Genetica **95:** 25–50, 1995.

Factor VIII, HIV and AIDS in haemophiliacs: an analysis of their relationship

Eleni Papadopulos-Eleopulos[1], Valendar F. Turner[2], John M. Papadimitriou[3] & David Causer[1]
[1]*Department of Medical Physics;* [2]*Department of Emergency Medicine, Royal Perth Hospital, Perth, Western Australia;* [3]*Department of Pathology; University of Western Australia*

Received 17 February 1994 Accepted 14 June 1994

There are three steps in the revelation of any truth: in the first, it is ridiculed; in the second, it is resisted; in the third, it is considered self-evident.
Arthur Schopenhauer

Abstract

In this review, the association between the Acquired Immune Deficiency Syndrome (AIDS) and haemophilia has been carefully examined, especially the data that have been interpreted as indicating transmission of the human immunodeficiency virus (HIV) to the recipients of purportedly contaminated factor VIII preparations. In our view, the published data do not prove the hypothesis that such transmission occurs, and therefore HIV cannot account for AIDS in haemophiliacs.

Introduction

Currently, it is accepted that many patients with haemophilia have become HIV infected and/or developed the AID clinical syndrome as a direct result of the transfusion of factor VIII preparations contaminated with this particular virus. That this is indeed the case requires proof:

1. of the existence of a unique, infectious retrovirus, HIV. (For a critical discussion of this issue see Papadopulos-Eleopulos, 1988; Papadopulos-Eleopulos, Turner & Papadimitriou, 1992; 1993a; 1993b);

2. of the existence of HIV in factor VIII preparations;

3. of the existence of HIV in haemophiliacs;

4. that HIV is necessary and sufficient for the decrease in T4 cells observed in haemophiliacs;

5. that HIV and a decrease in T4 lymphocytes are necessary and sufficient for the development of the clinical AID syndrome.

Factor VIII and HIV

Since factor VIII is made from plasma, as a first step in proving contamination of this blood product with HIV, evidence must be presented that infectious viral particles with morphological characteristics attributed to HIV are present in the plasma of 'HIV infected' individuals. Then it must be shown that HIV can survive (a) the time between blood collection and freezing of plasma; (b) the freezing and thawing itself; and (c) the process of manufacturing factor VIII from thawed plasma. In other words, as Jay Levy succinctly expressed in 1989, it is 'important to know whether retroviruses could survive the preparation involved in producing Factor VIII concentrates. Otherwise, AIDS in many haemophiliacs [a minority may have other risk factors] could not be explained' (Levy, 1989).

HIV in plasma

To date, there is no evidence of the existence in human plasma of particles with the morphological characteristics attributed to HIV even though the plasma of at least some 'HIV infected' individuals is claimed to contain such particles. Thus Levy, whose team

reported most often on the relationship between HIV and factor VIII wrote in 1988: 'Human Immunodeficiency virus in plasma or serum has been found in about 30% of specimens from seropositive persons, generally at a concentration of less than 10 IP/ml[12] [IP=infectious particles] (Levy, 1988). Reference 12 cited by Levy is a paper which he published in collaboration with Barbara Michaelis but this paper does not contain a description of the method used to show that (a) HIV seropositive (non-haemophiliac) plasma was infected with 'HIV particles'; (b) HIV was 'present in low titers' and (c) the particles were 'infectious'. Commenting on this and his colleagues' findings Levy wrote: 'These studies demonstrate further that not all seropositive individuals have virus recoverable from their PMCs and that isolation from serum is not a common event' [PMCs=peripheral blood mononuclear cells] (Michaelis & Levy, 1987). 'Thus, cell-free virus in body fluids is unlikely to be a meaningful source of HIV transmission' (Levy, 1988). At least one other eminent HIV/AIDS researcher is also of the opinion that HIV cannot be transmitted through '... products prepared from blood, such as albumin, plasma, protein fractions, or hepatitis B vaccine' (Blattner, 1989). If HIV cannot be transmitted through 'cell-free' body fluids (plasma) because it is not found in the plasma of 70% of seropositive individuals, and in the remaining 30% is 'generally at a concentration of less than 10 IP/ml', then it will be even less probable that factor VIII prepared from plasma can be a 'meaningful source of HIV transmission' since, even if HIV were present in a plasma collection, it would be diluted many times over during the process of factor VIII manufacture. This is because factor VIII is made by pooling plasma obtained from 2,000 to 30,000 individuals amongst whom at most there will be only a few HIV seropositives. Since factor VIII prepared from large batches of pooled plasma is ultimately shared amongst many haemophiliacs, the load of HIV for each haemophiliac will be substantially lower than 10 IP/ml.

Since 1989, detection of a 24,000 molecular weight protein (p24) in cell cultures (T cells from persons presumed to be infected) or co-cultures (of T cells from persons presumed to be infected, with T cells from normal individuals) has been used to quantify HIV in cells, 'cellular viremia' (Masquelier *et al.*, 1992). Detection of p24 in cultures of T cells from normal individuals with plasma from those presumed to be infected has been used to quantify HIV in plasma, 'plasma viremia' (Coombs *et al.*, 1989; Ho, Moudgil & Alam, 1989; Clark *et al.*, 1991). There are many reasons why p24 cannot be used to quantitate or even detect the presence of 'HIV infectious particles'. These include:

(a) there is ample evidence that the p24 protein is not HIV specific (Papadopulos-Eleopulos, Turner & Papadimitriou, 1993a and see below);

(b) there is no relationship between plasma viraemia, cellular viraemia (p24 in culture), and the titre of p24 in (uncultured) plasma (HIV antigenaemia). 'Only 45 percent of patients with plasma viremia had HIV p24 antigen in either serum or plasma' (Coombes *et al.*, 1989). 'Plasma p24-antigen titres before or after acidification did not show any significant correlation with quantification by tissue culture method' (Weber & Ariyoshi, 1992). Nor does correlation exist between the 'most specific' HIV antibody test, the Western blot (WB), and plasma p24. With methods which have a reported lower limit of sensitivity of 10–50 pg/ml, p24 can be detected in only 12% of HIV positive sera (Jackson, Sannerid & Balfour, 1989);

(c) 'Much of the viral protein secreted from HIV-infected cells is non-particulate, and the proportion of (for example) p24 in virions is a function of the viral genotype and the age of the culture. In extreme cases, less than one percent of the total p24 and gp120 present is in virions' (McKeating & Moore, 1991). [It must be pointed out that in the AIDS literature, the terms 'HIV', 'HIV isolation', 'pure particles', 'virus particles', 'virions' and 'infectious particles' have a variety of meanings and include all of the following, but most often without proof of the presence of a particle: (i) 'RNA wrapped in protein'; (ii) material from the cell culture supernatants which passes through cell tight filters but through which organisms such as mycoplasmas may pass; (iii) the pellet obtained by simple ultracentrifugation of the culture supernatant; (iv) recently, very often, detection in AIDS cultures of p24 (Papadopulos-Eleopulos, Turner & Papadimitriou, 1993a)].

In the process of preparing plasma for factor VIII extractions, great care is taken to exclude cells. Even if some cells are inadvertently present, it would be most unlikely that they would constitute 'a meaningful source of HIV transmission' since, like 'HIV particles', 'infected' cells present in the plasma of a seropositive donor would be diluted many times over by the plasma and cells from the manifold number of non-seropositive donors from which factor VIII is made.

Furthermore, according to some of the best known HIV experts:

(a) 'in early and intermediate stages of disease' (it is unlikely that individuals with advanced disease, AIDS, would be able to donate blood), the frequency of HIV infected cells in the blood as determined by the polymerase chain reaction (PCR) is 1/10,000 in the early stages, and between 1/10,000 and 1/1000 in the advanced stages (Pantaleo *et al.*, 1993);

(b) with the PRC one does not detect viral particles or even the whole viral genome, but only a small region, 'a gene at best' (Wain-Hobson, 1989);

(c) up to 99.9% of the 'HIV genomes' in the plasma may be defective (Sheppard, Ascher & Krowka, 1993): that is, one or several genes are absent.

Plasma processing and HIV

'Source plasma' obtained by plasmapheresis and fresh frozen plasma from whole blood donation are best suited for the preparation of factor VIII. The interval between collection and freezing of plasma is approximately six hours (van Aken, 1991). The plasma is kept frozen for lengthy periods, days to weeks, and is then thawed for processing.

Researchers from the Laboratory of Molecular Retrovirology, Georgetown University, took two blood samples from each of the ten HIV seropositive patients: 'One sample from each individual was processed immediately after phlebotomy to obtain plasma, and aliquots of this plasma were used at once to infect PHA-stimulated donor PBMCs as described. A second set of aliquots of this 'immediately processed' plasma was frozen at $-70°$ for 3 h, and then thawed and used to infect the same donor cells. Five of the ten immediately processed/immediately used plasma samples (50%) were positive for HIV-1 using the p24 antigen detection method, while all of the corresponding frozen aliquots were negative (0%). The second blood samples from each of the 10 patients was kept at room temperature for 3 h prior to plasma separation. Again, after processing, one aliquot was used for the infections while another was frozen and thawed before use. In this experiment, only one of the ten samples (10%) was culture positive after the 3 h delay and also after the one cycle of freezing and thawing' (Dewar *et al.*, 1992). Thus, although these workers determined the optimum conditions for 'HIV isolation' prior to conducting the above experiments, they could 'isolate' (detect p24 culture) HIV from only 10% of HIV^+ plasmas which were left at room temperature for three hours and from 1 of 20 (5%) HIV^+ plasmas which had been frozen for three hours. In 1984, Levy himself, using 'mouse retroviruses' reported that: 'The virus titre (10^8 IP) was not affected by mixing with cold (5°C) plasma. In contrast, incubation of the virus with plasma at 37°C for 30 min reduced its titre 100-fold. This finding accords with the report of complement-mediated lysis of retrovirus by human serum' (Levy, Mitra & Mozen, 1984). Other researchers have shown 'freeze-drying' parameters commonly employed under commercial conditions for the preservation of protein solutions are not favourable for survival of viral suspensions' (Damjanovic, 1987). However, Levy and his colleagues claim to have shown that a retrovirus 'can survive and remain infectious after procedures used in the preparation of factor VIII (FVIII), cryoprecipitate or concentrates'. According to these researchers, when HIV was 'added to human plasma (5°C), no reduction in virus titre was observed'. Cryoprecipitate made from the plasma contained 'a 10-fold reduced titre'. 'Purification of cryoprecipitate by acid and glycine precipitation and filtration to achieve a sterile FVIII filtrate resulted in a further 10-fold reduction in virus titre'. Lyophilisation of factor VIII filtrate 'lowered the infectious virus titre about 10-fold. When this lyophilised preparation was then heated, very low titre virus was detectable after 10, 24 and 34 but not after 48 h of heating' at 68°C. They concluded 'our results indicate that lipid-enveloped retroviruses (both mouse and human) if present in sufficient amount of plasma can be found in infectious form in FVIII lyophilised products ... heating lyophilised FVIII for 72 h at 68°C or the liquid product for 10 h at 60°C will eliminate infectious ARV [HIV] if it is not present in the plasma at more than 10^6 infectious particles/ml.' (Levy *et al.*, 1985). However:

1. Commenting on Levy and colleagues' findings Damjanovic wrote: it is 'surprising that HIV survived procedures used in the preparation of Factor VIII before lyophilization'.
2. Levy *et al.* performed their experiments by 'infecting' plasma with 10^5 IP/ml, while factor VIII which is administered to haemophiliacs is made from plasma pooled from thousands of individuals most of whom are not infected. Given that:
   (a) the plasma from which factor VIII is prepared contains very few or no particles per ml of plasma;
   (b) the technique employed to prepare factor VIII reduced by a thousand fold the concentration

of any infectious particles present, even before heat treatment; one would have to conclude that factor VIII prepared before 1985 could not contain sufficient HIV particles to be a 'meaningful source of HIV transmission';

3. Levy and his colleagues detected and quantified HIV particles 'by induction of reverse transcriptase activity in the culture fluids of normal human PMC maintained for up to 1 month after virus inoculation' (Levy *et al.*, 1985). In Levy's laboratory, PMCs were cultured with interleukin-2, polybrene and phytohaemagglutinin (PHA). To prove HIV infection, the activity of the enzyme reverse transcriptase (RT) was determined using the primer-template $An.dT_{15}$ (Levy, Mitra & Mozen, 1984). Detection of reverse transcription cannot be considered proof of the presence of a retrovirus, certainly not HIV, and in fact, the above template-primers can be copied by all cellular DNA polymerase (see below). Because of this, reverse transcription of the primer-template $An.dT_{15}$ cannot be used specifically to quantify or even detect HIV or any other retrovirus.

HIV in factor VIII

The belief, as Levy pointed out, that haemophiliacs develop AIDS because they become infected with HIV by receiving contaminated factor VIII can be entertained if and only if evidence exists which proves that:

1. factor VIII used to treat haemophiliacs is contaminated with HIV particles;
2. the particles are infectious.

A paper entitled 'Detection, quantification and sequencing of HIV-1 from the plasma of seropositive individuals and from factor VIII concentrates' published in 1991, is the only paper to claim proof of the existence of HIV in factor VIII used therapeutically (Zhang *et al.*, 1991). Using PCR the authors tested eight batches of factor VIII, all 'unheated and prepared before the introduction of donor screening for anti-HIV antibodies'. Two batches 'gave positive results; in one case with the *env* primers, the other with the *pol* primers'. In sequencing their 'HIV RNA' they found that the sequences were 'distinct from those of all published HIV isolates and from any sequences obtained previously in our laboratory'. Despite this, they interpreted their signals as HIV and in fact quantified the HIV and concluded: 'the calculated amount of HIV RNA in both batches of reconstituted factor VIII was only 2.5 copies per ml'. The minimum requirement for such an interpretation of a PCR signal (or hybridisation in general), is prior proof that the PCR primers and the hybridisation probes belong to a unique retrovirus, HIV, and that the PCR and hybridisation reactions are HIV specific. Detailed discussion of the evidence has been presented elsewhere (Papadopulos-Eleopulos, Turner & Papadimitriou, 1993a) that the specificity of the hybridisation signals in general and of 'HIV' PCR in particular have not been determined, and that the finding of viral RNA or DNA, even if proven to belong to a unique retrovirus HIV, is not proof of the presence of the viral particle. Some additional points are:

1. Most, if not all probes used for hybridisation assays, including the PCR probes and primers, are derived from the H9 (HUT78) or CEM cell lines. The H9 cell line originated from a patient with T4 cell leukaemia, a disease which Gallo claims is caused by a retrovirus similar to HIV, HTLV-1 (Gallo, 1986). In 1983 Gallo himself reported that HUT78 'contained HTLV proviral sequences' (HTLV=HTLV-I) (Wong-Staal *et al.*, 1983). Recently, a retrovirus has been 'isolated' from a non-HIV-infected CEM (SS) culture (Minassian *et al.*, 1993). Thus, the above cell lines contain at least one retrovirus, if not more (see below), even when not infected with HIV. Since even the well established method (Papadopulos-Eleopulos, Turner & Papadimitriou, 1993a) for retroviral isolation (but which to date has never been reported for HIV) cannot distinguish between retroviruses, one cannot be confident that the 'HIV' nucleic acid probes and PCR primers are indeed specific for HIV;
2. The normal human genome contains HIV and HTLV-1 sequences (Parmentier *et al.*, 1992; Schneider *et al.*, 1993);
3. The specificity for HIV of hybridisation assays in general, and PCR in particular, can be determined only by the use of a gold standard. However, according to one leading HIV/AIDS researcher, William Blattner, 'One difficulty in assaying the specificity and sensitivity of human retroviruses [including HIV] is the absence of a final "gold standard"' (Blattner, 1989).

