A CRITICAL APPRAISAL OF THE EVIDENCE FOR THE ISOLATION OF HIV

Eleni Papadopulos-Eleopulos[1] Valendar F.Turner[2] John M. Papadimitriou[3] David Causer[1]

1Department of Medical Physics, 2Department of Emergency Medicine, Royal Perth Hospital, Perth, Western Australia; 3Department of Pathology, University of Western Australia.

Corresponding author:

Eleni Papadopulos-Eleopulos
Department of Medical Physics
Royal Perth Hospital
Wellington Street
Perth 6001
Western Australia

Voice: int + 61 8 92242500
Fax: int + 61 8 92241138
email <vturner@westnet.com.au >

Note:
This paper was submitted to the Royal Australasian College of Surgeons in 1997. According to that journal it is editorial policy to "welcome personal views of surgeons on a variety of topics", and to publish papers on "current and controversial issues". Although both reviewers accepted the bulk of the scientific arguments and found the paper "interesting reading", they advised against publication because, in their view, an analysis of evidence for the isolation of HIV was of "no real relevance…to a surgical audience" or "would be of little interest or use to the majority of readers of the Australian and New Zealand Journal of Surgery".

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The real purpose of scientific method is to make sure Nature hasn't misled you into thinking something you don't actually know...One logical slip and an entire scientific edifice comes tumbling down. One false deduction about the machine and you can get hung up indefinitely.

Robert Pirsig. Zen and the Art of Motorcycle Maintenance

SUMMARY
According to the published work of the leading HIV/AIDS scientists, HIV has been isolated and is a unique, exogenously acquired retrovirus and the necessary and sufficient cause of AIDS. However, contrary to the decades old, traditional method of isolating animal retroviruses, the seminal and explicit evidence for the isolation of HIV is based on a notion of specific reactivity between presumed viral proteins present in cell cultures/co-cultures containing tissues derived from AIDS patients and antibodies present in human and animal sera. The utility of this process is evaluated and it is concluded there are many unresolved problems with such a paradigm, all of which have a direct bearing on surgical practice.

INTRODUCTION

A virus possesses two distinguishing properties, the first being a physical entity, that is, a microscopic particle of particular size and morphology, the second, the ability to generate identical progeny by synthetic processes occurring obligatorily within the cytoplasm of a living cell and not within the viral particle itself. Significantly, it is the latter attribute which defines a viral-like particle as infectious and substantiates a viral-like particle as a virus. The three subfamilies (*Oncovirinae*, *Lentivirinae* and *Spumavirinae*) of *Retroviridae* (Retroviruses) are "enveloped viruses with a diameter of 100-120 nM budding at cellular membranes. Cell released virions contain condensed inner bodies (cores) and are studded with projections (spikes, knobs)"[1]. All retroviral particles contain the enzyme reverse transcriptase (RT), an RNA dependent DNA polymerase which catalyses the synthesis of DNA contrary to the central dogma of biology, that is, in a direction "reverse" from DNA to RNA. According to retrovirologists, such DNA is then integrated into existing cellular DNA as a DNA "provirus". Furthermore, retroviral particles share the property of banding (concentrating) at a density of 1.16 gm/ml when centrifuged at high speeds in sucrose density gradients, a fact long used in their isolation.[2-3] Thus, in putatively infected tissues, retroviruses are identified by presenting evidence documenting purification (isolation) of retroviral-like particles followed by proof that such particles contain RT and are infectious. The steps necessary for such proof are founded on the invention of the ultracentrifuge and the electron microscope (EM) and were documented over the decades of animal retrovirus research that preceded the AIDS era. The method is a model of intelligibility and was thoroughly discussed at a meeting held at the Pasteur Institute in 1973, a meeting attended by several of the current HIV/AIDS scientific dignitaries.[2-3] The steps are:

1. Culture of putatively infected tissue.

2. Purification of particles by density gradient ultracentrifugation.

3. Electron micrographic examination of the 1.16 gm/ml sucrose density gradient revealing nothing else but particles exhibiting the morphological characteristics and dimensions of retroviral particles.

4. Proof that such particles contain reverse transcriptase.

5. Analysis of the particles' proteins and RNA and proof that these are specific for those particles.

6. Proof that the particles are infectious, that is, when pure particles are introduced into cultures or animals, identical particles are obtained as shown by repeating steps 1-5.

7. Proof that the particles are a property of putatively infected tissues and cannot be induced in control cultures, that is, tissues obtained from matched subjects and cultured under identical conditions differing only in that they are not putatively infected with the retrovirus. Apart from anticipating unexpected or unidentified factors, control cultures acknowledge the fact that unlike all other infectious agents, the normal human genome contains retroviral information from which retroviral RNA and proteins may be synthesised and which may further lead to the assembly of retroviral particles, that is, the expression of endogenous retrovirus. At present it is accepted that such retroviral DNA forms 1% of human DNA, that is, 30 million nucleotides (3,000 times the accepted length of the HIV genome) and that endogenous retroviruses are present "in all of us".[4] The expression of endogenous retroviral genomes may arise spontaneously[5] but may be significantly accelerated and the yield increased by conditions which induce cellular activation, the

same culture conditions which are obligatory to obtain "HIV" from cell cultures containing tissues derived from AIDS patients.[6-8] Significantly, according to both Montagnier and Gallo, regardless of being "infected with HIV", HIV replication including the production of particles, as well as the cytopathic effect "can only be observed in activated T4 cells. Indeed, LAV infection of resting T4 cells does not lead to viral replication or to expression of viral antigen on the cell surface, while stimulation by lectins or antigens of the same cells results in the production of viral particles, antigenic expression and the cytopathic effect".[9-10] Endogenously produced retroviruses are morphologically and biochemically indistinguishable from exogenous retroviruses and furthermore, new endogenous retroviral genomes may arise from rearrangements of existing cellular DNA caused by many factors, including pathogenic processes.[11-12] According to the eminent retrovirologist George Todaro, "the failure to isolate endogenous viruses from certain species may reflect the limitations of in vitro cocultivation techniques".[13]

