Finding Chinks in the Viral Armor: Influenza, AIDS and Antiviral Therapies

1 Natural Weapons of Mass Destruction

2 Beating Back the Enemy: Early Successes

Virus: “A piece of nucleic acid surrounded by bad news”

2 Identifying Targets for Antiviral Drugs

4 Not-so-good News About Antiviral Drugs

7 Fast Track: Basic Research Pays Off

8 Winner of the Race to Discover AIDS Virus? Patients

10 Using the Viral Map

12 A Familiar Enemy: Avian Flu

14 Readying the Scientific Arsenal
Acknowledgments

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COVER IMAGE: New concerns over a global pandemic of avian influenza, similar to that occurring in 1918 are leading to preventative elimination of poultry stocks in Asia. But thanks to scientists studying the fundamentals of how viruses work and how they are structured we now have the tools to combat viruses that cause influenza, polio, AIDS and other viral diseases. Decades of basic research in virology, biochemistry and molecular biology have identified targets for anti-viral drugs, a bench to bedside story which is impacting millions of lives worldwide. Cover photo by Reuters, soldiers photo courtesy of the National Museum of Health and Medicine, Armed Forces Institute of Pathology, and flu virus courtesy of National Institute of Allergies & Infectious Disease, National Institutes of Health, used with permission.
Finding Chinks in the Viral Armor:
Influenza, AIDS and Antiviral Therapies

Sylvia Wrobel, Ph.D.

Viruses just want to make more of themselves—and antibiotics can do nothing to stop them. But once scientists learned how different viruses are built and how they replicate, they had the tools they needed to build antiviral drugs to jam the process.

Natural Weapons of Mass Destruction

In 1918, as the war people hoped would end all wars was coming to a close, another enemy raised its head. World War I killed over 8.5 million people. The great influenza pandemic—a global epidemic—may have killed 50 million or more, an unknowable figure since most countries then didn’t have a way to keep such records and no laboratory test existed to determine if a person actually had the flu.

No vaccines, no antiviral treatments existed at that time. How could they? Scientists had not yet learned what caused the flu. Even the concept of viruses was vague, since no one had ever seen one. Without understanding the mutable, shape-shifter nature of the agent responsible for flu, no one knew why that particular flu was so lethal. In ordinary flu epidemics, the people most likely to die were the most fragile, the very young, or very old. The 1918 pandemic felled healthy people between 20 and 40, those with the strongest immune systems. More than half the deaths occurred within a 10-week period, between late September and early December. In the United States, life expectancy fell from 51 to 39 years almost overnight.

Today, scientists are watching to see if this old enemy is back, dressed in new clothes. Since first appearing in China in 1997, an avian flu virus has spread across Asia and steadily mutated, moving from wild birds to domesticated ones, and gaining the ability to infect other species. Between 2003 and 2005 alone, more than 100 humans are known to have contracted it and more than half have died. At present, most infections appear to be the result of direct exposure to infected birds. The virus may go away or disappear from the population, as viruses sometimes do. But if it continues to evolve, becoming efficient at human-to-human transmission like the ordinary flu viruses, the new virus will be unrecognizable by anyone’s immune system and humankind will be in for a difficult battle. The World Health Organization and developed nations, including the United States, have begun preparing for the worst-case scenario, stockpiling antiviral drugs in the hope of containing, or at least slowing, any pandemic that might emerge until scientists would have time to take the still evolving virus’s measure and develop a vaccine to fit it.

But if this and other viruses are evolving rapidly, so is science. In 1918, all that was known about viruses was that they must exist, since something that could slip unseen through the finest

Figure 1: 1918 Influenza Pandemic Affects WWI Soldiers in France: When the 1918 influenza pandemic swept the globe, killing millions and sickening many more, scientists knew very little about the virus causing the disease. Recent research indicates that the 1918 virus, which selectively felled health people between the ages of 20 and 40, was of avian origin (See sidebar “Digging for answers about the 1918 influenza virus”). Photo courtesy of the National Museum of Health & Medicine, Armed Forces Institute of Pathology.
filters was able to cause yellow fever. Virus is Latin for “poisonous slime.”

It would be 15 more years before scientists isolated the flu virus and, thanks to the invention of the electron microscope, actually got a look at this formerly invisible pathogen. And as they discovered how viruses are structured and replicate, they found some chinks in their viral armor.

**Beating Back the Enemy: Early Successes**

While antibiotics like penicillin appeared to be winning the battle against bacterial diseases, the early successes against viruses involved preventing infection, not treating it. Vaccination preceded the discovery of viruses. In the late 18th century, having noticed that milkmaids seldom had the pock-marked skin that marked smallpox patients, English country doctor Edward Jenner daringly inoculated a young boy with cowpox, a related but milder virus—then later inoculated him with fluid taken from a smallpox lesion. The child remained well. Such an experiment would never be permitted today, but the boy’s apparent immunity led to the inoculation of thousands worldwide. Two centuries later, armed with better understanding of both viruses and the immune system, scientists began to design highly specific vaccines with remarkable results.

In 1957, smallpox—one of the greatest killers in history—was rampant in 30 countries, causing 2 million deaths annually and terrible scarring, blindness, and disability for millions more. After a new vaccine was developed, an unprecedented global cooperation delivered 250 million vaccine dosages per year to reach all people at risk. Civil wars paused so people could be vaccinated. By 1977, smallpox apparently had vanished, except for highly-guarded containers in the U.S. Centers for Disease Control and Prevention and a laboratory in Russia.

Polio was another scourge against which new vaccines proved highly successful. Polio was the first virus that scientists were able to grow in tissue culture, so it could be harvested, purified, and crystallized, which allowed visualization of its molecular structure using electron microscopy, which was then a new technology. John Enders and two colleagues won the Nobel Prize for this achievement, which led directly to the engineering of first the Salk and then the Sabin vaccines. These vaccines eliminated—at least in the developed world—polio and its legacy of iron lungs and wheelchairs.

Such advances, together with unprecedented successes in treating bacterial diseases, led the U.S. Surgeon General in the late 1960s to declare that the war against infectious diseases had been won. It was time, he said in one speech after another, to turn attention and resources to conquering cancer and heart disease. Scientists, the very victors being cheered, were less sanguine. By this time they knew enough about viruses to know that their work had only just begun.

