Introduction

My interest in cancer goes back at least half a century.

As a teenager in the seventies, I recall looking at the stunning graphics in *Scientific American* that showed how cancer cells invade neighboring tissues, leaving a path of cellular destruction that portends an excruciating dance with death. I remember thinking that this terrifying aberration of our biology is part of who we are. I often wondered how our cells turn against us and why this devastating illness exists. I also remember thinking that I wanted to write about cancer one day.

Here we are, more than 50 years later. During a sizable chunk of that time, I had the privilege of participating in the development of medicines for the treatment of cancer, along with drugs for other grievous diseases, including hemophilia, anemia, osteoporosis, and rheumatoid arthritis. These medicines, called biopharmaceuticals (or biological drugs), are different from familiar drugs like Nexium and Lipitor, which are synthesized by combining chemicals in large vats to create pills for oral use by patients.

Biopharmaceuticals are manufactured in cell culture systems using equipment similar to what you might see at your local brew pub. Think animal cells instead of yeast (they are not all that different), and, instead of beer, a turbid solution, generally pale red in color, that contains, amongst the many living cells, precious biopharmaceutical proteins manufactured by those cells.

The names of some of these drugs might be familiar to some readers. Enbrel® and Humira®, which are competing medicines for rheumatoid arthritis and other autoimmune diseases, are used by millions of people around the world. These therapeutics cannot be administered orally because they are delicate protein molecules that would degrade in the highly acidic environment of the digestive tract. Rather, biopharmaceuticals are injected into patients, either by the intravenous route (into the vein), in the muscle (intra-muscular route), or under the skin (subcutaneously).

Currently, cancer therapeutics comprise the lion's share of the

biopharmaceutical drugs in development as we continue our lengthy battle with this most challenging and perplexing of human diseases, the "Emperor of All Maladies," as Dr. Siddhartha Mukherjee christened it in his scintillating 2010 biography of cancer. Decades of research have demonstrated that cancer is, from a biological standpoint, extraordinarily complex at the cellular and molecular levels. Moreover, every cancer is a "one-of-a-kind" affair, developed under a unique set of circumstances such that each tumor has an inimitable molecular fingerprint. The distinctive genetic, biochemical, and biological nature of cancer is also why achieving long-term clinical success is so difficult.

Now, more than ever, there is reason for hope in our battle with this terrifying disease. We live in a time when powerful new approaches show significant potential in the fight against cancer. Over the past few decades, scientists have assembled a comprehensive (but incomplete) scientific understanding of the molecular machinations of the human cell. As a result, we can now map, in exquisite detail, the aberrant molecular circuitry that drives the destructive growth of cancer cells.

From such a molecular understanding, the underlying biochemical defects permissive to tumor growth are being elucidated. The acquisition of this knowledge raises the possibility that the molecular switches that allow this life-threatening disease to spread throughout the body in the deadly process of metastasis can be turned off or, at the very least, controlled sufficiently to improve both the duration and the quality of life of cancer patients.

As a result of these insights into the nature of cancer, a dramatic shift is underway from the cancer therapeutic triad of surgery, radiation, and chemotherapy— "cut, burn, and poison"—to exciting new molecular approaches that harness the power of biotechnology to exploit the weaknesses of cancer cells. These developments include immunotherapeutics, new medicines that can stimulate the human immune system to seek out and destroy tumor cells that have escaped the continuous process of immune surveillance that guards us against disease.

By confronting cancer with biomolecules that can curtail its growth, it is now possible to realistically imagine a world in which a diagnosis of metastatic cancer is no longer, by default, the existential threat it represents today. Rather, the experience will be akin to that of patients with medically manageable chronic conditions such as diabetes and rheumatoid arthritis. While this malicious malady inherent to our biology cannot be eradicated, perhaps, at last, it can be tamed.

Over the past decade, clinical evidence has emerged that the new medical tools

described in this book are capable, in small subsets of fortunate patients with metastatic cancer, of achieving long-term remissions and even, on occasion, eradicating detectable cancer cells. Not long ago, the ability to develop and introduce therapeutic agents into clinical use that specifically and effectively target human cancer would have been found only in science fiction. In this century, the likelihood of breakthrough treatments for the most feared affliction of our time has never been more promising.

This is the age of molecular medicine—some have called it the "Bio-Century"—and its wonders await.

