CHAPTER 13

Frank Macfarlane Burnet & How Animals Make Antibodies

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INTRODUCTION

In the year following World War I, approximately two billion people caught the common flu, and between twenty and forty million people died. In one month, the flu killed 196,000 people in the United States alone. The flu's quick action is legendary. For example, four seemingly healthy women played bridge together one evening during the 1918 pandemic and all went to bed feeling fine, but three never woke up. They were victims of this unheralded rapid-action killer.

Many scientists conducted research on the flu virus in the 1920s and 1930s. During World War II, the search for an effective treatment became more intense because researchers feared another pandemic. Frank Macfarlane Burnet (Figure 13.1) was part of this intense anti-flu campaign. He was motivated by a powerful scientific curiosity to understand how the virus worked and a strong competitive streak, as well as a deeply humanitarian commitment to avert another pandemic at the end of the war. Though he never developed an effective treatment for the flu, Burnet had a tremendous impact on our understanding of the immune system and the nature of disease.

Burnet made two important discoveries regarding immune system function. First, he explained why the immune system doesn't attack an individual's own cells and tissues. Second, he helped develop the clonal selection theory of antibody activity.

Burnet's contributions were closely related to his social and intellectual background. During childhood, he gained a deep appreciation for ecological and evolutionary principles. Burnet used these perspectives in two related ways. First, he understood how disease-causing microorganisms, like other more familiar organisms, experienced their own struggle for existence within their environment—the human body. Second, he was able to make connections between ecological interactions within an ecosystem and immunological interactions within the body. He used Darwin's theory of natural selection as an analogy to interpret how the body can respond, or "adapt," to specific disease organisms.

FIGURE 13.1 Frank Macfarlane Burnet holding the first monkey paralyzed by an Australian polio virus. *Source:* Frank Macfarlane Burnet, *Changing Patterns: An Atypical Autobiography*, 1968.

BURNET'S EARLY YEARS

Frank Macfarlane Burnet was born in 1899 in a small town in Australia. His oldest sister was mentally retarded, perhaps as a result of birth complications, and required an inordinate amount of special care. Burnet's parents attempted to conceal her condition and the children were not allowed to bring friends to the house. His relatively isolated childhood may have led to Burnet's shy personality, which he still retained late in life when he was basking in the glow of scientific achievement.

As a result, Burnet spent a tremendous amount of time by himself, either reading books or wandering around outside, learning about the secrets of nature. He became an avid collector of all natural items, including rocks, butterflies, bird eggs, and freshwater mussels. But his greatest enthusiasm was reserved for collecting beetles, a passion that he shared with another naturalist, Charles Darwin. In his autobiography, Burnet admits that his passion for beetle collection, which peaked while he was in medical school, was probably a replacement for a social life that more outgoing men might enjoy at that age.

As a naturalist and proponent of Darwin's theories, Burnet extended Darwin's great struggle for existence to the microscopic world of disease. He was intellectually prepared to observe infectious agents competing for suitable hosts and responding adaptively to changes in their habitat. He took this unique perspective with him when he entered medical school and maintained it throughout his career.

AN ECOLOGICAL POINT OF VIEW

Burnet applied an ecological point of view to the world of microorganisms. Like Darwin, Burnet was impressed by the tremendous reproductive potential of all organisms, but because he focused his work on microorganisms, Burnet was able to appreciate the incredible reproductive potential of bacteria and viruses in the appropriate environment. In 1940, he argued that the application of the ecological point of view to understanding infectious diseases was the most important attitude change by microbiologists in recent history.

In Burnet's ecological point of view, disease-causing organisms—pathogens—are engaged in the same struggle for existence as other organisms. The difference is that pathogens often make their living *within* a host environment rather than *outside* it. In order to be successful, pathogens still need to deal with the basic necessities of life: food and reproduction. While gathering food and making offspring within their host's body, they must avoid being preyed upon by other organisms or, in the case of many pathogens, the host's immune system.

Burnet described how Australian scale insects invaded California citrus groves. These insects make a living by sucking out the juices from citrus trees, sometimes killing a tree within a year or two. Scale is not a serious problem in Australia, but by 1889, the California lemon crop was threatened with extinction. What was the difference between the two environments?

