

LECTURE 3

B Cells and Antibodies

REVIEW

Let's quickly review the material we covered in the last lecture. We talked about the complement system of proteins, and how complement fragments can function as "poor man's antibodies" to tag invaders for ingestion by professional phagocytes. In addition, complement fragments can act as chemoattractants to help recruit phagocytic cells to the battle site. Finally, the complement proteins can participate in the construction of membrane attack complexes that can puncture and destroy invading pathogens (e.g., certain bacteria and viruses).

The complement proteins are present in high concentrations in the blood and also in the tissues, so they are always ready to go. In addition, activation by the "alternative" (spontaneous) pathway simply requires that a complement protein fragment, C3b, bind to an amino or hydroxyl group on an invader. Because these chemical groups are ubiquitous, the default option in this system is death: Any surface that is not protected against binding by complement fragments will be targeted for destruction.

In addition to the alternative activation pathway, which can be visualized as grenades going off randomly here and there, we discussed a second pathway for activating the complement system that is more directed: the lectin activation pathway. In this system, a protein called mannose-binding lectin attaches to carbohydrate molecules that make up the cell walls of common pathogens. Then, a protein that is bound to the MBL sets off the complement chain reaction on the surface of the invader. So the mannose-binding lectin acts as a guidance system, which targets the complement "bombs" to invaders that have distinctive carbohydrate molecules on their surfaces.

We also talked about two professional phagocytes: macrophages and neutrophils. In tissues, macrophages have a relatively long lifetime. This makes sense because macrophages act as sentinels that patrol the periphery. If they find an invader, they become "activated." In this activated state, they can present antigens to T cells, they can send signals that recruit other immune system cells to help in the struggle – and they become vicious killers.

In contrast to sentinel macrophages, which reside beneath the surface of all the parts of our body that are exposed to the outside world, most neutrophils can be found in the blood – where they are on call in case of attack. Also, whereas macrophages are quite versatile, neutrophils mainly do one thing – kill. Neutrophils use cellular adhesion molecules to exit blood vessels at sites of inflammation, and as they exit, they are activated to become killers. Fortunately, these cells only live about five days. This limits the damage they can do to healthy tissues once an invader has been vanquished. On the other hand, if the attack is prolonged, there are plenty more neutrophils that can exit the blood and help out, since neutrophils represent about 70% of the circulating white blood cells.

The natural killer cell is another player on the innate team which is on call from the blood. These cells are a cross between a killer T cell and a helper T cell. Natural killer cells resemble Th cells in that they can secrete cytokines which affect the function of both the innate and the adaptive immune systems. And like CTLs, they can destroy some virus-infected cells. However, in contrast to CTLs, which select their targets by surveying peptides displayed by class I MHC molecules, NK cells specialize in killing cells that don't express class I MHC molecules – especially "stressed" cells that have lost class I MHC expression.

REVIEW (continued)

The innate system is programmed to react to “danger signals” that are characteristic of commonly encountered pathogens or pathogen-infected cells. Phagocytes, natural killer cells, and the complement proteins can attack immediately, because these weapons are already in place. As the battle continues, cooperation between players increases to strengthen the defense, and signals given off by the innate system recruit even more defenders from the blood stream. By working together, the players on the innate sys-

tem team provide a fast and effective response to common invaders.

The innate system also plays a crucial role in alerting the adaptive immune system to danger. In fact, as we begin now to discuss the adaptive immune system, you will want to be on the lookout for interactions between the innate and adaptive systems. I think you’ll soon appreciate that although its rapid response is crucial for our survival, the innate system does much more than just react quickly.

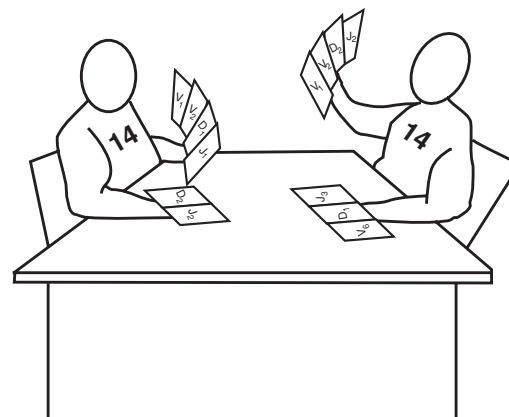
B CELLS AND ANTIBODIES

Microbes such as bacteria and viruses are always mutating. Just as mutations in bacteria can render them resistant to certain antibiotics, mutations also can change microbes in ways that make them better able to resist immune defenses. When this happens, the immune system must “adapt” by producing new counter-weapons. Otherwise, the mutated microbe may take over. Indeed, a chess match has been going on for millions of years in which the immune systems of animals constantly have been “upgraded” in response to novel weapons fielded by microbial attackers. The most striking upgrade of the immune system began about 200 million years ago, when, in fish, evolution led to the precursor of what might be called the “ultimate defense” – a system so adaptable that, in principle, it can protect against any possible invader. This defense, the adaptive immune system, has reached its most sophisticated form in humans. Indeed, without an immune system which can recognize and adapt to deal with unusual invaders, human life would not be possible.

In this lecture, we will focus on one of the most important components of the adaptive immune system: the B cell. Like all the other blood cells, B cells are born in the bone marrow, where they are descended from stem cells. About one billion B cells are produced each day during the entire life of a human, so even old guys like me have lots of freshly made B cells. During their early days in the marrow, B cells select gene segments coding for the two proteins that make up their B cell receptors, and these receptors then take up their positions on the surface of the cell. The antibody molecule is almost identical to the B cell receptors, except that it lacks the protein sequences at the tip of the heavy chain that anchor the B cell receptors to the outside of the cell. Lacking this anchor, the antibody molecule is exported out of the B cell (is secreted), and is free to travel around the body to do its thing.

