

## TYPE IV IMMUNOPATHOLOGY

**T CELL-MEDIATED IMMUNITY & DELAYED HYPERSENSITIVITY.** Delayed-type hypersensitivity (DTH) is an older term for T cell-mediated events that are considered undesirable or injurious; Type IV immunopathology is a somewhat newer term. When the very same mechanisms produce helpful immune responses, they are referred to as (T) cell-mediated immunity. Since we can't always decide whether a reaction is good or bad, it is most reasonable to just say "T cell-mediated" mechanisms. They are the only type of immunopathology (of Types I, II, III and IV) which do not require antibody or B cells. Since nothing in medicine is simple, real diseases often involve both Type IV and antibody-mediated phenomena; this isn't surprising, given that ► the underlying problem may be disordered immune regulation. As we'll see in several examples, there is a tendency for autoimmune conditions to begin with antibody (which can be asymptomatic) and then symptoms develop or worsen as T cell responses predominate.

Some examples where Type IV represents all or most of the mechanism:

- Rejection of allografts
- Graft-vs.-host disease (GvHD) - the reverse of allograft rejection
- A positive tuberculin skin test
- Resistance to *Mycobacterium tuberculosis*
- Resistance to fungal infections
- Contact dermatitis, e.g., poison ivy
- Chronic beryllium disease
- Many autoimmune diseases, e.g. multiple sclerosis
- Tumor immunity

**IMMUNIZATION AND EFFECTOR PHASES.** This is mostly a review of T cell mechanisms we've already considered, but now it's important that we have a clear understanding of the difference between the **initiation** of an immune response following first exposure to the antigen (immunization phase), and the **elicitation** of a reaction in a person who is already immunized (effector phase).

**Initiation.** Consider poison ivy, an example of contact dermatitis due to the oil of *Toxicodendron* (formerly *Rhus*) *radicans*. It contains the compound urushiol<sup>1</sup> which can penetrate intact skin and become associated with MHC on dendritic cells (either by binding directly to MHC, or by binding peptides which then get presented on MHC). The dendritic cell travels to the draining lymph nodes, where it presents its MHC plus antigen to the appropriate Th0 precursors, which develop into Th1 and Th17 cells. These begin to divide in the usual way, but by the time increased numbers of them are in the circulation, the antigen has been washed or worn off the skin, and there is no reaction. ► So at the time you became immunized (older word: "sensitized") you probably didn't know it happened.



*If leaves be three, it's time to flee.*

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<sup>1</sup> *Urushi* (漆), Japanese for lacquer. *Toxicodendron* species oil is the main ingredient in many traditional lacquers, which have to be applied carefully by experts. The Temple of the Golden Pavilion (Kinkaku-ji) in Kyoto, the most beautiful building in the world, is finished in urushi lacquer, covered by thick gold leaf.

**Elicitation.** Now imagine that, some months later, you take another walk through the forest and again encounter poison ivy plants. The oil rubs off on your skin and urushiol again associates with MHC on antigen-presenting cells. This time though, ► memory T cells from the expanded clones are throughout the body, and rapidly get activated in the area where the oil has been deposited. They secrete interferon- $\gamma$  which attracts and activates a large number of macrophages. The result is a *firm* red area of inflammation that, because of all the cellular events that need to take place, begins to be visible in 6 to 12 hours, and peaks at 24 to 48 hours, thus earning the label ► delayed- type hypersensitivity<sup>2</sup>. Breakdown of the skin often leads to ► blistering.

**Memory T cells** are persisting cells in a clone that was expanded by contact with antigen. The key thing is that there are more of them than in a naïve person. They also have ► a lower activation threshold, so that it takes less antigen for elicitation of a reaction than it did to immunize in the first place. Memory cells, with the surface marker CD45RO, can be distinguished from naïve Th cells which have CD45RA.

