TYPE II IMMUNOPATHOLOGY

TYPE II. This form of immunopathology is due to the actions of antibodies directed against a specific target tissue or cell; so it is one of the forms of **autoimmunity**. Type III immunopathology, as we've seen, may also be due to self-reactive antibodies, but the manifestation there is immune complex-based rather than specific tissue damage. T cell-mediated autoimmunity is referred to as Type IV immunopathology. As we've seen before, these conditions are often mixed.

We'll first discuss *how* antibodies damage or otherwise affect cells, and give some examples, before getting to *ways* the immune system might go wrong.

TYPE II PATHOGENIC MECHANISMS

1. "Stimulatory hypersensitivity." If the autoantibody happens to be directed against a cellsurface receptor, it may behave as an agonist, mimicking whatever hormone or factor normally works at that receptor. \blacktriangleright The best example of this is LATS (long-acting thyroid stimulator), found in the blood of most patients with hyperthyroidism. It is IgG antibody to the TSH (thyroidstimulating hormone) receptor on thyroid cells; when it binds to these receptors, it mimics TSH and causes the cell to secrete thyroid hormones. Of course, the normal feedback controls won't work in this case, so the result is \blacktriangleright hyperthyroidism, or **Graves' disease**.

Some people have **'inappropriate' tachycardia**, a fast heart rate without cardiac abnormalities. About half have been shown to have autoantibodies to the β -adrenergic receptor, which are stimulatory, like epinephrine; the effect can be reversed by the beta blocker propranolol. Most of the autoantibody-positive patients are women, as is often the case in autoimmunity¹.

2. Complement-mediated damage. Continuing with the receptor theme, in the disease **myasthenia gravis** antibody is made to the acetylcholine receptor at the neuromuscular endplate. This antibody may block transmission from nerve to muscle, or increase receptor turnover, but even more important is the destruction of the endplate by complement/phagocyte mediated mechanisms.

These are exactly the ones we are already familiar with from, say, bacterial immunity. Tissues can be damaged by \blacktriangleright lysis (red cells in autoimmune hemolytic anemia), by \triangleright phagocytosis (platelets in autoimmune thrombocytopenic purpura, ATP) or by \blacktriangleright release of the phagocytes' lysosomal enzymes and reactive oxygen species (probable in myasthenia gravis, and Goodpasture's disease, see below).

SOME ILLUSTRATIVE CONDITIONS

MYASTHENIA GRAVIS. A disease of progressive muscle weakness. Patients make antibody to myosin, and to the ACh receptor (AChR). The antibody to myosin may be useful in diagnosis (do immunofluorescence) but seems to play no role in pathogenesis, which brings us to a very

¹ Chiale PA, et al. 2006. Inappropriate sinus tachycardia may be related to an immunologic disorder involving cardiac beta adrenergic receptors. Heart Rhythm 3:1182-6.

important point in the discussion of autoimmunity: does the antibody you have identified actually cause the disease, or did something else cause the disease, and the antibody is just a—maybe irrelevant—sequel? \blacktriangleright The antibody to the alpha subunit of the AChR does the real damage, which is mediated by complement and neutrophils. The antibody to myosin may be the result of the release of sequestered myosin antigen, secondary to cell damage. A recent study² shows that the thymic transcription factor Aire (*see* T cells) drives the *thymic* expression of *CHRNA1*, the gene for the AChR alpha subunit. Two families were found to have an allele of the *CHRNA1* promoter that does not interact with Aire, so the protein is not expressed in patient's thymuses, and clones reactive with the AChR are not deleted by negative selection. They are therefore available to help B cells make antibody to the receptor.