**Virus: “a piece of nucleic acid surrounded by bad news.”**

One of the first things scientists noted, once they were able to actually see viruses, was how small they were: the smallest bacteria are bigger than the biggest viruses. A billion viruses easily fit in a drop of blood. Viruses differ greatly in structure and shape: rods, spheres, smooth, spiky. But the real surprise—the revelation that was to provide the targets for all antiviral drugs—was how they multiply and reproduce, a process called replication.

Bacteria have a life of their own. They contain all the equipment...

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1Frequently quoted definition coined by Nobel Laureate, Peter Medawar

*Breakthroughs in Bioscience*

*Figure 2: Human Poliovirus: Color enhanced, molecular graphic of a polio virus. The outer protein coat (capsid) of the polio virus is roughly spherical. It consists of 12 repeated pentagonal units, each of which consists of four different proteins. The tiny spheres represent individual amino acids. The capsid is highly resistant to heat and acid, making the virus stable in such harsh environments as sewage systems and the human gut. Infection is by the fecal-oral route, and may lead to the paralyzing disease poliomyelitis. Image by Jean-Yves Sgro, images at virology.wisc.edu/virusworld.*
and materials necessary to reproduce. Viruses, on the other hand, are like zombies. On their own, they are virtually lifeless, unable to replicate. While both viruses and bacteria have the genetic blueprint needed to make more of themselves, viruses need the machinery available inside a host cell to produce more viruses. Once inside the right plant or animal cell (or the right bacteria) viruses spring into action. And action for a virus means only one thing: turning their hosts into a factory for the mass reproduction of more of itself.

Unlike bacteria, which are fully-equipped for self-replication, a virus is honed to the bare essentials: a core of nucleic acid containing either DNA (deoxyribonucleic acid) or RNA (ribonucleic acid), depending on the type of virus, and a protective protein coat. Some viruses also contain a few enzymes, and some have an extra fat layer for added protection. That’s it. Since viruses don’t possess any of the machinery needed to make more of themselves, they must invade another cell, inject the virus’s own DNA or RNA, and take over the cellular machinery of the "host" cell.

Every virus has preferred target cells that it likes to invade. The AIDS virus looks for certain types of immune cells. Most strains of influenza virus infect various cells but only replicate in specialized cells of the lung that produce an enzyme needed for replication.

Before this invasion by the virus, the host cell was busy producing the proteins called for in the blueprint of its own genes. After take-over by the virus, the host cell is forced to produce the proteins called for in the virus’s genetic blueprint instead of those called for in the host cell’s own DNA. The result is a large number of viruses. When the exhausted hijacked cell is dead or dying, these new viruses break through the plasma membrane and look for another cell to infect. When they find it, they attach themselves to the cell’s surface, break through into the cell, and the cycle begins again.

And so the viral self-replication process continues, exponentially creating more viruses until one of several options happens. If the

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*In order to enter into the host cell, viruses actually hijack a protein present on the surface of the host cell and turn it into a specific receptor for the virus. These devilishly clever pathogens don’t need to sneak into a host, because they have a purloined copy of the key!*

**Breakthroughs in Bioscience**
patient is lucky, the body’s immune system wins out. If the virus overwhelms the body’s immune system, the virus kills the host. Some viruses, like the Herpesvirus, evolve to co-exist with human hosts, and enter a dormant or latent state, in which they live in relative harmony for the life of the host, although they may escape to do damage after a period of time. Or, if it’s one of the unlucky viruses for which science now has found an antiviral treatment, carefully selected chemical molecules halt one or more phases of the virus’s replication process.

Obviously, the best approach to interfering with viral replication begins as early as possible, through vaccination. The most effective vaccines often prevent infection so there is no disease and the virus never gains a foothold. Vaccines trick the vaccinated person’s immune system into “remembering” a virus’s antigens (the proteins on the surface of the virus) just as it would if the person had previously been infected with the disease. Then, should the virus present itself, the immune system can produce antibodies, which help to eliminate the virus, without delay.

In addition to smallpox and polio, vaccines are now effective against measles, yellow fever, rubella, mumps, and hepatitis A and B. That raises the question: why not AIDS? And why do we need a different flu vaccine every year?

The answer to both questions is that unlike smallpox, polio and other more stable viruses, viruses like HIV and influenza mutate constantly, thus becoming moving targets for vaccines and therapies. Furthermore, viruses like smallpox and polio affect only humans. Other viruses, such as the hantavirus and influenza, also affect animals, giving the virus a “reservoir” in which they can hide away and replicate, continuing to mutate, and later emerge to infect humans.

Vaccines against viral diseases remain the Holy Grail of virus control, but the challenges noted above—and the plight of people already infected for whom vaccination is too late—turned scientists’ attention toward the development of antiviral drugs.

Identifying Targets for Antiviral Drugs

The large herpes virus family includes herpes simplex 1 (cold sores), herpes simplex 2 (genital herpes), varicella-zoster (shingles and chickenpox), and Epstein-Barr (mononucleosis). The herpes simplex 2 virus causes painful, highly contagious sores on the genitals. As difficult as this is for all sufferers, the virus is most dangerous for those with weak immune systems, such as the elderly; newborns infected in the birth canal; and people whose diseases or medications lower their immune response.

Earlier compounds had been found to be active against specific herpes viruses, such as the one that causes the most common corneal infection in humans. Acyclovir was the first major antiviral drug to be developed, entering clinical trials in 1977 and becoming widely available in 1982. In the midst of an epidemic of genital herpes, Americans greeted the drug with enthusiastic headlines and gratitude. The discovery of acyclovir was built on a different scientific approach, one that won the 1988 Nobel Prize for Gertrude Elion and George Hitchings and heralded a new era of antiviral drug research.

What was different? Many early drugs in the history of medicine had developed out of folklore, like aspirin from willow bark; observation, like penicillin from mold; or random searches for active compounds. The new science of targeted antiviral therapy was built on understanding the fundamental, biochemical processes of viral replication and finding or designing medicines to interfere with that process without interfering with the fundamental, biochemical processes of normal, uninfected cells.

Living cells and viruses have one thing in common: the presence of nucleic acids. These molecules play a central role in the storage of genetic blueprints for replication and in the expression of this information through protein synthesis. The two primary forms of nucleic acids are DNA—the genetic blueprint—and RNA, which delivers the instructions coded in the DNA to the cell’s protein manufacturing sites.

