THE PAUL EHRLICH AWARD LECTURE

"THE PATH TO HUMAN RETROVIRUS DISCOVERIES"

ROBERT C. GALLO, MD
Professor and Director
Institute of Human Virology

of the

University of Maryland Biotechnology Institute
And
School of Medicine
University of Maryland, Baltimore
Baltimore, Maryland

March 14, 1999

Part I

APPRECIATION

Professor Meissner, Professor Kurth, Members of the Paul Ehrlich Foundation, members of the Board of Scientific Councillors, honorable guests, colleagues and friends: let me begin by expressing my deep gratitude to the Paul Ehrlich Foundation and to the members of the Scientific Council for this special honor to receive the Paul Ehrlich Prize. This is an award that makes me feel both fortunate and humble, since it honors the memory of the great Paul Ehrlich. No award can be given to any individual without recognizing the contributions of those who came before, the many colleagues who participated in the work, and the independent discoveries made by others.

Paul Ehrlich was gifted with knowledge, vision, and the capacity to bring his discoveries to practice which shaped the destiny of many scientific disciplines including chemotherapy, immunology, hematology, cytology and cancer research. His concept of receptors and ligands, which bind together in highly specific reactions, was introduced in 1898 and is both fundamental and central to our present-day biomedical research. He is truly the father of both pharmaceutical chemistry and of immunology. This, his birthday, is dedicated to his memory, and we pay homage to him for his seminal contributions to science and to human welfare.

Part II

THE BEGINNING

Today, I want to summarize briefly a scientific story that began with viruses and cancer and has initiated a cascade of new knowledge and discovery that followed in its wake. We now know that viruses play a role in about 20% of all human cancers, and recognition of their role, both in number and participation is increasing. Viruses that are involved in cancer are usually transmitted only by intimate contact and often require factors in addition to the virus, to cause the disease. Therefore, no cancer is transmitted by casual contact.

The science of virology is little more than a century old. Among the earliest viruses studied were some animal agents that cause cancers in animals. The predominant kind of viruses causing cancer in animal species belong to a family of agents we now call retroviruses. Peyton Rous discovered the first one in chickens at the beginning of the century, and though it produced a cancer of tissue called sarcoma, later it would become clear that retroviruses of animals mostly cause leukemias and lymphomas of blood cells. The genetic substance of retroviruses is ribonucleic acid or RNA, which shows high propensity for change, called mutation. Uniquely, these viruses can convert their RNA genome into deoxyribonucleic acid or DNA, giving them the advantage of establishing persistent infection with certainty. Indeed, we may call retroviruses the "kings of persistence". In their DNA form, the retroviruses insert and integrate their genes into the chromosomal DNA of the host cell, which is the storehouse of genetic information. Consequently, the cell is infected for its lifetime, and when the cell divides, the viral genes are replicated along with the cellular genes and transmitted to the daughter cells, thus rendering the host infected for life. Retroviruses are the only viruses that have also infected germ lines cells of animals and of man, in other words their oocytes and sperm cells. Therefore, they are also transmitted vertically from parents to their off-spring. Fortunately, these endogenous retroviruses, as we call them, in general do not appear to cause disease, at least not in man, as far as we know today.

Early in my career, I became interested in the biology of leukemias and lymphomas that are the principal cancers of human blood cells. Although no virus had been linked to a human leukemia, retroviruses had been shown to cause leukemias in many different animal species by the 1960's-70's. I believed that a study of these animal retroviruses and leukemias might provide useful basic information on the mechanisms involved in the development of human leukemias, even if such a virus never caused a human cancer. At the time, studies of cancers, – caused by animal retroviruses, was academically acceptable and would deserve support. However, by 1970 I decided to go a step further and attempt to find human retroviruses. If my colleagues and I succeeded, then we would seek to determine a role for them in human cancers such as leukemia. This was the challenge. At the time, however, study of virus in a human cancer was not an academically acceptableidea, and this caused a decade of opposition, laced withfrustration and difficulty. The reasons for lack of academic acceptability were many, most important being the failure for decades of many scientists to find such viruses. Then by the 1970's the U.S. National Cancer Institute initiated a program to find viruses of human cancers based on prior knowledge of the role of retroviruses as the cause of sarcoma in chickens by

Peyton Rous and of the leukemia of mice and cats by Ludwig Gross and William Jarrett respectively. After numerous fits and starts and spurious findings, it was decided administratively that there was no role for viruses in human cancers, and the program was stopped.