Chapter 1

In Search of the Magic Bullet

The idea that we carry an innate ability to resist disease dates to antiquity. The ancient Greeks observed that even in the face of a plague (likely smallpox or typhus) that decimated Athens in the fifth century B.C.E., some of the afflicted recovered and remained protected from the fatal effects of the deadly disease for years. Thucydides, the fifth century B.C.E. author of the definitive text on the Peloponnesian War, noted that these lucky individuals were protected from suffering the full measure of the disease: "The bodies of dying men lay one upon the other. . . [But] those who had recovered from the disease ... had now no fear for themselves; for the same man was never attacked twice—never at least fatally."[i]

The basis of microbial infection remained unknown until the middle of the nineteenth century, a product of the landmark achievements of two European scientists, German physician Robert Koch and French microbiologist Louis Pasteur. These towering figures in the history of biology showed that the great infectious diseases of the age were caused by specific types of microorganisms that live in the vast sea of invisible life surrounding us.

Koch's work on tuberculosis, anthrax, and cholera laid the foundation for our understanding of infectious diseases. According to his findings, encapsulated in what came to be known as *Koch's Postulates*, proof of infection with a specific microbial agent can be demonstrated if an organism isolated from an infected individual can be grown in the laboratory and subsequently shown to cause the same disease when introduced into an uninfected recipient.[ii] If these conditions are met, a relationship between the infectious agent and the disease it causes is unequivocally established.

Pasteur's breakthrough vaccinations for rabies, diphtheria, and anthrax in the late nineteenth century demonstrated the power of vaccination as a preventative agent against diseases caused by microorganisms. These achievements were based on the pioneering discoveries of British physician Edward Jenner nearly a century before.

Jenner had heard for decades that milkmaids bearing cowpox lesions on their hands and legs rarely contracted smallpox. Since cowpox caused a disease that is highly similar—but far milder in its effects—when compared to the more deadly smallpox, and some people exposed to smallpox remained free from its ravages, there had to be a natural capability to prevent the disease from taking hold in certain individuals. In a remarkable (and risky) human experiment in 1796, Jenner took some scrapings from

cowpox lesions he found on the hands and legs of a milk maiden named Sarah Nelms. Next, he placed the cowpox scrapings under the skin of an 8-year-old child named John Phipps, his gardener's son.[iii]

This phase of the experiment mimicked a procedure called *variolation*, in which scrapings from smallpox lesions were placed under the recipients' skin to prevent the disease. Turkish traders introduced variolation into Europe in the early 1700s and had practiced it in Asia for centuries. While the practice reduced the incidence of smallpox in the population, variolation infected about 2-3% of its recipients with smallpox, sometimes fatally.[iv]

Jenner's key idea was that it might be possible to protect against smallpox infection using material from the related disease, cowpox, without the risk of transmitting deadly smallpox to the recipients. Two months after young Master Phipps was inoculated with the cowpox-lesion-derived material, Jenner proceeded with the riskiest part of his experiment; he challenged the child by inoculating him with material from a fresh smallpox lesion. Amazingly, there were no ill effects at all. John Phipps did not even suffer the usual fever and malaise that routinely followed variolation.

This astonishing discovery happened at the end of the eighteenth century, more than a century-and-a-half before the discovery of antibodies, the powerful molecules of immunity that help protect us from disease. From this astounding result, Jenner confirmed his hypothesis: a small amount of diseased material can stimulate a protective response. With this enormous leap forward in preventing one of the deadliest infectious agents in the history of humankind, the science of vaccination was born.

Early in the twentieth century, medical science had advanced sufficiently to explore the biological and chemical bases of host immunity to infectious diseases. Many questions remained: How could we have immunity to a limitless set of substances (called *antigens*) in our environment that can elicit an immune response? How could we generate protective responses for such a large array of potential irritants?

These questions were the focus of the work of German physician Paul Ehrlich. Ehrlich was born in 1854 in Upper Silesia, Germany, in the southwest corner of modern-day Poland. Educated as a medical doctor, he recognized that the identification of the cell as the unit of biological life by German scientists Matthias Schleiden and Theodor Schwann in the middle of the nineteenth century had moved biology's central axis from the level of the whole organism in the nineteenth century to the level of the cell in the twentieth.

Ehrlich realized that to understand biology, we needed a way to look inside the cell to discover the secrets of the biological molecules responsible for cellular functions. He believed that the microscope, which allowed biologists to view cellular structures, but not the molecules that comprise them, had taken biology about as far as it could go in the quest to understand the living chemistry at the heart of cellular functions.