Many scientific discoveries are serendipitous and come from
Advice from an Unlikely Nobel Laureate

Acyclovir was only one of the successes in Gertrude Elion’s scientific career. The “pathways” approach she and colleague George Hitchings brought to developing drugs—an approach that relied on finding out how cells used chemistry to produce DNA—began well before the structure of DNA had been determined. Over the next 50 years, Elion played a major role in the development of “magic bullet” drugs that were among the first successful treatments for acute leukemia, gout, rheumatoid arthritis, and prevention of rejection following kidney transplantation. The thrill of seeing people get well who might otherwise have died cannot be described in words, she often said. The Nobel Prize was only the icing on the cake.

Were it not for her tenacity, however, the world might have lost one of its most brilliant scientists. After taking advantage of Hunter College’s free tuition program to graduate Phi Beta Kappa, with a degree in chemistry, then earning a master’s degree at night, the young daughter of Lithuanian and Polish immigrants was eager to enter the fray against cancer, which had taken her beloved grandfather. The death of her fiancée from bacterial endocarditis, an inflammation of the heart, reinforced her belief in the importance of scientific discovery; he had fallen ill only two years later, penicillin would have been available to cure the infection. But, as she later recalled, “the world was not waiting for me.” It was an unexpected shock. Women simply weren’t laboratory chemists, and laboratory directors feared having the first one in their lab would be “a distracting influence.” After seven years of working as a secretary and high school teacher, her desirability changed when many male chemists marched off to World War II. Her earliest jobs in the chemical industry involved tasks like determining acidity of pickles, but eventually she arrived at Burroughs Wellcome, where Dr. George Hitchings was trying to attack a variety of diseases by interferring with DNA synthesis. She had never heard of some of the words he tossed off so excitedly. But Hitchings was a supportive mentor, encouraging her to take on more and more responsibility in both chemistry and the rapidly expanding fields of biochemistry, pharmacology, immunology and eventually virology. Within five years she had synthesized a chemical component that inhibited growth in mouse leukemia and was the forerunner of the drug 6-mercaptopurine, still effective against cancer today.

Elion continued for years to feel a sense of failure that constraints of time and money meant that she had been unable to finish a Ph.D. Later, especially after 45 patents and 23 honorary degrees, including three doctoral degrees, she began to consider it a “badge of honor.” While young women—or young men, for that matter—told her they wanted to go into science but could never do it, she laughed and told them that of course they could. A lifelong mentor to the young, she always encouraged them to find work they loved, keep their eye on the goal, and never let anyone discourage them, remembering the words of Admiral Farragut: Damn the torpedoes, full speed ahead.

research designed to answer other questions. Elion and Hitchings were looking for differences in nucleic acid metabolism between normal human cells and cancer cells, bacteria and viruses. Like so much of the work on which early antiviral successes would be built, Elion’s and Hitchings’ initial efforts were aimed at blocking the growth of cancer cells. But in the 1970s, the scientists saw something that they realized had implications for viruses. A good antiviral target should be something as specific to the virus as possible. They recognized that as part of its replication process, the herpes virus produces an enzyme slightly different from the host’s normal enzyme.

Acyclovir is a nucleoside analogue, meaning it mimics a building block of DNA that the virus needs to reproduce. Cells infected by the herpes virus absorb more of the drug compound than do uninfected cells. Once the compound is inside the cells, the viral enzymes mistakenly add it to the growing strand of DNA. It’s as if a person added plaster in a recipe calling for baking soda. The DNA no longer works. Replication halts.

The Not-so-good News About Antiviral Drugs

The principle that produced Acyclovir, in which scientists targeted activities specific to a virus, would soon also produce AZT, the first drug developed against AIDS. But if Acyclovir demonstrated the promise of antiviral drugs, it also foreshadowed their limits.

For one thing, the dosage of Acyclovir had to be carefully controlled. Although the enzyme that Acyclovir targets is different from the cell’s enzyme, the two are still quite similar. Too little of the antiviral drug compounds will not be effective against the viral
enzyme; too much will start damaging the cell’s own DNA, a cost benefit ratio that would have to be taken into account for this and every drug. (Acyclovir manages this extremely well, and has proved one of the safest antivirals yet developed.)

Acyclovir also demonstrated the fact that, since antiviral drugs must be fairly specific, they may not work on all forms of the virus. Acyclovir worked best on genital herpes but did not work as well on other forms of this virus.

And finally, Acyclovir was not a cure-all. Although antiviral drugs can reduce the amount of virus in the body up to 1,000 times, no antiviral drug yet developed is able to inhibit virus replication completely. Acyclovir markedly shortens and limits the number of herpes outbreaks, but the virus remains able to retreat into the nervous system, biding its time before reappearing. Nonetheless, knowing how to interfere with the replication process was an enormous breakthrough in having some control over a virus, and development of Acyclovir permitted the development of new, similar drugs to treat other diseases in the big herpes family. The new information and insights provided by this breakthrough would soon be desperately needed to fight a new enemy.

If they can put a man on the moon, why is it so hard to come up with a vaccine or cure for the common cold?

It’s hard to know the enemy. The common cold can be caused by rhinoviruses, coronaviruses, adenoviruses, coxsackie viruses, orthomyxoviruses, paramyxoviruses, respiratory syncytial viruses—about 200 different contenders, some with subtypes of their own, arising from subtle changes in the proteins on their surfaces. Without expensive, time-consuming testing, it is hard to know which virus to blame (or treat) for any individual cold. Vaccine development also is difficult for the same reason people don’t develop broad immunity to colds: antibodies developed to one cold virus may not recognize the next one.

Timing is difficult. Antiviral therapies are most effective before or during the peak of viral replication. By the time the first cold symptoms appear, this time has often passed. Fever, aches, sneezing, coughing, runny noses and watery eyes are only collateral damage caused by the immune system’s mobilization of white blood cells and antibodies to route the cold virus. Slowing antiviral multiplication would help the immune system get its job done more quickly.

