MOLECULAR BIOLOGY OF THE CELL

Bruce Alberts • Dennis Bray
Julian Lewis • Martin Raff • Keith Roberts
James D. Watson

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"Long ago it became evident that the key to every biological problem must finally be sought in the cell, for every living organism is, or at sometime has been, a cell."

Edmund B. Wilson
The Cell in Development and Heredity
3rd edition, 1925, Macmillan, Inc.

Bruce Alberts received his Ph.D. from Harvard University and is currently a Professor in the Department of Biochemistry and Biophysics at the University of California Medical School in San Francisco. *Dennis Bray* received his Ph.D. from the Massachusetts Institute of Technology and is currently a Senior Scientist in the Medical Research Council Cell Biophysics Unit at King's College London. *Julian Lewis* received his D.Phil. from Oxford University and is currently a Lecturer in the Anatomy Department at King's College London. *Martin Raff* received his M.D. degree from McGill University and is currently a Professor in the Zoology Department at University College London. *Keith Roberts* received his Ph.D. from Cambridge University and is currently Head of the Department of Cell Biology at the John Innes Institute, Norwich. *James D. Watson* received his Ph.D. from the University of Indiana and is currently Director of the Cold Spring Harbor Laboratory. He is the author of *Molecular Biology of the Gene* and, with Francis Crick and Maurice Wilkins, won the Nobel Prize in Medicine and Physiology in 1962.

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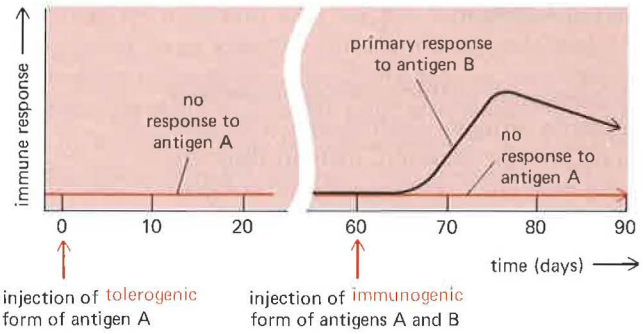

Figure 17–14 The experimental induction of immunological tolerance to a foreign antigen. The injection of a tolerance-inducing (tolerogenic) dose and/or form of antigen A (see text) not only fails to induce an immune response but also renders the animal specifically unresponsive to further injections of antigen A given in a form and dose that would normally induce a response. Note that the response to a different antigen, B, is unaffected.

spond to a particular antigen are eliminated; in other cases they survive, but their responses are specifically suppressed by a subclass of T cells known as *suppressor T cells* (see p. 997).

In summary, the binding of an antigen to its complementary receptors on a T or B lymphocyte can have any one of at least three consequences: (1) the lymphocyte may divide and differentiate to become an effector cell or a memory cell; (2) it may become tolerant; or (3) it may be unaffected by the encounter. The "decision" to *turn on, turn off,* or *ignore* depends largely on the nature and concentration of the antigen and upon complex interactions between different classes of lymphocytes and between lymphocytes and specialized macrophagelike *antigen-presenting cells,* which will be discussed in a later section. The decision also depends on the maturity of the lymphocyte. For example, newly formed B cells are highly susceptible to the induction of tolerance, while mature B cells are relatively resistant; this means that developing B cells with a high affinity for self molecules in their environment will become tolerant and never be activated.

Summary

The immune system evolved to defend vertebrates against infection. It is composed of billions of lymphocytes comprising millions of different clones. The lymphocytes in each clone share a unique cell-surface receptor that enables them to bind a particular "antigenic determinant" consisting of an arrangement of atoms on a part of a molecule. There are two classes of lymphocytes: B cells, which make antibodies, and T cells, which make cell-mediated immune responses.

Beginning early in lymphocyte development, those B and T cells with receptors for antigenic determinants on self molecules are eliminated or suppressed; as a result, the immune system is normally able to respond only to foreign antigens. The binding of a foreign antigen to a lymphocyte initiates a response by the cell that helps to eliminate the antigen. As part of the response, some of the lymphocytes proliferate and differentiate into memory cells, so that the next time that the same antigen is encountered the immune response is faster and much greater.

The Functional Properties of Antibodies[11]

The only known function of B lymphocytes is to make antibodies. A unique feature of antibodies, one that distinguishes them from all other known proteins, is that they can exist in millions of different forms, each with its own unique binding site for antigen. Collectively called **immunoglobulins** (abbreviated as **Ig**), they represent one of the major classes of proteins found in the blood, constituting about 20% of the total plasma protein by weight.

