OVERVIEW: HOW INNATE LEADS TO ADAPTIVE IMMUNITY

INTRODUCTION. This unit introduces 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 4 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 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 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.

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One 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 these 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, and about as many bacteria in your intestines as cells in your body. 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?

Soon after birth (much later if we’re born by Caesarian section, and later still if we’re not breast-fed) 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 World Health Organization and U.S. doctors have some differences of opinion.
**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 so 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 a 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 does an excellent job distinguishing between innocent and pathogenic invaders.

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 a little 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 point out some overall concepts in class.

**INNATE IMMUNITY: WHAT THE BODY IS TRYING TO DO.** It is crucial to know immediately 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 (that is, has receptors for) 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 made 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 in the IMMUNOMODULATORS and TUMOR IMMUNOLOGY units.

Most cells in the body have some ►pattern-recognition receptors, PRR, on their surface or on endosomal (internal) membranes. Among these are 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 that are 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.

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1 Cytokines are made by any cell, and influence the activity of neighbors that have the appropriate receptor; chemokines are small proteins that are chemotactic for various cells. There are LOTS of both of these families!
### Identified ligands (PAMPs and DAMPs)

<table>
<thead>
<tr>
<th>Toll-like receptor</th>
<th>Identified ligands</th>
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<tbody>
<tr>
<td>TLR-1/TLR-2</td>
<td>Tri-acyl lipopeptides (bacterial, mycoplasmal), soluble bacterial factors</td>
</tr>
<tr>
<td>TLR-2</td>
<td>Lipopeptides, zymosan (fungal), glycosylphosphoinositolis, glycolipids, lipoteichoic acid, porins, atypical LPS, HSP70 (host), lipoarabinomannan (LAM)</td>
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<tr>
<td>TLR-3</td>
<td>dsRNA (viral), RNA released from necrotic host cells</td>
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<tr>
<td>TLR-4</td>
<td>Endotoxin=Lipopolysaccharide (LPS), fusion and envelope proteins</td>
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<td></td>
<td>multiple host proteins including HMGB1, a nuclear protein, and fibrinogen cleavage products (thought to be involved in asthma)</td>
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<tr>
<td>TLR-5</td>
<td>Flagellin (the motor protein in bacterial flagella)</td>
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<tr>
<td>TLR-6/TLR-2</td>
<td>Di-acyl lipopeptide (mycoplasma)</td>
</tr>
<tr>
<td>TLR-7</td>
<td>ssRNA (influenza); Synthetic ligands: Imiquimod, Loxoribine (which stimulate innate inflammation and treat warts and some skin cancers)</td>
</tr>
<tr>
<td>TLR-8</td>
<td>ssRNA (mostly viral)</td>
</tr>
<tr>
<td>TLR-9</td>
<td>Unmethylated CpG sequences in DNA (mostly bacterial and viral, including herpes viruses)</td>
</tr>
<tr>
<td>TLR-10</td>
<td>Unknown; may actually suppress inflammation</td>
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These pathways seem 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 B. Verstak et al. Toll-like receptor signaling and the clinical benefits that lie within. Inflamm. Res. 56 (2007) 1–10.

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.
When body 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, see the image below. At a wound or infection 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. There are other PRR systems, and we’ll mention them later, too.

The DC is maybe the prettiest cell in the body
A BRIEF INTRODUCTION TO ADAPTIVE IMMUNITY
(read when you have time)

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 taking up and digesting; 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 huge number of different lymphocytes. This is very different from the handful of patterns recognized by innate immunity. Each of us has a very large number of different lymphocytes in our bodies. A small part of a large antigenic molecule, 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. The huge diversity of lymphocytes exists before antigen comes in. Antigens select the lymphocytes whose receptors they fit best. We’ll learn that mechanism in the antibody genes unit.

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. 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 become long lived memory cells, 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.

SNAPSHOT: THE TWO KINDS OF ADAPTIVE IMMUNITY. There are two kinds of adaptive lymphocytes: T and B. Their strategies are the same—the recognition and removal of foreign substances. Their tactics, however, are quite different.

T cells recognize antigens by means of their surface receptors, which see antigens presented by dendritic cells. 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 (cytokines made by a lymphocyte). These mediators call up a much-augmented inflammatory response. You begin to get better. We’ll have 2 units on T CELLS.

Another type of T cell, the CTL (cytotoxic T lymphocytes) 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, 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. They mature in the Thymus. 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 protect us from fungi, but 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**, help B cells get activated and make the 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.

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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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Helper T cells have molecules, called CD4, on their surface, which increase their affinity for antigen, and help get them activated. Killers have related molecules, CD8. We measure numbers of CD4 and CD8 cells in blood or tissues to evaluate immunity.

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. 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. When IgG binds an antigen it can activate a family of inflammatory and protective mediators called complement. We discuss this in the unit called Antibody Function.

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. 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.

**IgD** is inserted into B cell membranes as an antigen receptor, which seems to be its only biological role.

**IgA** is the most important class of antibody in the secretions like saliva and milk. In these secretions it’s associated with another chain called **Secretory Component**, which 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** plays a special role in protecting the body from parasites, like protozoa and worms. It is designed to trigger the release of various mediators from specialized **mast cells**. But it’s also the antibody associated with conditions like allergy and asthma.

In general, 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, the CTL class of T cells 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. Although usually a nuisance, asthma can be life-threatening, and several people die each year of anaphylactic shock, in which mast cells throughout the body release their histamine.

**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.

**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.

**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** happen when the antigen 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.
LAST THOUGHTS. 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? What are they?
- 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?
- Are genetically-modified immune cells going to be the treatment that cures most cancers?
- 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.

The following LOs will be covered in subsequent sessions. They are here now just to suggest the most important points to think about if you read the introduction to 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.