   In addition to the above mentioned problems there are many other difficulties associated with the establishment of an HIV gold standard for PCR/hybridisation studies (Papadopulos-Eleopulos, Turner & Papadimitriou, 1993a). One recently identified problem is the fact that there are

'striking differences' between the proviral DNA and cDNA in one and the same PBMC sample which 'could not be explained by either an artefact of reverse transcriptase efficiency or template selection bias' (Michael *et al.*, 1993).

4. The presently available evidence obtained without a gold standard suggests that the 'HIV hybridisation' is not HIV specific (Papadopulos-Eleopulos, Turner & Papadimitriou, 1993a). Some additional evidence:

   (a) to address the question whether the neuronal cells of patients with AIDS dementia complex are infected with HIV, 'the brains from 10 patients with AIDS and neurological evidence of viral encephalitis and the brains from 5 patients without HIV-1 infection' were examined using an HIV *gag* probe. 'The antisense riboprobe hybridized to cells known to be infected with HIV-1. It hybridised to HIV-1 infected A3.01 cells as well as splenic and renal lymphocytes obtained at autopsies from patients known to have AIDS. The probe did not, however, hybridize to neurones in the brain sections from 10 patients with AIDS ... Surprisingly, when we applied the control sense HIV-1 *gag* probe to the brain section from patients with AIDS, we observed specific hybridization to neuronal cells. Similarly, when brain sections from the five individuals not infected with HIV-1 were examined, the HIV-1 sense probe detected transcripts in neuronal cells. Our Northern blot analysis confirmed these results and demonstrated the presence of a 9.0-kb polyadenylated transcript in brain tissues' (Wu *et al.*, 1993). Thus, either the positive hybridisation signals obtained with the antisense probe are non-HIV-specific or, as the authors concluded, there is a neurone-specific 9.0-kb transcript that shows extensive homology with antisense gag HIV-1 sequences and that this transcript is expressed in neuronal cells of both HIV-1 infected and noninfected individuals;

   (b) the finding of positive PCR in eosinophils has been interpreted to 'suggest that eosinophils may act as host cells for HIV-1' (Conway *et al.*, 1992). However, 'Formaldehyde-fixed eosinophils nonspecifically bind RNA probes [HIV RNA] despite digestion with proteolytic enzymes and acetylation ... When preparations are treated with amounts of ribonuclease adequate to destroy viral RNA, the eosinophilic binding remains' (Natoli *et al.*, 1993);

   (c) negative controls and even buffers and reagents may give positive HIV PCR signals (Conway, 1990);

One cannot but agree with Shoebridge *et al.* that 'until further molecular biological and epidemiological studies are carried out, it will be unclear as to what detection by PCR of proviral HIV-1 DNA, even when shown to be HIV-1, really means' (Shoebridge *et al.*, 1991).

Infectious particles?

Even if it is proven beyond reasonable doubt that factor VIII preparations contain HIV particles, the particles could not be infectious. This fact is of such pivotal significance it is essential to review the mechanism of HIV infection as reported by leading HIV researchers. According to:

1. Weber and Weiss, 'The first step in any viral infection is the binding of the virus particle to a component of the host cell's membrane ... For some time it has been known that the binding takes place when CD4 interacts with an 'envelope' protein of the virus called gp120' (Weber & Weiss, 1988);
2. Moore and Nara, 'HIV infection of CD4+ cells is initiated by an interaction between its surface glycoprotein, g120, and the cellular antigen CD4' (Moore & Nara, 1991);
3. Mortimer, 'The gp120 surface protein interacts with CD4 receptors on T4 cells so that the viral RNA can be injected into the cell' (Mortimer, 1989);
4. Matthews and Bolognesi, 'First gp120 binds to the CD4 receptor on an uninfected cell; then gp41 becomes anchored in the adjoining membrane; next the two membranes begin to fuse, and the virus spills its contents into the cell' (Matthews & Bolognesi, 1988);
5. Redfield and Burke, 'Infection begins as a protein, gp120, on the viral envelope binds tightly to a protein known as the CD4 receptor on the cell surface' (Redfield & Burke, 1988);
6. Rosenberg and Fauci, 'The initial event in the life cycle of HIV is the high-affinity binding of the HIV envelope glycoprotein (gp120) to CD4 that is present on the surface of cells' (Rosenberg & Fauci, 1990);
7. Montagnier *et al.*, 'The gp120 is responsible for binding the CD4 receptor' (Gougeon *et al.*, 1993);

8. Haseltine and Wong-Staal, gp120 is 'crucial to HIV's ability to infect new cells' (Haseltine & Wong-Staal, 1988);
9. Callebaut *et al.*, 'The human immunodeficiency virus (HIV) infects lymphocytes, monocytes, and macrophages by binding to its principal receptor, the CD4 molecule, through the viral envelope glycoprotein gp120. The V3 loop of gp120 is critical for HIV infection' (Callebaut *et al.*, 1993).

Thus, there is general agreement that the HIV envelope protein gp120 is crucial for HIV infection. However, agreement also exists that 'gp120 is easily shed by virus and virus-infected cells' (Bolognesi, 1990). Gelderblom and his colleagues at the Koch-Institute in Berlin who have conducted the most detailed electron-microscopy studies of 'HIV particles' have shown that the knobs on the surface of the particles, where the gp120 is found, are only present in immature (budding) particles, which are 'very rarely observed'. 'Mature', cell-free particles do not have knobs, that is, gp120 (Hausmann *et al.*, 1987).

Regarding infection by retroviruses, as far back as 1983 Gallo pointed out that 'the viral envelope which is required for infectivity is very fragile. It tends to come off when the virus buds from infected cells, thus rendering the particles incapable of infecting new cells'. Because of this Gallo said, 'cell-to-cell contact may be required' for retroviral infection (Marx, 1983). Since gp120 is 'crucial to HIV's ability to infect new cells', and since gp120 is not found in the cell free particles, even if HIV particles are present in plasma or factor VIII preparations, they will be non-infectious.

One must also consider the possibility that factor VIII is contaminated with HIV-infected cells. Even if the plasma from which the factor VIII is made contains cells, since preparation of factor VIII entails (a) freezing and thawing which lyses cells; (b) sterilisation by filtration which excludes cells and the majority, if not all, of cellular fragments from the filtrate; it is most unlikely that factor VIII would be contaminated with cells. Furthermore, even if the filtrate were to contain some cellular fragments, they could not be a source of HIV because the synthesis and assembly of type C and type D particles, and *Lentiviruses*, require the presence of an intact cell. In conclusion, the lack of evidence of HIV particles in plasma, the use of non-specific methods to detect HIV in cultures, the lack of gp120, considered to be crucial for HIV infection in cell-free particles and the physical processes involved in processing plasma into factor VIII even before heating was introduced, make it impossible for factor VIII to be contaminated with infectious retroviruses. It is not surprising, therefore, that to date nobody has reported HIV particles in factor VIII preparations. Thus, on the available evidence, HIV infected factor VIII cannot be the explanation for AIDS in haemophiliacs. If factor VIII is not infected with HIV then it is mandatory to explain the cause of the 'HIV-related' phenomena: that is, positive antibody tests, HIV isolation, T4 cell decrease and AIDS observed in haemophiliacs.

HIV antibodies in haemophilia

By 1988 most, if not all haemophiliacs, (in the USA, Europe and Australia), were tested for HIV antibodies and the vast majority of those tested were reported as being positive. Based on the antibody tests, as far back as 1984, the CDC concluded that 'These serological data, indicating a high risk of exposure to LAV from heavy users of factor VIII concentrates, support the contention that LAV may be transmitted by some blood products' (Ramsey *et al.*, 1984). However, the specificity of the HIV antibody test for HIV infection have never been determined. According to Philip Mortimer, director of the Virus Reference Laboratory of the Public Health Laboratory Service, London, UK: 'Diagnosis of HIV infection is based almost entirely on detection of antibodies to HIV, but there can be misleading cross-reactions between HIV-1 antigens and antibodies formed against other antigens, and these may lead to false-positive reactions. *Thus, it may be impossible to relate an antibody response specifically to HIV-1 infection.* In the presence of clinical and/or epidemiological features of HIV-1 infection there is often little doubt, but anti-HIV-1 may still be due to infection with related retroviruses (e.g. HIV-2) which, though also associated with AIDS, are different viruses' [italics ours] (Mortimer, 1989).

The specificity of an antibody test, any antibody test, cannot be determined by 'clinical and/or epidemiological features'. In the case of the HIV tests, this practice may create several problems. Given the fact that the vast majority of individuals who test positive are asymptomatic, one must conclude that in these individuals, a positive HIV antibody test is a false positive. Furthermore, the 1993 AIDS definition permits the diagnosis of AIDS solely on the basis of a low T4 cell count and positive HIV serology. It has been estimated that the new AIDS definition will treble the number of AIDS cases compared to the 1987 AIDS definition (Brettle *et al.*, 1993), most of whom may

be expected to be asymptomatic, and thus a significant number of AIDS patients will have a false positive HIV antibody test. Even if a patient did have one of the AIDS 'indicator diseases' (none of which is new and some of which are common), because:

(a) haemophiliacs are exposed to 'an array of alloantigens (and infectious agents)' (Levine, 1985);
(b) gay men and intravenous users are also subjected to a wide variety of foreign antigens and infectious agents;
(c) all these groups are known to possess a plethora of antibodies directed against numerous non-HIV antigens;

one would expect cross-reactivity with 'HIV antigens' to be the rule rather than the exception and thus, in these groups, more than in any other, it will be difficult to conclude that a positive HIV antibody test signifies HIV infection and not cross-reactivity.

One cannot simultaneously use the presence of AIDS as proof of HIV infection, and conversely, the presence of a positive HIV test as proof that HIV is the cause of AIDS, as presently is the case. The specificity of an antibody test requires the use of the gold standard. A gold standard is an alternative, independent method of proving the presence or absence of the condition for which the test is to be employed and in the case of the HIV antibody tests the only admissible gold standard is HIV itself. However, the use of a gold standard has never been reported and may not even be possible (Papadopulos-Eleopulos, Turner & Papadimitriou, 1993a). This is a view shared by William Blattner: 'One difficulty in assessing the specificity and sensitivity of retrovirus assays is the absence of a final 'gold standard'. In the absence of gold standards for both HTLV-1 and HIV-1, the true sensitivity and specificity for the detection of viral antibodies remain imprecise' (Blattner, 1989). In fact, at present, there is ample evidence which suggests that the HIV antibody tests, even in the high AIDS risk group (gay men, IV drug users, blacks and haemophiliacs), may not be specific (Papadopulos-Eleopulos, Turner & Papadimitriou, 1993a). Some additional data related to haemophilia are:

(a) haemophilia patients have hypergammaglobulinaemia and hypergammaglobulinaemia correlates with HIV seropositivity (Brenner *et al.*, 1991);
(b) haemophilia patients have anti-lymphocyte antibodies (Daniel, Schimpt & Opetz, 1989);
(c) in one study, 12% of haemophiliacs were found to have HTLV-1 antibody, (the molecular weights of HTLV-1 and HIV-1 proteins are the same), 74% anti-cardiolipin antibodies, 28% anti-nuclear antibodies and 85% immune complexes (Matsuda *et al.*, 1993);
(d) HIV researchers accept that 'antilymphocyte, antinuclear and other autoantibodies' give rise to false positive HIV antibody tests (Biggar, 1986);
(e) in haemophiliacs, hepatitis B virus seropositivity is a predictor for HIV seropositivity (Brenner *et al.*, 1991);
(f) at least one other group with chronic liver disease, alcoholics, are known to have both false positive antibody tests and immune deficiency (Mendenhall *et al.*, 1986).

As has been already noted by, 1988, most haemophiliacs had already been found to be HIV seropositive. However, the test utilised by many researchers including Gallo, Blattner, Weiss, Montagnier and Chermann in papers published as late as 1990, was the ELISA (Melby *et al.*, 1984; Allain, 1986; Eyster *et al.*, 1987; Goedert *et al.*, 1989; Wagner *et al.*, 1990). Although before 1988 some researchers used WB to confirm the ELISA, the criteria used then to define a positive WB would not satisfy even the 'least stringent' criteria presently used to define a positive WB result (Lundberg, 1988). A few examples will suffice to illustrate this point:

1. 'Serological reactions with any combination of 18 kd, 25 kd and 41 kd proteins of LAV were scored as positive' (Jason *et al.*, 1985);
2. 'A positive Western blot test was defined as the presence of at least one band characteristic of antibody against an envelope protein (gp41, gp120, or gp160) and at least one other HIV-1 characteristic band' (Jackson *et al.*, 1988);
3. 'Serological reactions were scored as positive if there was reactivity with the 41-kD protein of HIV or reactivity with the 24-kD protein together with any one of several other HIV-associated proteins (18 kD, 31 kD, 51 kD, 55 kD, 65 kD or 110 kD)' (Lawrence *et al.*, 1989).

Thus, it is a distinct possibility that if haemophiliacs who have been tested using only ELISA, or even ELISA and WB prior to 1988, were reappraised, a significant proportion may no longer be classified as HIV seropositive.

In 1984, a number of researchers from the USA, including the well known retrovirologist Myron Essex, reported the finding of HIV antibodies in haemophiliacs and concluded: 'The present results suggest that exposure to HTVL-III is widespread in asymptomatic haemophiliacs', but also added 'However, it is pos-

sible that a significant proportion of asymptomatic haemophiliacs might be exposed only to inactivated HTLV-III rather than to the virus, owing to the manufacturing process involved in the preparation of commercial factor VIII concentrate' (Kitchen *et al.*, 1984). But the mere fact that some HIV antibody positive haemophiliacs have symptoms is not proof that they are infected with the virus. (As we have already mentioned, one cannot simultaneously use the presence of AIDS as proof of HIV infection, and conversely, the presence of a positive HIV test as proof that HIV is the cause of AIDS).

Later, the finding that haemophiliacs who received only heat treated factor VIII (van den Berg *et al.*, 1986; CDC, 1987) also became HIV seropositive was interpreted as evidence that these patients 'may not have been infected but rather immunized by preserved viral proteins' (Damjanovic, 1989; Jackson, 1989). As far back as 1985 researchers for the CDC wrote: 'It is possible that antibody to LAV is acquired passively from immunoglobulins found in factor VIII concentrates ... Likewise, it is possible that seropositivity is caused not by infectious virus but by immunization with noninfectious LAV or LAV proteins derived from virus disrupted during the processing of plasma into factor VIII concentrate' (Evatt *et al.*, 1985). Thus a positive HIV antibody test cannot be considered proof of HIV infection. Nonetheless, 'Because the virus has been isolated from lymphocytes of about 30% of antibody-positive asymptomatic haemophiliacs and because immune dysfunction has been progressive in other patients, it is believed that antibody positivity is indicative of infection instead of immunization in most, if not all, of the antibody-positive haemophiliacs' (Bretter *et al.*, 1988). According to other authors, 'Strictly speaking, detection of the virus is therefore necessary for diagnosis of an HIV infection in HIV-seropositive haemophiliacs' (Schneweis *et al.*, 1989). In conclusion, the presently available evidence does not prove that a positive HIV antibody test in haemophiliacs is proof of HIV infection.