Long before the AIDS era it was known that both tissues and tissue cultures contain many particles of many morphologies, some even retroviral-like but which are not viral. Some examples are "cellular fragments", and microsomes from disrupted cells and "membranous vesicles which enclose other cellular constituents including nucleic acids" especially when "inadvertent lysis of cells" cannot be avoided[12, 14-15] (HIV is claimed to be cytopathic and in several instances, "HIV" is obtained after deliberate lysis of cells). For such reasons, it is pivotal to strictly maintain and prove the duality of viruses in order that one is not misled by non-viral material. Thus, isolation of retrovirus-like particles is the *sine qua non* to prove uniqueness and infectivity and is also obligatory to prove which biological effects, if any, are caused by the virus and to source viral proteins and nucleic acids for evaluation as diagnostic agents.

It is important to appreciate the two fundamental obstacles to the interpretation of antibody/antigen reactivity. The first is that antibody molecules, even monoclonal antibodies, may not interact only with the inducing antigen but also with other antigens, that is, antibodies may "cross-react"[16-23] Indeed, there are instances where "cross-reactive antigen binds with higher affinity than the homologous antigen itself...The most obvious fact about cross-reactions of monoclonal antibodies is that they are characteristic of all molecules and cannot be removed by absorption without removing all reactivity...Even antigens that differ for most of their structure can share one determinant, and a monoclonal antibody recognizing this site would then give a 100% cross-reaction. An example is the reaction of autoantibodies in lupus with both DNA and cardiolipin". However, "It should be emphasised that sharing a "determinant" does not mean that the antigens contain identical chemical structures, but rather that they bear a chemical resemblance that may not be well understood, for example, a distribution of surface charges".[23] Since polyclonal antibodies are no more than composites of monoclonal antibodies these facts apply equally, if not more so, to polyclonal antibodies. It is of importance to note that HIV experts concede "cross-reactivity" as the reason for "indeterminate" antibody reactivity seen in the HIV Western blot (see below), as well as, for example, reactivity between monoclonal antibodies to the HIV p18 protein and dendritic cells in the lymphatic tissues of a variety of patients with a number of non-AIDS related diseases[24] and normal tissues taken from non-HIV infected individuals.[21] The second obstacle is that some stimuli cause the appearance of antibodies which are not directed against the stimulating agent. Indeed, these facts are extensively exploited in clinical medicine for the diagnosis of diseases such as syphilis, infectious mononucleosis and relapsing fever. In these diseases, *T. pallidum*, Epstein-Barr virus and *B. recurrentis* cause the appearance of antibodies reactive with ox-heart proteins, sheep and horse red blood cells and *Proteus* OXK and OX19 respectively. For these reasons, we must accept that

Nature imposes a fundamental limitation on our ability to extend the observation and measurement of antibody/antigen reactivity to the precise determination of the participating reactants. This applies even if one reactant, either antigen or antibody, is fully documented beforehand. Thus as in the case of mononucleosis for example, it is not axiomatic that the presence of antibody reactive with horse red blood cells (measured for example in the "Monospot") is synonymous with the presence of immunising antibody to horse red cells and thus to "infection" with horse blood. Thus, as each case arises, the specificity of an antibody/antigen reaction must be proven, especially when such reactivity is contemplated for diagnostic use in clinical medicine.

IN VITRO PHENOMENA CLAIMED TO PROVE HIV ISOLATION

In May 1983 Luc Montagnier and his colleagues from the Pasteur Institute published a paper in *Science* entitled, "Isolation of a T-Lymphotropic retrovirus from a patient at risk for Acquired Immune Syndrome (AIDS)"[25]. This is the paper which, since the resolution of the polemics between Montagnier and Gallo concerning allegations of misappropriation by the latter of the French virus sent to the US by the Pasteur Institute, is almost universally accepted as the first proof of the isolation and thus the existence of HIV. There it was shown that mitogen activated lymph node cell cultures from a gay man with lymphadenopathy were able to reverse transcribe the synthetic RNA primer-template $An.dT_{15}$. From this data Montagnier and his colleagues concluded that the lymph node cells of the gay man contained a retrovirus. (However, as long ago as 1973 it was known that mitogenic stimulation of normal lymphocytes causes reverse transcription of the same primer-template.[26-27]) The finding of the same activity in the supernatant of a co-culture of the same cells with lymphocytes from a healthy individual was considered proof that the retrovirus could be transmitted. In another experiment, supernatants from the co-cultures were added to two, three day old, mitogenically stimulated, umbilical cord lymphocytes cultures. After seven days "a relatively high titer" of $An.dT_{15}$ transcription was detected. This was considered proof not only of transmission but isolation as well. Subsequently, a supernatant from the "infected" cord blood lymphocytes was banded in a sucrose density gradient and tested with sera from the same and another patient with multiple lypmhadenopathies and from two healthy individuals. Three proteins, p24/25, p41/45 and p80 reacted with the serum from the original patient. "Electron microscopy of the infected umbilical cord lymphocytes showed characteristic immature particles with dense crescent (C-type) budding at the plasma membrane". There was no electron microscopic (EM) data on the material banding at 1.16 gm/ml but the authors concluded "A retrovirus belonging to the family of recently discovered human T-cell leukemia viruses (HTLV) but clearly distinct from each previous isolate, has been isolated from a Caucasian patient with signs and symptoms that often precede the acquired immune deficiency syndrome (AIDS). This virus is a typical type-C RNA tumor virus, buds from the cell membrane, prefers magnesium for reverse transcriptase activity, and has an internal antigen (p25) similar to HTLV p24"[25] Montagnier and his colleagues called their "virus" lymphadenopathy associated virus (LAV). (A year later, when it was realised that individuals who have antibodies which react with this "virus strain" did not rapidly progress to AIDS, the taxonomically distinct "typical type-C" retrovirus became a taxonomically distinct, typical *Lentivirus*, although the term *Lentivirus* observes a strict morphological description[28] for which no data was presented). It is significant that Gallo and his colleagues did not consider the Montagnier group data as proving "true isolation".[29]

