OVERVIEW: HOW INNATE LEADS TO ADAPTIVE IMMUNITY

INTRODUCTION. This unit is about the workings of the immune system. It begins with a look at the innate immune system, in enough detail for our current needs. Then there is a discussion of how, when the resources of innate immunity are insufficient, innate engages the adaptive immune response. The last few pages offer a survey of adaptive immunity, to help you begin to build up the "big picture"—what the immune system is, how all the parts fit together, how it keeps us well, and finally, how it can go wrong. Immunology is concerned with every aspect of the immune system. We try to understand how it operates so we can explain, and preferably prevent, or if necessary treat, problems.

►Today we'll spend most of the time on the innate immune system, which is important of itself, and also is necessary to activate the adaptive (T and B cell) immune system. So please read at least that part of the notes before class. The rest is a quick overview of what's coming: what T cells and B cells are there for. We'll cover some of this, but the notes are there primarily to help you orient yourself; we'll of course get into all that in subsequent weeks.

The problem with us humans is that we're just too delicious. The world crawls with microscopic creatures that would love to get inside and settle down. We're warm and wet and full of nutrients, and we're sociable and mobile, so we can easily spread our little visitors around.

In a war between the humans and the bugs, the bugs would surely win because we're badly outnumbered: there are a thousand times more bacteria on your skin than there are people on the planet. Due to small litters and long maturation times, humans are expensive to produce, so nature invests a vast amount of energy in protecting us from microorganisms. How that happens is the subject of this course.

Resistance to infection is layered like an onion. On the outside we have the skin and mucous membrane barriers. These are not as impervious as Teflon or tough as Kevlar, but they have a special property that the plastics can't match: they are constantly being replaced, the skin in about seven days, the gut lining in as little as three. They are rich in antibiotics: harmless skin *Staphylococcus epidermidis* can survive but dangerous *Staph. aureus* die within minutes of landing on the skin, done in by toxic proteins. There are numerous members of the **defensin** and **cathelicidin** families, small proteins made by epithelial cells or brought to them by white blood cells. They are positively charged (cathelicidin = CATionic HELIcal bacteriCIDal proteIN) so they bind to negatively-charged pathogen membranes, and then insert to form lethal pores.

> **ASK YOURSELF:** Our skin makes 5 g/day of these protective proteins. So do you think bathing is a bad idea?

The details are not all available but somehow we establish rich commensal floras in our guts and on our skin; ►most of the organisms are beneficial, and it doesn't do (and is pretty impossible) to try to remove them, for example by excessive washing with bactericidal soaps or by crazy 'detoxification' treatments. Washing hands after contact with any patient, or an ill colleague, or an icky doorknob, however, *is* a very good idea. Wash with soap and hot or cold water for at least 15 seconds. Purell®-type alcohol washes are good, too, and even better than soap for certain bacteria, although here the WHO and the U.S. doctors seem to disagree.

IMMUNITY. The next layer is called **innate immunity**. It's interesting to think of the innate immune system as one of our sense organs. Senses detect changes in the environment and make it possible for us to respond to them: We smell pizza and move towards it, we see a saber-toothed cat and climb a tree, we hear a strange noise and turn to investigate it. The immune system is our cellular and molecular-level sense. ►Its job is to **detect** intruders that have

ventured too deep into the body's structures, and then arrange for their **inactivation**, **destruction**, and **removal**. It makes a strong effort to distinguish between innocent and pathogenic invaders. But it can mistakenly react to harmless things such as pollens or foods.

When they hear 'immunity' most people think of antibodies and lymphocytes and vaccines—the **adaptive** response—and that's good; in fact up to a few years ago that's all we would consider in an immunology course. Now we realize that the innate response is older, and extremely important both on its own and as an activator of the adaptive response. So we'll cover innate immunity in some detail now, and follow up with an overview of adaptive immunity, which you'll do mostly on your own. We'll expand on it in later units, and I'll emphasize some overall concepts in class.

