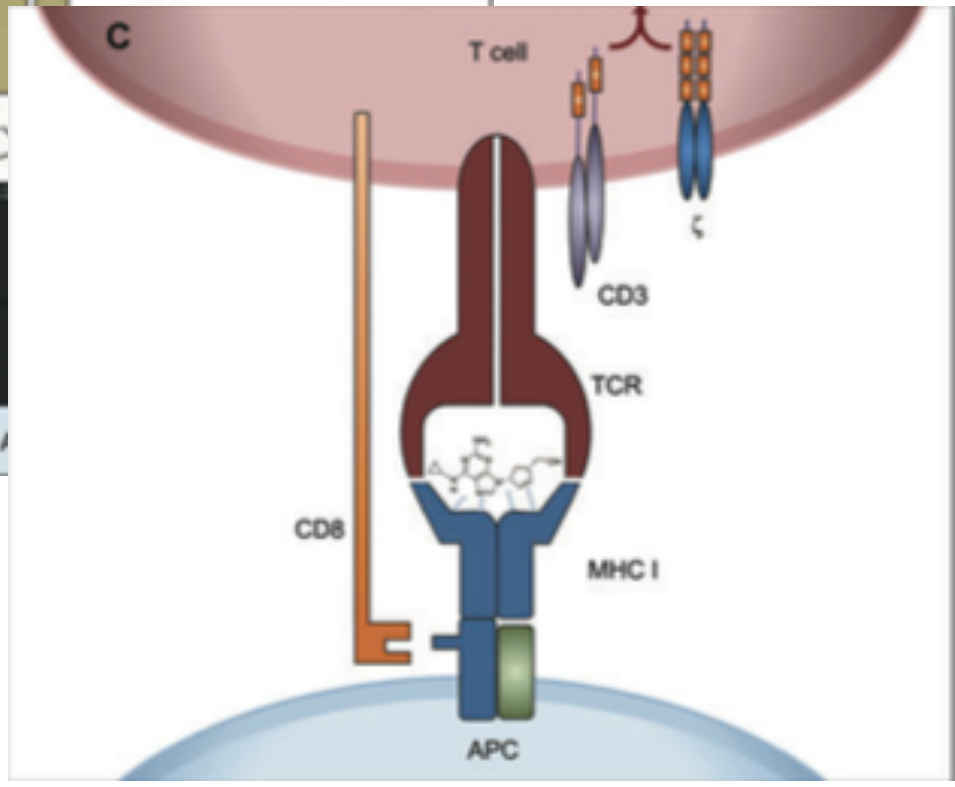




Prohapten:

Drug metabolites become chemically reactive and bind to proteins

Eg Co-trimoxazole

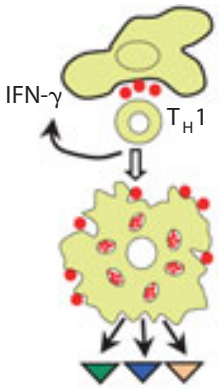
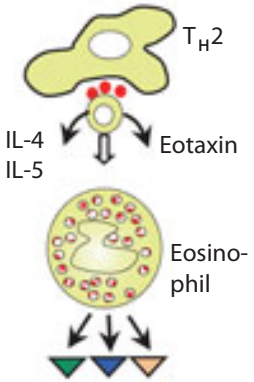
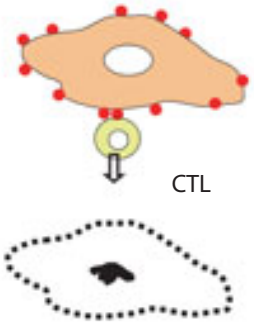
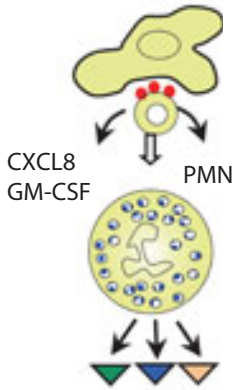


P-I complex:

Drugs directly interacting with HLA or T cell receptors (reversible binding) in a similar way to receptor-ligand interaction.
May account for reaction on first exposure to drug

Type IV

- Maculopapular exanthema, and delayed urticaria most common
- SCAR potentially life-threatening with high morbidity/mortality
  - SJS/TEN
  - DRESS
  - AGEP