A year later, in May 1984, Gallo, Popovic and their colleagues published four papers in *Science* in which they claimed to have isolated another retrovirus from AIDS patients. The Gallo virus was called human lymphotropic virus-III (HTLV-III). On the 23rd of April 1984, at a Washington press conference held two weeks before the *Science* papers were

published, Margaret Heckler, the then Health and Human Services Secretary, announced that Gallo and his co-workers had discovered the "probable" cause of AIDS and had developed a sensitive test to show whether the "AIDS virus" is present in blood. In 1985, the Pasteur Institute alleged that Gallo had misappropriated LAV in developing the blood test. The ensuing conflict, which reached the American courts, was eventually settled by a negotiated agreement signed in 1987 by Gallo, Montagnier, US President Reagan and French Premier Chirac. The agreement declared Gallo and Montagnier to be co-discoverers of the AIDS virus, presently known as the Human Immunodeficiency Virus. Nevertheless, the misappropriation conflict drew the attention of John Crewdson, an investigative journalist, and US Senator John Dingell. In November 1989, Crewdson published a lengthy article in the *Chicago Tribune* newspaper, "With allegations that Robert C. Gallo stole from French scientists the virus he discovered to be the cause of AIDS".[30] This led to a National Institute of Health (NIH) internal "inquiry" into the allegation with "an outside committee of expert but disinterested parties [led by Yale biochemist Frederic Richards] to oversee the activity of the internal panel".[31] Following the inquiry, which was viewed as a fact-finding mission, the Richards committee insisted on a "formal investigation...on suspect data in one of four seminal papers published by Gallo's lab in Science on 4 May 1984".[32] In the first paper, with Mikulas Popovic the principal author, "there appears to be differences between what was described in the paper and what was done".[30] A draft report of the formal investigation written by NIH Office of Scientific Integrity (OSI), was published in September 1991. In the draft report, Popovic is accused "of misconduct for misstatements and inaccuracies" that appeared in the paper, and that Gallo, as laboratory chief, "created and fostered conditions that give rise to falsified/ fabricated data and falsified reports". However, Gallo's actions were not considered to "meet the formal definition of misconduct".[33] The final draft report of the OSI, completed in January 1992, was immediately criticised by the Richards Panel as well as Senator Dingell. This led to a review of the OSI report by the Office of Research Integrity (ORI), which found Gallo guilty of scientific misconduct. Nonetheless, the scientific misconduct is said not to "negate the central findings of the [1984 Science] paper".[33-34]

In this first paper entitled "Detection, Isolation and Continuous Production of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and pre-AIDS", experiments were described in which "concentrated culture fluids harvested from short-term cultures of T-cells" from patients with AIDS or pre-AIDS were cultured with highly selected clones of a leukaemic cell line (HT). The data presented as proof of isolation of HIV were (i) RT activity in cell free supernatants and concentrated culture fluids after "purification" through a sucrose density gradient; (ii) immunofluoroescence of cells obtained by reactions with "Rabbit antiserum to HTLV-III" and "Patient serum (E.T.)"; (iii) electron microscopy showing the presence of extracellular particles. Reading this paper the impression is gained that the HT cell line was cultured with concentrated (supernatant) fluids originating from individual, AIDS patient, mitogen stimulated T-cell cultures. However, the OSI enquiry found that the HT cell line was cultured with concentrated fluids pooled initially from individual cultures of three patients and ultimately from the individual cultures of ten patients.[35] In evidence given to the enquiry the reason given was because none of the supernatants "individually was producing high concentrations of reverse transcriptase". In other words, Gallo and his colleagues did not regard the levels of RT from individual cultures as sufficient proof that the initial, individual specimens contained a retrovirus before they were mixed and added to the HT cell line. The Gallo investigation found the pooling of specimens to be "of dubious scientific rigor". One scientist described the procedure as "really crazy".[31] In essence it is no different from investigating an outbreak of a new type of pneumonia by culturing the sputum of each patient and, when no organisms are cultured, combining all specimens and reculturing.

