

TYPE I IMMUNOPATHOLOGY & PARASITE IMMUNITY

IgE AND PARASITES. ► Fighting parasites is the real reason we have Th2 cells and IgE. Worms are the most successful metazoan group; 4 out of 5 animals on land are microscopic nematodes! Our major parasites are single-celled protozoa, helminths (the general name for worms), and ectoparasites, which include things like ticks and mosquitoes¹. The closer your mouth is to the ground, the more likely you are to have parasites, and for wild-living rats IgE is the most important of the isotypes. The closer you live to the equator, the higher your chances are of having intestinal helminths, most of which are harmless. Worms in particular stimulate IgE responses. In fact, an old lab trick to get good IgE production in animals is to mix your antigen with adjuvant and ground-up roundworms. Recently it was shown that a particular protein of worms, incubated with dendritic cells, polarizes them such that they induce a strong Th2 response². What pattern-recognition receptor recognizes the worm protein (or more likely the protein-associated glycans) remains to be determined (it may not be a TLR).

► A person with a worm infestation will make both IgE and IgG against them. The IgG binds the worm or its ova, activates complement (to which worms are impervious) and C3a and C5a attract neutrophils. They arrive, seize the opsonized worm, and...nothing. Neutrophils lack a helminthocidal mechanism.

► That's where IgE comes in. As the worm sheds antigens they diffuse to nearby mast cells, which have become loaded with anti-helminth IgE. The IgE is cross-linked and the mast cells degranulate. Histamine causes gut smooth muscle contraction, and violent peristalsis can help expel worms. But the real action is in the late-phase response, where prostaglandins and leukotrienes are elaborated; as "ECF-A" they attract eosinophils in large numbers. Like other phagocytes, eosinophils have Fc receptors for IgG, which as we've noted is coating the worm. When an *eosinophil* engages an opsonized worm it release the contents of its granules, which include Major Basic Protein (the reason eosinophils bind the acidic dye eosin). MBP is highly toxic to helminths.

► The second part of the response to parasites is the Th2. Its close relative, the Th2-like Tfh, is busy in the lymph node helping B cells switch to IgE production. The Th2 itself goes out into the body like a Th1 cell does, and attracts both eosinophils and macrophages; but the suite of lymphokines a Th2 makes, IL-4, IL-5 and IL-13, turn the macrophages into alternatively activated M2 macrophages, the cells that heal damage and wall off M1-resistant invaders. This is fine and desirable in a parasite infection. If it becomes chronic, as in asthma, all that fibrosis is far from fine.

Eosinophilia in blood or sputum is a sign of either parasitic disease or severe Type I immunopathology.

TYPE I IMMUNOPATHOLOGY. Type I immunopathology involves IgE and mast cells, and is roughly equivalent to allergy; but the term allergy is widely misused to include even such obvious Th1 cell-mediated phenomena as delayed-type hypersensitivity and organ transplant rejection. Many people who are sure they have food

¹ A list of the most common protozoan and helminthic parasites is available as a Supplement on the web site (Supplementary material: Human endoparasites)

² Everts, B. et al. 2009. Omega-1, a glycoprotein secreted by *Schistosoma mansoni* eggs, drives Th2 responses. *J. Exp. Med.* 206: 1673-1680.

allergies do not; they have idiosyncratic reactions which are not IgE-based. In allergy studies antigens are usually called **allergens**.

For a long time the nature of allergies was mysterious³ and the different syndromes were not thought to be related. The first cogent experiments were done by Portier and Richet, surely the luckiest immunologists in history, as they worked in a lab aboard the yacht of Prince Albert of Monaco. They were trying to make a vaccine against the sting toxin of sea anemones, which inconvenienced the royal bathers. They injected dogs with the toxin, and expected those that survived (most, if the dose was low enough) to be protected against a second treatment. That is, they thought the “sensitization” would produce *prophylaxis*. What they got instead they called ***anaphylaxis***: the dogs experienced dyspnea and diarrhea, and went into shock, from which some died within 30 minutes. Further work on anaphylaxis earned Richet the Nobel Prize in 1913.

The serum of such sensitized dogs could transfer anaphylactic susceptibility to another dog. The ingredient in the serum that did it was named “reagin” and was shown to be specific for the allergen, and thus presumed to be a special kind of antibody. After years of claims and counterclaims, reagin was finally identified as IgE by Teruko and Kimishige Ishizaka in Denver⁴ in 1966. A patient with a rare IgE myeloma was identified the next year, allowing the structure of this isotype to be determined.

