HIV causes AIDS: Koch's postulates fulfilled

Guest editorial

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Abbreviations

CDC	Center for Disease Control
hPBL	human peripheral blood lymphocyte
LW	laboratory worker
SCID	severe combined immunodeficiency
SIV	simian immunodeficiency virus

Randy Shilts, in his chilling narrative of the early phases of the AIDS epidemic entitled "And the Band Played On" [1], suggested that a gay Canadian airline steward termed Patient Zero disproportionately spread human immunodeficiency virus (HIV) through multiple sexual liaisons during the American bicentennial celebration in 1976. Although this scenario may be apocryphal, the timing fits with the first outbreak of a 'gay cancer' (as AIDS was known in those days to reflect the association with multifocal Kaposi's sarcoma) diagnosed in 1981 in New York and Los Angeles. HIV was initially discovered in clusters of AIDS patients in 1983, and epidemiological data have provided a definite association of HIV and AIDS in multiple studies since 1983 [2–9]. The spread of AIDS through sexual contact, blood transfusion and contaminated syringes has led to a recent estimate of nearly four million AIDS cases worldwide and some 21 million people infected with HIV-1 or HIV-2 [10]. In the western world, HIV and AIDS are primarily concentrated among homosexual men, intravenous-drug abusers, transfusion recipients, and hemophiliacs who were infected by contaminated blood products before a program screening them for HIV was initiated in 1985. In sub-Saharan Africa, HIV is transmitted largely by heterosexual encounter and immune deficiency disease is extremely prevalent [11,12].

The causative agent for AIDS was shown to be HIV by a number of different observations, primarily epidemiological, that documented the presence of HIV or HIV antibodies (indicating exposure) in over 95% of AIDS patients throughout the world [2–10]. Most observers who have examined the clinical data are convinced that HIV does indeed lead to a gradual decline of the CD4+ T lymphocyte population (which is an important component of the immune response) leading to AIDS. In fact, both the scientific establishment and the executors of public policy have adopted the position that HIV causes AIDS and that avoiding exposure and infection would protect individuals from this incurable plague.

In spite of overwhelming evidence to the contrary, Berkeley molecular virologist Peter Duesberg (along with several high profile converts such as Nobelist Kary Mullis) continues his decade-long crusade to argue rhetorically (but with scant data) that HIV does not cause AIDS ([13–23]; interview with Peter Duesberg, Genre, Paris Edition, June 1994). Following acrimonious debate in both the scientific and popular press, Duesberg's latest offering, a 772 page polemic entitled 'Inventing the AIDS Virus' [20], outlines in elaborate detail the perceived problem with the tens of thousands of scientific papers and scientists who are persuaded that HIV is the etiological agent that causes AIDS. Duesberg postulates that recreational drugs, such as nitrate inhalants, antivirals such as zidovudine, and immune hyperstimulation are the true cause. Duesberg and his disciples have largely rejected epidemiological arguments for HIV being the cause of AIDS because of the 5% edges of statistical inference and the confusing panoply which represents the ever changing definition of what constitutes AIDS. For example, Kaposi's sarcoma can occur in persons with a relatively intact immune system, while most other AIDS-defining illnesses only occur in the setting of profound immunodeficiency. Add to this the imprecise definition of AIDS as it occurs in the developing world, where unexplained wasting and tuberculosis, which occur commonly in the absence of HIV, are major manifestations [11,12].

It is not surprising that their arguments become persuasive to some and that the high decibel rhetoric itself raises questions in others ([22–29]; London Sunday Times, March 21, 1993: *Epidemic of AIDS in Africa, a tragic myth.*). There is one fundamental observation, however, that cannot be ignored. The natural history of AIDS behaves like an epidemic, with abrupt emergence and spread of both disease and HIV prevalence in places and times where neither had occurred previously. From the earliest description of AIDS, a microbial pathogen was suspected because networks of cases could be linked via sexual contact, needle sharing, or by blood transfusion, all hallmarks of an epidemic mediated by an infectious agent [2–9]. Proposed alternatives such as recreational or prescription drugs are implausible, primarily because large numbers of AIDS patients (e.g. hemophiliacs, children of HIV-infected mothers, transfusion recipients) were never exposed to these drugs but had been exposed to HIV [30].

In this editorial we suggest that HIV now fulfills (while the anti-HIV school fails to fulfill) the time-honored Koch's postulates [31–33], which previously were invoked by Professor Duesberg as a tool to discredit the HIV hypothesis. In 1884 Robert Koch, discoverer of the anthrax bacillus, presented a group of conditions, termed Koch's postulates, that he reasoned should be fulfilled before concluding that a bacterial agent had caused a disease. Modifications have been suggested over the years to accommodate new technologies, particularly in relation to suspected viral pathogens [8,31–38], but the fundamental tenets, as listed here, have become a litmus test for the causation of epidemic disease: first, epidemiological association — the suspected cause is strongly associated with the disease; second, isolation — the pathogen can be isolated and propagated outside the host; and third, transmission pathogenesis — the transfer of the pathogen to an uninfected host (man or animal) leads to disease.