Viral isolation

In a paper published in the *Lancet* in 1984 entitled 'Isolation of a New Lymphotropic Retrovirus from two Siblings with Haemophilia B, one with AIDS', Montagnier and his associates were the first to describe 'isolation of HIV' from haemophiliacs. The T lymphocytes of the two children, one symptomatic and the other healthy, were cultured with, among other chemical agents, PHA, IL-2, polybrene and anti-human alpha-interferon. From the symptomatic sibling they reported the following findings:

1. In the *culture*, retrovirus-like particles;
2. In the material from the cultures which in density gradients banded at 1.16 gm/ml:
   (a) proteins which using the ELISA reacted with sera from a gay man with lymphadenopathy and several specimens of the patient's serum collected prior to the blood used for 'HIV isolation'. No serological data are given regarding the blood from which the HIV was isolated. However, the serum collected after treatment and clinical improvement was non-reactive. In WB analysis a p24/25 protein which banded at 1.16 gm/ml was found to react with the patient sera as well as with the serum from the gay man with lymphadenopathy. The same sera did not react with goat antiserum specific for the p24/25 of HTLV-1;
   (b) RT activity which 'showed a preference for poly-A-oligo-dT12-18 and poly-C-oligo-dG12-18 over poly-dA-oligo-dT12-18, a feature which usually distinguishes retroviral enzymes from cellular DNA polymerases. The maximum activity was obtained with Mg^{2+} over Mn^{2+} with poly-A-oligo-dT as template primer as previously described for human retroviruses such as HTLV or LAV'. They also reported the finding of 'viral' particles and RT in the culture from the second sibling. In the ELISA his serum reacted with LAV and $IDAV_2$ (immunodeficiency-associated virus $\equiv$ the material from the culture of the first patient which banded at 1.16 gm/ml). In the WB, the p24 of $IDAV_2$ was recognised by his serum. Montagnier and his colleagues concluded: 'Our findings are consistent with the hypothesis that retroviruses such as that found in our patients can be transmitted by way of plasma products' (Vilmer *et al.*, 1984).

Using similar methods, researchers from the CDC and the Children's Hospital of Los Angeles reported in 1985 the isolation of HIV from 6 of 19 healthy seropositive haemophiliacs (Gomberts *et al.*, 1985). In 1987, another group of American researchers reported the isolation of HIV from 16 of 66 (24%) haemophiliacs seropositive for HIV, but not from any of the six without HIV antibody. For this, patients' PBMC were co-cultured with cells from healthy seronegative donors that had been stimulated with PHA. To the co-cultures

they also added IL-2 and polybrene. The findings in the culture of:

(a) RT, 'An assay count of 10^4 cpm/ml (after subtraction of cellular polymerase activity) was considered positive for virus', using An.dT12-18 as template-primer;

(b) cells positive for viral RNA by cytoplasmic dot blot hybridisation;

were considered proof of HIV isolation (Andrews *et al.*, 1987).

Using the same co-culture techniques and conditions as the above authors, in 1988 Jackson *et al.* tested '75 unselected hemophiliacs to determine whether patients positive for HIV-1 antibody are actively infected rather than immunized by viral proteins in non-heat-treated factor VIII or IX concentrates'. An 'ELISA kit that primarily detects the core p24 antigen of HIV-1' was used to test the culture. The finding of two serial supernatant fluid samplings as positive, 'with the later sampling showing greater reactivity', was considered synonymous with HIV isolation. They reported HIV isolation from '55 (98%) of 56' haemophiliacs seropositive for HIV and concluded 'that antibody-positive hemophiliacs have been actively infected by HIV-1' (Jackson *et al.*, 1988).

In 1989 Schneweis *et al.* reported that between 1986 and 1988 they were able to 'isolate HIV' from 70 of 211 (33%) of haemophiliacs who were seropositive for HIV. 'After March 1988 an increase in sensitivity of virus isolation was attained by testing the supernatants of the culture for the presence of p24 antigen (p24Ag) instead of reverse transciptase (RT)' (Schneweis *et al.*, 1989). One year later the same authors 'isolated' HIV from 29 of 46 haemophiliacs (63%) (Wagner *et al.*, 1990). As can be seen, by HIV isolation is meant detection of one or more of the following phenomena: rarely, virus-like particles and positive hybridisation signals for 'viral' RNA, and most often RT and p24. Elsewhere we have presented evidence that detection of these phenomena cannot be considered synonymous with isolation. They can only be used for viral detection, and even then if, and only if, they are first shown to be specific for HIV. The above phenomena have been discussed in detail (Papadopulos-Eleopulos, Turner & Papadimitriou, 1993a) and it has been shown that none is specific for HIV or even for retroviruses. Below some additional points regarding virus-like particles, RT and p24 will be considered, (additional points regarding hybridisation have been presented above).

Virus-like particles

Although the origin and role of 'retrovirus particles' are not known, they are considered ubiquitous and this is especially the case in cell cultures and in pathological tissue. In 1969 Chopra and Feller, noticing that 'Virus like particles resembling the C-type [some classify HIV as a C-type] particles associated with mouse leukemia have been reported in human leukemic tissues by a number of investigators' reported that: 'These particles have been observed in the density gradient purified fractions of milk samples obtained from women having breast cancer and from milk of a normal woman with a family history of breast cancer. A few particles have also been detected in tissue-culture of a breast cancer biopsy' (Chopra & Feller, 1969). Levine *et al.* examined (blindly) plasma of leukaemic and healthy individuals: 'A specimen was considered positive if there were numerous double-membraned particles with dense nucleoid which were about 100uu in diameter and comparable to the type C particles described by Porter and Dalton. A specimen was designated as suspicious if particles were found which were morphologically similar to those in positive specimens, but were very few in numbers. Specimens with numerous but less typical particles and 'empty' particles were also considered suspicious. All other specimens were classified as negative ... In this study the problems of false positives was largely eliminated by using ultrathin sections of high speed plasma pellets'. They reported that 'Of 45 patients with myelocytic leukemia, five with acute and four with chronic myelocytic leukemia showed multiple virus-like particles. Seven additional patients had similar particles in lesser numbers or particles devoid of the dense nucleoid. In these 16 patients the particles were detected when the disease was untreated or not responding to therapy. Three patients with acute myelocytic leukemia and numerous virus-like particles in the florid leukemic phase showed no particles while in complete or partial remission. Numerous particles were found in the plasma of one patient with acute lymphocytic leukemia but were not found in samples from 14 patients with chronic lymphocytic leukemia. One suspicious sample was obtained from a patient with infectious mononucleosis but 14 other nonleukemic samples were negative' (Levine *et al.*, 1967). In 1972, virus-like particles with morphological characteristics similar to those ascribed to HIV by some researchers (*Lentiviruses*) were reported in cultures of human brain cells (Hooks *et al.*, 1972). By 1974, researchers from the Koch-

Institute in Germany, including Gelderblom, reported virus-like particles in HeLa cells, and Canadian researchers reported the same particles in cultures of marrow cells from leukaemic patients (Bauer *et al.*, 1974; Mak *et al.*, 1974; Watson *et al.*, 1974). In conclusion, particles with morphological characteristics ascribed to HIV are not specific to this virus.

Reverse transcriptase

Although at present some of the best known AIDS researchers consider RT as being the '*sine qua non*' of retroviruses, and regard the detection of reverse transcription in lymphocyte cultures from AIDS patients not only as proof of the presence of such viruses but of HIV itself, according to some of the best known retrovirologists, including the discoverers of RT, reverse transcription is a property of all cells, and is by no means confined to retroviruses (Temin & Baltimore, 1972; Varmus, 1987).

'Reverse transcriptase (RT) was first discovered as an essential catalyst in the biological cycle of retroviruses. However, in the past years, evidence has accumulated showing that RTs are involved in a surprisingly large number of RNA-mediated transcriptional events that include both viral and nonviral genetic entities ... the possibility that reverse transcription first took place in the early Archean' is supported by a number of facts and 'the hypothesis that RNA preceded DNA as cellular genetic material' (Lazcano *et al.*, 1992).

As has already been stated, when the HIV researchers Andrews and colleagues used RT for proving HIV isolation from haemophiliacs 'An assay count of 10^4 cpm/mL (after subtraction of cellular polymerase activity) was considered positive for virus'. However, the demonstration of higher levels of reverse transcription from the cells of haemophiliacs is not proof that the activity is due to HIV. How does one know that the higher activity of these cells is not due to:

(a) activation 'of cellular polymerase activity' by factor VIII itself or the many contaminants present in factor VIII preparations to which haemophiliacs are exposed?
(b) the many factors (PHA,IL-2, polybrene) to which the haemophiliacs' cultures are exposed?

Even if RT were a property only of viruses, it is not specific to retroviruses. According to Varmus: 'Reverse transcription was assigned a central role in the replication of other viruses [hepatitis B and cauliflower mosaic viruses] and in the transposition and generation of other kinds of eukaryotic DNA' (Varmus, 1988). 'The hepatitis B viruses (HBVs) are small DNA viruses that produce persistent hepatic infections in a variety of animal hosts and replicate their DNA genomes via reverse transcription of an RNA intermediate. All members of this family contain an open reading frame (ORF), 'P' (for *pol*), which is homologous to retroviral *pol* genes' [*pol*=polymerase] (Chang *et al.*, 1989). 'Hepatitis B virus (HBV) resembles retroviruses, including HIV, in several respects. In particular, both viruses contain reverse transcriptase, and replicate through and RNA intermediate'. Because of this, it has been suggested that hepatitis B infection should be treated with the same antiretroviral agents as HIV infection (Mitsuya & Broder, 1989). At present, evidence exists which shows that although the major target organ for hepatitis B virus is the liver, cells other than hepatocytes 'including peripheral blood lymphocytes and monocytes, may become infected with HBV' (Neurath, Strick & Sproul, 1992). Lymphocyte stimulation in general and PHA stimulation in particular is associated with production of hepatitis B virus from peripheral blood lymphocytes in patients infected with HBV including 'viral replication in chronic hepatitis B infection of childhood' (Vegnente *et al.*, 1991; Sarria *et al.*, 1993). It is of pivotal significance to note that 98% of HIV seropositive patients with haemophilia are infected with hepatitis B virus (Brenner *et al.*, 1991). It is also of interest to note that AIDS patients suffer frequently from bacterial infections and that 'bacteria too, have reverse transcriptases' (Varmus, 1989).

In 1989 Blattner wrote: 'Assays for reverse transcriptase, the unique viral enzyme, employ special oligonucleotide templates in the presence of magnesium. A characteristic profile of enzyme activity suggests the presence of a retrovirus, but false positivity arising from cellular enzyme activity or false negativity because of low reverse transcriptase level make this technique too unreliable for epidemiologic application' (Blattner, 1989).

However, there is no 'characteristic profile of enzyme activity' in haemophilia cultures/co-cultures and no 'special oligonucleotide templates' are used. To prove HIV infection, all researchers use the template-primer poly-A-oligo-dT12-18 ($An.dT_{15}$). However, this template-primer is not specific to retroviral RTs. As far back as 1972 Gallo and his colleagues showed that reverse transcription of the template-primer $An.dT_{15}$ can be achieved with material obtained from cultures of 'PHA stimulated (but not unstimulated) normal human blood lymphocytes', which in sucrose density gradi-

ents bands at 1.16 gm/ml (Gallo, Sarin & Wu, 1973). Not only is this template-primer not specific to retroviral RT, but all the cellular DNA polymerases, α, β and γ, can copy An.dT_{15} (Sarngadharan, Robert-Guroff & Gallo, 1978). In fact, in 1975, an International Conference on Eukaryotic DNA polymerase (Weissbach *et al.*, 1975) defined DNA polymerase γ, 'a component of normal cells' (Robert-Guroff *et al.*, 1977) 'found to be widespread in occurrence' (Sarngadharan, Robert-Guroff & Gallo, 1978), whose activity can be increased by many factors, including PHA stimulation (Lewis *et al.*, 1974), as:
the enzyme which 'copies An.dT_{15} with high efficiency but does not copy DNA well' (Weissbach *et al.*, 1975). Thus, reverse transcription, including that of the primer-template An.dT_{15}, cannot be considered specific to HIV or even to retroviruses.

The p24 protein

The p24 protein is considered to be coded by the HIV *gag* gene, that is, by the gene which codes the group specific antigens of retroviruses. As far back as 1974 Gelderblom and his colleagues wrote 'While the virus envelope antigens are primarily virus-strain specific, the bulk of internal proteins of the virion with molecular weight (mw) between 10,000 d and 30,000 d are group-specific (gs) for viruses originating in a given animal species (gs-spec. antigens). The major protein constituent of mammalian C-type oncornaviruses with a molecular weight in the range of 30,000 d was found to possess, besides gs spec. antigen, an antigenic determinant that is shared by C-type viruses of many mammalian species including monkeys and was thus termed gs interspecies (gs-interspec.) antigen' (Bauer *et al.*, 1974). As late as 1983 Blattner stated: 'It may be feasible to use viral antigen probes to look for cross-reactive antibodies, since certain viral proteins, particularly the polymerase and *gag* proteins, may be highly conserved between subtypes of virus' (Blattner, 1989). Thus, even if p24 were to be specific to retroviruses, it cannot be HIV specific. Indeed, apart from a joint publication with Montagnier in 1988 (Gallo & Montagnier, 1988) where it is claimed that p24 is unique to HIV, Gallo and his colleagues have repeatedly stated that the p24 of HIV and of two other human retroviruses, HTLV-1 and HTLV-II, which Gallo claims to have isolated from humans, immunologically cross-react (Wong-Staal & Gallo, 1985).

The whole blood cultures of 49/60 (82%) of 'presumably uninfected but serologically indeterminant individuals and 5/5 seronegative blood donors' were found positive for p24 (Schupbach *et al.*, 1992). The 'HIV proteins (p17, p24)' appear in the blood of patients (previously negative for all HIV markers) following 'transfusions of HIV-negative blood and UV-irradiation of the autoblood' (Kozhemiakin & Bondarenko, 1992). p24 is detected in a significant number (up to 36% of patients with systemic lupus erythematosus) (Barthel & Wallace, 1993). Detection of p24 has been also reported in organ transplant recipients. In one kidney recipient (the donor was negative for p24 antigen) who, three days following transplantation developed fever, weakness, myalgias, cough and diarrhoea, all 'Bacteriological, parasitological and virological samples remained negative [including HIV PCR]. The only positive result was antigenaemia p24, positive with Abbot antigen kits in very high titers of 1000 pg/ml for polyclonal and 41 pg/ml for monoclonal assays. This antigenaemia was totally neutralizable with Abbot antiserum anti-p24 ... 2 months after transplantation, all assays for p24-antigen became negative, without appearances of antibodies against HIV. Five months after transplantation our patient remains asymptomatic, renal function is excellent, p24 antigenaemia still negative and HIV antibodies still negative' (Vincent *et al.*, 1993). Using two kits, the Abbot and Diagnostic Pasteur, in one study p24 was detected transiently in 12/14 kidney recipients. Peak titres ranged from 850 to 200 000 pg/ml 7–27 days post-transplantation. Two heart and 5/7 bone marrow recipients were also positive, although the titres were lower and ranged from 140–750 pg/ml. Disappearance of p24 took longer in kidney (approximately 6 months) than in bone-marrow (approximately 4–6 weeks) recipients. According to the authors: 'This may be related to differences in immunosuppression therapy.' Discussing their findings they wrote: 'The observation of a 25–30 kD protein [the French researchers report p24 as p25] binding to polyclonal anti-HIV human sera after immunoblots with reactive sera raises several questions. This protein could be related to a host immune response to grafts or transplants ... Its early detection after transplantation might indicate the implications of immunosuppression therapy ... The 25–30 kD protein could therefore be compared with the p28 antigen recently described with human T-cell-related virus lymphotropic-endogenous sequence ... The characterization of this 25–30 kD protein may represent an important contribution to the detection of HIV-1 related endogenous retroviruses' (Agbalika *et al.*, 1992).