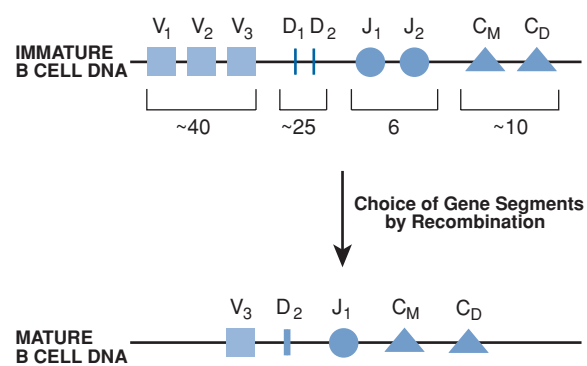
The B cell receptor

I want to tell you a little about the process of selecting gene segments to make a B cell receptor. I think you’ll find it interesting – especially if you like to gamble. **The B cell receptor is made up of two kinds of proteins, the heavy chain (Hc) and the light chain (Lc), and each of these proteins is encoded by genes that are assembled from gene segments.** The gene segments that will be chosen to make up the final Hc gene are located on chromosome 14, and each B cell has two chromosome 14s (one from Mom and one from Dad). This raises a bit of a problem, because, as we discussed earlier, each B cell makes only one kind of antibody. Therefore, because there are two sets of Hc segments, it is necessary to “silence” the segments on one chromosome 14 to keep a B cell from making two different Hc proteins. Of course, Mother Nature could have chosen to make one chromosome a “dummy,” so that the other would always be the one that was used – but she didn’t. That would have been too boring. Instead, she came up with a much sweeter scheme, which I picture as a game of cards with the two chromosomes as players. It’s a game of “winner takes all,” in which each chromosome tries to rearrange its cards (gene segments) until it finds an arrangement that works. The first player to do this wins.



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You remember from the first lecture that the finished Hc protein is assembled by pasting together four separate gene segments (V, D, J, and C), and that lined up along chromosome 14 are multiple, slightly different copies of each kind of segment.



The players in this card game first choose one each of the possible D and J segments, and these are joined together by deleting the DNA sequences in between them. Then one of the many V segments is chosen, and this “card” is joined to the DJ segment, again by deleting the DNA in between. Right next to the rearranged J segment is a string of gene segments (C_M, C_D , etc.) that code for various constant regions. By default, the constant regions for IgM and IgD are used to make the BCR, just because they are first in line. Immunologists call these joined-together gene segments a “gene rearrangement,” but it is really more about cutting and pasting than rearranging. Anyway, the result is that the chosen V, D, and J segments and the constant region segments all end up adjacent to each other on the chromosome.

Next, the rearranged gene segments are tested. What’s the test? As you know, protein translation stops when the ribosome encounters one of the three stop codons, so if the gene segments are not joined up just right (in frame), the protein translation machinery will encounter a stop codon and terminate protein assembly somewhere in the middle of the Hc. If this happens, the result is a useless little piece of protein. In fact, you can calculate that each player only has about one chance in nine of assembling a winning combination of gene segments that will produce a full-length Hc protein. Immunologists call such a combination of gene segments a “productive rearrangement.” If one of the chromosomes that is playing this game ends up with a productive rearrangement, that chromosome is used to construct the winning Hc protein. This heavy chain protein is then transported to the cell surface, where it signals

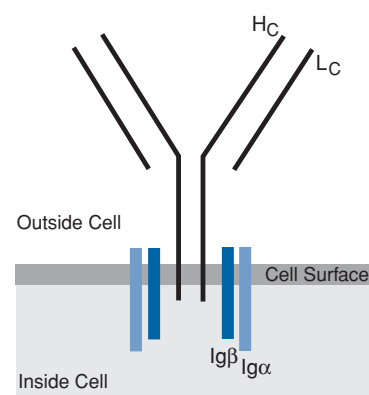
to the losing chromosome that the game is over. Exactly how the signal is sent and how it stops the rearrangement of gene segments on the other chromosome remain to be discovered, although it is thought to have something to do with changing the conformation of the cell’s DNA so that it no longer is accessible to the cut-and-paste machinery.

Since each player only has about a one in nine chance of success, you may be wondering what happens if **both** chromosomes fail to assemble gene segments that result in a productive rearrangement. Well, the B cell dies. That’s right, it commits suicide! It’s a high-stakes game, because a B cell that cannot express a receptor is totally useless.

If the heavy chain rearrangement is productive, the baby B cell proliferates for a bit, and then the light chain players step up to the table. The rules of their game are similar to those of the heavy chain game, but there is a second test which must be passed to win: The completed heavy chain and light chain proteins must fit together properly to make a complete antibody. If the B cell fails to productively rearrange heavy and light chains, or if the Hcs and Lcs don’t match up correctly, the B cell commits suicide. So **every mature B cell produces one and only one kind of BCR or antibody, made up of one and only one kind of Hc and Lc. However, because a mix and match strategy is used to make the final Hc and Lc genes of each B cell, the receptors on different B cells are so diverse that collectively, our B cells can probably recognize any organic molecule that could exist.** When you consider how many molecules that might be, the fact that a simple scheme like this can create such diversity is truly breathtaking.

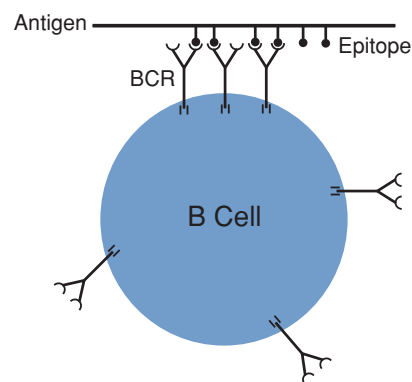
How the BCR signals

Immunologists call the antigen that a given B cell’s receptors recognize its “cognate” antigen, and the tiny region of the cognate antigen that a BCR actually binds to is called its “epitope.” For example, if a B cell’s cognate antigen happens to be a protein on the surface of the flu virus, the epitope will be the part of that protein (usually 6 to 12 amino acids) to which the BCR binds. When the BCR recognizes the epitope for which it is matched, it must signal this recognition to the nucleus of the B cell, where genes involved in activating the B cell can be turned on or off. But how does this BCR “antenna” send a signal to the nucleus that it has found its epitope? At first sight it would appear that this could be a bit of a problem, because, as you can see from this figure, the part of the heavy chain that extends through the cell membrane into the interior of the cell is only a few amino acids in length – way too short to do any serious signaling.



To make it possible for the external part of the BCR to signal what it has seen, B cells are equipped with two accessory proteins, $Ig\alpha$ and $Ig\beta$, which associate with the Hc protein and protrude into the inside of the cell. Thus, **the complete B cell receptor really has two parts: the Hc/Lc part outside the cell that recognizes the antigen but can't signal, and the $Ig\alpha$ and $Ig\beta$ proteins that can signal, but which are totally blind to what's going on outside the cell.**

To generate a signal, many B cell receptors must be brought close together on the surface of the B cell. When B cell receptors are clustered like this, immunologists say they are "cross-linked" – although the receptors really are not linked together. B cell receptors can be clustered when they bind to an epitope that is repeated many times on a single antigen (e.g., a protein in which a sequence of amino acids is repeated many times).



