

## TYPE III IMMUNOPATHOLOGY (IMMUNE COMPLEX DISEASE)

**THE TYPES OF IMMUNOPATHOLOGY.** The NIH recognizes 80 conditions of autoimmunity, though there are without doubt more. Between 5 and 8 persons in 100 in the USA have one of these conditions. If you add hypersensitivity (allergies) it could be as many as 25% of the population. As with all of disease, immunopathology depends upon three factors:  
 ► genetics, environment, and bad luck.

In 1963 Gell and Coombs tried to make sense of immunological diseases by grouping them by common mechanisms. They argued that there seemed to be only 5 pathological processes that caused all these conditions. It was an important advance, and is still useful as long as we keep in mind that ► they are just descriptions of mechanisms; and most actual diseases have been found to involve more than one mechanism. So, though it would be nice to have tables of Type I or Type IV diseases, what we'll see is that in rheumatoid arthritis, for example, there are well-defined autoantibodies (Type II), immune complexes (Type III), and destructive T cell immunity (Type IV). We will discuss the mechanisms with numerous examples to give you a flavor of the thinking in the world of immunopathology, and help you understand the fundamental interconnectedness of things and how the immune system is so dependent on effective self-regulation. If you have a special interest in any particular condition you will want to read beyond the limits of what we have space for here.

### THE MECHANISMS

**Type I:** Symptoms or pathology due to IgE antibody. Since the type of B-cell-helper Tfh cell that drives switching to IgE seems to be closely related to the Th2 cell, Th2-mediated events are often seen along with those caused by IgE.

**Type II:** Pathology due to IgG, IgM, or IgA antibody causing harm to self. In most cases this refers to autoantibodies. In the original Gell and Coombs classification, **Type V** was separate; but it is now folded into Type II, as it involves autoreactive antibody against surface receptors which happen to *stimulate* (rather than damage) the cell.

**Type III:** Pathology caused by the formation of immune complexes which are trapped in the basement membranes of blood vessels and activate complement, leading to inflammation and vasculitis. When Type III is chronic, T cell-mediated immunity (Type IV) tends to become increasingly important as part of the disease.

**Type IV:** Pathologic outcomes of normal or abnormal (including autoimmune) T cell responses, including both helper and cytotoxic cells.

**Chronic frustrated immune responses:** This is not part of the original classification; JJC made up the name. It refers to conditions in which the body is using the adaptive immune response to try to get rid of antigens *that it never can*. These include things like normal gut flora (as in Crohn disease), skin flora (psoriasis), chemicals (as in chronic beryllium disease), or foods (gluten in celiac disease). Some have used the essentially meaningless term 'autoinflammatory diseases' for these. In CFIR the antigen can be neither disposed of nor effectively walled off.

We'll start with Type III.

**TYPE III IMMUNOPATHOLOGY.** Of the 4 Gell and Coombs classifications of immunopathologic mechanisms, Type III might be the least intuitive. Why would complexes between antigen and antibody ever be anything but good? The answer relates to a phenomenon with which we are now quite familiar. The purpose of the immune system is to

*1. Recognize 2. Inactivate 3. Destroy 4. Remove*

foreign substances; and it does that, in large part, by invoking inflammation. So if events 1, 2, and 3 take place but 4 is delayed, inefficient, or impossible, prolonged, chronic or destructive inflammation may result.

**MECHANISMS.** Let's first consider a condition in which there are both antigen and antibody in the blood at the same time.

An 8-year old boy in rural Rwanda has had a sore throat for about a week, treated with rest, acetaminophen, and a liquid diet. On day 8 he complains of sore joints, develops a rash, and looks very ill. His urine output is minimal. His parents ask neighbors to help carry him to the roadway where a trucker takes them to the University hospital in Kigali.

What happened: He has Strep throat. At the same time that the infection was shedding antigen into the blood, the boy's immune system was similarly shedding antibody. Complexes between *Streptococcus* proteins and antibody form in the circulation. Since IgG is divalent, and the response is polyclonal, it will be easy to form large antigen-antibody complexes. These are readily cleared from the blood by the reticuloendothelial system<sup>1</sup> and cause no concerns. Small complexes (say one antibody with one or two antigens) are too small to activate complement, and they are similarly harmless. ► But complexes of just the right<sup>2</sup> size—we sometimes say 'about a million daltons'—are the problem here; they can activate complement but are below the size that is rapidly removed by the RES.

► These complexes have another disadvantage; they are too large to pass easily through the basement membranes of small blood vessels. [Remember, IgM itself, at 750,000 daltons, has trouble getting past the vascular endothelium.]