In the second paper Gallo and his colleagues claimed to have "isolated" HTVLV-III (HIV) from 26/72 (36%) of AIDS patients. The explanation for the low yield of virus isolation was, "many tissue specimens were not received or handled under what we now recognize as optimal conditions. This is particularly so for the samples received from late-stage AIDS patients. Such samples usually contain many dying cells". However, Gallo and his colleagues were widely regarded as world leading experts in culturing tissues for retroviruses and it is now claimed that in AIDS patients who have not received antiviral treatment, "plasma viral RNA levels...at baseline ranged from $10^{4.6}$ to $10^{7.2}$ molecules per ml".[36] (equivalent to $10^{4.2}$ to $10^{6.9}$ [approximately one hundred thousand to ten million] "virions" per ml[37]). In this paper the criteria listed for the isolation of HTLV-III were "more than one of the following": "repeated detection of a Mg^{2+} -dependent reverse transcriptase activity in supernatant fluids; virus observed [in the cultures, not at the density of 1.16 gm/ml] by electron microscopy (EM); intracellular expression of virus-related antigens detected with antibodies from seropositive donors or with rabbit antiserum to HTLV-III; or transmission of particles". By transmission of particles was meant detection of RT or particles in cultures of human umbilical cord blood, bone marrow or peripheral blood T-lymphocytes, cultured with supernatants from the "infected" cultures.[38] (It can be seen that the Gallo group method permitted instances of "isolation" of a retrovirus without the necessity of evidence for either particles or RT activity). In the third paper it was reported that from the supernatant of the "infected" cultures which, in sucrose density gradients banded at 1.16 gm/ml, two proteins, p41/45 and p24/25, reacted with various human sera. For this and no other reason it was claimed that "these molecules are the major components of the virus preparation. p24 and p41 may therefore be considered the viral structural proteins". (Of interest is the fact that Montagnier still regards p41/45 as the ubiquitous cellular protein actin and not an HIV protein.[25, 39] (Later, a number of other proteins present in either the material from culture supernatants banding at 1.16 gm/ml or just cellular extracts, and for no reason other than each reacted with antibodies present in sera from AIDS patients or those are risk of developing AIDS, were considered to be specific, HIV proteins). As far as morphology is concerned, the Gallo group reported that the HIV particle "is produced in high numbers from infected cells by budding from the plasma membrane. A possible unique feature of this virus is the cylindrical shaped core observed in many mature virions...HTLV-III is a true member of the HTLV family". (HTLVs are type C retroviral particles and are not *Lentiviruses)*. From their data Gallo and his colleagues claimed "isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS" and that their data "provide strong evidence of a causative involvement of the virus in AIDS". Two years later Gallo wrote that "The results presented in our four papers provided clearcut evidence that the aetiology of AIDS and ARC was the new lymphotropic retrovirus, HTLV-III".[40]

COMMENTS ON VIRAL ISOLATION

As far as "true isolation" is concerned, the only substantive difference between the Montagnier and Gallo group experiments was that the latter cultured putatively infected tissues with highly selected, "permissive" clones of the HT (HUT78) leukaemic cell line and claimed this provided "large amounts of virus for detailed molecular and immunological analyses".[29] Thus, apart from a qualitative difference, the Gallo group experiments are no different from those performed by Montagnier and his colleagues and it follows that neither the Gallo nor the Montagnier groups conducted true isolation, that is, isolation performed according to the customary, well established method where the first step is to obtain a retroviral-like particle free from all extraneous matter. It is also of pivotal significance that neither the Montagnier nor the Gallo group reported the use of proper controls (see above) in any of their experiments.

The word "isolation" is derived from the latin "*insulatus*" meaning "made into an island". It refers to the act of separating an object from all matter that is not that object. It is obvious that even by the most liberal of definitions, combinations of phenomena including transcription of RNA into DNA, retroviral-like particles, or antigen/antibody reactions in cultures or co-cultures or in material banding at 1.16 gm/ml, cannot be construed as proof of isolation of a retrovirus. They can be used only for the detection of a retrovirus but if and only if, they are first shown to be specific. This can only be proven by first isolating the retrovirus.

Reverse transcriptase

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 including HIV isolation.[41-43] However, according to some of the best known retrovirologists including its discoverers and Nobel Laureate and Director of the US National Institutes of Health, Harold Varmus, reverse transcriptases are present in all cells including bacteria.[15, 44-45] "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".[46] 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".[47] "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].[48] "Hepatitis B virus (HBV) resembles retroviruses, including HIV, in several respects. In particular, both viruses contain reverse transcriptase, and replicate through an RNA intermediate". Because of this, it has been suggested that hepatitis B infection should be treated with the same antiretroviral agents as HIV infection.[49] 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".[50] Lymphocyte stimulation in general and PHA (an agent employed in all cultures of tissues from AIDS patients), 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"[51-52] Furthermore, reverse transcriptase is estimated indirectly, that is, by detecting the DNA product of reverse transcription of RNA. However, normal cellular DNA polymerases are also able to transcribe RNA into DNA[53-54] and, in the Gallo group case, HUT78, the parental HT cell line, originated from a patient with "malignancies of mature T4 cells", a disease claimed by Gallo to be caused by HTLV-I, which Gallo also claims is a type-C retrovirus discovered by him and his colleagues in 1981.[55] In fact, in 1983, Gallo and his colleagues reported the presence of HTLV-I genomic sequences in the HUT 78 cell line.[56] Furthermore, as far back as 1973 it was known that leukaemic T-cell cultures reverse transcribe $An.dT_{15}$,[26] the synthetic RNA-template primer used in all HIV research. Given these data, how may one claim that reverse transcription of $An.dT_{15}$ is proof of detection not only of a retrovirus but of a unique retrovirus, HIV?

