

HUMORAL IMMUNOLOGY

We are surrounded by a sea of microorganisms that threaten to overwhelm us. This threat is made dramatically clear by the condition of AIDS, where the immune system is crippled by a slowly progressive infection extending over a period of years. In an AIDS patient, *opportunistic* infectious agents, including bacteria, viruses, and fungi, take advantage of the weakened immune system to invade and multiply unhindered. These are not exotic infectious agents rarely encountered in our environment, but rather widely distributed disease-causing agents that a healthy, functional immune system is able to control under normal circumstances.

How does the immune system manage this control? It does so in two ways, as detailed below: via **humoral** and **cellular** immunity. Humoral immunity depends on the immune system's ability to produce **antibody** molecules which bind and inactivate infectious agents. Cellular immunity involves the development of immune cells that are able to recognize, bind, and kill other cells that have previously been infected by foreign infectious agents.

THE HUMORAL IMMUNE SYSTEM

The word humoral refers to fluids (Latin - humors) that pass through the body. In this case, the fluids - both blood **plasma** (the non-cellular fluid portion of blood) and **lymph** (the plasma-like clear fluid that fills the space between cells and drains via lymph ducts to the lymph glands and eventually into the venous circulation) carry specific molecules, which are termed variously **antibodies** or **immunoglobulins**. These are the mediators of the humoral immune response. They recognize foreign infectious agents and help the immune system to eliminate them. They are the eyes and the ears of the immune system.

The nature of antigens and antibodies

Antibody molecules are complex proteins which have the ability to recognize and bind to the surface of a foreign infectious agent, like a virus or bacterium, and then neutralize it. This binding is **specific**, in that a given antibody molecule will only bind to one type of virus and not to all others. Moreover, in the case of a virus particle, the antibody will not bind to all parts of a virus, but only to specific chemical groups displayed by the virus which are termed **antigens**, or more precisely, **antigenic determinants**.

What is the chemical nature of the antigens that are displayed on the surface of a virus particle and recognized by antibody molecules? Almost all antigens are proteins or parts of proteins. An antigen might be composed chemically of an unusual, unique sidechain of sugars that is attached covalently to the coat protein of one virus but not other viruses.

In reality, however, actual antigens are almost always **oligopeptides** - short stretches or loops of amino acid sequence - which are exposed on the outer surface of viral protein molecules. The specific sequence of amino acids that constitute this oligopeptide antigen may be present uniquely on one particular type of virus and not present in any other protein found in a normal, uninfected cell or tissue. If this oligopeptide is indeed unique, then the antigen that it creates will provide a unique *signature* of the presence of the virus that distinguishes this virus from all other viruses and all other normal components of the uninfected cell or organism. Because virus particles are assembled from multiple copies (from sixty up to many hundred) of certain viral proteins, a given oligopeptide antigen may be displayed dozens or even hundreds of times on the surface of a virus particle.

All proteins are constructed as amino acid chains far longer than the short amino acid sequence forming an oligopeptide antigen. This means that a single protein molecule will be large enough to encompass a number of distinct oligopeptide subdomains, each one of which can serve as an antigen. Moreover, a

virus particle may be assembled from several distinct proteins, each of which has its set of oligopeptide antigens. Imagine, therefore, that a virus is covered with a number of distinct antigenic determinants, A, B, C, D, etc. Each of these determinants is composed of a distinct oligopeptide sequence, and each determinant, as mentioned above, may be displayed multiple times on the surface of a virus particle.

The immune system can produce a number distinct **antibodies**, each of which is able to specifically recognize one of these antigens. For example, antibody **a** may bind specifically to any one of the many copies of the antigen **A** on the surface of the virus particle; antibody **b** may bind to any one of the many copies of antigen **B** on the surface of the virus particle, and so forth. *Hence, each antibody molecule recognizes and binds tightly to a specific, complementary antigen displayed on the surface of the virus particle.* This very directed and targeted binding of an antibody to its complementary antigen represents its **binding specificity**. Some antibody molecules may be less specific, in that they may recognize and bind to an oligopeptide antigen that happens coincidentally to be shared by a number of otherwise unrelated proteins. (Since the uniqueness of an oligopeptide antigen is dictated by its sequence of amino acids, you can imagine that the same amino acid sequence may be present in a number of otherwise unrelated proteins).

