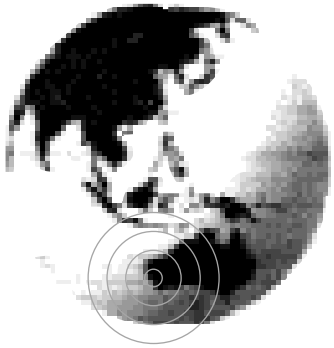
Why *no* whole virus?

Eminent AIDS-analysts, biophysicist ***ELENI ELEOPULOS*** *and colleagues, answer Peter Duesberg's criticism of their case that HIV has never been isolated*



We gratefully acknowledge Peter Duesberg's criticisms of our paper "HIV Isolation: Has it really been achieved?".[1] Before responding it will be useful to define some terms and objectives.

Virus

A virus has two distinct properties, one physical and the other behavioural. A virus is a microscopic particle able to generate exact copies of itself when placed inside a living cell, that is, the particle is infectious.

Those who espouse the viral theory of AIDS accept that a viral particle, and not a naked protein or DNA or RNA fragment, is transmitted from person to person and is both necessary and sufficient to induce the several dozen laboratory abnormalities and diseases that constitute the clinical AID syndrome.

Isolation

The essence of isolation is the separation of desired matter from all other matter not the object of concern.

Isolation of a putative viral particle is necessary to:

(a) document and analyse its constituents;
(b) conduct experiments in order to prove it is infectious and thus a virus;
(c) obtain reagents (proteins and nucleic acids) for diagnostic and other uses
(d) prove that the pathological effects, if any, are due to the virus and nothing else.

1 19 'HIV' genomes

"...the weakest point of the HIV-non-existentialists is their failure to explain the origin of "19 sequences encompassing the full-length, 10-kb-HIV-1 genome" and "19 full-length HIV genomes"".

(a) Let us repeat that the claim of the existence of "19 sequences encompassing the full length, 10-kb-HIV-I genome", "19 full-length HIV genomes" is not one of our making but that of the HIV experts we quote. The same experts accept that of the "19 full-length HIV genomes", no two are the same either in sequence or even in length;

(b) The question we set out to answer in our critique was not what is the origin of the 19 full-length HIV-1 sequences but does the presently available data prove that these sequences represent the genome of a unique, exogenous retrovirus, HIV? The answer, we repeat, is NO.

Nonetheless, although it was not our task to determine the origin of these sequences, we did present a number of alternative mechanisms that science offers as a "rational origin for such sequences" in addition to "viruses or other infectious agents".

2 Odds of assembly

"Remember the odds of coming up with even one nucleotide sequence of 9150 bp by chance are astronomically low, namely, 1 in 4^{9150} which is very close to 0."

It is apparent that we and Peter Duesberg are referring to two entirely different systems, one completely random and the other heavily biased by cell and culture conditions. True, the probability of assembling a particular sequence of RNA (DNA) of 9150 bases randomly selecting each of the four nucleotides is one in 4^{9150}. However, this statistical reasoning bears no resemblance to how nucleic acid polymers are assembled either *in vivo* or *in vitro* and thus on the probability of finding a particular unique sequence. That this is the case is best illustrated by Spiegelman's minivariant, a 220-nucleotide stretch of RNA of unique length and sequence which was discussed in our *Continuum* paper. The

probability of assembling such a unique RNA stretch by chance is 1 in 4220, also "very close to 0", yet, under certain conditions in the laboratory, the Spiegelman minivariant is frequently produced indicating that the assembly of nucleotides is anything but a random process. Furthermore, the 19 unique sequences do not have to be assembled from the four, individual nucleotides. They may result, for example, by recombination of:

(a) stretches of pre-existing cellular DNA sequences;

(b) stretches of DNA sequences of endogenous retroviruses which form 1% of the cellular DNA, a phenomenon accepted to take place quite frequently and to result in the assembly of novel genomes. It is also accepted that the conditions affect the recombination both qualitatively and qantitatively.

It is significant that as far back as 1985 both Gallo and Montagnier accepted that it is not possible to generate "HIV" and the effects attributed to it unless the cells are activated (stimulated) and that this year Chermann and his colleagues showed that the infected cultures contain fragments of the "HIV genome" but after PHA stimulation there is an increase in the "full-length genome" and a concomitant decrease in the fragments.[2] Whatever the odds may be of obtaining by chance the conditions necessary to generate "even one nucleotide sequence of 9150 bp", it is certain not 1 in 4^{9150}.