Studying the function and specificity of allergen-specific IgE was facilitated by a clever technique called “passive cutaneous anaphylaxis” in which serum from an allergic donor was injected intradermally in defined spots on the skin of a non-allergic volunteer. A day later, allergen could be injected into the same spots, resulting in an immediate wheal-and-flare reaction. Biopsies of the sites at various times gradually yielded the sequence of molecular and cellular processes.

► The basic mechanism is straightforward: IgE binds strongly to FcεR1 receptors on the surface of mast cells. When two adjacent IgE molecules so bound are cross-linked by allergen, the mast cell is signaled to release the contents of its characteristic granules, including histamine, which causes local or systemic vasodilation and increased permeability, itch, and gut and bronchial smooth muscle contraction.

SYNDROMES AND SHOCK ORGANS. What symptoms a person gets on exposure to allergen depends on the route of entry and other less-defined factors. In general, if the allergen is inhaled, the symptoms will be in the lungs; if allergen is in the air but not inhaled deeply, the symptoms may be in the oral, nasal, or conjunctival (eye) mucosa; if ingested, in the gut or (after absorption) skin; if administered systemically, the response may be systemic, too. Although Type IV antigens (e.g., poison ivy) that T cells see may penetrate intact skin, that is uncommon in Type I allergy.

³ **Surgeon:** “Allergies are undealt-with childhood issues. So if you have allergies, the treatment should be psychiatric. Something in your past is just hanging on...”

Intern: “Funny, I never had allergies until I came to the east coast.”

Surgeon: “Maybe it’s the nurses. The nurses are different.” (*Hopkins*, ABC-TV, 2008)

⁴ They were at Children’s Asthma Research Institute and Hospital, which became National Asthma Center, which merged with National Jewish Hospital, which became National Jewish Health. They named it IgE, it is said, because it reacted with the “E” antigen of ragweed.

Route of entry	Shock Organ	Syndrome
Intravenous	Blood vessels, skin, gut, lungs	Hives (urticaria ⁵), anaphylaxis, anaphylactic shock
Air exposure	Conjunctiva	Allergic rhinitis (“hay fever”)
Inhalation	Nasal mucosa	Allergic rhinitis (“hay fever”)
Inhalation	Lung	Bronchoconstriction, asthma
Ingestion	Oral mucosa	Oral allergy syndrome
Ingestion	Intestinal mucosa	Diarrhea, vomiting, anaphylaxis
Ingestion	Skin	Hives, eczema

Allergic (seasonal) rhinitis is also called hay fever, though there is no fever and hay does not cause it. It’s the runny nose and itchy eyes that Americans get in the August and September ragweed season; and other times from other pollens, or cats and dogs, and other things.

Eczema is chronic dry and easily irritated skin, itch, and rash. It tends to be self-worsening: itchy people scratch, and the microdamage to skin cells causes release of inflammatory cytokines, which further exacerbate the condition.

Oral allergy syndrome, OAS, is a recently-recognized special case of food allergy. This occurs almost immediately on putting the offending food in the mouth. The antigens seem to be able to pass through the mucous membranes of the mouth and gain rapid access to local mast cells, and eventually to mast cells at more distant sites. The symptoms include tingling lips and tongue, itching, and sometimes swelling of the lips. Though usually just a nuisance, it may lead to more severe and general symptoms and should not be ignored by either patients or their care providers. Studies have repeatedly shown that the foods that cause OAS contain proteins structurally similar to and cross-reactive with proteins in certain pollens. It is thought that the patient becomes sensitized (that is, makes IgE) in response to the pollen, which may or may not in itself trigger allergic symptoms. The food symptoms are oral because these particular antigens are destroyed in the stomach⁶.

