The U.S.S.R. and the origin of the AIDS virus

Could the HIV virus have been created, deliberately or accidentally, in a laboratory? John Grauerholz, M.D. presents the evidence for a new hypothesis.

Despite years of unprecedented levels of scientific research, no one knows where the AIDS virus comes from. Some have hypothesized that the virus made a mysterious "species jump" two to three decades ago from an animal to man, but they have been unable to find the animal reservoir. Others have hypothesized that the AIDS virus has been around for hundreds or thousands of years and underwent a mysterious mutation to a new lethal form 20-30 years ago. A third hypothesis is that the virus was generated spontaneously in diseased human tissue under extraordinary conditions.

Then, there are two additional hypotheses which suggest that the virus was created either deliberately as a biological warfare weapon or accidentally in a molecular biology laboratory of some sort. The sensational "biological warfare" theory was designed by the Russians, who have launched repeated international campaigns of charges that the AIDS virus was deliberately "cooked up" in the U.S. biological warfare program at Fort Detrick, Maryland. The laboratory accident thesis, perhaps in a Russian laboratory or perhaps by an East bloc scientist while on leave in a Western laboratory, has also been advanced. This paper examines that last hypothesis in some detail. To investigate that possibility I intend to provide the reader with the necessary background material which he or she may need on the subject.

This paper proposes a hypothesis on the origin of the human immunodeficiency virus (HIV), through a laboratory accident, which is consistent with the known biology of these viruses and with the published scientific literature. This hypothesis accounts for the apparent lack of an animal reservoir by demonstrating that such a reservoir in fact existed, and still does exist, and that its interaction with human beings most likely occurred, and still is occurring, in the laboratory.

The nature of the retrovirus problem

AIDS is a clinical complex or syndrome which results from the activation of a previously latent infection by a specific kind of virus known as a "retrovirus"—the human immunodeficiency virus (HIV). Viruses consist of a segment of genetic material, either DNA or RNA, wrapped in a coat of protein. They have been called "bad news in a protein coat," the bad news consisting of the genetic information encoded in the DNA or RNA. Most viruses simply take over control of the metabolic machinery of the infected cell and utilize it to produce multiple copies of the infecting virus with accompanying destruction of the cell. Some viruses establish latent infections which persist over time and some of them are capable of transforming normal cells into cancer cells.

Retroviruses are animal RNA viruses which are able to infiltrate into the reproductive material of the cell nucleus and transform the genetic instructions of the host cell. Unlike other RNA viruses, which always exist as RNA inside or outside of cells, retroviruses reproduce through a DNA intermediate inside the cell. This intermediate stage exists as either a closed circular double helix of DNA, or as an integrated DNA segment within the DNA of the host cell. If these integrated DNA sequences are present in germ cells, they can be passed to offspring produced from such germ cells. They can thus be passed from generation to generation, without being activated, in an unexpressed form.

The retrovirus family is divided into three subfamilies; the oncoviruses or RNA tumor viruses, the slow-acting or lentiviruses like Visna in sheep or AIDS in man, and the foamy viruses which form a multinucleated mass of protoplasm formed by the merging of cells (syncytium). The Human Immunodeficiency Virus (HIV) is a member of the lentivirus subfamily, a group of viruses which have been linked to the induction of arthritis, encephalitis, progressive pneumonia, and slow neurological diseases in certain species, primarily sheep, cattle, and goats.

Recombination between retroviruses

Different viruses infecting the same cell can interact and their genetic messages can be recombined, incorporating critical features of the two genetic blueprints together. Recombination can occur naturally, as in an individual simultaneously infected by two viruses, or in the laboratory. Over the past two-to-three decades scientists have learned how to recombine genetic messages in the laboratory. In the 1960s, molecular biologists mastered the rudiments of genetic recombination capabilities. In genetic engineering, critical features of one message can be transported into another cell or species artificially.

Retroviruses are RNA viruses in their extracellular form as free virus particles. Within an infected cell they exist as RNA in the cytoplasm or as the DNA intermediate in the nucleus, which is known as a "provirus." As RNA viruses they are capable of the rapid evolutionary change characteristic of RNA genomes (Reanney, 1982; Holland et al., 1982). This rapid evolution occurs because of a lack of the error, correcting enzymes which assure the fidelity of DNA replication. Combined with a high rate of replication, this results in rates of RNA genome mutation over a millionfold greater than the mutation rate of host cell DNA.