Nonetheless, scientists are making progress in “cures” for the common cold, using approaches that have proved successful against HIV and other viruses. Drugs under investigation include those able to interfere with replication by preventing rhinoviruses from shedding their protective jacket and unloading their payload of genetic information and enzymes; protease inhibitors; and decoy receptors to trick the cold virus into binding to them instead of the ones on the human cell. But even the best drugs will come with limitations and questions. How much will patients be willing to pay to shorten an already self-limiting sickness? What will be the cost-benefit ratio in terms of the effect of any drug on the host cells? Because colds are generally minor, the standard for acceptable potential damage and side effects will necessarily be set very high.

Why treating colds with antibiotics is a bad idea

First, it won’t help. All “anti-bug” drugs—whether against bacteria, viruses, parasites or fungi—are designed specifically for that “bug.” Antibiotics destroy the bacteria’s protective cell membrane, killing them outright, or interfere with how the bacteria metabolize, weakening the microbes sufficiently for the body’s immune system to finish the job. They have no effect on viruses. When healthcare providers do prescribe antibiotics for people with colds, it’s either because the patient has both a virus AND a bacterial infection or because the patient has insisted on getting “something” (whether it works or not) for the cold.

Second, inappropriate antibiotic use can contribute to drug resistance, a growing problem worldwide. Antibiotics won’t affect the cold virus, but they do affect the millions of bacteria present in humans at all time, sick or healthy. Most bacteria in this mix are helpful and play important roles in keeping harmful bacteria in check. Antibiotics change that balance (the reason yeast infections are able to grow or why people on antibiotics sometimes develop diarrhea). Small or inconsistent dosages of antibiotics, as taken during a cold, also can destroy the weakest of any harmful bacteria present in the body, leaving to multiply only the few mutant versions of the bacteria that are less responsive to antibiotics. This new strain of the bacteria is now “drug-resistant.” Some diseases once thought under complete control, such as tuberculosis, are now often “multi-drug resistant,” making it harder and harder to find drugs that work.
Fast Track: Basic Research Pays Off

Recognition of a frightening new epidemic in America began in the 1980s. This emerging disease seemed to arrive in waves, first affecting young gay men, then heterosexual men and women who injected drugs, then immigrants who were neither gay nor drug-users, then young men with hemophilia who depended on a clotting factor isolated from donor blood. Their sexual partners also became ill, as did some babies born to women with the disease and some people who had received blood transfusions. Word soon came that the disease was active in many other countries, with no respect for sexual orientation. The disease originally referred to as GRID—gay related immune disorder—was slowly renamed AIDS, acquired immune deficiency syndrome.

The symptoms of AIDS were puzzling: inexorable destruction of the immune system that left the person vulnerable to an onslaught of often ghastly opportunistic infections and cancers; uncommon forms of pneumonia, rarely seen fungal infections, and Kaposi’s sarcoma, a disfiguring cancer ordinarily seen only in very old men.

But what was causing it? Veteran researchers from the war against cancer rushed to this new battlefront, bringing an armamentarium that would make possible the identification and treatment of the virus causing AIDS.

Scientists often point out that even the most dramatic, seemingly out-of-the-blue scientific breakthroughs are often actually like the final brick placed in a wall. Scientific achievement is made possible by many thousands of discoveries accumulated steadily and quietly, with little or no fanfare, in hundreds of laboratories over years, by scientists asking questions that often seemed obscure at best, useless at worst. When AIDS hit the world, any hope of combating it depended on advances already made in at least four different areas.

**DNA structure.** In the early 1950s, as Watson and Crick described the structure of DNA, other scientists found that viruses like smallpox and herpes were essentially capsules of DNA, which held the genetic information needed to create more viruses. Later, researchers discovered that viruses like polio, hantavirus, yellow fever, Lassa and influenza, were filled not with DNA but with RNA, which also carries the genetic code.

**Virus structure.** As scientists had learned more about the structure of proteins and viruses, they could begin to identify different “docking” proteins protruding from the capsules of different viruses and used to lock onto and invade living cells – a vital part of both the virus’s infection and replication processes.

**Retroviruses.** At first, scientists assumed genetic information flowed only one way. DNA provided genetic instructions to RNA, which produced the proteins to carry out those instructions. The process of decoding DNA into its RNA message is called transcription. In the case of RNA viruses, no such synthesis of DNA was required; the RNA itself was the source of genetic information. But in the 1960s, Howard

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**What a Difference the Manner of Replication Makes: A DNA, RNA, and Retrovirus Primer.**

- DNA viruses, such as the herpes viruses, have a nucleic acid core composed of DNA, which contains all the genetic information about that virus. Once inside the host cell, the virus uses one of the host cell’s enzymes, a RNA polymerase, to transcribe its genetic information on how to make the proteins necessary for the virus to reproduce.

- RNA viruses, such as the influenza virus, have a nucleic acid core composed of RNA that already contains all the genetic information about that virus. That means the virus transcribes information from RNA, rather than from DNA. The enzyme involved in this process, called RNA polymerase, has to be encoded by the virus itself since the host cell does not make RNA from RNA. And since the viral polymerase does not have a proofreading mechanism like DNA polymerases do, the chance of mutation is greater.

- Retroviruses also have genetic information on the RNA but unlike their RNA cousins, retroviruses reproduce by making DNA copies of the RNA, using an enzyme called reverse transcriptase. Retroviruses tend to be error-prone because reverse transcriptase, like the RNA polymerase, lacks proofreading capability.
Temin, a researcher studying how viruses cause cancer in chickens, was surprised when a drug known to block DNA activity also inactivated viral replication in the Rous chicken sarcoma virus, a RNA virus. He realized this must mean that the RNA virus somehow was using DNA as part of the process to make new cells. In 1970, simultaneously with MIT researcher David Baltimore, Temin discovered that a new class of viruses, the retroviruses, used such a pathway, a discovery for which they won the Nobel Prize in 1975. A unique cellular enzyme, called reverse transcriptase (for which the retrovirus is named) uses the RNA as a template to make a DNA copy. The viral DNA is replicated with the host RNA whenever the cell divides for generation after generation. The reverse transcriptase enzyme thus serves as a footprint that scientists use to test whether a tissue has been previously infected with a retrovirus.