In initial critiques on the HIV–AIDS causality, its critics and defenders agreed that HIV failed to fulfill these postulates [2,8,13,16,17,34]. While the epidemiological association and the necessity to isolate the pathogen were clearly satisfied, demonstrating that HIV causes disease directly had been difficult. The defenders [8] added, however, that some other diseases for which pathogens were identified did not pass the stringent postulates either (e.g. typhoid fever, diphtheria, leprosy, relapsing fever), primarily because they could not be cultured *in vitro*. We now suggest that this caveat is no longer necessary because available data and evidence, summarized here, provide definitive fulfillment of Koch's postulates for HIV being the direct cause of AIDS.

Epidemiological association

The epidemiological concordance of HIV exposure and AIDS has been shown by a number of different studies that document the presence of HIV or HIV antibodies in over 95% of AIDS patients throughout the world [2–10]. The major categories of epidemiological associations include the following: very high prevalence of HIV or HIV antibodies among risk groups for AIDS (gay men, hemophiliacs, recipients of contaminated blood transfusions, intravenous-drug users) and very high prevalence of titers of HIV antibodies among AIDS patients (over 90%); studies of cohorts of gay men and intravenous-drug users showing that although behavior was similar within these groups, only those gay men and intravenous-drug users who were HIV antibody positive developed immunosuppression and AIDS; temporal and geographic concordance of the occurrence of AIDS and HIV spread in certain locales, notably India, Thailand and Myanmer (formally Burma); prompt and progressive depletion of CD4+ lymphocytes and subsequent AIDS diagnosis following HIV antibody seroconversion in the vast majority of infected hemophiliacs [39,40]; HIV antibody seroconversion in more than 90% of transfusion recipients of blood from donors who were HIV antibody positive, followed by prompt and progressive loss of CD4+ lymphocytes and the eventual development of AIDS [39–41]; transmission of HIV to approximately one-quarter of infants who are born to HIV-infected women, with AIDS developing only in HIV-infected infants and not in the three-quarters who were HIV-uninfected despite being born of similar women and into similar environments; and demonstration that the quantity of circulating plasma HIV-1 genomic RNA offers a precise prognosis for AIDS and AIDS mortality, far more accurate than CD4+ cell concentration [42,43]. Two recent prospective cohort studies of HIV-infected hemophiliacs provide a direct link of HIV infection to mortality, by showing a tenfold increase in death rate among those carrying HIV compared with uninfected patients, irrespective of the severity of hemophilia [44,45]. Since screening for HIV commenced, AIDS has virtually disappeared from hemophiliacs and transfusion recipients.

A particular concern of the anti-HIV school is the occurrence of AIDS-defining conditions in patients who are HIV antibody negative. The answer to this phenomenon is straightforward. HIV-induced AIDS is not the only way to develop immune deficiency in man. There are several hereditary disorders that result in immune collapse [46,47]. Certain drugs can cause immune suppression and there may also be other factors including infectious agents. Indeed there are many diseases with multiple equally effective causes: pneumonia — *Mycobacterium pneumoniae*, *Pneumocystis carinii* and mycoplasma; liver cirrhosis — hepatitis B, hepatitis C, congenital disorders and alcohol imbibition; and cancer — X irradiation, chemical carcinogens, genetic predisposition and cigarette smoke. There also are rare HIV-induced AIDS cases in which patients may lack the ability to mount an antibody response against HIV, due to hypogammaglobulinemia; one such case is now known to have a high plasma level of HIV RNA (5.3 log10 per ml; JJ Goedert, unpublished data).

Isolation of pathogen from AIDS patients

Multiple HIV isolates have been cultured from AIDS patients. Virus has been cultivated in fresh human T lymphocytes, macrophages and certain immortal tissue culture cell lines developed for *in vitro* propagation. The full genomes of many HIV isolates have been molecularly cloned and over 100 sequences (full or partial) have been reported and exist in sequence data banks [48]. It is true that virus isolation is sometimes not successful, but the presence of HIV can be demonstrated by polymerase chain reaction amplification of low abundance HIV genomes in most patients [42,49–51]. In fact the persistence of antibody titers for over a decade in AIDS patients is most likely explained by the continued stimulation of the humoral (antibody producing) immune system by low levels of sequestered virus. Otherwise, antibody titer would drop off as it does for other diseases when the pathogen is cleared or when antigenic challenge ceases following vaccination. The isolation component of Koch's

postulates for HIV has been repeatedly demonstrated since the discovery of HIV.