INNATE IMMUNITY: WHAT THE BODY IS TRYING TO DO. It is crucial to know *quickly* when something dangerous has come into the body, and mobilize defenses against it. The innate immune response is shared in part with much older creatures, for example invertebrates. It recognizes a few molecular 'motifs,' chemical structures found in pathogens but not in the host itself. For humans, such motifs would include components of bacterial cell walls (us eukaryotes don't have cell walls, of course), and distinctive microbial nucleic acid patterns.

►Innate immunity recognizes three sorts of things:

1. Foreign molecular motifs called pathogen-associated molecular *patterns* (**PAMPs**);

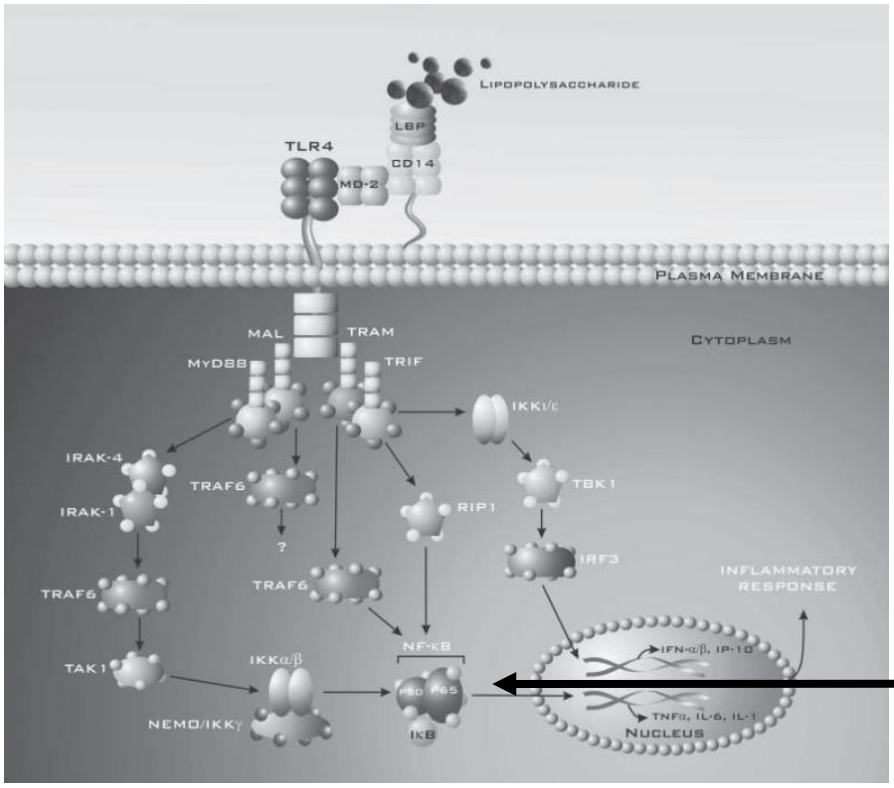
2. Stress or damage indicators expressed by body cells, usually called damage-associated molecular patterns (**DAMPs**);

3. The *absence* of certain 'self' marker molecules, which would certainly indicate a problem. This is done by NK cells, which we'll hear about later in the course.

Most cells in the body have some ►pattern-recognition receptors, **PRR,** on their surface or on endosomal (cytoplasmic) membranes. Among these are at least 10 (in humans) 'Toll-like receptors,' **TLR**, so called because of homology to the *Toll* gene in the fruit fly (which controls innate immunity in invertebrates, as well as early anterior/posterior differentiation). Each TLR can recognize a foreign molecular structure that we humans don't have; for example, TLR4 binds lipopolysaccharide (part of the cell wall of Gram-negative bacteria); TLR2 binds peptidoglycan (Gram-positive bacteria); and TLR3 binds double-stranded RNA, which many viruses make. The table that follows is for your interest and reference; don't memorize it!

When TLR—for example on blood vessel cells, or white blood cells passing by near an infected wound—bind the foreign pattern, signaling cascades are activated (see the Figure on the next page) that lead to the expression of factors that cause ►**inflammation**. The factors released by the stimulated cell are called **cytokines** and **chemokines**. Inflammation is defined for our purposes as increased blood vessel diameter, molecular stickiness, and leakiness, with an efflux of fluid and phagocytic white blood cells into the tissues. The intent is to quickly get defense and healing agents into the damaged or invaded area.