There are many reasons why the p24 detected in the sera and cultures of haemophiliacs, like the p24 detected in organ recipients may not be the protein of an exogenous retrovirus, HIV, but either a non-viral-protein or the protein of an endogenous retrovirus:

1. Like transplant recipients, haemophiliacs receive material derived from other humans;
2. Like organ transplant recipients, haemophiliacs are immunosuppressed (see below);
3. HIV cannot be 'isolated' unless the cultures are mitogenically stimulated (activated);
4. The normal human genome contains many copies of endogenous retroviral sequences (proviruses), 'including a complex family of HIV-1 related sequences' (Horwitz, Boyce-Janino & Faras, 1992), a 'large fraction' of which 'may exist within a host cell as defective genomic fragments. The process of recombination however may allow for their expression as either particle or synthesis of a new protein(s)' (Weiss *et al.*, 1982; Varmus & Brown, 1989; Cohen, 1993; Lower & Lower, 1993; Minassian *et al.*, 1993). Varmus describes the genetic behaviour of retroviruses as follows: 'During the virus life cycle, several interesting genetic and quasi-genetic phenomena may occur, especially if cells are infected by more than one virus: production of heterozygotic dimeric genomes, formation of pseudotypes at high frequencies (particles with core proteins and genome provided by one virus and envelope proteins by another), frequent deletions and nucleotide substitutions, and recombination between related, coinfecting viruses. [Recombination between retroviruses is surprisingly efficient but its mechanistic basis has not been resolved]' (Varmus, 1988).
5. Cultivation of normal 'non-virus' producing cells leads to retroviral production (expression), 'the failure to isolate endogenous viruses from certain species may reflect the limitations of *in vitro* cocultivated techniques' (Todaro, Benviste & Sherr, 1976). The expression can be accelerated and the yield increased by exposing the cultures to mitogens, mutagens or carcinogens, co-cultivation techniques and cultivation of cells with supernatant from non-virus producing cultures (Toyoshima & Vogt, 1969; Aaronson, Toduro & Schlonick, 1971; Hirsch, Phillips & Solnick, 1972). For HIV isolation, in most instances, all the above techniques are employed. Thus, even if 'true' (Popovic, Sarngadharan & Read, 1984) retroviral isolation can be achieved from the cultures/co-cultures of tissues from haemophiliacs, it would be difficult if not impossible to be certain that the retrovirus in question is an exogenous retrovirus which is acquired through factor VIII administration. For such evidence to be accepted as proof of the existence of HIV, the activation of an endogenous provirus or a provirus assembled by recombination of endogenous retroviral and cellular sequences would need to be rigorously excluded.

Thus, although AIDS researchers acknowledge that:

(a) plasma is 'unlikely to be a meaningful source of HIV infection';
(b) cell free particles in plasma lack the gp120 protein which is 'crucial to HIV's ability to infect new cells';
(c) factor VIII preparations are cell free;
(d) the physical processes employed in the manufacture of factor VIII even in the absence of heating, destroy both cells and viruses;

AIDS researchers claimed and continue to claim that 'HIV' has been 'isolated' from haemophiliacs. However, and in spite of this affirmation, the above data strongly signifies that the HIV phenomena (particles, RT, antibody-antigen reactions (WB), HIV-PCR-hybridisation) observed in patients with haemophilia, whatever they represent, are inconsistent with the parenteral acquisition of an exogenous retrovirus.

Lastly, 'HIV' has been 'isolated' from children with haemophilia:

(a) who had no other risk factor other than haemophilia;
(b) where each plasma unit from which factor VIII was made 'had been tested for HIV antibody, hepatitis B surface antigen and alanine aminotransferase, usually within 2 days after collection' (Remis *et al.*, 1990);
(c) where factor VIII was heat treated at 60°C for 30 hours (according to some authors HIV is 'completely inactivated in the samples within a few minutes', of heating (Hilfenhaus *et al.*, 1990));
(d) where the source plasmas from which the lots of factor VIII were made were retested 'within several months after donating factor VIII, and were found negative' (Neumann *et al.*, 1990; Remis *et al.*, 1990).

This is as close a proof as one can get that what has been called HIV infection in haemophiliacs is not caused by an exogenous retrovirus to which haemophiliacs have been exposed by the administration of factor VIII preparations.

T4 cells

It is generally accepted that in patients with haemophilia, HIV destroys T4 lymphocytes leading to acquired immune deficiency. Although this view has prevailed for ten years, at least one well known group of researchers of AIDS in haemophiliacs, that from the University of Bonn, questioned the above relationship between HIV and T4 cells as recently as 1990. 'It is not clear whether the virus-host interrelationship in HIV infections is regulated primarily by the virus or by the host; i.e., are $CD4^+$ cells depleted by non-viral mechanisms and does the virus adjust itself to the weakened defense? Or is the depletion of CD^+ cells the consequence of the spread and cytopathogenicity of virulent viral variants, which developed at random from avirulent precursors?' (Scheneweis *et al.*, 1990). Discussing their data a year earlier they concluded 'The results suggest that reactivation of HIV occurs when immune deficiency has become manifest' (Schneweis *et al.*, 1989). The question whether HIV leads to T4 cell depletion or conversely whether T4 cell depletion leads to 'HIV infection' (particles, RT in cultures, antigen/antibody reactions, 'HIV/PCR') can only be resolved by having direct evidence that HIV destroys the T4 cells of haemophiliacs. No such evidence exists. An indirect method of resolution is the examination of the chronological sequence of HIV infection and T4 cell depletion. Numerous reports from many well known researchers of AIDS in haemophiliacs have shown that T4 cell depletion precedes 'HIV infection':

1. Mortimer and his colleagues state, 'The OKT4 subset was reduced in both seropositive ($p < 0.01$) and seronegative ($p < 0.05$) haemophiliacs but there was no difference between seropositive and seronegative patients' (Moffat, Bloom & Mortimer, 1985);
2. Weiss and colleagues report, 'We have thus been able to compare lymphocyte subset data before and after infection with HTLV-III. It is commonly assumed that the reduction in T-helper-cell numbers is a result of the HTLV-III virus being tropic for T-helper-cells. Our finding in this study that T-helper-cell numbers and the helper/suppressor ratio did not change after infection supports our previous conclusion that the abnormal T-lymphocyte subsets are a result of the intravenous infusion of factor VIII concentrates per se, not HTLV-III infection' (Ludlam *et al.*, 1985);
3. Kessler and colleagues found, 'Repeated exposure to many blood products can be associated with development of T4/T8 abnormalities' including 'significantly reduced mean T4/T8 ratio compared with age and sex-matched controls' (Kessler *et al.*, 1983);
4. In 1984, Tsoukas *et al.* observed that among a group of 33 asymptomatic haemophiliacs receiving factor VIII concentrates, 66% were immunodeficient 'but only half were seropositive for HTLV-III', while 'anti-HTLV-III antibodies were also found in the asymptomatic subjects with normal immune function'. They summarised their findings as follows: 'These data suggest that another factor (or factors) instead of, or in addition to, exposure to HTLV-III is required for the development of immune dysfunction in haemophiliacs' (Tsoukas *et al.*, 1984);
5. By 1986 researchers from the CDC concluded: 'Haemophiliacs with immune abnormalities may not necessarily be infected with HTLV-III/LAV, since the factor concentrate itself may be immunosuppressive even when produced from a population of donors not at risk for AIDS' (Jason *et al.*, 1986);
6. In 1985 Montagnier (Montagnier, 1985) wrote: 'This [clinical AIDS] syndrome occurs in a minority of infected persons, who generally have in common a past of antigenic stimulation and of immune depression before LAV infection'.

Thus, haemophiliacs may develop immune deficiency before HIV infection, that is, HIV is not necessary for the decrease in T4 cells observed in haemophiliacs. Furthermore, to date, there is no evidence either from the haemophilia studies or from the studies in any other AIDS risk group, that HIV can induce immune deficiency (Papadopulos-Eleopulos *et al.*, 1994). In other words, HIV is neither necessary nor sufficient for the appearance of immune deficiency (decrease in T4 cells). However, there is ample evidence which shows that:

1. Decrease in T4 cells in AIDS patients is not due to destruction of T4 cells, but due to a change in T8 phenotype (Papadopulos-Eleopulos *et al.*, 1994);
2. There is no correlation between T4 numbers and the clinical syndrome in any AIDS risk group (Allain *et al.*, 1987; Papadopulos-Eleopulos *et al.*, 1994).

That is, decrease in T4 cell numbers is neither necessary nor sufficient for the appearance of the clinical syndrome.

Clinical and classification considerations

In 1981, high frequencies of Kaposi's sarcoma (KS), *Pneumocystis carinii* pneumonia (PCP) and a small number of other diseases induced by other opportunistic infectious agents, that is by agents which are ubiquitous but which usually produce clinical disease only when the host defense mechanisms are depressed, were observed in gay men in the United States.

Some of the gay men with KS or PCP were tested for immunological abnormalities and a significant number were found to have low numbers of T4 cells, 'cellular immune deficiency'. Because of this, the term gay related immune deficiency (GRID) was first used to describe the disease in these patients, but not long after this was changed to AIDS. In 1982, the CDC defined AIDS as

> 'illnesses in a person who 1) has either biopsy-proven KS or biopsy- or culture-proven life-threatening opportunistic infection, 2) is under 60, and 3) has no history of either immunosuppressive underlying illness or immunosuppressive therapy' (CDC, 1982a; 1982b). In addition to PCP, the 'serious OI' were 'meningitis, or encephalitis due to one or more of the following: aspergillosis, candidiasis, cryptococcosis, cytomegalovirus, nocardiosis, strongyloidosis, toxoplasmosis, zygomycosis, or atypical mycobacteriosis (species other than tuberculosis or lepral); esophagitis due to candidiasis, cytomegalovirus or herpes simplex virus; progressive multifocal leukoencephalopathy; chronic enterocolitis (more than 4 weeks) due to cryptosporidiosis; or unusually extensive mucocutaneous herpes simplex of more than five weeks duration'.

It must be pointed out that not one of the diseases which constituted AIDS, the AIDS indicator diseases, was new. What was new was the high frequency of these diseases in gay men (CDC, 1982a; 1982b). In the same year, Robert Gallo, Myron Essex and James Curran put forward the hypothesis that the cause of AIDS is a virus, the retrovirus HTLV-1 or a similar virus (Gallo, 1987). According to this theory, the retrovirus induced immune deficiency by causing the destruction of T4 cells, which in turn led to the appearance of KS, PCP and other OI, i.e. AIDS. In order to obtain evidence in support of the above theory, that AIDS was caused by an infectious agent, the CDC formed a task-force composed of 32 individuals, mainly physicians, which 'actively surveyed physicians in 18 major metropolitan areas in the United States by letter and telephone to inquire about Kaposi's sarcoma in persons under 60 years of age or opportunistic infections in patients without a known predisposing factor since January 1979 ... a formal request was made to state health departments to notify the CDC of illnesses suspected of fitting the [above] case definition' (CDC, 1982a). Since HTLV-1 was claimed to be transmitted by blood and blood products, patients with haemophilia became a specific target. In July 1982 the CDC reported the first three cases of '*Pneumocystis carinii* pneumonia among persons with Haemophilia A'.

The first patient was a 62 year old individual with a one year history of weight loss. The treatment and previous medical history were not given. In December 1981, following the development of cough and fever he was found to be 'lymphopenic, and chest X-ray revealed interstitial infiltrates compatible with viral pneumonia'. He was treated with corticosteroids resulting in an 'overall clinical improvement'. In January 1982 he presented with 'severe respiratory distress' and PCP was proven by open lung biopsy.

The second patient, 59 years old, with a history of weight loss, 'apthous-like ulcers and anterior cervical adenopathy beginning in October 1981', was diagnosed with oropharyngeal candidiasis in February 1982. No previous medical history or treatment was given. In May 1982 he was hospitalised 'with symptoms including nausea, vomiting, and recurrent fever. Pneumonia was diagnosed, and *P. carinii* and cytomegalovirus (CMV) were repeatedly identified from lung tissue or bronchial secretions'. He also had decreased T4 cell number, increased T8 cell numbers and a decreased T4/T8 ratio.

The third patient, 27 years old, with a history of fever and urinary frequency and urgency (treatment not given), was diagnosed with PCP in October 1981. In February 1982 he was treated with ketoconazole. By April he developed fever, splenomegaly, anaemia, lymphopenia, and *Mycobacterium avium* was grown from a number of tissues. He also had 'a reduction in absolute numbers of circulating T-cells'. Subsequently, he was found to have decreased T4 cells, increased T8 cells and a low T4/T8 ratio.

From these case histories it was concluded that 'the clinical and immunological features of these three patients are strikingly similar to those recently observed among certain individuals from the following groups: homosexual males, heterosexuals who abuse IV drugs, and Haitians who recently entered the United States. Although the cause of the severe immune dys-

function is unknown, the occurrence among the three hemophiliac cases suggests the possible transmission of an agent through blood products' (CDC, 1982a; 1982b).

As a consequence, the CDC 'notified directors of hemophilia centers about these cases and, with the National Hemophilia Foundation, has initiated collaborative surveillance'. In the same year, Ragni and colleagues found two haemophiliacs with decreased T4 and increased T8 cell numbers, elevated IgG and IgM levels and lymphadenopathy and concluded that their findings were 'consistent with the diagnosis of AIDS' (Ragni *et al.*, 1983).

By October 1983, the CDC had 23 reports of AIDS cases in haemophiliacs, 18 in the USA and 5 other countries, none with KS. Two of the above cases had other risk factors, one was an IV user, the other gay (Jason *et al.*, 1984). By the end of 1984, the number of haemophilia AIDS cases increased to 67 (Levine, 1985). By this time, Gallo's claim that AIDS in all risk groups – gay men, IV users, blood transfusion recipients, and haemophiliacs – is caused by a new retrovirus, HTLV-III, later renamed HIV, became generally accepted. By the end of June 1985, 80 haemophiliacs in the USA and five in the United Kingdom were reported with AIDS, none with KS (Jones *et al.*, 1985). At about the same time it became known that by 1982 the vast majority of haemophiliacs tested positive for HIV. 'Yet the attack rate of AIDS in hemophiliacs is not steadily climbing per reported period [in gay men it was increasing exponentially]. In addition, the last two reporting periods [last quarter of 1984, and first quarter of 1985 when HIV testing was introduced] contains a disproportionate number of patients with mild and moderate disease' (Levine, 1985). Indeed, as has been seen, the only clinical symptoms in the two patients reported by Ragini *el al.* consistent with the 'diagnosis of AIDS' was lymphadenopathy.

Some published reports represent the gallant efforts made by some researchers to prove that HIV infection in haemophiliacs, like HIV infection in gay men, leads to neurological complications. Researchers from the Royal Postgraduate Medical School in London reported two fatal haemophilia cases. The first patient exhibited 'lethargy, poor concentration, and difficulty with micturition. Examination disclosed diminished cognitive function and brisk reflexes. Computed tomography (CT) of the brain showed dilated lateral ventricles and widened sulci consistent with cerebral atrophy'. Four months later 'he was incontinent and had difficulty walking and showed signs of a pyramidal tract lesion'. One month later he was 'unable to walk, had paranoid delusions. Relentless neurological deterioration followed with painful spastic quadriparesis and convulsions'. The second patient showed 'weight loss, confusion, unilateral cerebellar dysfunction, and diplopia which was diagnosed clinically as an internuclear opthalmoplegia. A cerebral CT scan showed low attenuation areas in the white matter of the frontal lobes and also in the right parietal lobe'. The above clinical signs were followed by coma. Although no general or neuropathological examination was conducted in either of these patients (permission for autopsy was refused), both these cases of 'subacute encephalopathy' were attributed to HIV because the patients were HIV positive and had a low T4/T8 ratio (Rahemtulla, Durrant & Hows, 1986). Similarly, cerebral toxoplasmosis attributed to HIV, also without neuropathological examination, has also been reported in haemophiliacs (Esiri *et al.*, 1989).