The German physician called the various biological processes in the cell the "*partial cell functions*." He noted that "for a further penetration into the important, all-governing problem of cell life even the most highly refined optical aids will be of no use to us."[v] Thus, he issued a call for more sophisticated analytical instrumentation that would not come to fruition until after the Second World War, almost half a century later.

Recognizing that a true understanding of biological processes required that investigations go beyond

micro-anatomical descriptions to the underlying chemical mechanisms at play, Ehrlich noted, "Since what happens in the cell is chiefly of a chemical nature and since the configuration of chemical structures lies beyond the limits of the eye's perception we shall have to find other methods of investigation for this."[vi]

These scientific insights were remarkably predictive of the future of biological and medical science. Ehrlich demonstrated a penchant for prescience when he proclaimed, "This approach is not only of significant importance for a real understanding of the life processes, but also the basis for a truly rational use of medicinal substances."[vii] Herein lay his key insight: truly effective medicines must target specific biological processes rather than merely provide relief from symptoms. By understanding how medicines work—by investigating what scientists now call the *mechanism of action*—the drug development process can be guided by biological knowledge rather than by trial and error. This approach, Ehrlich realized, would require a detailed understanding of the biochemistry of the cell. Succinctly put, he proclaimed, "We have to learn to aim chemically."[viii]

Ehrlich's work on the neutralization of diphtheria and botulinum toxins by *anti-toxins* in the blood of infected individuals convinced him that the toxin and the anti-toxin must interact in a highly specific way. This specificity, he proposed, was rendered by precise interactions between the toxin and the anti-toxin mediated by what Ehrlich called *"side chains."*

He envisioned these side chains as chemical structures with individualized shapes. When the side chains of a toxin are complementary to those on an anti-toxin—that is, the side chains of one fit together in three-dimensional space with the side chains of the other—the toxin and the anti-toxin will latch onto each other in a firm chemical embrace.

We can think of analogies: a lock and a key or a pair of tessellating tiles that fit perfectly into each other. Ehrlich envisioned that if he could find an anti-toxin that perfectly fits in a specific way with a known toxin, it would be possible to neutralize the toxin.

In this vision, the anti-toxin was envisioned as a "magic bullet"—a specific, precise, and effective means to target a toxic substance in the body, bind to it, and thereby prevent the toxin from causing physiological harm. This was a powerful vision, and it would take decades of research to discover that the anti-toxins—Ehrlich's "magic bullets"—are proteins called *antibodies*. The antibodies, which are made by white blood cells called *B lymphocytes*, comprise only part of the extraordinarily complex system of immunity that protects us from disease.

Ilya Mechnikov was born in 1845 in a small village near Kharkiv, Russia, in modern-day Ukraine. Encouraged to study science by his mother, he was a natural science prodigy who lectured neighborhood children on botany and geology when he was six.[ix]

After studying biology at the city's university, Mechnikov collaborated with Russian zoologist Alexander Kovaleskyin—first in Naples, Italy, and then in St. Petersburg, Russia, where the two scientists fled following a cholera outbreak in southern Italy in 1865.[x] Mechnikov completed his doctoral studies in 1867, earning a Ph.D. in embryology.[xi]

While pursuing his studies in comparative embryology in 1882, Mechnikov was examining starfish larvae under a microscope. He had chosen the larvae of the genus *Bipinnaria* because they provide an excellent model system for biological study due to a convenient matter of their anatomy. *Bipinnaria* larvae are transparent, making it possible to peer inside them with a microscope and observe the movement of cells.

Mechnikov noticed that cells were moving inside the larvae engulfing particles of food. It occurred to him as he observed the cells engulfing the food particles that these cells might also be involved in protecting the larvae from microbes, microscopic organisms that can cause disease. "These wandering cells in the body of the larva of a starfish, these cells eat food ... but they must eat up microbes too!"[xii]

He devised a simple experiment in which he placed tiny thorns inside the larvae to assess whether the wandering cells would react to the presence of foreign substances. As predicted, the cells responded to the foreign bodies in their midst. "He noted that the cells within the larvae were no longer moving around aimlessly, but were instead aggregated around the foreign bodies, as if to drive them out."[xiii]

Mechnikov called the process in which the wandering cells engulf foreign matter *phagocytosis*, from the Greek words' *phage*, meaning "to eat," and *cyte* (from the Greek *ketos*), meaning "cell." These cells, which he named *phagocytes*, can engulf foreign matter.