**HTLV-1 and HTLV-2, the first human retroviruses.** In the 1970s, while head of the National Cancer Institute’s laboratory of tumor cell biology, Robert Gallo, was searching for retroviruses that would cause cancer in humans as they did in animals. He focused on bloodborne T-cells, an important part of the body’s immune system, and developed a growth factor that sustained cancerous T cells in cell cultures. This was important because the technique kept cells alive long enough to give scientists time to determine whether or not a retrovirus was present. In 1979, while examining the T cells from a young Alabama man with lymphoid cancer of the skin, Gallo and his colleagues were astonished to discover that they had at last found a human retrovirus. The following year, Japanese scientists were convinced a retrovirus was to blame for an unusual form of leukemia. When Gallo tested blood from these patients, he found the same retrovirus. Human T-Cell Leukemia Virus, or HTLV-1, infected a subset of T cells called CD4. When a second retrovirus was discovered that caused another form of leukemia, it was named HTLV-2.

Gallo had been one of many scientists who had benefited from the Nixon administration’s declaration of “war on cancer,” encouraged by the earlier discovery of oncogenes, genes that can cause a cell to develop into a tumor cell. That work continues today, of course—viruses are now believed to play a role in more than 15 percent of cancers, such as hepatitis B virus and liver cancer, or human papilloma virus and cervical cancer. But as so often happens in science, the information these cancer warriors obtained in one battle would prove invaluable in a new one.

**Winner of the Race to Discover the AIDS Virus? Patients**

And it was definitely a race, against time and between two scientific teams.

Scientists soon noted that AIDS patients were deficient in a specific subset of immune cells, called CD4 T cells. They concluded that whatever was causing AIDS selected CD4 T cells (as the host cells that were invaded) and then killed them. CD4 T cells, also known as helper cells, are involved in protecting the body against viral, fungal and other infections, signaling other cells in the immune system that an invader is present and that it is time for these immune cells to each perform their special protective functions. Destroying CD4 T cells is like destroying the conductor of the immune response orchestra.

As AIDS progressed, the number of CD4 T cells dropped significantly. Since CD4 T cells are the same cells infected by HTLV-1, Gallo theorized that the same virus might also be causing this new disease. Then Harvard virologist Max Essex showed that injection with HTLV damaged the immune system in a way that resembled AIDS.

In 1983, Pasteur Institute researcher Luc Montagnier cultured lymph gland tissue from a man with lymphadenopathy and found evidence of reverse transcriptase, the enzyme that is the hallmark of a retrovirus. He asked Gallo, by then head of a new National Cancer Institute Task Force on AIDS, to share antibody to HTLV-1 so he could see if it was the same virus. Gallo did.

In a highly contested competition, Montagnier is generally conceded to have won the race. His tests indicated the man’s immune deficiency disease was caused by a retrovirus in the same family as HTLV-1. The team began to isolate the virus from AIDS patients.
They called it LAV, for lymphadenopathy-associated virus. Shortly thereafter, Gallo and his team at NCI isolated the same virus, which they called HTLV-3. Eventually, this was changed to HIV, for human immunodeficiency virus, in recognition that the AIDS virus does not cause cancer directly, like the other HTLV-named viruses, but instead attacks the immune system so that it cannot effectively combat infectious disease or cancer.

Immediately, scientists began to decipher the newly identified virus’s genes. Then, using the viral genes that code for the individual proteins in the virus and recombinant molecular biotechnology (genetic engineering), they programmed bacteria to produce large quantities of each protein so they could be studied in detail. Looking for drug targets, they were able to quickly draw a map of the replication process and the roles various proteins play in this process.

In a short time, a blood test was developed to screen for the AIDS virus. SIV (simian immunodeficiency virus), a retrovirus similar to HIV, was found in monkeys, providing hints as to the original source of the virus and, most importantly, providing a way to test the antiviral drugs scientists were scrambling to create.

As the number of AIDS cases soared, money, both federal and private, began to flow to fight this frightening new disease, and the scientific advances resulting from this funding began to steadily rise. Many of the advances described here, however, were an unexpected byproduct of earlier support—such as NIH support for polio that meant AIDS researchers had the infor-

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**HIV: How the retrovirus for AIDS replicates**

1. The virus binds to any host cells containing two antigens, CD4 and a co-factor.

2. Once attached, it penetrates the cell and takes over, injecting its own dense core containing strands of RNA as well as about 10 different structural proteins and enzymes, including reverse transcriptase.

3. The process of creating new viruses with the same genetic information begins when the viral enzyme reverse transcriptase copies the virus's RNA to DNA, as any retrovirus does, then copies it again to make a double stranded helix.

4. The virus's newly copied DNA now enters the nucleus of the host cell, where another of the enzymes it brought with it splices it into chromosomes. That means whenever the host cell divides, it makes new copies of the viral DNA. (It also means that the virus has a place to hide, where the body's immune defenses can't find it. When the infection first takes place, the immune system begins to develop antibodies to face the invader. But by the time these antibodies are ready, the AIDS virus is hidden away, hiding its time until it reappears again in large numbers, wiping out much of the body's natural immunity.)

5. Now, the viral DNA is transcribed into RNA, which has two functions. One is to make viral proteins; the other is to package itself into new virions (single infective viral particles, or progeny, as scientists like to say). But in order for the virus's individual proteins to carry out their tasks, the long strand of joined proteins must first be separated or cleaved (cut) by a naturally occurring protein named protease that is encoded by the virus. Understanding of this process was based on earlier polio work involving proteases.

6. These new, fully-functional virions break through the host cell and seek out other cells to infect.
mation they needed about the proteases later found to be important in the AIDS virus or for the development of recombinant molecular biotechnology that later proved vital in quickly determining the genetic makeup of this new virus. The war against AIDS—and the hope for other medical conditions in search of a treatment—continued to gain ground from 1998 to 2003, when elected officials voted to double support to the NIH.

Using the Viral Map

Once HIV has entered the cell, there are four primary ways to keep it from multiplying. One can prevent the virus from attaching to the target cell. Alternatively, once HIV has entered the cell, one can block viral DNA or RNA replication. Thirdly, one can block assembly of the complete virus by interfering with processing of viral components. Finally, one might block exit of the newly formed virus from the cell. The earliest efforts to find antiviral drugs effective against AIDS took the second option: they targeted reverse transcriptase and blocked the replication pathway.

In 1987, less than four years after identification of HIV, the first FDA-approved antiviral treatment for AIDS came on market. Zidovudine, known as AZT, had been developed as a cancer drug but abandoned because of noxious side effects. The sure, rapid mortality rate of AIDS changed that equation.