Paradoxically, by the end of the 1970s, as the U.S. Virus Cancer Program was ending, it became evident for the first time that some other already well-known and common viruses of human beings were involved in causing certain human cancers. In this regard, the most important and long lasting contributions in my view were the linkage of some wart viruses (papilloma viruses) to cervical cancer in women by Harald zur Hausen in Germany, and the clarification of the mechanisms explaining how a common virus infection (the Epstein Barr virus) could sometimes be involved in inducing some human cancers, by George Klein of Sweden. In addition, at about the same time the Hepatitis B virus, known to cause serious inflammation of the liver, became suspect as a cause of liver cancer. Maurice Hilleman, member of the Scientific Council of the Paul Ehrlich Foundation and the most outstanding pioneer of life-saving vaccine development since the 18th century Edward Jenner, developed the first Hepatitis B vaccine that prevents infection and thus liver cancer.

Part III

DISCOVERIES

Even though a search for retroviruses in human cancer was not a popular endeavor, some scientists besides myself persisted in the quest for retroviruses of man, such as Kurth and Chandra here in Frankfurt. It seemed possible to me that the ease with which retroviruses had been found in cancers of animals was related to the animals being inbred or otherwise selected for a capacity to replicate leukemia viruses. Lack of such genetic manipulation in humans would leave an absence of facilitation, and it would be logical to assume that finding a human virus would be far more difficult than for a virus of animals. Added to this was the lack of an animal model that could detect such virus. A possible role of retrovirus in human cancer became more plausible when others showed that a retrovirus causing leukemia in cattle was present only in small amount and was very difficult to find. A further step toward plausibility was given when we and others succeeded in isolating retroviruses that caused leukemia in gibbon apes. This was a major discovery, since it revealed retrovirus cancer in primate species.

The tactic I decided to follow in order to find human retroviruses had two elements. First I would use a test for an enzyme, just discovered in 1970 by Howard Temin and David Baltimore, as a surrogate assay for the presence of a retrovirus. This enzyme, called reverse transcriptase, was found in all animal retroviruses. Within the same year my co-workers and I reported a similar activity in a few samples of human leukemic blood cells. However, it required several years of research by my colleagues and me to make this test sufficiently sensitive, specific, and reliable for its use as a marker for detecting a retrovirus in man. This was achieved by the mid-1970s, and we obtained some early findings that suggested presence of retroviruses in some human leukemia blood cells based on these tests, which gave us more encouragement.

However, this test could only give us strong suggestions for presence of a retrovirus. Because the blood cells would die within a few days, this approach does not permit isolation of the virus. The second and most challenging component of our quest was to discover or to develop a cell that would be susceptible to and would propagate a retrovirus *in vitro*. Such cells would be essential for characterizing the virus, for proving it was a human virus, and for obtaining evidence that it caused a cancer. I chose to use some human white blood cells (leukocytes) for this purpose because leukemias developed in white blood cells. My colleagues and I committed ourselves to a long-term goal of developing methods to grow human blood cells in cell culture in the laboratory. In the course of extensive testing, we made the seminal discovery of a new factor, a substance that would cause specific white blood cells, called T-lymphocytes, to divide and reproduce in the laboratory. We initially called the substance T-Cell Growth Factor, but it was later named Interleukin-2 or IL-2. This small protein enabled us to grow human T lymphocytes for the first time in the laboratory in 1976. As of now, this cytokine, IL-2, which is now used as a common laboratory tool for growing T cells, is also used for treating some patients with cancers as well as AIDS. Having developed the two new techniques, we applied them to the human leukemia problem, and we discovered and isolated the first human retrovirus in 1980, which we named Human T-Cell Leukemia Virus-1 or HTLV-1. Japanese workers independently isolated the same HTLV-1 the following year, 1981, and both our group and many in Japan were soon able to show that HTLV-1 was the cause of an unusual and very malignant form of adult leukemia involving T-cells. Later, others showed that the virus also caused a neurological disease similar to multiple sclerosis. Oddly, HTLV-1 was found chiefly in southern Japan, the south Pacific, and among some African tribes and their American and West Indian descendants. In 1982 we discovered and isolated a second human retrovirus causing leukemia that we named HTLV-2. The evidence indicates that these viruses first entered humans from old world monkeys, and that they are transmitted among human beings by blood transfusion, by sex, and by mother to infant through milk feeding. We also have considerable knowledge of how they cause leukemia.

In putting it all together our findings of the means to grow and to discover human leukemia retroviruses provided the tools for discovery of other human retroviruses, particularly the HIV agent that causes AIDS. It provides a typical example of how basic discoveries in science can eventually lead to very important medical interventions. In our example, there was a cascade of events in which our sensitive reverse transcriptase tests for a retrovirus in human cells and our discovery of a new cytokine called IL-2, collectively made it possible to grow T lymphocytes in the laboratory, to discover the first human retroviruses, and to establish their role in causing leukemias in man. These were the breakthroughs that opened many doors, and there was more to come.