When the scale insect invaded the new host, there were no natural predators, so the scale insect numbers increased dramatically, threatening to destroy their host. After ecologists successfully introduced a predatory species of ladybird beetle, the numbers of scale insects declined dramatically. Thus a balance was established, with a relatively low number of predators and prey within the habitat.

The ecological and evolutionary perspectives complement each other. Each pathogen is as much the product of adaptive evolution as is the host. Both the pathogen and host share an evolutionary history of living together. The evolution of the immune system is the adaptive response of the host species to generations of pathogens. Burnet argued that when two organisms have evolved together in a host/pathogen relationship, the long-term survival of the pathogen is best served by the development of a pattern of limited infection. Sufficient host material is eaten to keep the pathogen and its offspring alive, but the host is otherwise not seriously injured, allowing the pathogen a greater opportunity to infect new hosts. By killing the host, the pathogen destroys its own environment. This is a very unstable balance because genetic changes in the pathogen may lead to epidemic outbreaks of pathogen-induced disease.

PROBLEM

Burnet argued that pathogens benefit by keeping their hosts alive. Keeping a host alive is costly to the pathogen from an evolutionary perspective, however, because less of the host can be consumed and fewer pathogen offspring are produced. If a genetic mutation arises that allows a pathogen to be transmitted much more easily from one host to another, will the pathogen still benefit as much from keeping its host alive? In the long run, would the offspring of this pathogen be more or less likely to kill their hosts?

EARLY HYPOTHESES FOR ANTIBODY ACTIVITY

One of the most prominent scientists of the late 1800s was Paul Ehrlich, who established a technique for measuring the quantity of antibodies within the blood. Using this technique Ehrlich came to appreciate just how explosively antibodies proliferate following the introduction of an invading molecule or **antigen**. Ehrlich turned his attention to the question of how antibodies form in the body. According to his **side-chain theory** of antibody formation, the surface of a white blood cell bears receptors with a number of different types of side chains to which the antigens bind. Each white blood cell carries the full diversity of side chains that react to different incom-

