Without Miracles

4 The Immune System:

Selection by the Enemy

From Providence to Instruction

The extraordinary number of specific antibodies, including those against artificial antigens, defied the genetic origin originally propounded by Paul Ehrlich. A somatic, custom-made template mechanism seemed more logical.

--Debra Jan Bibel[1]

Selection

It follows that an animal cannot be stimulated to make specific antibodies, unless it has already made antibodies of this specificity before the antigen arrives. It can thus be concluded that antibody formation is a selective process and that instructive theories of antibody formation are wrong.

--Niels Jerne[2]

The previous two chapters described how Darwin's theory of cumulative variation and selection provides a naturalistic explanation for the fit of biological form and behavior observed in living organisms. But although the fit thus achieved is striking, natural selection is a very wasteful process in the sense that it depends on eliminating entire organisms, those less fit, from the evolutionary process. Working on large populations of organisms over long periods of time involving many generations, Darwinian natural selection is what is referred to as a *phylogenetic* process, since it results in new *phyla*, or new branches of the evolutionary tree of life. It would thus seem to be in an organism's own interest if it could somehow increase its fit to its environment during its lifetime, that is, be capable of *ontogenetic*[3] adaptation.

Indeed, individual organisms undergo ontogenetic adaptation in many ways. Muscles grow stronger with increased use, and animal and human behaviors change over time in functional, adaptive ways. These changes are readily observable, but the best understood ontogenetic adaptation involves the mammalian immune system.

Probably the best example of a puzzle of fit at the microscopic level is demonstrated by the antibodies produced by the mammalian immune system. For the immune system to be able to rid the body of antigens--foreign invaders in the form of chemical toxins, viruses, bacteria, cells, and tissues--there must be a precise fit between antibody and antigen. Attempts to explain this fit go back over 100 years and resulted in one of the most striking triumphs of the relatively new field of molecular biology.

A (Genetically) Providential Theory of Antibody Production

After the general acceptance of Louis Pasteur's germ theory of disease late in the nineteenth century, a number of scientists became interested in understanding the mechanisms responsible for the appearance of antibodies in an animal's blood after it had been infected with disease-producing bacteria. Antibodies were of special interest since they were known to protect the animal from subsequent infection by the same pathogenic bacteria.

Paul Ehrlich (1854-1915) was a German who in the 1890s developed a technique for estimating the quantity of antibodies in blood. He was intrigued by the explosive increase in antibody production after exposure to an antigen and attempted to account for this phenomenon by formulating his side-chain theory. According to this theory, the surface of white blood cells is covered with many side chains, or receptors, that form chemical links with the antigens they encounter. For any given antigen, at least one of these receptors would bind, stimulating the cell to produce more of the same type of receptor, which would then be shed into the blood stream as antibodies. According to Ehrlich's theory, an antibody could be considered an irregularly shaped, microscopic, three-dimensional label that would bind to a specific antigen but not to the other cells of the organism.[4] This analogy of antibodies as labels is used here since the antibodies themselves usually do not destroy the antigen, but label it, providing a molecular kiss of death for destruction of the antigen by complement proteins, macrophages, or other agents that either perforate the antigen's cell membrane or completely engulf the antigen. [5]

The major assumption of Ehrlich's theory, and one that makes it a type of providential theory, was that white blood cells possessed numerous genetically specified side chains, at least one of which would bind to any encountered antigen. That is, the information essential for the production of all possibly necessary antibodies was *provided* by the animal's genes. For this reason, this is known as a germ-line theory, the germ line referring to the entire set of genes (or *genome*) that is passed from an organism or pair of organisms to its offspring.

But the germ-line theory soon encountered a major difficulty. During the early 1900s, Karl Landsteiner (1868-1943) clearly demonstrated that there was apparently no discernible limit to the range of antibodies that an animal could produce. His finding that antibodies could even be produced in response to completely novel artificial substances revealed that the animal could not possibly possess in its finite genome the information required to produce an infinite number of all possibly necessary antigens. This led to the rejection of Ehrlich's theory and to the consideration of constructivist theories of antibody production. Such constructivist theories had to account for the fact that the immune system was not only adapted to the task of producing fit antibodies, but that it was adapt*ive* as well, able to create new puzzles of fit in response to completely unpredictable and novel antigens.