The Antigen-specific Receptors on B Cells Are Antibody Molecules[12]

As predicted by the clonal selection hypothesis, all of the antibody molecules made by an individual B cell have the same antigen-binding site. The first antibodies made by a newly formed B cell are not secreted; instead they are inserted into the plasma membrane, where they serve as receptors for antigen. Each B cell has approximately 10^5 such antibody molecules in its plasma membrane.

When antigen binds to the antibody molecules on the surface of a resting B cell, it usually initiates a complicated and poorly understood series of events culminating in cell proliferation and differentiation to produce antibody-secreting cells. Such cells now make large amounts of soluble (rather than membrane-bound) antibody with the same antigen-binding site as the cell-surface antibody and secrete it into the blood. While activated B cells can begin secreting antibody while they are still small lymphocytes, the end stage of this differentiation pathway is the large plasma cell (see Figure 17–4B), which secretes antibodies at the rate of about 2000 molecules per second. Plasma cells seem to have committed so much of their protein-synthesizing machinery to making antibody that they are incapable of further growth and division and die after several days of antibody secretion.

B Cells Can Be Stimulated to Make Antibodies in a Culture Dish[13]

Two significant advances in the 1960s revolutionized research on B cells. The first was the development of the **hemolytic plaque assay,** which made it possible to identify and count individual B cells secreting antibody against a specific antigen. In the simplest form of this assay, lymphocytes (commonly from the spleen) are taken from animals that have been immunized against sheep red blood cells (SRBC). They are then embedded in agar together with an excess of SRBC so that the dish contains a "lawn" of immobilized SRBC with occasional lymphocytes in it. Under these conditions, the cells are unable to move, but any anti-SRBC antibody secreted by a B cell will diffuse outward and coat all SRBC in the vicinity of the secreting cell. Once the SRBCs are coated with antibody, they can be killed by adding complement (see p. 988). In this way, the presence of each antibody-secreting cell is indicated by the presence of a clear spot, or *plaque,* in the opaque layer of SRBC. The same assay can be used to count cells making antibody to other antigens, such as proteins or polysaccharides, simply by coupling these antigens to the surface of the SRBC.

The second important advance was the demonstration that B lymphocytes can be induced to make antibody by exposing them to antigen in culture, where the cell interactions can be manipulated and the environment controlled. This led to the discovery that both T lymphocytes and specialized *antigen-presenting cells* are required for antibody production by B lymphocytes against most antigens; the cell interactions involved will be described in a later section of this chapter.

Antibodies Have Two Identical Antigen-binding Sites[11]

The simplest antibody molecules are Y-shaped molecules with two identical antigen-binding sites—one at the tip of each arm of the Y (Figure 17–15). Because of their two antigen-binding sites, they are said to be *bivalent.* Such antibody molecules can cross-link antigen molecules into a large lattice, as long as the antigen molecules each have three or more antigenic determinants

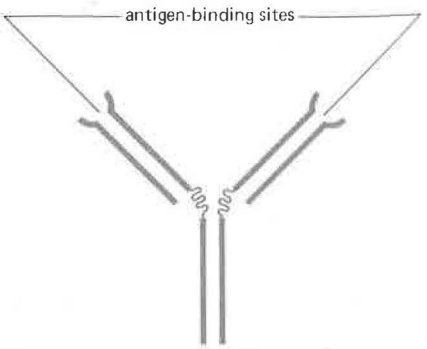

Figure 17–15 A highly schematic diagram of an antibody molecule with two identical antigen-binding sites.

(see Figure 17–30). Once it reaches a certain size, such a lattice precipitates out of solution. This tendency of large immune complexes to precipitate is useful for detecting the presence of antibodies and antigens, as we shall see later. The efficiency of antigen-binding and cross-linking reactions by antibodies is greatly increased by a flexible *hinge region* where the arms of the Y join the tail, allowing the distance between the two antigen-binding sites to vary (Figure 17–16).

The protective effect of antibodies is not due simply to their ability to bind antigen. They engage in a variety of biological activities that are mediated by the tail of the Y. This part of the molecule determines what will happen to the antigen once it is bound. Antibodies with the same antigen-binding sites can have a variety of different tail regions and, therefore, different functional properties.