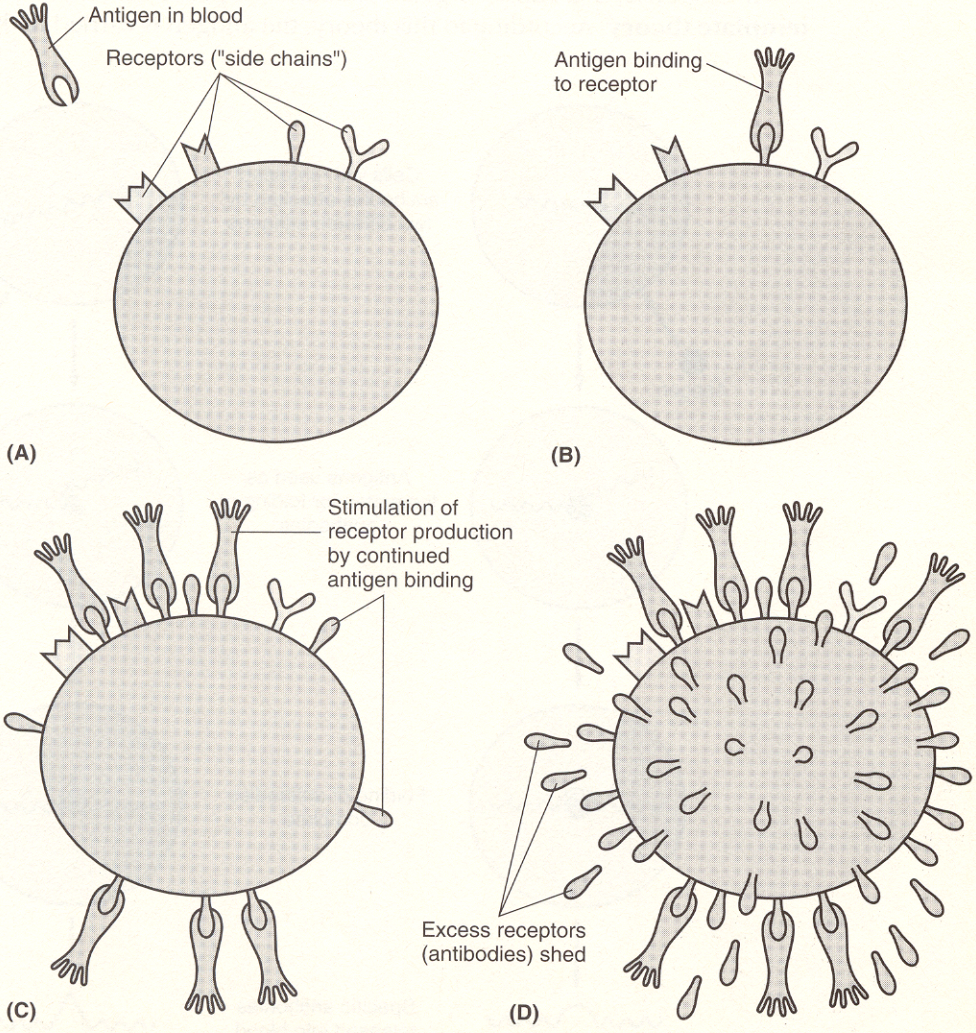

FIGURE 13.2 Ehrlich's side-chain theory. (A) Cell with many different types of side chains. (B) Antigen binds to a receptor, inducing formation of more receptors specific to the antigen. (C) More antigens bind to the receptors, inducing formation of more identical receptors. (D) Receptors are shed into blood as antibodies.

ing antigens. When the antigen is linked to the side chain, the white blood cell produces multiple copies of the correct receptor, which it dumps into the bloodstream as antibodies (Figure 13.2).

Organic chemists cast serious doubt on Ehrlich's theory based on the physical and molecular properties of these newly isolated antibodies. They found that animals could generate an almost unlimited number of antibodies, including antibodies that were specific to new, synthetic molecules. How could animals produce unique antibodies with side chains specific to molecules that had never existed on earth before? As the number of different types of known antibodies increased, it became clear that there was simply not enough space to fit all these different types of side chains on the surface of each white blood cell.

In response, a group of organic chemists proposed an alternative theory—the **template theory**. According to this theory, the antigen is carried in the body to the

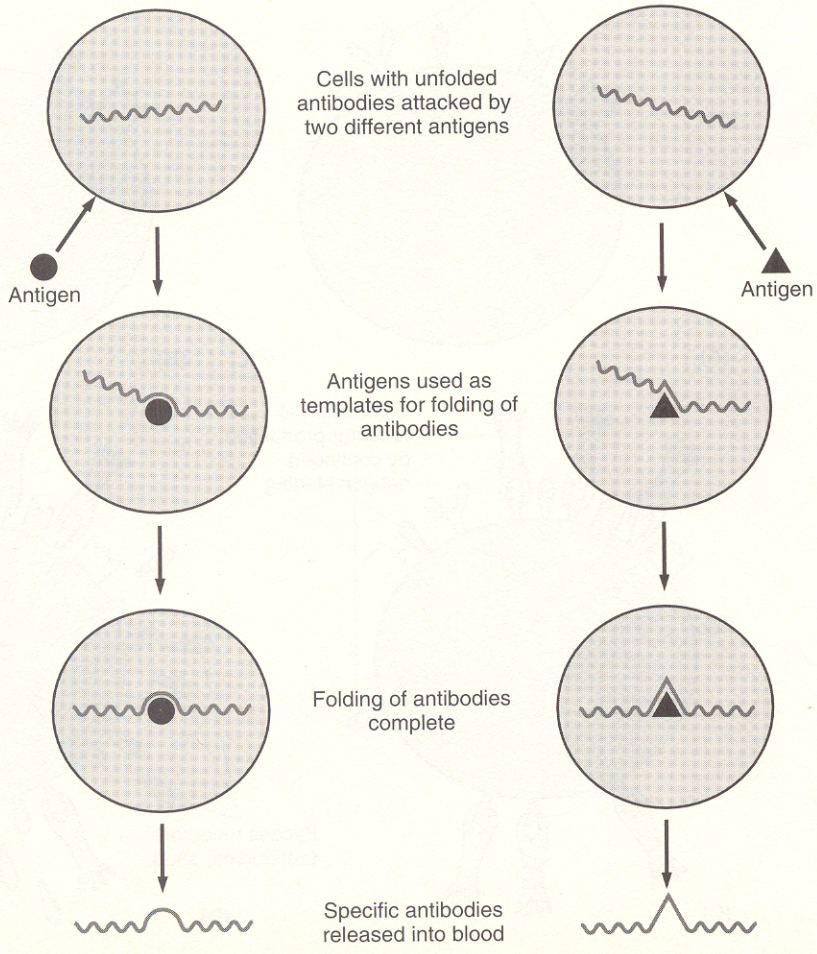

FIGURE 13.3 The template theory.

