

Human retroviruses in leukaemia and AIDS: reflections on their discovery, biology and epidemiology

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ABSTRACT

The study of retroviruses has had a profound impact by unveiling an unusual form of viral replication: the multiplication of RNA viruses *via* a proviral DNA, for which Jan Svoboda provided the experimental model over forty years ago. In 1970 Temin, Mizutani and Baltimore discovered that this group of viruses contains a unique enzyme catalysing the synthesis of a DNA copy of the viral RNA: reverse transcriptase (RT). The discovery of RT has itself had an enormous impact on molecular biology in general, but also stimulated many premature claims of its detection in human disease. Claims by Gallo's laboratory that the cytoplasm of human leukaemia cells contained RT proved to be unfounded, as did his report in collaboration with Weiss that myeloid leukaemia contained HL23 virus, this organism proving not to be human but a laboratory contaminant of three monkey viruses. Conclusive demonstration of a retroviral involvement in human leukaemia was first provided in 1981 by Hinuma and his associates, showing that adult T-cell leukaemia (ATL), a rare form of leukaemia endemic to south-west Japan, is caused by a new retrovirus (ATLV). Other publications in December 1980 and through 1981 claimed the discovery of a new human T-cell leukaemia virus involved in mycosis fungoides (MF) and Sézary's syndrome (SS). This virus was termed HTLV by Gallo. The nucleotide sequence of ATLTV is strongly conserved, that of my 1983 isolate from a black British ATL patient being practically identical with the Japanese virus isolates.

After AIDS was recognised in 1981 by Gottlieb and coworkers as a new human disease, several papers were published by Gallo and his associates during 1983–4, invoking the oncovirus responsible for adult T-cell leukaemia as the cause of AIDS. In 1983 the French scientist Barré-Sinoussi and her colleagues succeeded in isolating a new agent in the disease, a lentivirus, which they named LAV. The French immunologist Klatzmann and his colleagues discovered that LAV killed CD4+ T-cells, furnishing an explanation for the pathogenesis of AIDS and providing a mechanism for how AIDS developed. For some time Gallo continued to suggest leukaemia virus involvement, claiming that his independent isolate of the AIDS virus, termed HTLV-III, was closely related to HTLV-I (the Japanese ATLTV). Although this created considerable confusion among researchers for a period, the relationship was eventually disproved. Unlike ATLTV, whose nucleic acid sequence is very stable, the AIDS virus (now termed HIV by international agreement) is extraordinarily unstable, the sequences of independent HIV isolates being quite unique: this made it possible to establish conclusively that both HTLV-III and another independent isolate CBL-1, from Weiss' laboratory, were actually LAV isolates from the French laboratory.

It has been shown by Hayami and his associates that only African primates are infected with similar lentiviruses to HIV which explains why AIDS started in Africa. Further research has clarified the origin of HIV-1 to be a chimpanzee lentivirus and HIV-2 to be the sooty mangabey lentivirus, which began to spread in humans perhaps no more than fifty years ago. The infection has spread rapidly, primarily through sexual intercourse, but also by transmission through blood and its products as well as contaminated needles and syringes. Sexual intercourse has now spread the virus around the World; and there are probably some 70 million infected. 90% of those infected with HIV develop the deadly disease of AIDS within ten years of infection: the death toll from the disease has been enormous. By contrast, HTLV-1 has been infecting man in isolated areas probably for hundreds of years; but it has not spread widely. HTLV causes leukaemia in only less than 1% of those infected.

The prime mode of transmission of HTLV-1 is between mother and neonate; infections can be reduced by stopping breast-feeding by infected mothers. The isolation of HIV enabled screening tests to be developed for contaminated blood. However, due to the peculiar biology of HIV infection, unfortunately all efforts to develop an effective vaccine have so far failed.

Key words: retroviruses, adult T-cell leukaemia, oncoviruses, ATLV/HTLV, AIDS, lentiviruses, HIV.

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I. INTRODUCTION

The title of this article covers two entirely different diseases. Leukaemia is a cancerous growth of the white blood cells; the virus found to cause adult T-cell leukaemia (ATL) transforms thymus (T)-derived blood lymphocytes so that they proliferate uncontrollably; it is a very rare disease, the virus not being readily transmitted. By contrast, the viruses responsible for acquired immune deficiency (AIDS) have a deadly (cytopathic) effect on susceptible T-cells, and they are far more infectious: during the past twenty years, it is estimated, approximately 70 million people have become infected; well over 20 million have already died and the disease is continuing to spread rapidly. Two reasons justify reviewing these two diseases together.

The first is that the virus found to cause ATL and the two viruses that cause AIDS belong to the large family of retroviridae, enveloped RNA viruses that contain an enzyme reverse transcriptase (RT), that enables this group of viruses to replicate through a proviral DNA. The leukaemogenic virus belongs to the oncoviridae subgroup of retroviridae, while the AIDS-causing viruses belong to a distinct subgroup, the lentiviridae.

The second, historical, reason is that during the early years of AIDS research the causative agents of AIDS and adult T-cell leukaemia were persistently confounded, notably through numerous publications of Robert Gallo and his associates (Essex *et al.*, 1983*a*; Gallo *et al.*, 1983; Gelmann *et al.*, 1983; Schupbach *et al.*, 1984*a*) who claimed that the virus causing ATL was also involved in the development of AIDS. This overshadowed work by French researchers reporting the isolation of another retrovirus (Barré-Sinoussi *et al.*, 1983). Even after the viruses had been conclusively shown to belong to distinct subgroups, publications of the Gallo group continued to maintain that the agents were very closely related and warranted using HTLV-3, human T-cell leukaemia virus-3, as a designation for the AIDS virus (Sarnagadharan *et al.*, 1985), as if it were a variant of HTLV-1, the virus that causes adult T-cell leukaemia.

The reasons why this field of virus research developed in a rather unusual way had much to do with some of the personalities involved, and there remain many unsatisfactory aspects and unanswered questions about the early years of AIDS research. Unfortunately the significance of the matter extended beyond academic squabbling over scientific priority, because it was an early priority to introduce a screening test for the virus in dealing with AIDS. However the regulatory bodies responsible for validating a new screening test could not act before there was a consensus between scientists as to the causative agent: the continuing dispute delayed this process. Thousands of people who may otherwise have escaped will have been infected as a result.

Gallo's laboratory succeeded in propagating an isolate of the AIDS virus which has been subsequently proven scientifically to be one they had received from the Institute Pasteur in Paris (Montagnier, 1984) giving it the designation HTLV-3 instead of the French name, LAV (lymphadenopathy-associated virus). HTLV-3, signifies a variant of

human T-cell leukaemia virus and thus using this name initially implied that this was a different virus. A US patent for the screening test granted to Gallo generated lucrative royalties and Gallo has continued to receive numerous prizes as the co-discoverer of the virus that causes AIDS.

In this review I concentrate on the published record to evaluate the data on their scientific merit. The first part deals with the leukaemia viruses, because most of the fundamental biology and molecular biology of retroviruses was unravelled during early studies of those viruses.

II. LEUKAEMIA

In 1908 the Danish veterinarians Vilhelm Ellerman and Oluf Bang showed that leukaemia in chickens was transmissible by cell-free filtrates and was therefore presumably caused by a virus (Ellermann & Bang, 1908). This was the first virus-induced malignancy to be discovered. Then, in 1911, Peyton Rous showed that a sarcoma in chickens could be transmitted by a cell-free filtrate, indicating that it too was due to a virus (Rous, 1911); he also pointed out that the presumptive agent always caused the same type of tumour in the chick and must therefore carry specific genetic information. Subsequently it was shown that sarcoma viruses are essentially leukaemia viruses that have additional transduced cellular oncogenes. Rous was so much ahead of his time that for several decades his work was unrecognised; it was not until 1966, at the age of 86, that he received a Nobel Prize for the work he had done 55 years earlier.

The next major milestone was the discovery by Ludwik Gross in 1951 of the mouse leukaemia virus (Gross, 1951). This discovery changed the climate of opinion. Not only did it revive interest in chicken leukaemias and sarcoma, but it started a search for possible viral involvement in other animal leukaemias and sarcomas and the first extensive search for viruses involved in the development of the corresponding human malignancies. During the past 30 years, viruses have been found to be involved in the development of leukaemias in an increasing number of animal species. Notable discoveries have included viruses responsible for leukaemia in cats (Jarrett *et al.*, 1964) and cattle (Miller *et al.*, 1969). The first isolation and demonstration of a virus involved in the development of leukaemia in subhuman primates was accomplished in the gibbon (reviewed previously by Karpas, 1989). In 1981 the first conclusive evidence of the involvement of a virus in human adult T-cell leukaemia was published (Hinuma *et al.*, 1981).

The early studies of the animal leukaemias and sarcomata revealed that they contain RNA as their genetic material, and that they are produced in the cytoplasm of the infected cells whence they are usually released through the membrane of infected cells by a process which has been described as budding. The studies by the Czech scientist Jan Svoboda, who infected rats with the Rous sarcoma virus (RSV), enabled him to determine not only that the virus caused the development of a sarcoma in the infected rats but also, more fundamentally, to suggest that this RNA virus probably replicated through a proviral DNA. In contrast to

the chicken sarcoma that produced infectious RSV, the rat sarcoma did not yield infectious virus. However, by co-cultivating rat sarcoma cells with chicken cells infected with the leukaemia virus Svoboda was able to recover an infectious RSV (Svoboda, 1960, 1961, 1962, 1964). Those early experiments provided at least two fundamental observations. Firstly, that the sarcoma and leukaemia viruses were genetically closely related; and the leukaemia viruses could readily complement the defective genome of the sarcoma viruses. Thus an infectious sarcoma virus was basically a leukaemia virus containing an additional gene, the *Sarc* gene, that enabled the *in vitro* and *in vivo* transformation of fibroblasts. Secondly and most importantly, the persistence of the sarcoma genome in virus-negative rat XC cells demonstrated that the Rous sarcoma virus RNA genes must be retained in the cells in a proviral DNA form. This was long before reverse transcriptase (RT) was discovered. In order to prove his hypothesis Svoboda initiated with his Czech compatriots, Hill and Hillova an experiment to try to prove that the nuclear DNA of the RSV-transformed rat cells contained the proviral DNA of RSV. Regrettably the initiation of these experimental studies coincided with the Soviet block invasion of Czechoslovakia in 1968. Hill and Hillova fled to France but subsequently were able to restart their research in Paris. The results of their experimental studies confirmed that the genetic material of RSV was carried in an integrated proviral DNA within the nucleus of the RSV-transformed rat cells (Hill & Hillova, 1972). Chicken cells that were transfected with nuclear DNA from the RSV-transformed, but virus-negative rat cells yielded infectious RSV. Subsequently, we were able to extend those observations to mammalian retroviruses (Karpas & Milstein, 1973). The experimental studies of Hill and Hillova confirmed Svoboda's earlier pioneering observation and suggestion that the genetic material of RNA tumour viruses must be integrated within the nucleus of the infected cells in a DNA form. The explanation as to how an RNA virus can be transcribed into DNA was provided by Temin and Mizutani (1970) and independently by Baltimore (1970) when they showed that the chicken leukaemia viruses contain a unique enzyme, which Temin named RNA-directed-DNA-polymerase and was subsequently renamed Reverse Transcriptase (RT). Thereafter RNA viruses that contain RT were grouped together under the name 'retroviridae'. This group was further subdivided into three subgroups.