**Tuberculin skin testing.** The Mantoux skin test is the one most commonly used in the USA. In it, 0.1 mL of PPD—purified protein derivative, a standardized preparation of *M. tuberculosis* antigens—is injected intradermally. (It is necessary to see a skin “bubble,” because if not the injection has gone subcutaneously, and will diffuse away before the reaction can get established.) ► The antigen is taken up by local macrophages and dendritic cells, and presented on MHC Class II. If the subject has an increased number of anti-tuberculosis Th1 cells, they will come by and get stimulated, produce IFN $\gamma$ , and attract macrophages. The test is read at 48 hours, and the diameter of the induration (firm raised part) is measured; 15 mm is always positive, and 10 or even 5 mm can be called positive under certain conditions, for example if a person is partly immunosuppressed.

The induration is significant, since it represents a cellular infiltrate. You may remember that one activated Th1 can attract 1000 macrophages, so ► these, not Th1, would be the predominant cell you’d see if you biopsied the site at 48 hours.

The TB skin test emphasizes what we just said about memory cells: The dose of PPD needed to elicit a positive reaction in an *immune* person is far lower that would be required to initially immunize him or her. Therefore, it turns out that the tiny doses of ► **TB skin tests are not immunizing**, and they can be repeated regularly without the subject becoming positive. Memory cells are long-lived, and after immunization with vaccine or by infection you will stay skin-test positive for years, though not necessarily forever.

Exposure to many other environmental antigens can produce delayed-type hypersensitivity. So when we want to determine if a patient has normal T cell function, we perform skin tests just like the Mantoux test, using a panel of common antigens, which may include tetanus toxoid, *Candida* (yeast) extract, PPD, and Trichophyton (from a common skin fungus). Studies have shown that over 95% of adults will have a positive DTH response to at least one of these, so a negative panel suggests “**anergy**” and requires follow-up investigation.

**Immunization to TB antigens** normally happens during a primary infection, which is usually unapparent to the patient, so a positive routine skin test usually comes as a surprise. Exposure to certain of the 200 other species of *Mycobacteria* can sometimes cross-react to produce a false-positive skin test. In many countries, Bacille Calmette-Guérin (BCG) vaccine—it is attenuated bovine tuberculosis bacteria—is given to newborns, and most people so immunized have positive PPD skin tests due to cross-reaction.

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<sup>2</sup> Of course, a Type I skin reaction, which peaks in 15-20 minutes, is “immediate hypersensitivity.”

**T CELL-MEDIATED IMMUNITY IN VITRO.** The lab can do a variety of tests, whose principles should be familiar to us by now. Whole blood or white blood cells (you need both T cells and APC like macrophages) may be incubated with antigen in cell culture, and activation observed: one could count cell numbers for proliferation, look at cell size for activation (“blast transformation”), or at DNA synthesis using radiolabeled precursors. Cytokines released into the medium can be quantified. None of these is a routine test, however, except:

The **QuantiFERON®-TB Gold** test is new, very nice, and is preferred (2010, CDC) to skin testing when the subject has had BCG immunization. Purified *M. tuberculosis* proteins (with only human-specific, no cow cross-reactive epitopes) are added to a sample of whole blood, and after incubation, interferon- $\gamma$  is measured in the medium by a capture ELISA assay. Unlike the skin test, it remains negative in people vaccinated with BCG (the human epitopes do not cross-react with BCG) allowing you to distinguish infection from previous immunization.

**ASK YOURSELF:** What does this tell us about the antigenic relationship between *M. tuberculosis* and BCG?

**CYTOTOXIC T LYMPHOCYTES IN DTH.** These have been much less studied than Th1 in T cell-mediated immunity, because there is no in vivo test for them. They take part in most manifestations of T cell-mediated immunity, and are quite important in many autoimmune diseases, tumor immunity, and transplant rejection. To demonstrate their presence, we need a suitable target cell (for example, an antigen-presenting cell exposed to the antigen, or any cell infected by it, if that is possible; sometime, normal cells can be soaked in an epitope-sized peptide which associates directly with MHC without having to be processed.) These are then mixed with the patient’s T cells (or purified CD8 cells) and after several hours, target cell death is measured, usually by the release of intracellular contents.