site of protein formation, where it serves as a template upon which the antibody molecule is constructed. The antibody molecule is synthesized upon the surface of the antigen so that the molecular structure of the antibody is complementary to the antigen. The antibody, when released into the blood, would be a perfect fit to the antigen. Hence there was no need for an innate diversity of antibodies—instead, diverse antibodies were produced by the body in response to its experiencing new antigens (Figure 13.3).

Some biologists objected to the template theory. One reason was a distrust by biologists of the purely molecular approaches used by the chemists. Although they recognized the importance of chemistry, immunologists criticized the template theory because it ignored important biological phenomena associated with antibody activity.

Problems with the Template Theory

During much of his career, Burnet supported the template theory, but he realized that it had potentially damaging problems. One problem was that animals failed to produce antibodies to particular antigens under some specific conditions. For example, under normal circumstances an animal doesn't make antibodies to itself. The immune system can distinguish, in Burnet's words, between self and not-self, resulting in what we call **immunological tolerance**. The template theory had difficulty explaining how the immune system was able to make this discrimination.

Two experiments demonstrated that in very early stages of development, an animal may not yet have immunological tolerance. In one experiment, Burnet was unable to evoke an immunological response in chick embryos, despite trying to do so with three common types of antigens that caused powerful immunological responses in slightly more mature chickens. Thus there was a stage in development when chicks made no immune response to foreign antigens. In a second experiment, a colleague of Burnet's demonstrated that fraternal (nonidentical) twin calves exchanged blood through a common placenta, maintaining a mixture of two different types of blood antigens in the uterus without reacting to the antigens provided by the other calf's blood. Again, this provided evidence that, early in development, animals tolerated foreign antigens. Burnet's early work on immunological tolerance ultimately paved the way for developing successful techniques for organ transplants. Accordingly, he was awarded the Nobel Prize in 1960, along with the British zoologist, Peter Medawar.

During World War II, Medawar studied how skin grafts were accepted or rejected by burn victims of incendiary bombs. After the war, he used Burnet's findings as the basis for a series of experiments on mouse cells. Instead of studying antibody production, he used acceptance or rejection of a skin graft to measure immune response. When he grafted skin from three-week-old mice of one strain onto the skin of mice from a second strain, the graft was always rejected. But if he injected spleen cells from a mouse of the first strain into a newborn mouse of the second strain, then attempted the same skin graft after the mouse was three weeks old, the graft was accepted. As Burnet had predicted, the mice developed immunological tol-

erance of the graft as a result of exposure to the cells very early in development. The host treated the skin graft as "self" rather than foreign tissue.

Medawar's findings cast doubt on the template theory. According to the template theory, initial exposure to antigens stimulated production of specific antibodies. If this theory were correct, mice exposed to a foreign tissue early in life should reject a skin graft of the same foreign tissue three weeks later.

Burnet and his colleagues raised other equally important objections to the template theory. First, the effectiveness of antibodies seemed to improve over the course of the immune response. Second, it was becoming clear that antibody production continued after the antigen was no longer present in the circulation, leading to an unexplainable situation where nonexistent templates were producing antibodies. A final problem for the template theory was that the secondary response to the same antigen was characteristically much more rapid and intense than the initial (primary) response—that is, the immune system showed memory (Figure 13.4). Why should reintroducing the antigen (template) give rise to more antibody molecules than did the initial introduction of the template?

PROBLEM

Four problems for the template theory are (1) immunological tolerance, (2) increased effectiveness of antibodies, (3) continued response after antigens are no longer present, and (4) immunological memory. Revise the template theory in a way that could account for each finding.