At any time, there will be many identical copies of antibody molecule **a** floating around in the circulation. Any one of these may recognize and bind to the **A** antigens present on the surface of the virus particle, in effect coating the virus particle, and functionally inactivating it- the process of virus **neutralization**. This coating of antibody molecules may prevent the virus particle from adsorbing to the surface of target cells that it might otherwise infect, thereby blocking its infectivity. In addition, the antibody-coated virus particle is fair game for certain specialized cells of the immune system (such as **macrophages**) which can recognize antibody-coated virus particles, gobble them up, and degrade them (**phagocytize** them).

Tolerance

The complexity of foreign antigens that the immune system may experience suggests another difficult problem that it must solve. Our own bodies manufacture tens of thousands of distinct normal cell proteins, each of which has its own set of oligopeptides that might be recognized by antibodies produced by the immune system. Yet, it is unusual to detect antibodies in the plasma of a normal healthy individual that react with that individual's own normal proteins.

This means that the components of the immune system that are responsible for manufacturing antibodies are somehow able to distinguish foreign proteins and their associated antigens from the normal internal proteins that the body itself normally makes. In the language of immunology, the immune system is able to distinguish **Self** from **Non-Self**. That is, normal cell and tissue proteins are distinguished from foreign proteins brought in by an infecting agents such as a virus or a bacterium.

Having sorted out these two classes of protein antigens, the immune system focuses its energies on making antibodies that recognize only (or largely) the foreign proteins. In effect, the immune system **tolerates** the body's own complement of normal proteins by not making antibodies against these proteins. Such antibodies, if made, might cause the disruption or destruction of our own normal proteins and thereby disrupt normal cellular processes. On occasion, however, this specificity breaks down: the immune system inappropriately begins to make antibodies that react with normal cellular proteins - resulting in an **autoimmune** disease that may lead to the destruction of one of the body's tissues by its own immune system. For example, early onset diabetes is often due to the destruction by the immune system of the insulin-producing cells in the pancreas.

Antibody diversity

As an illustrative example of an infectious agent, we might take the poliovirus particle, which like other viruses, displays a series of oligopeptide antigens on its surface. By focusing exclusively on this single infectious agent, we sidestep and overlook one of the major problems in immunology: poliovirus is only one of many infectious agents that can invade the body. The immune system must be able to mount an effective defense against every one of them. How many different infectious, disease-causing agents - sometimes called **pathogens** - does the immune system need to defend us against? How many distinct antibodies does it need to make?

In fact, many hundreds of different pathogens, both viral and bacterial, may enter the body during a lifetime. Each of these pathogens may in turn display dozens of distinct antigens on its surface, against which our immune system will make highly specific antibodies. Thus, during the course of a lifetime our immune systems will manufacture many thousands, if not millions of distinct antibody molecules, each one of which is reactive specifically with a distinct antigen. How can our immune systems make so many distinct antibodies, each having a different reactivity toward a distinct antigen?

The structure of antibody molecules

To begin, an antibody molecule is a complex protein, formed as an assembly of several protein chains (refer to Purves, Fig. 19.11). Each antibody molecule - sometimes termed an **immunoglobulin** - has a region dedicated to its antigen-recognizing capabilities. This region, called the **variable** domain, is unique to that particular antibody molecule and is composed of amino acid sequences that are distinctive to that antibody. The antibody molecule also has a second region, its **constant** domain, that is standard hardware, shared commonly with all other antibody molecules. The constant domain is formed from amino acid sequences that create a standard structural scaffolding - a backbone - used by all antibody molecules. The constant domain is not involved in conferring antigen-recognizing specificity. Thus, antibody **a** has the same constant region amino acid sequence as antibodies **b, c, d** etc. What distinguishes them are their respective variable regions, the amino acid sequences of which are unique to each. Each variable region can be thought of as forming an **antigen-recognizing pocket** (or cavity) which is capable of binding to a specific antigen. The antigen can be thought of as a key which fits specifically into the antibody lock.

