Breakthroughs in Bioscience

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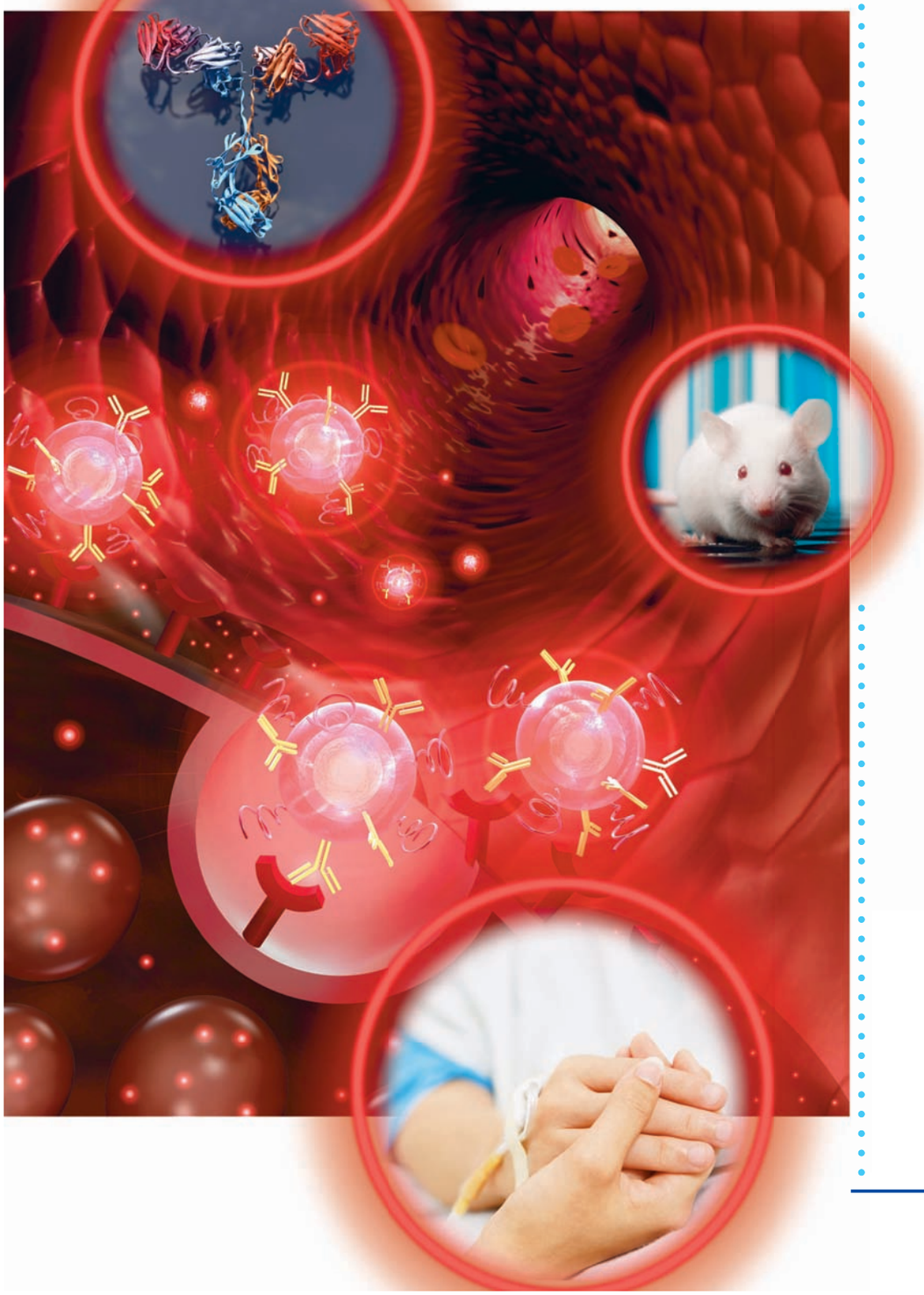

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Magic Bullets and Monoclonals: An Antibody Tale

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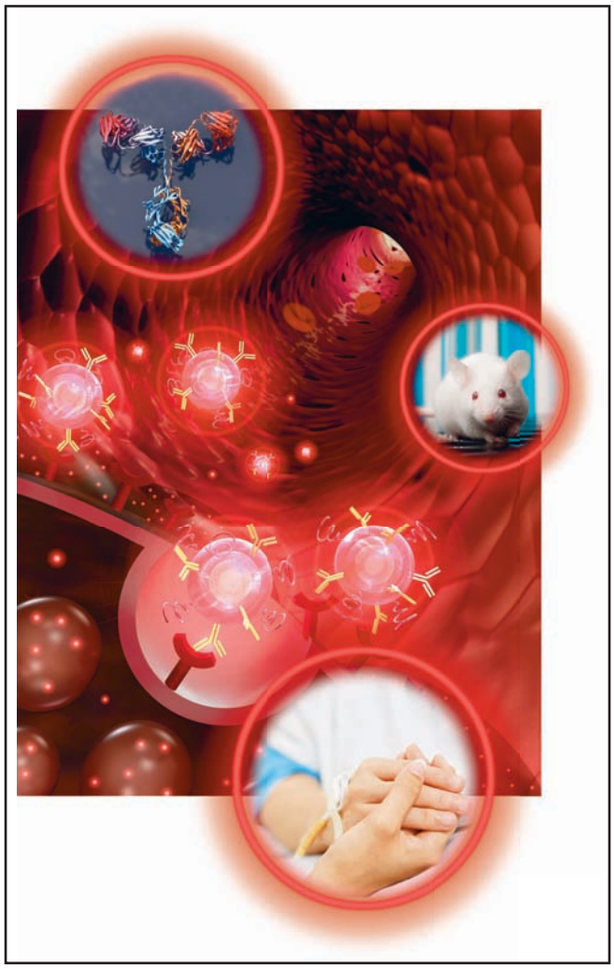


COVER: Nearly a century of basic discovery in immunology, cell biology, and cancer biology contributed to today's remarkable monoclonal antibody therapies, which are produced through human-mouse cell hybrids. Scientists spanning decades pieced together clues about the fundamental nature of antibodies, a critical part of our immune response, in order to develop today's monoclonal antibody drugs, used to treat cancer, auto-immune disorders, and blood clotting. *Credit: Science Photo Library and iStock Photo.*

Magic Bullets and Monoclonals:
An Antibody Tale

In 1890, the German scientist Emil Behring stunned his colleagues by showing that he could protect a guinea pig from the ravages of diphtheria by merely giving it a shot of blood (Figure 1). The blood had been purged of its cells, and was gathered from a guinea pig that had recently recovered from the disease. Behring's fellow scientists at the Institute for Infectious Diseases in Berlin were intrigued by this mysterious invisible substance in the blood that protected the guinea pig from diphtheria, but not from tetanus. One colleague, Paul Ehrlich, postulated that there must be chemicals in the blood that could seek out and destroy specific toxins like "magic bullets." This led to the suggestion that by harnessing these magic bullets, researchers could develop cures for all kinds of disorders.

It took a century for these magic bullets, in the form of monoclonal antibodies, to be developed as effective weapons in combating a number of diseases. There are now more than 20 monoclonal antibody-based drugs on the market, including several blockbusters, that are used by millions of people worldwide. These medicines can effectively treat disorders that don't respond to other treatments, such as several common deadly cancers, and debilitating cases of rheumatoid arthritis and inflammatory bowel disease. Hundreds more of these magic bullet drugs are being tested in people with the hope of providing innovative targeted treatments for an even wider range of disorders.