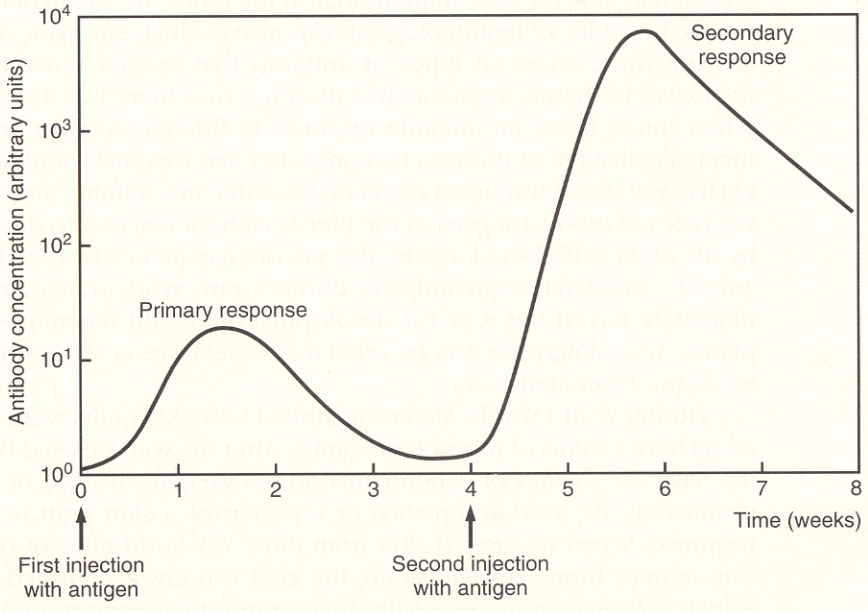

FIGURE 13.4 Differences in timing and magnitude of antibody formation in response to primary and secondary exposure to a given antigen.

Despite its problems, most scientists in the 1950s supported the template theory because it explained why antibodies are specific for a tremendous diversity of antigens. Burnet's objections did, however, make many scientists aware of difficulties with the template theory and paved the way for considering new solutions to these problems.

DEVELOPMENT OF A NEW THEORY

In 1955, Niels Jerne resurrected Ehrlich's side-chain theory in a slightly novel form, which he called the **natural selection theory** of antibody formation. He argued that diverse antibodies are normally formed by the animal in small quantities. When an antigen of a particular type enters the blood, it eventually encounters an antibody of appropriate specificity and is bound to it. This antigen/antibody complex then moves to specialized cells that reproduce the specific antibody in large numbers. These cells have the ability to faithfully reproduce whatever antibody is brought to them, though occasional mistakes are made. These mistakes may result in an antibody that binds even better to the antigen (is a better fit). Jerne had no definite opinion about when the initial population of antibodies was produced. But he did deal with the problem of immunological tolerance by suggesting that newly created antibodies that attach themselves to tissues in the animal's body are removed from circulation, and thus not available for reproduction.

PROBLEM

Reconsider the four problems raised by Burnet and his colleagues. Which does the natural selection theory successfully deal with? Which are still problematic?

Upon reading Jerne's article, Burnet had a mixed reaction. Personally, Burnet was annoyed with Jerne, who had recently attacked Burnet's book on immunology as being overly philosophical. From a biological perspective, Burnet was also concerned that Jerne's theory could not explain the origin of antibody diversity or how antibodies were replicated so accurately by the cells. He did, however, appreciate that Jerne's selection theory dealt with many of the template theory's problems. Burnet was also enthusiastic about the Darwinian analogy that ran through Jerne's theory, and, as a beetle collector with thousands of different species in his collection, he could easily imagine that a body could produce the innate diversity of natural antibodies required for Jerne's theory to work. This appreciation for diversity in biological systems distinguished him from most medical researchers.