Toll-like receptor SURFACE *ENDOSOMAL*	**Identified ligands (PAMPs and *DAMPs*)**
TLR-1/TLR-2	Tri-acyl **lipopeptides** (bacterial, mycoplasmal), soluble bacterial factors
TLR-2	Lipopeptides, **zymosan** (fungal), glycosylphosphoinositols, **glycolipids**, lipoteichoic acid, porins, atypical LPS, *HSP70 (host)*, lipoarabinomannan (LAM)
TLR-3	**dsRNA** (viral), *RNA released from necrotic host cells*
TLR-4	**Endotoxin=Lipopolysaccharide (LPS)**, fusion and envelope proteins (viral), HSP60 (bacterial), *multiple host proteins including HMGB1, a nuclear protein, and fibrinogen cleavage products*
TLR-5	**Flagellin**
TLR-6/TLR-2	Di-acyl **lipopeptide** (mycoplasma)
TLR-7	Synthetic ligands: Imiquimod, Loxoribine; **ssRNA** (influenza)
TLR-8	**ssRNA** (mostly viral)
TLR-9	**Unmethylated CpG** sequences in DNA (mostly bacterial and viral, including herpes viruses)
TLR-10	Unknown; may actually suppress inflammation

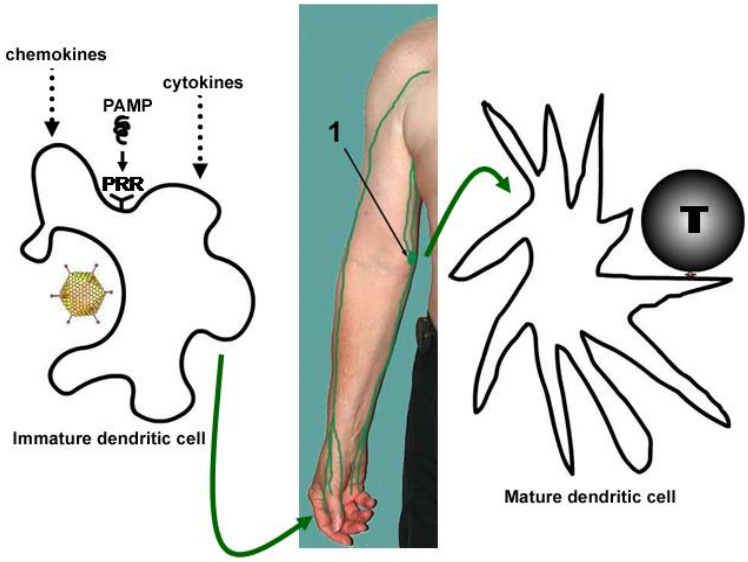


These pathways seem dizzyingly complex, but inflammation can save your life or kill you, and things like that need a lot of controls. All these factors offer possible targets for modulation by pharmacologists. In this example[1], LBP binds LPS, CD14 captures the complex allowing it to engage MD-2, which finally engages TLR4.

All TLR except TLR3 use the MyD88 and IRAK pathway (TLR3 uses IRF). **A main effect is to activate the mother of all inflammatory transcription factors, NF-κB.** This upregulates the production of cytokines.

[1] B. Verstak et al. Toll-like receptor signaling and the clinical benefits that lie within. Inflamm. Res. 56 (2007) 1–10.

As cells get damaged and stressed, they release certain of their internal molecules (the **DAMPs**), and some TLRs bind them, too, increasing the local inflammation. Thus even before the 'real' immune system gets involved the local area of damage is a hotbed of inflammatory mediators. Some of these mediators—especially the chemokines—are chemotactic for phagocytic white blood cells, which flow in from distant areas. You see this around a splinter, and of course it's worse if there is bacterial infection. ►The innate system is fast, but note that it can't *adapt* to totally new challenges—it only sees a few established patterns.