In addition to the high rate of mutation characteristic of RNA viruses, retroviruses have an extraordinarily high rate of recombination (Coffin, 1979). Their genomes are the result of recombination between molecules of different retrovirus, and possibly other RNA virus, genomes with very little physical linkage of genetic sequences. The probability of recombination between neighboring nucleotides in the genome has been estimated to be on the order of 10^3, or 100 times higher than bacteriophages considered to have an extremely high frequency of recombination. Crossing over occurs throughout the genome with little restriction.

Because of the high mutability, and hence instability, of retroviral genomes many deletions occur which result in production of defective viruses. Reacquisition of lost genetic information by simple recombination, in the presence of virus containing the deleted segments, occurs, resulting in production of complete virus (Stavnezer et al., 1986).

Recombinations between retrovirus genomes and host DNA: In addition to rapid evolution of the RNA genome and high frequency of recombination between retroviruses themselves, recombination between viral and host cell genetic information occurs. Such recombination occurs at the level of the integrated DNA provirus. This recombination occurs in situations involving viruses specific for one species grown in cells of another species and results in alteration of host range in such viruses (Aaronson 1971; Kotler et al., 1984). In such experiments mouse tumor viruses, for example, were grown in human cells and acquired new, genetically stable, surface antigens of human origin. These altered viruses were able to grow much more efficiently in human cells and, in fact, lost their ability to transform mouse cells. In another experiment an avian sarcoma virus grown in chicken cells was compared with the same virus grown in rat cells. The virus grown in rat cells was different from that grown in chicken cells and was demonstrated to contain genetic sequences homologous to the rat cell genome, indicating that that virus was a recombinant between the original virus and the rat cells in which it was grown.

Species jumps

Since retroviruses arose from cellular genetic material, recombination between different retroviruses and between retroviruses and cellular genetic material would be expected. Transmission of retroviruses originating in one species to another species, with subsequent propagation in the germ line of the second species has been documented (Todaro, 1980). In such cases, DNA homology (similarity in the sequence of nucleotides) between the acquired virus and the species of origin can be detected and can serve to measure evolutionary divergence and to estimate when the crossover occurred. Such sequence homology appears to indicate that the AIDS virus HIV-2, or HTLV-4, is related to the simian virus STLV-3, which infects African green monkeys. In such a case, it should be possible to infect these animals with this virus.

In the case of HIV-1, it is contended, no comparable, naturally occurring progenitor has so far been identified and the origin of the virus, in the words of Dr. Luc Montagnier, is "a mystery." Since HIV-1 is not an endogenous human virus (Howard Temin, personal communication) and no naturally occurring ancestor or animal reservoir has been identified, and no animal other than the chimpanzee can even be infected, where did HIV-1 come from? One possibility which must be seriously considered is that the virus could have arisen by recombination with other viruses and human cells in laboratory culture. In contrast to natural conditions, evolution is markedly speeded up in laboratory conditions and by the time such a virus was even recognized, it could well have evolved to the point that its origins would be obscure. For this to happen, though, would require extensive co-cultivation of human cells with an animal virus which already possessed the characteristics of the AIDS virus.

One such virus is the Maedi-Visna virus of sheep, which is closely related to HIV-1 and does cause progressive neurological disease, similar to AIDS dementia, and a progressive pneumonia, similar to the chronic lymphocytic interstitial pneumonitis (CLIP) seen in some HIV-infected individuals. It has been contended by some (Seale, 1986; Strecker, 1986; Siegal, 1986) that HIV was deliberately created as a recombinant between bovine leukemia virus (BLV) and Visna virus grown in human cells.

One problem with such an hypothesis is that it would require that a good deal more was known about lentiviruses than was reflected in the scientific literature of the period in which HIV-1 first arose, which was probably in the early sixties. My alternative hypothesis of laboratory origin requires no premeditation and is consistent with the biology of retroviruses and the prevalence of certain laboratory conditions.