(1) **Oncoviridae**: morphologically consisting of four distinct particle types: A-, B-, C-, and D-type. Many members of this group have been shown to be oncogenic in numerous species in nature, e.g. the C-types producing hematological malignancies (leukaemias/lymphoma) and sarcomata or B-types producing mammary carcinomas.

(2) **Spumaviridae**: also called foamy viruses, often isolated in primary cell cultures, causing cytoplasmic vacuolisation and cell degeneration in tissue culture. Morphologically, the foamy viruses resemble oncoviruses, but are not yet known to be the causative agent of any disease.

(3) **Lentiviridae**: also called slow viruses, often cytopathic to the cells in which they replicate. Viruses belonging to this group are known to cause slowly progressive disease in some

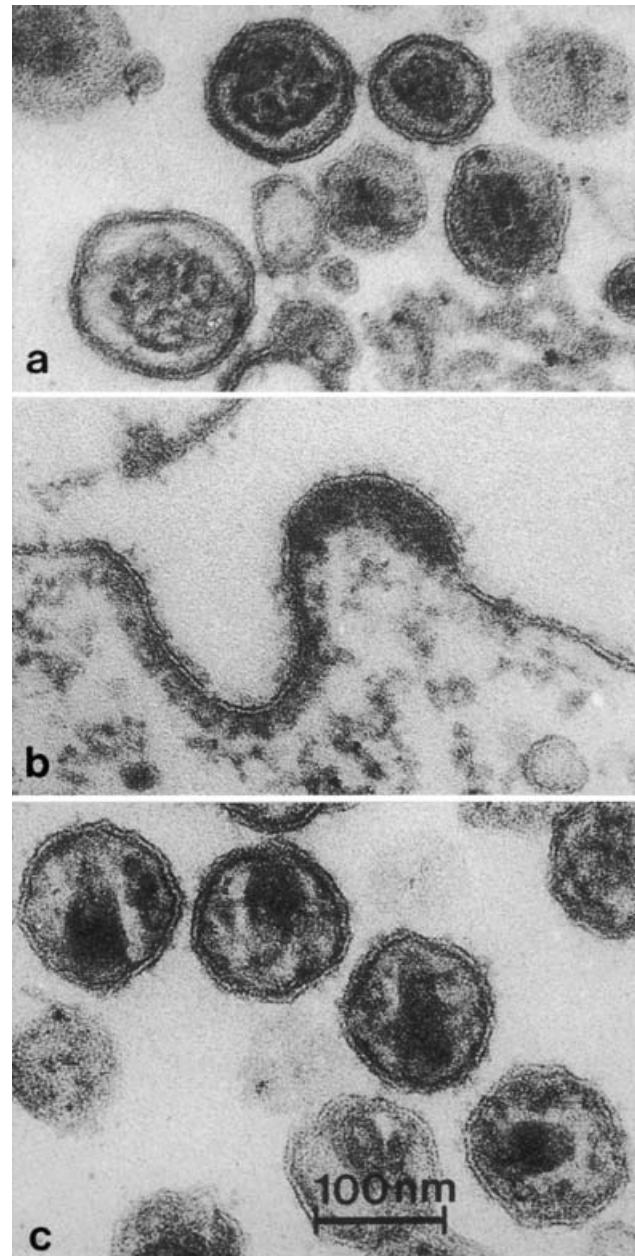


Fig. 1. Electron micrographs of ultra-thin sections of virus infected cells. (A) Cell infected with ATLV/HTLV-1 causing adult T-cell leukaemia. It has a C-type oncovirus morphology of an enveloped electron dense round core. (B) Budding of HIV-1 from the cell membrane. This stage of the virus cycle is indistinguishable from that of C-type oncoviruses. (C) HIV-1 with an enveloped bullet shaped central core which is typical of lentiviruses. Scale bar in C applies to all parts.

of the affected animals and AIDS in man. Morphologically they are distinct from any of the oncoviruses (Fig. 1).

(1) **Transmission of leukaemia viruses**

Under natural conditions, leukaemia viruses are transmitted by various modes in different animal species.

In some species, such as the domestic cat, leukaemia is a highly infectious disease, transmitted in much the same way that humans catch cold. A cat not previously exposed to the feline leukaemia virus (FeLV) becomes infected on contact with an infected cat secreting FeLV in its saliva. Some infected cats develop an immunity to the virus and remain protected from subsequent infection, others develop leukaemia and die. In other species, such as the mouse, the transmission is genetic. When mice from a strain with a moderate incidence of leukaemia are inbred, the incidence of spontaneous leukaemia in their offspring increases significantly. Continuous inbreeding between such mice results in strains in which almost all the mice eventually develop leukaemia. However, leukaemia does not appear in leukaemia-free strains of mice, even after continuous experimental exposure to the virus from the time of birth, including suckling from mice secreting the virus in their milk. In the domestic chicken under natural conditions, both contact and genetic transmission of leukaemia viruses can occur (Gross, 1970).

Despite close physical association between men and cattle and cats in whom leukaemia is transmitted by contact, epidemiological studies have not revealed any increase in the incidence of human leukaemia. Furthermore epidemiological studies on the incidence and possible transmission of leukaemia in man have not revealed any transmission by human contact. The vast majority of leukaemia patients are found to be the single case within their family or particular social surroundings (e.g. their school), although a few observations of a higher incidence of leukaemia within families have been reported [up to 13 cases in one large family (Gunz *et al.*, 1978)]. One epidemiological study from the United States presents evidence that when one identical twin develops leukaemia there is a one in five chance that the other twin will also develop the disease; but this was not confirmed in a similar study in Great Britain (Till *et al.*, 1967). Another study suggests that when leukaemia affects a non-identical twin, the other twin's chance of developing the disease is probably increased (Miller, 1967).

These observations do not suggest that direct genetic transmission of the disease is likely in humans, although they would not be incompatible with the existence of a transmissible predisposing agent. Further evidence for genetic predisposition is the higher incidence of the disease in certain groups of people with genetic abnormalities. There is a 20-fold higher incidence of leukaemia in children with Down's syndrome, a group noted for its lower resistance to infections generally.

Suggestive evidence that leukaemia in humans may be transmitted by viral genetic material has been reported by Fialkow *et al.* (1971) and by Goh & Klemperer (1977) from studies of leukaemic patients who had been treated by bone marrow transplantation. In this form of treatment both the leukaemic and the normal blood-forming cells of a patient are killed by cytotoxic chemotherapy or irradiation before a marrow transplant is given. The studies were conducted on female patients who were transfused with compatible bone marrow cells (those of a brother) to replace their own leukaemic cells. The transfused male cells repopulated the female patients' blood-forming organs, and the patients

went into remission. Analysis of the chromosomes of the circulating blood cells showed that they were of donor – in this case, male – origin. When the leukaemia recurred, months later, analysis of the chromosomes of the leukaemic cells showed them to be the male donor cells. Whereas the donors (brothers) remained healthy, their transplanted white blood cells (WBC) had become leukaemic in their sisters. Fialkow *et al.* (1971) suggested that an *in vivo* transmission of a virus had occurred.

In general, epidemiological studies point to a greater than average incidence of leukaemia in persons affected by genetic abnormalities or exposed to radiation. However, since most radiation-exposed individuals do not develop leukaemia, it is not unreasonable to suggest that if proviral DNA is an essential cofactor in the development of leukaemia, such genetic material is probably present only in a small percentage of the population.

Attempts to isolate a human virus by injecting leukaemic material into experimental animals have proved fruitless; therefore, investigations have been largely confined to studying human leukaemic cells *in vitro*. Repeated efforts by numerous researchers in the United States and Europe to isolate a retrovirus *in vitro* directly from fresh human leukaemic cells have been unsuccessful. However, the failure to isolate a virus directly from fresh leukaemic cells of the most common types does not rule out the possibility of viral involvement in the development of the disease. It has been shown in some animal leukaemias that leukaemic cells that do not release virus *in vivo* may eventually do so, after prolonged *in vitro* culture. The virus may be produced either spontaneously or on induction by a chemical agent such as iododeoxyuridine (IUDR), a halogenated pyrimidine nucleoside.

(2) Isolation of animal retroviruses from human leukaemic cells: HL23 virus

Following the discovery of RNA-directed DNA polymerase (reverse transcriptase) a number of reports on the isolation of retroviruses from fresh and *in vitro* cultured human leukaemic cells appeared. They subsequently turned out to be animal retroviruses and thus usually were accepted as accidental laboratory contamination. A human retrovirus designated as HL23 (for human leukaemia) virus was isolated from fresh leukaemic cells by Gallagher and Gallo (1975). In a second publication Weiss' group together with Gallo stated that 'although closely related, the HL23V-1 virus and SSV [simian sarcoma virus] are not identical ...' and that 'genomic differences do exist between HL23-1 (A-204) and SSV ...' (Teich *et al.*, 1975). Two groups of investigators (Ohino *et al.*, 1976; Okabe *et al.*, 1976), prompted by these publications, were able to establish that the virus in question was a mixture of several monkey retroviruses, the gibbon leukaemia virus and its sarcoma counterpart, simian sarcoma virus (SSV) as well as the baboon endogenous virus (BaEV), which is a distinct primate retrovirus. In further reports it was claimed that fresh human acute myeloid leukaemia cells contained baboon endogenous virus (BaEV) sequences (Wong-Staal, Gillespie & Gallo, 1976); another study by Weiss' group reported

that 'of 39 sera, 100% showed reactivity against baboon endogenous virus, whereas only 49% showed reactivity against simian sarcoma-associated virus.' (Kurth *et al.*, 1977). Two independent US teams of researchers were eventually able to show that the procedures employed by Kurth and Weiss gave rise to false positives (Barbacid, Bolognesi & Aaronson, 1980; Snyder & Fleissner, 1980).

(3) Human T-cell malignancies

The most common form of T-cell malignancy in man is acute lymphoblastic leukaemia (T-ALL). It affects mainly children, amounting to 5% incidence of the total number of human leukaemias, while mycosis fungoides (MF) and Sézary syndrome (SS) are chronic forms of T-cell leukaemia occurring mainly in advanced age, representing 2% of the human leukaemias. T-cell chronic lymphatic leukaemia is extremely rare. The above forms of leukaemia are non-endemic and occur worldwide. By contrast, adult T-cell leukaemia (ATL) is very rare in most parts of the world; but it is endemic in south-west Japan and in Japanese from that area who migrated to Brazil. It is also more common in some parts of Africa, and so occurs in some blacks of African origin in the USA, the Caribbean, and in some who migrated to the UK.