**CONTACT DERMATITIS.** This condition is also called contact hypersensitivity or contact sensitivity or, incorrectly, contact allergy; ‘allergy’ should be reserved for IgE-mediated events. The classic example of this is poison ivy, but many other chemicals can cause it; the main requirements are that they pass through intact skin to reach antigen-presenting cells, and they associate with MHC Class II. Metals like nickel (used in plated goods, including jewelry, watch straps, garters); chemicals like paraphenylenediamine, the only permanent hair dye; latex in gloves; topical antibiotics like neomycin and bacitracin; plants, including poison oak and poison sumac; soaps, detergents and industrial chemicals. How do you treat these? Avoidance, and topical steroid creams or ointments.

**HLA AND DRUG REACTIONS.** You would expect that there would be an association between HLA haplotype and drug reactions, and you’d be right. Here’s a very specific example.

A 27-year old man living with AIDS came to a clinic complaining of continual low fever, a rash on body and limbs, malaise, and variable GI, respiratory, and musculo-skeletal symptoms. A careful work-up revealed no current infections with opportunists that could explain these symptoms. He dated their onset to 5 months previously, when his medications were changed. The doctors decided to increase the dose of one of his antiretrovirals, abacavir, thinking his HIV might not have been adequately controlled. Within 2 weeks he returned to the emergency room with exacerbations of almost all of the symptoms, at which time a third-year medical student identified the problem.

Up to 8% of people who are given abacavir, a nucleoside reverse transcriptase inhibitor, for HIV, develop *abacavir hypersensitivity syndrome* which is quite awful and difficult to diagnose correctly. Nearly all people with the syndrome are HLA-B\*5701. We now test for this allele before offering the drug, a good example of “personalized medicine.”

Note that HLA-B\*5701 is Class I, not the Class II which is recognized by Th1. This drug reaction is predominantly a CTL problem. Work by P.T. Illing et al. in 2012 showed that abacavir changes the structure of HLA-B\*5701 so that it binds certain **self**-peptides that are not, of course, normally presented; the syndrome is actually a drug-induced autoimmune reaction!

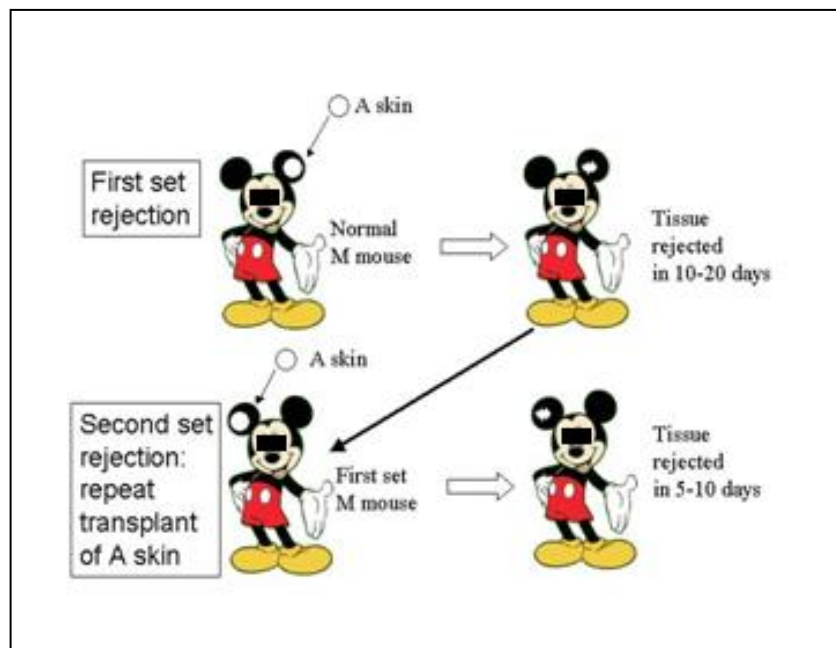
**ASK YOURSELF:** Can you remember another connection between HLA-B57 and HIV?