Figure 1 – Emil von Behring (1854-1917): In 1890, Behring helped to show that immunity from diphtheria could be achieved by an injection of blood serum from an infected animal. In 1901, he received the first Nobel Prize awarded in physiology or medicine for his work. *Credit: Science Source / Science Photo Library.*

But the path to the development of monoclonal antibody drugs was not as straight as you would think, and instead was a global adventure that depended on the unexpected findings of numerous curious scientists. These scientific explorers pursued such basic questions as what "disinfectants" lie in the blood?; how do antibodies work?; how can the body

create 10 million different antibodies uniquely suited to whatever foreign invaders it encounters?; and what causes antibodies to improve their ability to latch onto compounds over time? By seeking answers to these questions, researchers from multiple disciplines collectively assembled the basic building blocks that led to the development of the monoclonal antibodies that now underlie many diagnostic tests and important new drugs.

Blood, a natural disinfectant

Behring's amazing discovery of the curative powers of blood led to the first effective treatments for diphtheria and tetanus, and his being awarded, along with Shibasaburo Kitasato, the first Nobel Prize in Physiology or Medicine in 1901. But his research had humble origins—the quest to find the perfect disinfectant. A military man, Behring's main objective was to find a way to prevent the scourge of deaths stemming from infections of war injuries. He began his research career exploring in animals whether iodine-like compounds added to wound dressings or injected could help prevent or control infections by blocking the actions of bacteria.

Behring's research took a revolutionary turn, however, when he switched from exploring the power of various chemicals to disinfect to exploring the power of the blood to disable microbes. Curious to see if blood might have some of the same anti-bacterial properties as the synthetic chemicals he was testing, Behring ran tests in various animals to see if the addition of a clear, cell-free component of blood (serum) would affect the course of a disease, such as diphtheria (Figure 2). But this research had some frustrating moments—although some sera was remarkably effective at preventing or relieving animals of diphtheria, other sera had no effect. The blood from normal animals not infected with diphtheria, for example, had no effect, nor did the blood from an animal infected with tetanus. Only an animal that survived infection with diphtheria or its toxin could produce serum with any anti-diphtheria benefit.

Figure 2 – Importance of animal models: Animals such as guinea pigs, rabbits, horses, and mice were critical to the basic immunological discoveries that led up to today's monoclonal antibody therapies. *Credit: U.S. Department of Agriculture, Agricultural Research Service.*

This led Behring to conclude in his Nobel address "the internal disinfection that has been achieved is thanks to the fact that Nature herself has been taken as a guide…It is one of the most wonderful things imaginable to see how the supply of poison becomes the prerequisite for the appearance of the antidote in the poisoned living organism."

Behring called the natural antidote "anti-body" and left it up to other researchers to answer the intriguing puzzle of how exactly antibodies work, and of what they are comprised. But one man's frustration—the specificity of the disinfective properties of the blood—proved to be a source of inspiration to others working in this field. It led Ehrlich to

imagine that the blood must contain a myriad of antibodies capable of fitting around and blocking the action of foreign substances (antigens) the body encounters. He likened the structural affinity an antibody has to antigen to that of a key in a lock. Ehrlich's contemporary Karl Landsteiner, at the Pasteur Institute in Paris, tried to assess the chemical specificity of antibodies. He found that slight alterations in the structure of an antigen changed its three-dimensional shape enough so that it no longer bound strongly to the same antibody.

Like Everest to hill climbers

Many researchers eagerly embarked on a quest to discover the exact structure of antibodies and how they formed, so as to figure out how they worked so quickly and precisely to fight against invaders. But this quest was a long and difficult journey because blood contained a confusing jumble of surprisingly similar antibodies. Even an animal not actively fighting an infection will have one percent of its blood comprised of thousands of antibodies, which researchers could not distinguish with the technical tools at hand.

Those tools improved with the advent of spinning devices called ultracentrifuges in the 1930's, which were modeled after dairy cream separators. These devices separated groups of antibodies by size and shape. Filtering techniques developed in the 1940's separated antibodies by electric charge and/or size. But antibodies able to latch onto a specific microbe could be found in more than one class of antibodies grouped by size, shape, or charge. The large size of antibodies also made them difficult to decipher—they were at least 20 times the size of those protein molecules whose structures had been solved. As Gerald Edelman put it, the size of antibody molecules was "large enough to strike terror in the hearts of the structurally inclined protein chemist…

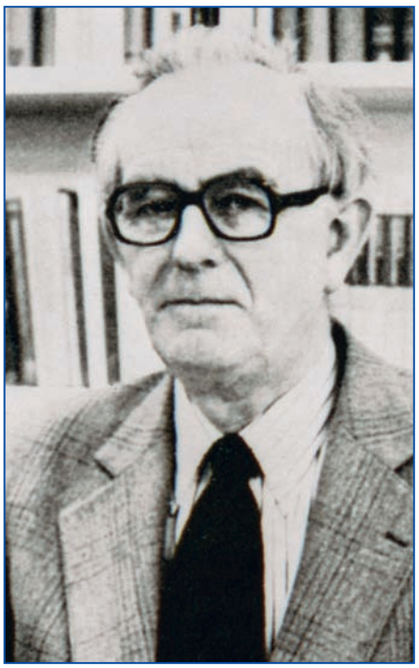


Figure 3 – Rodney P. Porter (1917-1985): Porter, a British immunologist, was awarded a Nobel prize in 1972 for his discovery of the chemical structure of antibodies. *Image from the National Library of Medicine.*

It seemed like Everest to local hill climbers. As if that were not daunting enough, the shape of the mountain—its specificity—seemed almost beyond grasp."

It took Edelman and another dedicated biochemist intrigued by the little bit that was known about antibodies to overcome these challenges, with the help of government funding for their research. Both researchers tackled the problem by breaking up antibody molecules into smaller components. By comparing what they discovered about those components, the investigators were able to formulate the overall shape and detailed structure of antibodies.

Rodney Porter (Figure 3) of the British National Institute for Medical Research, who later worked at St. Mary's Hospital Medical School in London, found it astonishing, as he put it, that the pool of antibodies found in the blood had an infinite ability to combine with any substance, yet superficially seemed to be a nearly uniform group of proteins. This confounded him and bolstered his determination to decipher antibody structure and function. In the late 1950's, he took an innovative approach to the problem. Instead of grappling with separating antibodies according to what they were able to attack, so as to separate and accumulate enough of a specific antibody to study, Porter instead assessed the general structural components of a group of different rabbit antibodies.

Porter used an enzyme from papaya to break apart these antibodies into three major sections that he separated for further study. This work, combined with later work by Edelman of the Rockefeller Institute in New York, revealed that the basic unit of antibodies is shaped like a Y. Despite the mixture of many anti-

bodies used in his studies, Porter found that the stem of the Y was remarkably consistent in the composition of its protein chains, whereas the V portion varied widely. The diverse nature of the V portion led Porter to suggest that it is the section of the antibody that locks on to the antigen.

Meanwhile, across the Atlantic, Edelman's medical background made him aware that certain cancers of the immune system produce an overabundance of a single specific antibody that can be easily isolated from the blood or, in incomplete form, from the urine of patients with these cancers. Such malignancies, known as myelomas, are tumors of the cells that make antibodies. (Only one specific antibody is overproduced in a patient because this is the antibody made by the original cell which became cancerous.) Different patients overproduced different antibodies and became useful sources of bulk quantities of unique antibody proteins.