(a) Adult T-cell leukaemia virus and disease

In 1977 the first study of an endemic form of leukaemia was reported from Japan (Uchiyama *et al.*, 1977). The Japanese described a form of thymus-derived (T-cell) leukaemia, clustering among patients in south-west Japan and characterised by pleomorphic malignant T-cells with highly lobulated nuclei. Since it occurred only in adults, it was named adult T-cell leukaemia (ATL). Thereafter, Miyoshi *et al.* (1979) reported the development of the MT-1 cell line which grew indefinitely *in vitro* from the leukaemic cells of one of the ATL patients. Hinuma *et al.* (1981) studied the MT-1 cell line reporting that it produced a C-type virus and that iododeoxyuridine (IUDR) significantly amplified the expression of the virus and its antigen during the growth of the cells. Most importantly, they showed that the MT-1 cells contained an antigen that reacted with sera from 100% of patients with ATL, and that 26% of healthy adults in the endemic area of south-west Japan had antibodies to the same antigen. By contrast, only a few (2/105) healthy adults from other parts of Japan had antibodies to the virus. The viral antigen reacting with the ATL sera was not detectable in B- or T-human lymphoid cell lines derived from non-ATL malignancies. They therefore named their virus adult T-cell leukaemia virus (ATLV), now more widely known as HTLV-1. This paper established a very strong case for a causal relationship of the Japanese ATLV with the corresponding disease. Seroepidemiological studies from Japan indicate that as many as one million Japanese may be infected with ATLV/HTLV, with most living in the endemic areas of south-west Japan. These studies also indicate that approximately 1 in 1000 of ATLV-infected males and 1 in 3000 of ATLV-infected females in Japan are likely to develop the disease (Y. Hinuma, personal communication).

At about the same time as the work of Hinuma *et al.* (1981) it was reported from Japan that ATLV can infect, transform and immortalise normal human T-cells following co-cultivation of leukaemic T-cells with cord white blood cells from non-infected fetuses (Miyoshi *et al.*, 1981) without the use of additives such as T-cell growth factor (IL-2). The following year, Yoshida, Miyoshi & Hinuma (1982) published the molecular characterisation of ATLV and provided the most important confirmatory evidence that ATLV is involved in the development of ATL, by detecting proviral DNA of ATLV in fresh leukaemic cells of each of the five patients with ATL used in the study. Each patient's leukaemic cells were shown to contain a single copy of ATLV. The site of integration of the provirus within the cellular DNA differed between different individuals, suggesting that there was no specific site of integration. There followed a complete nucleotide sequence of ATLV proviral DNA, showing that it was made up of 9032 nucleotides and contained, in addition to the *gag*, *pol* and *env* genes, a region designated pX (Seiki *et al.*, 1983).

The first, and to the best of my knowledge the only, complete nucleotide sequence of ATLV/HTLV-1 outside Japan was obtained in my laboratory by Karim Malik from a virus I isolated in 1983 from a patient of Caribbean origin with ATL in London. It revealed only 2.3% difference from the Japanese ATLV sequences (Malik, Even & Karpas, 1988). Unlike epidemiological studies of ATL in south-west Japan, other studies of ATL have so far failed to provide evidence of the existence of an endemic area in the Western hemisphere. An early report of ATL in blacks showed that seven were born in different states of North America and two were from the Caribbean (Bunn *et al.*, 1983). Screening for antibodies to human T-cell leukaemia virus (HTLV/ATLV) in the West Indies provided evidence of the virus' sporadic distribution. Only 3% (11/336) HTLV-serum positive individuals were recorded in St Vincent (Blattner *et al.*, 1982) and 1% (2/191) in Dominica (Hunsmann *et al.*, 1983). Large-scale movement of people from different tribes and locations in Africa and their subsequent dispersal in the West, mean that it is not surprising that no endemic area like that in southwest Japan has been found in the American continent. The failure to find one so far indicates that the virus, although exogenous, is not easily transmitted. As in Japan, it appears to be transmitted mainly from husband to wife (Tajima *et al.*, 1982) or from mother to child (Kumoro *et al.*, 1983). An example of intercontinental transfer of ATL and ATLV was documented in the case of a 49-year-old Japanese woman, born in the endemic area of south west Japan, who emigrated to the United States 26 years before developing ATL (Haynes *et al.*, 1983). The appearance of the disease and virus in the United Kingdom would be explained by two intercontinental transfers, from Africa to the West Indies, and more recently from the West Indies to Britain.

(b) Transmission of ATLV/HTLV

The natural modes of transmission are primarily from mother to baby *via* infected milk, and can also occur during sexual intercourse and *in utero*. The non-natural forms of

transmission are *via* contaminated syringes, and in recipients of blood transfusions. Retrospective study on the transmission of ATL/HTLV following blood transfusion showed that only 63.4% (26/41) seroconverted after transfusion of whole virus-infected blood, while none of the 14 recipients of ATL/HTLV-infected plasma seroconverted (Okochi, Sato & Hinuma, 1984). This indicates that cell-free virus is not infectious *in vivo*, an observation that has been confirmed also *in vitro*. Maternal milk of infected mothers usually contains ATL/HTLV-1 infected cells and is considered the main mode of transmission to the newborn. Furthermore, HTLV-1 infected cells have been detected in cord white blood cells (WBC) as well as in the peripheral blood of children who have not been breast-fed. Most infections during sexual intercourse are from male to female, probably *via* infected cells that are phagocytosed by vaginal macrophages.

(c) Pathogenesis

Most infections with the virus occur shortly before or after birth. Those infected develop both humoral and cellular immunity against the virus. This immune response appears to be very effective. Over 99% of those infected never develop the disease; even those who eventually develop leukaemia do so only many years after infection, hence the description adult T-cell leukaemia. In Japan the average age for ATL development is 52.7 years. It was initially recognised as a distinct form of leukaemia because of its unique pathology with malignant cells with highly lobulated nuclei. From the onset of symptoms the clinical deterioration is rapid and does not respond to treatment. Many patients have skin lesions such as erythroderma and nodule formation, as well as lymphadenopathy, hepatosplenomegaly and hypercalcaemia. Clinically, ATL appears to be the most aggressive form of T-cell malignancy with nearly 90% mortality within the first year after the onset of clinical symptoms. Even in those cases where remission is achieved its duration is very short.

Hinuma *et al.* (1982) observed that fresh leukaemia cells from ATL patients do not express the virus antigens. This suggests that the immune response against HTLV-1 is probably very effective; and only when malignant transformation occurs without the expression of viral antigen can the transformed cells escape immune surveillance and proliferate. The emergence of such cells is probably very rare: where malignancy has occurred the leukaemia appears to have arisen from a single cell, since the proviral DNA is monoclonally integrated in the entire cell population of a given individual. However, the site of the proviral integration within the chromosomal DNA differs between individual patients. Evidently there is no specific site of integration that leads to malignant transformation without viral antigens being expressed (Yoshida, Miyoshi & Hinuma, 1982). Karyotype analysis of malignant cells also reveals a range of chromosomal abnormalities.

Immunological phenotyping has shown that in most cases the malignant cells are virally transformed CD4+ T-cells. Serological studies show that all infected individuals possess throughout life high levels of anti-HTLV antibodies,

including neutralising antibodies; and it has been reported that cell-mediated immunity to HTLV-infected cells is present in the blood of healthy HTLV-infected individuals.

HTLV-1 can also cause neurological disorders known as HTLV-associated myelopathy (HAM) which is also called tropical spastic paraparesis (TSP) (Gessain *et al.*, 1985; Osame *et al.*, 1986) which are chronic demyelinating diseases.

(d) Molecular biology of HTLV

As outlined above, practically all the pioneering molecular studies on ATL/HTLV-1 have been carried out by Yoshida's group in Tokyo. The structure of the provirus genome is similar to proviruses of other oncoviruses. It contains the long terminal repeats (LTR) and the three genes *gag*, *pol* and *env*. The LTR contain the *cis*-acting sequences important in the regulation of viral gene expression. The full-length viral mRNA encodes the *gag*, *pol*, and protease gene products and is also utilised as the genomic RNA packaged into the virion (Fig. 2).

The *gag* gene encodes the virion core proteins initially synthesised as a precursor which is subsequently cleaved to form the mature matrix protein p19, capsid protein p24 and nucleocapsid p15. The viral protease cleaves the *gag* precursor to generate the mature viral core proteins. The *pol* gene encodes reverse transcriptase (RT), and RNase H integrase. The *env* gene encodes the surface glycoprotein gp46, which functions in receptor recognition and binding, and the transmembrane protein p21, which functions as the envelope anchor and in membrane fusion. Unlike most oncoviruses HTLV, like bovine leukaemia virus, contains the regulatory proteins Tax and Rex. The Tax p40 is involved in the initial stage of viral replication, functioning in the transcriptional activation of the viral LTR, and therefore is essential for viral replication. The Rex p27 functions post transcriptionally, regulating the mRNA of *gag*, *pol* and *env*. The Tax p40 is considered to be a key product during cellular transformation, since it also transactivates several cellular genes, some of which are implicated in cell transformation (for further details about the function of Tax and Rex see Green & Chen, 1994).

(e) Studies of adult T-cell leukaemia in the United Kingdom

It was in the United Kingdom that adult T-cell leukaemia (ATL) was first recognised to affect blacks (Catovsky *et al.*, 1982). The patients were of West Indian (African) origin, and to date not a single case of ATL has been diagnosed in British Caucasians. In 1983 I isolated the virus from two ATL patients in London (1010 and 1025 isolates) and later from two more ATL patients. Following co-cultivation of the patients' leukaemic cells with cord cells, the cord T-cells were transformed, immortalised and produced the virus (Karpas, Malik & Lida, 1987b). Our serological studies confirmed that ATL patients, whether Japanese or blacks, have antibodies to all the structural proteins of the virus. In contrast to the high prevalence (over 25%) of antibodies to ATL in south-west Japan, none of 70 serum samples obtained from healthy blacks of Caribbean origin in London contained antibodies to the virus.

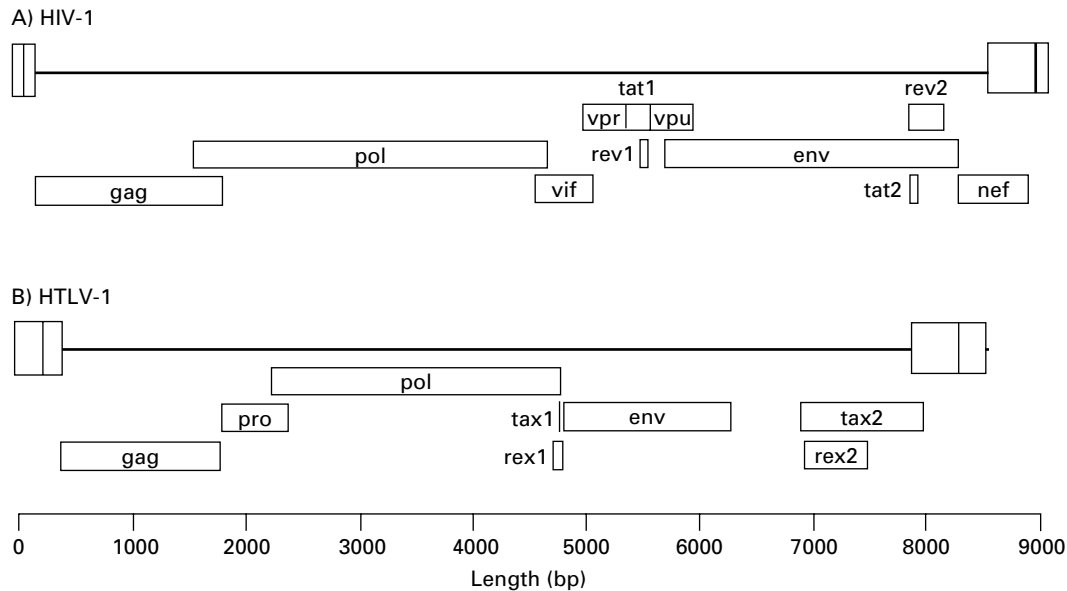


Fig. 2. Genomic organisation of the lentivirus HIV-1 and the oncoretrovirus HTLV-1. In addition to *gag*, *pol* and *env* both retroviruses encode a variety of accessory proteins. The HIV-1 Tat and Rev proteins are responsible for transcription activation and transport of unspliced mRNA, respectively. Vif aids productive infection in certain cell types. Vpr encodes a cell cycle arrest function and also aids transport of preintegration complexes into the nucleus. Nef reduces the level of CD4 on the surface of infected cells, whereas Vpu promotes degradation of newly synthesised CD4 and thereby aids virus assembly. The HTLV-1 accessory proteins Tax and Rex are analogous to HIV-1 Tat and Rev in that Tax stimulates transcription and Rex promotes the transport of unspliced mRNA to the cytoplasm.