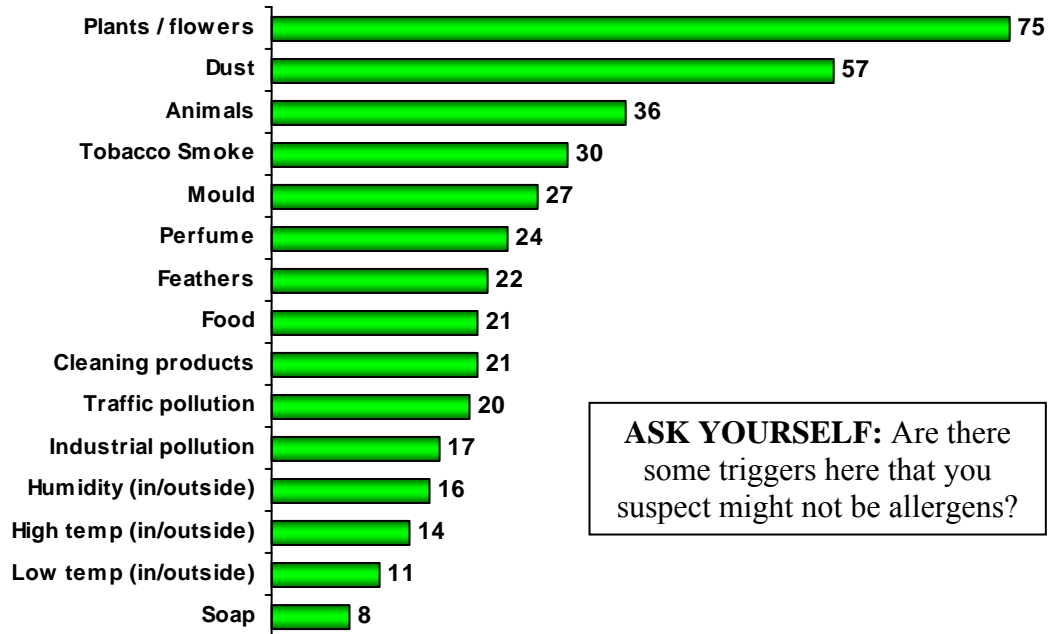
Asthma is both bronchoconstrictive and inflammatory, and no longer considered a mild disease. The inflammatory changes require specific treatment, with the aim of maintaining good control now, to avoid fibrosis in the future. Although low-income people are at lower risk of developing asthma (*see* Old Friends Hypothesis,) they usually do worse when they have the disease, since it requires the close monitoring and treatment to which they have inadequate access; and they often live in places with polluted air.

HYPER IgE SYNDROME. Also called Job’s Syndrome for the cutaneous manifestations and extreme suffering. This autosomal dominant condition is fortunately rare, and seems to be due to an inability to make IFN γ effectively. As you would suppose, thinking of Th1-Th2 sibling rivalry, this leads to poor Th1 responses and a predominance of Th2 cells. The signs and symptoms include very high serum IgE, skin abscesses, and fungal or *Pseudomonas* pneumonia.

⁵ Urticaria from *Urtica*, the stinging nettle plant, whose leaf hairs actually contain histamine!

⁶ This description is reproduced from an article by JJ Cohen in the 2007 McGraw-Hill Encyclopedia of Science and Technology.

ALLERGENS. This graph of triggers of allergic rhinitis, self-reported by the indicated fraction (%) of 3,500 European respondents⁷ is similar to what is seen in North America. Note that not all of these triggers are, or contain, allergens; non-specific symptom triggers are discussed below.



► Cross-reactions are common in atopic disease. For example, a person with T cell-mediated contact dermatitis to latex (gloves, catheters, condoms) may have symptoms of IgE-mediated oral allergy to avocados, bananas, or chestnuts, all of which contain a cross-reactive antigen. Cantaloupes cross-react with ragweed; people with ragweed rhinitis can have cantaloupe oral allergy syndrome, for example.

INCIDENCE. Allergic disease are among the most common conditions suffered by humans, a clear indication that this is a normal mechanism going slightly wrong. Estimates as high as 20% of all people have been published; 15% seem more likely to experience allergic symptoms at some time in their lives. ► There is a strong genetic component; risk of developing allergy climbs to 35% if a newborn has one allergic parent, and to 65% with two such parents. Allergic seasonal rhinitis is the most common, followed by food allergy and eczema in children, and by asthma. In 1995 the CDC estimated that 10% of the US population had had at least one asthma attack. The incidence of asthma nearly doubled in the US from 1980 to 1995. A lot of that increase is real, not just better diagnosis. Happily, deaths from asthma are decreasing every year.

⁷ Patient Voice Allergy Survey, 2005;

<http://www.efanet.org/enews/documents/AllergysummaryreportFINALdata220605-onsitea.doc>

THE ATOPIC STATE. Atopy, atopic disease, and allergy are all terms of somewhat vague definition⁸. It now means “prone to develop any of the range of allergic syndromes.” An atopic individual may begin life as an infant with eczema (also called atopic dermatitis), go on to have allergies to milk, fish, or eggs, and develop asthma in middle school or hay fever in college. Allergy (Greek “other response”) is roughly the same as atopy. It is an atypical immune response to environmental antigens, eventually becomes characterized by increased reactivity or hypersensitivity of the end-organs to inflammatory mediators and irritants.