Edelman realized that the large quantities of a specific type of antibody produced by these tumors would enable him to do the biochemical studies needed to figure out antibody structure. He used various chemical scalpels recently developed by Swedish biochemist Pehr Edman to cut apart the tightly knit antibody molecule into more components than Porter's papaya enzyme had done. He then used a new labeling technique, developed by his chemist colleagues Stanford Moore and William Stein at Rockefeller Institute, to mark the amino acid building blocks of the protein chains that make up the antibody so as to determine their order in the chains.

By 1969, Edelman and his colleagues had deciphered the entire chemical structure of an antibody (Figure 4), the largest molecule to be biochemically unraveled to that point. His analyses revealed that two linked protein chains comprised each half of the Y of an antibody divided lengthwise. Edelman's comparisons of the chemical structures of the different antibodies produced by different patients with myeloma also revealed that, as Porter found, the structure of the base of the antibody was virtually identical among individuals, but the V section varied from patient to patient. Later, studies revealed that it is indeed the V portion of the antibody that latches on to an antigen.

But one of the "most satisfying conclusions" that emerged from his structural analysis of antibodies, according to Edelman, was that it explained how the body could make the millions of antibodies needed to latch on to all the foreign compounds it might encounter during a lifetime. Different combinations of the protein building blocks called amino acids, which make up the

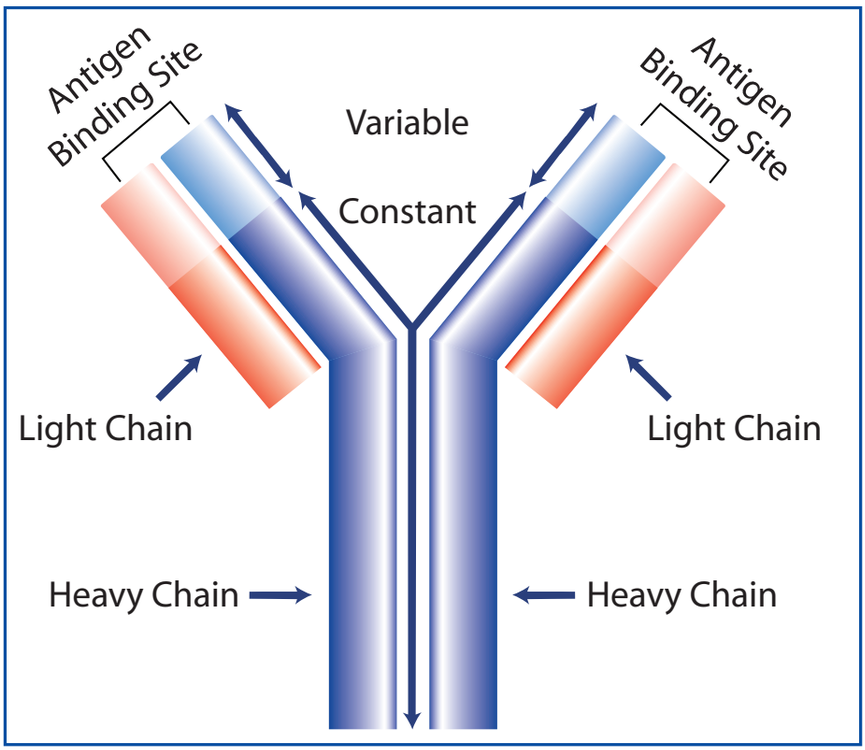


Figure 4 – Structure of an antibody: This Y-shaped protein is produced by B-lymphocyte white blood cells as part of the immune response. Each antibody consists of four polypeptides (protein pieces), two smaller "light" chains and two larger "heavy chains." The amino acid sequence in the tips of the Y (variable region) varies widely among different antibodies and is what confers the antigen specificity to each antibody. *Figure designed by Corporate Press.*

portions of the V section of an antibody that bind an antigen, could generate the needed antibody diversity. (These minute differences in the combination of amino acids were not significant enough to discernibly affect the size or shape of antibodies, which is why at first glance, they all appeared so similar.) Both Porter and Edelman were awarded the Nobel Prize in Physiology or Medicine in 1972 for their pioneering work on antibody structure.

B-cell brainteaser: Explaining the antibody response

Still left unexplained was how the body is able to quickly produce an abundance of the specific antibody needed to disable a specific antigen, or as Behring put it, provide an antidote to the poison almost immediately after it enters the body. But this was eloquently explained by a theory formulated by Danish immunologist Niels Jerne and improved by Australian virologist Sir Frank Burnet. They applied Charles Darwin's ideas about natural selection of animal species in the environment.

In their "natural selection" theory, Jerne and Burnet suggested that each antibody-producing white blood cell, called a B cell, can only make one specific antibody. Each of these B cells coats itself with the unique antibody it produces, and then patrols the blood or other body tissues. When a B cell encounters its matching antigen—the antigen that fits best into the uniquely shaped crevasse within the V region of the antibody jutting from its surface—the binding of the antigen to the B cell's antibody triggers the B cell to create clones (multiply) and rapidly secrete an arsenal of its antibodies (Figure 5).

During this rapid duplication process, the cells make subtle genetic mistakes that affect the structure of the antibodies they produce. Some of these changes enable the antibody to bind more tightly to the antigen. This tighter