Transmission pathogenesis in animal models or in man

The postulate of transmission pathogenesis cannot be fulfilled by epidemiological data, but instead is a requirement for direct empirical evidence. Ethical consideration precludes experimental transmission to uninfected human patients, making verification difficult. Therefore, this has been the most controversial Koch postulate with respect to HIV and AIDS. However, we now believe that this postulate can be considered to have been accomplished by five graphic examples: two in human transmission and three in animal models. A combination of comparative genetic analysis, pathogenic documentation and phylogenetic inference provides dramatic and conclusive evidence for the causative association of HIV and AIDS. The five examples are documented below.

Accidental infection of laboratory workers with HIV-1 led to AIDS

Three laboratory workers (LWs) who became infected with HIV-1 (strain HTLV-IIIB) while working with the same strain are now being monitored for clinical disease ([52]; W Blattner, M Reitz, G Colcough, S Weiss, abstract PO-801-0876, International Conference on AIDS, Bethesda, MD, June 1993). None of these workers had other risk factors for AIDS (i.e. homosexuality, transfusion recipience, intravenous-drug use) and virus was isolated and sequenced from each [53]. The sequence divergence between LW virus envelope genes and clonal HTLV-IIIB is ≤3%, which is the same genetic distance from LAV-LAI to HTLV-IIIB, viral strains now agreed by all to have been derived from a single patient [54,55]. This low level of sequence divergence is equivalent to the variation observed between HIV-infected infants and their mothers, and threefold less than the extent of variation of HIV between unconnected patients [56]. One patient (LW-1) was discovered to be HIV antibody positive in a screen of research technicians working in direct contact with HIV [52], and the other two reported acute exposure incidents (W Blattner, M Reitz, G Colcough, S Weiss, abstract PO-801-0876, International Conference on AIDS, Bethesda, MD, June 1993): LW-2 had a puncture wound while handling a centrifuge used for HIV concentration and LW-3 had contact with concentrated virus through facial and mucous membranes. Two of the patients were infected in 1985 and one in 1991. One patient, LW-2, developed *Pneumocystis carinii* pneumonia, an AIDS-indicator disease, in 1991. All three have shown marked CD4+ cell depletion; two have dropped below 200 per mm^3, the cutoff for AIDS diagnosis by the Center for Disease Control (CDC) [57]. The LWs provided cogent examples of accidental transmission of a phylogenetically verifiable strain of HIV (HTLV-IIIB) to three different individuals at different times, with each displaying immune cell (CD4+) depletion and with two developing AIDS in the absence of other risk factors.

The Florida dentist case

In 1990 the testimony of Kimberly Bergalis before the US Congress captivated the public with the horrible prospect of transmission of HIV from health care providers. Bergalis was HIV positive and was dying of AIDS, but she claimed to have had no risk factors (no drugs, no sexual activity and no blood transfusions). She believed that her infection came from her dentist, Dr David Acer of Jensen Beach, Florida [58,59]. Acer was diagnosed with symptomatic HIV infection in 1986 and developed AIDS in September 1987, manifested by Kaposi's sarcoma and a drop in CD4+ T lymphocyte count to below 200 per mm^3. For the next two years he continued his practice. Prompted by Ms Bergalis' accusation, he published an open letter to his patients in a local newspaper, urging them to be tested for HIV antibodies. Over 1100 of his patients were tested by the Florida Department of Health and Rehabilitative Services, and ten of these tested positive for HIV-1 antibodies. Four of the ten HIV-positive patients reported high risk behavior, one patient was indeterminant, five had no risk factors identified to the CDC (except multiple visits to Dr Acer for invasive dental procedures such as extractions and root canal therapy after he developed AIDS).

Whether Dr Acer had somehow infected his patients became a volatile issue with both legal and public health implications [58,59]. The answer came with a comprehensive phylogenetic analysis of a 300 base pair C2–V3 region of HIV envelope gene isolated from the dentist, from the ten HIV positive patients and from a group of HIV infected individuals from the Jensen Beach area, who had no known connection with Dr Acer. The results, which were analyzed by two different research groups, were dramatic in their support of the dental transmission scenario [60–62]. The five patients that reported no risk factors plus the single indeterminant patient had HIV sequences that were genetically as close to the dentist and to each other as are HIV-1 isolates from the same patient or as is virus from infants infected by their mothers. The virus gene sequences from the four patients with other risk factors and from the local controls had divergent envelope sequences reminiscent of unconnected patient isolates.