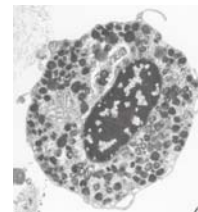
MECHANISMS: IgE PRODUCTION. IgE production is Tfh/IL-4 dependent, so these Tfh resemble Th2 cells. Development of symptomatic levels of IgE is slow; allergic people, when they move to a new continent, take about 7 years to show symptoms to the common allergens of the new environment. Multiple exposures to the allergen usually boost the immune response, resulting in symptoms that are increasingly severe. Patients may report that they can tolerate a snack of shrimp, for example, but burst out in hives when they make a meal of it.

Genome-wide single-nucleotide polymorphism studies in allergy and asthma have not yet yielded much in the way of new insights, and there is no smoking gun; rather, many loci have weak associations with these conditions, including transcription factors, cytokines, and end-organ specific transcripts. We are left, for now, with the notion that some of the propensity to make excessive amounts of IgE to environmental antigens is genetically determined, and much of the rest is due to conditions during early postnatal development.

MECHANISMS: THE IMMEDIATE REACTION. IgE binds to the main mast cell and basophil IgE receptor, FcεRI, with an association constant of 10^{-10} , which is so strong that the off-rate is expressed in months. Thus plasma IgE levels are very low (about 150 ng/mL); any secreted IgE is almost immediately bound by receptors. Highly allergic people can have IgE concentrations over 1 mg/mL, but that is unusual and total IgE levels are of limited use in diagnosis or prognosis.

IgE-loaded mast cells are triggered to release the contents of their granules when two adjacent IgE molecules are cross-linked by allergen, which must therefore be at least divalent. ► This explains why a normal individual may have IgE on her mast cells, but since the IgE represents the products of many weakly-activated clones, the chances of 2 adjacent IgEs being specific for 2 epitopes on the *same* allergenic protein are small; whereas in an allergic person, a few clones are responding strongly, and statistics will often put 2 IgEs from those clones side by side.

Mast cells in tissues and basophils in the blood are similar, though for the specialist, not identical; they come from the same bone marrow progenitors, but express different transcription factors. They have in common large basophilic granules⁹. Degranulation releases histamine, heparin, enzymes, and TNF. These are preformed in the granules so their action is very rapid; within 15 minutes of the intradermal injection of an allergen, a positive wheal-and-flare



⁸ Atopic means “out of place,” and so was originally one of the words doctors coin to mean “we don’t know.” Others are “idiopathic,” “agnogenic,” “primary,” and “essential.”

⁹ “Mast” is German for “food [like acorns] stored away,” as it seemed to early observers that mast cells might be stuffed with storage granules.

response (a “hive” or urticaria) is very obvious. ► Of the granule components the clinically most important is histamine, which will cause itch, blood vessel dilation, and leakiness. Its half-life in tissues is only a minute, so fortunately the reaction is transient.

MECHANISMS: THE LATE-PHASE REACTION. The activated mast cell also initiates a series of enzymatic steps: phospholipase PLA₂ cleaves arachidonic acid from membrane phospholipids, and arachidonic acid can be converted by the cyclooxygenase pathway to prostaglandins, and by lipoxygenase to leukotrienes. ► These active compounds initiate inflammation, constrict bronchioles, and are together called “eosinophil chemotactic factor of anaphylaxis” or ECF-A, because they are particularly good at attracting eosinophils. Cytokines are also released by the activated mast cell.

Thus Type I reactions are often observed to have two phases. The immediate reaction is due to histamine, and can be blocked effectively by antihistamines, which are receptor antagonists. The late phase, beginning perhaps 4 to 10 hours later, is not affected by antihistamines, as it depends on prostaglandins, leukotrienes, and cytokines. Since it is the important feature in the bronchoconstriction of asthma, it is not surprising that antihistamines have only a minor role to play in that condition. Similarly, atopic dermatitis (eczema), which seems to be chronic late-phase Type I immunopathology, needs anti-inflammatory rather than histamine-blocking therapy, though antihistamines can help if itching is prominent. Chronic Type I reactions often show, on biopsy, infiltration with Th2 cells and eosinophils. The eosinophils are attracted by ECF-A from mast cells, and by IL-4 released by Th2. They are there, of course, because the body is convinced the allergen is actually a protozoan or worm.