The introduction of 'mild and moderate disease' as indicating AIDS, which commenced in the last quarter of 1984, coincided with the acceptance of HIV as the cause of AIDS in all risk groups and the redefinition of AIDS by the CDC. Before this date practically all AIDS was KS and PCP. According to the 1985 CDC definition, 'a case of acquired immunodeficiency syndrome (AIDS) is an illness characterized by:

I. one or more of the opportunistic diseases listed below (diagnosed by methods considered reliable) that are at least moderately indicative of underlying cellular immunodeficiency; and

II. absence of all known underlying causes of cellular immunodeficiency (other than LAV/HTLV-III infection) and absence of all other causes of reduced resistance reported to be associated with at least one of those opportunistic diseases.

Despite having the above, patients are excluded as AIDS cases if they have negative result(s) on testing for serum antibody to LAV/HTLV-III, do not have a positive culture for LAV/HTLV-III, and have both a normal or high number of T-helper (OKT4 or LEU3) lymphocytes and a normal or high ratio of T-helper to T-suppressor (OKT8 or LEU2) lymphocytes. In the absence of test results, patients satisfying all other criteria in this definition are included as cases' (WHO, 1986).

A number of additional AIDS indicator diseases were added to the 1982 definition. These included: lymphoma limited to the brain, disseminated histoplasmosis, isosporiasis and non-Hodgkin's lymphoma. Although HIV was accepted as being the cause of

AIDS in all AIDS risk groups, there were significant differences between the groups. For example:

1. If HIV is the cause of AIDS in all the above risk groups, one would expect the rate of conversion to clinical AIDS in all HIV positive individuals to be the same. This is not the case. In a cohort of gay men in the USA, the three year actuarial progression rate was 22% (Moss *et al.*, 1988). In a cohort of haemophiliacs, the annual incidence of AIDS ranged from zero during the first year after seroconversion to 7% during the eight year follow up with an eight year cumulative rate of 13.3% (Goedert *et al.*, 1989). In the United Kingdom three percent of haemophilia patients developed AIDS by three years after seroconversion and 7% by five years post seroconversion (Darby *et al.*, 1989);
2. The clinical syndrome in haemophiliacs is different from that in gay men. KS, one of the two most significant and frequent diseases in gay men, for whose explanation the HIV hypothesis was put forward, is practically non-existent in haemophiliacs. Such is also the case with oral hairy leukoplakia (Greenspan & Greenspan, 1989).

To determine the forms of neuropathological and systemic pathology in HIV positive haemophiliacs as compared to other HIV positive subjects, Esiri *et al.* examined the brains of 42 HIV seropositive HIV individuals. Among these were 11 haemophiliacs and 29 gay men. Four of the haemophiliacs were classified as having AIDS, as were the majority of the gay men. 'The prevalences of opportunistic infections of the central nervous system were significantly higher in non-haemophiliacs (cerebral toxoplasmosis 23% (7), progressive multifocal leucoencephalopathy 10% (3), and cerebral cytomegalovirus infection 19% (6) in the non-haemophiliacs *v* no cases in the haemophiliacs). The prevalences of fresh and old intracranial haemorrhages and cirrhosis of the liver were significantly higher in the haemophiliacs (fresh intracranial haemorrhage 45% (5), old intracranial haemorrhage, 36% (4) and cirrhosis of the liver 27% (3) in the haemophiliacs *v* no cases in the non-haemophiliacs)'. Discussing their results Esiri and colleagues wrote: 'The rarity of opportunistic infections in the central nervous system and elsewhere in haemophiliacs is in keeping with many of them dying at an earlier (pre-AIDS) stage in the development of HIV associated immunodeficiency than do most subjects with HIV infection. [To the contrary, as has been stated above, HIV seropositive haemophiliacs live longer than HIV seropositive gay men]. Consistent with this suggestion is Hilgartner's view that the pattern of disease due to HIV infection in haemophiliacs differs from that in other groups at high risk, and from the observation of Darby *et al.* that a substantial burden of fatal disease occurs among haemophiliacs who are positive for HIV and not formally diagnosed as having AIDS. If our cases of haemophiliacs are representative of others much of this fatal disease would seem to be accounted for by cerebrovascular and liver disease' (Hilgartner, 1987; Esiri *et al.*, 1989; Darby *et al.*, 1990).

Once again, it is of pivotal significance to note that even in the early years of the recognition of AIDS, it was agreed that in haemophiliacs, there was 'an immunodeficiency independent of HTLV-III infection' (Hollan *et al.*, 1985; Madhok *et al.*, 1986). That is, haemophiliacs have 'known underlying causes to cellular immunodeficiency (other than LAV/HTLV-III), HIV'. Thus, according to the 1985 AIDS definition, haemophiliacs cannot be AIDS cases. Furthermore, although a prerequisite of the diagnosis of AIDS in the 1985 definition was a positive test for HIV, of all AIDS cases reported in the two year period 1985–1987 in New York City and San Francisco, which constituted approximately one third of all AIDS cases in the USA, 'less than 7% have been reported with HIV-antibody test results' (CDC, 1987).

Like the 1982 definition, the 1985 required the diseases which constituted AIDS, the AIDS 'indicator' diseases, to be definitely diagnosed. However, the New York State Health Department found that although 13% of 1329 AIDS cases reported by the beginning of 1987 did have a positive HIV antibody test, clinically these individuals' symptoms were suggestive of AIDS but were not definitely diagnosed. In a similar study researchers from the CDC and the Departments of Health in New Jersey, Puerto Rico, Boston, Washington, D.C. and Connecticut found that approximately 11% of cases had a presumptive diagnosis because, according to one AIDS epidemiologist 'Many physicians are familiar enough with AIDS now that when they see a young man with pneumonia, they can make a reasonable presumptive diagnosis [of PCP] without resorting to biopsy,' (Anonymous, 1987). Thus a significant number of reported AIDS cases did not meet either the 1982 or the 1985 AIDS definition.

To accommodate the non-compliance with the 1985 AIDS definition, the CDC claimed that their 1985 definition made it 'unnecessarily difficult to diagnose' AIDS, and thus it underestimated the number of AIDS cases. In 1987, the CDC yet again redefined AIDS. The 1987 definition permitted reporting of Acquired

Immunodeficiency (AIDS) cases even if there was no evidence of immune deficiency or of a definite diagnosis of at least some of the AIDS indicator diseases. More importantly, although the definition considered HIV to be the sole cause of AIDS, individuals could be reported as AIDS cases even when there was evidence against HIV infection. The major features of the 1987 definition are:

I. Without laboratory evidence of HIV infection (patients not tested for HIV or if tested the results were inconclusive), the 1985 indicator diseases 'if reliably diagnosed and other causes of immune deficiency are ruled out', (that is, immunosuppressive therapy $\leq$ 3 months before the onset of the indicator disease, a small number of neoplastic diseases diagnosed $\leq$ 3 months after diagnosis of the indicator disease, an even a smaller number of congenital immunodeficiency diseases), 'are still accepted as a diagnosis of AIDS'.

II. 'Regardless of the presence of other causes of immunodeficiency in the presence of laboratory evidence of HIV infection':
A. Twelve new AIDS indicator diseases, when definitely diagnosed, indicate AIDS. These include:
(i) extrapulmonary tuberculosis;
(ii) wasting syndrome, that is, involuntary weight loss of $>$ 10% of body weight and either chronic diarrhoea (at least 2 stools per day for $\geq$ 30 days) or chronic weakness and documented fever (for $\geq$ 30 days, intermittent or constant);
(iii) HIV encephalopathy (schizoid behaviour, general fatigue, malaise, diminished cognitive function) (Gomperts, 1990);
(iv) bacterial infections, multiple or recurrent (any combination of at least two within a 2-year period) of the following types affecting a child less than 13 years of age: septicaemia, pneumonia, meningitis, bone or joint infection, or abscess of an internal organ or body cavity (excluding otitis media or superficial skin or mucusal abscesses) caused by *Haemophilus, Streptococcus,* (including *Pneumococcus*, or other pyogenic bacteria.
B. The diseases listed below, even if presumptively diagnosed, indicate AIDS:
'1. Candidiasis of the oesophagus.
2. Cytomegalovirus retinitis with loss of vision.
3. Kaposi's sarcoma.
4. Lymphoid interstitial pneumonia and/or pulmonary lymphoid hyperplasia (LIP/LPH complex) affecting a child $<$ 13 years of age.
5. Mycobacterial disease (acid-fast bacilli with species not identified by culture), disseminated (involving at least 1 site other than or in addition to lungs, skin, or cervical or hilar lymph nodes).
6. *Pneumocystis carinii* pneumonia.
7. Toxoplasmosis of the brain affecting a patient $>$ 1 month of age.' For example:
(i) presumptive diagnosis of candidiasis of the oesophagus is:
'A recent onset of retrosternal pain on swallowing; **AND** oral candidiasis diagnosed by the gross appearance of white patches or plaques on an erythematous base or by the microscopic appearance of fungal mycelial filaments in an uncultured specimen scraped from the oral mucosa'.
(ii) presumptive diagnosis of KS is:
'A characteristic gross appearance of an erythematous or violaceous plaque-like lesion on skin or mucous membrane'
(WHO, 1988).

III. If there is 'laboratory evidence against HIV infection', that is, the laboratory tests for HIV infection are negative, but the patient has all above mentioned causes of immunodeficiency excluded and either:
(a) *Pneumocystis carinii* pneumonia diagnosed by a definite method OR;
(b) any of the 1985 AIDS indicator disease diagnosed by a definite method AND;
(c) a T4 cell count $<$ 400/mm^3
the patient has AIDS.

Thus the 1987 AIDS definition legitimised the reporting of a person as suffering from Acquired Immune Deficiency Syndrome, accepted to be caused by HIV when;

1. Evidence of HIV infection was 'not performed or gave inconclusive results', or even when all tests were negative, i.e. when there was definite evidence that the patient was not infected with HIV;
2. The absence of any evidence of immune deficiency and even when the cause of immune deficiency could have been other than HIV;
3. Both in the absence of HIV infection and immune deficiency.

In 1987, it was known that many indicator diseases of the 1985 definition, including KS and PCP, were difficult to diagnose both clinically and histopathologically (CDC, 1981; Follansbee *et al.*, 1982; Hughes, 1987; Beral *et al.*, 1990). Yet the definition permitted a person to be reported as suffering from AIDS, even when the indicator diseases were presumptively diagnosed, i.e. on the basis

of non-specific findings. In fact, the 1987 definition allowed so many degrees of freedom that nearly anybody, especially those belonging to a 'risk group', could be reported as an AIDS case. This can best be illustrated by the following example:

In 1992, 'The AIDS Reporting System was searched for all persons who had been given a diagnosis of AIDS' by investigators from the CDC (Smith *et al.*, 1993). In seven reported haemophilia 'AIDS' cases:

(a) the HIV tests were all negative;

(b) not one of the patients had an AIDS indicator disease: four had common diseases especially frequent in haemophiliacs: haematomas, hepatitis C infection, thrombocytopenia and oral herpes. Two patients were asymptomatic;

(c) all patients had a low ($< 300/mm^3$) T4 cell count.

Interestingly, in 1987, merely by redefining AIDS, there was a sharp increase in AIDS cases in all AIDS risk groups including haemophiliacs. In 1988, there were 552 cases in haemophiliacs of which 31 were known to be gay and 12 drug users (Koerper, 1989). Not only did the 1987 AIDS definition fail to solve the major problems arising from the 1982 definition, but by then other problems associated with the HIV hypothesis of AIDS in haemophiliacs became apparent:

1. It is accepted by all HIV researchers that heating factor VIII preparations destroys HIV. Yet AIDS has been diagnosed in haemophiliacs who exclusively received heat treated factor VIII (CDC, 1987);
2. Unlike other AIDS risk groups, in haemophilia, thrombocytopenia and older age are risk factors for the development of AIDS (Eyster *et al.*, 1987). However:

   (a) it is well known that older age is associated with both immune deficiency and increased incidence of OI and malignancies. Indeed, of the first three cases reported to the CDC with PCP in haemophiliacs in 1982, one of these was not considered to be an AIDS case because he was 62 years of age;

   (b) before the AIDS era the rate of thrombocytopenia in haemophiliacs was significantly different ($p < 0.0003$) than the normal population. Eyster *et al.* examined data collected by the Hemophilia Study Group from 1975 to 1979 on 1551 patients, 'To determine whether there was clinical or laboratory evidence to suggest an abnormality of immunoregulation in person with haemophilia before the recognition of AIDS'. Twenty-six of 518 (5%) patients whose platelet number were determined were found to be thrombocytopenic (four developed idiopathic thrombocytopenic purpura [ITP]) and 9.3% (94/1013) had lymphocytopenia (Eyster *et al.*, 1985);

   (c) to elucidate 'The attention given to infectious diseases in haemophiliacs', which has 'given rise to the concept of a novel form of "immunosuppression" in this population group', Aronson obtained data from the National Center for Health Statistics USA regarding primary and associated causes of death in haemophiliacs. For the years 1968–79, 949 deaths were recorded, '2 patients had candidiasis listed as the primary cause of death. 66 deaths were related to pneumonia (10 primary, 56 associated) usually from unidentified organisms. Many of these pneumonia-associated deaths occurred in the younger age groups (25/66 [38%] were in patients below the age of 45 while only 8% of pneumonia deaths in the normal male population are below the age of 45)'. Aronson concluded 'it seems possible that many of the unspecified pneumonias in haemophiliacs in the past would be classified today as AIDS' (Aronson, 1983). 'Death reports from the United States Vital Statistics System and from the hemophilia center survey' for the above period 1968–1979 were also analysed by workers from the CDC and the National Hemophilia Foundation. They found that 'an average of six deaths were reported to the National Center for Health Statistics annually in 1968–1979 for conditions which could possibly be related to AIDS' (Johnson *et al.*, 1985). Of 89 haemophilia deaths in the UK between 1976 and 1980, 11 (12%) died of unknown causes, 4 (4.5%) of unspecified pneumonias and 7 (8%) of neoplasms (Rizza & Spooner, 1983).