Like a number of antivirals developed soon after, AZT inhibits the process of reverse transcription; it is incorporated into the viral DNA and prevents the HIV enzyme reverse transcriptase from completing its job of converting the virus’s RNA to DNA. When AZT is present, the replication process comes to a halt.

AZT brought many patients back from death’s door, leaving both clinicians and patients smiling in amazement as the drop in the patients’ CD4 T cell counts slowed, even stabilized. The results were so promising that behavioral scientists expressed concern that young people might view the magic pills as a reason to no longer worry about the sexual behaviors that place them at risk for AIDS.

But HIV was not so easily dismissed. The ever-mutating virus frequently stopped responding to AZT, sometimes less than a year after treatment began.

This was not surprising to scientists because they already had seen evidence of some resistance to Acyclovir, even though the DNA-based herpes simplex virus is far more stable than its RNA and retrovirus cousins like the virus causing AIDS. As shown in the DNA, RNA, and retrovirus replication primer on page 7, retroviruses have no “proofreading” mechanism for copying genetic information, as do other viruses. This means they are even more susceptible to making cumulative errors that change the gene sequence in ways that can result in a significant reduction in a drug’s effectiveness. Very soon, HIV had become drug resistant to AZT.

Fortunately, other scientists had been exploring another route to slowing viral multiplication: stopping manufacture of proteins needed by the virus. This strategy evolved from fundamental knowledge about how proteins are synthesized by cells and how protein precursors mature into their final functional forms. With this knowledge from basic science in hand, the scientists targeted protease, the enzyme used by the HIV virus to “activate” proteins by cleaving them at very specific sites so they could assemble new viruses.

The first protease inhibitor, Saquinavir, was approved by the FDA in 1995, in near record time, and two others—Ritonavir and Indinavir—followed a few months later. The speed of this drug development was spectacular. The knowledge that made it possible was gleaned from literally hundreds of person years of research on how proteases work—much of it done before this application could have been envisioned. Recognizing that the virus was likely to become resistant to any drug given it, clinicians began to give patients “cocktails” made of the new protease inhibitor and the older reverse transcriptase inhibitor. The idea was that inhibiting different stages of the replication process simultaneously would have a more powerful

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1In theory, another way is also possible: to prevent HIV from entering the cell by blocking entry via the CD4 cells the virus chooses. However, this approach has not been successful when tried in clinical trials.

Breakthroughs in Bioscience
effect and thus make it more difficult for the virus to mutate sufficiently to avoid responding to therapy.

Once again the new cocktails had a Lazarus effect. People who had already written their wills and given away their books got up from their beds and went back to work. For some patients, levels of the virus in their blood, called viral loads—the best way to monitor the effects of therapy—fell too low to be measured. Between 1996 and 1998, deaths caused by AIDS dropped more than 70 percent in the U.S.

New developments continue. The cocktail combinations have become more effective and far easier to take, often being combined in one pill. In 2003, the FDA approved a drug using yet a third approach. Fusion inhibitors inhibit the fusion (or entry) of the virus into the host cell membrane, preventing infection of uninfected cells. The drug, called Enfuviride, is a peptid (a small, protein fragment). Unfortunately, this means it cannot be taken orally or it would be digested before it could take effect. Instead, it must be injected. This, plus its steep price, means it is used primarily for patients who have become resistant to all other available drugs.

As of winter 2005, the FDA has approved 27 adult antiviral AIDS drugs. AIDS is no longer among the top ten causes of death in the United States nor a frequent cause of death in developed nations. But scientists are not complacent. Genomic assays—tests to detect changes in the virus’s genomic sequence and thus determine if a virus is becoming less responsive or “resistant” to the drug being used for treatment—have become a standard part of patient manage-
ment. And the devastating rise of AIDS in poorer countries calls out for easier, cheaper treatments.

**A Familiar Enemy With Changing Fingerprints: Avian Flu**

The first scientists studying influenza viruses must have felt as if they were opening some devious puzzle – or a Pandora’s box. There was no single influenza virus. Three types of virus, influenza A, B, and C, cause flu in humans. The most common, influenza A, changes constantly, sometimes within the same flu season, throwing the immune system into confusion and thwarting the best laid plans of vaccine manufacturers.

The Influenza A virus produces two different proteins: HA (hemagglutinin), with 15 subtypes of its own, and NA (neuraminidase), with nine subtypes. Hemagglutinin is responsible for the first step in infection, the binding of the virus to the target cell. Neuraminidase is necessary for the release of newly-formed virus particles from the infected cell after the virus has replicated. Since the virus is an RNA virus, it is highly mutable, and the HA and NA proteins on the surface of the virus can be expected to change slightly from year to year, in a process called antigenic drift. Virus strains are named for the combination of protein subtypes. For example, H1N1 stands for Hemagglutinin 1, Neuraminidase 1. H1N1, H1N2 and H3N2 are strains currently in general circulation among infected humans.

As a new flu strain begins to circulate, scientists involved in vaccine development rush to type the proteins in the new variant in order to make a corresponding vaccine. In 2003, at least 292 million doses of influenza vaccine were distributed worldwide. When vaccines are in limited supply, as they were in 2004, following a production problem, priority is usually given to those at most risk, including healthcare workers, the elderly, and people with impaired immune systems. Since the strains of one year’s flu are usually close enough to that of the previous year, many people retain some immunity from year to year.

Very occasionally, however, an abrupt, major shift occurs in the viral genes causing an antigen *shift* as opposed to the usual, more gradual antigen *drift*.

**Flu Season**

Along with death and taxes, people who live in temperate climates have another certainty in their lives. Every year, usually in late fall and winter, the “flu season” will arrive on some unspecified date, spread rapidly through the community with a peak at about three weeks, then linger on for another three or four weeks before subsiding. The season varies; it can begin as early as October and may not end until May. But it always comes. Every year, from 5 to 20 percent of Americans will be infected with the flu virus of the year, which will be different enough from the previous year’s virus to overcome any left over immunity. On average, some 200,000 will require hospitalization and 36,000—mostly the very old, very young, or those with weakened immune systems—will die.

