THE AIDS RETROVIRUSES

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Although the first animal retrovirus, avian leukosis virus, was discovered at the beginning of this century, the involvement of retroviruses in human disesases has been only recently recognized, and it will probably dominate——through the AIDS epidemic——the end of this century.

My interest in viruses began more than 30 years sgo, but I really decided working on retroviruses——called at that time RNA tumor viruses——in 1965. Not much was known then about the molecular biology of these viruses : their genetic material, assumed to be RNA, was ill-defined, and their mode of replication was completely ignored.

At the beginning of the 1970s, two important discoveries were really a breakthrough not only for the knowledge of retroviruses themselves, but also for modern molecular genetics and studies on cancers : that by Temin and Baltimore of the reverse transcriptase, the enzyme which allows transcription of the viral RNA into DNA, the latter becoming integrated into the host cell DNA, and the first demonstration by Hill and Hillova that proviral DNA was infectious and contain all the genetic information of the virus.

At that time, a number of laboratories engaged themselves in a search for human retroviruses associated with cancer, following the models shown by laboratory or domestic animals. This search was unsuccessful, while the work on transforming retrovirus led to the discovery of modified cellular genes involved in cancers, the oncogenes. Only a rare form of leukemia, first described in Japan, adult T cell leukemia, was found associated with a real human retrovirus, HTLV-I, first described by my colleague which is honoured today, Dr. R. Gallo and his group, and also discovered and well studied in this country by Drs. Miyoshi, Hinuma, Yoshida and their coworkers.

In 1979, with my associates J. C. Chermann and F. Barre-Sinoussi, I started application to the search of human retroviruses of an observation we made in mouse retroviruses : like other viruses, cells infected by retroviruses are making interferon. If we suppress the action of interferon by antibodies raised against interferon, the virus replication is significantly increased.

But even using this improvement we could not detect any retrovirus in many short term primary cultures of human cancer cells. We had only indirect evidence of possisle involvement of retroviral DNA sequences in some breast cancers, so called at "poussées évolutives". Interestingly, such sequences were not only found in the tumor cells but also in the cultured blood T lymphocytes of the patients. This led me to undertake T lymphocyte cultures (with anti-interferon serum and T cell growth factor) from many cancerous patients. The same method was applied to AIDS patients or patients with persistent lymphadenopathy, a sign which sometime precedes by years the occurence of AIDS. We were lucky enough to isolate from the first lymph node culture of a patient with lymphadenopathy, the lymphadenopathy associated virus, LAV, renamed later HIV-I. Much to our surprise, the virus appeared antigenically very different from the only known human retrovirus at that time, HTLV-I. We realized that the virus we had discovered was new and distantly related to the equine infectious anaemia virus, a lentirus of the horse associated with severe, sometime fatal anaemia. During the year 1983, I set up a team of researchers and clinicians and we designed studies aimed at demonstrating the involvement of this new retrovirus in AIDS. Besides the virologists I already named, I like to ackowledge the important contribution of the immunologists, J. C. Gluckman and D. Klatzmann, the clinicians, W. Rozendaum, C. Griscelli and E. Vilmer, the medical virologists, F. Brun-Vezinet and C. Rouzioux, the epidemiologist, J. B. Brunet.

During the year 1983, we were able to isolate

LAV-like viruses from authentic cases of AIDS in patients belonging to the main at risk groups : homosexuals with multiple partners, hemophiliacs, I. V. drug users, Central Africans. We set up ELISA and RIPA tests to detect antibodies not only in patients, but also in healthy carriers of the virus.

We detected a cytopathic effect of the virus in T lymphocytes, and showed that only T4 lymphocytes could be infected. By March 1984, we had fair evidence that the new virus was the causative agent of AIDS. This is not to diminish the important contributions of Dr. Gallo and his coworkers. Collaborative work made obvious that the HTLV-III described by them corresponded to the same viral entity than LAV.