NON-SPECIFIC TRIGGERS. People with allergic asthma will react to the specific allergen, for example cat dander, but also to irritants in polluted air, to which they are not specifically allergic. This hyperreactivity is sometimes called “twitchy lungs.” When asked to inhale a very dilute aerosol of histamine, asthmatics will begin to show bronchospasm at far lower concentrations than do people without the condition. This is why asthmatics must be careful, and may have to stay indoors, on heavily polluted days, and why respiratory disease hospitals ban smoking and the wearing of perfume.

Dry air can trigger bronchospasm in hyperreactive lungs; this is probably the true reason for exercise-induced asthma, as panting dries the airways. Viral infections of the mucosa likewise stimulate nerve endings and can trigger an asthma attack. Some people have mast cells that are easily triggered to release histamine by mild trauma, such as stroking the skin with a pen or stick; the extreme form is called dermographism. Others may have mast cells triggered by cold¹⁰, and have to take special precautions outdoors in winter. There are surely genetic polymorphisms responsible for these behaviors but they are not yet identified.

There is a syndrome of asthma, nasal polyps, and aspirin intolerance, which seems to be based on abnormal prostaglandin production. Aspirin exposure in these patients can cause severe, even fatal asthma attacks. This is not a Type I allergic condition.

¹⁰ I have seen a patient with a square hive caused by holding an ice cube to her skin; below it was a perfect trickle-shaped hive!

DIAGNOSIS. In allergy, **history** is central to diagnosis and the best allergists are the best detectives. What is causing this child to wheeze at night? If necessary, the parents must empty the bedroom, clean it extensively, and put the child to bed on the floor on a plastic sheet. If symptoms do not recur, then a pillow can be added, and a mattress, and toys, and so on, until the symptoms are triggered and the offender identified. Specialist units have environmental chambers where allergens can be introduced in a double- or single-blind fashion, in the air or in orally ingested capsules. The time of year of symptoms is significant, and daily pollen counts are a valuable resource for the patient and the clinician. Family history, as described above, is also crucial.

Skin testing plays a useful role. It is easy to conduct a skin prick test; a drop of allergen extract is placed on the forearm or back and a fine hypodermic needle or special lancet is used to just prick the epidermis through the drop. The test areas are observed at 15 or 20 minutes and the results recorded as diameter of the central raised wheal/ diameter of the flare (e.g., 5/15 mm). Testing with buffer is necessary to control for skin hyperreactivity. All patients should be observed for 20-30 minutes after skin testing. ► A positive skin test does not necessarily mean that your symptoms are due to that allergen; your level of sensitivity may be subclinical even with a positive test, or your symptoms may come from something else that cross-reacts with the test extract.

The pioneering radio-allergo sorbent test or RAST has been replaced by an improved lab test called **CAP-FEIA** (a Pharmacia name; CAP because it is done in a sort of capsule, + Fluorescent Enzyme ImmunoAssay). It is usually standardized in large laboratories, to which the clinician sends a sample of the patient's serum. CAP-FEIA is similar in concept to the ELISA. An allergen is fixed to a capsule, the patient's serum added and then unbound proteins washed away, and the presence of bound IgE is revealed by quantifying the binding of enzyme-tagged antibodies against human epsilon heavy chains. The enzyme substrate used is not fluorescent, but becomes so after cleavage. The result is reported in arbitrary units; a score above a certain level correlates well with symptoms. ► CAP testing is completely safe, unlike skin tests which have some risk. It is mostly used as part of the diagnosis of food allergies.

Asthma is defined as a reversible bronchoconstrictive disease with progressive inflammation leading to fibrosis. It is easily tested for by **spirometry**, the measurement of air flow. The patient blows into a mouthpiece connected to an apparatus that measures air flow rates and volumes. Various measures can be made; the most often used is the FEV1, which is the volume of air that can be forcibly exhaled from full lungs in 1 second. It is measured as a baseline and then the patient inhales a bronchodilator and repeats the test; a significant improvement in FEV1 indicates a bronchoconstrictive condition. For a long time it was observed that this value would inevitably decline in asthmatics, even with good bronchodilator treatment. Now we know that the late-phase reactants and Th2 cells present in the lung are pro-inflammatory, and untreated chronic inflammation inevitably leads to fibrosis, which is irreversible. So inhaled glucocorticoids are added early to the asthma treatment regimen; they are scarcely absorbed and can be used, with monitoring, in growing children without the serious side effects associated with systemic steroid treatment.