3 Viral genome

"The non-HIV-existentialists also fail to realize that available isolation efforts have already adequately identified the 9150 bases as the genome of a virus".

In our extensive search of the HIV literature we could not find even one reference, (although it is possible we may have missed some), in which the HIV genome was reported to of 9150 nucleotides. The closest length was reported Montagnier's group who, in 1984, reported it to be 9.1 to 9.2 kbases and, in 1985, as 9193 bases.[3,4] If the 9150 base DNA is the genome of a virus then an absolutely necessary but not sufficient condition is that the virus in all infected individuals will have a length of 9150 bases. Yet, two HIV genomes of the same length have yet to be reported. More importantly, the length of an RNA (DNA) fragment, no matter how often such a fragment is detected, provides no information regarding its origin. The only way to prove it belongs to a unique virus is to isolate a viral particle and demonstrate it has a genome of 9150 bases. This has not been done and the "available isolation efforts" do not contain even suggestive evidence let alone proof that a 9150 base long RNA is a constituent of a particle, any particle much less a viral particle.

4 Koch's postulate

"In order to isolate a given infectious agent, one needs no more than to isolate it from all other, possible contaminating, infectious agents, this is in fact Koch's second postulate".

At the Xth International Medical Congress held in Berlin in 1890, in response to the question as to how to obtain irrefutable proof that a bacterium caused a specific disease, Robert Koch included in his answer the requirement that "The pathogen must be isolated and bred in adequate numbers in pure culture."[5] Apart from omitting the second part, "bred in adequate numbers in pure culture", Peter Duesberg's definition of isolation is somewhat obscure. Can a physician for example, claim to have isolated his patient with hepatitis by placing him in a room with patients who may have coronary artery disease, fractures or appendicitis, but none of whom have infectious diseases? In fact, in 1987,[6] Peter Duesberg himself defined the second Koch postulate as, "it [the pathogen] must be isolated from the host and grown in pure culture", that is, in the absence of "all other, possible contaminating" agents including non-infectious agents.

5 Re-isolating 'HIV'

"Montagnier's original isolate of HIV from extracellular fluids is an example. Indeed, Montagnier's isolate appears to meet functional standards of isolation adequately, because two of the world's leading retrovirologists, Robert Gallo of the NIH and Robin Weiss of the Chester Beatty have re-isolated only HIV from Montagnier's virus stock. If Montagnier's virus had been grossly contaminated by other viruses or microbes those would have been found by Gallo and Weiss".

There is no evidence in Montagnier's "original isolate" which proves isolation of a virus no matter how liberal a definition one applies to the word "isolation". As far as "functional isolation" is concerned, suffice it to say:

(a) In 1983, like Gallo in 1984, Montagnier reported HIV as a "typical type-C RNA tumor virus"[7] having a characteristic "cylindrical core".[8] By 1985 it was reported that the nucleotide sequences between Montagnier's first HIV isolate, LAV-1 BRU and Gallo's first isolate, HTLV-IIIB, "differ by less than 1% overall".[9] Even though Montagnier had sent supernatant(s) from LAV-1 "infected" culture(s) to the Gallo laboratory, "with the express understanding that it could be used for biomedical, biological and molecular biological studies", neither Montagnier nor Wain-Hobson considered such differences as proving Gallo's HTLV-IIIB was LAV-1 BRU and in February 1986 wrote, "Thus there is only a single AIDS retrovirus, and LAV, HTLV-III and ARV represent different isolates of the same virus" (italics ours).[9]

Indeed, if there "is only a single AIDS retrovirus", a unique retrovirus, then genomic differences of "less than 1%" should be the rule, not the exception. However, unexpectedly, not long afterwards it was discovered that "If you were to test two HIV-positive people at random and analyse the genetic material of their strains, they would differ, on average, by about 13 per cent".[10] As

a result, the French accused Gallo of misappropriating their strain which they had sent to him in 1983. In other words, Gallo's isolate of HTLV-IIIB was not a "different isolate" of HIV but LAV-1 BRU which Gallo transmitted to the permanent cell line HT (HUT78). At the same time they suggested that HIV-1 including their LAV-1 BRU is not a "typical type-C RNA tumor virus" but "possibly a lentivirus", that is, a taxonomically distinct retrovirus containing a conical core. Although there was no proof, this suggestion was soon accepted as fact by almost everybody apart from Gallo's group which for many years insisted that HIV belonged to the same family as HTLV-I and that it is a type-C particle. Furthermore, as already mentioned, the length of LAV-1 BRU was reported to be 9.1 to 9.2 kb (9193) while that of HTLV-IIIB as 9749.[11] By 1991, Gallo et al including Chermann presented evidence including sequence analysis, which showed "that Gallo's IIIB did not come from the Pasteur Institute".[10, 12]