While Burnet was thinking about Jerne's paper, researchers in his institute were beginning to produce evidence that white blood cells carried some immunological properties. These findings, linked with Jerne's paper, helped Burnet formulate the concept of **clonal selection**. He proposed that each cell, based on its genetic composition, produces characteristic receptors on its surface that are complementary to an antigen. If the receptors bind a foreign antigen, the cell is induced to proliferate. Each immunologically active cell (lymphocyte) is genetically constrained to produce

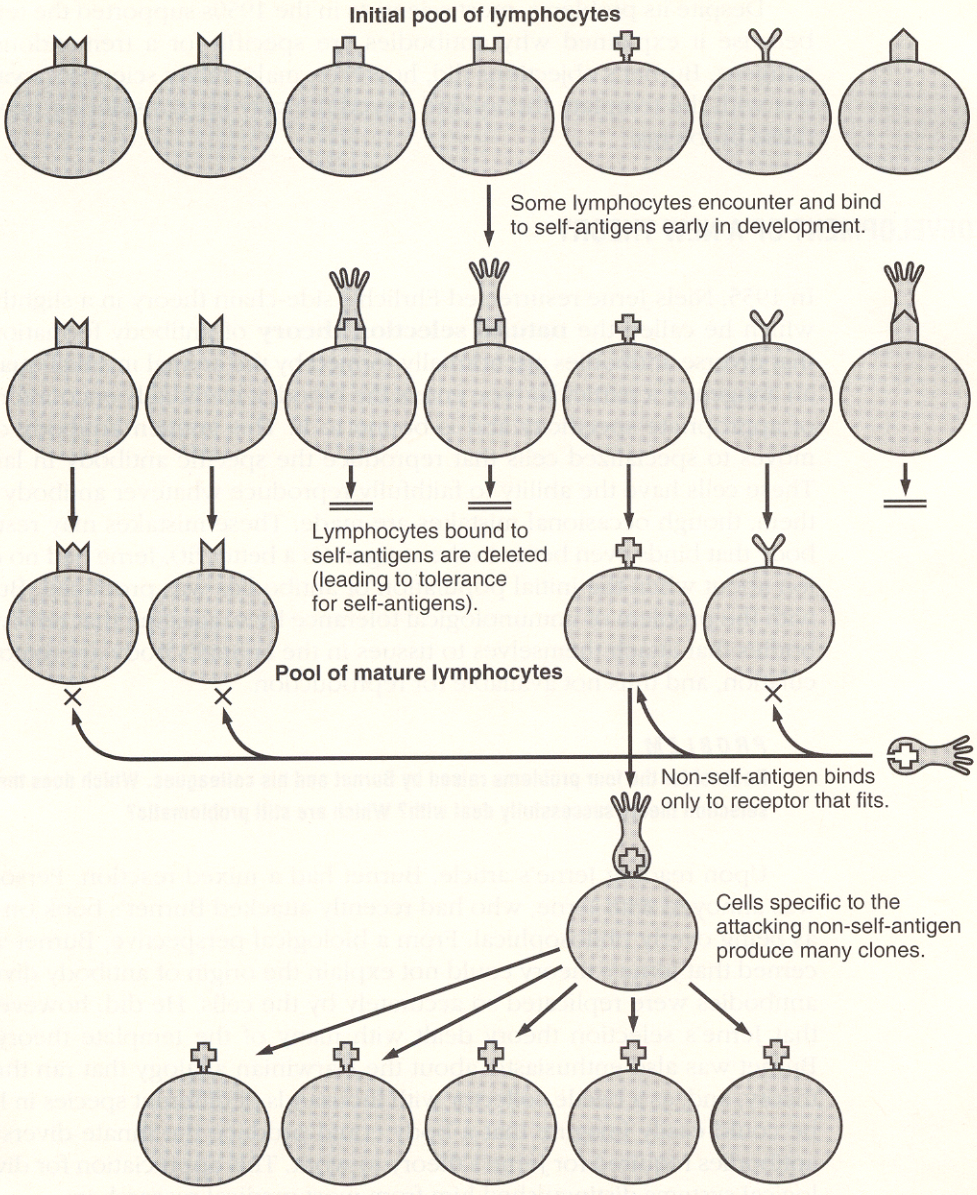

FIGURE 13.5 Clonal selection theory. Each class of lymphocyte present in the body before encountering an antigen has a distinct receptor for a specific antigen. Lymphocytes with receptors that bind to self-antigens are eliminated early in development, assuring tolerance of self-tissue. A mature lymphocyte, upon binding an antigen, is stimulated to go through a series of mitotic divisions, yielding a clone of identical progeny, all with receptors specific to that particular type of antigen. Some of these progeny develop into effector cells that eliminate the antigens, while others mature into memory cells, which remain in circulation, ready to respond to the next challenge by the same type of antigen.

only one kind of receptor on its surface. After successful reproduction, the receptors on the surface are then shed into the blood as antibodies (Figure 13.5).