The problem of generating many distinct antibody genes

The above discussion provokes a major question that puzzled immunologists for several decades: How do cells of the immune system have enough genes to specify thousands and likely hundreds of thousands of distinct protein-encoding sequences? In theory, the information for making all of these antibodies could be encoded in the germline, i.e. in hundreds of thousands of distinct genes, each of which directs the immune system to synthesize a distinct antibody molecule having its own unique variable region. But given the fact that the genome has approximately 30 thousand genes to begin with (the vast majority of which are used for functions unrelated to immunity), this hardly seems a likely solution.

There is a second problem with having the germline carry all the genes made by an organism (e.g. a human) in its lifetime: How can the germline genome **anticipate** pathogen that might infect an individual during his or her lifetime. How could hundreds of thousands of distinct coding sequences be evolved and encoded in our genome in order to prepare, well in advance, for all future infectious events, particularly when novel pathogens that our ancestors never experienced are continually invading the human population?

In fact, it is now known that only a relatively small number of DNA segments - numbering in the hundreds - are responsible for the vast diversity of antibodies. Hence, hundreds of genes are used to make millions, even billions of distinct antibody molecules, each having its own amino acid sequence.

How is this possible? The solution is to exploit somatic mutational mechanisms to generate **antibody diversity** anew, each time a mammal goes through its normal embryonic and post-embryonic development. The germline configuration of antibody genes is tinkered with, rearranged, and combined in many different ways to create a myriad novel genes. By randomly mixing these several hundred sequences in various **combinatorial** ways, an immune system is able to generate a vast number of distinct antibody-encoding genes.

Until now, we have portrayed the human genome as a fixed set of genes that is preserved unaltered throughout one's life unless struck accidentally by damaging mutations. Now we propose a dramatically different scenario: the immune system purposely rearranges and mutates a specific subset of genes during our lifetime, creating configurations of DNA sequence that did not exist at the moment of conception.

Before the immune system begins to develop, these DNA segments are separated from one another along a specific chromosomal region. (see Purves Figs. 19.20, 19.21) As the immune system develops, however, these various segments are pasted, cut, and spliced together in various combinations to create a vast array of distinct configurations of fused genes, each able to encode a distinct antibody molecule characterized by a distinct variable region with associated specific antigen-combining properties. By combining a relatively small number of pre-existing, germline sequences in different arrays, the immune system can make trillions of different, distinctly structured antibody molecules. We will discuss the molecular mechanics of this in more detail below.

The cells that make antibodies

In order to understand antibody production, we need to move up from molecules back to cells and resolve the following question: What types of cells are responsible for creating this antibody diversity? In fact, the **B lymphocytes** do much of this rearranging. These cells are the less-differentiated precursors of the **plasma cells**, which secrete antibody encoded by the now-rearranged genes. Antibody-secreting plasma cells manufacture antibodies in their endoplasmic reticulum and secrete them by exocytosis into the blood plasma. The combined antibody secretion of billions of plasma cells results in the diverse array of antibody molecules present in soluble form in our blood plasma.

Once again, we encounter a puzzle. Does each **B cell** make hundreds of thousands of distinct antibody molecules, or is each **B cell specialized** to make only one type of antibody molecule? Here we get a clue from the bone marrow cancer called **myeloma**, which is a malignancy of the B cell lineage. In the plasma of myeloma patients, instead of the usual, approximately equal mixture of hundreds of thousands of different antibody molecules, ***one distinct type of antibody molecule will often predominate above all others!*** Thus, this one antibody type (with its own distinctive variable region/antigen recognition domain) may actually represent the majority of the antibody molecules in the blood plasma, rather than only one-hundred-thousandth (or far less) of the complex mixture of distinct antibody molecules in the blood plasma.