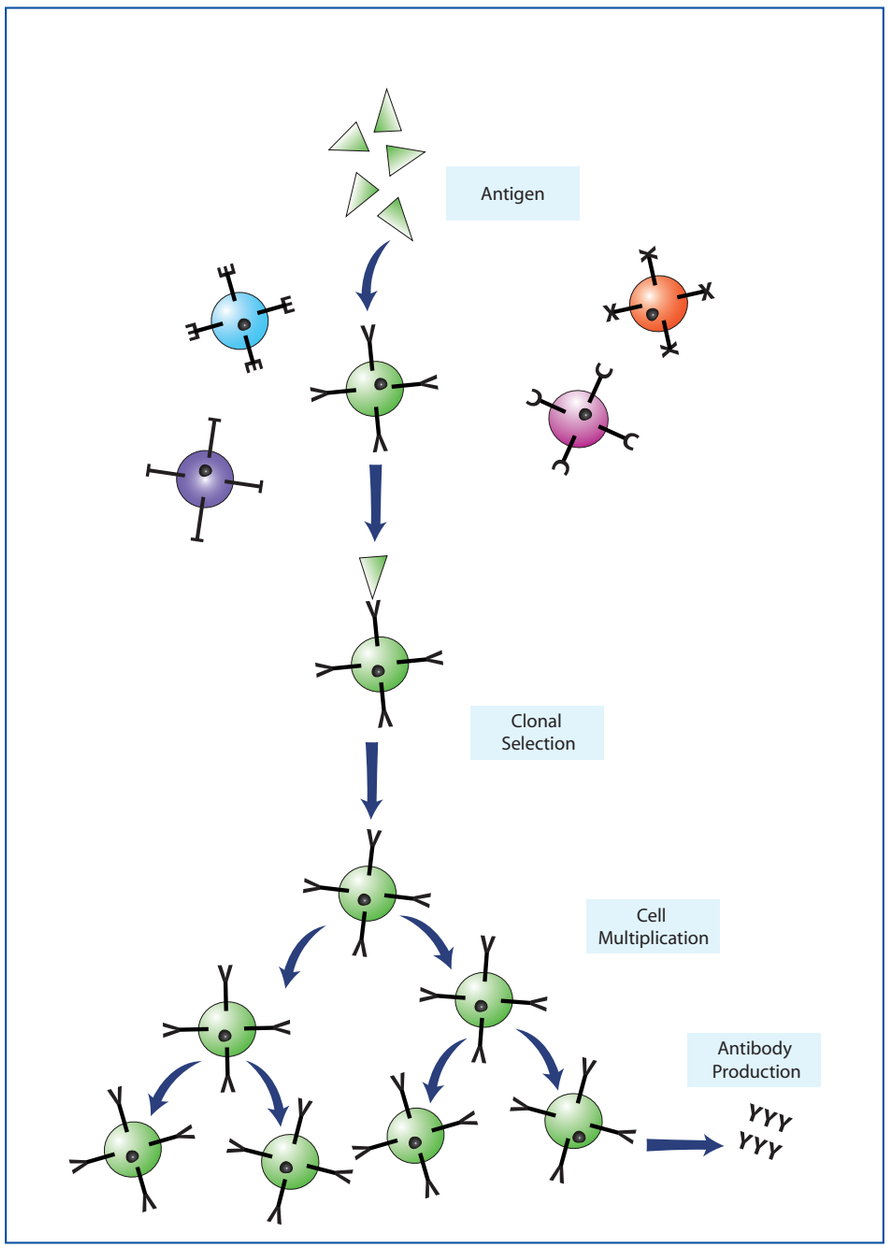
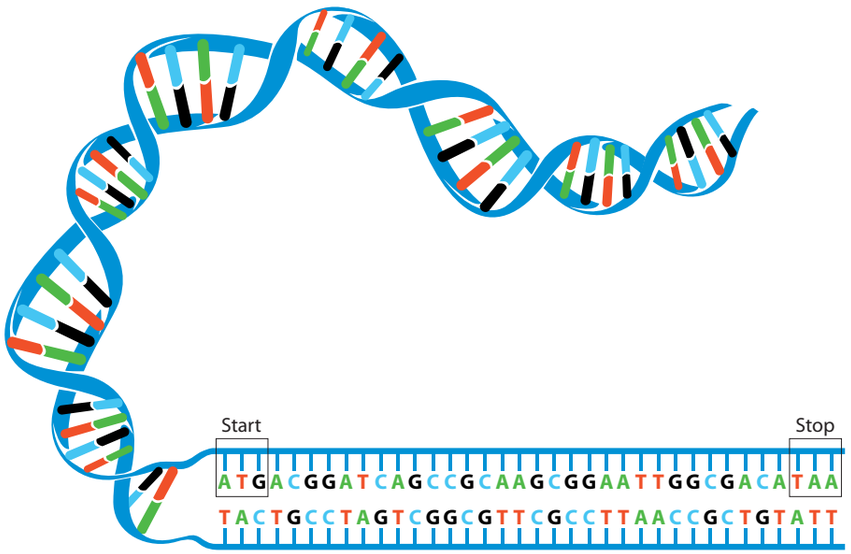


Figure 5 – Clonal selection: Each B cell has antibody receptors corresponding to a single type of antigen. When the B cells encounters and binds to the antigen it recognizes (clonal selection), it triggers the B cell to multiply, creating many clones of itself. This new army of clones rapidly releases an arsenal of specific antibodies. *Figure designed by Corporate Press.*

binding leads to greater expansion of the clones of cells producing this antibody. A repetition of this scenario over many successive cell divisions ultimately causes an explosion in the number of cells producing the highest antigen-binding ability (antibody affinity). This could explain why antibodies to a specific toxin or microbe dramatically increase in the blood after the foreign invader enters the body, and within a short period of time evolve such that they can latch on more strongly to antigens, thereby triggering the foreigner's destruction by other components of the immune system. This process is called antibody affinity maturation.

Support for the natural selection theory first came in 1958, when Nobel Prize-winning molecular biologist Joshua Lederberg and his colleague Gus Nossal showed in rabbits that each B cell can produce only one specific antibody. More evidence for the now well-accepted natural selection theory has accumulated over the decades. Both Burnet and Jerne won Nobel Prizes for their creative vision of how antibody production is generated and improved over time.

But as often is the case in basic scientific research, when one puzzle is solved, another one arises. The chemical explorations undertaken in the first half of the 20th century explained antibody structure and even pointed to the regions of the antibody responsible for its ability to bind to the many different compounds



TTT Phenylalanine TTC TTA Leucine TTG	TCT TCC TCA Serine TCG	TAT Tyrosine TAC TAA Terminator TAG	TGT Cysteine TGC TGA Terminator TGG Tryptophan
CTT CTC Leucine CTA CTG	CCT CCC Proline CCA CCG	CAT Histidine CAC CAA Glutamine CAG	CGT CGC Arginine CGA CGG
ATT Isoleucine ATC ATA Methionine ATG (Initiator)	ACT ACC Threonine ACA ACG	AAT Asparagine AAC AAA Lysine AAG	AGT Serine AGC AGA Arginine AGG
GTT GTC Valine GTA GTG	GCT GCC Alanine GCA GCG	GAT Aspartic GAC GAA Glutamic GAG	GGT GGC Glycine GGA GGG

Three "letter" combinations encoded in DNA correspond to different amino acids, the building blocks of protein.

Figure 6 – Protein is made of sequences of amino acids encoded in DNA: Three letter sequences of nucleotides or bases (the building blocks of DNA) correspond to particular amino acids (the building blocks of protein). This is how DNA serves as a genetic blueprint for the building of proteins by cellular machinery. See Breakthroughs in Bioscience article "Genetic Research: Mining for Medical Treasures" for more detail on how DNA is translated into protein. *Figure designed by Corporate Press.*

that the body encounters. And biologists had offered a plausible explanation for how antibodies are made in response to a bodily intrusion by an antigen. But both sets of advances created a new puzzle: how could the body genetically code for the millions or more antibodies it needed to have on hand to defend itself from potential intruders?

As the biochemists had shown, an antibody is composed of several protein chains each comprised of amino acids. Genetic studies done in bacteria at that time suggested that all proteins

are made by cellular machinery using a continuous section of DNA as a pattern or blueprint. This pattern specifies the order of the amino acids in a corresponding protein by using an instruction code made from DNA compounds called bases, which are abbreviated A,T, C or G. The DNA sequence TAC, for example, codes for the amino acid tyrosine, while AAC codes for leucine (Figure 6). Just as a string of words spell out a complete sentence, it was assumed that a contiguous sequence of DNA base triplets spelled out a complete protein. It was also assumed that every gene produced only a single protein.

But studies then suggested humans only had about 100,000 genes in their DNA. (More recent estimates are closer to 30,000.) How could 100,000 genes create millions of antibodies, in addition to providing all the proteins needed to build a body and make it function properly? This paradox was said to be one of the most vexing problems in biology at the time, and it was while trying to solve this new puzzle, that monoclonal antibodies serendipitously arrived on the scene.

Mystery of diversity

Two competing theories were formulated to explain this paradox in immunology. One theory was that within each sperm and egg, and all the resulting cells of an embryo and then the adult, was an enormous number of genes needed to make millions of antibodies. Most experts familiar with modern genetics thought this was unlikely, as the known number of genes were estimated to be far fewer than the number of antibodies a person was thought able to produce. Another theory was that the genes in B cells changed (mutated) during development to form the many antibodies. But if this were the case, why would the portion of an antibody gene that coded for the constant region in the stem of the Y stay virtually the same in all antibodies, while the part of the same gene that coded for the variable portions in the ends of the V mutate wildly?