Why is there a flu season? Despite what your grandmother may have told you, people do not develop flu (or colds) as a reaction to colder temperatures. Many experts believe the reason lies in the fact that people tend to spend more time indoors with each other during the fall and winter—school begins, sporting events move indoors—thus giving the virus a better chance to spread. As people develop immunity to the new version of the virus, it begins to fade.
Digging for Answers about the 1918 Influenza Virus

At the time the 1918 flu pandemic encircled the globe, killing more people in a short period of time than any disease ever known, no one even knew what a virus was, much less how to isolate and preserve it. During the flu pandemics of 1957 and 1968 and again in 1997 with concerns about a potential avian flu pandemic, scientists have yearned to be able to ask questions of that earlier killer—where it came from, what happened to make it so different than ordinary flu, what clues it held that would help fight new and emerging deadly viruses.

Last fall, when Dr. Jeffrey Taubenberger of the Armed Forces Institute of Pathology in Washington and colleagues at the Institute and the University of Iowa published an analysis of the full genome sequence of the deadly virus, fellow scientists hailed the achievement as the biggest virology breakthrough in decades. The Washington Post referred to it as one of the most astonishing technical feats in the history of science, “the viral equivalent of bringing back dinosaurs in the fictional Jurassic Park.”

Simultaneously, Terrence Tumpey and colleagues announced they had used this genomic “recipe” to recreate the virus and infect mice with it, working in the highly secure laboratories of the Centers for Disease Control and Prevention. Wise move. The virus proved far more lethal than they had expected; within six days, all the mice had died, compared to no deaths from a strain of current human flu. Four days after infection, the dead mice’s lungs had 39,000 times more virus particles than lungs of mice infected with ordinary flu.

Some questions were answered immediately.

First of all, the 1918 influenza was a bird flu, pure and simple—the most bird-like of all mammalian flu viruses, said Taubenberger—that had jumped directly from birds to humans with very few changes. The 1957 and 1968 flu pandemics, by contrast, had been caused by viruses that were a mix of avian and human flu viruses, brought about when the two flu viruses infected the same person at the same time.

Second, this ability to move directly from bird to human appeared to be the result of only a handful of genetic mutations, perhaps as few as 25 or 30 changes in the 4,400 amino acids in the viral proteins.

But that is just the beginning of the new knowledge now possible, say the researchers. For example, pinpointing genetic mutations that enabled the bird flu virus to move easily among humans will help scientists recognize other bird viruses that could trigger a pandemic and design better vaccines and antiviral drugs to fight them.

Much credit for the huge achievement—the first time scientists have ever resurrected and recreated any ancient pathogen—must go, of course to the steady, often undramatic accumulation of knowledge, tools and technologies in molecular biology. But chalk up part of the credit to the sheer doggedness of scientists themselves, because it took a lot of digging to find that long-vanished virus.

In the early 1950s, the digging was by shovel in the permafrost of Alaska, where mass graves of the indigenous Inuit people killed by the 1918 pandemic lay buried in permanently frozen ground beneath the tundra. Johan Hultin, a young Swedish graduate student, reasoned that the virus might still be present there. Using missionary records, he identified an Inuit village where 72 of the 80 residents had died within five days in November 1918 and been buried in the frozen ground. Hultin arrived by the newly opened Alaska Highway and was given permission to exhume by village elders eager to help in the development of vaccines against future such horrors. Together with two Iowa professors and a paleontologist working in the area, he dug through three feet of tundra and gravel, then three feet of permafrost, to obtain tissue samples from bodies with evidence of pulmonary hemorrhage, the tell-tale mark of influenza. Getting the samples back to the mainland was an adventure story of its own, but in experiment after experiment using the technology of the time Hultin was unable to recover the virus. However, the search for the virus—and Hultin’s part in it—had not ended.

In the mid 1990s, pathologist Taubenberger also began digging, searching for evidence of the virus in slides of lung tissue from soldiers who had died in the pandemic and been given military autopsies. The postage-sized pieces of tissue were filed away as part of the Armed Forces Institute of Pathology’s 3 million pathological specimens. Like Hultin, Taubenberger’s first excavations came up empty-handed. Then he found viral particles in the tissue of two young soldiers. The virus had long since degraded; what remained were bits of RNA that encoded the virus’s eight major gene segments. Using polymerase chain reaction (PCR) technology, he expanded these fragments into enough material to match against genetic primers from other viruses. Reassembling the fragments, he published a report on the first of the virus’s eight gene segments.

In Seattle, Hultin, now a retired pathologist, read the report with excitement. He wrote Taubenberger that he knew where there might still be frozen organs from 1918 victims. Go, said Taubenberger, please. Two weeks later Hultin, now in his 70s, set off for Alaska. Again with permission from the village elders and help from a local crew, he opened the mass grave. Many of the bodies had decomposed in the forty-plus years since he had first excavated but the body fat of one very heavy woman appeared to have protected her from the permafrost’s periodic thaws. In the Armed Forces Institute of Pathology laboratories, her tissue samples yielded yet more fragments of the virus’s RNA strands. What had once been a failed experiment had become an essential part of an extraordinary successful one. Eight years later Taubenberger had painstakingly pieced together the virus’s genome.

Let the scientific questions begin.
Antigen shift happens when viruses swap or “reassort” the influenza virus’s eight separate gene segments. Influenza A can infect birds, pigs, horses, seals, whales, and other animals as well as humans. Its natural hosts—its reservoir—are wild birds. Because pigs can be infected with both avian and mammalian viruses, including human strains, they can serve as both hosts and mixing bowls where a new combination of virus can be created, causing human disease. That’s why scientists carefully watch swine flu. But swine may not be the only mixing bowls. Recent evidence cited by the CDC suggests that for at least some of the 15 different avian influenza virus subtypes circulating in bird populations, humans themselves might serve as a mixing bowl. And recent recreation of the 1918 flu virus found it had been able to go straight from bird to humans, with no mixing of viruses (see box “Digging for Answers About the 1918 Influenza Virus”).

When a new influenza strain is created, with a protein or protein combination not before seen by contemporary humans, no one has any natural immunity to the new variant. If it spreads easily from human to human, a global pandemic occurs, ignoring all borders. Such antigen shifts caused global pandemics in 1918 (the “Spanish flu,” A H1N1), 1957 (the “Asian flu,” A H2N2), and 1968 (the “Hong Kong flu,” A H3N2)."}

Will such an antigen shift happen again? Yes, but no one knows when. However, scientists are closely watching, and preparing for, any change in avian flu H5N1 that might allow it to spread from human to human.