When a tumor develops, all of the cells in the tumor are the lineal descendants of a common, founding ancestor. These cells represent a **cell clone**. If each B cell in the blood were specialized to make only one type of antibody, and one of these became malignant and spawned a large cohort of descendants, then the blood would be filled with these descendants, each secreting the same antibody into the plasma ***-exactly the situation we see in myeloma.*** Therefore we conclude that each normal B cell is indeed specialized to make only one kind of antibody.

In a normal individual, his/her B cell population represents a **polyclonal** mixture of hundreds of thousands of different sub-populations, **subclones** of B cells. Each B cell subclone is composed of

many identical cells, all making the identical antibody molecule. In a patient suffering from myeloma, one of these subclones has expanded so enormously that it now constitutes 10% or even 50% of the total B cells in circulation.

Development of an immune response

We all know that we can acquire immunity against an infecting virus such as poliovirus. This immunity may protect us for years, decades, or often for our entire lives. How is this immunity, which depends on the body's ability to make antibodies that recognize poliovirus, connected with the above scheme which depicts the immune system as consisting of hundreds of thousands of distinct cell clones (subpopulations), each specialized to make a specific antibody molecule?

The first time a virus infects a person, it multiplies relatively unhindered for a day or two until the immune system begins slowly to produce antibodies to combat it. Only after a protracted struggle does that person's immune system succeed in overwhelming the invading virus through production of massive amounts of antibody molecules that specifically recognize the invading virus. Therefore, the immune system is poorly prepared for the initial onslaught by the virus and its response is delayed by several days. Only after this delay can the immune system finally generate the antibodies needed to recognize and neutralize the invading virus particles.

A dramatically different outcome is seen the second time that this individual is attacked by this strain of virus. On this occasion, the immune system reacts almost instantaneously with an **antibody response** that is much quicker and results in the release of a hundred- or a thousand-fold greater concentrations of antibody molecules reactive with this virus (see Purves Fig. 19.8). This suggests that the B cells of the immune system have some type of **long-term memory** of an earlier exposure to a specific antigen (or set of antigens), and thus have developed the ability to mount a rapid and intense response to the second infection. This increased, highly potent secondary response creates a state of **immunity** in an individual. The initial infection **immunizes** the individual against a secondary infection by creating a large cohort of B cells that are able to mount a rapid, almost instantaneous response to the virus the second time this virus tries to attack.

Clonal expansion

We have recognized two major functions of the B cells: different B cell clones make distinct antibody molecules and B cell clones remember exposure to an antigen and mount a response years later that creates long-term immunity.

The **secondary response** (the ability to respond to a second attack by the virus years or decades later) provides some clues to how B cells accomplish both functions. The phenomenon of the secondary response must depend on the fact that in an individual immunized against a specific antigen, ***the number of B cells specialized to make antibody reactive to that antigen is able to persist for long times and is now much larger than when the individual initially experienced the antigen.*** Thus, one of the underlying mechanisms of immunity depends on the ability of the immune system to vastly expand the clone of cells that is able to recognize a particular (viral) antigen after that antigen is introduced into the body. This expansion, which occurs through the multiplication of cells in this clone of B cells, results in a much larger B cell/plasma cell population and thus increased amounts of antibody. In addition, this clone of cells can sit around for years producing this specialized antibody, thereby maintaining the concentration of antibody molecules at a reasonably high level in the blood plasma. These cells can also respond with great rapidity to the secondary challenge by the virus by expanding even further and very rapidly.

We conclude that a **foreign antigen can directly stimulate the multiplication of cells capable of making antibody against such antigen**. The case of myeloma is a deregulated exaggeration of this. In myeloma, the clonal expansion of a given B cell clone is driven by its own internal oncogenes rather than by the presence of a foreign antigen that it may recognize.

We must now postulate a mechanism of **antigen-driven clonal expansion**. Early in life, we start out with an enormously diverse population of B cell clones, each of equal size, each specialized to make an antigen-specific antibody molecule. The number of such clones may be very large - many billions. The number of these clones may reflect the attempts by the immune system to make as many different antibody molecules as is possible, each made by one of these B cell clones.