(b) In January 1991 Weiss stated that he "cannot exclude the possibility" that his isolate, CBL1, is either Montagnier's LAV-1 BRU or Gallo's HTLV-IIIB. The reasons given were:

(i) nucleotide sequences representing 2,443 nucleotides (one quarter of the "HIV genome") in env, tat, and nef, showed that CBL1 "has 98.0% amino acids in common with LAV-1 BRU and 97.8% with HTLV- IIIB (BH10 clone), whereas the similarity in the same regions between BH10 and BRU is 98.3%. The tat sequence was most variable, with 94.2% of the CBL1 sequences identical to both BRU and BH10";[13]

(ii) "...monoclonal antibodies raised against CBL1 gag proteins do not distinguish between CBL1, BRU and IIIB".[13]

However:

(i) the genomic differences reported by Weiss are greater than "the less than 1%" differences reported between LAV- 1 BRU and HTLV-IIIB;

(ii) should not the antibodies raised against one strain of HIV react with all the other strains? If different strains of HIV can be distinguished by an antibody test then how can one perform HIV antibody tests without having an antibody test for each strain?

(iii) a few months later other British researchers reported that "CBL-1 and HTLV-IIIB show striking differences in their biological properties".[14]

Given the above data it is not possible to claim that Gallo and Weiss re-isolated Montagnier's virus. In fact, the groups do not agree between themselves as to the crucial questions of which samples were given and received, and even less as to which samples were sequenced.[10,12]

In addition, there are two basic scientific reasons which make it impossible for Gallo and Weiss to "have re-isolated only IIV from Montagnier's virus stock":

(i) To isolate HTLV-IIIB Gallo used the H9 (HUT78) cell line. However, evidence exists that the H9 cell line releases retrovirus-like particles even when not "infected with HIV''.[15] Furthermore, the HUT78 cell line was established from a patient with "malignancies of mature T4 cells" , a disease which, according to Gallo, is caused by the exogenous retrovirus, HTLV-I. Indeed, as far back as 1983, he claimed to have shown that the HUT78 cell line contained HTLV-I proviral sequences.[16] Weiss obtained his "CBL-I material" from the leukaemic cell line CEM, a cell line shown to harbor retrovirus even when not infected with "HIV".[17]

(ii) One aspect on which all HIV experts concur is that gpl20 is indispensable for HIV infectivity. Suffice it to quote from Jay Levy's and Wain-Hobson's most recent papers. "The likely steps in HIV infection are as follows. The CD4-binding site on HIV-1 gpl20 interacts with the CD4 molecule on the cell surface. Conformational changes in both the viral envelope and the CD4 receptor permit the binding of gpl20 to another cell-surface receptor, such as CCR5. This second attachment brings the viral envelope closer to the cell surface, allowing interaction between gp41 on the viral envelope and a fusion domain on the cell surface. HIV fuses with the cell. Subsequently, the viral nucleoid enters into the cell, most likely by means of other cellular events. Once this stage is achieved, the cycle of viral replication begins"[18] (italics ours). "HIV-encoded gpl20 recognizes the host-encoded CD4 receptor. This interaction leads to a conformational chage in gpl20, allowing it to bind to a second receptor, CCR-5...At some point to be defined, the amino acid terminus of gp41 is uncovered allowing penetration of the host cell membrane and fusion of the viral and host cell membranes. Stripped of its lipid protection, the capsid complex moves into the cytoplasm, and reverse transcription is initiated''.[19]

We could find no data regarding the type of "material" Weiss received from Montagnier. The samples received by Gallo "were cell-free supernatants of LAV cultures". However, as Hans Gelderblom and others have attested, cell-free viral particles do not contain knobs, (spikes), that is, gpl20.[20, 21] This makes it impossible not only for Gallo to "have re-isolated only" HIV-I BRU but even to have transmitted it to his cultures.
Given the facts that:

(i) AIDS patients and those at risk are diagnosed as infected with many agents. These include cytomegalic inclusion virus, Epstein- Barr virus, herpes simplex virus and Hepatitis B virus. The latter is present not only in hepato-cytes but like, "HIV", also in T-lymphocytes.[22, 23] It is also accepted that most cultures contain Mycoplasma;[29]

(ii) To "infect" their cultures, Montagnier, Gallo and Weiss did not use "pure HIV" or even the material which from the cultures banded in sucrose density gradients at 1.16 gm/ml, but "cell-free" culture fluids;

it would have been a miracle, if they had looked, for "Montagnier's virus" to have not "been grossly contaminated by other viruses or microbes" and for Gallo and Weiss not to have found such agents irrespective of which strain of "HIV", Montagnier's or theirs, they had "re-isolated".

6 All cells have RNA

"...viruses can also be isolated as infectious nucleic acids from infected cells".

Viruses are not mere nucleic acids. Neither can the introduction of nucleic acids into cells and their reproduction be considered as proof for viral infection. If:

(a) one starts with a presumption, but no proof, that a cell is infected with a unique retrovirus;

(b) chooses from its RNA a fragment of arbitrary length, and calls it retroviral RNA

(c) inserts the RNA (cDNA)into a cell and reproduces the same RNA (cDNA) and interprets this as infection;

(d) construes (a)-(c) as proof of isolation of a unique retrovirus;

then, given the fact that the same steps can be achieved with any cellular RNA (DNA), one would have no choice but to consider every single fragment of cellular RNA (DNA) as retroviral, and that all cells are nothing more than an assembly of retroviruses.

7 Others

"...such infectious nucleic acids initiate replication of virus in uninfected cells from which new virus particles are subsequently released".

This may be the case with the genome of other infectious agents but this has never been shown for the genome of HIV.

8 Cloning

"...infectious HIV DNA has been isolated from infected cells several times by molecular cloning".

This matter has been discussed at length in our Continuum paper. Suffice here to stress two points:

Retroviruses are not "cloned, infectious HIV DNA of 9150 bases" but "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)".[25] Furthermore, such particles share the physical property of banding at a density of 1.16 gm/ml in sucrose density gradients, a fact long used in their isolation . Cloning of a virus is defined as obtaining EXACTLY the same virus by introducing its genome into a cell. However, to date, nobody has reported such particles by "cloning, infectious HIV DNA of 9150 bases", or DNA of any other length. In fact, nowhere in the HIV literature can one find particles which have "a diameter of 100-120 nm" AND which are "studded with projections (spikes, knobs)", let alone such particles banding at 1.16 gm/ml in sucrose density gradients. Since cloning is a process leading to the production of an exact copy of whatever object one starts with, how can one claim cloning of something before there is proof that it ever existed?

SUMMARY

What does one have to do and how hard does one have to plead in order to obtain answers to fundamental questions regarding a retrovirus which has menaced the world and in whose name hundreds of thousands of people have died or been poisoned?

For example:
1. How is it possible to transmit a cell-free retrovirus, "HIV", when it is accepted that:

(i) gp 120 is absolutely necessary for the virus to enter the cell and for the "cycle of viral replication to begin";

(ii) to date nobody has reported the existence of cell-free particles with the dimensions of retroviral particles possessing knobs, that is, gp 120?

2. How can one claim that AIDS patients and those at risk are infected with a unique retrovirus, HIV, when to date nobody has even reported in fresh, cultured tissue, or tissue co-cultures, particles fulfilling the principal morphological and physical characteristics of retroviral particles?

We agree with Peter Duesberg that "the cause that unites us all" is finding a solution to AIDS. With this our aim we were among the first to put forward non-infectious factors as agents to explain AIDS in gay men and furthermore we were the first to propose a non-infectious theory with a unifying mechanism to explain the development of AIDS in all risk groups.[26] Indeed, our theory also predicts a non-infectious explanation for the phenomena which others have inferred as "isolation" of a novel retrovirus from AIDS patients. However, once HIV was accepted as the causative agent, we realised that the single most important obstacle in finding the explanation for AIDS is the belief in HIV. For this reason, from the beginning and unlike anybody else, we have critically analysed the data which claim proof for the existence of a unique, exogenous retrovirus, HIV, in AIDS patients and have always maintained that no such proof exists.

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