The strongest association (OR > 1,000) between HLA alleles and drug-induced hypersensitivity has been detected for carbamazepine<sup>3</sup> in the Han Chinese population. The association is also in Thai, Malay, and Indian populations, but not in Caucasians. The allele is HLA-B\*1502 and the correlation is specifically with a nasty condition called Stephens-Johnson Syndrome, or similar though nastier Toxic Epidermal Necrolysis, both of which are probably CTL-dominated forms of Type IV immunopathology of skin.

**GRAFT REJECTION.** The study of the rejection of foreign tissue grafts is of great importance in medicine. Rejection is a complex phenomenon eventually involving most or all of the specific immune and nonspecific amplifying elements of the immune system. Allograft immunity shows specificity and memory:

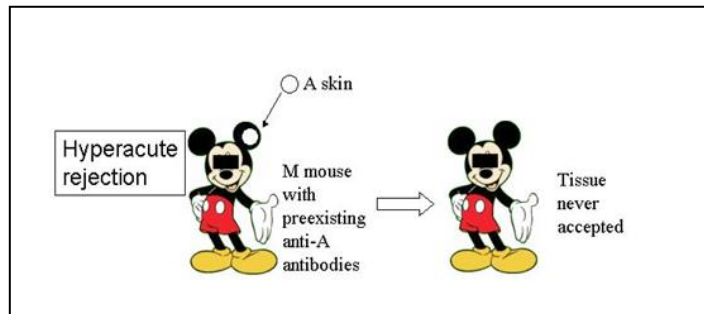
**The first set reaction.** A skin graft from mouse strain A to strain M is rejected in 10-20 days. Remember that 5-10% of the recipient's T cells will be able to react with the foreign MHC, even before grafting, because some foreign MHCs look like self MHC + a peptide. It is these cells that cause graft rejection in 10-20 days. But as this process proceeds, the recipient's response to A's MHC is boosted, and it develops even more anti-A Th1 and CTL.

**The second set reaction.** Another A skin graft is placed on same M recipient. It is rejected in 5-10 days. This is a secondary response resulting from T cell memory developed during the first exposure. It is specific: a first graft from unrelated strain C will be rejected in 10-20 days.



<sup>3</sup> Used to treat seizures, nerve pain, and bipolar disorder.

**Hyperacute or “white graft” reactions.** If you keep putting A grafts onto B, eventually they will be rejected even before they heal in, that is, they stay white and bloodless. This is due to the development of *antibodies* to histocompatibility or other cell-surface antigens.



Hyperacute rejection is common when xenografts (from another species) are attempted. It's usually because of pre-existing antibody to ubiquitous carbohydrate epitopes which are present in the foreign species but not in the human. In this regard they are similar to the ABO blood group antigens, which we'll discuss later. People are going so far as to try to breed transgenic pigs that lack these carbohydrates, as potential organ donors for human patients.

**AUTOIMMUNE DISEASES.** Many conditions are clearly autoimmune, and T cells are involved in the pathogenesis. Some of these conditions also involve autoantibodies, and thus there is both Type II and Type IV immunopathology. Which comes first, or is most important? That is still controversial. For example, **multiple sclerosis**, the demyelinating disease in which T cell reactivity to an autoantigen (myelin basic protein) was first shown, responds to therapies directed at T cells, such as the humanized monoclonal antibody natalizumab. ► But it also responds to the B cell-depleting monoclonal rituximab.

**ASK YOURSELF:** Do you have suggestions for how depleting B cells could make a Th-dependent disease better?

How can one make an immune response against one's own brain? This is a critical question. The brain is, in fact, ► antigenic in its own, but not immunogenic. As long as you keep it in your head, you should not have a problem. Even if T cells find their way into the normal brain, they will not be stimulated, because that would require (at least) professional antigen-presenting cells, an innate response, and cell damage; common in skin but not in the well-defended brain.