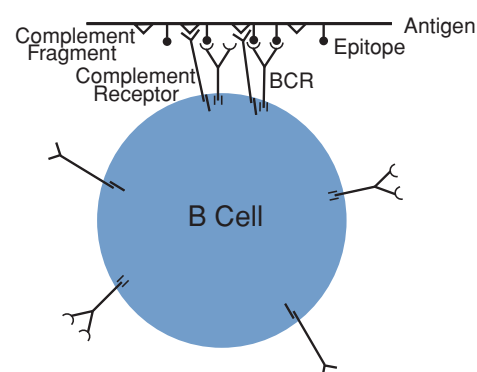
Cross-linking of B cell receptors also can result when B cell receptors bind to epitopes on individual antigens that are close together on the surface of an invader. Indeed, the surfaces of most bacteria, viruses, and parasites are composed of many copies of a few different proteins. So if a B cell's receptors recognize an epitope on one of these proteins, lots of B cell receptors can be clustered.

Indeed, the requirement for cross-linking is one way B cells focus on common enemies. Finally, B cell receptors can also be brought together by binding to epitopes on antigens that are clumped together (e.g., a clump of proteins). Regardless of how it is accomplished, cross-linking of B cell receptors is essential for B cell activation. Here's why.

The tails of the $Ig\alpha$ and $Ig\beta$ signaling molecules interact with enzymes inside the cell. When enough of these interactions are concentrated in one region, an enzymatic chain reaction is initiated which sends a message to the nucleus of the cell saying, "BCR engaged." So the trick to sending this message is to get lots of $Ig\alpha$ and $Ig\beta$ molecules together – and that's exactly what cross-linking of B cell receptors does. **The clustering of BCRs brings enough $Ig\alpha$ and $Ig\beta$ molecules together to set off the chain reaction that sends the "BCR engaged" signal. So BCR cross-linking is key.**

You remember from the last lecture that fragments of complement proteins (e.g., C3b) can bind to (opsonize) invaders. This tag indicates that the invader has been recognized as dangerous by the innate immune system, and alerts innate system players like macrophages to destroy the opsonized invader. It turns out that antigens opsonized by complement fragments also can alert the adaptive immune system. Here's how.

In addition to the B cell receptor and its associated signaling molecules, $Ig\alpha$ and $Ig\beta$, there is another protein on the surface of a B cell that can play an important role in signaling. This protein is a receptor that can bind to complement fragments which are decorating an invader. Consequently, for an opsonized antigen, there are two receptors on a B cell that can bind to the antigen: the BCR which recognizes a specific epitope on the antigen, and the complement receptor that recognizes the "decorations." When this happens, the opsonized antigen acts as a "clamp" that brings the BCR and the complement receptor together on the surface of the B cell.



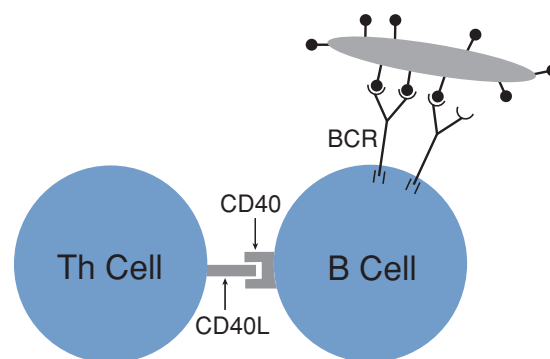
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When the BCR and the complement receptor are brought together in this way by opsonized antigen, the signal that the BCR sends is amplified greatly. What this means in practice is that the number of BCRs that must be clustered to send the “receptor engaged” signal to the nucleus is decreased at least 100-fold. **Because the complement receptor can have such a dramatic effect on signaling, it is called a “co-receptor.”** The function of this co-receptor is especially important during the initial stages of an attack, when the amount of antigen available to cross-link B cell receptors is limited. **Recognition of opsonized invaders by the B cell co-receptor also serves to make B cells exquisitely sensitive to antigens that the innate system has identified as being dangerous. This is an excellent example of the “instructive” function of the innate system. Indeed, the decision on whether an invader is dangerous or not is usually made by the innate, not the adaptive, system.**

How B cells are activated

To produce antibodies, B cells must first be activated. Most B cells have never encountered their cognate antigen, and these cells are called “naive” or “virgin” B cells. An example would be a B cell that can recognize the smallpox virus, but which happens to reside in a human who has never been exposed to smallpox. In contrast, B cells that have already encountered their cognate antigen are called “experienced.” There are two ways that naive B cells can be activated to defend us against invaders. One is completely dependent on the assistance of helper T cells (T cell–dependent activation), and the second is more or less independent of T cell help (T cell–independent activation).

Activation of a naive B cell requires two signals. The first is the clustering of the B cell’s receptors and their associated signaling molecules. However, just having its receptors cross-linked is not enough to fully activate a B cell – a second signal is required. Immunologists call this the “co-stimulatory” signal. In T cell–dependent activation, this second signal is supplied by a helper T cell. The best-studied co-stimulatory signal involves direct contact between a B cell and a Th cell. On the surface of activated Th cells are proteins called CD40L. When CD40L plugs into (ligates) a protein called CD40 on the surface of a B cell, the co-stimulatory signal is sent, and if the B cell’s receptors have been cross-linked, the B cell is activated.



The interaction between these two proteins, CD40 and CD40L, is clearly very important for B cell activation – humans who have a genetic defect in either of these proteins are unable to mount a T cell–dependent antibody defense.

In response to certain antigens, virgin B cells can also be activated with little or no T cell help, and this mode of activation is termed T cell–independent. What these antigens have in common is that they have repeated epitopes which can cross-link a ton of B cell receptors. A good example of such an antigen is a carbohydrate of the type found on the surface of many bacterial cells. A carbohydrate molecule is made up of many repeating units, much like beads on a string. If each “bead” is recognized by the BCR as its epitope, the string of beads can bring together many, many BCRs.