Thus, AIDS-like diseases in an appreciable number of haemophiliacs were reported before the AIDS era, 1980. A high frequency of reporting, or even true incidence of these diseases in this group since 1980 may be due to a number of factors other than HIV:

1. Underreporting of specific causes of death in haemophilia patients before 1980. In the above mentioned survey by workers from the CDC and

the National Hemophilia Foundation it was found that 'The number of deaths reported among factor VIII-deficient patients in the hemophilia treatment survey decreased from 26 deaths and 24 deaths in 1978 and 1979 respectively, to 18 and 19 deaths in 1980 and 1981, respectively. The number of deaths then more than doubled, with 53 deaths reported for 1982. The two- to three-fold increase in deaths in 1982 include the first five reported of immunodeficiency, an increase in deaths assigned to haemorrhage unrelated trauma, and an increase in deaths unrelated to AIDS or hemophilia. The sharp increase in deaths across all categories is most likely due to underreporting of deaths, as a result of hemophilia treatment centers inability to identify deaths in previous years' (Johnson *et al.*, 1985);

2. The increased reporting of PCP in haemophiliacs may be due to a true increase of the incidence of PCP or due to:
   (a) under-diagnosis of PCP in this population before 1980 as a result of:
   (i) lack of awareness; one searched for PCP only in immunosuppressed patients, but before 1980 no one was aware that haemophiliacs were immunosuppressed (no immunological tests were carried out in this population);
   (ii) the inadvisability, in haemophiliacs, of performing invasive procedures which were required for definitive diagnosis;
   (b) in the AIDS era, overdiagnosis of PCP after 1980, that is, pneumonias of unknown aetiology are presumed to be PCP. Even when pneumonias are 'definitely diagnosed' as PCP, this may not be the case: 'one might expect to find *P. carinii* in the fluids from bronchoalveolar lavage of about 40 percent of patients with AIDS who present with symptomatic pneumonia caused by other organisms' (Hughes, 1987). However, regarding the method for definite diagnosis of PCP, the CDC definition states: '*Pneumocystis carinii* pneumonia (on histology, or microscopy of a "touch" preparation, bronchial washings or sputum)' (WHO, 1986);
3. Over-diagnosis of AIDS cases. For example, between July 1986 and June 1987, the CDC had 3001 death certificates 'that indicated HIV infection/AIDS', but only 85% met the CDC AIDS definition (CDC, 1991);
4. Increased life span of patients with haemophilia. PCP 'in infants and children with congenital immunodeficiency did not evolve until after the development of antibiotic therapy which allowed these children to live long enough to develop a non-bacterial infection' (Burke & Good, 1973). That this may also be the case in haemophiliacs is suggested by the following:
   (a) in haemophiliacs the risk of AIDS is directly related to age. In one USA study, eight-year cumulative incidences of AIDS in HIV positive haemophiliacs have been found to be as follows: 1–11 years old, 3%; 12–17 years old, 9.2%; 18–25 year old, 14.9%; 26–34 years old, 19% and 35–70 years old, 29.7% (Goedert *et al.*, 1989). The CDC also reported that the AIDS patients are 'older than the other haemophilia treatment center patients ($p < 0.005$), with a median age of 34 years' (Johnson *et al.*, 1985);
   (b) before the AIDS era, the life span of patients with haemophilia was much lower than that of the rest of the population, in 1972 the median age was 11.5 years. This compared with a median age of 26.8 years for the USA male population in 1970. As a result of treatment with factor VIII the median age increased to 20 years in 1982, and was 25 years in 1988 (Johnston *et al.*, 1985; Koerper, 1989);
5. 'Because of advances in medical practice' in the last few decades, there has been an increase in the incidence of immunosuppression and OIs. This may especially be expected in haemophiliacs since steroids have been used for the treatment of joint problems (Muller, 1960), for factor VIII inhibitors (Lian, Larcada & Chiu, 1989) and for ITP (Eyster *et al.*, 1985), all of which are present in haemophilia patients more often than in the general population. Joint problems have also been treated with other immunosuppressive agents such as radioactive gold or technetium (Fernandez-Palazzi, de Bosch & de Vargas, 1984). In fact, before the AIDS era, 'Pretreatment with antihistamines, corticosteriods and adrenergic agents was recommended for all patients with hemophilia being treated at home' with factor VIII (Helmer *et al.*, 1980);
6. HIV positive individuals including haemophiliacs are treated with AZT. The toxic effects of AZT have repeatedly been stressed by Lauritsen (1990) and Duesberg (1992). Here, only some of these properties, especially those of significance to haemophiliacs, will be mentioned:
   (a) bone marrow failure including anaemia, neutropenia and thrombocytopenia (Callaham,

1991). Many patients require blood transfusion within weeks of commencing AZT. It is important to note that 'the frequency of lymphocytopenia and thrombocytopenia was increased in multitransfused factor VIII-deficient hemophiliacs before the advent of AIDS' and that the latter is a contributing factor in the development of AIDS in haemophilia (Eyster *et al.*, 1985). Furthermore, haemophiliacs with thrombocytopenia 'usually need treatment with drugs as zidovudine, corticosteroids or immunoglobulins, which interfere with the immune system' (Mannucci *et al.*, 1992).

(b) peripheral neuropathy;

(c) myopathy: 'up to one-third of patients taking the drug for more than a year, at a dose of around 1g daily, develop myopathy'. It is manifested clinically as symmetrical proximal weakness, usually preceded by and associated with myalgia, together with muscle wasting. This leads to difficulty in walking and patients become wheelchair or bed-bound (Lane *et al.*, 1993). The toxic effects on muscle eventually lead to heart and other cardio-vascular and pulmonary problems. Since the major long term disabilities in haemophiliacs, irrespective of AIDS, are musculoskeletal disease (Levine, 1985), the above toxic effects of AZT are of particular interest in this group of individuals.

(d) In the 1960s, before the AIDS era, AZT was developed as an agent to treat neoplasms. All drugs presently used to treat cancer are known to be immunosuppressive and to lead to the appearance of OI. They are also known to be carcinogenic (Papadopulos-Eleopulos, 1982). Before the AIDS era animal evidence showed that AZT is no exception (Callaham, 1991) and the widespread use of AZT in HIV positive individuals in the AIDS era has shown that this is also the case in humans. Thus lymphomas develop in 9% 'of AZT-treated AIDS patients, with Kaposi's sarcoma, pneumonia and wasting disease' within one year of commencing therapy and it has been calculated that the 'annual lymphoma risk of AZT recipients is about 30 times higher than that of untreated HIV-positive counterparts' (Duesberg, 1992).

(e) AZT induces liver damage and may cause hepatic failure and death (Touchette, 1993). This is of particular interest in haemophiliacs who, regardless of their HIV status, can suffer from chronic liver disease, which may also 'contribute to AIDS-related diseases', and since the introduction of factor VIII for the treatment of bleeding, has become the leading cause of death in haemophiliacs (Eyster *et al.*, 1985; 1987).

7. Factor VIII. As Levine has pointed out: 'To understand the occurrence of AIDS in haemophilia, it is important to recognize that each vial of factor VIII concentrate will contain, depending on manufacture and lot number, a distillate of clotting factors, alloantigenic proteins, and infectious agents obtained from between 2,500 and 25,000 blood or plasma donors. Until recently, of all the protein injected in 'factor VIII preparations', factor VIII accounted for only about 0.03–0.05% of the total. The rest included: albumin, fibrin(ogen), immunoglobulins and immune complexes (Eyster & Nau, 1978; Mannucci *et al.*, 1992). Even the recent 'high-purity' factor VIII contains 'potentially harming proteins' such as isoagglutinins, fibrin(ogen), split products, immunoglobulins and, when monoclonal antibodies are used for factor VIII preparation, murine protein in addition to albumin (Beeser, 1991).

Factor VIII was first introduced in the late 1960s. 'In 1975, the average patient received an estimated 40,000 units of factor VIII per year (a unit being the equivalent of 1 mL of fresh frozen plasma as to factor VIII content). By 1981, the average patient was consuming 60,000 to 80,000 units per year' (Levine, 1985). The introduction of factor VIII led to a dramatic decrease in haemophilia deaths from bleeding but it also had some harmful effects including myocardial ischaemia, visual disturbances, headache, dyspnoea, bronchospasm, hypotension and anemia (Eyster & Nau, 1978; Kopitsky & Geltman, 1986; Beeser, 1991). As previously stated, factor VIII preparations contain immunoglobulin which may produce systemic reactions such as pruritus, chills, fever, tremor, flushing, malaise, nausea, vomiting, back pain and joint pain (van Aken, 1991). Before the AIDS era, no immunological studies were carried out in haemophiliacs but subsequently, as has been mentioned, in 1985 Eyster *et al.* showed that frequency of lymphocytopenia and thrombocytopenia was increased in haemophiliacs prior to the AIDS era (Eyser *et al.*, 1985). More recently performed immunological studies, including determination of T4 cell numbers, led to the generally accepted view that factor VIII itself is immunosuppressive. Recently, researchers from the UK showed that progression to AIDS in HIV seropositive haemophiliacs

is determined by abnormalities induced by factors other than HIV, all of which existed before seroconversion (Simmonds *et al.*, 1991). In other words, HIV is not sufficient for the development of AIDS in patients with haemophilia.

In conclusion, HIV is not necessary for the development of AIDS in patients with haemophilia. Nonetheless, since:

1. According to the new 1993 CDC AIDS definition, any individual who is HIV seropositive and who has one ('the lowest accurate, but not necessarily the most recent') T4 cell count less than 200 cells/μL, irrespective of the clinical situation even if asymptomatic, has AIDS (CDC, 1992) and,

2. (a) most haemophiliacs test positive for HIV (but AIDS experts accept that in haemophiliacs a positive antibody test does not prove HIV infection);
(b) most haemophiliacs have a low number of T cells (but AIDS experts accept that in haemophiliacs the immune deficiency may be caused by factors other than HIV);

in the future, by definition, virtually all haemophiliacs will die from no other disease but AIDS caused by HIV.

Note added in proof

After this paper was accepted for publication the CDC forwarded the authors a copy of its fact sheet (CDC 1994) on HIV transmission. Given the perilous future for haemophilia patients enshrined in the CDC's 1993 AIDS definition and cognisant of the fact that factor VIII has long been supplied as a freeze-dried powder which may spend many weeks or months waiting use, it is incomprehensible that the CDC would also, in 1994, communicate the following experimental data and conclusion: "In order to obtain data on the survival of HIV, laboratory studies have required the use of artificially high concentrations of laboratory grown virus ... the amount of virus studied is not found in human specimens or anyplace else in nature, ... it does not spread or maintain infectiousness outside its host. Although these unnatural concentrations of HIV can be kept alive under precisely controlled and limited laboratory conditions, CDC studies have shown that drying of even these high concentrations of HIV reduces the number of infectious viruses by 90 to 99 percent within several hours. Since the HIV concentrations used in laboratory studies are much higher than those actually found in blood or other body specimens, drying of HIV-infected human blood or other body fluids reduces the theoretical risk of environmental transmission to that which has been observed – essentially zero". It is thus inexplicable, given their own data, that the CDC continues to regard patients with haemophilia at risk for HIV infection via contaminated factor VIII concentrates and enigmatic that another explanation for 'HIV' and AIDS in haemophiliacs has not been sought.

Acknowledgements

We would like to thank all our colleagues and especially Richard Fox, Wendy Erber, Michael Ristow, Stephan Lanka, Alfred Hässig, Neville Hodgkinson, Christine Johnson, Fabio Franchi, Cecily Metcalf, Philip Adams, Barry Page, Livio Mina, Gary James, Iris Peter, the staff of the Royal Perth Hospital Library and the clerical staff of the Department of Medical Physics. We also thank Harvey Bialy, Udo Schülenk, Charles Thomas, Gordon Stewart, James Whitehead and Katrina Prastidis for continual encouragement, and Peter Duesberg for inviting us to submit this paper to *Genetica*.

We especially thank Michael Verney-Elliot, Joan Shenton and Bruce Hedland-Thomas for their help and motivating encouragement.

References

Aaronson, S.A., G.J. Todaro & E.M. Scholnick, 1971. Induction of murine C-type viruses from clonal lines of virus-free BALB/3T3 cells. Science 174:157–159.

Agbalika, F., F. Ferchal, J.P. Garnier, M. Eugene, J. Bedrossian & P.H. Lagrange, 1992. False-positive HIV antigens related to emergence of a 25–30 kD protein detected in organ recipients. AIDS 6:959–962.

Allain, J.P., 1986. Prevalence of HTLV-III/LAV antibodies in patients with hemophilia and in their sexual partners in France. NEJM 315:517.

Allain, J.P., Y. Laurian, D.A. Paul, F. Verroust, M. Leuther, C. Gazengel, D. Senn, M.J. Larrieu & C. Bosser, 1987. Long-term evaluation of HIV antigen and antibodies to p24 and gp41 in patients with hemophilia. NEJM 317:1114–1121.

Andrews, C.A., J.L. Sullivan, D.B. Brettler, F.E. Brewster, A.D. Forsberg, S. Scesney & P.H. Levine, 1987. Isolation of human immunodeficiency virus from hemophiliacs: correlation with clinical symptoms and immunologic abnormalities. J. Pediatr. 111:672–677.

Anonymous, 1987. CDC definition inadequate to handle growing cases, scientists complain. AIDS Alert July:120–121.

Aronson, D.L., 1983. Pneumonia deaths in haemophiliacs. Lancet II:1023.

Barthel, H.R. & D.J. Wallace, 1993. False-positive human immunodeficiency virus testing in patients with lupus erythematosus. Sem. Arthrit. Rheumat. 23:1–7.

Bauer, H., J.H. Daams, K.F. Watson, K. Molling, H. Gelderblom & W. Schafer, 1974. Oncornavirus-like particles in HeLa cells. II. Immunological characterization of the virus. Int. J. Cancer 13:254–261.

Beeser, H., 1991. Characterization of highly purified factor VIII products. Ann. Hematol. 63:126–130.

Beral, V., T.A. Peterman, R.L. Berkelman & H.W. Jaffe, 1990. Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection? Lancet 335:123–128.

Biggar, R.J., 1986. Possible nonspecific associations between malaria and HTLV-III/LAV.NEJM 315:457.

Blattner, W.A. 1989. Retroviruses. pp. 545–592, in Viral Infections of Humans, 3rd Edition, edited by A.S. Evans, Plenum Medical Book Company, New York.

Bolognesi, D., 1990. Vaccine development for HIV infection. J. Am. Acad. Dermatol. 22:1295–1299.

Brenner, B., S. Schwartz, E. Ben-Porath, I. Tatarsky, D. Varon & U. Martonwitz, 1991. The prevalence and interaction of human

immunodeficiency virus and hepatitis B infections in Israeli hemophiliacs. Isr. J. Med. Sci. 27:557–561.

Brettle, R.P., S.M. Gore, A.G. Bird & A.J. McNeil, 1993. Clinical and epidemiological implications for the Centers for Disease Control/World Health Organization reclassification of AIDS cases. AIDS 7:531–539.

Brettler, D.B., A.D. Forsberg, P.H. Levine, C.A. Andrews, S. Baker & J.L. Sullivan, 1988. Human immunodeficiency virus isolation studies and antibody testing. Arch. Int. Med. 148:1299–1301.

Burke, B.A. & R.A. Good, 1973. *Pneumocystis carinii* infection. Medicine 52:23–50.

Callaham, M.L., 1991. Prophylaxis with zidovudine (AZT) after exposure to human immunodeficiency virus: a brief discussion of the issues for emergency physicians. Ann. Emerg. Med. 20:1351–1354.

Callebaut, C., B. Krust, E. Jacotot & A.G. Hovanessian, 1993. T cell activation antigen, CD26, as a cofactor for entry of HIV in $CD4^+$ cells. Science 262:2045–2050.

CDC, 1981. Kaposi's sarcoma and *Pneumocystis* pneumonia among homosexual men—New York City and California. MMWR 30:305–307.

CDC, 1982. Epidemiological aspects of the current outbreak of Kaposi's sarcoma and opportunistic infections. NEJM 306:248–252.

CDC, 1982. *Pneumocystis carinii* pneumonia among persons with hemophilia A. MMWR 31:365–367.

CDC, 1982. Update on acquired immune deficiency syndrome (AIDS)—United States. MMWR 31:507–514.

CDC, 1982. Update on Kaposi's Sarcoma and Opportunistic Infections in Previously Healthy Persons-United States. MMWR 31:294–301.

CDC, 1987. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. JAMA 258:1143–1154.

CDC, 1987. Survey of non-U.S. hemophilia treatment centers for HIV seroconversions following therapy with heat-treated factor VIII concentrates. MMWR 36:121–124.

CDC, 1991. Mortality attributable to HIV infection/AIDS-United States, 1981–1990. MMWR 40:41–44.

CDC, 1992. 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults. MMWR 41:1–19.

CDC, 1994. Facts about the human immunodeficiency virus and its transmission. CDC HIV/AIDS Prevention. January.

Chang, L.J., P. Pryciak, D. Ganem & H.E. Varmus, 1989. Biosynthesis of the reverse transcriptase of hepatitis B viruses involves de novo translational initiation not ribsomal frameshifting. Nature 337:364–368.

Chopra, H.C. & W.F. Feller, 1969. Viruslike particles in human breast cancer. Texas Rep. Biol. Med. 27:945–953.

Clark, S.J., M.S. Saag, W.D. Decker, S. Campbell-Hill, J.L. Roberson, P.J. Veldkamp, J.C. Kappes, B.H. Hahn & G.M. Shaw, 1991. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. NEJM 324:954–960.

