

Chapter 2. Antibodies

An iddy-biddy antibody
Just nanometers long
Saved the butt of a sumo man
Hundreds of kilos strong

Anonymous

The main elements of the immune system are firstly antibodies, secondly cells that synthesize antibodies, and thirdly cells and molecules that regulate the production of antibodies. Immunology is thus about antibodies in much the same way that chemistry is about molecules. The most abundant type of antibody is called IgG or immunoglobulin G. (The words antibody and immunoglobulin ("Ig") are synonymous.) IgG is a large Y-shaped protein molecule, consisting of more than 20,000 atoms, and has a molecular weight of about 150,000 times the mass of a hydrogen atom. Each antibody consists of a constant part (C region) and two variable (V) regions. The immune system makes a vast number of different antibodies, that differ from each other in their V regions. The two V regions of a single antibody are identical, but the V regions of any two randomly chosen antibodies are almost always different from each other. Each antibody molecule is a specialist; its V region defines its specialty.

The diversity of antigens is matched by a diversity of antibodies

We do not know how many different antibody molecules one person or one mouse makes, but it is known to be a large number. Typical guesses are in the range of 10^6 to 10^8 . The large number of different antibodies made by each immune system ensures that whatever bacterium, virus or other foreign substance comes along, antibodies are available that can deal with it.

In fact antibodies are able to recognize not only a great variety of bacteria and viruses, but also completely harmless substances that an experimentalist might decide to inject into an animal. These include for example proteins and polysaccharides that are foreign to that animal. Substances that induce the production of specific antibodies are called antigens. Antigens used by immunologists in experiments include harmless substances such as egg albumin, red blood cells from another species, and many other synthetic compounds an immunologist decides to synthesize, many of which have never existed previously in nature.

Each V region is capable of binding to only a small fraction of all possible antigens. An antibody binds only to antigens that are roughly complementary

to some part of the antibody's V region. We say that the antibody is specific for those antigens.

The V regions of antibodies specific for a particular foreign organism (say "organism A") bind to the surface of the organism A. We call these antibodies "anti-A antibodies". Furthermore, following infection, much larger numbers of anti-A antibodies are synthesized, which then coat A, leading to it being killed or eliminated from circulation. More details concerning this process will be described in the next chapter on clonal selection.

Lymphocytes are the main cells of the immune system, and are responsible for both synthesizing antibodies and regulating the activities of other lymphocytes, including the regulation of the production of antibodies. Lymphocytes are found in several organs, namely in the spleen, in lymph nodes, in the thymus, in bone marrow and in the blood. They are roughly spherical cells with a diameter of about 7 to 10 microns. Blood typically contains about 10 milligrams of antibody per ml (this is about 4×10^{16} antibody molecules per ml) and about 10^6 lymphocytes per ml. Lymph nodes and the thymus consist almost entirely of lymphocytes, while spleen consists mainly of lymphocytes and red blood cells. An adult human has a total of about 10^{12} lymphocytes and about 10^{20} antibodies.

There are two main classes of lymphocytes called B cells and T cells. B cells make antibodies, and T cells regulate the production of antibodies by B cells. T cells can help B cells make antibodies, and they can suppress B cell responses. One of the most important challenges facing immunologists has been to determine how T cells perform these functions. Some T cells are also capable of killing other cells; they are then called "cytotoxic T lymphocytes". We will however be focusing on the role of T cells in helping and suppressing B cell responses.

Table 2-1 summarizes the roles played in the immune system by various cell types. Table 2-2 lists some parameters for antibodies in the blood, and Table 2-3 lists some parameters for lymphocytes.

Antibody structure

The IgG antibody is a protein consisting of four polypeptide chains: two identical "light chains" (214 amino acid building blocks each) and two identical "heavy chains" (about 440 amino acids each). Antibodies are constructed using a building block concept. The blocks are compactly folded sections called "domains." Each domain has a molecular weight of about 12,500. A heavy chain of IgG consists of a polypeptide with four domains, namely three constant (C) domains and one variable (V) domain, linked together as $V_{H1}-C_{H1}-C_{H2}-C_{H3}$. The light chain consists of one C domain and one V domain, similarly linked and labelled V_L and C_L . So IgG has a total of twelve domains.

