

## TYPE IV IMMUNOPATHOLOGY

**T CELL-MEDIATED IMMUNITY & DELAYED HYPERSENSITIVITY.** Delayed-type hypersensitivity (DTH) is an old term for T cell-mediated events that are considered undesirable or injurious. When the very same mechanisms produce helpful immune responses, they are happily referred to as 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" or "Type IV" 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 participation; this isn't surprising, given that the underlying problem may be disordered immune regulation.

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
- Berylliosis
- 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 in this context it's very 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 to the appropriate 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 ("sensitized") you probably didn't know it happened.



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

<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 what is said to be 20 tonnes of gold.

**Elicitation.** Now imagine that, some weeks 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 come by quickly, and get activated in the area where the oil has been deposited. They secrete interferon- $\gamma$  and IL-17 which attracts and activates a significant 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. 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 immunize him or her. Therefore, **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 may 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, mumps antigen, PPD, streptococcal proteins, 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 other species of *Mycobacteria* can sometimes cross-react to produce a positive skin test. In many countries, Bacille Calmette-Guérin (BCG) vaccine—it is attenuated bovine tuberculosis bacteria—is used, and most people so immunized have positive PPD skin tests.

**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 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.

The **QuantiFERON-TB** test is new, very nice, and is preferred (2010, CDC) to skin testing when the subject has had BCG immunization. Purified *M. tuberculosis-specific* proteins are added to a sample of whole blood, and after incubation, interferon- $\gamma$  is measured in the medium by an ELISA assay. Unlike the skin test, it remains negative in people vaccinated 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 they are more recently discovered and there is no in vivo test for them. They probably take part in many manifestations of T cell-mediated immunity, and may be quite important in the autoimmune diseases. 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.** Also called contact hypersensitivity or contact sensitivity. 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 then associate with MHC Class II. Metals like nickel (used in cheap 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 DTH REACTIONS.** You would expect that there would be an association between HLA haplotype and DTH reactions, and you’d be right. This is probably true in all cases, even where it has not yet been studied. A couple of interesting examples may suffice. First: up to 8% of people who were given **abacavir**, a nucleoside reverse transcriptase inhibitor, for HIV, developed systemic DTH which is quite difficult to diagnose correctly. “Symptoms of a hypersensitivity reaction to abacavir include combinations of fever, rash, constitutional symptoms, gastrointestinal tract symptoms, and respiratory symptoms that become more severe with continued dosing.”<sup>2</sup> In the quoted study, nearly all people with DTH to abacavir were HLA-B\*57:01.

Note that HLA-B\*57:01 is Class I, not the Class II which is recognized by Th1. Possible explanations for the DTH association are: (1) B\*57:01 is in linkage disequilibrium with the real culprit, an HLA-D allele that has not yet been identified; (2) an abacavir-modified peptide derived from the protein chains of HLA-B\*57:01 is processed and presented on HLA-D (this phenomenon has been observed in other conditions.)

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<sup>2</sup> S. Mallal et al (2008) HLA-B\*5701 Screening for Hypersensitivity to Abacavir. *NEJM* 358: 568-579.

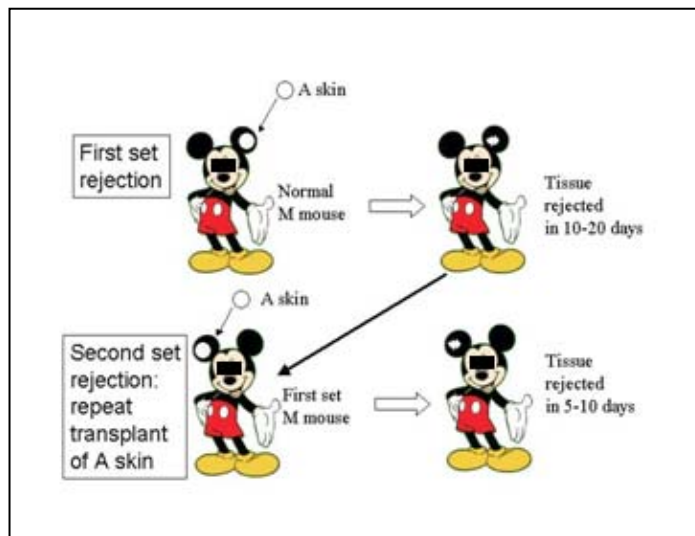
**ASK YOURSELF:** Can you remember another connection between HLA-B57 and HIV?