The cross-linking of such a large number of BCRs can partially substitute for co-stimulation by CD40L, and can cause a B cell to proliferate. But to be fully activated and produce antibodies, a naive B cell must receive a second signal. For T cell–independent activation, this second key is a “danger signal” (e.g., the battle cytokine, IFN- γ), which is a clear indication that an attack is on. What this means is that if a B cell has receptors that can recognize a molecule with repeated epitopes like, for example, your own DNA, it may proliferate, but fortunately, no anti-DNA antibodies will be produced. The reason is that your immune system is not engaged in a battle with your own DNA, so there will be no danger signals to provide the necessary co-stimulation. On the other hand, if the innate immune system is battling a bacterial infection, and a B cell’s receptors recognize a carbohydrate antigen with repeated epitopes on the surface of the bacterial invader, that B cell will produce antibodies – because danger signals from the battlefield can supply the second key needed for complete B cell activation. Of course, as

is true of T cell–dependent activation, **T-cell independent activation is antigen specific: Only those B cells whose receptors recognize the repeated epitope will be activated.**

One advantage of T cell–independent activation is that B cells can jump right into the fray without having to wait for helper T cells to be activated. The result is a speedy antibody response to those invaders that can activate B cells independent of T cell help. But there is something else important going on here. Helper T cells recognize only protein antigens, so if all B cell activation required T cell help, the entire adaptive immune system would be focused on proteins. This wouldn't be so great, because many of the most common invaders have carbohydrates or fats on their surface that are not found on the surface of human cells. Consequently, these carbohydrates and fats make excellent targets for recognition by the immune system. So **by allowing some antigens to activate B cells without T cell help, Mother Nature did a wonderful thing: She increased the universe of antigens that the adaptive immune system can react against to include not only proteins, but carbohydrates and fats as well.**

In addition to T cell–dependent and T cell–independent activation of B cells, there is another, “unnatural” way that B cells can be activated. In this case the antigen, usually called a mitogen, binds to molecules on the B cell surface that are not B cell receptors, clustering these molecules. When this happens, BCRs that are associated with these molecules also can be clustered. In contrast to T cell–dependent and T cell–independent activation, this “polyclonal” activation does not depend on the cognate antigen that is recognized by the BCR – the BCR just comes along for the ride. In this way, many different B cells with many different specificities can be activated by a single mitogen. Indeed, mitogens are favorite tools of immunologists, because they can be used to activate a lot of B cells simultaneously, making it easier to study events that take place during activation.

One example of a mitogen is the highly repetitive structure that makes up the surface of certain parasites. During a parasitic infection, the molecules that make up these structures can bind to receptors (mitogen receptors) on the surface of B cells and cluster them – and when the mitogen receptors are brought together in this way, the cell's BCRs also are clustered. The result is polyclonal activation of B cells. But why would the immune system want to react to a parasitic attack by activating B cells whose BCRs do

not even recognize the parasite? The answer is that this is not something the immune system was designed to do! By activating a bunch of B cells that will produce irrelevant antibodies, the parasite seeks to distract the immune system from focusing on the job at hand – destroying the parasitic invader. So **polyclonal activation of B cells by a mitogen actually is an example of the immune system gone wrong** – a subject we will discuss at length in another lecture.

Once B cells have been activated, and have proliferated to build up their numbers, they are ready for the next stage in their life: maturation. Maturation can be divided roughly into three steps: “class switching,” in which a B cell can change the class of antibody it produces; “somatic hypermutation,” in which the rearranged genes for the B cell receptor can undergo mutation and selection that can increase the affinity of the BCR for its cognate antigen; and the “career decision,” during which the B cell decides whether to become an antibody factory (a plasma cell) or a memory B cell. The exact order of these maturation steps varies, and some B cells may skip one or more steps altogether.

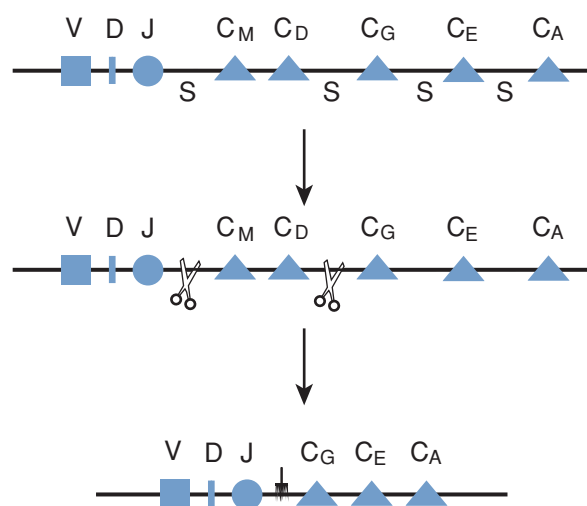
Class switching

When a virgin B cell is first activated, it produces mainly IgM antibodies – the default antibody class. B cells also can produce IgD antibodies. However, IgD antibodies represent only a tiny fraction of the circulating antibodies in a human, and it is unclear whether they actually perform any significant function in the immune defense. You remember that an antibody's class is determined by the constant (Fc) region of its heavy chain – the “tail” of the antibody molecule, if you will. Interestingly, the same heavy chain messenger RNA (mRNA) is used to make both IgM and IgD, but the mRNA is spliced one way to yield an M-type constant region and another way to produce a D-type tail.

As a B cell matures, it has the opportunity to change the class of antibody it makes to one of the other antibody classes: IgG, IgA, or IgE. Located just next to the gene segment on chromosome 14 that encodes the constant region for IgM are the constant region segments for IgG, IgE, and IgA. So all that a B cell has to do to switch its class is to cut off the IgM constant region DNA and paste on one of the other constant regions (deleting the DNA in between). Special switching signals that allow this cutting and pasting to take place are located between constant region segments on chromosome 14. For example,

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here's what happens when a B cell switches from an IgM constant region (C_M in this drawing) to an IgG constant region (C_G):



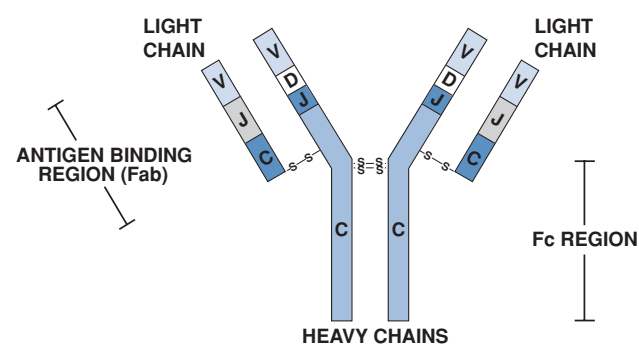
The net result of this switching is that although the part of the antibody that binds to the antigen (the Fab or "hands" region) remains the same, the antibody gets a new tail. This is an important change, because it is the constant region tail which determines how the antibody will function.