Cohen, J., 1993. Unlikely Recruit: Andrew Leigh Brown. Science 260:1264.

Conway, B. 1990. Detection of HIV-1 by PCR in Clinical Specimens. pp. 40–45, in Techniques in HIV Research, edited by A. Aldovini and B.D. Walker, Macmillan, New York.

Conway, B., P. Baskar, L.J. Bechtel, J.C. Kaplan, M.S. Hirsch, R.T. Schooley & S.H. Pincus, 1992. Eosinophils as host cells for HIV-1. J. Arch. Virol. 127:373–377.

Coombs, R.W., A.C. Collier, J.P. Allain, B. Nikora, M. Leuther, G. Gjerset & L. Corey, 1989. Plasma viremia in human immunodeficiency virus infection. NEJM 321:1626–1631.

Damjamovic, V., 1987. What makes human immunodeficiency virus (HIV) resistant to dry heat inactivation. J. Hosp. Infect. 10:209–210.

Damjanovic, V., 1989. Haemophiliacs with HIV seroconversion associated with heat-treated products: are they actively infected? JAMA 261:1275.

Daniel, V., K. Schimpf & G. Opelz, 1989. Lymphocyte autoantibodies and alloantibodies in HIV-positive haemophilia patients. Clin. Exp. Immunol. 75:178–183.

Darby, S.C., R. Doll, B. Thakrar, C.R. Rizza & D.R. Cox, 1990. Time from infection with HIV to onset of AIDS in patients with haemophilia in the UK. Stat. Med. 9:681–689.

Darby, S.C., C.R. Rizza, R. Doll, R.J.D. Spooner, I.M. Stratton & B. Thakrar, 1989. Incidence of AIDS and excess mortality associated with HIV in haemophiliacs in the United Kingdom: report on behalf of the directors of haemophilia centres in the United Kingdom. Br. Med. J. 298:1064–1068.

Dewar, R.L., M
.D. Sarmiento, E.S. Lawton, H.M. Clark, P.E. Kennedy, A. Shah, M. Baseler, J.A. Metcalf, H.C. Lance & N.P. Salzman, 1992. Isolation of HIV-1 from plasma of infected individuals: an analysis of experimental conditions affecting successful virus propogation. J. Acquir. Immune Defic. Syndr. 5:822–828.

Duesberg, P.H., 1992. AIDS acquired by drug consumption and other noncontagious risk factors. Pharmac. Ther. 55:201–277.

Esiri, M.M., F. Scaravilli, P.R. Millard & J.N. Harcourt-Webster, 1989. Neuropathology of HIV infection in haemophiliacs: comparative necropsy study. Br. Med. J. 299:1312–1315.

Evatt, B.L., E.D. Gomperts, J.S. McDougal & R.B. Ramsey, 1985. Coincidental appearance of LAV/HTLV-III antibodies in hemophiliacs and the onset of the AIDS epidemic. NEJM 312:483–486.

Eyster, M.E., M.H. Gail, J.O. Ballard, H. Al-Mondhiry & J.J. Goedert, 1987. Natural history of human immunodeficiency virus infections in hemophiliacs: effects of T-cell subsets, platelet counts, and age. Ann. Int. Med. 107:1–6.

Eyster, M.E. & M.E. Nau, 1978. Particulate material in antihemophiliac factor (AHF) concentrates. Transfusion September-October:576–581.

Eyster, M.E., D.A. Whitehurst, P.M. Catalano, C.W. McMillan, S.H. Goodnight, C.K. Kasper, J.C. Gill, L.M. Aledort, M.W. Hilgartner, P.H. Levine, J.R. Edson, W.E. Hathaway, J.M. Lusher, F.M. Gill, W.K. Poole & S.S. Shapiro, 1985. Long-term follow-up of hemophiliacs with lymphocytopenia or thrombocytopenia. Blood 66:1317–1320.

Fernandez-Palazzi, F., N.B. de Bosch & A.F. de Vargas, 1984. Radioactive synovectomy in haemophiliac haemarthrosis. Follow-up of fifty cases. Scand. J. Haematol. 33:291–300.

Follansbee, S.E., D.F. Busch, C.B. Wofsy, D.L. Coleman, J. Gullet, G.P. Aurigemma, T. Ross, W.K. Hadley & L. Drew, 1982. An outbreak of *Pneumocystis carinii* pneumonia in homosexual men. Ann. In. Med. 96:705–713.

Gallo, R.C., 1986. The First Human Retrovirus. Sci. Am. 255:78–88.

Gallo, R.C., 1987. The AIDS virus. Sci. Am. 256:38–48

Gallo, R.C. & L. Montagnier, 1988. AIDS in 1988. Sci. Am. 259:24–32.

Gallo, R.C., P.S. Sarin & A.M. Wu, 1973. On the nature of the Nucleic Age and RNA Dependent DNA Polymerase from RNA Tumor Viruses and Human Cells. pp. 13–24, in Possible Episomes in Eukaryotes, edited by L.G. Silvestri, North-Holland Publishing Company, Amsterdam.

Goedert, J.J., C.M. Kessler, L.M. Aledort, R.J. Biggar, W. Abe Andes, G.C. White II, J.E. Drummond, K. Vaidya, D.L. Mann, M.E. Eyster, M.V. Ragni, M.M. Lederman, A.R. Cohen, G.L.

Bray, P.S. Rosenberg, R.M. Friedman, M.W. Hilgartner, W.A. Blattner, B. Kroner & M.H. Gail, 1989. A prospective study of human immunodeficiency virus type 1 infection and the development of AIDS in subjects with hemophilia. NEJM 321:1141–1148.

Gomperts, E.D., 1990. HIV infection in hemophiliac children: clinical manifestations and therapy. Am. J. Ped. Hematol/Oncol. 12:497–504.

Gomperts, E.D., P. Feorino, B.L. Evatt, D, Warfield, R. Miller & J.S. McDougal, 1985. LAV/HTLV III presence in peripheral blood lymphocytes of seropositive young hemophiliacs. Blood 65:1549–1552.

Gougeon, M.L., A.G. Laurent-Crawford, A.G. Hovanessian & L. Montagnier, 1993. Direct and indirect mechanisms mediating apoptosis during HIV infection: contribution to in vivo CD4 T cell depletion. Immunol. 5:187–194.

Greenspan, J.S. & D. Greenspan, 1989. Hairy leukoplakia and other oral features of HIV infection. pp. 449–465, in AIDS Pathogenesis and Treatment, 1st Edition, edited by J.A. Levy, Marcel Dekker Inc., New York.

Haseltine, W.A. & F. Wong-Staal, 1988. The molecular biology of the AIDS virus. Sci. Am. 259:34–42.

Hausmann, E.H.S., H.R. Gelderblom, P.R. Clapham, G. Pauli & R.A. Weiss, 1987. Detection of HIV envelope specific proteins by immunoelectron microscopy and correlation with antibody titer and virus neutralizing activity. J. Virol. Meth. 16:125–137.

Helmer, R.E., J.B. Alperin, J.W. Yunginger & J.A. Grant, 1980. Anaphylactic reactions following infusion of factor VIII in a patient with classic hemophilia. Am. J. Med. 69:953–957.

Hilfenhaus, J.W., J.P. Gregersen, S. Mehdi & R. Volk, 1990. Inactivation of HIV-1 and HIV-2 by various manufacturing procedures for human plasma proteins. Cancer Detect. Prev. 14:369–375.

Hilgartner, M.W., 1987. AIDS and hemophilia. NEJM 317:1153–1154.

Hirsch, M.S., S.M. Phillips & C. Solnik, 1972. Activation of Leukemia Viruses by Graft-Versus-Host and Mixed Lymphocyte Reactions in Vitro. Proc. Nat. Acad. Sci. 69:1069–1072.

Ho, D.D., T. Moudgil & M. Alam, 1989. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. NEJM 321:1621–1625.

Hollan, S.R., G. Fust, K. Nagy, A. Horvath, G. Krall, K. Verebelyi, E. Ujhelyi, L. Varga & V. Mayer, 1985. Immunological alterations in anti-HTLL-III negative haemophiliacs and homosexual men in Hungary. Immunol. Lett. 11:305–310.

Hooks, J., C.J. Gibbs, H. Chopra, M. Lewis & D.C. Gajdusek, 1972. Spontaneous transformation of human brain cell grown in vitro and description of associated virus particles. Science 176:1420–1422.

Horwitz, M.S., M.T. Boyce-Janino & A.J. Faras, 1992. Novel human endogenous sequences related to human immunodeficiency virus type 1. J. Virol. 66:2170–2179.

Hughes, W.T., 1987. *Pnuemocystis carinii* pneumonitis. NEJM 317:1021–1023.

Jackson, J.B., 1989. Haemophiliacs with HIV seroconversion associated with heat-treated products: are they actively infected? (Reply). JAMA 261:1275.

Jackson, J.B., K.J. Sannerud & H.H. Balfour, 1989. Comparison of two serum HIV antigen assays for selection of asymptomatic antigenemic individuals into clinical trials. J. Acquir. Immune Defic. Syndr. 2:394–397.

Jackson, J.B., K.J. Sannerud, J.S. Hopsicker, S.Y. Kwok, J.R. Edson & H.H. Balfour, 1988. Hemophiliacs with HIV antibody are actively infected. JAMA 260:2236–2239.

Jason, J.J., S. McDougal, R.C. Holman, S.F. Stein, D.N. Lawrence, J.K.A. Nicholson, G. Dixon, M. Doxey & B.L. Evatt, 1985. Human T-lymphotropic retrovirus type III/lymphadenopathy-associated virus antibody. JAMA 253:3409–3415.

Jason, J.M., B. Evatt, T.L. Chorba & R.B. Ramsey, 1984. Acquired immunodeficiency syndrome (AIDS) in hemophiliacs. Scand. J. Haematol. 33:349–356.

Jason, J.M., J.S. McDougal, G. Dixon, D.N. Lawrence, M.S. Kennedy, M. Hilgartner, L. Aledort & B.L. Evatt, 1986. HTLV-III/LAV Antibody and Immune Status of Household Contacts and Sexual Partners of Persons with Hemophilia. JAMA 255:212–215.

Johnson, R.E., D.N. Lawrence, B.L. Evatt, D.J. Bregman, L.D. Zyla, J.W. Curran, L.M. Aledort, M.E. Eyster, A.P. Browstein & C.J. Carman, 1985. Acquired immunodeficiency syndrome among patients attending hemophilia treatment centers and mortality experience of hemophiliacs in the United States. Am. J. Epidemiol. 121:797–810.

Jones, P., P.J. Hamilton, G. Bird, M. Fearns, A. Oxley, R. Tedder, R. Cheingsong-Popov & A. Codd, 1985. AIDS and haemophilia, morbidity and mortality in a well defined population. Br. Med. J. 291:695–699.

Kessler, C.M., R.S. Schulof, A.L. Goldstein, P.H. Naylor, N.L. Luban, J.F. Kelleher & G.H. Reaman, 1983. Abnormal T-Lymphocyte Subpopulations Associated with Transfusions of Blood-Derived Products. Lancet 1:991–992.

Kitchen, L.W., F. Barin, J.L. Sullivan, M.F. McLane, D.B. Brettler, P.H. Levine & M. Essex, 1984. Aetiology of AIDS-antibodies to human T-cell leukaemia virus (type III) in haemophiliacs. Nature 312:367–369.

Koerper, M. 1989. AIDS in hemophilia. pp. 79–95, in AIDS Pathogenesis and Treatment, 1st Edition, edited by J.A. Levy, Marcel Dekker Inc., New York.

Kopitsky, R.G. & E.M. Geltman, 1986. Unstable angina associated with factor VIII concentrate therapy for hemophilia A. Ann. Int. Med. 105:215–216.

Kozhemiakin, L.A. & I.G. Bondarenko, 1992. Genomic instability and AIDS. Biochimiia 57:1417–1426.

Lane, R.J.M., K.A. McLean, J. Moss & D.F. Woodrow, 1993. Myopathy in HIV infection: the role of zidovudine and the significance of tubuloreticular inclusions. Neuropath. App. Neurobiol. 19:406–413.

Lauritsen, J., 1990. Poison by prescription–The AZT story. Asklepois Press, New York.

Lawrence, D.N., J.M. Jason, R.C. Holman, P. Heine & B.L. Evatt, 1989. Sex practice correlates of human immunodeficiency virus transmission and acquired immunodeficiency syndrome incidence in heterosexual partners and offspring of U.S. hemophiliac men. A.J. Hematol. 30:68–76.

Lazcano, A., V. Valverde, G. Hernandez, P. Gariglio, G.E. Fox & J. Oro, 1992. On the early emergence of reverse transcription: theoretical basis and experimental evidence. J. Mol. Evol. 35:524–536.

Levine, P.H., 1985. The acquired immunodeficiency syndrome in persons with hemophilia. Ann. Int. Med. 103:723–726.

Levine, P.H., J.S. Horoszewicz, J.T. Grace, L.S. Chai, R.R. Ellison & J.F. Holland, 1967. Relationship between clinical status of leukemic patients and virus-like particles in their plasma. Cancer 20:1563–1577.

Levy, J.A., 1988. The transmission of AIDS: the case of the infected cell. JAMA 259:3037–3038.

Levy, J.A., 1989. AIDS Pathogenesis and Treatment. Marcel Dekker, Inc., New York.

Levy, J.A., A.D. Hoffman, S.M. Kramer, J.A. Landis, J.M. Shimabukuro & L.S. Oshiro, 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. Science 225:840–842.

Levy, J.A., G. Mitra & M.M. Mozen, 1984. Recovery and inactivation of infectious retroviruses added to factor VIII concentrates. Lancet II:722–733.

Levy, J.A., G.A. Mitra, M.F. Wong & M.M. Mozen, 1985. Inactivation by wet and dry heat of AIDS-associated retroviruses during factor VIII purification from plasma. Lancet I:1456–1457.

Lewis, B.J., J.W. Abrell, R.G. Smith & R.C. Gallo, 1974. Human DNA polymerase III (R-DNA): Distinction from DNA polymerase I and reverse transcriptase. Science 183:867–869.

Lian, E.C.Y., A.F. Larcada & A.Y.Z. Chiu, 1989. Combination immunosuppressive therapy after factor VIII infusion for acquired factor VIII inhibitor. Ann. Int. Med. 110:774–778.

Lower, R. & J. Lower, 1993. Endogenous retrovirus sequences in human teratocarcinoma cell lines. J. Acquir. Immune Defic. Syndr. 6:738.

Ludlam, C.A., C.M. Steel, R. Cheingsong-Popov, D.B.L. McClelland, J. Tucker, R.S. Tedder, R.A. Weiss, I. Philip & R.J. Prescott, 1985. Human T-Lymphotropic Virus Type-III (HTLV-III) Infection in Seronegative Haemophiliacs after Transfusion of Factor VIII. Lancet II:233–236.

Lundberg, G.D., 1988. Serological Diagnosis of Human Immunodeficiency Virus Infection by Western Blot Testing. JAMA 260:674–679.

Madhok, R., A. Gracie, G.D. Lowe, A. Burnett, K. Froebel, E. Follett & C.D. Forbes, 1986. Impaired cell mediated immunity in haemophilia in the absence of infection with human immunodeficiency virus. Br. Med. J. Clin. Res. 293:978–980.

Mak, T.W., J. Manaster, A.F. Howatson, E.A. McCullough & J.E. Till, 1974. Particles with characteristics of leukoviruses in cultures of marrow cells from leukemic patients in remission and relapse. Proc. Natl. Acad. Sci. USA 71:4336–4340.