TREATMENT. If you understand the pathophysiology, the treatment is logical.

Avoidance. The cornerstone of treatment, but getting patient compliance is problematic. Given the choice, many families would rather get rid of the patient than the cat.

Antihistamines. These are effective for the early, histamine-dependent phase, and work well if symptoms are acute.

Epinephrine. A powerful sympathomimetic catecholamine that constricts blood vessels and relaxes bronchial smooth muscle, it is the first line of treatment in emergencies. People with significant allergic symptoms like airway constriction or anaphylaxis carry preloaded epinephrine injection units.

New York Times—

Ray Shaw, a retired president of Dow Jones & Company, who went on to build a family of local newsweeklies in 40 cities around the country, died Sunday (19 July 2009) in Charlotte, N.C. He was 75.

The cause was complications of a severe allergic reaction to a wasp sting he received a day earlier, according to his son Whitney Shaw.

Theophylline can be given by inhaler. A phosphodiesterase inhibitor, it raises intracellular levels of cyclic AMP in bronchial smooth muscle, causing relaxation. It is no longer a first-line drug, and is used only occasionally.

Glucocorticoids. Excellent treatment when their effects can be kept local, for example in pulmonary inhalers or as ointments; they are risky with multiple side effects when used systemically. They inhibit the production of arachidonic acid from phospholipids and thus block both PG and LT synthesis. They also induce apoptosis in eosinophils.

Leukotriene inhibitors. New drugs are available which either inhibit LT synthesis or block their binding to receptors. They can be superb additions to the treatment of asthma. [COX-2 inhibitors and NSAIDS do not play a role in treatment, as by blocking prostaglandin (PG) synthesis they shunt arachidonic acid into the equally-harmful leukotriene (LT) pathway.] Montelukast (Singulair[®]) is the most commonly used receptor antagonist. Zileuton (Zyflo[®]) is the main LT synthesis inhibitor.

LABAs (long-acting beta-2 agonists) rapidly reduces bronchoconstriction. They are most often given in a combination with an inhalable steroid. Fluticasone/salmeterol (Advair[®]) is the dominant drug in the market, with sales of about \$8 billion annually. Budesonide/formoterol (Symbicort[®]) is rapidly catching up.

IgE blocker. For treatment of moderate to severe asthma in people 12 and older who do not respond adequately to inhaled steroids, it is possible to use a monoclonal antibody against IgE. There is so little IgE in the blood that it can all be effectively mopped up by the mAb, which is called omalizumab (Xolair[®]).

Immunotherapy. “Allergy shots” are dilute solutions of allergen extracts, given subcutaneously once or twice a week with increases in the concentration as tolerated. When the maximal dose is reached shots are given monthly. About 75% of people with seasonal rhinitis say they have an easier season after a course of immunotherapy. Shots are now available for insect venoms, too. Treatment may go on for several years. The mechanism is not clear; many think that the route of administration favors IgG production, and that the IgG effectively traps and clears the allergen before it can reach IgE-loaded mast cells. Others believe that repeated antigen exposure desensitizes mast cells. Lately, an increase in Treg has been observed. Many centers are testing, and some are using, oral allergen administration for desensitization to foods, mostly in kids.

Learning Objectives for Immunopathology Type I

1. Define:
 - atopic
 - immediate hypersensitivity
 - allergy, allergen
 - anaphylaxis
 - asthma
 - hives, wheal-and-flare reaction
2. State the approximate incidence of atopic diseases in the general population, and in individuals with allergic parents.
3. Describe the mechanism of IgE-mediated hypersensitivity in terms of: IgE attachment to basophils or mast cells; reaction with allergens; mediator release; effects of mediators on target tissues and cells.
4. Discuss the features that the various atopic diseases have in common which justify lumping them together.
5. Discuss the reasons for using glucocorticoids in asthma treatment.
6. Discuss intradermal skin tests with reference to procedure, safety and specificity.
7. Discuss specific immunotherapy of allergic disease, considering duration of effect, risk of anaphylaxis, and percent of patients obtaining significant relief.
8. Describe the immediate allergic reaction and the late-phase reaction in terms of:
 - time course of the reaction
 - mediators involved
9. Discuss the roles of IgG, IgE, M2 macrophages, and eosinophils in helminth immunity.