A further test of his theory involved placing fungal spores in water fleas of the genus *Daphnia*. Mobile cells in the flea could also engulf the spores. Obviously, these cells played a role in protecting the organism from infection. Further experiments with higher organisms, such as rabbits, convinced him that he had discovered a general mechanism of immunity present in all multi-cellular organisms. Extrapolating to humans, he noted, "Our wandering cells, the white cells of our blood—they must be what protects us from invading germs."[xiv]

The Russian scientist had found a powerful, innate defense against infection, a means for the body to neutralize potential microbial threats. "Where natural immunity is concerned, and man enjoys this in respect of a large number of diseases, it is a question of the phagocytes being strong enough to absorb and make the infectious microbes harmless."[xv]

Shortly after the discovery of phagocytosis, a German scientist named Emil von Behring made another profound discovery. Von Behring had worked directly with Robert Koch, and near Paul Ehrlich, at the Institute for Infectious Diseases in Berlin. He applied that strong scientific foundation to his studies of diphtheria, a bacterial illness that posed a serious and potentially lethal threat to children in the early twentieth century.

Von Behring found that he could remove all the cells from a sample of an infected animal's blood (cell-free blood is called *serum*), infuse the infected animal's serum into the bloodstream of an uninfected animal, and thereby protect the uninfected animal from a challenge with the causative agent of diphtheria, the bacterium *Corynebacterium diphtheria*.[xvi] This serum transfer experiment demonstrated that a substance in an infected animal's bloodstream could protect against diphtheria infection. Known as an anti-toxin by biologists at the time, the agent, later called an antibody, was (we now know) a protein that can bind to a specific target on a foreign substance in the body—in this case, to a target on the surface of the bacterial cells.

Von Behring received the first Nobel Prize in Physiology or Medicine in 1901 for demonstrating that

the immune response was not solely a matter of phagocytic action by Mechnikov's wandering cells. As a result of von Behring's work, a heated debate ensued in the biological community about whether immunity was a matter of cellular activity (phagocytes) or, alternatively, whether anti-toxins (antibodies) in the blood provided protection against microbes.

With Ilya Mechnikov as a major proponent, the former idea was called the cellular basis of immunity. The latter idea, supported by the work of von Behring and Ehrlich, was known as the "humoral" basis of immunity in recognition of the role of blood—one of the humors (bodily fluids) described by Hippocrates—in providing protection against infectious microbial organisms.[xvii] As it turned out, both sides had equal merit.

In awarding the 1908 Nobel Prize in Physiology or Medicine to Mechnikov and Ehrlich for their groundbreaking work on immunity, the Nobel committee equally recognized the critical importance of both immune mechanisms. This view would be strengthened during the following century of investigation, which clearly showed that the cellular and humoral immunity mechanisms work together in a highly coordinated fashion to regulate the immune response.

The discoveries of Ehrlich, Mechnikov, and von Behring on the nature of immunity launched the science of immunology. Their work revealed the presence of two major subsystems called *innate immunity* and *adaptive immunity*. These two subsystems, the "arms" of the immune system (in common biological vernacular), interact with each other through complex molecular signaling networks to coordinate the overall immune response.

As the name implies, we are born with the elements of innate immunity already in place and on the job. Our skin, the body's largest organ (by surface area), is the primary layer of protection against invasion. Immune cells called *neutrophils* and *macrophages* circulate throughout the body to kill and engulf microbial invaders. Innate immunity is a generalized response triggered by exposure to foreign substances, regardless of their identity or origin. The innate response does not need to develop over time; innate immunity is triggered without requiring previous exposure.

The other arm of immunity, the adaptive immune response, requires (as the name implies) that the system learns over time to distinguish antigens (proteins or chemical substances bound to proteins) originating inside our bodies from those derived from foreign sources. Throughout our lives, the cells of adaptive immunity continually sample the antigens in the body, learning to distinguish foreign antigens from our own and thereby guarding against potential threats that require an immediate response.

In the adaptive arm, the first exposure to a specific antigen, a process that immunologists call *priming*, does not trigger a significant response. Rather, it trains the system to respond to subsequent exposures to the antigen. Once primed, adaptive immunity is ready to respond when re-exposed to the priming antigen. Herein lies the basic principle of vaccination, in which a virus or piece thereof trains the immune system to respond in the event of a future infection by that virus.