If the innate response can't completely handle the problem (we guess that it usually can) then the ***adaptive*** immune system, the 'real' one, is called into play. It's a little slower to get started but its potential is greatly superior. ►Here's the connection between the two responses: there are special phagocytic cells at the interfaces between the body and the world—skin, lung, other mucous membranes—called **dendritic cells** (**DC**) because their membranes are highly branched and folded. At our wound site, immature DC get activated by the soup of cytokines and chemokines, and take up anything they can, including foreign molecules derived from the invaders. ►It's impossible to have a good adaptive response if there isn't an innate response to prime the pump (some vaccines have innate immune stimulators—*adjuvants*—added for this reason). The activated DC leave the local area and travel in the lymphatics to the nearest (draining) lymph node (**1** in the diagram), where they 'show' what they've eaten to lymphocytes (thus they are also referred to as 'antigen-presenting cells,' **antigen** being the name for things recognized by adaptive immunity). In the lymph node, there is just the right balance of B cells, T cells, and DC to get an adaptive immune response going. Adaptive immune responses can't develop in the periphery—that's why it's so important for antigen to be brought to the organized lymphoid tissues by dendritic cells; once triggered and expanded in the node, though, immune cells and molecules leave and become distributed throughout the body. We will, of course, hear more about all this.

CELLS OF THE ADAPTIVE IMMUNE SYSTEM. Two kinds of cells divide the work of the immune system between them: lymphocytes and phagocytes. Lymphocytes are specialized for the *recognition* of foreignness and phagocytes are specialized for *eating*; that's what their name means.

THE ADAPTIVE IMMUNE RESPONSE. To do their job of recognition, the lymphocytes have surface receptors that bind the antigenic molecule whose 'shape' fits best. A lymphocyte may have a hundred thousand receptors on its surface, but all are identical; so each cell can recognize only one foreign shape. To be able to recognize all potential antigens, then, we need a lot of diversity: a huge number of different lymphocytes. This is very different from the handful of patterns recognized by innate immunity. People have estimated that the human species might be able to make as many as a hundred thousand billion (10 to the 14th power) different antigen receptors, so each of us must have a very large number of different lymphocytes in our bodies. Only a small part of a large antigenic molecule, perhaps 10 to 20 amino acids, called an ►**antigenic determinant** or **epitope**, fits into the lymphocyte's receptors. The fit is conceptually

like a lock and key; if it's good, the lymphocyte can become activated. An antigen may encounter millions of lymphocytes before it meets one whose receptor it fits. Note that lymphocyte diversity exists *before* antigen comes in. We don't make lymphocytes to fit an antigen; antigens select the lymphocytes they fit best. We'll learn that mechanism later.

►After antigen is bound and certain other conditions are met, the resting ('naïve') lymphocyte is activated, and begins to proliferate, so that a **clone** of identical cells is produced. The number of cells able to recognize that particular antigen is expanding; in fact, doubling about every 6 to12 hours. As the clone expands some of its cells differentiate and begin to secrete proteins which get the immune response rolling. Once the clone is big enough, we have enough cells and molecules to fight the infection (if that's what got the process started) and we begin to recover. Many lymphocytes are long lived, so that the factory remains expanded, and the next time we encounter that organism we can usually overwhelm it before it establishes a beachhead; in other words, we are **immune** (►we have immunologic memory.) This happens either after a real infection, or after the administration of a vaccine. And we not only have more **memory cells**, they are easier to activate next time, so they can respond to small amounts of antigen.

SNAPSHOT: THE TWO KINDS OF ADAPTIVE IMMUNITY. Two kinds of lymphocytes have already been mentioned: T and B. Their strategies are the same—the recognition and removal of foreign substances. Their tactics, however, are quite different. The reason for this has to do with the jobs each population has been assigned. B cells protect the extracellular spaces of the body—the tissue fluids, blood, secretions—by releasing **antibodies** into these fluids. T cells themselves survey the surfaces of the body's cells, looking for ones that have parasites within them or that are dangerously changed or mutated.

T cells recognize antigens by means of their surface receptors, ►which see antigens **presented** by the newly-arrived DC. When a T cell does so, it's activated, proliferates, and the daughters travel throughout the body until they reach places where antigen has invaded. There they are restimulated by local antigen-presenting cells and release a family of short-range mediators called **lymphokines** (Definition: cytokines made by a lymphocyte). These mediators call up a much-augmented inflammatory response by attracting and activating monocytes and macrophages, which are specialized for phagocytosis and destruction. You begin to get better.