Table 2-1. Immune System Cells

blood cells = white cells and red cells

white cells = immune system cells

(red cells = the oxygen distribution system)

white cells = lymphocytes and non-specific cells

lymphocytes = diverse cells, each with its specific receptor (V region)

non-specific cells = cells without antigen-specific receptors (e.g. macrophages)

lymphocytes = T cells and B cells

T cells = lymphocytes that do not make antibodies

B cells = lymphocytes that make antibodies

T cells = helper T cells, suppressor T cells and cytotoxic T cells

helper T cells = cells that help B cells make antibodies

suppressor T cells = cells that inhibit B cells from making antibodies

cytotoxic T cells = cells that kill other cells directly (cell-cell contact)

Table 2-2. Concentration of IgG antibodies in blood:

[IgG] = 10 mg/ml = 1% by weight = 10^{-4} M = 4×10^{16} molecules/ml

Concentration of a single specificity, if present at 1 in 10^6 = 10^{-10} M

Table 2-3. Lymphocytes

Number of lymphocytes in a person	10^{12}
Number of lymphocytes in a mouse	10^9
Number of specific receptors on lymphocytes	10^5
Diameter of a resting lymphocyte:	7 to 10 microns
Fraction of lymphocytes that are B cells	50%
Fraction of lymphocytes that are T cells	50%

As shown schematically in Figure 2-1, the twelve domains consist of three clusters of four domains each, with each cluster consisting of two domains from two polypeptide chains. The two heavy chains of IgG are connected to each other by disulphide bridges, each involving two cysteine residues. The domain clusters are connected to each other by relatively flexible polypeptide chain sections, that are susceptible to being cut by proteolytic enzymes.

Different enzymes cleave the IgG molecule in different places. For example the enzyme papain cuts the IgG molecule into three pieces, each consisting of four domains (Figure 2-2). Two identical clusters so obtained contain the V regions and are called "Fab fragments." Fab stands for "fraction antigen binding." The third cluster consists entirely of C domains, and is called the "Fc fragment." This historically determined name stands for "fraction crystallizable." We are now able to obtain crystals of Fab fragments, but in the early days only the Fc fraction could be crystallized. That was because the Fab fraction of antibodies purified from blood consist of a mixture of many different V regions, that naturally failed to crystallize due to that diversity. In the meantime we are able to produce monoclonal antibodies (antibodies of a single specificity) in large amounts. Since the V regions of a given monoclonal antibody are all identical, their Fab fragments can be crystallized and the detailed structure determined. Each Fab fragment consists of two V domains, one on the heavy chain and one on the light chain, and two C domains.

Figure 2-1. The twelve domains of an IgG antibody molecule, namely two each of V_H , C_{H1} , C_{H2} , C_{H3} , V_L and C_L . After J. A. Gally and G. M. Edelman (1972) *Annu. Rev. Genet.* 6, 1-46.

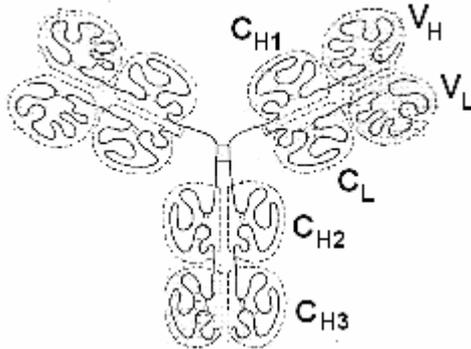


Figure 2-2 The enzyme papain splits the antibody molecule into two Fab fragments and an Fc fragment.

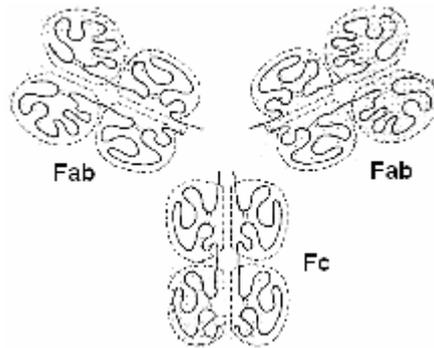
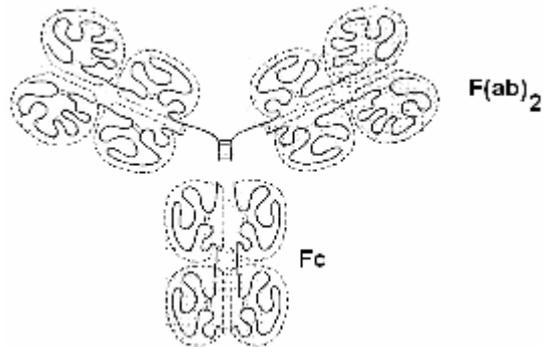


Figure 2-3. The enzyme pepsin splits the antibody molecule into an $F(ab)_2$ fragment and an Fc fragment.