Following antigen exposure, the cellular constituents of the adaptive immune system primed by

previous antigen exposure go into production mode, generating a humoral (antibody) response to the antigen by antibody-producing white blood cells (B lymphocytes). In addition, an adaptive cellular response is stimulated, characterized by the rapid activation of immune cells called *T cells* (*T lymphocytes*) that are specific for the antigen. These activated T cells can kill foreign cells (for example, bacteria and viruses). Known as the "cellular soldiers" of adaptive immunity, T cells circulate throughout the body following antigen stimulation in search of the foreign antigen that launched them into action.[xviii]

Von Behring's experiments conclusively demonstrated that immunity engendered by serum transfer is specific to the organism that caused the disease in the animal from which the serum was taken. Thus, the transfer of serum from an animal infected with diphtheria can protect against a subsequent challenge with the bacteria responsible for diphtheria, but not against the bacteria responsible for botulism (and vice versa).

Ehrlich's side chain theory proposed that this specificity was related to the molecular characteristics of the antigens on the surface of infectious organisms. In turn, the chemical properties of the antigens' side chains provided specific binding sites for the anti-toxins' side chains, which fit snugly in three-dimensional space with specific structural features of the antigens.

Given the observed specificity of the response, and the complexity of this process at the level of molecular structure, how was it possible that the body can recognize and generate a specific response to the millions of antigens present in the environment? Stated in the terms used by modern-day immunologists, what mechanisms are at play in providing the vast repertoire of antibodies that can be elicited by antigen stimulation?

According to Ehrlich's theory, the answer resided in the presence of a limitless array of chemical structures on the surfaces of the anti-toxin-producing cells (later renamed *antibody-producing cells*) in the circulation. Ehrlich reasoned that for these cells to manufacture an anti-toxin for a toxin that is present in the bloodstream, they must have a way of identifying the chemical side chains on circulating toxins. Envisioning a mechanism that might explain how the anti-toxin-producing cell recognizes and responds to the toxin, Ehrlich proposed that the side chains of the molecules on the surfaces of the anti-toxin-producing cells must be the same as those on the anti-toxin produced by that cell.

The rationale for this proposal is as follows: If the side chains on the surface of the cell fit together with those of the toxin, and the anti-toxin made by the cell has the same side chains as those on the toxin-binding structure on the cell surface, then the side chains on the anti-toxin produced by that cell will also fit with those on the toxin. This format provided a ready answer to the question of how the anti-toxin-producing cell creates a molecule with side chains that can bind to the side chains of the toxin amidst the extraordinarily complex biochemical milieu of bodily tissues.

At the time, not only was the biochemical structure of anti-toxins unknown, the identity of antibodies as members of a family of related protein molecules involved in immunity had not yet been established. Ehrlich had no concept of the existence of cell surface proteins on each B lymphocyte that are, in fact, the antibodies produced by that cell. Paul Ehrlich's proposal was, therefore, unadulterated

genius.

It is unfathomable to this twenty-first century biochemist how Ehrlich made such a leap beyond what was known at the time. In the complete absence of any data supporting his contention, Ehrlich formulated a prescient hypothesis on the biological basis of toxin/anti-toxin specificity decades before the nature of antigens and antibodies was revealed.

Refinements of Ehrlich's side chain theory did not emerge for half a century. In 1955, British immunologist Niels Jerne proposed that the existence of a vast array of pre-existing antibodies in the serum was responsible for antibody diversity. Once an antibody finds an antigen with which it forms a tight biochemical "fit," Jerne reasoned, the presence of the antibody-antigen complex stimulates the B cell that produced that antibody to divide. He called his idea the *natural selection theory* of antibody production.

The main problem with the natural selection theory as formulated by Jerne was the lack of an explanation for how a B cell can sense when its antibody molecules are bound to antigens in the circulation. One could propose that following antigen binding, a signaling event takes place between the circulating antibodies and the antibody-producing cells. However, there was no evidence for this mechanism, nor was there a conceivable explanation why this might be so.

This mystery was solved shortly thereafter, in 1960, when Australian immunologist Frank Macfarlane Burnet modified Jerne's natural selection theory. Burnet proposed that the antigen-recognizing protein sticking out of the membrane on the surface of the antibody-producing cell *is* the antibody produced by that cell. This idea harkened back to Ehrlich's concept that the anti-toxins with their toxin-specific side chains resided on the cell surface.