Virus-like particles

The finding of particles with the appearances of retroviruses is not proof that such particles are retroviruses and even less proof that such particles are a particular retrovirus. It is important to note that apart from the EMs published by the Montagnier and Gallo groups of non-banded culture material classifying HIV particles as *Oncovirinae* type C and *Lentivirus* particles, data of numerous authors place HIV particles within the type A and type D genera of the subfamily *Oncovirinae*.[57-62] Thus, the leading HIV experts cannot agree into which subfamily of retroviruses HIV belongs and have described HIV as a member of two subfamilies and three genera of *Retroviridae*. Moreover, retroviral-like particles are ubiquitous. In the 1970s, such particles were frequently observed in human leukaemia tissues,[63] cultures of embryonic tissues and "in the majority if not all, human placentas".[64] (Montagnier obtained EMs of HIV particles from umbilical cord blood lymphocytes). Type-C particles are found in "fish, snakes, worms, pheasant, quail, partridge, turkey, tree mouse and agouti"[65] as well as in "tapeworms, insects...and mammals".[28] In addition, cell cultures of tissues from AIDS patients contain particles of numerous sizes and morphologies including viral-like particles but in no such cultures are there particles which display both principal morphological characteristics of retroviruses, that is, "a diameter of 100-120 nm" AND surfaces which "are studded with projections (spikes, knobs)"[66-70], the latter unanimously claimed to be essential for the replication of retroviruses.[71] Virtually every cell-free (mature) "HIV particle" is devoid of knobs and thus of gp120 which is considered to be the constituent protein and an absolute necessity for infectivity. In one of their latest publications, Hans Gelderblom and his colleagues from the Koch Institute in Berlin, who have conducted most of the EM on HIV, estimate that immediately after being released from the cell membrane, "HIV particles" possess an average of 0.5 knobs per particle which are lost over time but also pointed out that "it was possible that structures resembling knobs might be observed even when there was no gp120 present, i.e., false positives".[72] Notwithstanding, nowhere in the vast HIV/AIDS scientific literature is there even one EM of a banded supernatant showing which, if any of the numerous particles observed in cultures of tissues from AIDS patients, is present at the sucrose density of 1.16 gm/ml. Also, and of particular significance, in an *in vivo* study conducted by O'Hara and colleagues from Harvard, "HIV particles" were found in 18/20 (90%) of patients with enlarged lymph nodes attributed to AIDS. However, identical particles were also found in 13/15 (87%) of patients with enlarged lymph nodes not attributed to AIDS and at no risk for developing AIDS. These data led the authors to conclude, "The presence of such particles does not, by themselves indicate infection with HIV".[73]

Antibody/antigen reactions

As discussed above, neither Montagnier nor Gallo and their respective colleagues employed the decades old method for proving the existence of a novel retrovirus. However, five years before the AIDS era, Gallo accepted that even the discovery of a retroviral-like particle containing RT is not proof that the particle is a retrovirus. In 1976 he wrote, "Release of virus-like particles morphologically and biochemically resembling type-C virus but apparently lacking the ability to replicate have been frequently observed from leukaemic tissue".[63] Perhaps for this reason, in his research into the phenomena which are claimed to constitute the evidence for the isolation of HIV from AIDS patients and those at risk, Gallo and his colleagues regarded antibody/antigen reactivity as crucial. Thus, to prove HIV isolation, both Montagnier and Gallo and their colleagues employed sera from patients and rabbits which they claimed contained "specific reagents".[40] The latter is especially enigmatic. Rabbits do not develop HIV infection or AIDS and if such specific antibodies were to exist they could only be produced by immunisation of rabbits with pure HIV or, as the first Gallo group paper reported, "from rabbits infected repeatedly with disrupted HTLV-III". If rabbits were immunised with pure virus, why should it be necessary

to produce specific reagents to define the isolation of virus that had already been isolated? That an antibody/antigen reaction cannot be use to prove the existence of a new virus is accepted by Donald Francis, a researcher who with Gallo, played a significant role in developing the theory that AIDS is caused by a retrovirus.[74] In 1983, Francis, then the chief collaborator of the AIDS Laboratory Activities, US Center for Disease Control (CDC) and former chief of the WHO smallpox program, speculated on a viral cause for AIDS: "One must rely on more elaborate detection methods through which, by some specific tool, one can "see" a virus. Some specific substances, such as antibody or nucleic acids, will identify viruses even if the cells remain alive. The problem here is that such methods can be developed only if we know what we are looking for. That is, if we are looking for a known virus we can vaccinate a guinea pig, for example, with *pure virus*...Obviously, though, if we don't know what virus we are searching for and we are thus unable to raise antibodies in guinea pigs, it is difficult to use these methods...we would be looking for something that might or might not be there using techniques that might or might not work"[75] (italics ours).

Rationally, as surgeons deem the identity of their patients' tissues and organs contingent upon removal from their patients' bodies, the identity of HIV proteins can only be defined by their removal from purified retroviral-like particles shown to be a retrovirus. However, as the experiments of Montagnier and Gallo clearly reveal, this was not the case for HIV. Rather, a set of proteins not even known to be embodied in any kind of particle, were defined viral specific by virtue of their interaction with unknown antibodies. (Neither is there evidence that "HIV RNA" has been extracted from purified particles shown to be a virus). Indeed, nowadays "HIV isolation" is obtained by observing a reaction between just one "HIV" protein, p24, and a commercially available "HIV" antibody. However, p24 is not specific to HIV.[76-80] The non-specificity of the p24 antigen test is so obvious that it is accepted by no less an authority on HIV testing than Philip Mortimer and his colleagues from the UK Public Health Laboratory Service, "Experience has shown that neither HIV culture nor tests for p24 antigen are of much value in diagnostic testing. They may be insensitive and/or non-specific".[81] Even if the reverse were true, the production of an antibody/antigen complex is not virus isolation. Could such a complex be used to study the morphology of an HIV particle or determine the biological properties of HIV or extract RNA for analysis? If this method is virus isolation then consistency demands we regard the pregnancy test as placental isolation. The most recent situation using p24 monoclonal antibody to "isolate" HIV is best illustrated by a large, World Health Organisation study. Between 1992-93, 224 specimens were collected in Brazil, Rwanda, Thailand and Uganda from "HIV positive" individuals. Serostatus was first confirmed in the country of origin and then at the "centralized laboratories responsible for confirming serology, virus isolation, virus expression, and distribution of reagents (George-Speyer-Hans Chemotherapentisches Forschunginstitut (GSH) in Frankfurt, Germany; National Institute for Biological Standards and Control (NIBSC) in London, United Kingdom,; and DAIDS/NIAID in Bethesda, Maryland, United States". In this study the isolation rate was reported to be 37%,[82] a figure not significantly different from that reported by Gallo in 1984. Furthermore, in a study published in 1991, researchers from the University of Washington tested 192 patients "with confirmed Western blot antibodies to HIV", for p24 antigen as well as p24 antibodies. "HIV antigen was detected in 64 (33%) while anti-p24 was detected in 70 (33%). The frequency of HIV antigen in CDC class II, IVa and IVc was 31%, 39% and 30% respectively. For anti-p24 these were 58%, 41% and 25% respectively". However, according to the US Food and Drug Administration and in the United Kingdom (as well as in particular instances under the CDC and Consortium for Retrovirus Serology Standardization criteria), antibodies to p24 are an absolute necessity for seropositivity. This means that by these criteria, 59% of patients "with constitutional disease (formerly AIDS related complex or ARC)" class IVa and 75% of patients with AIDS are not infected with HIV.[83]