Part IV

APPLICATION

The last part of this story has to do with the application of our findings to discoveries of additional viruses, for thedevelopment of means for elucidation of the mechanisms by which these viruses induce disease, and for intervention in therapy and in prevention of human disease. Our first application was the development of a blood test for the HTLV's causing leukemias

which is now mandatory for screening all blood for transfusions in the U.S. and Japan with present considerations by Europe. We did not guess, however, that these breakthroughs would soon be applied to diseases of greater importance. It was at that time that the Acquired Immunodeficiency Syndrome (AIDS) was beginning to appear in the U.S. and Europe, and there was desperate need to discover its cause. The technologies and concepts developed for human leukemia found immediate application to studies of AIDS.

How did AIDS begin? It is speculated that, similar to the origin of HTLV's, a virus resembling HIV first entered humans by contacts with monkeys and chimpanzees. This probably happened many times before, involving hunters who cut themselves, or pet owners who were bitten, as possible examples. A few of these monkey and chimpanzee viruses, -- now known as Simian Immune Deficiency Virus or SIV, adapted well to human cells, initially with limited spread. Societal changes in Africa that followed World War II gave means for spread of the virus through urbanization which brought with it increased sexual contacts, group intravenous drug use, and transport of contaminated blood products for medical purposes from one nation to another. In effect, the rainforest of Central Africa had "come to town". By the 1960's, the virus was spreading widely, and by the 1970's it was Out of Africa!

It is of importance that if the AIDS epidemic had to come, it came at a very bad time. There was little organized capability for researchers to handle it, since it had become policy that epidemics were things of the past, and institutions charged with the responsibility to investigate epidemics were minimally staffed and ill equipped to meet the challenge.

It is of equal importance that if AIDS had to come, it came at a good time, since we had just achieved the scientific insight and the technologic capability to be able to apply the knowledge and tools of human retrovirology to the problem. Therein lies a tale of discovery of the AIDS virus and intervention against it by application of epidemologic and chemotherapeutic procedures.

AIDS was first described in 1981 by physicians in the U.S. in case reports of homosexual men with unusual infections that were associated with a decrease in special immune cells, subsets of the T-lymphocytes called CD4 T cells, and sometimes this immune abnormality was accompanied by a strange tumor called Kaposi's sarcoma. Groups of persons at high risk to the disease were soon identified. The acronym H, H, H, H was used that stood for homosexual men, Haitians, heroin addicts, and hemophiliacs. AIDS was more common at that time in these groups, but nonetheless was found only in a fraction of each group. Because of the complexity and the diversity of AIDS as an infectious disease, as a cancer, and as an entity of unknown etiology, no one organization or group of individuals was capable or held responsible to solve the problem. Many ideas were proposed as to what the cause might be. They included extremely irrational and even harmful ideas such as:

The devil causing trouble,
God competing with the devil and punishing people, and
"life style", an idea which says there is really no specific cause. These notions, of course, were without merit.

Rational ideas that were proposed needed to be sorted out, since they included several kinds of common viruses and certain other microbes such as Mycoplasma and fungi.

The idea that my colleagues and I generated and proposed in 1982 was that AIDS was caused by a new human retrovirus. The logic for a retroviral cause of AIDS derived from earlier observations of retroviruses in diseases of animals. Max Essex of Harvard and William Jarrett of Glasgow had noted that the retrovirus of cats, which caused cat leukemia, could mutate to a form of the virus which caused serious immune deficiencies in cats. A supporting idea was a logical extension of the prior studies of HTLV leukemia. For example, Takatsuki had noted that the disease produced by the HTLV agent in Japan was sometimes associated with immune suppression, and work in my laboratory had shown that the HTLV's, which targeted T cells, could impair their immune function in *in vitro* tests in the retroviruses of both animals and man could target T cells and could cause immune dysfunction in them. Other important observations were the epidemiological results showing that HTLV's were transmitted by blood, sex, and mother-to-infant, just as AIDS was being shown to be transmitted, and the findings that there was a high prevalence of AIDS in Africa and Haiti where HTLV infections were also abundant. Why not another retrovirus then as the cause of AIDS as well?

Our first experiments with tissues from patients with AIDS began in the spring of 1982. Our working hypothesis was that AIDS might be caused by a new human retrovirus, one that was likely related to the HTLV's. The basic element of this hypothesis eventually was proved true. AIDS was ultimately shown to be caused by a new retrovirus but one not very closely related to the HTLV's. In exploring the concept, we followed the same protocols that led us to discovery of the HTLV's five years earlier. From 1982 to early 1983, our ideas were supported by occasional hints of positive findings, though shrouded with frustrating confusion. We were soon aware that our confusion arose from the fact that some of the specimens being studied were from patients who were doubly infected with both HTLV and the virus of AIDS. The serological tests for HTLV are highly specific, and we soon found them to be insensitive for detecting an AIDS virus.