Burnet described two advantages to his revision of Jerne's theory. First, it seemed to deal with all the problems of the template theory and with his objections to Jerne's natural selection theory. Immunological tolerance arises because entire clones of lymphocytes are simply deleted very early in development if they match the tissue of the individual (Figure 13.5). The binding abilities of antibodies improve over time because the antigens act as agents of selection, "choosing" cells with receptors that are literally the best fit, and inducing them to proliferate. Once the lymphocytes have gone through a series of reproductive cycles, they will make antibodies even after the antigen is gone by continuing mitosis and shedding their receptors. Finally, the secondary response is more rapid and powerful than the primary response because there are more antigen-specific cells in the blood as a result of the initial antigenic attack.

The second attraction of this theory for Burnet was more philosophical, reflecting his admiration for Charles Darwin. His theory of immune response was analogous to Darwin's theory of natural selection. Furthermore, as a microbiologist, he presented a theory demonstrating that the microscopic world behaved in much the same way as the macroscopic world more familiar to most naturalists. Burnet saw the immune response as a Darwinian world in miniature, with the lymphocytes forming a population within a community, undergoing differential survival and reproduction in relation to their individual fitness. In this case fitness is determined by how well the receptors on the surface of the lymphocytes "fit" to the antigen that enters the environment. Thus fitness, as in Darwin's world view, reflects changes in the environment. In addition, accidental changes (mutations) in the genetic makeup of the cells introduce novel variation into the population, serving as raw material for selection.

Despite the tremendous success Burnet was enjoying, he still retained some of his early childhood insecurities. He had already proposed two incorrect hypotheses of antibody function, both of which involved template types of mechanisms. Thus he published his clonal selection theory in an obscure Australian journal so as not to embarrass himself too badly if he was wrong once more. Additionally, he had a great deal of patriotism for his homeland and wanted this idea to see the first light of day in Australia.

Burnet and Jerne became admirers of each other's work. Burnet considered Jerne the most intelligent living immunologist, and when Jerne received a Nobel Prize in 1984, he sent Jerne a congratulatory letter stating that their joint theory was more deserving of a Nobel Prize than was the tolerance research for which he and Medawar had shared the prize. Jerne, in addressing a symposium on antibodies, congratulated Burnet for stimulating a great proliferation of immunologists and, later, for hitting the nail on the head with his clonal selection theory.

☐ EPILOGUE

Application of Burnet's ecological point of view is a departure point for problems that are challenging medical researchers today. For example, smallpox is a horrible disease that causes weeping sores, high fever, and, often, death. As a result of

extensive vaccination programs, the last case of smallpox was reported in 1978. Why can medical researchers develop effective preventions or treatments for some diseases yet be frustrated in their efforts against others, such as influenza or AIDS? Why are some viral diseases more difficult to control than others?

Some viruses are particularly hardy and can withstand long periods of time away from the host. Others are effective travelers, moving from host to host with relative ease. Finally, viruses vary in the speed with which they evolve, with some types changing their genetic identity very frequently.

The smallpox virus is one of the largest and most resilient viruses known, as it can live apart from its host for decades. A medical researcher recently published a paper in which he recommended that archaeologists who worked on mummies should be vaccinated against smallpox, given the virus's ability to survive in cool, dry conditions for many years. It is also easily transmitted from one host to the next. The virus is genetically very stable, however, which made it susceptible to eradication by the major worldwide vaccination effort.

In contrast, the influenza virus, which so interested Burnet, has a mutation rate about 100 times greater than many other viruses. This high mutation rate exists because the genetic material of the flu virus is RNA. In contrast to DNA viruses like smallpox that have effective repair systems, RNA viruses cannot correct copying errors during the process of replication. Each flu outbreak is caused by a virus with slight changes in its surface antigen, rendering it unrecognizable to previously formed memory cells and insensitive to immunity produced by vaccines made the previous year.