Antibody/antigen reactivity is specific for a viral infection if and only if such reactivity occurs exclusively in the presence of a particular virus. This means that assertions concerning specific reactivity must be based on the use of virus isolation as a gold standard, that is, specific reactivity can only be adduced *after* virus isolation. This being the case, specific reactivity cannot be used as a premise to prove the existence of a virus if one must first isolate the virus to prove the premise upon which isolation is contingent. However, while it may prove demanding to verify specificity, several data may signify the reverse, that is, in the scientific literature there are many examples of reactivity to “HIV” where the absence of HIV infection is considered most likely:

1. Lundberg and his colleagues from the US Consortium for Retrovirus Serology Standardization reported that 127/1306 (10%) of individuals at "low risk" for AIDS including "specimens from blood donor centers" had a positive HIV Western blot by the "most stringent" of the US criteria, that is, the presence of antibodies to p24, p32 and gp41 or gp120/160.[84]

2. In 1992, Jorg Shupbach, the principal author of the third and co-author of the fourth of the 1984 papers published by Gallo's group on HIV isolation, reported that the whole blood cultures of 49/60 (82%) of "presumably uninfected but serologically indeterminate individuals and 5/5 seronegative blood donors were found positive for p24".[79]

3. According to the AID vaccine Clinical Trials Group,[85] "The presence of p24 band was common among low-risk, uninfected volunteers and complicated the interpretation of the Western blot test results".

4. If the p24 band in the WB is considered the result of specific reactivity, then approximately 30% of individuals who are transfused with HIV negative blood become infected with HIV as a result.[76]

5. A 40 year old, male, HIV antigen negative, heterosexual donor of Rh negative blood was given six 5ml injections of donated Rh positive serum, administered at 4 day intervals. His "wife and child were seronegative on HIV ELISA". The donor serum "was shown to be negative on HIV antibody and antigen ELISA". "Blood taken after the first immunization was shown to be negative on HIV antibody ELISA and immunoblot assay. After the second immunization a weak signal on ELISA, slightly above the cut-off level, was monitored. After the third immunization the signal was strong and immunoblot revealed distinct interaction with p17 and p55 proteins. An even stronger signal was monitored after the fifth immunization. Interaction with p17, p31, gp41, p55 and some other proteins was evident".[86]

6. 11/208 (5%) of healthy blood donors and 10/50 (20%) of patients with measles, mumps, herpes simplex, dengue and other viral illnesses had either a p24 or p18 band on the HIV Western blot test.[87]

7. In 1991, Elizabeth Dax and associates from the US National Institute on Drug Abuse HIV reanalysed 1985 Western blot strips of sera originally obtained from intravenous drug addicts in 1971-72. (The actual sera themselves were not available for retesting). Ten persons "with potentially positive WB patterns, when the more specific 1985 criteria were used", were traced. One patient had died from a motor vehicle accident and there were "no lymphoreticular changes at autopsy, and a thorough retrospective analysis provided no evidence of either current substance abuse of HIV infection". Of the nine living addicts, two could not be assessed clinically, seven were not chronically ill, (one was in prison but in

good health, one had been successfully discharged from a methadone program, one was enrolled in a methadone program, another sporadically consumed illicit drugs). "The two former patients whose 1971-72 WB results were most strongly reactive had current ELISA and WB assays that were negative. The immune function parameters were inconsistent with immune suppression". Their data led the authors to conclude, "it is possible that antibodies to a nonpathogenic virus would have disappeared during the 17 to 18 years...followup. Although this potential cannot be ruled out, it is more likely that the earlier results were false positives...definitive evidence of HIV infection in the United States' addict population as early as 1972 is still lacking".[88] If positive ELISAs and Western blots in drug addicts who do not develop AIDS are false-positives, then how does one know that this is not also the case in those who do develop AIDS? It goes without saying that one cannot use the antibody tests as proof that HIV is the cause of AIDS and that AIDS is proof that the antibody tests are HIV specific.

8. 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".[89]

9. In 1991 Kion and Hoffman injected non-HIV-infected mice with T-lymphocytes from another strain of non-HIV-infected mice. The recipient mice developed antibodies to the HIV gp120 and p24 proteins.[90]

10. Researchers from the University of Rome injected healthy mice with an extract of *E. coli*. Following the injection the mice developed antibodies to the p120 and p41 proteins of HIV (V. Colizzi et al., personal communication).