Basement membranes are a nonliving layer under the endothelial lining cells of blood vessels. They are made up mostly of type-IV collagen and some other proteins and proteoglycans. They support the endothelial cells and form a (variably porous) barrier to the passage of large molecules and cells.

► So the intermediate complexes get stuck in basement membranes. There, they activate complement by binding C1q and initiating the classical complement cascade. C3a and C5a attract neutrophils, which arrive and release a variety of inflammatory factors, including the proteases cathepsin G and elastase; and hydrogen peroxide, which by activating metalloproteinases also contributes to the proteolytic degradation of the basement membrane. C3a and C5a will, as anaphylatoxins, release histamine and other mediators from mast cells

**ASK YOURSELF:** The skin rash seen in immune complex disease is commonly on the lower legs—shin and calf. Can you suggest a reason why that would be?

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<sup>1</sup> The reticuloendothelial system (RES) is a mysterious entity, named long ago. It basically includes all the macrophage-class phagocytes of the tissues, especially those in the liver and spleen. One good way to think about it is this: They are the cells that would be black if you injected India ink (carbon suspension) intravenously and waited about a day. If you decide to try this, use Pelikan, not Higgins, ink, which in my experience tends to be toxic.

<sup>2</sup> That is, wrong.

Simple biophysics says that complexes will be trapped most in capillary beds where there is most filtration of blood; that is, where there is net outflow of fluid. For example, the capillaries of any location that must be kept wet qualify:

Location	Reason
Joints	Make lubricating synovial fluid
Pleura	Make pleural fluid to keep the lungs inflated
Peritoneum	Peritoneal fluid keeps your guts from squeaking
Skin	Due to evaporation there is net outflow from skin capillaries
Choroid plexus in brain	Makes cerebrospinal fluid
Kidney	Filters your plasma volume 65 times/day

If immune complexes can lodge in basement membranes because of their size, and cause inflammation based on the Fc part of the antibody they contain activating complement, you might infer that the nature of the antigen is irrelevant, and all immune complex conditions would have a similar constellation of symptoms; ► and you'd be, for the most part, right.

The symptoms you would expect (looking at the above Table) are: Arthralgia/ arthritis; pleurisy/ pleural effusion; sterile peritonitis; skin rash, especially on the lower legs; central nervous symptoms, including confusion or even dementia; and glomerulonephritis. Another way of saying all this is: ►widespread small vessel vasculitis.

Of course nothing in nature follows the rules all the time, so there are some antigens that, because of their charge or other chemical characteristics, preferentially lodge in certain basement membranes, which are not all chemically identical. Complexes of antibody and hepatitis (B, C) virus proteins tend to get stuck in the basement membranes of medium arteries, causing the variant condition **polyarteritis nodosa** (of which, however, most cases are idiopathic, that is, the cause is unknown.)

**ANTIGENS OFTEN ARE EXOGENOUS.** Exogenous antigens have to be present in sufficient amounts to form harmful complexes at the time when antibody is also present in sufficient concentration. ► Practically, this means the antigen may have been given in a large quantity, so a lot is still present when antibody is made; or the antigen may be in a depot, so it persists; or it may be self-replicating. Let's look at examples.

**ONE-SHOT SERUM SICKNESS.** Soon after doctors began to use rabbit and horse antisera to treat infectious diseases, von Pirquet and Schick [1906; *Die Serumkrankheit*<sup>3</sup>] described a nasty side effect of the treatment. About 10-14 days after the administration of a dose of animal serum, fever, malaise, rash and itch, and arthralgia develop. Hives may be observed. Examination will reveal tender lymphadenopathy, and urinalysis may show red blood cell casts and protein. Inflammatory markers in the blood will be increased (C-reactive protein, erythrocyte sedimentation rate) and total hemolytic complement decreased as it is used up

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<sup>3</sup> An excellent book; Schick translated it into English in 1951 (Williams & Wilkins) but it's hard to find nowadays outside a library.

You can think of these events as an immunoprecipitation reaction with time, rather than space, as the variable. Shortly after injection of the animal serum (given intravenously) the situation is antigen excess, with no complexes. Then the patient starts to make antibody against the foreign horse proteins. As antibody production increases, exponentially, a time will come where equivalence between the antibody in circulation and the remaining antigen is achieved, and large complexes form. Just before that, though, there will be complex formation in relative antigen excess, so they are smaller than optimal-proportions complexes; in fact, some will be just the size to get lodged in basement membranes. ► That is the time that symptoms begin. They persist a week or more, until enough antibody has been made to form the large complexes that are readily cleared by the RES. Treatment in those days was anti-inflammatory, careful fluid management, hope, and time.

