Overview Of Allergy Testing Methods
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Inhalant Allergy Mechanism
- Antibody (Ab): allergen-specific IgE
- Binds to specific receptors on mast cells and/or basophils
- Antigen (Ag) prepared by antigen-presenting cells (APC)
- Ag bridges adjoining IgE molecules
- Mast cell degranulates, releasing preformed and newly formed mediators -> symptoms

Electron micrograph of IgE receptor

Methods of Testing
- Measuring allergen-specific IgE
  - Bound to mucosal mast cells: direct challenge
  - Bound to skin mast cells: skin test
  - Free in circulation: RAST, ELISA
- Measuring total IgE

Skin Testing
- Humoral (IgE)
  - Antigen + Sensitized mast cell = mediator release
  - Immediate wheal (edema) and flare (erythema)
- Cytokines (chemoattractants) and cells
  - Late phase, 4-6 hours later
- Cellular
  - Delayed reaction, 1-2 days later

Types of Skin Testing
- Patch test
- Epicutaneous tests
  - Scratch test
  - Prick test
  - single prick test
  - multiple prick devices
- Intradermal (Percutaneous) tests
  - Single dilution intradermal
  - Dilutional intradermal testing (eg, SET)

History of Skin Testing
- Blackley (1865), first skin test: scratch test
- Adapted for allergy, Smith, Walter (1909, 1917)
- Schloss (1912), intradermal allergy testing
- Cook (1915), quantitation of intradermal tests
- Lewis & Grant (1924), prick-puncture testing

Methods of Testing
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Patch Testing

- Introduced by John Draize (1940)
- Allergen is applied to skin under occlusion and is observed for delayed reaction (generally type IV)
- Indications:
  - Contactants
  - Chemicals
- Disadvantage: nonspecific response

TRUE patch test kit w/ template

Scratch Testing

- Produce scratch
- Apply extract
- Amount variable
- Read results
- Non-specific reactions common
- False positive and negative results
- Very technique-dependent

No longer advocated as testing method

(1987, AMA Joint Council on Scientific Affairs)

Prick (Prick-Puncture) Testing

- Prick skin through drop of extract
  - Tent skin to prick it, or
  - Use specific puncture instrument
  - Numerous variations exist
  - Antigen amount not quantified
  - Somewhat technique-dependent
- Read results and compare with positive (histamine) control wheal & flare
  - Several grading systems
- Safe for highly sensitive patients

Tip of Morrow-Brown needle

Prick Testing

- Advantages:
  - Rapid
  - Relatively safe
  - Good correlation with intradermal tests
- Disadvantages:
  - Lacks standardization and quantification
  - Misses low sensitivity responses
  - Affected by tester proficiency and interpretation

Prick-puncture testing

Prick Testing: Multiple Prick Devices

- More sensitive than single prick method
  - Strong positive roughly equivalent to #3 or #4 dilution IDT endpoint
- Less technique dependent than single prick
- Enables testing for numerous antigens more rapidly and efficiently
  - Up to 8 antigens per device
- Ex: Multi-test II, Quintest

Intradermal Testing (Conventional)

- Utilizing skin test syringe (short bevel), needle introduced bevel down
- Antigen injected to form wheal of specific size
- Wheal measured
- Most sensitive method of skin testing
- More antigen introduced than with prick test
Intradermal Testing (single dilution)

- Advantages:
  - Moderately sensitive
  - Reproducible
- Disadvantages:
  - Subjective interpretation
  - Qualitative only
  - Significant variation in wheal size and erythema

Intradermal Titration Skin Testing

- Philips (ca 1926)
- Levine & Coca 1926
- French Hansel 1936
  - Tested with 1:10 dilutions
- Herbert Rinkel 1952
  - Used 1:5 dilutions

Intradermal Dilutional Skin Testing

- Three methods
  - 1:10 dilutions: Some general allergists
  - 1:5 dilutions: ENT allergists
  - 1:3 dilutions: FDA, IUIS (for antigen standardization)

Why Use 1:5 Dilutions?