An Instructionist Theory of Antibody Production

The first well-known theory that attempted to account for the immune system's ability to produce antibodies in response to novel antigens appeared in 1930 with Breinl and Haurowitz's introduction of the template instruction theory,[6] which was further developed and advocated by Nobel prize-winning chemist Linus Pauling.[7] The template theory attempted to explain that antibodies could be made to bind with any novel antigen because they were produced by direct contact with antigens. By proposing that the antigens themselves served as models for antibody production, the template theory was able to account for the seemingly limitless range of antibodies. To pursue the label analogy, if Ehrlich's innatist side-chain theory could be likened to an innately determined set of labels designed so that at least one would stick to any given antigen, the template theory saw the immune system

as supplied with the knowledge of a *procedure* whereby labels would be custom-made for each antigen in the same way a tailor makes a suit of clothes using the customer as a template. The template theory could therefore be considered *instructionist* since it did not require innate, germline information for the production of all antibodies, but rather required only a general label-construction procedure for building antibodies using the antigens themselves as the required source of instructions.

Although one version or another of the instructionist template theory remained influential for over 20 years, it too encountered a number of serious difficulties. In the 1950s Danish immunologist Niels Jerne noted several immunological findings that the theory could not explain.[8] First of all, it could not account for the increasing rate of antibody production during the initial immune response. If the antigen itself was necessary for the production of each antibody, how could the antibodies so quickly outnumber the templates? Second, it could not explain the memory of the immune system by which a second exposure to a given antigen results in a much more rapid production of antibodies than does the initial contact. If the antigen itself served as a template, its total elimination by the immune system would also necessarily entail destruction of templates, so a second exposure to the same or similar antigen should be accompanied by an immune response no different from the first. Third, since it was thought that antibody cells were quite short lived, the template theory could not account for the fact that antibodies continued to be present long after the antigen had been eliminated from the body. Finally, the theory did not explain the fact that the antibodies produced during the latter stages of an immune response are usually more effective in binding with the antigens (are better-fitting labels) than the antigens initially produced.

It was therefore clear that in attempting to account for the formation of new antibodies, the instruction-based template theory ran into a number of rather serious problems that motivated scientists to pursue other explanations.

A Selectionist Theory of Antibody Production

Jerne's paper provided not only arguments against the template theory but also an alternative theory that in some respects resembled Ehrlich's original side-chain theory. Jerne's natural-selection theory of antibody production stated that a mammal initially possesses a relatively small number of antibodies. The successful binding of an antibody to an antigen triggers the antibody to produce a large number of copies of itself. In this way, a preexisting antibody is effectively *selected* by the antigen, which stimulates the chosen antibody to produce a multitude of clones. Australian virologist and immunologist Sir Frank Macfarlane Burnet (1899-1985) further developed the theory using the term clonal selection to describe it.[9]

Much research still has to be done to understand fully the complex dynamics of the immune system, but the Jerne-Burnet clonal-selection theory of antibody production is generally accepted. Although its major characteristics are not difficult to understand, we have to go into a bit more detail concerning the immune response to appreciate its selectionist functioning.

First, instead of thinking of an antibody as either binding or not binding to an antigen, we must appreciate that antibodies have a very wide range of affinity (that is, attraction or binding power) to a given antigen.[10] Thus whereas a well-fitted antibody will almost always bind to an encountered antigen for which it is well fit, another less well-fitted antigen may also bind with the same encountered antigen, although its rate of binding may be considerably less than 100%. In contrast, an antibody that is completely unfit for binding with a given antigen will seldom, if ever, bind to the antigen.

Second, instead of every antigen having a single "handle" (called a *determinant* or *epitope* by immunologists) for the antibody to grasp, they all have many such determinants, each of which is *different* and thus can be bound by a different type of antibody. These determinants correspond to small patterns of molecular structure on the surface of the antigen.[11]

The large number of determinants on each antigen effectively increases the likelihood that the immune system will be able to produce an antibody that will bind to any antigen introduced into the body. It still has to create a staggering number of different antibodies to ensure its effectiveness, however, a diversity that includes up to 10 billion B lymphocyte cells, each able to produce more than 100 million different antibody proteins. And since a person has only about 100,000 genes, there is simply no way our genes could specify each and every one of these proteins.[12]

The answer to this enigma required a radical reconceptualization of genes and how they function. Until the mid-1970s it was a generally accepted principle of biology that one gene always leads to the synthesis of one and only one protein, and that the genetic makeup of an organism remains constant throughout its life. (Relatively rare and usually harmful mutations do occur due to genetic copying errors during cell division or exposure to irradiation or other environmental mutagens.) But in 1976 at the Basel Institute for Immunology, Susumu Tonegawa discovered that antibody genes are not inherited complete, but rather as fragments that are shuffled together to form a complete gene that specifies the structure of a given B lymphocyte and the antibodies it produces (Tonegawa received a Nobel prize in 1987 for his discovery). In addition, as the DNA segments are combined to form the complete B lymphocyte gene, new DNA sequences are added at random to the ends of the fragments, ensuring even more antibody diversity.[13] In this manner:

> The receptors used in the adaptive immune response are formed by piecing together gene segments, like a patchwork quilt. Each cell uses the available pieces differently to make a unique receptor, enabling the cells collectively to recognize the infectious organisms confronted during a lifetime.[14]

This active, random reshuffling of immunoglobulin genes, together with the insertion of random DNA sequences during the recombination process, is responsible for the diversity of antibody receptors attached to each B cell. Such diversity virtually ensures that at least one antibody, although perhaps not fitting perfectly, will be able to bind with at least one of the many determinants presented by a new antigen.