Another type of T cell are specialized for killing any body cell that they recognize as containing abnormal molecules, which may be the result of damaging mutations, or the products of intracellular pathogens like viruses.

B cells also arrange for the phagocytosis and destruction of foreign materials. Like T cells, they recognize antigens via surface receptors, and become activated and proliferate. They then release ►soluble versions of their receptors, namely **antibodies**, which go out to do the work.

Summarizing: Although all resting lymphocytes look the same, and pretty uninteresting at that, you now see that there are two main types: T and B. B lymphocytes secrete antibodies; T lymphocytes secrete short-range mediators called lymphokines, a subset of cytokines.

T LYMPHOCYTES. Let's expand just a bit on T lymphocytes, or T cells for short, so called because, although they begin their development in the bone marrow, they mature in the **T**hymus. There are 2 main classes of T cells: Helpers and Killers. There are 5 specialized subtypes of helpers:

Type 1 Helper T cells, **Th1**, recognize antigen and make a lymphokine that attracts thousands of **macrophages**, the heavy-duty phagocytes, to the area where antigen has been recognized. This intense inflammation can wipe out a serious infection—or a transplanted kidney.

Th17 Helper T cells, **Th17**, are similar to Th1 in that their main role is to cause focused inflammation, although they are more powerful than Th1. They have been implicated in many serious forms of autoimmunity.

Type 2 Helper T cells, **Th2**, stimulate macrophages to become 'alternatively activated,' able to function in walling-off pathogens and promoting healing, a process that usually takes place after the pathogen-killing Th1 response. They are very important in parasite immunity.

Follicular Helper T cells, Tfh, are stimulated by antigen and migrate from T cell areas of lymph nodes into the B cell follicles, where they help B cells get activated and make the IgM, IgG, IgE and IgA antibody subclasses.

Regulatory T cells, Treg, make cytokines that suppress the activation and function of Th1, Th17, and Th2 cells, so they keep the immune response in check. They are part of the Th family.

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Cytotoxic or **killer T cells**, **CTL** for short, destroy any body cell they identify as bearing a foreign or abnormal antigen on its surface.

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Th1, Th2, Th17, Tfh, and Treg have a molecular marker, called **CD4**, on their surface, which increases their affinity for antigen, helps get them activated, and also serves us as a convenient tag for their identification. Killers have a related marker, **CD8**.

T CELL-MEDIATED IMMUNITY STARTS. Imagine you've been exposed to a virus. The virus enters the body, makes a small local infection, and soon encounters, sticks to and is taken up by a dendritic cell. Infection and local damage stimulate the innate immune response; as the affected cells release chemokines and cytokines, inflammation results which helps activate the dendritic cells. Within its phagocytic vacuoles the antigen is partially digested. Peptides derived from it are ►loaded into special antigen-presenting molecules called MHC Class II and recycled to the cell surface. The dendritic cell now travels via lymphatics to a lymph node or the spleen, where T cells are in abundance. The receptors of helper T cells are designed to recognize antigen that has been eaten, processed by dendritic cells, and loaded onto MHC Class II ('presented'). The T cells become activated and begin dividing rapidly; in a few days each may give rise to thousands of daughters. They leave the node and travel around the body until they encounter antigen. Inflammation results, and you begin to get better.

KILLER T CELLS. Cytotoxic T cells also examine the surfaces of incoming dendritic cells for presented antigenic fragments; in this case, they are looking for fragments on a ►different class of antigen-presenting molecule, called MHC Class I, which is not only on dendritic cells, but on *all* cells. The appropriate clones of CTL get expanded and the daughters circulate in large numbers throughout the body. When one of the daughters of a stimulated CTL binds a cell showing the same peptide, it delivers a 'lethal hit,' signaling the target cell to commit suicide by activating an internal self-destruction process (apoptosis). The activated killer T cell can then kill other infected cells. This is a great way to eliminate infected cells.

> **ASK YOURSELF:** How can it be better for the immune system to kill an infected cell than to just let the virus kill it? After all, in both cases, the cell is dead.

If a person is ***immune*** to a particular antigen, for example a virus, that means he or she had a previous encounter with it—either during an actual illness, or by vaccination, or by contact that did not lead to illness. The responsive T cell clones are already expanded, and there are many memory cells.