Serum sickness can happen—fortunately rather rarely—after treatment with murine, chimeric, or humanized monoclonal antibodies. Polyclonal rabbit or horse antithymocyte (effectively, anti T cell) globulin is used in tissue transplantation, and can also cause serum sickness; this risk is mitigated by affinity purification, which lowers the dose of foreign protein by getting rid of irrelevant stuff; and by chopping off and discarding the Fc part, using only F(ab) or F(ab<sub>2</sub>).

**ASK YOURSELF:** Would you think there was any advantage to removing the Fc portions of these therapeutic antibodies? Disadvantage?

The condition can also occur with hapten-sized monovalent antigens. As you know, these are not immunogens; but some drugs can couple themselves to proteins, which then serve as carriers, making the antigenic drug immunogenic. For example, penicillin acts by covalently coupling to bacterial cell wall-forming enzymes. In some people it also complexes to their own proteins. ► Penicillin-specific B cells bind the penicillin epitope, the protein is taken up and broken down to peptides in endosomes, and then a penicillin-modified peptide is loaded onto MHC class II. This is presented to Th cells, and if the right Tfh is present, the B cell will be driven to make anti-penicillin; first IgM, then IgG, and if luck is not with the patient, IgE. This is the basic mechanism for IgG-mediated immune complex disease to penicillins (often seen after a large depot injection) as well as true IgE-mediated penicillin allergy, which is a Type I immunopathology.

Among other types of exogenous antigens, ► serum sickness-like symptoms can be seen in patients with viral infections, especially hepatitis, where lower-leg rash, malaise, fever and arthralgias persist until the viral load is reduced.

**BACK TO ACUTE POST-STREPTOCOCCAL GLOMERULONEPHRITIS.** This is one of two immune complications of infection with Group A Beta Hemolytic *Streptococcus pyogenes*. ► Symptoms begin 10-14 days after infection (strep throat, scarlet fever, impetigo of skin) and are typical of Type III, with the kidney—the great filter—being the most affected site. Signs and symptoms include nausea and vomiting, fever, malaise, hypertension, reduced urine output, hematuria, joint pain and rash. Serum complement levels are decreased. Diagnosis is by history and renal biopsy. Treatment is symptomatic and supportive, and includes adequate antibiotics. ► The disease is usually self-limiting and recovery can be uneventful with proper care. It is seen nowadays mostly in developing countries, where treatment with antibiotics may be delayed or unavailable, and it can still be fatal.

**HYPERSENSITIVITY PNEUMONITIS.** Farmer's lung is the best-known example in North America. It is caused by exposure to thermophilic *Actinomyces*, filamentous bacteria which are found in moldy hay and silage. After chronic exposure by inhalation, the farmer develops serum IgG antibodies. Then one day, perhaps when the hay has been unusually damp and is now drying and aerosolizing spores, she inhales enough antigen that antigen-antibody complexes form in the lungs as the mold proteins diffuse through the alveoli into the capillaries. ► Complement and neutrophils cause the symptoms; this is often called an 'allergic' disease but it is Type III, not Type I. An acute attack will start 4 to 8 hours after the exposure, with shortness of breath, a dry cough, malaise, fever, and tachycardia. Most episodes are rather more chronic, with similar but milder symptoms, to which arthritis is sometimes added.

With time, ► T cell-mediated inflammation begins and eventually predominates in more serious cases. There are Th1 cells present, and granulomas also become apparent, suggesting Th2 cell involvement as well. So Type III evolves into Type IV, and gets more destructive and harder to treat<sup>4</sup>.

Many other inhalants can cause hypersensitivity pneumonitis; there are dozens of these in the literature.<sup>5</sup> Air conditioners and hot tubs can be mold sources. Diagnosis is by history, complement levels, sometimes finding immune complexes in the blood, and if necessary, bronchoalveolar lavage and biopsy. Treatment is with inhaled steroids, and avoidance.

**ARTHUS REACTION.** Named for Nicholas Arthus (1862-1945), long-time professor at Lausanne, this is the *local* manifestation of immune complex disease. The most common example is the result of a booster immunization. The subject has some pre-existing antibody to the immunogen (it may be cross-reactive) so that when the antigen is deposited by injection, complexes immediately begin to form locally. They activate complement, which attracts neutrophils; the symptoms become noticeable by about 4-6 hours, and last a day or so. ► It is the cause of fussiness in vaccinated children, and your sore arm after your flu or tetanus boosters.