The proteolytic enzyme pepsin cleaves IgG differently, to produce an Fc fragment and two Fab fragments that remain attached to each other via disulphide bonds. The latter are then called $F(ab)_2$ fragments (Figure 2-3). Fab fragments (one V region) and $F(ab)_2$ fragments (two V regions) can be used to demonstrate that the mechanism for stimulating lymphocytes involves the cross-linking of receptors, as described in chapter 9.

Antigen structure

Anything that is able to induce an immune response is an antigen. Proteins foreign to a particular animal are often used as experimental antigens for studying the immune system. For example, bovine serum albumin (BSA), a protein obtained from cattle, has been used to induce the production of anti-BSA antibodies in mice. BSA would not induce an immune response in cattle, because it is not a foreign substance for cattle.

The interaction between an antibody and an antigen is typically a non-covalent interaction that involves complementarity in a broad sense. The antigen and the corresponding specific antibody typically bind to each other via some combination of various weak interactions, namely van der Waals bonds, ionic interactions, hydrogen bonds, and hydrophobic bonding.

Epitopes

A part of an antigen that has complementarity with the V region of an antibody specific for that antigen is called an epitope or antigenic determinant. A given antigen can have several epitopes. For example, when a foreign protein is injected into a mouse, several different antibodies are typically produced that bind to various epitopes on the surface of the protein.

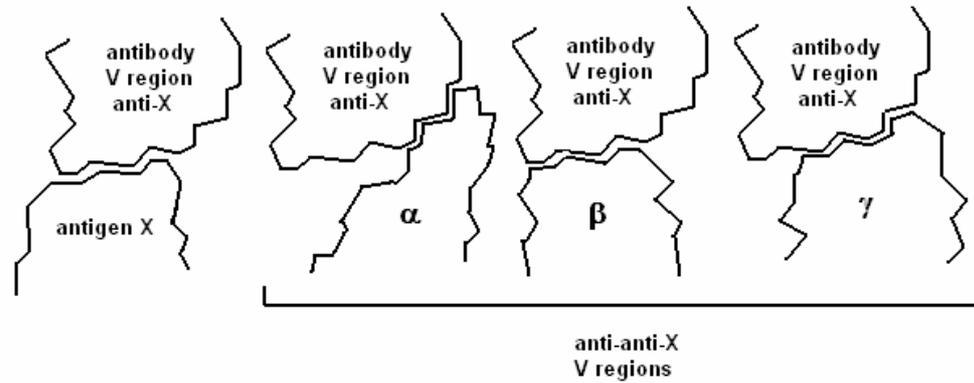
Anti-antibodies

Anti-antibodies are antibodies specific for other antibodies. They can be obtained by purifying a particular antibody, for example mouse antibodies specific for an antigen X ("anti-X") and immunizing (say) a guinea pig with the antibodies. The guinea pig is likely to respond by making two broad classes of antibodies. The first class comprises antibodies to the constant part of the mouse antibodies. These are called anti-mouse immunoglobulin, or more succinctly "anti-mouse Ig". The second class of antibodies are anti-V region antibodies, where the V region is anti-X. Hence we can call them anti-anti-X antibodies. Figure 2-4 shows the complementarity in shape between part of an antigen X and the V region of an anti-X antibody. Also shown are three examples of anti-anti-X antibodies.