ANTIBODY CLASSES AND THEIR FUNCTIONS

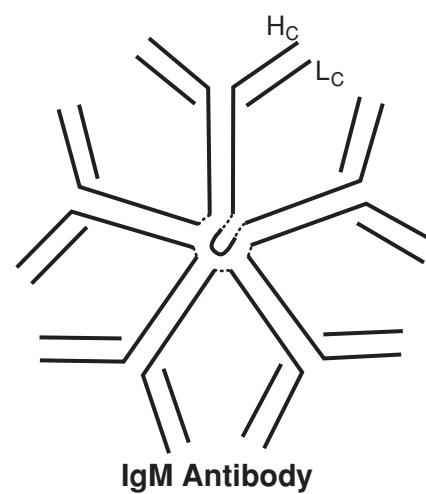
Let's take a look at the four main classes of antibodies: IgM, IgA, IgG, and IgE. As you will see, because of the unique structure of its constant (Fc) region, each antibody class is particularly well suited to perform certain duties.

IgM antibodies

IgM antibodies were the first class of antibodies to evolve, and even "lower" vertebrates (my apologies to the animal rights folks) have adaptive immune systems that produce IgM antibodies. So it makes sense that in humans, **when naive B cells are first activated, they mainly make IgM antibodies.** You may remember from the first lecture that an IgG antibody looks roughly like this:



Well, an IgM antibody is like five IgG antibody molecules all stuck together. It's really massive!



Producing this huge IgM antibody early during an infection is quite smart, because IgM antibodies are very good at activating the complement cascade (immunologists call this "fixing complement"). Here's how it works.

In the blood and tissues, some of the complement proteins (about 30 of them!) get together to form a big complex called C1. Despite its size, this complex of proteins cannot activate the complement cascade, because it's bound to an inhibitor molecule. However, if two or more C1 complexes are brought close together, their inhibitors fall off, and the C1 molecules can then initiate a cascade of chemical reactions that produces a C3 convertase. Once this happens, the complement system is in business, because, as you remember from the last lecture, the C3 convertase converts C3 to C3b, setting up an amplification loop that produces more and more C3b. So **the trick to activating the complement system by this "classical" (antibody-dependent)**

pathway is to bring two or more molecules of C1 together – and that’s just what an IgM antibody can do.

When the antigen-binding regions of an IgM antibody bind to an invader, C1 complexes can bind to the Fc regions of the antibody. Because each IgM antibody has five Fc regions close together (this is the important point), two C1 complexes can bind to the Fc regions of the same IgM antibody, bringing the complexes close enough together to set off the complement cascade. So the sequence of events is: **The IgM antibody binds to the invader, several C1 molecules bind to the Fc region of the IgM antibody, and these C1 molecules trigger the complement chain reaction on the invader’s surface.**

The reason antibodies are so useful in this regard is that some clever bacteria have evolved coats which resist the attachment of complement proteins. However, B cells produce antibodies which can bind to essentially any coat a bacterium might put on. Consequently, antibodies can extend the range of the complement system by helping attach complement proteins to the surface of wily bacteria. This is a nice example of the innate immune system (the complement proteins) cooperating with the adaptive immune system (IgM antibodies) to destroy an invader. In fact, the term *complement* was coined by immunologists when they first discovered that antibodies were much more effective in dealing with invaders if they were “complemented” by other proteins – the complement proteins.

The alternative (spontaneous) complement activation pathway that we talked about in the last lecture is totally nonspecific: Any unprotected surface is fair game. In contrast, the classical or antibody-dependent activation pathway is quite specific: Only those antigens to which an antibody binds will be targeted for complement attack. In this system, the antibody identifies the invader and the complement proteins do the dirty work.

Certain “subclasses” of IgG antibodies also can fix complement, because C1 can bind to the Fc region of these antibodies. However, IgG antibodies are real wimps, with only one Fc region per molecule. So bringing two C1 complexes close enough together to get things started requires that two molecules of IgG bind very close together on the surface of the invading pathogen – and this is only likely to happen when there is a lot of IgG around. So, **early in an infection, when antibodies are just beginning to be made, IgM antibodies have a great advantage over IgG antibodies, because they fix complement so efficiently. In addition, IgM antibodies are very good at “neutralizing” viruses by binding to them and preventing them**

from infecting cells. Because of these properties, IgM is the perfect “first antibody” to defend against viral or bacterial infections.

IgG antibodies

IgG antibodies come in a number of different subclasses that have slightly different Fc regions and therefore different functions. For example, one subclass of IgG antibodies, IgG3, fixes complement better than any other IgG subclass. Likewise, the IgG1 subclass is very good at binding to invaders to opsonize them for ingestion by professional phagocytes. This is because macrophages and neutrophils have receptors on their surfaces that can bind to the Fc portion of IgG1 antibodies once those antibodies have bound to an invader.

Natural killer cells have receptors on their surface that can bind to the Fc region of IgG3 antibodies. As a result, IgG3 can form a bridge between an NK cell and its target by binding to the target cell (e.g., a virus-infected cell) with its Fab region, and to the NK cell with its Fc region. Not only does this bring the NK cell close to its target cell, but having its Fc receptors bound actually stimulates an NK cell to be a more effective killer. **This process is called “antibody-dependent cellular cytotoxicity” (ADCC). In ADCC, the NK cell does the killing but the antibody identifies the target.**

Like IgM antibodies, IgG antibodies also are very good at neutralizing viruses. However, IgG antibodies are unique in that they can pass from the mother’s blood into the blood of the fetus by way of the placenta. This provides the fetus with a supply of IgG antibodies to tide it over until it begins to produce its own – several months after birth. This extended protection is possible because IgG antibodies are the longest-lived antibody class, with a half-life of about three weeks. IgM antibodies have a half-life of only about one day.

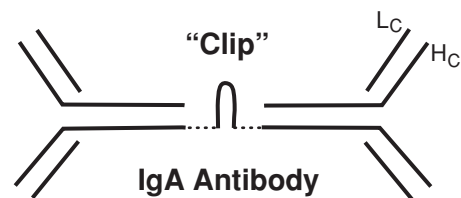
The G in IgG stands for *gamma*, and IgG antibodies are sometimes called *gamma globulins*. If there is a possibility that you have been exposed to an infectious agent, say hepatitis A virus, your doctor may recommend that you get a gamma globulin shot. These shots are prepared by pooling together antibodies from a large number of people, at least some of whom have been infected with hepatitis A virus, and are therefore making antibodies against the virus. The hope is that these “borrowed” antibodies will neutralize most of the virus to which you have been exposed, and that this treatment will help keep the viral infection under control until your own immune system can be activated.