Soviet retrovirus research

Soviet research on the ability of Rous sarcoma virus, a tumor-causing retrovirus of chickens, to cause tumors in other animals dates from at least the late 1950s (Zilber et al., 1957, 1958). By the mid-1960s, Soviet scientists had demonstrated that Rous sarcoma virus could cause tumors in a number of primate species (Zilber, Lapin, et al., 1965, 1966). In 1967, Lapin et al. reported causing leukemia in monkeys by injecting blood from human leukemia patients, and subsequently passing the disease from animal to animal by a filtered preparation. The filtered agents (viruses) reacted with sera taken from human leukemia patients. In 1968, Adzhigitov, Lapin et al., published a report on the possibility of culturing a virus causing leukemia in monkeys.

Work on human cells was obviously under way during this period, and in 1970 Lapin and Iakovleva published an article on the viral nature of human leukemia. In 1972, Zhdanov et al. published a paper on the isolation of a leukovirus (a leukemia-causing virus) from a continuous human cell line. The virus was a C-type retrovirus (like the AIDS virus) which did not react to mouse or avian leukovirus antisera and showed some annealing of its reverse transcribed DNA to RNA from the spleen of a leukemic patient. However, the last sentence of the paper contains the comment, "Another possibility is that a virus derived from cattle had been introduced into the culture with the bovine serum used in many culture passages." A subsequent paper (Zhdanov et al., 1973) on isolation of oncornaviruses (tumor viruses) from continuous human cell cultures again notes; "Besides a human origin, they may be contaminants arising from the calf serum."

Zhdanov's concern about contamination of his serum was well founded, since cattle viruses, including leukemia viruses (Khoklova and Rakhmanin, 1970), are widespread in the Soviet Union. It is quite obvious that the Soviets had, in their usual ham-handed manner, unwittingly anticipated Geogiades' 1978 demonstration that human leukemia cells could be infected with bovine Visna virus (BVV), now known as bovine immunodeficiency virus (BIV), and had been doing so for years.

It appears that viruses weren't the only contaminants in Soviet cultures. In May 1972, Nixon negotiated an agreement for biomedical cooperation with the Soviet Union. In November, a group of American cancer researchers presented their Soviet colleagues with a set of animal tumor viruses. In return the Russians presented the Americans with six cultures of cancer cells, all of which contained viruses which the Soviets suspected were the cause of the malignancies.

It turned out that all six cultures, from six different Russian cancer patients, were all the same cells from an American black female who died in 1951, Henrietta Lacks of Baltimore, Maryland. These cells, known as HeLa cells, were the first successfully cultured human tumor cell line and are used all over the world. They have been in use since 1952 and are particularly tenacious cells, capable of prolonged survival, and will tend to take over any culture which they contaminate, and eliminate the original cells. It is probable that HeLa contamination is widespread in the Soviet Union, where facilities and technique in many areas of biological research are quite crude. One consequence of this sloppiness was an outbreak of leukemia in the baboon population of the Sukhumi Monkey Colony, which started in 1967 and continued for several years (Lapin, 1976).

Since 1971, a virus which looks exactly like a tumor-causing retrovirus has been observed in some HeLa cells. This so-called HeLa virus is widespread in human cell cultures in Europe (Gelderblom, 1976), but uncommon in cultures from the United States. Since the Soviet Union acquires its reagents in Europe, it is not unreasonable to suspect that this virus is contaminating the HeLa cells which are contaminating its cell cultures.

Given the crude state of Soviet virology in terms of facilities and the documented clumsy contamination of cultures by both viruses and cells, it would appear that Soviet virus research facilities function as a culture medium for recombination and generation of altered virus species regardless of any intent on the part of the researchers involved. The Soviets obviously grew human cells in cultures contaminated by known, and unknown, retroviruses, including the most likely candidate as a precursor of HIV-1, and then presented the products of their sloppy technique as valid scientific discoveries. Likewise contamination of vaccines would be a major problem in such a situation.

Contamination of vaccines would account for the widespread prevalence of HIV-1 in Africa among the general population. Since most of the vaccines used in Africa originate in Europe, this would explain why HIV infection in the

United States was initially confined to certain groups. It would also indicate that the conditions for such contamination were more prevalent in parts of Europe and the Soviet Union than in the United States. Thanks to the recent emphasis on budget cutting in the United States, similar conditions can be expected to develop in more and more laboratories here. But the point is that during the period in which the AIDS virus can be presumed
1960s, conditions in U.S. and Western European laboratories were much better than in Soviet and Eastern European laboratories. The probabilities were therefore greater that an accidental recombinant could arise, and go undetected, in Soviet laboratories and the evidence would indicate that this indeed occurred.