This become still more apparent when, at the end of 1984, both viruses were cloned and sequenced. Here came the surprise: besides the classical genes seen in all retroviruses, the AIDS virus has some new genes involved in the regulation of its expression and possibly with deleterious effect in cells. The groups of W. Haseltine and F. Wong-Staal could identify a gene having a transactivation effect on the other viral genes (TAT). We recently identify a possible role of another gene, F, in the transduction of signals to the lymphocytes. Sequencing of others isolates, especially of two viruses isolated from African subjects, showed us the extent of the large genetic variability of this type of virus, particularly in the envelope gene.

A still more distant virus, actually a new type called now HIV-2 was isolated by us at the end of 1985 from an AIDS patient living in West Africa. Since then, many isolates of HIV-2 have been made, and its association with AIDS recognized.

HIV-2 has similar biological properties, and we could show that for both HIV-1 and HIV-2, the virus envelope binds to the same receptor, the CD4 molecule. The main difference between the two viruses lies in their genetic sequence (more than 50% difference) and it is amazing how Nature has found two solutions for the same biological properties. Actually, HIV-2 is close, but not identical to a simian virus associated with an AIDS-like syndrome in macaques, and may be derived from this virus.

The isolation of HIVs has opened the way to the elimination of their transmission by blood transfusion and blood products, the design of therapeutics which look promissing (AZT) and vaccines. However there is little hope that we could very quickly find the right solution for treatment and vaccination.

There are still many gaps between our knowledge on the virus at the molecular level and the pathophysiology of the disease. It is particularly difficult to understand why a chronic infection of a relatively small number of T4 lymphoytes and macrophages can lead to an irreversible disorder of the whole immune system, and to a fatal issue, even in patients apparently cured of their opportunistic infections or cancers.

Understanding this phenomenon is a prerequisite for designing rational therapeutics.

Similarly we do not know the reason of the long incubation period which lies between the exposure to the virus and the occurence of clinical signs. Does the virus change during this period ? What is the role of immunogenic stimulation ?

The problem of vaccine is also a very difficult one. Apparently the virus is constructed to have the vital parts of its envelope protected against antibody or cytotoxic response. Trying to make those parts immunogenic is critical for obtaining protective vaccines.

Therefore much remains to be done in fundamental research about the AIDS virus ! My colleagues and I will make any effort to contribute to the eradication of this dreadful disease.

HUMAN VIRUSES OF THE LATE 20TH CENTURY: THEIR ROLE IN CANCER AND AIDS

Robert C. Gallo

AIDS is the most serious pandemic of our time, effecting all society and impacting on our legal structure, economics, medical care, research, and our feelings about each other and ourselves. It poses a novel challenge to the strength of society, to democratic values, to our way of life, and to our abilities to work together. Although only first recognized in the U.S. in 1981, remarkable progress was made in a few years. Thus, between 1982 and 1984 : A retrovirus theory was proposed and the virus identified, proven to be the cause of AIDS, mass produced in continuous culture,(thereby providing specific reagents for virus typing and large scale sero-epidemiology), and an accurate blood test was developed which prevented the virus from infecting many thousands of additinal people. Also, the viral genes were molecularly cloned and expressed, the variation of isolate to isolate discovered, the various genes identified, the presence of the virus in the brain found, the entire viral genome sequenced and the beginning of antiviral treatment with some benefit developed.