GOODPASTURE'S DISEASE. This uncommon condition involves formation of autoantibodies to lung and kidney basement membranes (BM) (which are the collagenous non-living connective tissue framework upon which the endothelial cells of capillaries sit). ► There is an epitope on the antigen (Type IV collagen) shared between the BM's of these two organs; other organs are not involved. The patients have persistent glomerulonephritis, and pneumonitis with pulmonary hemorrhages. This was the first human autoimmune disease in which the antibody was *proved* to cause the condition: kidneys were removed from a patient who had died of Goodpasture's, the antibody eluted from them (low



pH breaks antigen-antibody bonds), purified, and injected into a chimpanzee, who came down rapidly with typical Goodpasture's syndrome. In Goodpasture's the antibody is directed against the basement membrane, not trapped as clumps, so the staining by immunofluorescence is sharp and 'linear,' not 'lumpy-bumpy' as it is in Type III, immune complex conditions.



Type II, linear: Goodpasture's



Type III, lumpy-bumpy: Lupus

² Giraud, M. et al. 2007. An IRF8-binding promoter variant and AIRE control CHRNA1 promiscuous expression in thymus. Nature 448: 934-937.

DRESSLER'S SYNDROME. Most people who have a heart attack will make some autoantibody which reacts with heart. This seems to do them no harm. Dressler's is a syndrome of persistent cardiac pain, fever, malaise, and pericardial effusion seen after heart attack (and commonly after heart surgery) which seems directly related to an immune response to pericardial or myocardial antigens. A better name might be post-cardiac injury syndrome. Treated with anti-inflammatory agents, it usually gets better as the heart heals.

RHEUMATIC HEART DISEASE. Defined as heart disease occurring shortly after a streptococcal infection, for example a 'strep throat.' There is very good evidence that it is due to ► cross-reaction between a Group A *Streptococcus* M-protein antigen and a structure on the heart's endothelial lining, probably laminin on heart valves, followed by neutrophil-mediated tissue destruction. **Rheumatic fever** is the same disease with more widespread manifestations, including in the skin and CNS. (Poststreptococcal *glomerulonephritis*, on the other hand, is Type III immunopathology, due to complexes between antibody and strep antigens trapped in the kidney.)

AUTOIMMUNE THROMBOCYTOPENIC PURPURA (ATP). Until recently this was called idiopathic thrombocytopenic purpura. These patients have bleeding abnormalities due to destruction of platelets (thrombocytes) by autoantibody; ► the platelets are opsonized and their destruction, mainly in the spleen, is rapid. Platelets are needed for blood clotting. (Treatment: suppress the immune system and/or remove the spleen.) ATP is often seen in young healthy people some weeks after a viral infection; in older people, in association with many other autoantibodies; and in people treated with certain drugs; all of which might suggest to you, when you've read through these notes, the sorts of processes that could be going on.

AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA). Like ATP, this may follow a viral infection, or be associated with an autoimmune syndrome, or cancer. ► Many drugs, such as penicillin, methyldopa, chlorpromazine and quinidine, can induce AIHA, usually temporarily. In the rare condition paroxysmal cold hemoglobinuria (PCH), the patient experiences hemolysis after exposure to cold. It is due to an autoantibody which only binds to red cells at about 15° C.

ASK YOURSELF: Your fingers get to 15° C, so in PCH red cells could agglutinate in skin capillaries and be lysed when they move to warmer regions where complement is activated. But your lymph nodes don't get that cold; so how could B cells ever be stimulated to make this antibody?

SYSTEMIC LUPUS ERYTHEMATOSUS. This is the classical multifactorial autoimmune disease, with both genetic and environmental causes and triggers; but we still don't understand it. Many think it could be a disorder of the management of apoptotic cells, which are normally recognized and taken up for reprocessing via receptors that do not activate phagocytes to become antigen-presenting. If by some accident uptake was mediated by $Fc\gamma R$, they say, the activated phagocytes might then be able to break normal self-tolerance. There are a number of different mouse models in which most or all animals get lupus-like symptoms of immune-complex glomerulonephritis (Type III) and a variety of autoantibodies (Type II); the best known of these are the NZB x NZW F1 hybrid mice. Even in this simple model, many genes are implicated. The incidence in mice and people is considerably greater in females, an estrogen effect. People with lupus may make antibody to: nuclear and nucleolar proteins; DNA; RNA; erythrocytes; clotting factors; platelets; skin; and T cells. Antibody to double-stranded DNA is pathogenic, and may explain not just the kidney disease but also the characteristic facial butterfly rash, with local

immune complex formation near sun-damaged, DNA-releasing skin cells. In the US the incidence of SLE is 1/3500; it is lowest in people of European origin..