Into the body comes an invading poliovirus particle, which now stimulates the few cells that have the pre-existing ability to make anti-poliovirus antibody molecules to start growing. This allows us to deduce another fact: the ability of a specific B cell to make an antibody reactive with a poliovirus antigen gives this B cell the special ability to proliferate whenever poliovirus infects the body. Poliovirus provokes the multiplication of this B cell selectively. Conversely, poliovirus has no effect on the proliferation of all the other B cell clones that happen to make antibodies reactive against other, unrelated (non-poliovirus) antigens.

Once members of a specialized B cell clone have multiplied, resulting in a general increase in the population size of that clone (sometimes termed **clonal expansion**), this clone may remain expanded for the lifetime of the organism, secreting its specialized antibody molecule. Before the poliovirus infection, B cells making antibodies that recognize poliovirus might represent only one-millionth of all the B cells in the body. After a poliovirus infection, one in every 10 or hundred B cells may make anti-poliovirus antibodies because these particular cells are representatives of cell clones that have undergone enormous clonal expansion in response to a poliovirus challenge.

This scenario depicts millions, even billions of distinct B cell clones sitting around in the body, awaiting a stimulus from an infecting viral or bacterial pathogen. What has made each one of these clones so distinct and different from all others? As you can deduce from the above discussion, each of these clones has rearranged its antibody genes into a novel configuration. Most rearranged antibody genes encode antibody molecules that will prove useless in the life of an individual, since such antibody molecules will react with antigens this individual will never encounter in his/her lifetime. But by rearranging antibody genes to make a vast array of distinct antibodies, that person's immune system is prepared for all eventualities, i.e. all possible foreign antigens that might be encountered in a lifetime.

The other side of this coin relates to tolerance - the ability of the immune system to recognize normal antigens that are indigenous to the body. Although the mechanisms of tolerance are not totally worked out, it appears that early in the development of the immune system, a program of **clonal deletion** operates, in which B lymphocyte clones that happen to produce antibodies recognizing normal indigenous "self" antigens are suppressed or wiped out, leaving behind only those B cell clones that have the capacity to recognize "non-self" i.e. foreign antigens.

Detailed structure of antibody molecules

What is the structure of the antibody genes that enables them to encode such a diverse array of antibody molecules to begin with? First, let's examine the structure of the most common antibody molecule in the circulation, the immunoglobulin γ , also called IgG or gamma globulin. It is actually a **tetramer** of 4 protein chains, 2 **heavy chains** and 2 **light chains** (Purves, 19.11). The two heavy chains are identical to one another, and the 2 light chains are identical to one another. As we discuss in more detail below, the **V regions** of these protein chains form the pockets in the antibody molecule that are specialized to recognize and bind antigen.

The V region of one heavy chain combines with the V region of one light chain to produce a single antigen-binding site. Therefore, together, there **are actually 2 antigen-binding sites on a single IgG antibody molecule**. The heavy chain and the light chain are encoded by two different genes on different chromosomes. A given antibody-producing cell therefore makes an antibody molecule by expressing two heavy chains encoded by a heavy chain gene and two light chains encoded by a light chain gene and then assembling these four protein chains into a single antibody molecule, which is subsequently secreted by the cell.

Now let's look at the structure of a heavy chain gene. (Purves Fig. 19.19). The gene is enormous, encompassing many hundreds of kilobases of DNA. The DNA of this gene can be divided up into hundreds of distinct sub-domains/segments: In humans there are ~300 V (variable) segments, 10 D (diversity) segments, and 4 J (joining) segments in this one gene.

In an early B cell during development of the immune system (and prior to any clonal expansion), each of the early B cell precursors randomly picks one of its many V segments, one of its many D segments, and one of its J segments and fuses them together end-to-end to create a fused VDJ reading frame that constitutes the variable region of the antibody gene. These particular V, D, and J segments lie on the same chromosomal stretch of DNA; their fusion is achieved by deleting all the DNA sequences that happen to lie between them.