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IgA antibodies

Here's a question for you: What is the most abundant antibody class in the human body? No, it's not IgG. It's IgA. This is really a trick question, because I told you earlier that IgG was the most abundant antibody class in the blood – which is true. It turns out, however, that we humans synthesize more IgA antibodies than all the other antibody classes combined. Why so much IgA? Because **IgA is the main antibody class that guards the mucosal surfaces of the body**, and a human has about 400 square meters of mucosal surfaces to defend. These include the digestive, respiratory, and reproductive tracts. So although there aren't a lot of IgA antibodies circulating in the blood, there are tons of them protecting the mucosal surfaces. Indeed, about 80% of the B cells that are located beneath these surfaces produce IgA antibodies.

One reason IgA antibodies are so good at defending against invaders that would like to penetrate the mucosal barrier is that each IgA molecule is like two IgG molecules held together by a "clip."



The clipped-together tail structure of IgA antibodies is responsible for several important properties of this antibody class. **This clip functions as a "passport" that facilitates the transport of IgA antibodies across the intestinal wall and out into the intestine, and their unique tail structure also makes IgA antibodies resistant to acids and enzymes found in the digestive tract.** Once inside the intestine, IgA antibodies can "coat" invading pathogens and keep them from attaching to the intestinal cells they would like to infect. In addition, whereas each IgG molecule has two antigen-binding regions (two "hands"), the "dimeric" IgA molecule has four hands to bind to antigens. **Because they are dimeric, IgA antibodies are very good at collecting pathogens together into clumps that are large enough to be swept out of the body with mucus or feces.** In fact, rejected bacteria make up about 30% of normal fecal matter.

All together, these qualities make IgA antibodies perfect for guarding mucosal surfaces. Indeed, **it is the IgA class of antibodies that is secreted into the milk of nursing**

mothers. These IgA antibodies coat the baby's intestinal mucosa and provide protection against pathogens that the baby ingests. This makes sense, because many of the microbes that babies encounter are taken in through their mouths – babies like to put their mouths on everything, you know.

Although IgA antibodies are very effective against mucosal invaders, they are totally useless at fixing complement, because C1 won't bind to an IgA antibody's Fc region. Again we see that **the constant region of an antibody determines both its class and its function.** This lack of complement-fixing activity is actually a good thing. If IgA antibodies could initiate the complement reaction, our mucosal surfaces would be in a constant state of inflammation in response to the pathogenic and nonpathogenic visitors that continuously assault our mucosal surfaces. And, of course, having chronically inflamed intestines would not be all that great. So IgA antibodies mainly function as "passive" antibodies that block the attachment of invaders to cells that line our mucosal surfaces, and usher these unwanted guests out of the body.

IgE antibodies

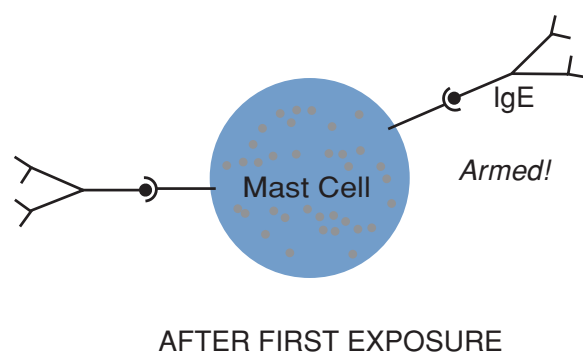
The IgE antibody class has an interesting history. In the early 1900s, a French physician named Charles Richet was sailing with Prince Albert of Monaco (Grace Kelly's father-in-law). The prince remarked to Richet that it was very strange how some people react violently to the toxin in the sting of the Portuguese man-of-war, and that this phenomenon might be worthy of study.

Richet took his advice, and when he returned to Paris, he decided, as a first experiment, to test how much toxin was required to kill a dog. Don't ask me why he decided to use dogs in his experiments. Maybe there were a lot of stray dogs around back then, or perhaps he just didn't like mice. Anyway, the experiment was a success and he was able to determine the amount of toxin that was lethal. However, many of the dogs he used in this first experiment survived, because they didn't receive the lethal dose. Not being one who would waste a good dog, Richet decided to inject these "leftovers" with the toxin again to see what would happen. His expectation was that these animals might have become immune to the effects of the toxin, and that the first injection would have provided protection (prophylaxis) against a second injection. You can imagine his surprise then, when all the dogs died – even the ones that received tiny amounts of toxin in the second injection! Since the first injection had the opposite

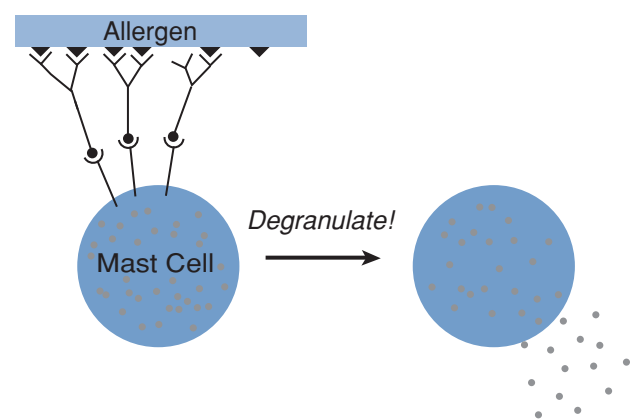
effect of protection, Richet coined the word *anaphylaxis* to describe this phenomenon (*ana-* is a prefix meaning “opposite”). Richet continued these studies on anaphylactic shock, and in 1913, he received the Nobel Prize for his work. I guess one lesson here is that if a prince suggests you should study something, you might want to take his advice seriously!

Immunologists now know that **anaphylactic shock is caused by mast cells degranulating**. Like macrophages, mast cells are white blood cells that are stationed beneath all exposed surfaces (e.g., beneath the skin or the mucosal barrier). As blood cells go, mast cells are very long-lived. They can survive for years in our tissues, lying in wait to protect against invaders. Recently, it has been learned that mast cells play a role in the innate defense against bacteria by phagocytosing opsonized bacteria, and by giving off cytokines that recruit neutrophils and other immune system cells to the site of a bacterial infection. However, the most famous function of mast cells is to protect against infection by parasites that have penetrated the barrier defense. Stored safely inside mast cells are lots of granules that contain all kinds of pharmacologically active chemicals, the most famous of which is histamine. When a mast cell encounters a parasite, it dumps the contents of these granules (i.e., it “degranulates”) onto the parasite to kill it. Unfortunately, in addition to killing parasites, mast cell degranulation also can cause an allergic reaction, and in extreme cases, anaphylactic shock. Here’s how this works.