In the following group of diseases, both autoantibodies and autoreactive T cells are implicated in the pathogenesis, so they are mixed Type II and Type IV mechanisms. Sometimes, as we've already seen, Type III is also involved.

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; \blacktriangleright it is IgM anti-IgG! It is a useful biomarker, but may not actually cause much joint damage. There are other antibodies involved, as well as T cells, so the pathogenesis is complex (and the etiology is unknown). Although 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.

Several groups have conducted genome-wide single nucleotide polymorphism screens of RA patients, and the loci identified are interesting: *HLA-DRB1* (one of the β 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 5th component of complement;) *TRAF1* (a modifier of signal transduction through proinflammatory TNF receptors;) and *PAD14* (a deiminase that converts arginine in proteins to citrulline.) This last is intriguing, since antibodies to citrullinated peptides seem to be absolutely 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.

HASHIMOTO'S THYROIDITIS. An inflammatory disease of the thyroid in which there is very good evidence for both T and B cell immunity to various thyroid antigens, including thyroglobulin. The antibodies to thyroid antigens are destructive, not stimulatory. Histologically the thyroid is infiltrated by T cells. It may be that T cell damage releases normally sequestered antigens, to which antibodies are made, worsening the condition. The result is hypothyroidism.

OTHER DISEASES. There are often autoantibodies found in conditions that are known to be T cell-mediated; this indicated that immunity may be generally dysregulated. In celiac disease, there is an antibody to tissue transglutaminase that is very useful for diagnosis, and in Type 1 (childhood) diabetes several antibodies to islet-associated antigens are seen which provide prognostic information; but in neither of these conditions are they thought to be pathogenic, so diabetes and celiac disease are considered under Type IV immunopathology.

MECHANISMS. We don't know in most cases what causes a breakdown of the body's '*horror autotoxicus*' rule³; here are eight possible mechanisms. You could probably think up others.

³ Paul Ehrlich (1854-1915) believed the immune system would be horrified to harm itself; maybe it is, but it does so anyway.

1. Emergence of a **forbidden clone.** A clone might somehow escape the normal clonal abortion mechanism, and mature so that encounters with antigen immunize it. This seems to be probable in myasthenia gravis.

2. **Illicit help.** Suppose you had anti-self B cells that hadn't been aborted. They would not necessarily get you into trouble if the self antigens were T-dependent, and you did not have antiself T helper cells. This actually seems to be the case for most antigens. But: suppose that a foreign antigen were to couple to the self antigen. ► The anti-self B cell could bind and ingest self, and carry the foreign antigen along with it. Then foreign epitopes might be presented to a Tfh cell on the B cell's Class II MHC. The B cell



would have received both necessary signals (from its receptor and from the T cell) and become activated.

ASK YOURSELF: Can you see how this mechanism is very similar to what goes on when you use a conjugate carbohydrate-protein vaccine?

3. **Cross-reaction** between a foreign antigen and a self antigen. We've been talking about this one since the beginning. Undoubtedly important, and it would become more so if only we could identify the antigens that get things started. By the time the patient develops clinical symptoms, the triggering antigen may be long gone, with the process being maintained by autoimmune responses to normally-sequestered antigens released from damaged cells.

4. Release of a **sequestered antigen.** Note that in the special case of sequestered antigens, the antigen cannot get out into the general system, and therefore is not normally immunogenic, ▶ but if an immune response *does* get initiated, then the response can usually get into the place where the antigen was sequestered. Example: some adult men who get mumps end up sterile. The virus breaks down the blood/testis barrier, allowing immunization to sperm antigens. Because these are not yet expressed in children, some sperm-reactive B and T cells never got aborted.