Although the genetic conclusions were not without controversy ([63–65]; CBS 60 Minutes: *Kimberly's story*. CBS Incorporated; June 19, 1994), the dental transmission explanation for the six implicated patients has been supported by five different empirical measures of genetic/phylogenetic relatedness [60–62]: low sequence divergence of six patients and dentist (average 3.4–4.9%) compared with larger distances (11–13%) among unrelated isolates; phylogenetic clustering of dentist and six patient isolates in maximum parsimony analysis, bootstrap support = 80%; dentist and patients sharing a rare eight

amino acid residue signature; phylogenetic alignment of dentist and each patient when tested individually; and increase in phylogenetic confidence using a weighted parsimony method to account for inequivalence of nucleotide substitution. The dentist, Ms Bergalis and two other patients have died of AIDS, and one of the three remaining HIV-infected patients has progressed to AIDS on the basis of CD4+ cell count. The genetic analysis of HIV-1 genomic sequences provides a compelling case for the dentist being the source of HIV-1 infection leading to AIDS in the six patients, fulfilling the transmission pathogenesis postulate for HIV-1 causing AIDS.

HIV-2 causes AIDS in baboons

Certain strains of HIV-2, a less pathogenic strain of HIV (in humans) limited to West Africa and India [66], replicate and establish persistent viremia when inoculated into yellow baboons (*Papio cynocephalus*) [67]. In a recent report three of five HIV-2-infected baboons showed a depletion of CD4+ cells and AIDS-like pathology [67]. These observations provide *prima facie* evidence for the transmission of AIDS pathology to an animal model by a human HIV strain.

HIV causes immune deficiency in SCID mice

Some mouse strains with a mutation at the *SCID* (severe combined immunodeficiency) locus are blocked in developmental differentiation of B and T lymphocyte lineage progenitors [68,69]. Implantation of human fetal lymphoid tissue or human peripheral blood lymphocytes (hPBLs) reproducibly reconstitutes a functional human immune system in the SCID mice that produces proper human responses to immunogenic challenge for up to six months. Inoculation of these hPBL–SCID mice with several HIV strains results in productive infection (with subsequent virus isolation), and with four HIV-1 strains, reproducible CD4+ T lymphocyte depletion occurs [70–72]. The fastest depletion occurred within 2–4 weeks after incubation. Thus, in this combined animal and human lymphoid organ transplant model, HIV strains reproducibly mediate human CD4+ T lymphocyte depletion, the hallmark of AIDS.

Simian immunodeficiency virus causes AIDS in monkeys

Simian immunodeficiency virus (SIV) was originally discovered in 1985 as a lentivirus with immunological cross-reaction to HIV in Asian macaques housed in US primate centers [73]. Since then over 25 SIV isolates have been recovered from a dozen primate species and characterized in terms of prevalence, pathogenesis and genome sequence analysis [74–76]. From a genetic standpoint they are the closest viral relatives of HIV; SIV has the same ten functional genes as HIV and the DNA sequence similarity is very high for all ten genes. Phylogenetic studies of HIV and SIV group genomes show that HIV-1 and HIV-2 are close relatives of SIV_{CPZ} and $SIV_{SM\text{-}MAC}$, respectively, leading to the conclusion that the human immunodeficiency viruses have emerged from a simian virus ancestor within the past few hundred years [76–79]. The striking similarity of genome organization (same genes) and genomic sequence homology (fraction of DNA sequence matching) plus the phylogenetic intercalation of human and nonhuman primate lentivirus offer strong evidence that SIV and HIV share a very recent common ancestry [74].

At least 12 SIV strains induce AIDS (within one year) when cultured in adapted cell lines and transferred as tissue culture supernatant to simian species (largely Asian macaques). Here, AIDS is defined by the depletion of CD4+ T lymphocytes and several indicator opportunistic infections, namely, *Pneumocystis carinii* pneumonia, *Mycobacterium avium*, oral candidiasis, meningoencephalitis, and lymphoma [80–82]. Two pathogenic SIV strains ($SIV_{MAC\Delta 239}$, SIV_{AGM90}) have been molecularly cloned and the molecular clones have been used to induce AIDS in macaques [80–82]. Control macaques inoculated with saline, with inactivated $SIV_{MAC\Delta 239}$ or with $SIV_{MAC\Delta 239}$ carrying mutations/deletions in the *nef* gene failed to induce AIDS in the same macaque species [80,83,84]. Because SIV strains cause AIDS in monkeys and because they are the closest phylogenetic relatives of HIV, they provide an animal model fulfillment of Koch's transmission postulate.

Conclusions

The HIV–AIDS debate has continued beyond what is reasonable for an academic exercise. The data summarized above provide an overview of a mosaic of scientific data which prove conclusively that HIV-1 causes AIDS. It is our hope that the dangerous diversion which this debate has fostered will cease because of the potential for harm which could lead those at risk to become infected by ignoring prevention messages, and those infected from benefiting from the advances in therapy. The debate should cease and all energies should be put towards finding the ultimate proof of causation, the development of an effective vaccine and curative treatment.

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