But here is a fascinating and sad story<sup>4</sup>: An interpreter and a public health nurse noticed neurological symptoms in several workers in a **meat-packing plant** in Minnesota in 2007. On further study 18 workers at 3 plants were diagnosed with progressive inflammatory neuropathy, or PIN, with weakness, partial paralysis, and peripheral nerve root demyelination. All the affected workers were employed at “head tables” where their job was to blow the brains out of pig heads using compressed air. The workers wore protective goggles but nothing over their noses or mouths as they blew the brains out, creating aerosols.

If brain is removed from one mouse, mixed with innate-immunity stimulating adjuvant, and injected into a syngeneic mouse, the recipient will come down with what is called experimental autoimmune encephalitis. This is considered a useful, though not perfect, model for multiple sclerosis; and it seems to be pretty much what the poor meat packers are suffering from. ► So the bottom line here is that if you make brain into an immunogen by presenting its antigens to your immune response in the proper way (that is, so it can be picked up by dendritic cells and carried

<sup>4</sup> MMWR 8 February 2008.

to lymph nodes) then you will make activated T cells and they will have no trouble entering and attacking the corresponding organ, even if we have always thought that cells there were “sequestered” from the immune system, for example behind the blood-brain barrier.

**MOLECULAR MIMICRY.** Several groups have studied the way myelin basic protein peptides sit in MHC Class II, and analyzed the distribution of positive and negative charges over the surface that would contact a T cell receptor. Using the information to scan a database of microbial proteins they have found several cases where a viral peptide, whose sequence is not necessarily the same as the MBP sequence, but which has close structural similarity, that is, distribution of charges and hydrophobicity, acts as a strong stimulator of clones of T cells derived from MS patients. Could a prior infection with such a virus produce activated T cells that could then enter the brain and attack the myelin there? It would appear to be so: It’s been shown in mice that Theiler’s murine encephalomyelitis virus infection can trigger MS-like, myelin-specific, autoimmunity.

**HASHIMOTO THYROIDITIS** is characterized by a destructive attack by T cells on thyroid antigens. ► Almost 1.5 million people in the US have the disease, the most common cause of **hypothyroidism**. Like many autoimmune diseases, it has a familial tendency; and families with it also have increased incidence of other autoimmune diseases, like type 1 diabetes, vitiligo, and gluten-sensitive enteropathy (celiac disease). It is about 5 times more prevalent in women than men. Although most investigators think the T cells are pathogenic, Hashimoto also involves anti-thyroid antibodies, whose presence is commonly used to confirm the diagnosis. A variety of environmental agents have been proposed, to explain the huge increase in incidence in certain regions (notably, Sicily).

**SJÖGREN SYNDROME** is said to be the second most common autoimmune disease, but that’s only an estimate as it is difficult to diagnose; symptoms are highly variable until the characteristic dry eyes and mouth develop, which can take years. It is an autoimmune reaction against exocrine glands, especially those that secrete tears and saliva; little is known about its etiology, and pathogenesis seems to involve CTL. Like all these conditions, Sjögren has genetic and environmental predispositions.

**TYPE 1 DIABETES.** Autoimmunity is strongly implicated in Type 1, or juvenile, or insulin-dependent diabetes mellitus (T1D). Depending on the technique used, antibody to  $\beta$ -cells (the islet cells in the pancreas that produce insulin) can be detected in the serum of over 90% of patients at the time of diagnosis (normals, fewer than 2%). In fact, if you study children at genetic risk of T1D, they have antibodies for years before developing hyperglycemia. Tissue obtained at autopsy from an occasional patient early in the disease show antibody and typical inflammatory responses. Although antibody is a useful marker, and B cells may play a role as APCs to the harmful T cells, T cells are dominant in the pathogenesis of T1D. It brings up many interesting questions; for example, should antibody-positive people be treated even before diabetes develops? Studies have tried to induce oral tolerance by feeding such kids small doses of insulin; it is thought to induce insulin-specific Treg in the gut, which, it’s hoped, could travel to the pancreas.