An out-of-the-box theory was needed to explain this conundrum: in the 1960's, William Dreyer and Claude Bennett of the California Institute of Technology put forth their two-gene, one antibody hypothesis. They suggested that one gene for the constant region of the antibody is somehow combined with one of hundreds to thousands of possible genes coding for the variable region to form a specific antibody. But this radical theory, which called for split genes, was scoffed at by many in the scientific community who held to the dogma of one contiguous gene equals one protein, apparently not considering that what happens in bacteria may not also happen in mammals.

At this point, a Japanese molecular biologist Susumu Tonegawa entered the fray (Figure 7). He also was skeptical of the Dreyer-Bennett theory, but realized that the power of the new genetic tools that enabled researchers to sequence genes (spell out their sequence of A,C,T, and G bases) and pinpoint their location on a DNA strand could shed some light on this heated debate. Like Edelman, Tonegawa's work was aided by the use of single-specificity antibodies generated by myeloma tumors. These tumors

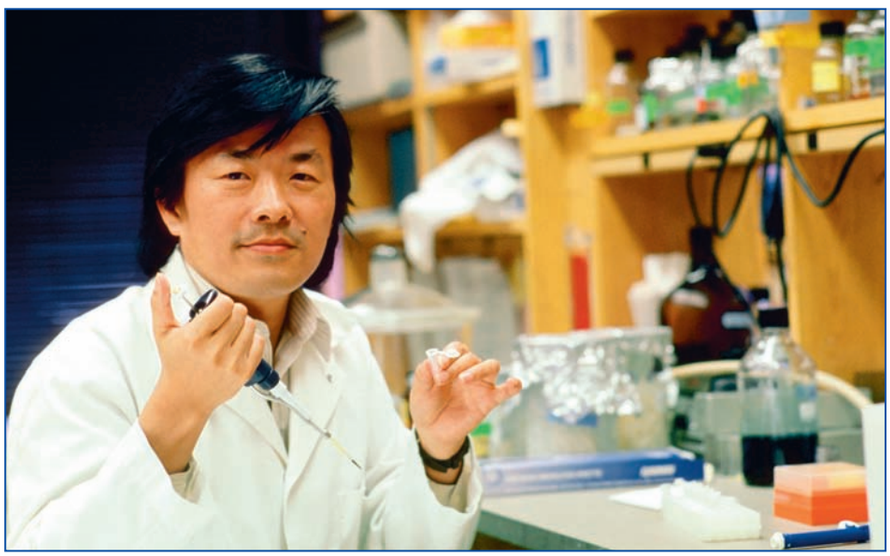


Figure 7 – Susumu Tonegawa: Susumu Tonegawa, Director of the Picower Center for Learning and Memory at M.I.T., won the 1987 Nobel Prize for his discovery of the genetic origin of antibody diversity. *Credit: Allen Green / Science Photo Library.*

came from Michael Potter of the National Cancer Institute, who had an array of experimentally induced mouse myelomas that he used to study the genetic triggers of these cancers.

In 1976, while working at the Basel Institute for Immunology (BII) in Switzerland, Tonegawa was pleasantly surprised to discover that, in mice, the portion of the DNA that encodes the constant-region stem of Y-shaped antibodies is indeed separated from the DNA that encodes the variable upper V section, just like Dreyer and Bennett proposed. This meant one continuous stretch of DNA could not produce an antibody. He also showed that these two sections of DNA were widely separated on a chromosome in mouse embryos but arranged close together in mouse antibody-producing tumor cells, suggesting that some genetic shuffling had occurred. And in between the constant and variable coding sections was a new unexpected section, which he named J for joining section.

Subsequent studies by Tonegawa at the BII and later at the Massachusetts Institute of Technology, as well as those of Leroy Hood of California Institute of Technology and colleagues, revealed that during the development of a B cell, several different sections of DNA--what one researcher describes as a "kit of components"--are stitched together to form the pattern for the assembly of a specific antibody, with intervening DNA sequences lost in the process.

With this set up, there are tremendous avenues for antibody diversity:

- There are several to hundreds of DNA sequence options for each section of coding DNA that is spliced together, which multiplies the possible combinations of amino acids determining antibody structure considerably;
- Where these sections are joined can also vary enough to affect the structure of the resulting antibody;
- The B cell's genetic machinery makes mistakes in the splicing process, especially in the joining regions. These mistakes can also affect the structure of the antibody that forms from the pattern; and
- The main chains that comprise an antibody are made separately from different patterns, making even more combinations.

The end result is that millions of combinations are possible, with each combination generating its own unique antibody (Figure 8). As Potter put it, "Mother Nature pulled out all the stops" to generate antibody diversity. At last researchers had hit upon an explanation for how millions of antibodies could form from a small kit of genetic components. Tonegawa received the Nobel Prize for Physiology or Medicine in 1987 for his elegant work.

A major advocate for the notion that genetic changes in antibody cells enable both antibody diversity and affinity maturation was Argentinean biochemist Cesar Milstein of the publically supported Medical Research Council (MRC) in London (Figure 9). It was while trying to provide proof for this theory, that he and German immunologist Georges Kohler created compounds that would revolutionize medicine.

Failure that leads to success

Milstein wanted to analyze the rate and nature of mutations of antibody-producing cells to see how the mutations affected an antibody's ability to bind to antigen. For his studies, he used Potter's mouse myeloma tumors to make cell cultures he fondly tended like they were his children, one fellow researcher noted. But his experiments led to a frustrating dead end. Contrary to Milstein's expectations, using these cultures he wasn't able to show high mutation rates in the variable regions of the antibodies these cells produced using these culture. This failure mainly stemmed from the fact that his myeloma cell cultures, which had the longevity needed for laboratory studies, produced abnormal antibodies that were rather wimpy when it came to binding with antigen.

Meanwhile, in Switzerland, Kohler was having the opposite problem, while also trying to validate the important role mutation played in antibody structure. Kohler was working with cultured B cells, which had great antigen-binding capacity but quickly died out, foiling