A second example: people exposed to dust from beryllium compounds (miners, metalworkers) may develop **berylliosis**, a chronic pneumonia with similarities to sarcoidosis or tuberculosis, which results in lung fibrosis. In these people it is known that beryllium associates with certain HLA-D alleles, particularly HLA-DP1. All suspect alleles have a critical glutamine at position 69 of the HLA Class II  $\beta$  chain, which is in the antigen-binding cleft and coordinates with the Be, positioning it to be recognized by a T cell receptor. Both Th1 and Th2 cells are involved.

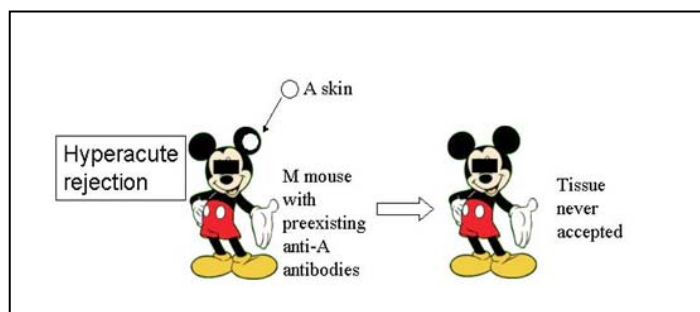
**GRAFT REJECTION.** The study of the rejection of foreign tissue grafts is of great importance in medicine. Rejection is a complex phenomenon eventually involving most or all of the specific immune and nonspecific amplifying elements of the immune system. Allograft immunity shows specificity and memory:

**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.



**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. 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. It seems that 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, and depends on the disease. 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 owner, but not immunogenic. So 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>3</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 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.

**Hashimoto's 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 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's also involves anti-thyroglobulin antibodies, whose presence is commonly used to confirm the diagnosis.

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<sup>3</sup> MMWR 8 February 2008.

**SJÖGREN'S 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. 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.

**JUVENILE 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 that produce insulin) can be detected in the serum of over 90% of patients at the time of diagnosis (normals, fewer than 2%). 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 implicated in the pathogenesis of T1D. It brings up many interesting questions; for example, should newly-diagnosed T1D patients be treated with immunosuppressive drugs? A fascinating study of 15 young patients was reported recently<sup>4</sup>. Within 6 weeks of diagnosis, their bone marrow hematopoietic stem cells were mobilized and purified from blood, and then returned to them after they had undergone a short course of cyclophosphamide and anti-thymocyte globulin, to destroy most of their immune systems. At 7 to 35 months of follow-up, only one patient required insulin. Has their dysregulated immune response been reset? Do newly-developed T and B cells regard damaged islet cells as normal self, and so ignore them? (Something similar has been done in the Netherlands with juvenile rheumatoid arthritis<sup>5</sup>.)

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 thought to be the 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 a very similar unusual Class II MHC molecule, H2 I-A<sup>g7</sup>.

**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.

**GRAFT VERSUS HOST REACTIONS.** If a non-identical graft contains T cells (and, except for corneas, they usually do, because they contain blood) 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*. The host is a lot bigger than the graft, and usually rejects the grafted lymphocytes before they can begin to mount a serious reaction.

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<sup>4</sup> Voltarelli, JC, et al. 2007. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA 297:1568-1576.

<sup>5</sup> Brinkman DM, et al. 2007. Autologous stem cell transplantation in children with severe progressive systemic or polyarticular juvenile idiopathic arthritis: long-term follow-up of a prospective clinical trial. Arthritis Rheum. 56:2410-21.

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.
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.

**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.) 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 lost 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 does not respond well to conventional therapy, one treatment is to give patients huge 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 all the bone marrow) therapy. Many studies have shown that leukemia patients who receive bone marrow from themselves, or receive 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 T cells. So it is assumed that somehow, graft-versus-leukemia is an important part of the success of the bone marrow transplant. 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.

**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 the terminology could be in 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 uncontained infection is widely disseminated (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 have 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 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. Discuss in principle how T cell immunity could be measured in vitro.
6. Explain why TB skin tests can be administered repeatedly to the same subject.
7. Differentiate between a first-set and second-set graft rejection.
8. Define hyperacute rejection.
9. Discuss how autoimmunity can result from environmental exposure to tissues that cross-react with human organs.
10. Speculate on the role of HLA alleles in autoimmunity and chronic inflammatory diseases.
11. Define molecular mimicry.
12. Discuss the requirements for graft-versus-host disease to occur.