Periodic influenza pandemics arise from two major causes. First, the influenza virus has a segmented genome, with each of the eight loosely bound segments responsible for producing one or two viral proteins. The loose connection between segments allows them to come apart and rearrange with segments from other nearby viruses. If they recombine with segments from viruses in other animals, there can be significant changes to the surface antigens (antigenic shift) that result in the new virus being completely unrecognized by the immune system of the human host it encounters. Second, humans provide an environment in which this genetic reassortment is very likely to occur. Chickens, pigs, and ducks are excellent vectors for the virus, as they harbor the virus within their guts but don't get sick from it. Particularly in China, chickens, ducks, and pigs are commonly raised together; all are likely to get infected with human flu virus, and if they are concurrently harboring the virus from their own species, this form of reassortment may occur. With the recent increase in fish farming in China, which involves feeding hen feces to pigs and fertilizing the fish ponds (which are also duck ponds) with pig manure, there is cause for serious concern that the frequency of gene reassortment will increase over the next few years. Given that ducks are excellent long-distance migrants, they may spread the virus throughout the world very quickly.

As a final example, the HIV virus, which causes AIDS, is relatively fragile outside the body, losing its infectious properties within two hours of exposure to the air. It is also very difficult to transmit, requiring the bodily fluids of a host individual to enter into another person's tissues. HIV, however, has two features primarily responsible for its deadly effect on humans. First, it infects cells of the immune system, seriously restricting the immune system's ability to mount any serious defense. Second,

once the viral RNA enters the host cell, it manufactures a double strand of viral DNA that inserts itself into the nucleus of the host cell. There it remains, often for many years, until activated by factors at this point still unknown to immunologists.

There are several implications to this unusual mode of reproduction. Once the HIV genetic material is integrated into the host DNA, the immune system does not appear to recognize it as a foreign antigen. The long latency period between initial infection and symptoms of serious illness increases the chance that the infected person will transmit the disease to one or more hosts. Additionally, being an RNA virus, HIV has a very high mutation rate; in fact, an individual may have more than one kind of HIV variant as a result of mutation events that occurred after the initial infection.

Burnet's evolutionary perspective is now being adopted by medical researchers to combat a new and potentially very deadly problem—that of the evolution of antibiotic resistance. Fifty years ago, Burnet warned the medical community that antibacterial drugs should be used with great caution, as their unsupervised use was creating a novel environment selecting for new strains of pathogens. Today, the medical community is finding that many of its most potent antibacterial drugs are no longer effective against the new strains of antibiotic-resistant bacteria that have adapted to their novel environment.

QUESTIONS AND ACTIVITIES

1. What does this case show about the following aspects of doing biology?
   — the use of analogies
   — importance of perspective and background
   — usefulness of a natural history background
   — consideration of alternative scientific theories
   — communication within the scientific community
2. Burnet uses the example of ladybird beetles, scale insects, and lemon trees as an analogy to how the immune system functions. Which characters within the immune system are analogous to ladybird beetles, scale insects, and lemon trees? How do they interact with each other? How does this analogy help you understand the immune system?
3. Burnet freely credited Jerne with stimulating the clonal selection theory. In contrast, Jerne did not even cite Ehrlich in his original paper. How would Jerne have known about Ehrlich's work? Propose some reasons that could account for why Jerne did not cite Ehrlich. What does this case reveal about how theories build upon previous theories?
4. The central dogma of molecular biology states that DNA makes RNA, which makes protein. Given that antibodies are protein (globulin) molecules, is Jerne's natural selection theory compatible with the central dogma? Why or why not?
5. Regarding natural selection of organisms and clonal selection of lymphocytes, answer the following:
   a. What is being selected?
   b. What is meant by "fit"?

c. What is the role of mutation?
d. What is the role of diversity?

In what ways does this analogy help you understand how the immune system functions?

6. Propose a set of practical guidelines for dealing with the increase in antibiotic resistance in disease organisms. In your answer, consider the following information.
   a. Some types of antibiotics are broadscale, affecting many types of bacteria, while others are very specific.
   b. Some ranchers routinely put antibiotics in cattle feed.
   c. Today, many antibiotics must be given in much larger doses than previously in order to be effective.
   d. Recently, several people died in hospitals from antibiotic-resistant infections that they picked up in the hospital.

SUGGESTED READING

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