In view of a putative relationship between HIV and HTLV we screened sera from 400 HIV-positive homosexuals in London for antibodies to HTLV-1/2 using the cell test (Karpas *et al.*, 1985). Only two were positive. Both were born in the United Kingdom, one was black of Caribbean origin, the other white with a black sexual partner. Our molecular hybridisation studies showed no cross-hybridisation between HTLV-1 and HIV (Karpas *et al.*, 1987*b*). Sera from patients with other haematological malignancies or multiple sclerosis did not possess antibodies to HTLV-1.

(f) *Mycosis fungoides/Sézary's syndrome/human T-cell leukaemia virus (HTLV)*

In 1979 van der Loo and associates reported the morphological development of a C-type virus in lymph nodes of patients with mycosis fungoides and Sézary syndrome (MF/SS), both indolent T-cell leukaemia/lymphomas. In their biochemical study (van der Loo *et al.*, 1979), they found that the particles banded at a density of 1.16 g ml^{-1} and contained reverse transcriptase activity. The second report on the presence of retroviruses in human T-lymphoid cells was in December 1980. Poiesz *et al.* (1980) reported the presence of a C-type virus that contained reverse transcriptase activity in a T-cell line established by Gazdar *et al.* (1980) from a patient diagnosed as having mycosis fungoides. The virus was named human T-cell leukaemia virus (HTLV). It is not mentioned whether the patients who gave rise to HTLV had antibodies to the virus.

Careful study of the work on HTLV shows two distinct periods in the published studies. The first is between December 1980 and the early part of 1982. During this period, it was repeatedly claimed that HTLV was involved with MF/SS only. The early published work on HTLV was devoted to the study of reverse transcriptase of the virus (Rho *et al.*, 1981) and to its structural proteins (Kalyanaraman *et al.*, 1981). A separate paper dealt with the molecular hybridisation studies (Reitz *et al.*, 1981). Using a wide range of retroviruses, it was shown that HTLV failed to hybridise with any of the other retroviruses tested. Likewise, the HTLV complementary DNA (cDNA), was also tested against DNA preparations from a wide range of animal species but failed to show any significant level of hybridisation, including human DNA preparations from six normal and one ovarian adenocarcinoma tissues. The failure to show any level of hybridisation led Reitz *et al.* (1981) to conclude that HTLV is not an endogenous human virus. However no single hybridisation study of HTLV with fresh leukaemic cells from patients with T-cell malignancy was described. Despite that, several subsequent publications by the same group refer to the Reitz *et al.* (1981) paper as providing essential molecular evidence to link their HTLV with ATL. The second isolation of HTLV was reported by Poiesz *et al.* (1981) from a patient diagnosed as having Sézary syndrome. The paper included hybridisation studies between the cDNA of HTLV and the patient's fresh leukaemic cell DNA, but reported only 17% hybridisation. This value is similar to the 16% that was considered negative in hybridisation between a cDNA probe of HTLV and

DNA from simian sarcoma virus by Reitz *et al.* (1981). In the Poiesz *et al.* (1981) paper, controlled hybridisation with DNA from normal human tissue gave values of 3–11%. This report was followed by an independent serological study in which the sera of only two of 23 patients with T-cell malignancy and one of 66 normal persons were shown to have antibodies to HTLV (Kalyanaraman *et al.*, 1981). A turning point in the studies of HTLV occurred in 1982 (Catovsky *et al.*, 1982), when it was recognised in the United Kingdom for the first time that some leukaemic black patients of West Indian origin had adult T-cell leukaemia. Hinuma *et al.* (1981) had reported that sera of Japanese patients with MF did not contain antibodies to adult T-cell leukaemia virus (ATLV). Our serological studies of British MF/SS sera failed to detect antibodies to ATLV. Nevertheless, it was still reported up to 1983 (Popovic *et al.*, 1983) that HTLV was involved in mycosis fungoides and Sézary syndrome. A paper by Gallo's group, claiming a common site of integration for HTLV in the leukaemic cells was later retracted when Yoshida's group provided molecular evidence that there was no common site of integration (Hahn *et al.*, 1983). To date there is no evidence for a retroviral etiology of MF/SS.

(g) HTLV-II

The first report on the isolation of HTLV-II appeared in 1982 (Kalyanaraman *et al.*, 1982). It was obtained from the Mo T-cell line, which was derived in 1976 from the spleen of a patient with a rare T-cell hairy cell leukaemia. A second isolate of HTLV-II was reported in 1986 from a patient with a typical hairy cell leukaemia (Rosenblatt *et al.*, 1986). Hairy cell leukaemia is an uncommon form of B-cell leukaemia, representing approximately 1% of all leukaemic cases. Very few cases of hairy cell leukaemia with T-cell phenotype have been reported. The serological studies with HTLV-II Mo virion in an immunoprecipitation assay showed that the sera from the two T-cell hairy cell patients that gave rise to the two isolates (HTLV-II Mo and HTLV-II NRA) reacted only with the p24 antigen. This observation (Rosenblatt *et al.*, 1986) is unlike the reaction of sera from patients with ATL (whether Japanese or blacks) who have antibodies to the other viral structural proteins, including the envelope glycoproteins (gp61). To date there is no evidence that this virus is involved in the development of any disease. Nucleic acid sequence of the HTLV-II genome revealed that it is 70% homologous with HTLV-I which explains the serological cross reaction.

III. AIDS: A NEW DISEASE

In the late 1970s/early 1980s physicians in Florida, New York and California, who were looking after patients with sexually transmitted illnesses, noticed an increasing number of young male homosexuals with a range of opportunistic infections and malignancies which had not been seen before in this age group. The most frequent were pneumocystis carinii pneumonia (PCP), oesophageal candidiasis,

toxoplasmosis of the brain, Kaposi sarcoma and non-Hodgkin's lymphoma as well as other unusual manifestations. In 1981, Gottlieb *et al.* published a detailed study of four young patients (29–33 years of age) who had PCP, candidiasis and multiple viral infections, from whom cytomegalovirus (CMV) was isolated. The authors also noted that the patients were anergic, lymphopenic and had an acquired immune deficiency syndrome (AIDS). Since most early cases of this new syndrome were initially diagnosed in homosexuals, some suggested that it might be the outcome of their 'life style'. Others suggested that it was punishment inflicted by God particularly on the homosexuals but on the promiscuous society in general. AIDS began during the period of the Cold War, so some even suggested that the CIA had created the viruses by genetic engineering. Some prominent research doctors put forward the idea that 'AIDS is an opportunistic infection and Kaposi sarcoma results from secondary immune stimulation' (Levy & Ziegler, 1983).

(1) The viral origin and the background to claims about priority

When it became apparent that the disease was transmissible, a wide range of known microorganisms was suggested as the agent. The first report of the isolation of a new human virus as the causative agent was by a French group from the Pasteur Institute in Paris (Barré-Sinoussi *et al.*, 1983). This early paper reported the first 'Isolation of a T-lymphotropic retrovirus from a patient with AIDS', indicated that their isolate was 'clearly distinct from each previous isolate', and that 'type-specific antisera to HTLV-1 do not precipitate the proteins of the new isolate'. They also noted that the 'viral core proteins were not immunologically related to the p24 and p19 proteins of HTLV-1'. Their electron micrographs demonstrated a virus budding from the cell membrane which was distinct from HTLV-1 but similar to lentiviruses. Simultaneously three articles by Gallo and his associates were published on the involvement of the leukaemogenic virus HTLV-1 in AIDS (Essex *et al.*, 1983 *b*; Gallo *et al.*, 1983; Gelmann *et al.*, 1983), a topic which was further amplified in the same issue of *Science* in an article entitled 'Human T-cell leukaemia virus linked to AIDS' (Marx, 1983).

One month later in June 1983, the French team submitted a second paper entitled 'Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T-lymphocytes' to the London office of *Nature* (L. Montagnier, personal communication). The manuscript conclusively showed that the virus involved in AIDS belonged to 'a new group of human retroviruses that differ from human T-cell leukaemia virus (HTLV-1)'. It also made a most significant observation that the virus was cytopathic to helper T4 (CD4) cells, providing for the first time an explanation of how and why AIDS developed. Regrettably, the manuscript was rejected. The resulting delay before it was finally published (Klatzmann *et al.*, 1984) in *Science*, meant that its medical and scientific audience had been denied crucial information for nearly a year.

During a Cold Spring Harbour (CSH) Laboratory meeting in 1983 on 'Human T-cell leukaemia/lymphoma viruses', Montagnier presented a paper on September 15 on 'A new human T-lymphotropic retrovirus: characterisation and possible role in lymphadenopathy and acquired immune deficiency syndromes'. This presentation contained similar data to those in the rejected *Nature* manuscript and further provided evidence that their virus isolate, in contradistinction to HTLV-1, belonged to the lentivirus subfamily of retroviruses. It included extensive serological and immunological data, electron micrographs showing typical lentivirus morphology, and evidence that the viral isolate (named LAV) propagated *in vitro* and was cytopathic to the helper CD4 lymphocytes, causing cells to fuse with giant cell formation (Montagnier *et al.*, 1984). Twelve days after Montagnier had made this presentation Gallo wrote 'I have never seen the virus that Luc Montagnier described and I suspect that he might have a mixture of two' (Gallo, 1983), and in further publications throughout 1983–1984 maintained the involvement of HTLV-1 in AIDS. Subsequently Gallo's group reported on the 'First isolation of HTLV-III', stating that they 'had evidence for the presence of a new retrovirus in AIDS and ARC [AIDS-related complex] patients long before the LAV particles were sent to us and even before the publication of the results by Barre-Sinoussi *et al.* in 1983' (Gallo *et al.*, 1986).

Subsequent research established that the different AIDS virus nucleic acid sequences are extremely polymorphic, making it feasible to identify unequivocally the precise origin of different HIV isolates. This first revealed that HTLV-III was in fact LAV because of their unique identity (Rabson & Martin, 1985), and then that another isolate, originating from the Chester Beatty Laboratory (CBL) and designated CBL-1 virus (Weiss *et al.*, 1985) was also LAV (Aldhouse, 1991). The virus had been supplied to both laboratories at various times by the French team (L. Montagnier, personal communications).