Once an antibody is selected by an antigen by binding, it stimulates the B lymphocyte to which it is attached to divide and make exact copies of itself. Some of the selected clones remain as circulating B lymphocytes and as such serve as the immune system's memory. Increased numbers of these cells provide for a faster immune response to subsequent infections and establish the immunity that follows many infections and vaccinations. Other selected clones stop dividing, grow larger, and turn into plasma cells whose sole function is to produce large numbers of free antibodies to fight the current infection.

The clonal-selection theory explains the great diversity of antibodies and the ability of the immune system to bind with completely novel antigens. It also provides an account for Jerne's first three findings, noted above, in his criticism of the template theory. The theory as described so far, however, still fails to account for the finding that the antibodies produced during the latter stages of the immune response are more effective in binding with the antigens than the antibodies initially produced. This fine-tuning of antibodies is accomplished by another mechanism that also changes the genetic makeup of the antibodies--the random mutation of the genes within the B cell clones. "By altering individual nucleotide bases the mutations fine-tune the immune response, creating

immunoglobulin genes whose products better match the antigen."[15]

We therefore see that the clonal-selection process of antibody production has a number of noteworthy characteristics. First, it is constructive in that the actual structure of the antibodies is not explicitly included in the genome. Thus, antibodies are quite unlike other physiological structures (such as livers, eyes, and noses) whose basic design depends on what is believed to be a fixed set of genes.[16]

Second, the structure of antibodies is determined by what appears to be an essentially blind process. This blindness shows itself at three levels: the random recombination of immunoglobulin genes as B lymphocyte cells are formed, the random insertion of DNA segments into the recombined gene, and the consequent blind hypermutation of the clone B cells to fine-tune them to the antigen. Thus, the immune system does not attempt to predict the antibody structure that will bind with an antigen, but rather uses a type of "shotgun" approach that sends in a diverse army to meet the invaders. Almost all of these produced antibodies will turn out to be quite ineffective in binding with the antigens, but the diversity of this army virtually ensures that at least one of them will be effective. Indeed, studies have shown that if antibodies are produced blindly, the probability that a novel antigen will be recognized is virtually assured if very many antibody types are present.[17]

Finally, the immune system is designed so that only those antibodies that are able to bind with the antigen are reproduced and remembered the next time the same or similar antigen invades the animal. Antibodies that are not successful leave no offspring and therefore soon become extinct, to be replaced by the estimated one million new B lymphocytes produced in the bone marrow every second.[18]

But we still have to account for another important ability of the immune system--that it does not attack the cells and products of its host body. Although it was first thought that the immune system was provided this information in the germ line, research has now demonstrated that the immune system in fact *learns* to distinguish self from nonself. The process by which it does so will not be described in detail here, but it is virtually a mirror image (with an important reversal) of how it produces antibodies to recognize invaders. That is, mature lymphocytes are triggered to reproduce and mutate when they encounter a foreign antibody; however, immature lymphocytes that form while the animal is still in utero or shortly after birth "go through a stage when binding of their receptors causes them to die. Self-reactive cells are killed before they have a chance to proliferate and damage their host." [19] This is the clonal-deletion theory of how the immune system learns tolerance to self, and was first proposed by Joshua Lederberg in 1959.

To summarize, the clonal-selection theory states that a very large number of unique B lymphocytes to which are attached antibody receptors are always circulating throughout the body. Their great diversity results from the random recombination of immunoglobulin gene fragments and random insertion of DNA sequences as the B cells develop. This blind diversity of B cells virtually ensures that at least one will produce an antibody that will bind with any antigen that makes its way into the organism. The binding of a B cell's antibody with an antigen stimulates the cell to divide and produce clones, with successive generations of reproducing clones resulting in an exponential rise over time in the number of circulating antibodies of the selected type. Some of the B cell clones remain in circulation to form the immune system's memory of the antigen. Others terminally differentiate, forming plasma cells that produce large numbers of antibodies that fight the current infection. Finally, as the B cell clones reproduce they undergo a high rate of somatic mutation that, when combined with the continued selection pressure exerted by the antigen, fine-tunes the fit of the antibodies to the antigen. A similar process of variation and selection of immature B cells (although now with selected cells eliminated rather than reproduced) accounts for the immune system's ability to tolerate the cells and products of its host body.