Idiotopes

When Jerne formulated the network hypothesis he drew a distinction between the part of the V region of an antibody that is recognized by other V regions and a part of the V region that ostensibly recognizes other V regions. He called

Figure 2-4. The V region of an antibody that is specific for an antigen X has a shape that is complementary to the shape of the antigen (left). This complementarity results in the anti-X antibody binding specifically to the antigen X. Anti-anti-X antibodies have V regions with complementarity to some part of the anti-X antibody. Three examples are shown. The V regions of anti-anti-X antibodies can bind to a part of the anti-X antibody that is separate from the part involved in anti-X binding to X (case α), to the same part of anti-X as that to which X binds (case β), or to a site that partly overlaps with the part of the anti-X V region where anti-X binds to X (case γ).



the recognizing part of the antibody the "paratope" (the "lock" in the lock-and-key picture of enzymology), and he called a part that is recognized an "idiotope". In other words, idiotopes are epitopes on the V regions of antibody molecules. Anti-anti-X antibodies then have paratopes that bind to idiotopes on anti-X antibodies, where X is an antigen. In the symmetrical network theory that is introduced in chapter 10, the asymmetry implicit in distinguishing between paratope and epitope, and thus between recognizing and recognized parts of the V region, is of little or no significance. In that theory an epitope and a paratope are complementary shapes on two V regions, and labelling one the paratope and the other the epitope is no more and no less valid than the converse labelling. This is an important simplification.

Idiotypes

Antigenic determinants that are associated with the V regions of antibodies of a particular specificity (idiotopes) were reported by Jaques Oudin and his colleagues in France⁷ and by Henry Kunkel and his colleagues in the U.S.A⁸

⁷ J. Oudin and M. Michel (1963) Une nouvelle forme d'allotypie des globulins du serum de lapin, apparemment liée a la fonction et a la spécificité anticorps, C. R. Acad. Sci., 257, 805-808.

already in 1963. Ten years later Jerne presented the network hypothesis, in which he defined the idiotype of an antibody as the set of idiotopes present on the V region of the antibody. In the context of the symmetrical network theory (chapters 10 to 17), the distinction between idiotope and paratope disappears, and for interactions between antibodies the terms idiotype and V region can usually be used interchangeably. Antigen-specific regulation that is related to idiotypes is called idiotypic regulation, and the anti-anti-X antibodies mentioned above are called antiidiotypic antibodies.

Internal images

If an antiidiotypic antibody (anti-anti-X) resembles an antigen X, it is called an internal image of X. In Figure 2-4 the case β anti-anti-X antibody is an internal image antibody. Not all antiidiotypes resemble the antigen, since X and the antiidiotype can have complementarity to different parts of an anti-X antibody, as shown for example in case α of Figure 2-4. Intermediate cases between these two extremes can be expected to occur, as shown in case γ of Figure 2-4.

Isotypes

In addition to the enormous heterogeneity in the variable regions of antibodies, there is a more limited heterogeneity in their constant parts. There are five main classes of antibodies, called isotypes, that have various functions, and the heterogeneity in function is the result of differences in the constant parts of their heavy chains. The five isotypes are called IgG, IgM, IgA, IgE, and IgD. In the development of network theory we have been concerned initially mainly with IgM and IgG. IgM is important as a first line of defense and IgG is important in immune memory.

Heavy chains

As shown in Figure 2-2, IgG consists of four polypeptide chains, namely two identical "light chains" and two identical "heavy chains," with each chain having a C region (constant part) and a V region (variable part). IgM, IgG, IgA, IgE, and IgD are distinguished from each other by having different heavy chain constant regions. The heavy chains of IgG, IgM, IgA, IgE and IgD are called the γ , μ , α , ϵ and δ chains respectively. The differences between these chains are associated with differences in the functions of the five classes of antibodies.

⁸ H. G. Kunkel, M. Mannik, and R. C. William (1963) Individual Antigenic Specificities of Isolated Antibodies, *Science*, 140, 1218-1219.

IgM

Serum IgM molecules consist of five IgG-like molecules joined together with an extra chain called the "J chain", to yield a molecule with ten V regions and a molecular weight of about 900,000 (Figure 2-5). IgM is the first line of immunological defense in the sense that the first antibodies to be made in response to an antigen are typically IgM. A single IgM molecule, together with a set of enzymes called complement, is able to kill a cell (Figure 2-6). This plays an important role in the symmetrical network theory, as we will see in chapter 10. The concentration of IgM antibodies typically present in blood (200 micrograms/ml in mice) is small compared to that of IgG (10mg/ml).

IgG

Single IgG antibody molecules are unable to kill a cell; at least two IgG antibodies are needed. The two IgGs bind next to each other on a cell's surface and bind complement components to initiate the complement mediated killing process (Figure 2-7).⁹ IgG can also kill cells by a different mechanism, that utilizes non-specific cytotoxic accessory cells¹⁰ instead of complement. This process, called antibody dependent cellular cytotoxicity (ADCC) involves the Fc part of the IgG molecule and a receptor for Fc on the non-specific accessory cells.