B CELLS. B cell receptors see antigen alone, and do not require the simultaneous recognition of an associated MHC molecule ("presentation") the way T cells do. When a B cell binds antigen, it is activated to proliferate and differentiate (usually after help from a Tfh cell.) A fully differentiated B cell is a **plasma cell,** a protein-production factory. It releases soluble versions of its receptor, called **antibodies**. Antibodies bind to the corresponding antigen, and this may be enough to neutralize a toxin, or prevent a microorganism from binding to its target cell.

There are 5 classes of antibodies.

IgG, short for Immunoglobulin G, is the most abundant antibody in blood. Two adjacent IgG molecules, binding an antigen such as a bacterium, cooperate to activate ►**complement**, a system of proteins that enhances inflammation and pathogen destruction (much more on this later).

The complement system is very important in disease resistance, and its various components can do different things. Some can lyse (burst) a bacterium by making holes in its membrane. Others diffuse away from the site where antibody is interacting with antigen, and attract phagocytic cells, in this case predominantly polymorphonuclear neutrophils, or PMNs. This is useful in disposing of many kinds of antigens.

IgG is the only class of antibody that passes the placenta from mother to fetus in humans, and so is very important in protecting the newborn until it can get its own IgG synthesis going.

IgM is a large polymeric immunoglobulin. It's even better at activating complement than is IgG, and is the first antibody type to appear in the blood after exposure to a new antigen. It is replaced by IgG in a week or two.

IgD is the main form of antibody inserted into B cell membranes as their antigen receptor, which seems to be its only biological role.

IgA is the most important class of antibody in the secretions like saliva, tears, genitourinary and intestinal fluids, and milk. In these secretions it's associated with another chain called Secretory Component, which it acquires from epithelial cells during the process of being secreted. Secretory Component makes it resistant to digestive enzymes. IgA plays an important role as the first line of defense against microorganisms trying to gain access to the body through the mucous membranes.

IgE is designed to attach to **mast cells** in tissues. Thus attached, when it encounters antigen, it will cause the mast cell to make prostaglandins, leukotrienes, and cytokines, and release its granules which contain powerful mediators of inflammation like **histamine**. These mediators produce the symptoms of allergy, which range from hay fever and hives to asthma and anaphylactic shock, depending on the site of antigen entry and dose. All this is inconvenient; but the *real* role of IgE is in resistance to parasites, such as worms.

ANTIBODY FUNCTION IN DISEASE. The first time an antigen enters the body at the mucous membranes, it may penetrate to the local lymphoid tissues where there are T and B cells; the environment there favors the production of IgA and, in certain people, IgE too. The IgA is secreted and local immunity is established. Oral polio vaccine, for example, favors the formation of mucosal IgA antibodies to polio virus, which trap viruses before they can even enter the body.

If the antigen penetrates further into the body, it reaches local lymph nodes or the spleen, and there the environment favors first IgM production, and then IgG, which bind up pathogens as they circulate.

When most antigens enter the body, there will be both T and B cell responses to them. Some will be more important than others for that particular antigen. We'll consider this as the course progresses.

In general, though, antibody is important for combating extracellular pathogens like the bacteria *Staphylococcus*, *Streptococcus*, and *Hemophilus*. It is also important for neutralizing toxins like tetanus toxin, and will block the spread of virus in the blood (but once the virus gets into cells, CTL will be needed to get rid of it.)

INTRODUCTION TO IMMUNOPATHOLOGY: THE COMMON MECHANISMS

Type I immunopathology is seen in patients who make too much IgE to an environmental antigen, which is often innocuous like a pollen or food. More than 10% of the population have allergic symptoms. Although usually a nuisance, asthma can be life-threatening, and several people die each year of anaphylactic shock, in which the mast cells throughout the body are suddenly degranulated and release their histamine. A bee sting or certain foods can do it. We don't know why some people are allergic; it is partly genetic, and if both your parents are allergic your risk of developing allergies goes up to over 60%.