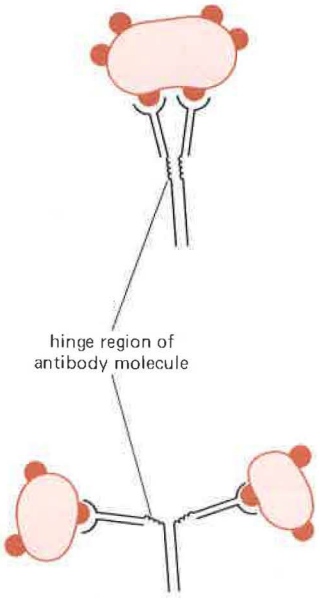

Figure 17–16 The hinge region of an antibody molecule improves the efficiency of antigen binding and cross-linking.

An Antibody Molecule Is Composed of Four Polypeptide Chains—Two Identical Light Chains and Two Identical Heavy Chains[14]

The basic structural unit of an antibody molecule consists of four polypeptide chains, two identical **light (L) chains** (each containing about 220 amino acids), and two identical **heavy (H) chains** (each usually containing about 440 amino acids). The four chains are held together by a combination of noncovalent interactions and covalent bonds (disulfide linkages). The molecule is composed of two identical halves in which both L and H chains contribute almost equally to the two identical antigen-binding sites (Figure 17–17).

The proteolytic enzymes papain and pepsin split antibody molecules into different characteristic fragments: *papain* produces two separate and identical **Fab** (*f*ragment *a*ntigen *b*inding) **fragments,** each with one antigen-binding site, and one **Fc fragment** (so called because it readily crystallizes). *Pepsin,* on the other hand, produces one **F(ab′)$_2$ fragment,** so called because it consists of two covalently linked F(ab′) fragments (each slightly larger than a Fab fragment); the rest of the molecule is broken down into smaller fragments (Figure 17–18). Because F(ab′)$_2$ fragments are bivalent, they can still cross-link antigens and form precipitates, unlike the univalent Fab fragments.

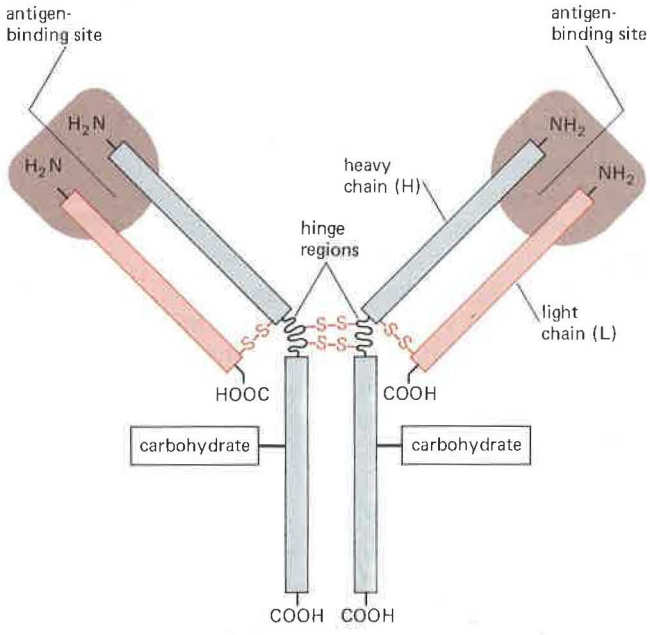

Figure 17–17 Schematic drawing of a typical antibody molecule composed of two identical heavy (H) chains and two identical light (L) chains. Note that the antigen-binding sites are formed by a complex of the amino-terminal regions of both L and H chains, but the tail region is formed by H chains alone. Each H chain contains one or more oligosaccharide chains of unknown function.