Mannucci, P.M., A. Gringersi, R. de Biasi, F. Baudo, M. Morfini & N. Ciavarella, 1992. Immune status of asymptomatic HIV-infected hemophiliacs: randomized, prospective, two-year comparison of treatment with a high-purity or an intermediate-purity factor VIII concentrate. Thrombosis and Haemostasis 67:310–313.
bitem Marx, J.L., 1983. Human T-cell virus linked to AIDS. Science 220:806–809.

Masquelier, B., R. Faivre, M.C. Paty & H.J.A. Fleury, 1992. HIV1 quantitation in infected patients: a comparison of cell viraemia and R-HEV. Res. Virol. 143:17–22.

Matsuda, J., K. Gohchi, M. Tsukamoto, N. Saitoh & T. Konshita, 1993. High prevalence of anti-cardiolipin antibody, C1q-, C3d- and mRF-IgG immune complexes, and anti-nuclear antibody in hemophiliacs irrespective of infection with human immunodeficiency virus type 1. J. Acquir. Immune Defic. Syndr. 6:1120–1124.

Matthews, T.J. & D.P. Bolognesi, 1988. AIDS vaccines. Sci. Am. 259:98–105.

McKeating, J.A. & J.P. Moore, 1991. HIV infectivity. Nature 349:660.

Melbye, M., R.J. Biggar, J.C. Chermann, L. Montagnier, S. Stenbjerg & P. Ebbesen, 1984. High prevalence of lymphandenopathy virus (LAV) in European haemophiliacs. Lancet II:40–41.

Mendenhall, C.L., G.A. Roselle, C.J. Grossman, S.D. Rouster & R.E. Weener, 1986. False Positive Tests for HTLV-III Antibodies in Alcoholic Patients with Hepatitis. NEJM 314:921–922.

Michael, N.L., G. Chang, P.K. Ehrenberg, M.T. Vahey & R.R. Redfield, 1993. HIV-1 proviral genotypes from the peripheral blood mononuclear cells of an infected patient are differentially represented in expressed sequences. J. Acquir. Immune Defic. Syndr. 6:1073–1085.

Michaelis, B.A. & J.A. Levy, 1987. Recovery of human immunodeficiency virus from serum. JAMA 257:1327.

Minassian, A., M. Merges, R. Garrity, K. Nagashima, W.P. Tsai, S. Oroszlan & P. Nara, 1993. Induction of a SMRV-like retrovirus from a human T-cell line after treatment with the mutagen ethylmethyl-sulfonate. J. Acquir. Immune Defic. Syndr. 6:738.

Mitsuya, H. & S. Broder, 1989. Antiretroviral chemotherapy against human immunodeficiency virus (HIV) infection: perspective for therapy of hepatitis B virus infection. Cancer Detect. Prev. 14:299–308.

Moffat, E.H., A.L. Bloom & P.P. Mortimer, 1985. HTLV-III antibody status and immunological abnormalities in haemophilic patients. Lancet I:935.

Montagnier, L., 1985. Lymphadenopathy-Associated Virus: From Molecular Biology to Pathogenicity. Ann. Int. Med. 103:689–693.

Moore, J.P. & P.L. Nara, 1991. The role of the V3 loop and gp120 in HIV infection. AIDS 5:S21–S33.

Mortimer, P.P., 1989. The AIDS virus and the AIDS test. Med. Internat. 56:2334–2339.

Moss, A.R., P. Bacchett, D. Osmond, W. Krampf, R.E. Chaisson, D. Stites, J. Wilber, J.P. Allain & J. Carlson, 1988. Seropositivity for HIV and the development of AIDS or AIDS related condition: three year follow up of the San Francisco cohort. Br. Med. J. 296:745–750.

Muller, G., 1960. Interstitielle plasmacellulare Pneumonie nach Corticosteroidbehandlung. Frankfurter Zeitschrift Pathologie 70:657–675.

Natoli, C., F. Dianzani, F. Mazzotta, E. Balocchini, P. Peirotti, G. Antonelli & S. Icaobelli, 1993. 90K Protein: A New Predictor Marker of Disease Progression in Human Immunodeficiency Virus Infection. J. Acquir. Immune Defic. Syndr. 6:370–375.

Neumann, P., M. O'Shaughnessy, R. Remis, C. Tsoukas, D. Lepine & M. Davis, 1990. Laboratory evidence of active HIV-1 infection in Canadians with hemophilia associated with administration of heat-treated factor VIII. J. Acquir. Immune Defic. Syndr. 3:278–281.

Neurath, A.R., N. Strick & P.S.O. Sproul, 1992. Search for hepatitis B virus cell receptors reveals binding sites for interleukin 6 on the virus envelope protein. J. Exp. Med. 175:461–469.

Pantaleo, G., C. Graziosi, J.F. Demarest, L. Butini, M. Montroni, C.H. Fox, J.M. Orenstein, D.P. Kotler & A.S. Fauci, 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. Nature 362:355–358.

Papadopulos-Eleopulos, E., 1982. A Mitotic Theory. J. Theor. Biol. 96:741–758.

Papadopulos-Eleopulos, E., 1988. Reappraisal of AIDS: Is the oxidation caused by the risk factors the primary cause? Med. Hypotheses 25:151–162.

Papadopulos-Eleopulos, E., V.F. Turner & J.M. Papadimitriou, 1992. Oxidative Stress, HIV and AIDS. Res. Immunol. 143:145–148.

Papadopulos-Eleopulos, E., V.F. Turner & J.M. Papadimitriou, 1993a. Is a Positive Western Blot Proof of HIV Infection? Bio/Technology 11:696–707.

Papadopulos-Eleopulos, E., V.F. Turner & J.M. Papadimitriou, 1993b. Has Gallo proven the role of HIV in AIDS? Emerg. Med. [Australia] 5:113–123.

Papadopulos-Eleopulos, E., V.F. Turner, J.M. Papadimitriou, D. Causer, B. Hedland-Thomas & B. Page, 1994. A critical analysis of the HIV-T4-cell-AIDS hypothesis. (This issue).

Parmentier, H.K., D.F. van Wichen, F.H.J. Gmelig Meyling, J. Goudsmit & H.J. Schuurman, 1992. Epitopes of human immunodeficiency virus regulatory proteins tat, nef, and rev are expressed in normal human tissue. Am. J. Pathol. 141:1209–1216.

Popovic, M., M.G. Sarngadharan & E. Read, 1984. Detection, Isolation and Continuous Production of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and pre-AIDS. Science 224:497–500.

Ragni, M.V., J.H. Lewis, J.A. Spero & F.A. Bontempo, 1983. Acquired Immunodeficiency-like syndrome in two hemophiliacs. Lancet I:213–214.

Rahemtulla, A., S.T.S. Durrant & J.M. Hows, 1986. Subacute encephalopathy associated with human immunodeficiency virus in haemophilia A. Br. Med. J. 293:993.

Ramsey, R.B., E.L. Palmer, J.S. McDougal, V.S. Kalyanaraman, D.W. Jackson, T.L. Chorba, R.C. Holman & B.L. Evatt, 1984. Antibody to lymphadenopathy-associated virus in haemophiliacs with and without AIDS. Lancet II:397–398.

Redfield, R.R. & D.S. Burke, 1988. HIV infection: the clinical picture. Sci. Am. 259:70–78.

Remis, R.S., M.V. O'Shaughnessy, C. Tsoukas, G.H. Growe, M.T. Schechter, R.W.H. Palmer & D.N. Lawrence, 1990. HIV transmission to patients with hemophilia by heat-treated, donor-screened factor concentrate. Can. Med. Assoc. J. 1247–1254.

Rizza C.R. & R.J.D. Spooner, 1983. Treatment of haemophilia and related disorders in Britain and Northern Ireland during 1976-80: report on behalf of the directors of haemophilia centres in the United Kingdom. Br. Med. J. 286:929–933.

Robert-Guroff, M., A.W. Schrecker, B.J. Brinkman & R.C. Gallo, 1977. DNA polymerase γ of human lymphoblasts. Biochem. 16:2866–2873.

Rosenberg, Z.F. & A.S. Fauci, 1990. Immunopathogenic mechanisms of HIV infection: cytokine induction of HIV expression. Immunol. Today 11:176–180.

Sarngadharan, M.G., M. Robert-Guroff & R.C. Gallo, 1978. DNA polymerase of normal and neoplastic mammalian cells. Biochim. Biophysica. Acta. 516:419–487.

Sarria, L., L, Gallego, B. de las Heras, M. Basaras & R.S.O. Cisterna, 1993. Production of hepatitis B virus from peripheral blood lymphocytes stimulated with phytohemahhlutinin. Enferm. Infect. Microbiol. Clin. 11:187–189.

Schneider, T.S., A.B. Beck, C. Ropke, R. Ullrich, H.P. Harthus, M. Broker & G. Pauli, 1993. The HIV-1 Nef protein shares an antigenic determinant with a T-cell surface protein. AIDS 7:647–654.

Schneweis, K.E., A. Ackermann, A. Friedrich, J.P. Kleim, K. Kornau & R. Ruff, 1989. Comparison of different methods for detecting human immune deficiency virus in human immunodeficiency virus-seropositive hemophiliacs. J. Med. Virol. 29:94–101.

Schneweis, K.E., J.P. Kleim, E. Bailly, D. Niese, N.N. Wagner & H.H. Brackman, 1990. Graded cytopathogenicity of the human immunodeficiency virus (HIV) in the course of HIV infection. Med. Microbiol. Immunol. 179:193–203.

Schupbach, J., J.B. Jendis, C. Boni, J. Boni & Z. Tomasik, 1992. False-positive HIV-1 virus cultures using whole blood. AIDS 6:1545–1546.

Sheppard, H.W., M.S. Ascher & J.F. Krowka, 1993. Viral burden and HIV disease. Nature 364:291.

Shoebridge, G.I., L. Barone, A. Wing-Simpson, B.H. Bennetts, B.N. Nighingale, W.J. Hensley & P.A. Gatenby, 1991. Assessment of HIV status using the polymerase chain reaction in antibody-positive patients and high-risk antibody-negative haemophiliacs. AIDS 5:221–224.

Simmonds, P., D. Beatson, R.J.G. Cuthbert, H. Watson, B. Reynold, J.F. Peutherer, J.V. Parry, C.A. Ludlam & C.M. Steel, 1991. Determinants of HIV disease progression: six-year longitudinal study in the Edinburgh haemophilia/HIV cohort. Lancet 338:1159–1163.

Smith, D.K., J.J. Neal, S.D. Holmberg, 1993. Unexplained opportunistic infections and CD4+ T-lymphocytopenia without HIV infection. NEJM 328:373–379.

Temin, H.M. & D. Baltimore, 1972. RNA-Directed DNA Synthesis and RNA Tumor Viruses. Adv. Vir. Res. 17:129–186.

Todaro, G.J., R.E. Benveniste, & C.J. Sherr, 1976. Interspecies Transfer of RNA Tumor Virus Genes: Implications for the search for 'Human' Type C Viruses. pp. 369–384, in Animal Virology, edited by D. Baltimore, A.S. Huang and C.S. Fox, Academic Press Inc., New York.

Touchette, N., 1993. HBV-drug deaths prompt restudy of similar antivirals. J. NIH Res. 5:33–35.

Toyoshima, K & P.K. Vogt, 1969. Enhancement and Inhibition of Avian Sarcoma Viruses by Polycations and Polyanions. Virol. 38:414–426.

Tsoukas, C., F. Gervais, J. Shuster, P. Gold, M. O'Shaughnessy & M. Robert-Guroff, 1984. Association of HTLV-III Antibodies and Cellular Immune Status of Hemophiliacs. NEJM 311:1514–1515.

van Aken, W.G. 1991. Preparation of plasma derivatives. pp. 323–333, in Principles of Transfusion Medicine, edited by E.C. Rossi, T.L. Simon and G.S. Moss, Williams and Wilkins, Baltimore.

van den Berg, W., J.W. Ten Cate, C. Breederveld & J. Goudsmit, 1986. Seroconversion to HTLV-III in haemophiliac given heat-treated factor VIII concentrate. Lancet 1:803–804.

Varmus, H., 1987. Reverse Transcription. Sci. Am. 257:48–54.

Varmus, H., 1988. Retroviruses. Science 240:1427–1435.

Varmus, H. & P. Brown, 1989. Retroviruses. pp. 53–108, in Mobile DNA, edited by D. E. Berg and M.M. Howe, American Society for Microbiology, Washington D.C.

Varmus, H.E., 1989. Reverse transcription in bacteria. Cell 56:721–724.

Vegnente, A., S. Guida, A. Lobo-Yeo, L. Alviggi, H. Daniels, P. Toscano, V. Nuzzo, G. Mieli-Vergani & D.S.O. Vergani, 1991. T lymphocyte activation is associated with viral replication in chronic hepatitis B virus infection of childhood. Clin. Exp. Immunol. 84:190–194.

Vilmer, E., C. Rouzioux, F. Vezinet Brun, A. Fischer, J.C. Chermann, F. Barre-Sinoussi, C. Gazengel, C. Dauguet, P. Manigne & C. Gricelli, 1984. Isolation of new lymphotropic retrovirus from two siblings with Haemophilia B, one with AIDS. Lancet I:753–757.

Vincent, F., L. Belec, D. Glotz, V. Menoyo-Calonge, A. Dubost & J. Bariety, 1993. False-positive neutralizable HIV antigens detected in organ transplant recipients. AIDS 7:741–742.

Wagner, N., R. Bialek, H. Radinger, M. Becker, K.E. Schneweis, H.H. Brackman & D. Niese, 1990. HIV-1 infection in a cohort of haemophiliac patients. Archiv. Dis. Childhood 65:1301–1304.

Wain-Hobson, S., 1989. HIV genome variability in vivo. AIDS 3:S13-S18.

Watson, K.F., K. Molling, H. Gelderblom & H. Bauer, 1974. Oncornavirus-like particles in HeLa cells. III. Biochemical characterization of the virus. Int. J. Cancer 13:262–267.

Weber, J. & K. Ariyoshi, 1992. Lack of correlation between acidified HIV p24 antigen and plasma viraemia. AIDS 6:428–429.

Weber, J.N. & R.A. Weiss, 1988. HIV infection: the cellular picture. Sci. Am. 259:80–87.

Weiss, R., N. Teich, H. Varmus & J. Coffin, Ed. 1982. RNA Tumor Viruses. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory.

Weissbach, A., D. Baltimore, F. Bollum, R. Gallo & D. Korn, 1975. Nomenclature of eukaryotic DNA polymerases. Science 190:401–402.

WHO, 1986. Acquired Immunodeficiency Syndrome (AIDS) WHO/CDC case definition for AIDS. Wkly. Epidem. Rec. 61:69–76.

WHO, 1988, Acquired Immunodeficiency Syndrome (AIDS) 1987 revision of CDC/WHO case definition for AIDS. Wkly. Epidem. Rec. 63:1–8.

Wong-Staal, F. & R.C. Gallo, 1985. Human T-lymphotropic retroviruses. Nature 317:395–403.

Wong-Staal, F., B. Hahn, V. Manzuri, S. Colombini, G. Franchini, E.P. Gelmann & R.C. Gallo, 1983. A survey of human leukaemias for sequences of a human retrovirus. Nature 302:626–628.

Wu, T.C., M.D. Kanayama, R.H. Hruban, W. Whitehead & B.K. Raj, 1993. Detection of neuron-specific 9.0-kb transcript which shares homology with antisense transcripts of HIV-1 gag gene in patients with and without HIV-1 infection. Am. J. Pathol. 142:25–31.

Zhang, L.Q., P. Simmonds, C.A. Ludlam & A.J. Leigh Brown, 1991. Detection, quantification and sequencing of HIV-1 from the plasma of seropositive individuals and from factor VIII concentrates. AIDS 5:675–681.