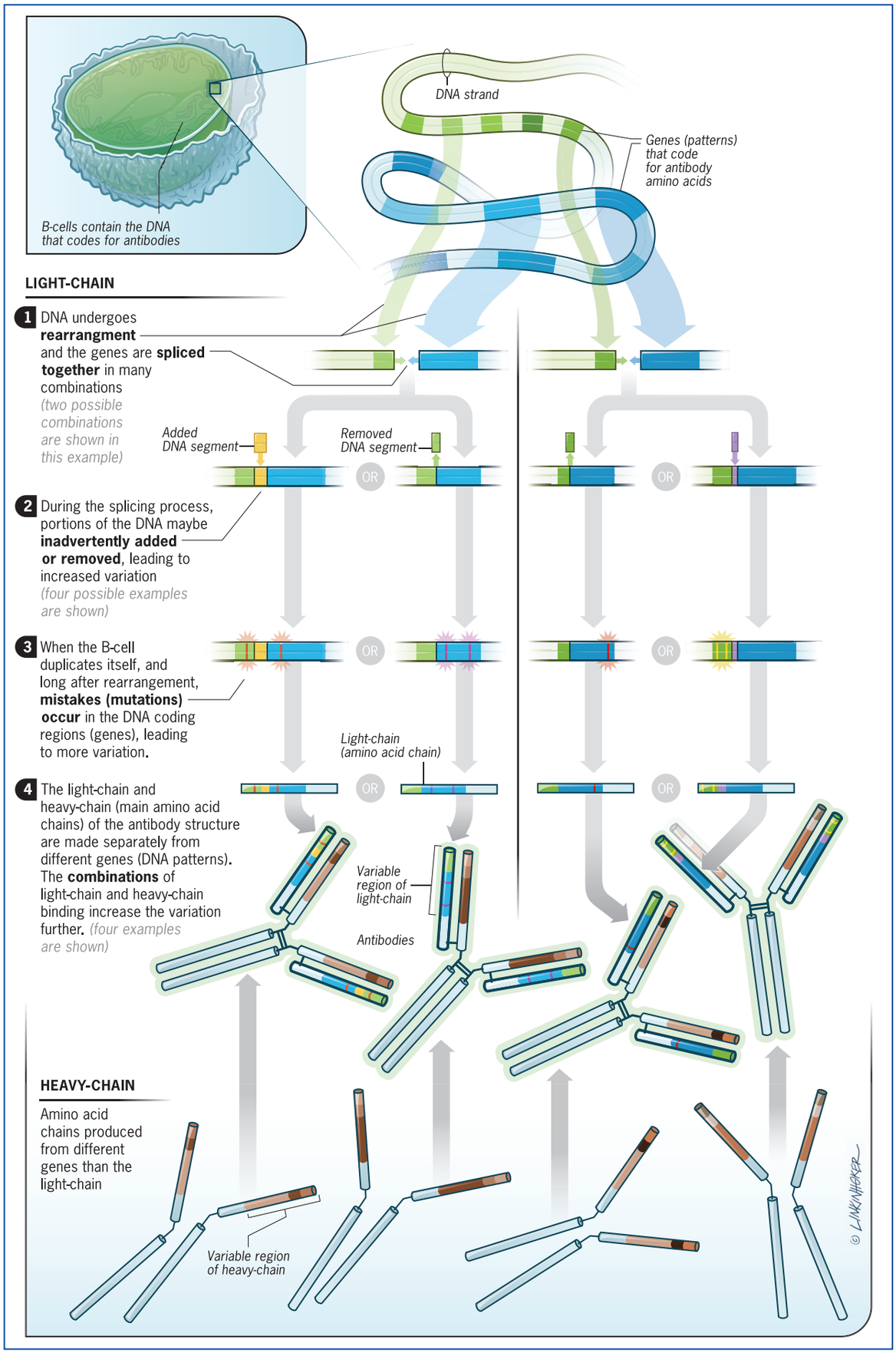


Figure 8 – Avenues for antibody diversity: This figure illustrates four different ways in which diversity of antibodies occurs, allowing the body to react specifically to innumerable antigens. *Designed by Michael Linkinhoker, Link Studio, LLC.*

Figure 9 – Cesar Milstein (1927-2002): While studying mechanisms for how antibody diversity is generated, Argentinean scientist Milstein pioneered the fusion technique for producing hybridomas and monoclonal antibodies. For his work, he won a 1984 Nobel Prize. *Credit: Dr. Rob Stepney / Science Photo Library.*

his experiments. After hearing Milstein give a talk about his research, Kohler came to work in his lab. The two realized that by combining the longevity of myeloma cells with the antigen-binding capacity of antibodies made by normal B cells, they could have more fruitful explorations of how B cells generate antibodies that tightly bind to a wide variety of antigens.

At the time, a technique for fusing cells had come onto the scientific scene. The two researchers used this technique to fuse antibody-producing B cells from mouse spleen to mouse myeloma cells. The mice were previously injected with a specific antigen, so they would produce large amounts of antibodies directed against the antigen. A special culture medium developed by Jerne was then used to select those fused cells producing the antibody to the injected antigen. Much to their surprise and pleasure, after years of failed experiments, the cell fusion worked! With this technique they could abundantly produce pure (monoclonal) antibodies that only bound to the specific antigen, with which they injected the animals (Figure 10).

With his monoclonal antibodies in hand, Milstein put a research student to work using them to detect mutation rates in antibody producing B cells. But those experiments failed and as Milstein was fond of saying, "If we didn't get what we wanted, we had to learn to love what we got." He put his antibody diversity experiments aside to explore the usefulness of monoclonal antibodies in other areas of basic research, and medicine. He recognized, along with Kohler, that the specificity and large production capacity of monoclonal antibodies would make them useful probes. They could be combined with a fluorescent dye or other signaling molecules to detect specific molecules of interest, such as microbial or cancer cell antigens, or hormones. Hundreds of compounds in the body that had previously eluded researchers' attempts to find and measure them suddenly became detectable and quantifiable. Scientists also discovered many new compounds by making monoclonal antibodies to them.

In collaboration with a number of other scientists, Milstein showed the usefulness of monoclonal antibodies in distinguishing blood and tissue types needed for organ transplants and blood transfusions. The researchers also used the antibodies to better diagnose leukemia, more quickly diagnose pregnancy, which led to home pregnancy testing kits, and to purify and concentrate the infection-fighting compound interferon, which is found in extremely minute concentrations in the body.

Milstein also realized that monoclonal antibodies have the capacity to uncover previously unknown cellular components, and by putting them to use in this way they tremendously accelerated the pace of basic research in immunology and other fields (much more so than Milstein's previous experiments did!) For example, different types of white blood cells have different

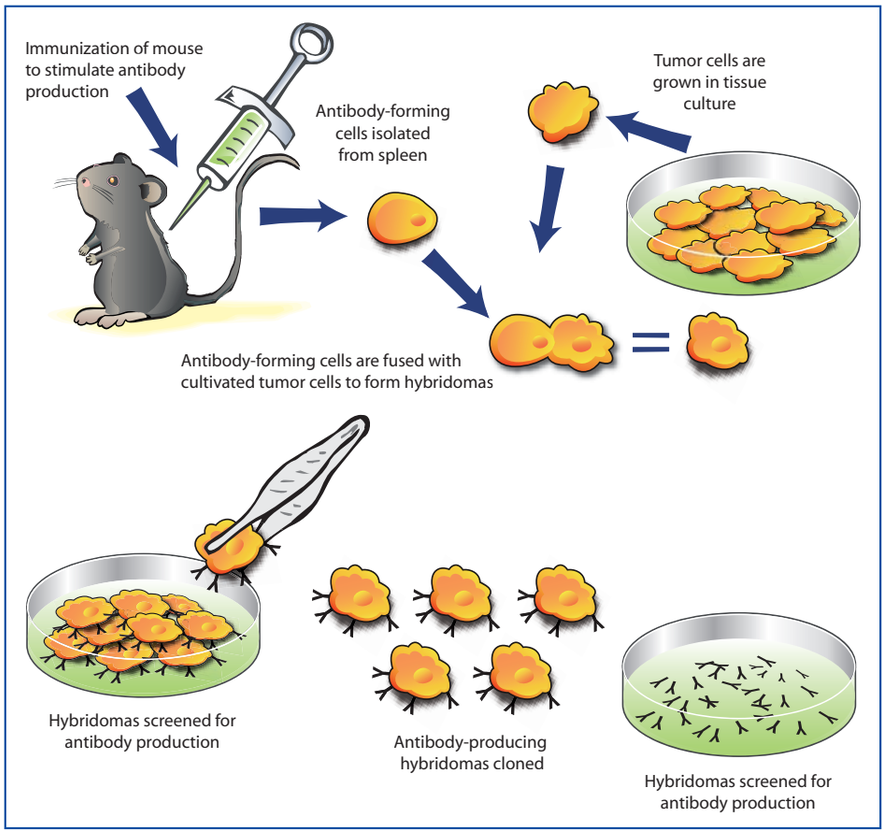


Figure 10 – Monoclonal antibody production: To produce monoclonal antibodies, first a mouse is immunized (injected) with the antigen of interest to begin production of antibodies against that antigen. The antibody producing cells are then isolated from the mouse's spleen. Meanwhile, tumor cells known as myelomas are grown in culture and then fused with the isolated spleen cells to form a new type of cell called a hybridoma. Hybridoma cells are then grown in culture and then tested individually to see if they are producing antibodies against the antigen of interest. These hybridomas are then cloned and cultured, becoming miniature factories for pure, monoclonal antibodies, which can be isolated. *Figure designed by Corporate Press.*