There is a strong HLA association between T1D and HLA-DR3 or HLA-DR4. These are in linkage disequilibrium with HLA-DQ2 and HLA-DQ8, respectively. The DQ genes are the real problem; they have unusual amino acids placements in the antigen-binding groove that allows ready presentation of islet cell-associated peptides. The best animal model, the NOD mouse, has an unusual Class II MHC molecule (H2 I-A<sup>g7</sup>) which is very similar to DQ8.

**RHEUMATOID ARTHRITIS.** This is probably the most common autoimmune disease, affecting more than 1 in 100 Americans. It is the ‘inflammatory arthritis.’ (Osteoarthritis, the ‘degenerative arthritis,’ where the joints wear out, is even more common.) RA affects women more than men, and usually attacks the smaller joints, especially those of the fingers, first. The initial evidence that it was autoimmune came with the discovery of rheumatoid factor (RF), which can be detected by adding the patient’s serum to microscopic beads coated with normal human IgG. RF makes the beads agglutinate; ► it is IgM anti-IgG! It is a useful biomarker, but may not actually cause much joint damage. It’s been difficult to identify a pathogenic antibody (unlike the case in lupus), nevertheless RA can respond extremely well to rituximab, a monoclonal antibody against the CD20 on the surface of B cells, which effectively depletes them from the body. The antibodies appear much earlier than the T cells, which correlate better with disease activity, as they do in T1D.

Several groups have conducted genome-wide single nucleotide polymorphism screens of RA patients, and the loci identified are interesting: *HLA-DRB1* (one of the  $\beta$  chain genes of HLA-DR, associated of course with antigen presentation, in this case maybe autoantigen;) *PTPN22* (a tyrosine phosphatase involved in T cell signaling;) *C5* (the 5<sup>th</sup> component of complement;) *TRAF1* (a modifier of signal transduction through proinflammatory TNF receptors;) and *PADI4* (a deiminase that converts arginine in proteins to citrulline.) This last is intriguing, since antibodies to citrullinated peptides seem to be specific to RA, though their role in pathology is not known. Most of the pathogenesis of RA seems to be due to T cells.

Air pollution, and especially smoking, is an important RA risk factor. It is known to increase the citrullination of proteins in the lung.

**GRAFT VERSUS HOST REACTIONS.** If a non-identical graft contains T cells (and, except for corneas, they usually do, because they contain blood or have tissue spaces where leukocytes can be hiding) there is a perfectly good possibility that these cells will recognize HLA antigens of the recipient (host) as foreign, and so the *graft will try to reject the host*. Normally, the host has a lot more T cells than the graft, and will usually reject the grafted lymphocytes before they can begin to mount a serious reaction.

So: for **graft-versus-host disease (GvHD)** to result, ► the following three conditions must be met:

1. The graft must contain immunocompetent T cells (even bone marrow has mature T cells in it).
2. There must be at least one antigen in the **host** which the **graft’s** T cells can recognize (so, no worries with identical twins.)
3. The host must be relatively immunoincompetent or unable for genetic reasons to recognize the graft’s MHC antigens, otherwise the graft would be rejected too rapidly.

**ASK YOURSELF:** Quick concept check: In a family of inbred mice, where the parents (P) are S and Q, and the babies are SxQF1, will you get GvH disease if you inject S or Q T cells into an F1? Will you get GvHD if you inject F1 T cells into S or Q?

**Acute GvHD.** This develops in 2 to 10 weeks after bone marrow transplantation in humans. The symptoms include a nasty maculopapular skin rash; diarrhea; hepatic inflammation with jaundice; and infections (probably due to immunosuppression, as Tregs try to control the raging immune activation.) The treatment is with anti-inflammatory drugs like corticosteroids and, paradoxically, with immunosuppressives.