An antigen (e.g., the man-of-war toxin) that can cause an allergic reaction is called an allergen. On the first exposure to an allergen, some people, for reasons that are far from clear, make lots of IgE antibodies directed against the allergen. Mast cells have receptors on their surface that can bind to the Fc region of these IgE antibodies, and when this happens, the mast cells are like bombs waiting to explode.



On a second exposure to the allergen, IgE antibodies that are already bound to the surfaces of mast cells can bind to the allergen. Because allergens usually are proteins with a repeating sequence, the allergen can cross-link many IgE molecules on the mast cell surface, dragging the Fc receptors together. This clustering of Fc receptors is similar to the cross-linking of B cell receptors in that bringing many of these receptors together results in a signal being sent. However, here the signal says “degranulate,” and the mast cell dumps its granules into your tissues.



Histamines and other chemicals released from mast cell granules increase capillary permeability so that fluid escapes from the capillaries into the tissue – that’s why you get a runny nose and watery eyes when you have an allergic reaction. This is usually a rather local effect, but if the toxin spreads throughout the body and triggers massive degranulation of mast cells, things can get very serious. In such a case, the release of fluid from the blood into the tissues can reduce the blood volume so much that the heart no longer can pump efficiently, resulting in a heart attack. In addition, histamine from the granules can cause smooth muscles around the windpipe to contract, making it difficult to breathe. In extreme cases, this contraction can be strong enough to cause suffocation. Most of us don’t have to worry about being stung by a Portuguese man-of-war, but for some people – those individuals who make lots of IgE antibodies in response to bee toxin – a bee sting can cause fatal anaphylactic shock.

This brings us to an interesting question: Why are B cells allowed to switch the class of antibody they make anyway? Wouldn’t it be safer just to stick with good old IgM antibodies?

Let’s suppose you have a viral infection of your respiratory tract (e.g., the common cold). Would you want

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to be stuck making only IgM antibodies? Certainly not. You'd want a lot of IgA antibodies to be secreted into the mucus that lines your respiratory tract to bind up that virus and remove it from your body. On the other hand, if you have a parasitic infection (worms, for example), you'd want IgE antibodies to be produced, because IgE antibodies can cause cells like mast cells to degranulate, making life miserable for those worms. So **the beauty of this system is that the different classes of antibodies are uniquely suited to defend against different invaders.**

ANTIBODY CLASS	ANTIBODY PROPERTIES
IgM	Great Complement Fixer Good Opsonizer First Antibody Made
IgA	Resistant to Stomach Acid Protects Mucosal Surfaces Secreted in Milk
IgG	OK Complement Fixer Good Opsonizer Helps NK Cells Kill (ADCC) Can Cross Placenta
IgE	Defends Against Parasites Causes Anaphylactic Shock Causes Allergies

Now suppose Mother Nature could arrange to have your immune system make IgA antibodies when you have a cold and IgE antibodies when you have a parasitic infection. Wouldn't that be elegant? Well, it turns out that this is exactly what happens! Here's how it works.

Antibody class switching is controlled by the cytokines that B cells encounter when switching takes place: Certain cytokines or combinations of cytokines influence B cells to switch to one class or another. For example, if B cells class-switch in an environment that is rich in IL-4 and IL-5, they preferentially switch their class from IgM to IgE – just right for those worms. On the other hand, if there is a lot of IFN- γ around, B cells switch to produce IgG3 antibodies that are very effective against bacteria and

viruses. Or, if a cytokine called TGF- β is present during the class switch, B cells preferentially change from IgM to IgA antibody production – perfect for the common cold. So **to insure that the antibody response will be appropriate for a given invader, all Mother Nature has to do is to arrange to have the right cytokines present when B cells switch classes.** But how could she accomplish this?

You remember that helper T cells are “quarterback” cells which direct the immune response. One way they do this is by producing cytokines which influence B cells to make the antibody class that is right to defend against a given invader. To learn how Th cells know which cytokines to make, you'll have to wait for the next two lectures, when we discuss antigen presentation and T cell activation. But for now, I'll just give you the bottom line: **In response to cytokines made by Th cells, B cells can switch from producing IgM antibodies to producing one of the other antibody classes. This makes it possible for the adaptive immune system to respond with antibodies tailor-made for each kind of invader – be it a bacterium, a flu virus, or a worm.** What could be better than that?!

SOMATIC HYPERMUTATION

As if class switching weren't great enough, there is yet another amazing thing that can happen to B cells as they mature. Normally, the overall mutation rate of DNA in human cells is extremely low – only about one mutated base per 100 million bases per DNA replication cycle. It has to be this low or we'd all end up looking like Star Wars characters with three eyes and six ears. However, in very restricted regions of the chromosomes of B cells – those regions that contain the V, D, and J gene segments – an extremely high rate of mutation can take place. In fact, mutation rates as high as one mutated base per 1,000 bases per generation have been measured. We're talking serious mutations here! This high rate of mutation is called “somatic hypermutation,” and it occurs after the V, D, and J segments have been selected – and usually after class switching has taken place. So **somatic hypermutation is a relatively late event in the maturation of B cells. Indeed, B cells that still make IgM antibodies usually have not undergone somatic hypermutation.**

What somatic hypermutation does is to change (mutate) the part of the rearranged antibody gene that encodes the

antigen-binding region of the antibody. Depending on the mutation, there are three possible outcomes: The affinity of the antibody molecule for its cognate antigen may remain unchanged, it may be increased, or it may be decreased. Now comes the neat part. It turns out that for maturing B cells to continue to proliferate, they must be continually re-stimulated by binding to their cognate antigen. Therefore, because those B cells whose B cell receptors have mutated to higher affinity are stimulated more easily (because their B cell receptors bind better), they proliferate more frequently than do B cells with lower-affinity receptors. And because B cells with high-affinity receptors proliferate more frequently, the result of somatic hypermutation is that you end up with many more B cells whose B cell receptors have high affinity for their cognate antigen.