On the basis of the above numbers, one calculates that there are, combinatorially speaking, $300 \times 10 \times 4 = 12,000$ distinct V regions possible.

In fact, the number is much, much higher. The joining of the V to D to J chains is imprecise - very sloppy. Therefore, at the joining points, codons will be created and alternative reading frames generated. Moreover, the antigen-combining pocket is formed from the V regions of two antibody chains, (the heavy and the light chain), each of which contributes half of the structure. Therefore, the number of possible pockets equals the number of possible V regions generated by variability of the heavy chains **times** the number of possible V regions generated by variability of the light chains - together creating as many as 10^{11} possible combinations! Each one of these combinations may be represented in a single B lymphocyte -leading to the possible existence of as many as 10^{11} distinct types of B lymphocytes and 10^{11} distinct B cell clones in the body. The actual number of B cells in the body may be higher, because each B cell clone, prior to clonal expansion, may contain many, identical B cells in it. (However, the number of B cells can't be much higher since there are only about 3×10^{13} cells in the body!)

Thus, when a poliovirus invades the body, it will stimulate proliferation of those B cells to that, through random accidents of gene rearrangement, happen to produce antibody molecules that recognize a poliovirus antigen. These B cells will now expand clonally and will now be well represented among all B cells. Some progeny of the stimulated B cells will become plasma cells secreting large amounts of antibodies, some of the progeny will become long-lived memory cells important to lifetime immunity.

Supplementary reading: Additional sources of antibody complexity

There are two additional dimensions of antibody diversity. **First**, is the fact many generations of B cells are required to fight a viral infection in our bodies. In these dividing cells, there is a DNA region within the variable domain termed the **hypervariable region**, is subjected to **somatic mutation**, often point mutations that further diversify the V region DNA sequences. Those members of the initially expanded cell clone that happen to produce (as a consequence of these random hypervariable somatic mutations) even better (i.e. more tightly binding) antibody, are more likely to bind antigen, and therefore more likely to undergo further clonal expansion. With prolonged antigenic stimulation, the subsequent generations of B cells produce excellent antibody through trial and error due to subtle variations on the initially developed variable region. Thus at the level of biochemistry, the antibody molecules made late in an infection bind antigen much more tightly than do antibodies made early in the immune response. In the end, there are not just 10^{11} distinct V regions (see above), but many orders of magnitude more, since the initial 10^{11} combinations are then subject to subtle somatic mutations.

Earlier we implied that there is only one constant region and a virtually infinite array of variable regions, each forming a distinct antigen-binding pocket. In fact, there is not just one constant region DNA sequence, but a series of constant region DNA sequences arrayed along the chromosome downstream of the fused VDJ segment. Initially the VDJ primary RNA transcript splices to the segment that is immediately downstream of it, resulting in an IgM mRNA and IgM protein molecule. Later on, this segment will be deleted from the chromosomal DNA, which now allows an RNA transcript of the VDJ region to be spliced directly to a new constant segment, yielding an IgD mRNA and antibody, etc. IgM is made early in the immune response, whereas later one often sees various gamma globulins made like IgE, IgA, etc.

The immune system uses different **classes of antibodies** to carry out different functions. For example, one class of antibodies, the **IgM class**, is used as transmembrane receptors that stud the surface of early B cells and are used by these B cells to sense the presence of antigen in the extracellular space. This in turn signals the proliferation of the B cell, leading to clonal expansion, and eventually, to **class switching**, in which the descendants of this cell will now delete their □ constant segment and use the same variable, antigen-binding segments to form secreted IgG antibody molecules (from their □ constant region segments). Most circulating antibodies in the mature immune response (following repeated immunizations for example) will be of the **IgG class** (the so-called gamma globulins). But antibodies like IgA and IgE classes will be used in special situations, like secretion into the gut or in the teardrops. Thus, a given antigen-binding pocket can be combined with any of 8 constant regions for different specialized applications throughout the body.