Type II immunopathology is autoimmunity, due to antibodies which can react against self. There are a number of ways this can come about: For example, if a foreign antigen happens to look like a self molecule, the response to the antigen may accidentally 'cross-react' with self. And if an antigen sticks to certain cells in the body, the immune response may destroy the cells as innocent bystanders. The mechanism is what we observed in normal antibody immunity: antibody binds, complement is activated, phagocytes are attracted, and they attempt to eat the antigen.

> **ASK YOURSELF:** How do you think we treat these diseases? What might be some side effects?

Type III immunopathology can occur whenever someone makes antibody against a soluble antigen. Immune complexes of antigen and antibody are usually eaten by phagocytes, but if they are a bit too small for that, they may instead get trapped in the basement membranes of capillaries they circulate through. The trapped complexes activate complement and the usual inflammatory response occurs, with the tissue damaged as an innocent bystander. No matter what the antigen is, the symptoms tend to be the same: arthritis, glomerulonephritis, pleurisy, rash. Foreign antigens that cause Type III include drugs like penicillin when given in large doses, and foreign serum, such as horse antiserum to rattlesnake venom (in fact, the syndrome is often called *serum sickness*.) More troublesome is when the antigen is internal, as part of an autoimmune process. Thus people with systemic lupus erythematosus, SLE, make antibody to their own DNA, some of which can always be found free in blood.

Type IV immunopathology is T cell mediated, and can be autoimmune, or more commonly innocent bystander injury. For example, in tuberculosis most of the destruction in lungs is T cell-mediated, not bacterium-mediated. In acute viral hepatitis, most of the liver destruction is by killer T cells, just doing their job.

Chronic frustrated immune responses is JJ's term for conditions in which the antigen is not "self," but is something you can't get rid of: your own gut bacteria, for example, (inflammatory bowel disease) or, unless you stop eating it, gluten (celiac disease). Your body is the field on which the immune system and the antigen are in chronic battle.

AIDS. There are many other diseases with immunological overtones. AIDS is the most devastating new disease. You can see why in the context of the mechanisms we've been discussing. The AIDS virus, HIV-1, infects Th cells because its envelope glycoprotein, gp120, binds to the CD4 molecules they have on their surface. Inside, it uses its enzyme, reverse transcriptase, to copy its RNA into DNA which becomes inserted into the cell's own DNA. It then is latent, and seems to become reactivated when the T cell is activated by antigen, leading to a progressive loss in Th cells that, as you can imagine, is a critical blow to all branches of immunity.

THE END (OF THE BEGINNING). You can see that we understand quite a lot about the mechanisms of immunity. What we are having trouble figuring out is the regulation of the system, and these are some of the big questions, to which I'm sure you could add some of your own:

- How many genes regulate the immune response?
- Why does it go wrong in one person but not another?
- What are the consequences of a *slight* deficiency in immunity?
- How significant are the effects of emotions and stress on immunity?
- How can we stimulate specific immune responses in unresponsive patients?
- How can we turn off bad immune responses without turning off the whole system?
- Can we disguise tissues so that the immune system does not regard them as foreign? Can we clone such tissues?
- How do we convince otherwise-intelligent people that immunization is good, not bad, for their children?
- Can we make an effective vaccine against HIV? Malaria? TB? *Cancer*? *Alzheimer disease*?

Learning Objectives for Innate to Adaptive Immunity

Note: At this early stage your ability to respond to these objectives will probably be a bit incomplete or vague; don't worry about that, as the course progresses you can revisit these objectives to see how your knowledge has become more sophisticated, and how more confident you feel about addressing the objectives..

1. Define: Pattern-recognition receptor, pathogen-associated molecular pattern, damage-associated molecular pattern, Toll-like receptor, and recognize their abbreviations.
2. List some *common* foreign patterns recognized by TLR.
3. Identify the final transcription factor that is most commonly activated in inflammation.
4. Define cytokine and chemokine.
5. Describe the function of the innate immune response.
6. Name the cell that forms the bridge between innate and adaptive immunity.
7. Discuss briefly the function of T cells.
8. Discuss briefly the function of B cells.
9. Describe briefly the chief properties of IgG, IgM, IgA, and IgE.
10. Give examples of immunopathology.
11. Discuss some of the big problems still to be solved in immunology.