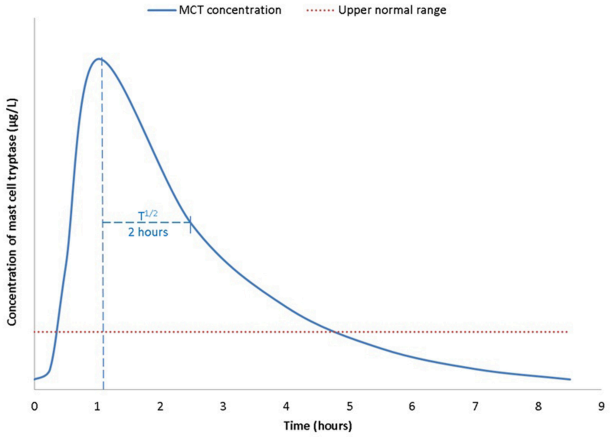
Type IVa	Type IVb	Type IVc	Type IVd
IFNγ , TNFα (T_H1 cells)	IL-5, IL-4/IL-13 (T_H2 cells)	Perforin/ granzymeB (CTL)	CXCL-8, IL-17 GM-CSF (T cells)
Antigen presented by cells or direct T cell stimulation	Antigen presented by cells or direct T cell stimulation	Cell-associated antigen or direct T cell stimulation	Soluble antigen presented by cells or direct T cell stimulation
Macrophage activation	Eosinophils	T cells	Neutrophils
 Chemokines, cytokines, cytotoxins	 Cytokines, inflammatory mediators		 Cytokines, inflammatory mediators
Tuberculin reaction, contact dermatitis (with IVc)	Chronic asthma, chronic allergic rhinitis Maculopapular exanthema with eosinophilia	Contact dermatitis Maculopapular and bullous exanthema hepatitis	AGEP Behçet disease

Hausmann et al 2012

Diagnosis of allergy

- Essentially clinical diagnosis
  - Suggestive clinical features + likely trigger
- Anaphylaxis def^{n}:
  - “A serious, life-threatening, generalized or systemic hypersensitivity reaction” (WAO)
- Serum tryptase clinically useful if considering anaphylaxis or severe immediate hypersensitivity
- Consider FBC – neutrophilia common

Tryptase



- Marker of mast cell turnover, or activation
- Most abundant preformed mediator in mast cells
  - Present in very small amounts in basophils
- Mast cells contain α and β tryptase:
  - α tryptase constitutively released (increased in systemic mastocytosis)
  - β released with mast cell activation (anaphylaxis)
  - Assay measures both α and β, therefore important to take peak sample (1-4 hrs after onset of event), and baseline (>24hrs after) and compare.
- Persistent elevation with level >20ug/L is minor criteria for systemic mastocytosis

Beck et al, Front Immunol 2019

Tryptase

- Serum tryptase level has sensitivity of ~70% and therefore normal level does not rule out anaphylaxis
- Most clinically useful change is 20% +2ug/L from the baseline level.
- Limited utility for food triggered anaphylaxis
  - Basophils in gut have less tryptase
- Sensitive to handling – specimen must be tested immediately or frozen down.
- Tryptase testing batched in lab, therefore results may take up to a week or more.

- Remember - diagnosis of anaphylaxis is CLINICAL and therefore do not rely on tryptase testing.

Other useful investigations

- FBC
  - Neutrophilia common in immediate hypersensitivity
  - Peripheral eosinophilia in DRESS and some other SCAR
- LFT, UEC
  - Abnormalities in DRESS, AGEP
- Laryngoscopy
  - Useful acutely in those with less typical symptoms of throat swelling
- Histology if rash

Identification of trigger

Testing options

- Immediate hypersensitivity
  - Serum specific IgE (RAST)
  - Skin testing
    - Skin prick / intradermal
  - Basophil activation test
  - Challenge
- Delayed hypersensitivity
  - Skin testing
    - Delayed read SPT / IDT
    - Patch testing
  - Lymphocyte transformation test
  - HLA testing
  - Challenge

Immediate hypersensitivity

Serum specific IgE testing (sIgE)

- Fluoroenzymeimmunoassay (FEIA)
- Measures Immunoglobulin E targeting specific allergens
  - Typically peptides
- Only a marker of sensitization, not clinical allergy
  - Provides no indication of mast cell reactivity to tested allergen
  - Common for atopic individuals to have detectable sIgE to a variety of inhaled allergens regardless of symptoms.
    - Critical to interpret in clinical context

Serum specific IgE testing (sIgE)