In 1986 the International Committee on the Nomenclature of Viruses renamed the AIDS virus Human Immunodeficiency Virus (HIV). Prior to this time, numerous publications refer to HTLV-III as the causative agent of AIDS or use the dual designation LAV/HTLV-III or HTLV-III/LAV.

(2) Origin and spread of AIDS

There is now little doubt that AIDS began in Africa (Karpas, 1990). Not only is the disease widely spread in sub-Saharan Africa, but only in Africa are there monkey species naturally infected with lentiviruses related to HIV as was first shown by Ohta *et al.* (1988). Although the first lentivirus was isolated from a macaque (and therefore named SIV_{MAC}), that animal was probably infected in captivity with SIV_{SMM}, for which the African sooty mangabey monkey (SMM) is the natural host.

It is obviously not possible to estimate the exact date when the first person became infected with a primate lentivirus, but the question of when and how the present epidemic began to spread is more tractable. Extensive European contacts with black Africa go back five centuries,

accompanied by the forceful transfer of millions of Africans to the New World. Arabs similarly had contact with West and East Africa over a span of hundreds of years and also engaged in slave traffic. Yet there is no evidence for the existence of HIV in Europe, the Americas or Arabia during the past century or even the first half of the twentieth century, suggesting strongly that the widespread HIV infection in Africa is a recent event.

The earliest serum sample that was found to contain antibodies to HIV-1 was collected in 1958 from Zaire. The earliest case of HIV-1 positive serum outside Africa was reported from Norway, that of a sailor who probably became infected in the mid-1960s and who, together with his wife and third daughter, died of what was retrospectively diagnosed as AIDS (Froland *et al.*, 1988). He may have become infected in an African port and, infecting his wife before or during her third pregnancy, indirectly transmitted the disease to their third child, leaving the two older daughters disease-free.

The epidemiological evidence thus points to the spread of HIV infection from Africa after the Second World War. The spread seems to coincide with the widespread use of syringes and needles from the West, at the time vaccination programmes were being promoted and antibiotics were being introduced. (The early generation of antibiotics came only as injectable medicines and, even now, injectable medication is the treatment of choice in many third-world countries.) It also coincides with a post-war period of greatly improved transport and extensive migration of African peoples.

It is worth noting that HIV is not the first monkey virus to infect man: in 1934, Sabin and Wright reported the isolation of the herpes virus simiae (B virus) from the brain of a laboratory worker who died after a bite by an apparently healthy macaque rhesus monkey. There have since been more than a dozen such cases. Herpes virus simiae seems to be the macaque counterpart to human herpes simplex virus; in macaques, infection is latent and benign. But on transfer to man herpes virus simiae is rapidly fatal. This eliminates the infected host before he or she has a chance to infect others; so this virus does not spread in man. Lentiviruses in the African monkeys also appear to be benign in their natural hosts and are deadly only when transmitted to other species, man or even other monkeys, e.g. the macaque. However, the long latent period associated with lentiviruses, lasting many years after infection, permits multiple transmission before there is any evidence of disease.

Studies of the genomic sequences of the primate retroviruses have revealed 82–89% homology between HIV-2 and the lentivirus (SIV) that has been isolated from a laboratory macaque and from West African sooty mangabeys from the region where HIV-2 is endemic. SIV_{SMM} is therefore thought to have given rise to HIV-2. It was predicted that another primate would be found harbouring the lentivirus precursor of HIV-1 (Karpas, 1987), and lentiviruses similar to HIV-1 have been isolated from chimpanzees with 85% homology in their nucleic acid sequence and the same genomic organisation as HIV-1 (Huet *et al.*, 1990). Studies of numerous HIV-1 isolates have shown most to

belong to one major group, M (Myers, MacInnes & Korber, 1992). This is subdivided into ten subtypes (A–J) on the basis of their nucleic acid sequences.

In 1997 a French team reported the isolation of a new and distinct subtype of HIV-1. It failed to react in those enzyme-linked immunological assay (ELISA) test kits that contain only few of the HIV-1 antigens but reacted with tests that contain the entire virus. Its nucleic acid sequence, although closely related to HIV-1, was found to be distinct from the M group; and it was therefore designated HIV-O for 'outliner' (Peeters *et al.*, 1997). Other isolates of this subgroup were found in Cameroon or could be traced to an infection originating there. Thereafter Simon *et al.* (1998) have identified another new HIV-1, distinct both from M and O groups, which was also recovered from an AIDS patient in Cameroon. Phylogenetic studies show that this new isolate (YBF-30) branches either with SIV cpz-gab or between SIV cpz-gab and HIV-1 group M; while the structural genes and Tat, Vpr and Nef are about equidistant from those of HIV-1 group M and SIV cpz-gab. Since YBF-30 is as distinct from SIV cpz-gab as it is from HIV-1 group M, it can be considered as a prototype strain of a new HIV group.

The most plausible explanation for the recent appearance of two new strains of HIV-1, HIV-O and HIV YBF-30, is continuing cross-over of viruses from primate hosts. It is not unreasonable to suggest that the three subgroups of HIV-1 (M, O and YBF-30) originated from different chimpanzee subspecies.

(3) HIV infections

In the West, AIDS was initially prevalent in homosexuals and drug abusers. However, most of the infected live in third-world countries, mainly in sub-Saharan Africa, India and Thailand. Most infections worldwide occur during sexual intercourse. Needles and syringes are also an important route of virus transmission. The relatively early spread of HIV infections in Scotland, Italy and Spain is attributed to the high rate of needle and syringe sharing between drug users, and in the early days of the epidemic, many haemophiliacs became infected by receiving injections of HIV-1-contaminated Factor VIII.

(a) Sexual infection

HIV-1 has been detected in both seminal and vaginal fluids and within cells and the cell-free fluid. These observations explain why the major route of HIV infections is sexual and why its spread is so difficult to stop. HIV is not an easily transmissible virus and the rate of transmission during sexual intercourse is very low between healthy heterosexuals. But since sexual intercourse is a common activity, most negative partners of an HIV-infected individual will eventually also acquire the infection. The following factors can amplify the frequency of transmission:

Sexually transmitted diseases affect both the infected and the non-infected partner. In the non-infected partner, the normal intact epithelium of the penis or vagina resists HIV infection. However lesions caused by microorganisms such

as *Neisseria gonorrhoeae* or ulcers caused by herpes simplex facilitate entry of HIV. It has been estimated that the receptive partner during anal sexual intercourse is about 100-fold more likely to become infected as compared to vaginal sex due to the presence of abrasions in the anal canal.

Macrophages usually migrate to the surface of lesions whether in the vagina or the penis. The function of macrophages is to phagocytise and kill invading microorganisms and following sexual intercourse, to digest heterologous cells whether in the semen or the vaginal fluid. Unfortunately macrophages are infectable by HIV. They can also become infected following the phagocytosis of HIV-infected cells. HIV does not readily kill macrophages; these therefore can continue their immunological functions, travelling to regional lymph nodes and initiating immune responses. In so doing, they probably spread HIV infection throughout their migratory route, and in particular transfer it to the lymph nodes where large numbers of CD4+ T-cells are present and highly susceptible to the virus. The reaction to the early spread of HIV to the lymph nodes manifests itself as lymphadenopathy; but this often regresses once the body mounts an effective cellular and humoral immune response.

In the infected individual a large number of HIV-infected cells is present in the genital fluid. The presence of other sexually transmitted diseases, such as genital ulcers, increases this number of cells. It has also been suggested that inflammation of the penis could explain the observation that transmission is higher in uncircumcised than in circumcised men.

(b) Transmission by blood and its products

The largest group in this mode of infection is the injectable drug users who contaminate the syringes with their infected blood. This form of infection occurs whenever syringes and needles are reused.

Before the development of screening tests for HIV a significant number of individuals became infected by contaminated blood. After screening tests were introduced, blood and blood products became safer. HIV infection following a blood transfusion now only occurs if the blood donor has been very recently infected, i.e. before detectable antiviral antibodies have appeared in their blood. The most important blood product to be affected by HIV infection was Factor VIII. Many haemophiliacs who received it regularly became infected by contaminated products. However even before the screening tests were developed, the preparations were made safe by heating at 56 °C for 30 min.

Accidental infection occurs also following needlestick injuries with HIV-contaminated blood. The incidence of infection following injury has been estimated at approximately 1 per 300–400 incidents.

(c) Transmission to the newborn

HIV transmission from mother to child occurs in up to 60% of infants born to infected mothers, taking place by direct

infection of the foetus or during birth. Infection of non-infected babies can also occur *via* breast milk. This makes it important to determine the HIV status of babies born to HIV-infected mothers, so that the HIV-negative babies can be prevented from breast feeding.

(d) *Saliva and other body fluids*

Although it has been reported that both free HIV and virus-infected cells have been demonstrated in saliva, there were only low levels of infectious virus.

A study of over 600 incidents of bites failed to detect virus transmission but one case of HIV transmission following a bite has been reported (Vidmar *et al.*, 1996). HIV has also been demonstrated in cerebrospinal fluid.

(4) Cellular tropism

(a) *Primary receptors*

For successful infections the virus needs to lock on to appropriate receptors on the surface of vulnerable cells. CD4+ helper T-cells are the prime target of HIV infection (Klatzmann *et al.*, 1984; Montagnier *et al.*, 1984), thus CD4 is the primary receptor for this virus. Subsequent studies have, however, shown that the CD4 receptor alone is not sufficient, nor is it the only way for HIV to enter cells.

(b) *Secondary receptors*

Chemokine receptors were found to act as coreceptors for the entry of HIV into cells. The first-named CXCR-4 acts as a coreceptor for HIV-1 T-cell tropic strains. Subsequently other molecules named CCR-5, CCR-3 and CCR-2b were found to act as coreceptors for macrophage tropic HIV-1 strains (Berger, 1997).

(c) *Infection of CD4 negative cells*

Numerous cell types throughout the body are infectable with HIV notwithstanding the absence of the CD4 receptor. They range from cells in the brain, intestine and skin, to cells in the heart, kidney as well as other organs (Levy, 1998).

(5) Events following HIV infection

Once the virus enters the cell it uncoats in the cytoplasm. The viral reverse transcriptase (RT) and RNase H synthesise a copy DNA (cDNA) and subsequently the double-stranded DNA provirus integrates within the cell's chromosomes. Integration of the provirus within the cell DNA is random; but it is thought to be essential for the cell to synthesise HIV. HIV RNA is synthesised in the nucleus while the structural proteins are synthesised in the cytoplasm of the infected cells, assembly of the virus takes place at the cell membrane. The viral RNA is incorporated into the protein capsid that buds from the cell surface, coated with the viral envelope proteins.

Many individuals do not develop any noticeable symptoms directly following infection with HIV; however, it is estimated that at least 50% of those infected develop

flu-like-symptoms. The appearance of clinical manifestations following infection with HIV probably depends on the strain of the virus, dose, route of infection and individual variation. These determine not only the wide spectrum of clinical symptoms, but also the great variation in the length of time it takes before these appear. The symptoms can consist of fever, head and muscle aches, sore throat, swollen lymph nodes, rash, diarrhoea, as well as other symptoms including even encephalitis. During this acute phase the individual also becomes viraemic. The major symptoms usually last 1–3 weeks, while lymphadenopathy and lethargy can last for several months. Thereafter the infected individuals usually remain physically well for many years. Antibodies to the virus can appear within a couple of weeks but on rare occasions take several months before becoming detectable in the blood. Disease progression to AIDS occurs in approximately 20% of the infected within five years, and in 70% within ten years. In approximately 10% of those infected it can take longer than ten years.