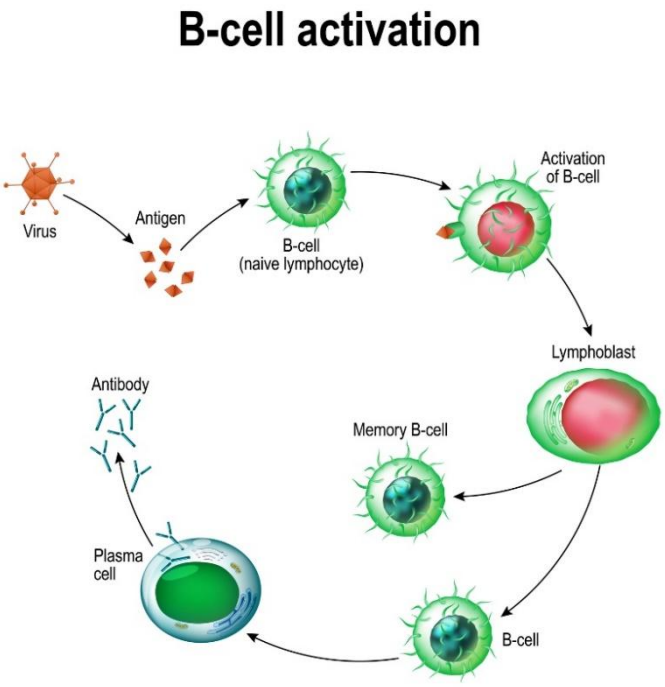

Figure 1. Activation of B cells by antigens

Burnet's solution to the question of B cell activation is shown in Figure 1. He proposed that once an antigen found an appropriate molecular fit with a surface protein on a B cell (which is the antibody produced by that cell), the cell would be stimulated to divide. This would produce many copies of the B cell that manufacture the same antibody as the originally stimulated B cell. In this way, large numbers of antibody molecules are created and subsequently secreted from the B cells into the circulation. Since this idea was based on the concept that a stimulated B cell can be copied (cloned) to make many copies, Burnet called his proposal the *clonal selection theory* of antibody production.

In addition to antibody-producing B cells (called *plasma cells*), another type of B cell, called a *memory B cell*, is created following antigen exposure. While activated B cell levels decay over time, memory B cells are responsible for long-term immune recognition. These two cell types are derived from the same ancestral activated B cell (called a *lymphoblast*) during B cell activation.

Once formed, memory B cells can be activated upon further antigen exposure, such that they rapidly proliferate and create new antibody-producing plasma cells. Memory B cells are found both in lymph nodes and in the circulation, and they persist long after the cessation of antibody production by plasma cells.[xix]

As our knowledge of adaptive immunity accumulated, a mystery remained. How could the genome possibly code for the millions of types of antibodies needed to cover the environment's tremendous diversity of antigens?

The first piece of this puzzle was solved by the mid-1970s with the advent of the *recombinant DNA revolution*. A series of discoveries in molecular biology provided, for the first time in human history, a technique for inserting genes of interest into bacteria, yeast, and mammalian cells. Thus, living cells could be used as factories for manufacturing the proteins encoded by the genes. These new techniques in molecular biology enabled clever investigators to unravel the mechanism of antibody diversity, providing compelling evidence that Burnet's clonal selection theory was correct.

As Burnet predicted, antibody molecules are indeed expressed on the surface of *naïve* (unstimulated) *B cells*, which are then selected for proliferation following binding of an antigen to a surface antibody on the B cell. As the selected B cell proliferates, the genes responsible for antibody production undergo rearrangements that lead to the refinement and maturation of the antibodies to increase their antigen specificity. In this process, called *affinity maturation*, multiple generations of refined antibody molecules are generated to increase the tendency of the antibody to bind to the antigen that stimulated the response.

With the tools of genetic analysis wrought by recombinant DNA technology—that is, the ability to isolate, amplify (make many copies), and sequence the DNA—the mystery of antibody diversity was unraveled, and the structure of antibodies was elucidated. While some of our proteins are comprised of a single chain of amino acids—a single *polypeptide*, in the language of biochemistry—other proteins are comprised of two or more distinct polypeptide chains. Such is the case for antibodies. Protein analysis work performed in the 1960s showed that *immunoglobulin G (IgG),* the major antibody type found in human serum, is comprised of four polypeptide chains: two copies each of two different polypeptides called the *heavy chain* and the *light chain*.