These nicknames often reflect where the flu was first detected. In the case of the Spanish flu, however, the name may have come about because Spain, not a combatant in World War I in 1918, was one of the few countries where uncensored news reports of the rapid spread of the disease appeared.

Readying the Scientific Arsenal

Scientists first recognized and typed H5N1 in 1997, when this avian flu moved from its natural wild bird host, which never became ill from the virus, into Hong Kong’s booming domestic poultry market, causing massive numbers of deaths among chickens. Eighteen humans also developed severe respiratory disease from the same influenza strain. Within three days, Hong Kong destroyed its entire domestic poultry population, about 1.5 million birds, a move that may have averted a pandemic. Since then, however, isolated human cases have been documented in other Asian countries.

The H5N1 influenza strain is of particular concern to scientists for three reasons. First, like the 1918 flu virus, it has gained the ability to be transmitted from the natural carrier, the bird, directly to humans, skipping the pig step seen in ordinary flu. Second, the virus appears highly lethal, killing more than 20 times as many of those infected as did the 1918 pandemic. And third, the rapid mutation of the influenza virus raises concern that it will mutate in a way that allows humans to easily transmit the virus to each other. The widespread distribution of the virus makes the chance of such a mutation unusually high. Virus replication and infectivity are at their peak before symptoms appear. Unlike SARS, there is no early
telltale fever. With H5N1 loose among humans, a new influenza pandemic would be underway, no further away from anyone than a plane flight. It would spread with amazing speed.

If that happens, the good news is that science has a far greater arsenal of tools in place today than ever before to meet such a threat—beginning with large, global surveillance programs by the World Health Organization and the U.S. Centers for Disease Control and Prevention, already closely monitoring this and other viruses. (See SARS box for evidence of how scientists worldwide can work together.)

Scientists around the world are working together to select the virus strains offering the best protection against the emerging pandemic so that vaccine development would begin immediately. Not knowing what the virus will look like, an exact vaccine can’t be made ahead of time. In the weeks needed for manufacture and distribution of a new vaccine, many people would likely be infected. That means that antiviral drugs will be the first line of defense.

Four antiviral drugs—amantadine, rimantadine, oseltamir and zanamavir—have been approved by the FDA for treatment of the ordinary human flu. If taken within two days of getting sick, these drugs can reduce symptoms, shorten time of illness, and make the person less contagious. The first three drugs also have been approved for prevention, and currently are used to control flu outbreaks in nursing homes, hospital wards, even cruise ships. Like other antivirals, these target the replication process.

- Amantadine (created in the 1960s) and its more recent, less toxic cousin rimantadine are entry-blocking drugs that work to prevent the type A influenza virus from binding with the surface of the healthy lung cells.

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Science and SARS

Viruses and other pathogens take advantage of globalization to move quickly from country to country. But increasingly, global cooperation is striking back. Just look at what happened with SARS.

In the early months of 2003, clusters of an unusual and often lethal pneumonia-like illness began to appear in Hong Kong and Vietnam. As patients were admitted to hospitals, struggling to breathe, large numbers of doctors and nurses in those hospitals began to fall ill, some dying. A flight attendant who had stayed in the Hong Kong hotel where early cases occurred became sick after traveling to Singapore; more than 100 cases in that city later would be linked to her infection. Toronto reported cases, also linked to international travel.

In March, the World Health Organization gave the puzzling new illness an official name—“severe acute respiratory syndrome”, or SARS—and called on leading laboratories to join a global network, sharing information by secure website and email, to find what was causing SARS and to develop a diagnostic test. The fact that no known or identified bacteria or virus had so far been found strongly suggested that a completely new pathogen might be to blame.

One week later, scientists at the CDC and in Hong Kong announced the isolation of a new coronavirus. Working around the clock, scientists used green monkey kidney cells to grow cells from a throat culture of a patient with SARS in order to reproduce the RNA of the virus. Within days, scientists had the sequence of the viral gene and compared it to all previously characterized coronaviruses: the SARS virus was different from any known human pathogen. Antibodies were isolated from SARS patients, making possible a diagnostic test. Scientists in the Netherlands were able to infect monkeys with the virus and produce similar symptoms, providing evidence the new virus was indeed the cause of SARS. Less than a month had gone by since the WHO had become aware of the new illness. The understanding of pathogens and the availability of complex genomic technology were essential to the discovery, but the speed with which it happened was made possible only by an unprecedented collaboration of 13 laboratories in 10 countries. After slightly fewer than 9,000 cases worldwide, the SARS virus appears to have retreated from spreading among humans. Science is responding with watchful waiting, ready, should it return, to quickly identify the virus and look for chinks in its armor.
Zanamivir and oseltamivir are neuraminidase inhibitors. If the neuraminidase found on the surface of both influenza A and B viruses is blocked, then new viruses are unable to break through the host cells to infect other cells. Since neuraminidase appears fairly constant across a wide range of flu strains, drugs inhibiting it stand a better chance of working as the virus evolves.

Early studies indicate that the H5N1 virus may already be resistant to amantadine and rimantadine but that oseltamivir, better known by its trade name Tamiflu, appears to work against the avian virus as it exists now. Tamiflu is the drug that countries are now stockpiling, while scientists continue to monitor the viruses for resistance. Thanks to the previous advances, scientists will be able to rapidly isolate and type whatever virus will come, modifying antivirals as needed, but it will take the combined effort of science, industry, and government to win this newest war.

Given the growing body of knowledge science has developed, and the growing partnerships between the National Institutes of Health, academic and private research, and pharmaceutical companies, many new antivirals are likely to be developed. For example, scientists now are studying the body’s own first-line defenses against viruses such as those for HIV and influenza, looking for new strategies to prevent these and other viral infections from progressing.

Scientists are also asking exciting new questions about the relationship of viruses to cancer and nervous system diseases, with some investigators speculating that a virus or viruses may be a factor in diseases such as multiple sclerosis. Even as science proceeds, history shows us the answers now being sought are likely to answer questions not yet posed and to prepare for emerging health threats not yet recognized. Finding more chinks in the viral armor will require weapons, vaccines or antiviral drugs, made specifically by applying the knowledge gathered through decades of research discovery, as well as new tools developed through research into the basic principles of immunity and infectious disease. This fundamental inquiry will provide the new findings that can be used in the future for designing vaccines and drugs for the inevitable emerging diseases of the future.