antigens on the surface of their cell membranes, and these telltale markers change as the cells mature and carry out different key functions. But pinpointing these cell membrane proteins had been challenging, in part because they are present in cells in minuscule amounts.

When mice are injected with whole cells, however, their B cells produce antibodies to all cellular components, no matter how rare, including the distinctive cell membrane antigens. By fusing these B cells to myeloma cells and linking them to a detectable marker, researchers used the resulting monoclonal antibodies to probe cells for previously undiscovered surface antigens. This use of Milstein and Kohler's monoclonal antibodies set off an exciting explosion of research that led to a better understanding of the different white blood cell types in the immune system and how they functioned.

One practical outcome of this field was the development of the first monoclonal antibody drug for cancer. This drug got its start when a young oncologist hoping to cure non-Hodgkin's lymphoma teamed up with an unlikely partner—a former classmate who was trying to make a blockbuster mouthwash to prevent tooth decay.

Monoclonal antibodies that target cancer

Lee Nadler was working on a clinical fellowship at the Sidney Farber Cancer Institute, (which later became the Dana Farber Cancer Center), when he heard of Milstein and Kohler's monoclonal antibodies and thought they would be the perfect tool to discover the B cell types that give rise to various immune system cancers called non-Hodgkin's lymphoma. At this point, basic research had revealed that most of these cancers get their start when genetic flaws crop up during the duplication of B cells. These flaws stop the cell's development so it never matures and keeps dividing, wreaking havoc in the body due to its uncontrolled growth.

Nadler thought that he could use monoclonal antibodies as basic science tools to better know his enemy—to define in what stage of development lymphoma tumor cells were in. Armed with this information, he would then be able to distinguish the tumors for better diagnosis and treatment. By knowing what cell markers these tumor cells had, he reasoned he could also develop a monoclonal antibody therapy that only targeted the tumor cells, while sparing most other white blood cells needed to fight infections.

At the time, Nadler's friend Phil Stashenko, who had a Ph.D. in immunology as well as a dental degree, was unsuccessfully trying to make monoclonal antibodies against the bacteria that cause tooth decay with the aim of creating a mouthwash that could prevent cavities. Nadler convinced Stashenko, then at the Harvard Dental School, to drop his project and help him make monoclonal antibodies that could uncover new cell markers for B cells.

The two researchers made remarkable progress in a short period of time. Within a few years after Milstein and Kohler first reported they had concocted monoclonal antibodies, Nadler and Stashenko and colleagues made monoclonal antibodies in their own lab, and then used them to discover a B cell antigen that was produced by B cells in most non-Hodgkin's lymphomas. (Eventually, all four known human B cell-specific antigens were discovered in Nadler's laboratory.) Nadler then became the first in the world to test a monoclonal antibody on a patient, when in 1979 he gave a monoclonal antibody that targeted a B cell antigen to a 54-year old man who had advanced lymphoma unresponsive to other treatments.

The treatment was a failure.

Although the patient did not develop any severe reactions to the treatment, it did not improve his condition. Blood and tissue samples revealed that there was minimal binding of the monoclonal antibody to tumor tissue, and that the researchers were foiled by the same immune system they hoped to redirect to the tumor! The experimental drug was made completely from mouse cells that were perceived as foreigners worthy of an antibody attack. This attack by the patient's own antibodies blocked the monoclonal antibody's actions, and primed it for destruction before reaching its tumor target. In addition, the weak binding of the mouse monoclonal antibody didn't seem to trigger an important immune defense, called the complement system, which normally would have led to the destruction of antibody-targeted tumor cells.

Figure 11 – Rituximab: Rituximab, which is marketed under the names Rituxan and MabThera, is a monoclonal antibody used to treat non-Hodgkin's lymphoma and B cell leukemia. It is currently being studied for its use in treating some autoimmune diseases, to help prevent the rejection of transplanted organs, and also to treat rheumatoid arthritis. *Credit: Dr. P Marazzi / Science Photo Library.*

This finding led Darrell Anderson and his colleagues at IDEC Pharmaceuticals Corporation in San Diego to use recombinant DNA techniques to develop a monoclonal antibody that combines a mouse (antigen-binding) V portion of the antibody with a human constant stem portion. That way the antibody is able to activate the human complement system directed at the tumor cells, yet still bind to the appropriate antigen. They called their part mouse and part human monoclonal antibody rituximab (Rituxan) (Figure 11).

Magic bullets, at last

Rituximab passed its clinical tests on patients with non-Hodgkin's lymphoma with flying colors. Initial results showed the drug shrank the tumors in half of patients with advanced disease that no longer responded to standard chemotherapy. Even better results occurred when the drug was combined with standard chemotherapy and used in younger patients with less advanced disease. In these patients, rituximab boosted the rate of complete remissions by more than 25 percent. After 3 years, 79 percent of patients who received rituximab were alive and free of any signs of the cancer, compared to 59 percent of the chemotherapy-only patients, one study found.

Rituximab targets B cells in a stage of development other than that of those actively secreting antibodies, and the drug doesn't latch onto stem cells that give rise to infection-fighting cells. So the drug does not increase risk of infection as much as standard chemotherapy. The agent does, however, kill normal B cells as well as tumor cells, a feature that has led to its application to treat certain autoimmune diseases (see below). It also has fewer and less severe side effects than traditional cancer drugs that are toxic to any dividing cells, including those that line the stomach and hair follicles, and bone marrow cells that produce the blood cells. This causes nausea and hair loss and decreased blood cell counts. By contrast, the most common side effects from rituximab are flu-like malaise, shortness of breath and/or a drop in blood pressure. These can usually be relieved by reducing the infusion rate, and are almost always limited to the first course of therapy.

Rituximab first entered the market in 1997 as a lymphoma treatment, and its success led to an explosion of therapeutic monoclonal antibodies that researchers developed and tested in the clinic (Table 1). Even rituximab was tested further to see what effects it might have on autoimmune disorders, which foster destruction of normal tissues, often due to overactive B cells. The Food and Drug Administration currently has approved the use of rituximab in the treatment of rheumatoid arthritis patients not relieved by standard therapy. The drug has also shown promise in the treatment of psoriasis, type 1 diabetes, multiple sclerosis, autoimmune thrombocytopenia, and is often used to prevent immune rejection after transplant.