IgG (Figure 2) comprises about 80% of the antibody population in human serum. The remaining

types (*classes*) of antibodies, called IgM, IgA, IgD, and IgE, each have a distinct architecture, with different numbers and orientations of the polypeptides that form the overall structure. The four polypeptide chains that comprise IgG are bound together in a Y-shaped molecular construct that is connected by bonds (called *disulfide bonds*) between a sulfur atom in the amino acid cysteine on one heavy chain and another sulfur atom in an adjacent cysteine on the other heavy chain (thereby creating a *disulfide bridge*). This region of the antibody, called the *hinge*, provides flexibility around a stable rotational axis. There are four IgG sub-types (*sub-classes*), called IgG1, IgG2, IgG3, and IgG4, each with a characteristic disulfide bond structure.[xx]

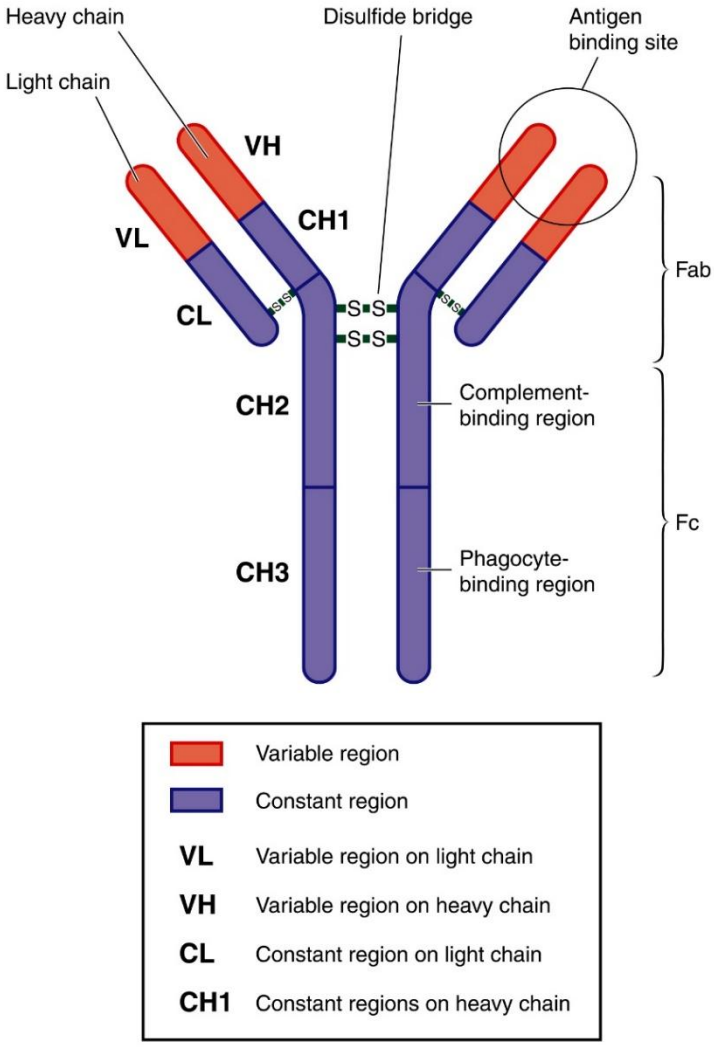

Figure 2. The structure of human IgG

The heavy (H) chain contains both variable (V_H) and constant (C_H) sections (called *domains*); similarly, the light (L) chain has both variable (V_L) and constant (C_L) domains. The Y-shaped IgG

structure has two identical antigen binding sites, as shown at the top of Figure 2. These antigen-binding sites, comprised of sequences with significant variability from antibody to antibody, consist of sections of the V_H and V_L domains of the heavy and the light chains, respectively.

If the antibody binds its target antigen with binding sites at the top of the arms of the Y-shaped structure (the sequences above the hinge form the antibody's *Fab region*), what is the function of the sequences below the hinge, known as the *Fc region*?

The Fc region is responsible for stimulating what are known as the *effector functions.* These functions are elicited by the binding of sequences on the Fc region of the antibody to specific proteins on the surfaces of immune cells to stimulate the destruction of a target cell (e.g., a bacterium). As the name implies, the constant regions of the antibody where the effector function binding sites reside (e.g., complement- and phagocyte-binding regions) are similar in sequence from antibody to antibody of the same class (e.g., IgG) and sub-class (i.e., IgG1, IgG2, IgG3, or IgG4). Each IgG sub-class has a distinct effector function profile. In addition to stimulating cell killing by immune cells, an effector function called *FcRn binding* allows IgGs to remain in the circulation for up to about three weeks.[xxi]

To provide for antibody diversity, the heavy chain and the light chain are assembled from information encoded in multiple immunoglobulin genes. These genes can be "mixed and matched" to create multiple protein sequences from the individual heavy and light chain genes. The biochemical processing of the genetic sequences that code for immunoglobulins is unusually imprecise during the assembly of the various immunoglobulin genes. This provides multiple sequence variants during assembly that increase antibody sequence diversity.