By using somatic hypermutation to make changes in the antigen-binding region of a BCR, and by using binding and proliferation to select those mutations that have increased the BCR's ability to bind to antigen, B cell receptors can be "fine-tuned." The result is a collection of B cells whose receptors have a higher average affinity for their cognate antigen. This whole process is called affinity maturation.

So B cells can change their constant (Fc) region by class switching, and their antigen-binding (Fab) region by somatic hypermutation – and these two modifications produce B cells that are better adapted to deal with invaders. Both of these changes are controlled by cytokines that are provided mainly by helper T cells. As a result, B cells that are activated without T cell help (e.g., in response to carbohydrates on the surface of a bacterium) usually don't undergo either class switching or somatic hypermutation.

B CELLS MAKE A CAREER CHOICE

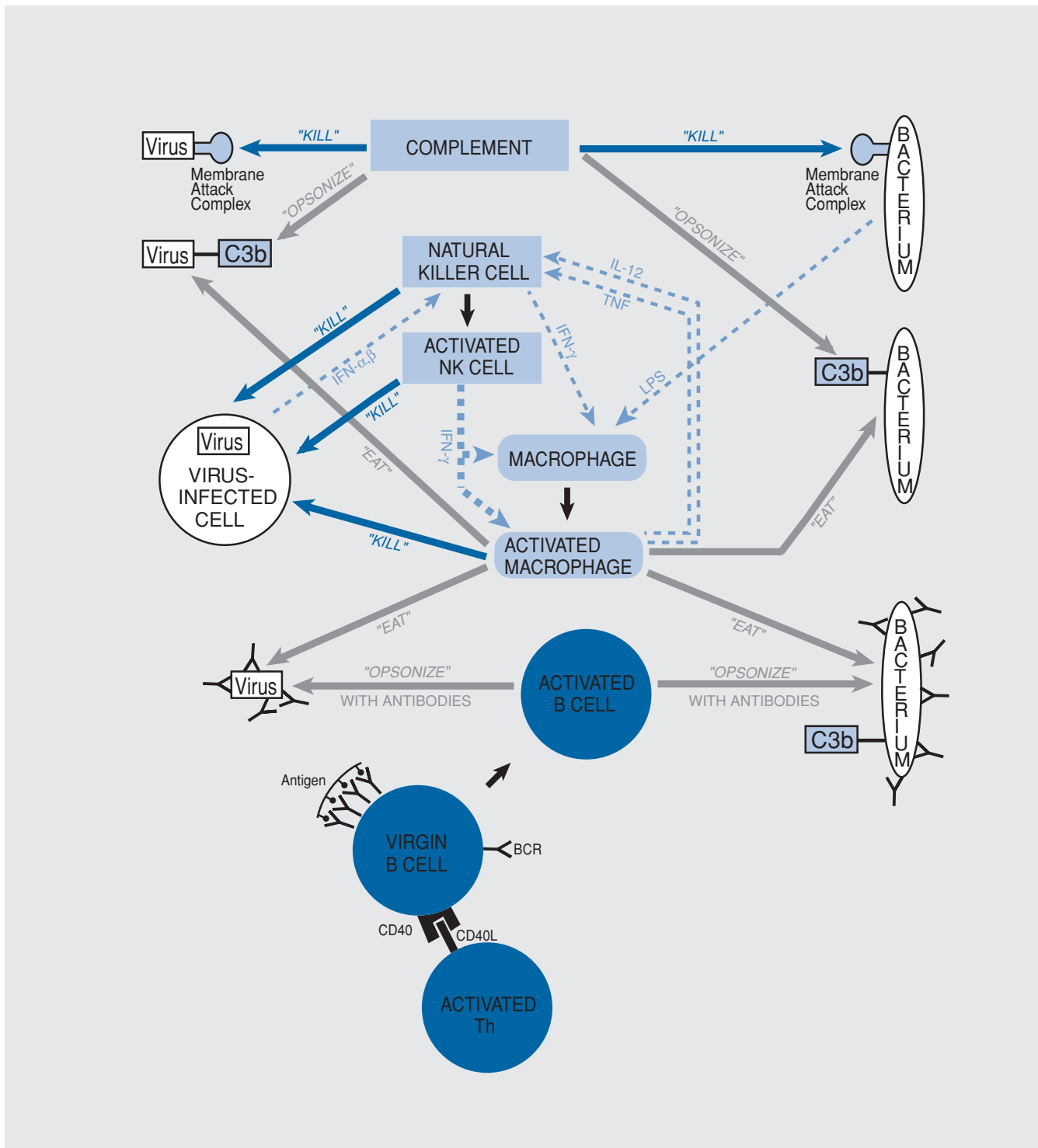
The final step in the maturation of a B cell is the choice of profession. This can't be too tough, because a B cell really only has two fates to choose between: to become a plasma cell or a memory cell. **Plasma cells are antibody factories.** If a B cell decides to become a plasma cell, it usually travels to the spleen or back to the bone marrow, and begins to produce the secreted form of the BCR – the antibody molecule. Some plasma B cells can mass produce 2,000 of these antibodies each second. However, as a result of this heroic effort, these plasma B cells only live for a few days. The fact that one plasma B cell can make so many antibody molecules enables the immune system to keep up with invaders like bacteria and viruses which multiply very quickly.

Although the B cell's other possible career choice – to become a memory B cell – may not be quite so dramatic as the decision to become a plasma cell, it is extremely important: It is the memory B cell that remembers your first exposure to a pathogen, and helps defend you against subsequent exposures. Immunologists haven't figured out how a B cell "chooses" to become either a memory cell or a plasma cell. However, they do know that the interaction between the co-stimulatory molecule, CD40L, on the surface of a helper T cell and CD40 on the B cell surface is important in memory cell generation. Indeed, **memory B cells are not produced when B cells are activated without T cell help.**

SUMMARY FIGURE

Our summary figure now includes the innate immune system from the last lecture plus B cells and antibodies.

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THOUGHT QUESTIONS

- 1** B cells are produced according to the principle of clonal selection. Exactly what does this mean?
- 2** Describe what happens during T cell–dependent activation of B cells.
- 3** Describe “fail-safe” systems that are involved in B cell activation.
- 4** Describe how B cells can be activated without T cell help.
- 5** Why is T cell–independent activation of B cells important in defending us against certain pathogens?
- 6** Describe the main attributes of IgM, IgG, and IgA antibodies.
- 7** Why do class switching and somatic hypermutation produce B cells that are better able to defend against invaders?
- 8** Why do most mast cells wait until their second exposure to an allergen before they degranulate? Hint: Think about the timing.