**ANTIGENS CAN BE ENDOGENOUS.** Endogenous antigens can be involved in immune complexes, in which case the etiology is autoimmunity for some reason (often unknown) but the pathogenesis is Type III. Because of the underlying autoimmune problem, though, you'd expect these syndromes might be mixed, with not just Type III but also Type II (antibody-mediated) and Type IV (T cell-mediated) pathology.

**IgG SUBTYPES IN IMMUNE COMPLEXES.** IgG<sub>4</sub> neither adheres to Fc receptors nor activates complement very well, so when it forms complexes they are inert. Thus they can block the activity of complexes made with other IgG subclasses; this particularly interests people who study the next two conditions.

**RHEUMATOID ARTHRITIS.** ► The oldest known autoantibody is 'rheumatoid factor,' RF, often present in the blood of patients with rheumatoid arthritis, and some other conditions. It is an IgM antibody patients make to their own IgG. This causes a Type III manifestation in a complicated disease which also has elements of Type II and Type IV pathology. The levels of RF correlate only slightly with disease activity, so it is probable that the other mechanisms are more important in pathogenesis, though the presence of RF is useful in diagnosis.

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<sup>4</sup> This transition from antibody-mediated to more severe T cell-mediated pathology is not uncommon, and is seen for example in type 1 diabetes and rheumatoid arthritis.

<sup>5</sup> The quaintest is probably 'thatched roof worker's lung.' Note that popcorn worker's lung, which you would guess to be an example, is actually bronchiolitis obliterans caused by the artificial butter flavoring diacetyl.

**SYSTEMIC LUPUS ERYTHEMATOSUS, SLE.** This is another disease with important Type III and Type II manifestations, but it's currently thought that the Type III issues are most significant. The key Type III problem arises from the patients' tendency to make IgG antibody to double-stranded DNA (that is, the native form). Since they also make antibodies to histones H2, H3 and H4, some think that the immunizing antigenic complex may be the nucleosome (the primary unit of DNA compaction, wherein about 180 bp wrap around a histone core). Nucleosomes are generated during apoptosis, so many have looked for abnormal apoptosis (unlikely) or abnormal disposal of apoptotic cells that gets them into an antigen-presenting compartment (recently shown to be the case.) ► The dsDNA immune complexes deposit preferentially in the kidney, making glomerulonephritis the most important SLE problem. 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 prevalence of SLE is 1/3500; it is lowest in people of European origin.

**ASK YOURSELF:** Antibodies don't cross plasma membranes to get at nuclei, so how come there are complexes of antibody and double-stranded DNA in the blood?

**IGA NEPHROPATHY,** first described in 1968, is the most common form of primary glomerulonephritis in the world, and the leading reason for kidney transplantation. It may be asymptomatic for years, which is why, on diagnosis, it is frequently found to be far advanced. Or it presents as recurring episodes of hematuria. ► It is characterized immunohistochemically by the co-deposition of IgA and IgG in the renal glomerulus.

The IgA in kidney deposits is of the IgA1 subclass. IgA1 has an unusually long hinge region, and in IgA nephropathy the terminal sugars of carbohydrate chains normally found in the hinge are missing, creating a new carbohydrate epitope that ends in N-acetyl-galactosamine. Unfortunately, cross-reactive antibodies to this epitope are common in human plasma, as it is a frequent epitope on bacteria and viruses. (We will hear about this kind of antibody later, in Immunohematology.) ► The antibodies, which are IgA or IgG, bind the underglycosylated IgA1, and the complexes get trapped in the renal glomerulus<sup>6</sup>. The cause of the underglycosylation of the IgA1 is not known, but a hint is that this condition often is first diagnosed following an infection, and many microorganisms make sugar-cleaving glycosylases. Chronic tonsillitis, for example, is associated with IgA nephropathy. But a heritable defect in some patients' ability to add the correct terminal sugars has also been observed, as is linkage to HLA alleles.

**ASK YOURSELF:** How, do you think, could chronic tonsillitis lead to IgA nephropathy?

**DIAGNOSIS OF TYPE III.** As we have seen, history is the important clue in most cases.

Blood tests will often reveal a low **total hemolytic complement** (CH<sub>50</sub>) level. This is measured by adding dilutions of the patient's serum to antibody-coated sheep red blood cells, and observing the maximum serum dilution that will still cause 50% of the cells to lyse; comparison is made to a normal standard. ► Curiously, some people who, for genetic reasons, have low levels of complement components C1 or C4 develop lupus. It may be that they have difficulty clearing immune complexes in the normal, efficient way, and thus are at increased risk for disease; so it is often a good idea to measure individual C components as well as CH<sub>50</sub>.