Rather than relying on monoclonal antibodies to trigger the immune system to destroy tumor cells, some researchers took a more aggressive approach. These investigators linked monoclonal antibodies that target the B cell marker found in most lymphoma cells to a radioactive chemical, so that the monoclonal antibody can mediate tumor destruction directly without the help of complement by delivering its deadly radioactive baggage to malignant tissues. The drug ibritumomab (Zevalin) is made in this manner, and researchers have shown it shrinks tumors in more than three-quarters of patients who receive it along with rituximab, with some responses lasting more than five years. This drug came on the market in 2002.

The Pharmaceutical Research and Manufacturers of America reported, in 2006, that U.S. companies had 160 different monoclonal antibodies in testing or awaiting regulatory approval to enter the market. Drug companies find they can bring new drugs made with monoclonal antibody technology to the market much quicker than those using traditional techniques, thus explaining why many of the drugs that entered the market in the last decade have been monoclonal antibody drugs. These include such widely used medicines as trastuzumab (Herceptin) for breast cancer, bevacizumab (Avastin) for colorectal, breast, brain, and lung tumors, infliximab (Remicade) for rheumatoid arthritis and inflammatory bowel disease, and abciximab (Reopro), which is used to prevent blood clots. Monoclonal antibodies are also showing promise for shepherding various treatments into the brain, which currently is impenetrable to many drugs.

One expert estimated that it costs only two million dollars to develop a monoclonal antibody for clinical testing, versus twenty million for that of a traditional drug. Because monoclonal antibodies tend to be less toxic, they are also more likely than traditional drugs to clear regulatory hurdles and enter the market. However, like nearly all drugs, monoclonal antibody drugs do cause some side effects in some people. But these drug reactions are rarely serious enough that they limit the use of monoclonal antibody drugs. For example, by disabling an immune defense that is overactive in some disorders, some monoclonal antibody drugs have caused some serious infections in some people. Heightened vigilance for infection can often prevent this complication. Some side effects of monoclonal antibody drugs are thought to be due to the body's reaction to their mouse components. Aimed at preventing these reactions, researchers continue to improve monoclonal antibodies by making nearly or completely humanized versions.

But it is not just monoclonal antibody technology that is leading to medical advances. Thanks to government-funded research, there have also been stunning advances in basic biology research that have uncovered the key compounds these monoclonal antibody drugs target. For example, without understanding that a compound made by the immune system called tumor necrosis factor (TNF) is the kingpin of the debilitating inflammation seen in rheumatoid arthritis, there would be no infliximab, which is comprised of monoclonal antibodies that target TNF. Some physicians view infliximab as a miracle drug for their patients' rheumatoid arthritis because, unlike other treatments, it provides significant relief of symptoms in more than three-quarters of patients who take it, with some people reporting symptom relief within just hours of receiving the medicine. Many of these patients no longer respond to other rheumatoid arthritis drugs. Infliximab has also proven to be a highly effective treatment for inflammatory bowel disease, as well as for psoriasis, and is being tested as a treatment for other autoimmune disorders.

Similarly, basic research that uncovered that the growth of certain types of breast cancer are fueled by a specific growth factor led to the monoclonal antibody drug trastuzumab, which targets that growth factor's receptor on tumor cells. In the quarter of the women with breast cancer who have heightened activity of this receptor in their tumors, trastuzumab has proven remarkably effective at preventing disease recurrence, when combined with effective chemotherapy.

There has also been a productive play between monoclonal antibody diagnostics and therapeutics. Monoclonal antibody probes have fostered a better molecular understanding of a number of disorders that, in turn, has led to the development of monoclonal antibodies to treat the panoply of disorders from allergies and asthma to diabetes, Alzheimer's disease, and cardiovascular disorders. And of course if it wasn't for the basic research on antibodies that had been pursued by chemists, immunologists, virologists and other investigators around the world, monoclonal antibodies wouldn't be here today. Cesar Milstein humbly sums up what led to these magic bullets in his Nobel lecture:

Therapy	Disease / Condition Treated
Infliximab *Brand name: Remicade*	• Rheumatoid arthritis • Chrohn's disease
Rit uximab *Brand names: Rituxan and MabThera*	• Non-Hodgkin's lymphoma
Etanercept *Brand name: Enbrel*	• Rheumatoid arthritis
Abciximab *Brand name: Reopro*	• Prevents blood clots in coronary angioplasty
Trastuzumab *Brand name: Herceptin*	• Specific kind of breast cancer
Bevacizumab *Brand name: Avastin*	• Several types of cancer
Basiliximab *Brand name: Simulect*	• Rejection of kidney transplants
Paliviziumab *Brand name: Synagis*	• Respiratory syncitial virus (RSV) infections in children
Alemtuzuab *Brand name: Campath*	• B cell leukemia

Table 1— Some examples of monoclonal antibody therapies currently in use and the conditions treated

"The hybridoma [monoclonal antibody] technology was a by-product of basic research. Its success in practical applications is to a large extent the result of unexpected and unpredictable properties of the method. It thus represents another clear-cut example of the enormous practical impact of an investment in research which might not have been considered commercially worthwhile, or of immediate medical relevance. It resulted from esoteric speculations, for curiosity's sake, only motivated by a desire to understand nature."

Interestingly, Milstein's research came full circle by the end of his life, having gone from basic to applied research, and then back to basic research. Remember his previously failed use of monoclonal antibodies to detect the mutations that occur in antibodies as they evolve in response to the presence of an antigen? With better techniques, Milstein eventually was able to use his monoclonal antibodies to show this evolution does occur in the antigen-binding regions of the antibodies, and he reported his results in a paper he submitted just a week before he died in 2002. So not only did Milstein create monoclonal antibodies, but he helped show how "Nature guides the internal disinfection," as Behring put it. His marriage of basic to applied research led to revolutionary new approaches in both biology and medicine—magic bullets in shots that were heard around the world.

The Breakthroughs in Bioscience series is a collection of illustrated articles that explain recent developments in basic biomedical research and how they are important to society. Electronic versions of the articles are available in html and pdf format at the Breakthroughs in Bioscience website at:

www.faseb.org

Biographies

Margie Patlak writes about biomedical research and health from the Philadelphia region. She has written for Discover, Glamour, Physician's Weekly, Consumer Reports on Health, The Washington Post, Los Angeles Times, Dallas Morning News and numerous other publications. She also writes frequently for the National Institutes of Health and the National Academy of Sciences. This is her sixth article in the Breakthroughs in Bioscience series.

Dan L. Longo, Ph.D. is Scientific Director at the National Institute of Aging at the National Institutes of Health (NIH) and a Senior Investigator in the Lymphocyte Cell Biology unit, where his research interests encompass the regulation of growth fraction in tumor cells, tumor-induced immunosuppression, and clonal selection of B-cells, among other topics. After completing medical school at the University of Missouri, Columbia and internal medicine training at the Peter Bent Brigham Hospital and Harvard Medical School in Boston, he obtained fellowship and laboratory training at NIH and has been there for 26 years. Before becoming Scientific Director, NIA in 1995, Dr. Longo was the Director, Biological Response Modifiers Program, and Associate Director, Division of Cancer Treatment, National Cancer Institute, Frederick, Maryland. He is the author of over 600 articles and book chapters. He is an editor of Harrison's Principles of Internal Medicine, and Cancer Chemotherapy and Biotherapy. He is an associate editor of Journal of the National Cancer Institute and Clinical Cancer Research and he sits on the editorial boards of six other peer-review journals. He has been cited by Good Housekeeping as one of the "Best Cancer Doctors in America" and listed in Best Doctors in America.

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