► There is a phenomenon in autoimmunity called 'epitope spreading.' Early in the disease antibodies are made to just one or two epitopes of some 'self' protein. With time, more epitopes, and more proteins are involved. Does tissue damage gradually reveal more sequestered antigens?

5. **Passive antibody.** In a child with hemolytic disease of the newborn (*see* Immunohematology); in a patient getting a mismatched transfusion; in a child of a mother with myasthenia gravis or SLE. A rare child of a lupus mother may be born with heart block due to cross-reactive antibodies.

6. **Innocent bystander.** A common mechanism, in which there is damage to normal tissue which happens to be associated with or infected by the antigen, which is truly foreign.

7. **Polyclonal activators.** We know compounds, like plant lectins and bacterial endotoxins, which nonspecifically activate T or B cells. In some infections, it may be possible that such polyclonal activators are released, resulting in the non-specific activation of many clones, among them self-reactive ones. What if a self-antigen somehow got coupled to a pathogen-associated molecular pattern that could stimulate a TLR—might that drive an autoimmune response?

8. **Failure of regulatory mechanisms.** A proper balance between Th1, Th17, Tfh, Th2, and Treg activity assures that immune responses are appropriate. Does this balance get perturbed in some way, so that some responses are exaggerated, and eventually self/non-self discrimination breaks down? This is an area of intense speculation lately. Some recent experiments that cause major shifts in T cell balance look very promising as therapy, if they can make the **translational jump** from the lab or the Phase I trial into safe general use. We'll talk more about this in the last of our immunopathology discussions.

TESTS. For diagnosing a Type II condition we look for antibody directed against the antigen or tissue in question. Antibody can be detected in the patient's serum by any of the tests you've already heard about, including ELISA.

In general the hallmark test is immunofluorescence. You can do a *direct* test, looking for antibody that is already in the patient's tissues, if you happen to have a sample of the patient's *tissues*:



Or, if you only have the patient's *serum*, you can look for antibody in it by an *indirect* immunofluorescence test, using normal human tissue (autoantibodies are almost always tissue specific but not individual-specific):



3. Labeled anti-IgG reveals that Abs in patient's serum bound GBM
2. Patient's serum with Anti-GBM antibodies
1. Normal kidney; no Ab in it

ASK YOURSELF: If the blood-brain barrier were to break down accidentally, would your brain then be foreign to your immune system? (Another way of thinking about this is, do you think your brain is a tolerogen to your immune system, or a potential immunogen which your immune system has not yet had a chance to see?) Do you think you would make an (auto)immune response? Would that be harmful?

Learning objectives for Type II Immunopathology and Regulation

1. Describe the molecular and cellular details of the immunologic mechanisms by which tissue damage occurs in a Type II ('cytotoxic antibody') reaction.

2. Give an example of a Type II mechanism disease of muscle, kidney, heart, red cells, platelets, thyroid, and pancreatic islets.

3. Describe the fluorescent antibody tests which would allow you to make the diagnosis of Goodpasture's Syndrome, given: patient's kidney biopsy, normal kidney biopsy, patient's serum, and fluoresceinated goat antisera to human IgG and complement.

4. Distinguish between the 'lumpy-bumpy' and 'linear' immunofluorescent patterns in terms of the most probable immunopathologies they represent.

5. Describe how you could tell, using fluorescent antibodies and biopsies of patient's kidney, if Type II or Type III immunopathology was involved. Name the antibodies you would use and the fluorescent patterns you would see.

6. Given patient's serum, fluorescent antibody to human immunoglobulins, and slices of normal kidney, describe how you could tell if the patient's glomerulonephritis was due to Goodpasture's disease or SLE.

- 7. Describe how antibody-mediated tissue damage could result from: The innocent bystander phenomenon. Cross-reaction of a foreign antigen with self. Coupling self antigen with a foreign antigenic 'carrier'. Exposure of a sequestered antigen.
- 8. Identify 'Rheumatoid Factor' and describe its molecular nature.
- 9. Describe the Type II mechanism of Graves' Disease.
- 10. Discuss how the Aire gene is involved in preventing autoimmune disease.