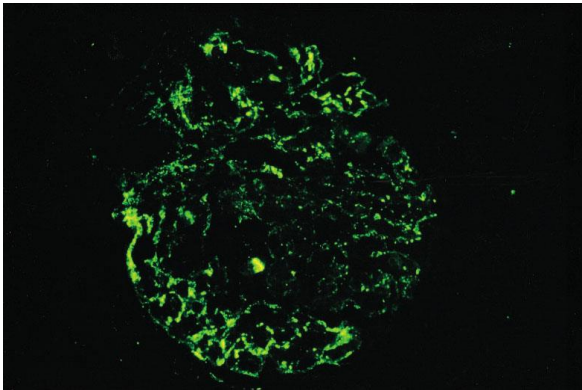
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<sup>6</sup> Although the blood level of IgA is only a third of that of IgG, we actually make more IgA each day, as its half-life is only about 5 days; so there is always plenty of fresh new IgA to make into complexes.

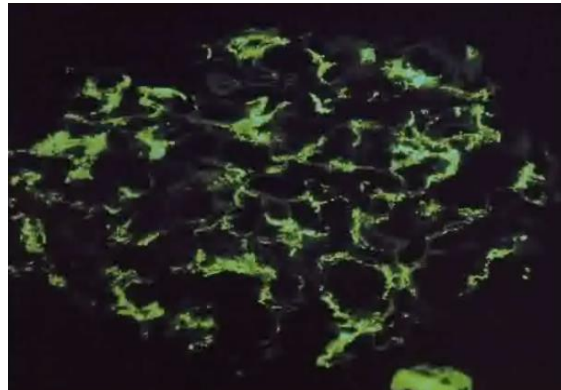
**Immune complexes** may be detectable in the blood. There are a variety of clever tests to show them, for example by their ability to bind to and agglutinate beads to which activated complement C1q is coupled. A common older test is to look for ‘**cryoglobulins**,’ which are seen as a fluffy precipitate when the serum is kept 24 hours in the refrigerator (immune complexes are less soluble in the cold.) These are ‘mixed’ cryoglobulins, being antigen + antibody; single-component cryoglobulins are the monoclonal product of an occasional clone of malignant B cells.

**Rheumatoid factor** is readily tested for by adding the patient’s serum to IgG coated microbeads; IgM anti-IgG will agglutinate the beads.

**Renal biopsy.** Thin sections of the kidney, containing at least one glomerulus, are placed on a slide and overlaid with fluorescein-labeled goat antibodies to human immunoglobulin classes or subclasses. After incubation the slide is rinsed and examined under an ultraviolet microscope. The basement membrane is visualized as the site of tiny clumps of antigen-antibody complex, in a pattern that is called ‘**lumpy-bumpy.**’ A fluorescent antibody to complement will usually reveal the same pattern. So, one would guess, would an antibody to the antigen, but we rarely know what the antigen is.



**Lupus glomerulonephritis, lumpy-bumpy**



**IgA nephropathy, lumpy-bumpy**

**TREATMENT** in all these conditions is anti-inflammatory and sometime immunosuppressive. If a patient has hives (urticaria), they may be a result of the anaphylatoxic release of histamine, and respond to antihistamine treatment. In the worst cases, plasmapheresis (replacement of plasma with return of red and white blood cells) can be helpful as a temporary measure.

## Learning Objectives for Type III Immunopathology

1. Arthus reaction and serum sickness are local and general manifestations of immune complex disease; describe the mechanism of inflammation and tissue damage. Discuss why this could reasonably be called 'innocent bystander injury.'
2. State the approximate size at which immune complexes get stuck in basement membranes.
3. Describe 'one-shot' serum sickness.
4. Name the types of tissues in which deposition of immune complexes is most likely to occur, and discuss why that is so.
5. Discuss the immunological mechanism of a typical Type III disease involving exogenous antigen.
6. Discuss how urticaria (hives) could result from interaction of antigen with IgG antibody.
7. Name 4 different kinds of human immune complex disease or problem and indicate a type of antigen involved in each condition.
8. Discuss the meaning of finding a fluffy white precipitate in a patient's serum after a day in the refrigerator. Include the name used for such precipitates, the most likely composition, and the interpretation of the phenomenon.
9. Define rheumatoid factor and discuss its components.
10. Discuss the pathogenesis of post-streptococcal glomerulonephritis. Describe the diagnosis of this condition by fluorescent antibody technique, and name the pattern of resulting fluorescence.
11. Discuss the pathogenesis of hypersensitivity pneumonitis, for example Farmer's Lung.