It is of importance that Luc Montagnier, Jean-Claude Chermann, and their co-workers in France were also working to find a cause for AIDS. In 1983 they published their finding of a new retrovirus in a patient who had an enlarged lymph node but did not have AIDS. The virus gave only temporary growth in cell culture, and this made it impossible for them to characterize the virus in detail and to provide credible evidence that the virus causes AIDS, as they themselves pointed out in their corresponding publication.

We were being extremely cautious in making claims of a retrovirus link to AIDS in the absence of definitive evidence. By the summer-autumn of the same year, (1983), we had isolated and propagated many isolates of a new retrovirus from AIDS patients as well as from people who were at high risk to AIDS. The Montagnier virus was ultimately found to be a member of the same retrovirus group of agents that we had been able to propagate freely and to study in the laboratory. My co-worker, Mika Popovic, and my senior technician, Betsy Reed, had succeeded in the first continuous and large-scale production of HIV in the laboratory studies using several of our isolates. By early 1984, a specific and sensitive blood test for these viruses was developed in our laboratory. This provided a critical breakthrough and one that was

urgently needed. Utilization of the new test in studies of virus isolates from 48 AIDS patients and the testing of blood for specific antibodies to the new virus in hundreds of people made it possible for us to establish definitively that the retroviruses we had found were indeed the cause of AIDS. This was announced in April and our 4 papers were published in May 1984.

The development of the blood test found immediate and important application as a test for diagnosing AIDS and as a means for detecting and preventing use in humans of contaminated blood and blood products. The technology for the test was licensed to industrial firms which made it quickly available for routine use all over the world. The test allowed the epidemic to be described and monitored for the first time, and it was critical in expanding the data that linked HIV to AIDS.

There were other important applications of these early discoveries. The capacity to grow T cells profusely and to propagate the viruses in them allowed scientists to test for drugs that might block HIV infection. Beginning in the mid-1980s we had our first glimpse of the possibility for therapy. AZT, a drug that had been produced by Burroughs-Wellcome laboratories, was first shown by Samuel Broder to inhibit HIV in our cell culture systems. Studies in the clinic revealed some benefits to patients. Though eventually found not able to prolong life, AZT nonetheless, has had an important legacy. Since that time new, important drugs that were combined with AZT have been introduced for use in patients with HIV and AIDS with great benefit.

The work on retroviruses and human diseases in the late 1980's to the early 1990's might be called years of maturation and refinement of the field. The field had expanded enormously. We began studies to elucidate the pathogenesis of Kaposi's sarcoma that often occurs in AIDS patients, and this work revealed the importance of cytokines and of other chemical messengers in inducing and maintaining this tumor.

In the more recent period we discovered that certain messengers of the immune system, called chemokines, block HIV replication. We believe these and some related substances, such as Reinhard Kurth's Interleukin-16, IL-16, are central to the variable resistance to HIV infection exhibited by different people, and that they also play an important role in determining the progression to AIDS in people already infected with HIV. These findings may have clinical applications in the future.

Part V

THE ENDING

What of the future? In the industrial world effective therapy is available for most. However, we must improve the anti-HIV chemotherapy to make them more sustainably effective. More important, HIV has now infected close to 50 million people, cumulatively, with 16,000 new infections every day, a number that will likely continue to increase. AIDS is now the most lethal infectious disease worldwide, killing at least 4 million human beings in 1999, more than tuberculosis and malaria. It is estimated that 90% or more of infected people receive

no therapy because of both cost and logistical problems. This is intolerable. We must develop drugs that are effective, but simple and cheap enough to permit universal use in all nations. The hope is that one day a vaccine will be made that will provide the solution to AIDS through prevention of infection by the right stimulus to the immune system. Though I am encouraged by some recent vaccine research findings, nonetheless I must tell you that in my view there is no vaccine within the immediate future that is likely to be effective.

The entire history of the retroviruses, from their discovery and elucidation of their properties and linkage to disease, to their treatment and attempts at preventing infection from them, has been an exciting voyage into the unknown. However, the ship has not yet arrived at its destination and will be afloat for many more years with many more ports of call.

I close by returning to Paul Ehrlich. Though Paul Ehrlich died more than eighty years ago his thoughts are very much alive, and, in fact, contribute more to biomedical research today than in his life-time. This is reminiscent of the writing by a 19th century American poet, Edwin Arlington Robinson, who once said,

"Ich werde mehr zu sagen haben,

wenn ich erst tot bin."

Or in his native tongue:

"I shall have more to say

when I am dead".

Thank you again.

END