- Indications:
  - Urticaria, angioedema, anaphylaxis with suggestive trigger
  - Suspected food allergy
  - Immediate drug reaction
    - Only limited number of drugs available:
      - Penicillins
      - Cefaclor
      - NMBA
      - Latex, chlorhexidine
  - Likely allergic asthma or allergic rhinitis:
    - Perennial – HDM, Cat, Dog
    - Seasonal – Rye, Bermuda, Tree
  - Skin test contraindicated (bad asthma, eczema or antihistamines) or unavailable

- Can do mixes or single allergen – single allergen superior (better sensitivity and provides more useful information) especially if considering food allergy
- Native, component and recombinant sIgE available
  - May assist in differentiating cross reactive sIgE (e.g. hymenoptera allergy, peanut allergens)
  - Useful in tracking development of tolerance to some allergens

Serum specific IgE testing (sIgE)

- Limitations
- Drug allergy:
  - Only a restricted number of drugs available for sIgE in routine diagnostic testing
    - Penicillin (Pen V, Pen G, amox, amp)
    - Cephalosporins (cefaclor only)
    - Chlorhexidine
    - Latex
    - Neuromuscular blocking agents
  - Poor sensitivity for drug allergy
- Caution if low positive sIgE and very high total IgE
  - ? False positive
  - Unclear what level of IgE cut-off should be used for this
    - >1000-2000 kU/L

Skin testing

- Skin prick / Intradermal
- Small amount of allergen introduced in the epidermis (SPT) or superficial dermis (IDT) to interact with specific IgE bound to local mast cells
- SPT - Prick skin with small lancet through droplet of allergen solution
  - can also do 'prick-prick' test for fresh food
- IDT – small amount (0.02-0.05ml) of allergen solution (typically drug) injected
- Mediators are released leading to a "wheal and flare" reaction
- Low risk
  - Caution required:
    - Incident reaction severe
    - Poorly controlled asthma
- Must be done by skilled operators and interpreted correctly
- Indication:
  - SPT: Food, drug, venom, vaccine allergy,
  - IDT: Drug, venom, vaccine allergy
- Sensitivity and specificity are high when done correctly

Skin prick testing

- Correlation of wheal size and RISK of reactivity on challenge, but data mainly in children:
  - 95% PPV of reaction on challenge:
    - Peanut SPT >=8mm or sIgE of 34 kUA/L
    - Egg SPT >=4mm or sIgE of 1.7kUA/L
    - Sesame SPT >=8mm
- Unclear if this holds true in adults

- No correlation with SEVERITY of reaction

Basophil activation test

- Research only
- In vitro assay
- Patient's PBMC collected, and incubated with allergen
- Flow cytometry to determine degree of cellular activation
- Time-critical, logistically very difficult to set up in routine diagnostic laboratory.
- Requires experience operators
- Can be used for food, inhaled allergens,

Challenge

- Gold standard for determining allergy
- Graded doses of presumed allergen given at intervals
  - Patient observed
- Ideally interval should be slightly longer than incident response to allow a reaction to be noted prior to administering next dose.
- Highest risk of all investigations for immediate hypersensitivity
- Food, drugs most commonly assessed.
- Ideally undertake skin testing prior to challenge if possible.

Delayed hypersensitivity testing

Patch Testing

- Contact dermatitis
- Can be performed with a vast array of different compounds or series, e.g.True Test
- Compounds mixed with paraffin and applied to the skin in a small metal chamber.
- Site is observed for a reaction on Day 3 and Day 7
- Typically performed by Dermatology
- Sensitivity varies depending on compound, indication and time since incident reaction

Lymphocyte transformation test

- In vitro test
- Research only
- Drug hypersensitivity
- Can measure T-cell proliferation, activation or cytokine excretion in response to stimulus (incubation with suspect drugs)

Challenge

- ?????
- Generally severe reactions are considered absolute contraindications.
- May be considered in some drug reactions, especially those with absolute need for the medication, e.g. TB

- Not a path we typically advised for delayed hypersensitivity reactions.

HLA testing

- Certain HLA (MCH) demonstrated to carry greatly increased risk of reaction to certain drugs
  - SJS/TEN
  - DILI
  - Exanthema
- Strong relationship with ethnicity
- Typically HLA Class 1 alleles (interact with CD8+ cytotoxic T-cells)
  - Thought to be associated with SCAR development through P-I concept interaction, therefore no prior exposure needed)
- HLA-B*57:01 – Abacavir
- HLA-B*57:01 – flucloxacillin DILI
- HLA-B*15:02 – CBZ
- HLA-B*58:02 – Allopurinol
- Identification doesn’t confirm reaction, only increased risk.

- Thank you

- katherine.nicholls@mh.org.au if any questions!