Bovine retroviruses

In order to provide the reader with some generalized background on the way in which the relevant viral recombinations could have occurred through a laboratory accident, I shall now present an overview of the development of the field in the last decades.

Bovine Immunodeficiency-like Virus. In 1972 Van Der Maaten et al. reported on the isolation of a virus similar to Maedi-Visna virus from cattle with persistent lymphocytosis (elevated numbers of white blood cells known as lymphocytes). This virus produced syncytia (cell fusion with resulting multinucleated cells) in cell culture, but was distinct from bovine syncytial virus (BSV) and bovine leukemia virus (BLV) (see below).

This virus, which causes persistent lymphocytosis, lymphadenopathy (swollen lymph glands), lesions in the central nervous system, progressive weakness and emaciation (similar to the "slim disease" seen in HIV-infected Africans) was isolated from white blood cells of cattle and called bovine Visna-like virus (BVV).

Subsequently, human cell cultures, derived from leukemic bone marrow, were successfully infected with this virus (Geogiades et al., 1978 and apparently the Soviets much earlier, although they didn't realize it). More recent studies (Gonda et al., 1987) indicate that this virus has a morphology most similar to the human immunodeficiency virus (HIV) and serological analyses demonstrate conservation of antigens between major core proteins of bovine Visna-like virus (BVV) and human immunodeficiency virus (HIV). Shared antigenic determinants with other pathogenic lentiviruses were also observed. Nucleotide sequence analysis of the highly conserved RT domain of molecular proviral clones and the serological data show that this virus is a novel lentivirus related to HIV and other lentiviruses. Gonda et al. now propose to name bovine Visna-like virus (BVV) bovine immunodeficiency-like virus (BIV) to indicate its biological similarity and genetic relationship to HIV.

Visna virus itself causes a slowly evolving neurological disease of sheep and a progressive pulmonary inflammation, almost identical to the chronic lymphocytic interstitial pneumonitis (CLIP) which occurs in humans infected with HIV. Visna virus has been shown to grow in choroid plexus cells of sheep, calves, and in sheep liver and kidney cells (Sigurdsson et al., 1960; Thormar, 1961, 1963). In addition infective virus has been obtained from infected choroid plexus cells of dogs, cats, pigs and humans (Thormar et al. 1962).

Visna virus can multiply and produce cytopathic changes in cultures of bovine (cattle) and porcine (pig) origin (Harter et al. 1968). Hybridization studies show a substantial amount of homology between HIV, Visna, caprine arthritis-encephalitis virus, and equine infectious anemia viruses (Gonda et al., 1986).

Contamination of fetal calf serum

Fetal calf serum, or fetal bovine serum (FBS), is a major component of almost all cell culture and tissue culture media. The serum is obtained by bleeding fetal calves, allowing the blood to clot, and then centrifuging it to remove the red cells and clot. The resulting serum is pooled and may or may not undergo additional purification. Virus contamination of such sera has been a recognized problem since the late 1960s at least (Molander et al., 1968). In one study (Kniazeff et al., 1975) 25% of 20 lots of FBS, pretested by suppliers and considered to be virus free, were found to contain endogenous bovine viruses. The techniques of detection were relatively crude and would not have detected latent or slow acting viruses. A number of investigators have reported spontaneous induction or production of retrovirus-like particles in human cell lines. These viruses resemble bovine Visna-like virus (BVV), now designated bovine immunodeficiency-like virus (BIV) and grow in human diploid cell lines (Demidova et al., 1975).

In view of the documented ability of retroviruses to alter their host range in tissue and cell culture, specifically the ability of BIV to infect human cells, it is entirely possible, and indeed probable, that a form of BIV with human cell tropism could have arisen in human cells grown in BIV-infected culture media. The recently isolated immunosuppressive virus of cats, FTLV (feline T-lymphotropic lentivirus, Pedersen et al., 1987) is morphologically similar to HIV and BIV and could well have arisen in feline cells grown in BIV-contaminated serum.

Why the origin of AIDS is significant

In conclusion, I would like to emphasize that the more accurate a hypothesis as to the origin of AIDS, the more clues science would have in combatting its spread. *Executive Intelligence Review* welcomes responses from readers who may have some clues to this issue. In exchange, we shall send our correspondents the 39 references to this article which cannot be printed for space reasons.