Antibody Production as a Microcosm of Darwinian Evolution

Even this much abbreviated and simplified account of the clonal-selection functioning of the immune system reveals it to be a remarkable microcosm of Darwinian evolution with the three major principles of superfecundity, variation, and natural selection each playing an essential role. Superfecundity is evident in that the immune system produces far more antibodies than will be effective in binding with an antigen. In fact, it appears that the majority of produced antibodies do not play any active role whatsoever in the response of the immune system. Natural (and blind) variation is provided by the variable gene regions responsible for the production of a highly diverse population of antibodies. And selection occurs, as only antibodies able to bind with an antigen reproduce.

The similarity between adaptive biological evolution and the production of antibodies is even more striking when one considers that the two central processes involved in the production of antibodies, genetic recombination and mutation, are the same ones responsible for the biological evolution of sexually reproducing species. We have seen that the recombination of immunoglobulin genes underlies the large diversity of the antibody population, and the mutation of these genes serves as a fine-tuning mechanism.[20] In sexually reproducing species, the same two processes are involved in providing the variations on which natural selection can work to fit the organism to the environment. Thus cumulative blind variation and natural selection, which over many millions of years resulted in the emergence of mammalian species, remain crucial in the day-to-day survival of these species in their ceaseless battle against microscopic foreign invaders.

A final similarity between the functioning of the immune system and biological evolution is worth noting--the evolution of our knowledge of how each operates. Our understanding of the fit of organisms to their environment has progressed from a providential explanation to an instruction-based (Lamarckian) one to a purely selectionist (neo-Darwinian) account. The same stages of thought can also be seen in biology's attempt to account for the puzzle of fit of antibody to antigen.

Ehrlich's side-chain theory can be considered providential in that the organism's genes were believed to provide all the knowledge necessary to construct antibodies that would be able to fit all the antigens the organism would ever encounter. Since this knowledge was considered to be the result of past evolutionary selection, the genetically providential theory does not lead to the same problem of ultimate origins that the supernaturally providential theory of the origin of species encounters. However, it did run into difficulties when it was found that the immune system could produce antibodies that fit novel antigens never encountered before, either in the organism's own lifetime or in that of its ancestors.

The appreciation of the immune system's ability to adapt to a changing environment of novel antigens led to the instruction-dependent template theory of antibody formation in which the environment (in the form of antigens) somehow transmitted instructions for the formation of close-fitting antibodies to the B lymphocytes. This theory, however, did not account for many of the characteristics of the immune system that were observed subsequently. In addition, no transmission of information from antigen to antibody was ever observed.

Given the problem of accounting for adaptive change without recourse to instructionist theories, it should perhaps not be surprising that the field of immunology would eventually hit on the same solution that Darwin had discovered, even if it took an additional century. By combining the basic principles of superfecundity, blind variation, and selection that explain the adaptation of organism to environment, the clonal-selection theory provides an understanding of how the immune system can produce antibodies adapted to its environment of novel antigens. This it does without recourse to providential or instructionist explanations. But whereas adaptive

biological evolution proceeds by cumulative natural selection *among* organisms, research on the immune system has now provided the first clear evidence that ontogenetic adaptive change can be achieved by cumulative blind variation and selection *within* organisms.

[1]Bibel (1988, p. 178).

[2]Jerne (1967, p. 201).

[3]Ontogeny, or ontogenesis, refers to the development and growth of an individual organism from embryo to adult.

[4]Ehrlich (1900).

[5]Tonegawa (1985, p. 105).

[6]Breinl & Haurowitz (1930).

[7]Pauling (1940). Pauling won the Nobel prize for chemistry in 1954 and the Nobel peace prize in 1963, the only person ever to win two unshared Nobel prizes.

[8]Jerne (1955).

[9]Burnet (1957).

[10]Jerne (1951, 1975).

[11]Ada & Nossal (1987, p. 52); Tonegawa (1985, pp. 104-105).

[12]Janeway (1993, p. 75).

[13]Tonegawa (1983, 1985).

[14]Janeway (1993, p. 73).

[15]Tonegawa (1985, p. 110).

[16]Recent research suggests that the recombination of somatic genes may be in-volved in the development of the mouse brain (Matsuoka et al., 1991) and therefore may be involved in other mammalian organs as well.

[17]Perelson & Oster (1979).

[18]Farmer, Packard, & Perelson (1986, p. 190).

[19]Marrack & Kappler (1993, p. 87).

[20]It should be noted that recombination of immunoglobulin genes involved in the production of antibodies differs somewhat from the recombination of parental genes in sexual reproduction. In the former, nucleotides can be inserted and deleted at random from the recombined immunoglobulin gene. This adds an important additional source of diversity in the generation of antibodies (see Janeway, 1993, p. 75; Kallenbach et al., 1992).