11. In 1991, Strandstrom and colleagues reported that 72/144 (50%) of dog blood samples "obtained from the Veterinary Medical Teaching Hospital, University of California, Davis" tested in commercial Western blot assays, "reacted with one or more HIV recombinant proteins [gp120--21.5%, gp41--23%, p31--22%, p24--43%]".[91]

Although they are obtained devoid of a gold standard, the above examples are persuasive for the existence of non-specific and/or cross reacting "HIV" antibodies. If this is not the case then all the above must represent instances where HIV infection has been acquired by unknown and/or most unusual means. However, since dogs, laboratory mice and healthy blood donors or human injected with HIV negative blood or even their own blood are not believed to be at risk from HIV infection of the development of AIDS, HIV cannot be regarded as the necessary and sufficient cause AIDS. On the other hand, if there are non-specific HIV antibodies then antigen/antibody reactivity cannot be used as a method of HIV detection or even less HIV isolation. This is the case even if all antigens, both infectious and non-infectious were to stimulate the production of only one antibody and each antigen/antibody pair only reacted with each other. Under these circumstances it is still not possible to prove the origin of either antigen in a culture or an antibody in serum of a patient from such a reaction. This especially the case in AIDS since both AIDS patients and cell cultures derived from their tissues may harbour multiple infections agents including hepatitis B, Epstein-Barr and cytomegalic inclusion viruses, mycoplasma, fungi and mycobacteria. Furthermore, the cultures contain cellular proteins and even amongst those which band at 1.16 gm/ml, only 20% are considered to be "HIV proteins".[92-93] It is also well known that sera from AIDS patients as well as individuals from the AIDS risk groups have raised levels of immunoglobulins including autoantibodies and have antibodies to a plethora of antigens.[94] In fact, anti-lymphocyte auto-antibodies are present in 87% of HIV seropositive patients and their levels correlate with clinical status.[95-96] Thus, the existence of

non-specific "HIV" antibody reactivity makes it impossible to determine what proportion, if any, "HIV" antibodies signify HIV infection and thus the correlation between HIV antibodies and AIDS cannot be used as proof that HIV is the cause of AIDS.

THE DIAGNOSIS OF HIV INFECTION *IN VIVO*

The assumption of specific reactivity also underlies the serological determination of HIV infection in all individuals regardless of risk group status. Furthermore, the correlation between such reactivity and the presence or the development of AIDS in certain groups of individuals remains the only *in vivo* evidence that HIV is the cause of AIDS. There are two antibody tests in common use.[97] The first is the HIV ELISA where a mixture of proteins banding at 1.16 gm/ml is incubated with patient serum and reactions are read by measurement of a colour change in the solution. In most countries of the world an initial reactive ELISA is repeated and if the second ELISA is also reactive a second test, the HIV Western blot is performed (the UK is a notable exception in use of the latter). In the Western blot, the proteins are first electrophoretically isolated in a nitrocellulose strip. When serum is added and the strip developed, individual protein/antibody reactions are visualised as a series of horizontal bands. The HIV ELISA is accepted as highly sensitive but possessing insufficient specificity to definitely diagnose individuals as HIV seropositive. On the other hand, a positive Western blot is deemed highly specific and is regarded as synonymous with HIV infection.

Apart from problems with reproducibility[84-98] HIV Western blot is not standardised[84] and its specificity has not been determined by comparison with the gold standard of HIV isolation.[94-99] According to the world literature, (Figure 1), the criteria for a positive HIV Western blot vary extensively according to which country, institution or laboratory is performing the test.[39, 84, 100-102] For example, antibodies reacting with any two of gp41, gp120 or gp160 are considered a positive Western blot in central Africa but this would not be positive in Australia or most of the USA. An individual positive under the criteria of the US Center for Disease Control or Food and Drug Administration would not be deemed positive in Australia. Between 1983-89, amongst the 4955 gay men participating in the Multicenter AIDS cohort prospective study with the aim to "Identify and quantify the correlates of HIV seropositivity" in men seropositive at entry and "Detect and quantify factors associated with seroconversion" in men seronegative at entry, a single "strong", including a p24 antibody band, was deemed a positive HIV Western blot. However, this result would not be positive anywhere else in the world, including Africa.[103] Accordingly, the criteria for HIV infection in one place are deemed indeterminate for HIV infection in others. Since a positive Western blot is said to be caused by specific HIV antibodies and since "The least likely explanation for an indeterminate western blot is that the individual is infected with HIV", and the "most likely explanation is that the patient being tested has antibodies that cross react with one of the proteins of HIV",[22] one must conclude that non-specific HIV antibodies in some countries or institutions metamorphose into specific antibodies in other countries or institutions. These data stretch the bounds of credulity and cannot be reconciled with the known behaviour of any conventional virus.[104] They are also incongruent with the acceptance of a universal HIV Western blot which "has a specificity of roughly 99.9993%"[105] Indeed, if mice injected with foreign cells and foreign proteins and humans injected with their own or foreign blood develop "HIV" antibodies but are not infected with HIV, why should gay men, intravenous drug users and haemophiliacs, who are all exposed to foreign cells and/or foreign proteins, not also develop "HIV" antibodies and not be infected with HIV?

For over two decades the virology literature has held a caveat illustrating the problematic nature of reliance on specific reactivity to define novel retrovirus. In the mid 1970s, Gallo