(6) Genetic heterogeneity of HIV

As soon as the complete nucleic acid sequences of HIV-1 isolates were published it became clear that each independent isolate was distinct from every other, with at least 6–10% differences. When it became widely known and accepted that each HIV-1 isolate had its own 'fingerprint', it became clear that the viruses HTLV-IIIB (Gallo *et al.*, 1984) and CBL-1 (Weiss *et al.*, 1985) were identical to the French isolate, LAV (Aldhouse, 1991).

There are three groups M, N and O and several sequence subtypes of HIV-1 (A to J). Each subtype differs from the others in amino acid composition by at least 20% in the envelope region and 15% in the gag region. Within each subtype the difference in the envelope can be up to 10% and in the gag region by up to 8% (Myers *et al.*, 1996).

(7) Immune response to HIV infection

The immune response to HIV can be compared to that of a live viral vaccine. It explains why most HIV-infected individuals remain well for years. Other viruses that establish lifelong infection, such as herpes viruses, tend to remain latent in the body and the only other exogenous retrovirus known to be capable of infecting humans, the adult T-cell leukaemia HTLV-1, causes disease in less than one in a thousand of the of infected individuals.

In man infection with HIV is probably never latent, because the virus appears to mutate continuously in every infected individual due to its highly error-prone reverse transcriptase (RT) which lacks the proofreading capabilities of other DNA polymerases. This has two consequences: (1) in nearly every infected individual, despite a vigorous immune response that is protective for many years, eventually one or more mutants emerge that manage to evade the immune response and lead to disease progression and death; (2) in drug-treated individuals, a drug-resistant virus emerges and treatment fails to halt disease progression. The continuous mutations of the replicating virus cannot be the only reason for the very high mortality of HIV infection

in man, because the viruses HIV-1 and HIV-2 do not cause disease in their natural hosts, the chimpanzee and the sooty mangabey monkey, respectively. Disease occurs only when the viruses cross species.

In addition to its high mutation rate, HIV can also evade the immune response by direct cell-cell contact through fusion between infected and non-infected cells: the virus can be transferred without being exposed to agents of the immune response, such as neutralising antibodies. This is facilitated by the affinity of viral glycoproteins expressed on the surface of infected cells for CD4 molecules on neighbouring uninfected cells. Probably this process is particularly important in the lymph nodes, where presentation of foreign antigen to lymphocytes by cell-cell contact is an essential step in initiating immune responses. Consistent with this, it is observed that the more disease progresses, the more lymph node architecture becomes disrupted and germinal centres involute and degenerate to a state of follicular lysis.

(a) Cell-mediated immunity

Early after infection with HIV, cell-mediated immune responses can be detected in infected individuals. They include several types of cells such as cytotoxic T-cells (CTL) and natural killer (NK) cells. In addition there are also CD4+ cytotoxic T-cells which can kill HIV-infected or non-infected cells; CD4+ T-cells respond to the infection by producing cytokines that enhance the antiviral immune response. The response of the cytotoxic CD8+ T-cells is directed at the virus-infected cells; the response is human leukocyte antigen (HLA)-restricted, antigen-specific and requires cell-to-cell contact. It is a very efficient way of HIV suppression (Levy, 1998). CTL and NK cells are usually found at high levels during the period of well-being and decline with disease progression. However the study of long-term survivors has revealed HIV-infected individuals who have lost most of their CTL activity but remain well and with a relatively high CD4+ T-cell count (Cao *et al.*, 1995). It is probable that when CTL are lost a high level of neutralising antibodies can by itself delay disease progression.

(b) Humoral immunity

Most infected individuals develop antibodies that are readily detectable in the blood within a few weeks of exposure to the virus. By 1985, a range of assays for the detection of such antibodies had been developed to screen for infected individuals and, in particular, to reduce the incidence of infection through contaminated blood transfusion or blood products. The development and introduction of such tests for the presence of antiviral antibodies greatly reduced the spread of HIV.

Most test methods can give false positive readings, so it is important to check any positive reading by a screening assay with a confirmatory test. Usually the Western blot (WB) test is used for confirmation. However, this is rather an expensive and time-consuming test (requiring overnight incubation); many third world countries are not in a position

regularly to confirm positive readings obtained by the routine screening methods such as ELISA. Since nearly 90% of the HIV infected live in third-world countries, this means that the majority of positive reactions are unchecked.

In 1985, I developed an alternative test method to provide a simple, inexpensive, rapid screening test which contains its own controlled confirmatory test; and when HIV-2 was isolated, cells infected with this virus were added to permit, in a single controlled test for specificity, the determination of whether the individual is infected with HIV-1 or HIV-2 (Karpas *et al.*, 1987*a*). The cell test can also be used to titre the level of antiviral antibodies (Karpas *et al.*, 1990*a*).

The cell test showed that most of the healthy HIV-infected individuals have a very high level of anti-HIV antibodies whereas, in contrast, patients who progressed to AIDS had a low level of antibodies that decreased further with disease progression. Studies of such sera with Western Blot correlate with the cell test titration studies. We have shown that the sera from the healthy HIV infected individuals form a thick band of antibodies for each of the viral structured proteins. In contrast, the sera from the AIDS patients are missing numerous antibodies and even the antibodies that are present are at low concentrations (Karpas *et al.*, 1990*b*).

(c) Neutralising antibodies

The HIV proteins that are involved in viral neutralisation by antibodies are part of the enveloped gp120 and gp41. The most important neutralising domain of gp120 is found in the central portion of the third variable region (V3 loop) which is located in the middle of the gp120. We have assayed for the presence of neutralising antibodies in over 100 healthy HIV-1 infected individuals and without exception found that the sera contained significant levels of such antibodies (Abelian *et al.*, 2001).

One of the earliest studies trying to explore and explain the differences in the immunological state between healthy HIV-infected individuals and AIDS patients was carried out by Karpas *et al.* (1985). Our studies have demonstrated that healthy HIV-1 infected individuals who were not viraemic had high levels of neutralising antibodies against the virus and a CD4+ T-cell count within the normal range while AIDS patients with very low numbers of CD4+ T-cells and high levels of HIV-1 were devoid of neutralising antibodies and had low levels of other antiviral antibodies (Karpas *et al.*, 1988).

Studies from the USA of long-term survivors have also found high levels of neutralising antibodies (Cao *et al.*, 1995; Pantaleo *et al.*, 1995). Although polymerase chain reaction (PCR) assays for the presence of HIV-1 RNA in the plasma revealed significant levels of RNA in some individuals, the biological assay for viral infectivity failed to reveal the presence of infectious virus, suggesting that the HIV-1 in the bloodstream had been inactivated by the neutralising antibodies. In addition, this indicates that PCR does not distinguish between infectious (live) and neutralised (killed) virus. We have recorded similar observations with AIDS patients who were treated with passive immunotherapy

(PIT). AIDS patients before the infusion of hyperimmune plasma were HIV-1 viraemic as monitored by the isolation of infectious virus from the plasma. After the infusion of hyperimmune plasma, infectious virus could not be isolated but many remained PCR positive.

(d) *Antibody-dependent cellular cytotoxicity (ADCC)*

Some of the antibodies to the viral envelope (gp120 and gp41) which bind to the infected cells induce ADCC with natural killer (NK) cells. A study of the role of ADCC has shown association between the healthy state and the presence of this cell-mediated lysis; and the loss of ADCC is associated with disease progression (Baum *et al.*, 1996).

(8) Development of cancer in HIV-disease

During the asymptomatic period before there is substantial damage to the immune system, there is no increase in the incidence of malignancies. However, once a significant decrease in the number of CD4+ T-cells has occurred, the incidence rises, and the malignancies are in general much more aggressive than those found in non-infected individuals. Many of the malignancies are induced by other viruses. Kaposi's sarcoma (KS), which is the most common, is thought to be caused by human herpes virus-8 (HHV-8). B-cell lymphomas are also much more common in AIDS patients with an approximately 60–100-fold increase compared to the general population. Increases are also seen in long-term surviving patients who have been treated by anti-HIV drugs over prolonged periods probably due to the immunosuppressive effects of their drugs. Approximately 60% of the B-cell lymphomas are thought to be caused by the Epstein-Barr virus (EBV), a distinct herpes virus. This is the causative agent of infectious mononucleosis; but it does not cause malignant proliferation in immunocompetent individuals. Anal carcinomas and cervical cancer which are also increased in AIDS patients are thought to be due to the involvement of human papilloma virus (HPV).

(9) Anti-HIV drugs

(a) *Reverse transcriptase inhibitors*

The first drug that was approved for use in people with HIV disease was azidothymidine (AZT), a chemical developed years earlier as an anti-cancer drug but abandoned because of its high level of toxicity. The toxicity is due to the incorporation of AZT in place of thymidine into newly synthesised DNA, thereby blocking further cell division. Erythroblasts, producing red cells, are the most actively dividing cells in the body and use the largest part of the nucleotide pool for cell division; so not surprisingly, an early study of bone marrow in patients who had been receiving AZT revealed that all developed anaemia with a varying degree of other white blood cell deficiencies.

AZT inhibits HIV replication by blocking the viral RT and there is no doubt that initially the effect is very dramatic. In the early short-term trials, AZT appeared to be beneficial. However, within a few weeks to a few months of AZT treatment, replication-competent, AZT-resistant

HIV strains emerge followed by disease progression. A placebo-controlled trial, lasting two years, revealed that AZT did not improve survival and was associated with more side-effects. In the British/French Concorde trial which involved 1700 patients and lasted three years, follow-up revealed a statistically significant increase of deaths in the AZT treatment arm as compared to those in the placebo (J. Derbyshire, personal communication 1994). The other nucleotide analogues that have been approved for use, such as ddC and ddI, are also highly toxic and of short-term benefit. A combination of two anti-RT drugs is claimed to be superior to the use of one, but has not been compared directly to placebo. Moreover follow-up revealed that 74% of the patients dropped out owing to toxicity, while mortality was delayed rather than prevented (Scott, 1996). Non-nucleotide anti-RT drugs have been developed and are less toxic, but virus-resistant mutants develop rapidly.