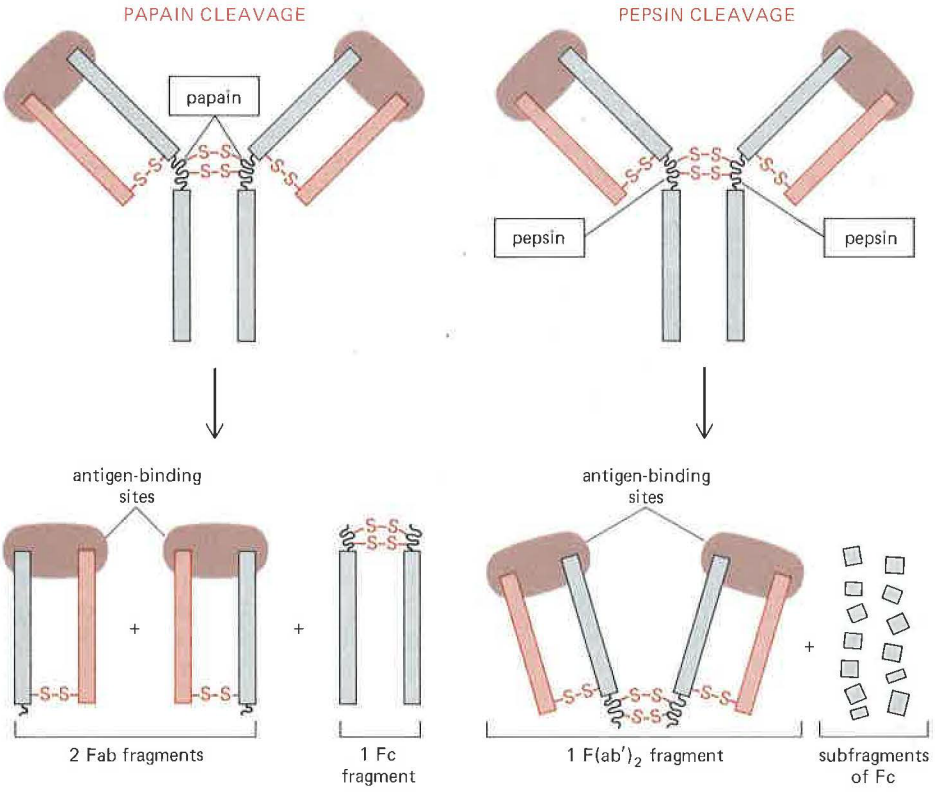

Figure 17–18 The different fragments produced when antibody molecules are cleaved with two different proteolytic enzymes (papain and pepsin) provided important clues for the investigators who determined the four-chain structure of antibodies.

Neither of these fragments has the other biological properties of intact antibody molecules because they lack the tail (Fc) region that mediates these properties.

There Are Five Different Classes of H Chains, Each with Different Biological Properties[11,15]

In higher vertebrates, there are five different *classes* of antibodies, IgA, IgD, IgE, IgG, and IgM, each with its own class of H chain—α, δ, ϵ, γ, and μ, respectively; IgA molecules have α-chains, IgG molecules have γ-chains, and so on (Table 17–1). In addition, there are a number of subclasses of IgG and of some of the other immunoglobulins. The different H chains impart a distinctive conformation to the tail regions of antibodies and give each class characteristic properties of its own (Figure 17–19).

IgG antibodies constitute the major class of immunoglobulin in the blood. They are copiously produced during *secondary* immune responses. The Fc region of IgG molecules binds to specific receptors on phagocytic cells, such as macrophages and polymorphonuclear leucocytes, thereby increasing the efficiency with which the phagocytic cells can ingest and destroy infecting microorganisms that have become coated with IgG antibodies produced in response to the infection (Figure 17–20). This is only one way in which IgG molecules combat infection. As well as binding to phagocytic cells, the Fc region of IgG can bind to and thereby activate the first component of the *complement system*, which under these circumstances unleashes a biochemical attack that kills the microorganism (see p. 988).

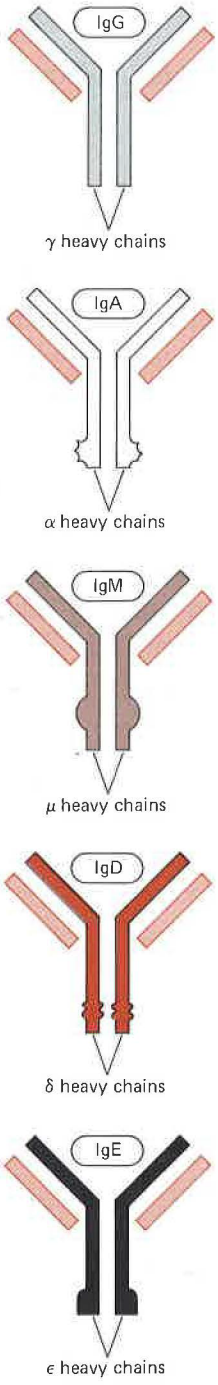

Figure 17–19 Highly schematic diagram showing how each different class of antibody has a distinctive class of H chain that imparts a distinctive conformation to its tail, or Fc region.