If the AIDS virus had became a problem 20 years earlier, we would not know the cause nor would we have a blood test, and perhaps we would by now be in pursuit of even more bizarre hypotheses than some of those first proposed. In the 1960's we could not distinguish T cells from B cells, nor grow T cells, nor clone and analyze genes, nor biochemically detect a retrovirus (only describe one by electron microscopy). The key advances that made our rapid progress possible were : the discovery of reverse transcriptase by Temin and Baltimore in 1970 based predominantly on Howard Temin's ideas and work ; the development of reverse transcriptase as a sensitive and specific assay for retroviruses, between 1970-75 by the late S. Spiegelman and coworkers and by my colleagues ; our discovery and use of interleukin-2 (IL-2) to grow T cells (1976); the development of monoclonal antibodies by G. Kohler and C. Milstein——their use by many groups for defining cell types, especially for typing lymphocytes ; the development of gene cloning technology, particularly by S. Cohen and H. Boyer ; and gene sequencing and other nucleic acid techniques pioneered in Great Britain by F. Sanger and coworkers. The discovery, study, and use of the first human retroviruses (HTLV-I and HTLV-II) and their linkage to disease by my group and by several Japanese groups (notably T. Takatsuki, M. Yoshida, O. Miyoshi, Y. Hinuma, our late friend Y. Ito, and more recently the group of T. Sugimura, M. Miwa and K. Shimotohno) were also critical to much of our thinking about the cause of AIDS and how to approach the problem as a retroviral disease.

Despite the rapidity of progress we are in urgent need for additional practical advances in anti-viral therapy, supportive care, and vaccine development. Anti-viral treatment has come out of fundamental work on the virus replication cycle. Like other retroviruses, HIV contains reverse transcriptase, the DNA polymerase enzyme which catalyzes formation conversion of viral DNA from the viral RNA after infection. This led to a search for DNA polymerase inhibitors, and positive results with one AZT, provided a psychological stimulus to the field because it proved to a very pessimistic and wary scientific community that something could be done. AZT helps, but it is not the answer. More and better compounds working by similar and different mechanisms are needed. A recent major advance is the production of soluble CD4 proteins. From basic studies of viral infectivity we learned that the CD4 molecule on the surface of the T4 lymphocytes is the receptor for the AIDS virus. This molecule has been made in large amounts by recombinant DNA technology, purified and solubilized. The free CD4 complexes to the viral envelope and thereby competes with the cell for virus binding and blocks

entry of the virus into the cell. Much future work will focus on inhibition of virus expression, particularly on interfering with the function of two of the five "accessory" genes of the HIV-genome, genes which Wong-Staal and co-workers have shown are essential for HIV replication.

Although public education is vital to the AIDS efforts, it is not sufficient. The problem in central Africa and among intravenous drug abusers will require much more. Thus, an effective vaccine is really needed, but at this time it is not in sight. The problems are many. HIV attacks the immune system ; it integrates its genetic information into the chromosomal DNA of the cell, and may be latent until (in the case of the T4 cell) the cell is immune activated——thus, escaping from the immune system for long periods ; it enters the brain ; shows genetic change (no two isolates are identical), and this change often leads to a difference in biological behavior including the ability to escape the immune system. In addition, the only animal models available are difficult to work with, hard to get, and when inoculated with virus they do not get disease. Nonetheless, we have made progress in defining viral epitopes for induction of neutralizing antibodies and epitopes for T cell immunity, as well as defining regions of the envelope which are constant. Also, although a very impressive immune response has not been easy to obtain in the animal models, certain vaccine protocols with the viral envelope used by my collaborator, Dr. Daniel Zagury of the University of Paris, on himself have shown that a substantial immune response in man to a vaccine candidate is possible. Of course, the answer to the key question "Is he protected against infection with HIV?" is unknown.

In addition to vaccine development and our continued work on HTLV-I and HTLV-II, my co-workers and I are now chiefly concerned with the question of HIV origin and pathogenesis. How many AIDS viruses are there? Where did they come from? Why now? What percent of infected people get AIDS? Is there any genetic resistance or susceptibility? Are there other environmental co-factors or other viruses which play a role? How does the brain get infected? What is the mechanism of the depletion of the T4 lymphocytes? How do the cancers(especially the Kaposi sarcoma) develop in HIV infected people?