(b) *Protease inhibitors and highly active antiretroviral therapy (HAART)*

Protease which is coded by the *pol* gene of HIV, autocatalyses its own precursor protein and then cleaves other polypeptides to bring viral proteins into functional units. Protease inhibitors are less toxic than AZT but when used alone, the virus quickly develops drug-resistant mutants. However when a protease inhibitor was used together with two RT inhibitors it marked the first significant progress in anti-HIV treatment. The combination of drugs has been named highly active antiretroviral therapy (HAART). Following the initiation of HAART treatment approximately 80% of AIDS patients improved clinically; and coincidentally their CD4+ T-cell counts increased and the plasma viral load dropped significantly or even disappeared (Hogg *et al.*, 1997). The length of the beneficial effects of HAART differs between the individual patients and ranges from a few months to several years. For some the toxic side effects are more pronounced than for others. In most individuals who can tolerate the drug combination over prolonged periods, a wide range of pathological conditions develops due to toxicity, many of them, such as lipodystrophy, have never been seen before in AIDS while liver damage and vascular conditions are common. As a result the HAART treatment of AIDS patients has changed from combating opportunistic infections to reducing toxic side effects. Earlier optimistic forecasts in the press and in AIDS meetings suggesting that triple therapy should make it possible to eradicate HIV have been misleading. According to a recent study of a group of patients who have been treated successfully for up to 30 months with triple therapy, replication-competent HIV-1 was routinely isolated despite the fact that even the plasma assay for HIV-1 PCR was negative (Finzi *et al.*, 1997; Wong *et al.*, 1997).

After varied periods of HAART treatment in many patients there is a significant increase in the PCR plasma viral load without a decrease in the CD4+ T-cell count and without clinical deterioration. We assayed for infectious HIV-1 in the plasma from such patients but failed to detect any infectious virus. In order to try to explain this

observation we undertook the molecular characterisation of the plasma HIV-1 genes of such patients. Our molecular studies revealed significant deletions in several of the viral genes which could explain why the plasma HIV-1 is not infectious (Saurya, Lichtenstein & Karpas, 2002*a, b*, 2003*a, b*).

Some AIDS researchers suggested that drug treatment should be initiated early in the course of HIV infection (Ho, 1995) but so long as the available drugs have only a limited period of effectiveness, and are toxic, that may be misguided. In most HIV-infected individuals, the immune system manages to limit the damage caused by the virus for many years – far longer (on average nine years) than any drug cocktails available that have the added disadvantage of being toxic. It seems more prudent that drug treatment should begin only when the virus begins to cause accelerated damage: namely, when the CD4+ T-cell count drops below 400 cells mm⁻³.

(10) Passive immunotherapy (PIT)

After our early study demonstrated that healthy HIV-infected individuals had high levels of neutralising antibodies, while AIDS patients had none, we investigated the possibility of using passive immunotherapy as a form of treatment in AIDS. This began in 1985, transfusing blood plasma from healthy HIV-1-infected individuals to AIDS patients (Karpas *et al.*, 1985). The open, uncontrolled trial giving 500 ml plasma at monthly intervals to AIDS patients at a late stage of the disease provided some evidence of benefit when the patients were treated for two years (Karpas *et al.*, 1988; Karpas, 1990*b*). We also treated three pregnant HIV-positive women (two with AIDS) who delivered normal HIV-negative babies. Although an anecdotal observation, this suggested that PIT might be beneficial. A more rigorous study of 28 women receiving PIT during pregnancy provided safety and pharmacokinetic data: PIT was tolerated without any adverse haematological, blood chemistry or immunological effects; but the effectiveness of PIT could not be established because the women were treated simultaneously with AZT, which itself reduces HIV transmission (Fletcher *et al.*, 1997; Lambert *et al.*, 1997). A short-term study on PIT in AIDS patients in the USA recorded HIV neutralisation and a reduced frequency of opportunistic infections (Jackson *et al.*, 1988). Thereafter two independent larger-scale, double-blind, placebo-controlled trials of PIT in patients with advanced disease were conducted in the USA (Levy, Youvan & Lee, 1994) and France (Vittecoq *et al.*, 1995). The USA trial concentrated on mortality and found improvement in the group of patients with a CD4+ T-cell count exceeding 50 mm⁻³. The French trial involved 86 AIDS patients, half of whom received plasma rich in antibodies against HIV-1 from healthy HIV-1 infected individuals, while the other half in the placebo arm received plasma from the healthy HIV-negative individuals. This study examined the frequency and timing of new AIDS-associated infections and found statistically significant benefit in the group receiving PIT as compared to the group receiving plasma from HIV-negative individuals.

There is evidence that the effects of PIT are dose-related: when AIDS patients received only 250 ml of plasma on a monthly basis there was no benefit (Jacobson *et al.*, 1993; Levy *et al.*, 1994; Vittecoq *et al.*, 1995). For patients with advanced disease, 500 ml of plasma per month is probably the minimum requirement. Our limited experience with a few pre-AIDS patients suggests that the lower 250 ml dose may be adequate to maintain clinical remission in those with AIDS-related complex (ARC) and a CD4+ T-cell count of approximately 250 cells mm⁻³.

Unfortunately, double-blind placebo-controlled trials have not been able to muster financial support in the UK.

(11) Neutralising monoclonal antibodies

Numerous groups, including our own (Karpas, Dremucheva & Czepulkowski, 2001) have been trying to generate human monoclonal antibodies that might be used in immunotherapy of AIDS patients. Several authors reported the successful development of protective monoclonal antibodies when tested in macaques (Baba *et al.*, 2000; Mascola *et al.*, 2000). However, the genetic variability between HIV isolates implies that a combination of several monoclonal antibodies is likely to be needed to be effective therapeutically. This, in turn, will mean that future immunotherapy with monoclonal antibodies will probably be far more expensive than plasma from healthy HIV-infected individuals.

(12) Therapeutic plasmapheresis

Passive immunotherapy cannot be carried out without plasmapheresis of healthy HIV-positive donors. A significant question is therefore the effect of repeated plasma donation on the progress of their own disease. A study over a two-year period of 26 HIV-1 positive healthy plasma donors monitored substantial increases in their levels of circulating CD4+ T-helper cells, as compared to a similar group of healthy asymptomatic HIV-infected individuals who were submitting routine blood specimens through the same clinic, but were not donating (Bainbridge *et al.*, 1997). Most donors seemed to be moving towards stabilising their CD4+ helper T-lymphocyte levels rather than losing cells. The status of the immune system in donors, assessed by taking other immunological changes into account as well, appeared to be moving away from AIDS, where non-donors were progressing towards AIDS. This raises the possibility that plasma donation could be beneficial in itself to infected individuals, at least before disease progression has begun to be apparent. Other studies of regular plasma donors support the notion that plasmapheresis is not detrimental and may prove therapeutic (Cummins *et al.*, 1991; Stricker & Goldberg, 1995; Blick *et al.*, 1998). We have followed an increasing number of plasma donors who have been donating continuously for 3–7 years without a decline in numbers of CD4+ T-cells or antibody level or other signs of disease progression (Abelian *et al.*, 2001). The mechanism of these effects is not understood. Defining it might help us to understand why HIV overcomes the immune system, and could open up new avenues for the development of therapeutic strategies against this deadly virus.

(13) Combining strategies to prolong the latent period

The foregoing studies of PIT suggest several novel approaches that might be employed for prolonging the disease-free period in HIV-infected individuals.

Plasmapheresis, it appears, may be therapeutic for healthy HIV-infected individuals. It is non-toxic and does not promote the development of drug-resistant mutants. This suggests that it could be a treatment of choice for healthy HIV-positive individuals.

In the later, asymptomatic period when there is an increase in T-cell activation and the T-cell count drops to 350–450 cells mm⁻³, low-dose PIT might delay disease progression without causing toxicity.

Once the CD4+ T-cell count has fallen below 300 cells mm⁻³ or AIDS has developed, a combination of PIT with current anti-HIV drug cocktails might be used to limit the rise of drug-resistant mutants, if the drugs can be withdrawn intermittently under PIT cover. Anti-HIV plasma has a broad spectrum of specificities that can neutralise infectious HIV from the circulation of all patients tested, irrespective of HIV strain. It might deny drug-resistant mutants the opportunity to emerge. It could also reduce the toxicity of the drugs as suggested earlier (Karpas, Ash & Bainbridge, 1998).

Ninety per cent of HIV-infected individuals live in countries where the average annual income is less than the monthly cost of triple therapy. For these people, PIT is an affordable potential treatment, indeed the only one. In many of the bigger cities and towns of sub-Saharan Africa some 20–30% of healthy blood donors test positive for HIV. At the moment their blood is discarded. It could, however, readily be used as a source of plasma to treat AIDS patients. Only a modest extra cost would be required to prepare plasma for PIT within the existing blood transfusion facilities by trained personnel.

(14) Vaccines

The development of effective antiviral vaccines which prevent the onset of the corresponding virally induced diseases is the most important weapon in the fight against pathogenic viruses. The successful antiviral vaccines can be sub-divided into inactivated (killed) or live attenuated vaccines. The poliomyelitis virus is a good example for both types of vaccines. The inactivated virus vaccine was produced first by Jonas Salk and the live attenuated vaccine was developed subsequently by Albert Sabin. Both vaccines are highly effective in protecting against the development of poliomyelitis. Neither vaccine prevents subsequent infection by a pathogenic virus, but the immunity they confer prevents the pathogenic virus from spreading to the central nervous system (CNS) thereby preventing clinical disease. Vaccines to other viruses also do not prevent infections but confirm protection. In the case of polio, the development of effective vaccines has led to near-global eradication of poliomyelitis.

Once a virus had been shown to be the causative agent of AIDS, it was therefore inevitable that enormous efforts should be spent on the search for an effective vaccine.

However, there are several reasons why after 20 years from the initiation of research in this field there are still no effective vaccines against HIV in sight. A major reason is that vaccines usually do not prevent infection; and in the case of HIV, a retrovirus, its biology is such that once it infects even a few cells it establishes a permanent infectious site. Macrophages probably phagocytose HIV-infected cells which are often present in infected semen or in the vaginal mucosa of infected individuals even in the presence of antiviral antibodies and cytotoxic T-cells. Macrophages are not readily killed by the virus; they pick up the infection, travel to lymph nodes, interact as antigen-presenting cells with lymphocytes, and aid its spread throughout the body. Another reason is that the virus is able to infect cells through cell–cell contact by way of cell fusion, avoiding exposure to antiviral antibodies and cellular immunity following earlier vaccination with structural proteins of HIV. It can explain why vaccinated individuals who developed humoral and cellular immunity were not protected.

In the absence of any novel approach to vaccine development, there is no immediate prospect of an effective vaccine against HIV in the foreseeable future. Prevention of infection with HIV remains for the time being the only strategy to limit the spread of AIDS.

(15) HIV-2

HIV-2 was discovered at the Institute Pasteur in Paris. It was isolated from West African patients who were sent to Lisbon, Portugal for treatment. The treating physician realised that the patients suffered from symptoms and illnesses indistinguishable from AIDS. However, when repeated assays for the presence of antibodies to HIV were negative he sent two of the patients to the hospital at the Institute Pasteur; this led to the isolation of a lentivirus morphologically indistinguishable from HIV. Subsequent cloning and sequencing analysis revealed that the newly isolated virus differed by more than 55% from the first HIV, explaining the lack of cross-reactivity in the patients' serum with the known AIDS virus (HIV-1) (Clavel *et al.*, 1986). It was therefore decided to name the first virus HIV-1 and the newly isolated virus HIV-2. Several studies have suggested that it takes longer for HIV-2-infected individuals to develop AIDS than those infected with HIV-1. Epidemiological studies suggest that HIV-2 originated in West Africa, where many infected individuals are found in Senegal, Guinea Bissau, Gambia and Cape Verde Islands. In other parts of the world, the incidence of HIV-2 infection is much lower than HIV-1 except in Portugal, which possessed several West African colonies where many Portuguese lived in close relationships with the local population.