**Chronic GvHD.** This develops in months to years, even in patients with a perfect HLA match; therefore it is probably against minor histocompatibility antigens. With a lot of chronically activated T cells pouring out cytokines, regulation is compromised and autoimmunity can become an issue.

As we talked about in the case of David, the boy with SCID, removing the T cells from the bone marrow may prevent acute GvH disease. Oddly, this often results in a poorer engraftment of the bone marrow's stem cells. It's possible that a few activated T cells make hematopoietic-stimulating growth factors that improve graft success.

**'Graft-versus-leukemia reaction'.** For leukemia that has stopped responding to conventional therapy, one treatment is to give patients large doses of drugs or radiation, which would in themselves probably be fatal. One then can take marrow from the best matched allogeneic donor one can find, and give it to the patient after the high-dose ("myeloablative," because it destroys the bone marrow) therapy. Many studies have shown that leukemia patients who receive stored, pre-treatment bone marrow from themselves, or T-depleted allogeneic marrow, have the fewest GvH symptoms; but they also have a higher rate of leukemia relapse compared to those who get allogeneic marrow that still has some T cells. So it is assumed that somehow, a "graft-versus-leukemia" reaction is an important part of the success of the bone marrow transplant. Possibly the GvL is against leukemia-specific tumor antigens. Now more centers are using less harsh pretreatment, and trying to optimize the GvL effect while minimizing GvH. This is a difficult tightrope to walk, especially since we still don't fully understand what's going on.

**Th2 CELLS IN IMMUNOPATHOLOGY.** Th2 cells are found in the periphery in certain inflammatory and infectious states, ► especially asthma and chronic worm infestation. They activate macrophages (alternatively activated or M2) which produce fibrosis under these chronic conditions; and also attract eosinophils which, in excess, make inflammation more intense. So although this raises the question of whether allergy and asthma might really be T cell diseases, for now most people refer to asthma as a Type I condition because of the role played by IgE. But as more studies show the central role of Th2 cells, and minor contributions of IgE in established asthma, the classification is due for a change.

*Mycobacterium leprae*, the causative organism of leprosy, has strange effects on dendritic cells, with the result that some people make strong Th1 responses against *M. leprae*, and others make Th2 responses. Both get immunopathology as the organism is extremely difficult to clear from the body. ► Those with Th1 responses get tuberculoid leprosy with large skin and nerve lesions, but they control the infection. ► If the response is dominated by Th2 the initially-uncontrolled infection is widely disseminated in many small granulomas (lepromatous leprosy)).



## Learning Objectives for Type IV Immunopathology

1. Define Type IV immunopathology.
2. Describe the cellular and molecular events following intradermal injection of tuberculin antigen into a person who has cell-mediated immunity to it. Justify calling the process 'delayed hypersensitivity'. Characterize the cells that would be seen in a 48-hour biopsy of the site with regard to whether T cells or macrophages predominate.
3. Explain why a person usually has no observed symptoms when first exposed to a "contact sensitizer" poison ivy.
4. Discuss how a chemical or small peptide might not need to be processed through an antigen-presenting cell to be presented by that cell to T cells.
5. Describe the problem that HLA-B\*5701 people may have with the HIV drug abacavir.
6. Discuss in principle how T cell immunity could be measured in the laboratory.
7. Explain why TB skin tests can be administered repeatedly to the same subject.
8. Differentiate between a first-set and second-set graft rejection.
9. Define hyperacute rejection and explain the mechanism.
10. Discuss how autoimmunity can result from environmental exposure to tissues that cross-react with human organs.
11. Speculate on the role of HLA alleles in autoimmunity and chronic inflammatory diseases.
12. Define molecular mimicry.
13. Discuss the requirements for graft-versus-host disease to occur.
14. Define the graft-versus-leukemia phenomenon.