No one knows the origin of HIV with certainty. We do know that an epidemic began in central Africa, Haiti and the U.S. about the same time ; that some European clinicians believe rare cases were seen in the early 1960s in rural central Africa ; and that like HTLV-I and HTLV-II, there are African monkeys infected with related retroviruses. We know that many sera from the 1970s and rare earlier ones are positive. My thinking is that like HTLV-I , HIV-1 and HIV-2 are old infections of man, but unlike HTLV-I , they were remarkably restricted to a very small geographic area and group. Probably social-economic changes caused what was once rare to spread concentrically, and with the major changes in 20th century habits, to many other areas of the world(widespread travel,increased sexual promiscuity, I.V. drug abuse, and the wide dissemination of blood and blood products for medical use). Thus, what was rare and isolated became common and global.

For practical purposes, there is really but one true AIDS virus(HIV-1). Of course, its heterogeneity creates problems for vaccines but not for diagnosis(blood tests). HIV-2, does, however, create a small problem because it can be missed in the HIV-1 blood test on a rate occasion(HIV-1 blood test will detect more than 90% of HIV-2 infections). HIV-2 has not spread like HIV-1, and obviously it is not nearly as pathogenic. Strikingly and strangely it is relatively confined to West Africa where it was first discovered by Essex and Kanki when they tested such sera for antibodies reactive with a monkey virus known as SIV.

We now know that SIV is closely related to HIV-2. Isolates of HIV-2 from Montagnier's group, and later from Sweden and our group have shown that HIV-2 has genomic variation from isolate to isolate comparable to HIV-1.

Many key aspects of the epidemiology of HIV-1 can be illustrated with a recent single study of I.V. drug addicts in the New York-New Jersey areas. These studies demonstrate: (1) I.V. drug abuse continues to be a major cause of spread by both leukemia viruses (HTLV-I and HTLV-II) plus AIDS virus(HIV-1); (2) many are double infected(HTLV-I and HIV-1); (3) there are sharp differences in prevalence over very short distances and among different groups.

There are no indications of genetic susceptibility to HIV and no known necessary co-factors. About 35% of infected people now seem to progress to frank AIDS, but a true estimate of progression will take more time.

From *in vitro* studies we learned that immune activation of a T4 cell leads to virus expression and spread. We assume the same is true *in vivo* so we caution against unnecessary vaccines and poorly thought out immune stimulation therapy, and we are concerned about other chronic infections which activate T cells. Recently, we discovered a new human herpes virus (HBLV or human harpes virus-6, HHV-6). We have shown HBLV can infect T4 cells and lead to their killing. Many HIV positive people have very high antibodies to HBLV. Therefore, we are now attempting to find out whether HBLV is a co-factor in some or all cases of AIDS, i.e., augmenting the T4 depletion.

T4 lymphocytes are not the only major target for HIV-infection. In 1984 we found that macrophage and related cells are also infected. Based on our analyses of brain sections we suggested that the macrophage may bring virus to the brain. Recently, we found that very minor changes in the viral genome can lead to differential cell tropism. The infected T4 cell is killed by HIV only when the virus is expressed and released not on entry or integration, and the killing appears to depend upon some internal interaction of the viral envelope with CD4.

During the past two years we have also focused on the origin and pathogenesis of the epidemic Kaposi sarcoma (KS) associated with HIV-1 infection. Recently, several exciting advances have been made: (1) we developed both a long term *in vitro* and *in vivo* model of human KS; (2) studies with these models indicate that KS is an autocrine and paracrine growth factor (and other cytokine releasing) abnormality and not necessarily a true malignancy. Abnormal amounts and abnormal release of several cytokines were found which we think can explain the entire pathophysiology of KS, and suggests new approaches to its control.

In conclusion, latent, slowly acting viruses typified by retroviruses, are an increasing problem for 20th century man with AIDS as the most obvious threat. Yet the combined forces of biomedical technology have the means to solve most of the medical scientific problems. It is less certain whether society (all of us) can handle the rest.