IgG responses are strongly associated with immune memory. This means that if an animal makes an IgG response it will typically respond more strongly to a second exposure to that antigen than it did following the first exposure. This stronger secondary response does not occur if the first response involves the production of only IgM antibodies. The immune response to many antigens consists of an IgM response followed by an IgG response.

IgA

IgA is a class of antibody that is specialized to deal with infections in regions that are particularly exposed to the external environment. This is called "mucosal immunity". IgA is found in excretions such as saliva and tears, and is present in serum at a low concentration similar to that of IgM. IgA is typically present as a dimer of two IgG-like molecules.

⁹ T. Borsos and H. J. Rapp (1965) Complement fixation on cell surfaces by 19S and 7S antibodies. *Science* 150, 505-506.

¹⁰ Non-specific in this context means specific for neither the antigen nor the V region of another lymphocyte. Non-specific aspects of regulation are discussed in chapter 7.

Figure 2-5. Schematic diagram of an IgM molecule. The J chain links five identical IgG-like molecules with ten identical V regions.

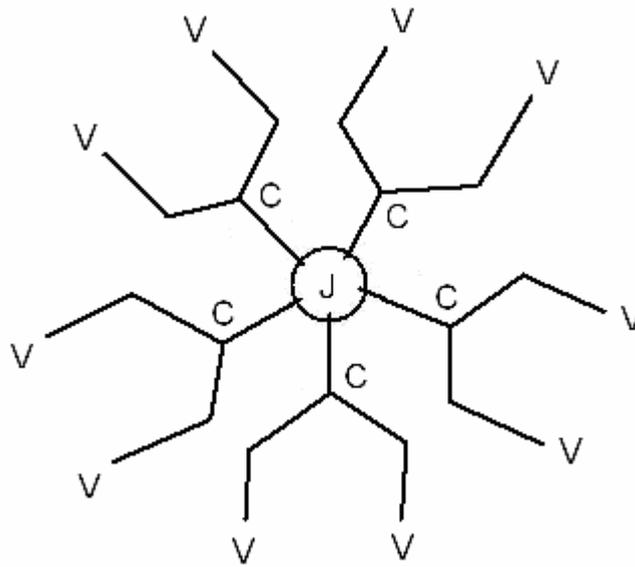


Figure 2-6. IgM killing. A single IgM molecule bound to a target cell together with the set of enzymes called complement ("C") suffices to kill a cell.

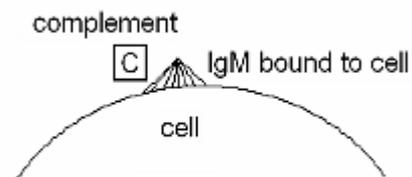
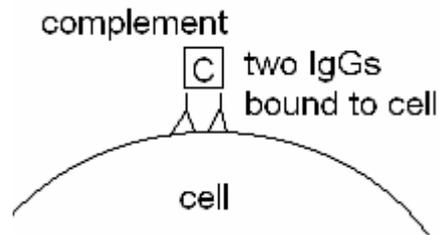


Figure 2-7. Two IgGs next to each other on the cell surface are required for the complement enzymes to be activated and then for the cell to be killed.



IgE

IgE antibodies cause us great discomfort; they are involved in allergies. If we make IgE antibodies specific for a substance X, we are allergic to X. We do not know why we make these antibodies; there is no known advantage to making them. Hence their existence is puzzling, if not paradoxical, in the context of the framework that everything in the system has to have a purpose.

IgD

The final class of antibodies is IgD, which is present as a receptor on some B cells. B cells typically express IgM, IgG or IgD as specific receptors, or some combination of these.

Allotypes

Animals of the same species but unrelated strains typically have different antibody genes, with the result that if antibodies purified from the serum of strain A (not selected for any particular specificity) are injected into an animal of strain B, the B animal may make anti-antibodies that recognize strain A antibodies, especially C region epitopes thereof that differ between the two strains. We then say that strains A and B have different allotypes, meaning different strain-specific, serologically detectable epitopes on their antibodies. ("Serologically detectable" means detectable by antibodies that are specific for the epitopes).