- 1:10 dilutions are faster, but do not give a precise enough endpoint
- 1:3 dilutions are accurate, but take too long
- 1:5 dilutions provide the best compromise between speed and accuracy
  - Rinkel, 1952

Intradermal Dilutional Testing (Skin Endpoint Titration)

- Intradermal testing with progressively stronger (five-fold) concentrations of antigen, starting with anticipated non-reacting dose
- Wheat which initiates progressive positive whealing signals safe starting point for IT
- Most reproducible method of skin testing
  - Used in antigen standardization (3-fold dilutions)
  - Highly quantitative

Skin Whealing Responses During Titration

- Intradermal injection of 0.01 ml of an inert solution (diluent) will form a 4 mm wheal, which normally spreads to 5 mm diameter
- Intradermal testing with an antigenically active substance at the lowest reactive concentration will cause a 5 mm wheal to enlarge at least 2 mm in diameter (7 mm wheal or greater)
- Intradermal testing with the next stronger 5-fold concentration will result in a further 2 mm wheal enlargement (9 mm wheal or greater)
Factors Affecting Whealing Response

- Degree of sensitization of cutaneous mast cells
  - Recent exposure
  - Prior immunotherapy
- Area of body tested
  - upper back > lower back > upper arm > lower arm
- Age of patient
  - pediatric, geriatric patients may be less sensitive

Factors Affecting Whealing Response

- Local axonal reflexes
  - Separate tests by 2 cm
- Concomitant foods
- Circadian rhythms
  - Most sensitive 7:00-11:00 PM
- Dermatopathology
  - Dermatographism
  - Eczema
- Medications

Medication Effect on Skin Whealing

- Suppress whealing; omit 48-72 hours
  - Antihistamines
  - All forms
  - Tricyclic antidepressants
  - Do not significantly affect whealing
  - Corticosteroids
  - Leukotriene modifiers
  - Bronchodilators
  - Decongestants
  - NSAIDS

Skin Test Controls

- Positive control (is the skin able to react?)
  - Histamine 0.0004 mg/ml
  - 4 mm wheal should grow to 7 mm or greater
- Negative control (reaction to physical trauma?)
  - Diluent
  - 4 mm wheal should grow to 5 mm or less
- Glycerine control (reaction to 2% glycerine?)
  - 2% glycerine (equivalent to #2)
  - Wheal formed by #2 antigen concentrations should exceed the size of this wheal by 2 mm
  - Similar method, using 10% glycerine, for #1 concentrations

Normal Whealing Response

- 5 mm = NEGATIVE WHEAL
- 7 mm = ENDPOINT
- 9 mm = CONFIRMATORY WHEAL

Abnormal Whealing Response

- 5 mm
- 7 mm
- 9 mm
- 15 mm
Technique for Intradermal Dilutional Testing (IDT)

Positive (histamine) and negative (diluent) controls
- ID injection of 0.01 cc of dilute antigen (anticipated non-reacting dilution) to form 4 mm wheal
- Wheal grows to 5 mm diameter by physical spreading
- Read wheal size at 10 mins; do not measure erythema
- Inject increasing concentrations (1:5) until wheal 2 mm larger than negative is produced (“endpoint”)
- Wheal from next stronger concentration should be at least 2 mm greater still in diameter (“confirming wheal”)
- The endpoint is defined as: “the first positive wheal that leads to progressive whealing”

Intradermal Dilutional Testing

Advantages:
- Very safe, even "in season"
- Very sensitive, even for low sensitivities
- Very few false positives or false negatives
- Quantitative measurement with positive and negative controls
- Safe guide to starting therapy
- Reproducible

Disadvantages
- Time consuming
- More supplies required
- More technical expertise of tester
- May produce aberrant skin test responses, necessitating retesting (or in vitro confirmation)
  - (Flash, hourglass, plateau)

Mechanics of Intradermal Dilutional Testing (IDT)

Patient assessment, informed consent
Choose antigens
Controls (histamine, diluent, glycerine)
Anticipated non-reacting concentration
  - Normally #6
  - May use #4 if not a “brittle” patient
Asthma, beta-blocker, prior reactions
Continue testing until endpoints determined
Calculate vial composition

Practical:

Prick-Testing Using Multi-Test