Further antibody sequence diversity is added by the presence, in the antigen-binding sequences of the variable (V_H and V_L) domains, sequences that are prone to rapid mutation (these are called *hypervariable regions*). The combination of processes described above that contribute to the diversity of antibody sequences provides the capability to confront an unlimited variety of antigens during our lifetimes.

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[i] D Masopust, V Vezys, EJ Wherry, and R Ahmed, A brief history of T cells. *Eur J Immunol* 37: S103–110 (2007).

[ii] The final requirement is to isolate the organism from the newly infected individual and successfully grow it in pure culture that is unadulterated by other infectious agents.

[iii] S Reidel, Edward Jenner and the history of smallpox and vaccination. *BUMC Proceedings* 18:21–25 (2005).

[iv] ibid.

[v] P Ehrlich, Partial Cell Functions. *Nobel Prize Lecture*, December 11, 1908.

[vi] ibid.

[vii] ibid.

[viii] K Strebhardt and A Ullrich, Paul Ehrlich's magic bullet concept: 100 years of progress. *Nature Rev Cancer* 8: 473-80 (2004).

[ix] Racine, Valerie, Ilya Ilyich Mechnikov (Elie Mechnikov) (1845-1916). *Embryo Project*

Encyclopedia (2014-07-05). ISSN: 1940-5030 http://embryo.asu.edu/handle/10776/8018.

[x] ibid.

[xi] SY Tan and MK Dee, Elie Mechnikov (1845-1916): discoverer of phagocytosis. *Singapore Med J* 50(5): 456-7 (2009).

[xii] ibid.

[xiii] ibid.

[xiv] ibid.

[xv] II Mechnikov, On the present state of the question of immunity in infectious diseases. *Nobel Prize Lecture*, December 11, 1908.

[xvi] Upon removal of all the cells from the blood, the remaining liquid, called plasma, contains proteins that participate in the clotting of blood. In this protein mixture we find the critical clotting protein Factor VIII (factor 8), a deficiency of which causes the most abundant form of hemophilia (Hemophilia A). The clotting proteins are removed from the plasma to create serum.

[xvii] The other humors were black bile, yellow bile, and phlegm.

[xviii] The requirement for training of the adaptive response explains why newborns have a limited capability to respond to antigens, and why breastfeeding infants is an important means for instilling immunity via maternal antibodies that are passed from the milk to the infant.

[xix] Herein we find one of the issues with using antibody levels in assessing the duration of the immune response after vaccination. The more time that passes after the first antigen exposure (priming), the fewer antigen-specific antibodies we find in the blood due to decay of the activated B plasma cells. This measurement tells us nothing about the status of the B memory cells, which are found in the lymph nodes after antigen priming in both B and T cell lineages.

[xx] Figure 2 depicts human IgG1, which has two disulfide bridges in the hinge. IgG2 has a more complex disulfide bond structure, with four disulfide bridges. Most human antibody therapeutics are members of either the IgG1 or IgG2 sub-class (IgG3 has 11 hinge disulfides, and IgG4 has 2).

[xxi] Amino acid sequences in the C_H2 domains of IgGs contain binding sites that mediate the antibody "half-life"—a measure of how long the antibody remains in the circulation—as well as sequences that interact with protein receptors on T cells, macrophages, and other immune cells that participate in the immune response. The effector functions are elicited by interactions between antibody-antigen complexes and receptors on immune cells. Activation of these effector functions, called antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP), results in the death of targeted cells by cytotoxic (killer) T cells (in ADCC) and activated phagocytic cells, such as macrophages (in ADCP). The third type of effector function, called *complement activation*, involves the interactions of complement proteins in the blood with the Fc domains of antibody-antigen complexes at cell surfaces. These interactions result in the creation of a *membrane attack complex* that punctures the membrane and destroys the cell. The complement system is a critical component of innate immunity. As noted in the text, the FcRn receptor is a "salvage" receptor that prevents rapid degradation of antibodies and thereby prolongs their residence time in the body. The lengthy residence time of antibodies explains (at least in part) why they have been so successful as therapeutic agents.