The main antigenic differences between HIV-1 and HIV-2 are in the envelope glycoproteins which are responsible for much of the reactivity with antibodies. By contrast, antibodies to the gag and pol proteins generally cross-react. As a consequence, the cross-reactivity of HIV-2 with HIV-1 sera is inconsistent, the sera of some individuals showing very low, those of others showing high, cross-reactivity. Like HIV-1, the individual sequences of independent isolates of HIV-2 differ substantially with every isolate having a distinct

restriction enzyme profile (Tristem *et al.*, 1989; Tristem, Hill & Karpas, 1991). Overall, the genome of HIV-2 is very similar to that of HIV-1 except for the absence of vpu and presence of vpx genes. We have shown that vpx is likely to be a duplication of vpr (Tristem *et al.*, 1990). The genome of HIV-2 is very similar to that of the virus that has been isolated from the West African sooty mangabey monkey (SIV_{smm}), which is thought to be the natural host of the HIV-2 precursor. In fact HIV-2 from the D-group is more similar to SIV_{smm} than to other HIV-2. Although SIV does not appear to cause any disease in the sooty mangabey it is highly pathogenic to the macaque, causing acute AIDS-like illness and death. For this reason the macaque has been used extensively for vaccine research.

IV. BLIND ALLEYS IN RETROVIRUSES OF HUMAN LEUKAEMIA AND AIDS

The development of this area of research, at least in its early phases, has perhaps been more than normally plagued by misleading and conflicting reports.

(1) Reverse transcriptase in human leukaemia

After Temin & Mizutani (1970) and Baltimore (1970) discovered RNA-directed DNA polymerase (reverse transcriptase) in avian and murine retroviruses, numerous papers were published asserting that similar reverse transcriptase (RT) activity was present in fresh human leukaemic cells from patients with acute lymphoid leukaemia (e.g. Gallo, Yang & Ting, 1970). This paper triggered an extensive study in numerous research centres, including our own, attempting to confirm the presence of the viral enzyme in human leukaemic cells. Over several years and a large number of scientific publications, no confirmation for such viral reverse transcriptase activity in the cytoplasm of fresh human leukaemic cells has been found by other investigators. The polymerase activity detected in the leukaemic cells is now believed to be cellular, not viral. Michael Bishop in his 1980 review article on the molecular biology of retroviruses concludes: 'Reverse transcriptase has not yet been identified with any assurance in uninfected cells, indeed it may be unique to retrovirus' (Bishop, 1980).

(2) Adult T-cell leukaemia and HTLV

Several papers have claimed that HTLV provirus sequences were present in fresh leukaemic T cells of acute lymphoblastic leukaemia (ALL) and Sézary syndrome (Kalyanaraman *et al.*, 1981, 1982; Gallo & Reitz, 1982). Experimental evidence to support such claims has not yet been provided.

HTLV provirus was reported to have common site of integration in malignant ATL cells, this was later retracted (Hahn *et al.*, 1983).

The first publication from the USA on the infective nature of HTLV (Ruscetti *et al.*, 1983) claimed that only cells from relatives of patients could be infected by the virus

and reported the obligatory dependence of: 'the addition of TCGF for growth'. Japanese work dating from 1981 showed no such dependence and that the Japanese virus (ATLV) can transform and immortalise the T-cells of unrelated donors (Miyoshi *et al.*, 1981). Our isolates from black ATL patients likewise infected and transformed T-cells from unrelated donors and grew without the addition of IL-2 (Karpas *et al.*, 1987*b*).

A reported homology between class 1 HLA and HTLV (Clarke, Gelmann & Reitz, 1983) was unsupported (Jennings & Even, 1984).

In 1985, a major article in *Nature* claimed the involvement of HTLV in the development of the disease of multiple sclerosis (MS) (Koprowski *et al.*, 1985). We, and others have failed to detect an association between HTLV and MS (Hauser *et al.*, 1986; Karpas *et al.*, 1986).

By 1987 it had been more or less universally accepted that HTLV-1 belongs to the oncoviridae, while HIV belongs to the lentiviridae. Nevertheless, Manzari *et al.* (1987) claimed the isolation of a new human retrovirus from T-cell lymphoma/leukaemia, HTLV-V, related to both HTLV-1 and HIV. However, their illustrated electron micrographs are distinct from any known retrovirus and the cells proliferating *in vitro* do not appear to be the patient's malignant cells.

Gallo has frequently claimed the discovery of the T-cell growth factor TCGF/IL-2 and its mandatory presence for the first isolation of both the adult T-cell leukaemia and AIDS viruses. Both viruses were in fact discovered without the use of IL-2 and numerous malignant human T-cell lines have been developed without its use since 1967. Credit for the discovery of this important cytokine should however probably be attributed to Doris Morgan, a research worker in Gallo's laboratory (Morgan, Ruscetti & Gallo, 1976).

(3) HTLV-IV

Daniel *et al.* (1985) isolated a simian immunodeficiency virus (SIV) from a macaque monkey with AIDS-like symptoms. Another group subsequently reported isolating a new human immunodeficiency virus from West Africa, which they named HTLV-IV (Kanki *et al.*, 1986). Thereafter it was shown that this resulted from a laboratory contamination of the human cells with Daniel's SIV strain (Desrosiers *et al.*, 1987; Mulder, 1988).

(4) The domestic history of early AIDS research

Montagnier (1984) records in a *Nature* letter that his laboratory 'supplied Dr Gallo with LAV first isolate on 17 July and 23 September 1983. According to Dr Gallo, the first sample failed to grow in his laboratory, but the second did'. The letter also states: 'In our earlier investigations, we received from Dr Gallo only HTLV-1 reagents, which were useful to show that LAV was not related to HTLV-1'. It appears that after the second shipment of LAV to Gallo's laboratory, the Czech virologist Mikulas Popovic succeeded in growing the French isolate in the Hut-78 T-cell line. On 12 December 1983 Gallo submitted another paper to

Science, accepted 12 March 1984 (Schupbach, Sarngadharan & Gallo, 1984b), regarding the involvement of HTLV-1 in AIDS; so probably not before early 1984 did Popovic reveal his ability to propagate LAV. It would have been within only a few weeks of this event, therefore, that Gallo submitted to *Science* (30 March 1984) four manuscripts on the successful study of an AIDS-virus isolate, which he named HTLV-III (later shown to be the French LAV). The papers were published in one issue (Gallo *et al.*, 1984; Popovic *et al.*, 1984; Sarngadharan *et al.*, 1984; Schupbach *et al.*, 1984b). They were followed one week later in the same journal by an article reiterating the involvement in AIDS of the oncovirus HTLV-1 without mentioning the discovery of HTLV-III (Schupbach *et al.*, 1984a). Their electron micrograph illustrating HTLV-III is now known to show LAV (Connor, 1987). The four articles variously claimed 'antigenic cross reactivity with HTLV-I and -II', 'nucleotide sequences of HTLV-III had been found to be related to HTLV-I and -II'. 'HTLV-III is related to HTLV-I and II and by all criteria, this new virus belongs to the HTLV family of retroviruses' and HTLV-III 'share[s] many properties with other HTLV isolates'. These claims which link AIDS to a leukaemogenic virus, were later disproved as was the claim about the genetic homology between HTLV-1 and HTLV-III (Arya *et al.*, 1984).

None of the four papers acknowledged the LAV AIDS-virus isolates provided by the French scientists the previous year, or attributed the derivation of 'their' T-cell line – used to propagate the virus – correctly to its originator. Termed HT and H9 in the papers, it was actually the Hut-78 T-cell line, established by Gazdar in another laboratory (Gazdar *et al.*, 1980).

V. CLOSING REFLECTIONS

The early studies on human leukaemia and immunodeficiency viruses are marked by a number of authoritative but misleading claims. Gallo reported that the cytoplasm of human leukaemia cells contained reverse transcriptase, which later proved to be incorrect. In 1975 Gallo and Weiss stated that they had isolated a human leukaemia virus, HL23 virus, but this was shown later to have resulted from laboratory contamination by three primate retroviruses. In 1980 Gallo claimed to have isolated a human T-cell leukaemia virus (HTLV), but did not present positive evidence that this was a human virus. During 1983–4 Gallo and his associates published several papers asserting that the human leukaemia virus, HTLV-1, was the agent involved in the development of AIDS. This was eventually disproven but meanwhile the attention of many scientists was misdirected, wasting time and resources that could have been put to far better use.

Justice was not done to the real discoverers of the AIDS virus and its mode of action. There was an unfortunate delay in the acceptance for publication of early compelling evidence from the French team that they had discovered the genuine cause of AIDS in 1983.

Science as a body of knowledge is eventually self-correcting and the nature and many of the details of this

disease are known now accurately. Why should the tortuous history of its discovery matter now? I believe that when regarded as an enterprise, Science is *not* always self-cleaning. The history of AIDS research involves huge rewards, unscrupulous ambition, disregard for common principles of scientific conduct, battles over priority leaving injustice uncorrected, and terrible consequences in the wider world. It is not an exaggeration to say that AIDS research was squandered for the best part of a year, delaying the development of an acceptable test for HIV, a year in which thousands of individuals contracted an infection that they may otherwise have avoided. Some of this has percolated through to the non-scientific public and provoked a number of articles, books and documentary programmes; further reference may be made to Connor (1987), Connor & Kingman (1988), Shilts (1987) and Crewdson (2002).

VI. CONCLUSIONS

(1) Jan Svoboda, in his early studies of the tumour-causing Rous sarcoma virus (RSV) developed transformed cells that led him to suggest that retroviruses replicate through a proviral DNA. This was extended in 1970 by Temin, Mizutani and Baltimore's discovery that this group of viruses contained reverse transcriptase (RT), an enzyme catalysing the synthesis of a copy DNA of the viral RNA. The spuma- and lenti-viruses were subsequently also found to contain RT and are therefore grouped as retroviruses.

(2) Despite extensive research to uncover cancerous retroviruses in man, to date only adult T-cell leukaemia (ATL) has been found to be caused by a retrovirus. The first conclusive evidence was provided in 1981 by Hinuma and his associates in Japan. In 1982 it was established that the same virus caused ATL in blacks of African origin.

(3) AIDS appears to have started in Africa in the middle of the twentieth century. Chimpanzee lentivirus gave rise to HIV-1 and sooty mangabey lentivirus to HIV-2. The introduction of the needle and syringe probably facilitated the transfer of these viruses from primates to humans and among humans.

(4) The human immunodeficiency-causing virus (HIV) was discovered early in 1983 at the Pasteur Institute in Paris by Francoise Barré-Sinoussi and her colleagues. In France it was also discovered by David Klatzmann and his colleagues that HIV killed CD4+ T-cells, thereby providing an explanation for the development of AIDS. The same team was also first to isolate the second AIDS-causing lentivirus HIV-2.

(5) There is no cure for HIV-AIDS. However a protease inhibitor used together with two RT inhibitors provides significant beneficial treatment prolonging life but with cytotoxic side effects. This combination of drugs has been named High Active Antiretroviral Therapy (HAART).

(6) Owing to the peculiar biology of HIV infection, all efforts to develop a vaccine have failed.

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