and his colleagues reported the isolation of the first human retrovirus, HL23V, from patients with leukaemias. In fact, the evidence for the "isolation" of HL23V surpassed that of both HTLV-I and HIV in at least two aspects. Unlike the case for HIV, Gallo's group: (i) reported the detection of reverse transcriptase activity in fresh, uncultured leucocytes;[63] (ii) published an electron micrograph of virus-like particles banding at the sucrose density of 1.16 gm/ml.[106] Following the discovery of HL23V, some researchers attempted to determine its prevalence utilising antibody tests[107] while others were interested in determining the specificity of the antibody reactions. The former included two of the best known HIV experts, Reinhard Kurth and Robin Weiss, and their colleagues who, for this purpose used "the simian sarcoma-associated helper virus (SSAV) and the M7 strain of baboon endogenous virus (BEV) to survey human sera for specific antibodies. Also included is a virus (HL23V-1) originally isolated from cultured peripheral blood leukocytes of a patient with acute myelogenous leukemia. HL23V-1 was shown to comprise a mixture of two viruses, one closely related to SSAV, the other to BEV" and found that "A survey of human sera from healthy individuals revealed the presence of naturally occurring antibodies that react in radioimmunoprecipitation assays with proteins of mammalian type-C viruses" including the internal (*gag*) and envelope (*env*) proteins of HL23V, SSAV and BEV. It was concluded that, "The serological studies presented here and by others provide indirect evidence that the infectious mode of transmission remains a real possibility in humans, and suggests that infection with an oncornavirus [retrovirus] may be extremely widespread".[108] Three years later, in 1980, two research groups,[109-110] one from the Laboratory of Cellular and Molecular Biology, National Cancer Institute and the other from the Laboratory of Viral Oncology, Memorial Sloan-Kettering Cancer Center, using the "viral glycoproteins", found that the antibodies present in human sera which reacted with these proteins were "directed against carbohydrate structures" and concluded that "The results are consistent with the idea that the antibodies in question are elicited as a result of exposure to many natural substances possessing widely cross-reacting antigens and are not a result of widespread infection of man with replication competent oncoviruses". In 1981 Gallo accepted the evidence that the antibodies which reacted with proteins of HL23V were directed not against the proteins "but against the carbohydrate moieties on the molecule that are introduced by the host cell as a post-transcriptional event, and which are therefore cell-specific and not virus-specific".[111] This discovery was of such significance that today nobody, not even Gallo, considers HL23V as being the first human retrovirus, or even a retrovirus. In fact, in 1981 Gallo and his colleagues next reported the isolation of HTLV-I and claimed it as the first human retrovirus.[111]

There is now ample evidence that antibodies to cell wall antigens such as the lipoarabinomannans of mycobacteria which "may share common epitopes with HIV", as well as to the mannans contained in all fungi (*Candida albicans*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Histoplasma capsulatum* including *Pneumocystis carinni*[112-114]), cross-react with what are regarded as all the HIV proteins.[115-118] Furthermore, one hundred per cent of AIDS patients (even those with "No candida clinically") have *Candida albicans* antibodies[119] and the vast majority of AIDS cases (88% of AIDS cases diagnosed between 1988 and 1992 had one or more fungal or mycobacterial infections[120]) including *Pneumocystis carinni* pneumonia, candidiasis, cryptococcosis, coccidioidomycosis, histoplasmosis, tuberculosis or *Mycobacterium avium-intracellulare* disease. In light of these data how can a positive antibody test in such individuals be claimed as proof of HIV infection and at the same time, proof that these diseases are caused by HIV?

As in the case of the extinct HL23V, the existence of non-specific reactivity to “HIV” counsels an urgent revision of the contemporary paradigm for identifying novel retroviruses

in humans. There is a persuasive argument to return to the traditional method in order to determine whether or not there are such entities as specific HIV proteins and antibodies and whether or not we have been misled by the compilation of phenomena inferred as isolation of the human immunodeficiency virus. Such a conclusion gains considerable momentum from study of the epidemiological data[121-124] which proves beyond all reasonable doubt that the only risk factor associated with the development of "HIV" seropositivity and AIDS (apart from that associated with the use of both parenteral and oral[125] illicit drugs) is passive anal intercourse in both men and women. In other words, "HIV" seropositivity and AIDS, like pregnancy, but unlike all other sexually transmitted diseases, can be sexually acquired but not sexually transmitted.

NOTE ADDED IN PROOF

Prior to late March 1997 no group of HIV researchers had published even a single electron micrograph (EM) of material banding at the density of 1.16 gm/ml in a sucrose density gradient, the fraction which all HIV/AIDS researchers regard as “containing viral antigen and/or infectivity” and “to contain a population of relatively pure virus particles” and from which “viral” proteins and “viral” RNA are obtained for diagnostic and other purposes.[126] The first EMs of such material banded in sucrose density gradients appear in two publications, one Franco/German[126] and the other from the US National Cancer Institute (NCI).[127] The Franco/German EMs are from the 1.16 gm/ml sucrose density whereas it is not possible to tell from which density the NCI data originate. The data from both studies reveal that the vast majority of the material is "non-viral", "mock" virus, cellular "microvesicles", that is, the banded material is regarded by these two research groups as virtually all cellular. Furthermore, although data from animal retrovirus experiments leads to the expectation that the band will contain millions of retroviral-like particles filling an EM to the exclusion of all other particles or material, there are only a minority of “HIV” particles in the EMs. (The authors express this data as a “co-purification” of such particles with the cellular material). However, neither research group provides any proof that the “HIV” particles are a specific retrovirus or even a retrovirus. Furthermore, measurement of the “HIV” particles shows that the majority are not spherical, none has a diameter is less than 120nM and in fact many particles have diameters exceeding twice that admissible for a retrovirus. Assuming the material was banded to equilibrium and the NCI group also sought particles at the correct retroviral density then all particles found by both groups must have a density of 1.16 gm/ml. Averaging the major and minor diameters of the “HIV” particles and assuming all particles are spherical reveals that the Franco/German particles are 1.14 times larger than *bona fide* retroviral particles and the NIC particles 1.96 times larger. These data translate into volumes 50% and 750% greater respectively. Since density is the ratio of mass to volume the Franco/German particles and the NCI particles must therefore have correspondingly higher masses. Given the maximum diameter of retroviral particles and the fact that such particles contain a fixed mass of RNA and protein, it appears untenable that the particles which both groups regard as HIV are the same particle or a retroviral particle. The only other explanation for these data is that the electron micrographs are not from the 1.16 gm/ml band in which case one must redefine the buoyant density of retroviruses and abandon the notion that the 1.16 band is HIV. In addition, no particle appears to have projecting surface knobs which, according to the HIV experts, are an absolute requirement for a virus-like particle to be infectious, that is, to be a virus particle.[128]

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