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## "HIV" genome, clones and sequences

We agree with Brian Foley who wrote in his rapid response entitled "Re: A further plea for references on HIV purification" (10 July 2003) that "A viral genome can indeed be cloned, sequenced and studied independent of any 100% pure preparation of viral particles". However, if you want to claim cloning of a new, unique retrovirus X then you must have beforehand its genome which at present can only be obtained by purifying the retrovirus X.

Brian Foley then wrote: "When asked to name one virus that has ever met their conditions, they listed two (the Bryan high titer strain of Rous sarcoma virus, and the Moloney Murine Sarcoma Virus) which are mixtures of two or more viruses and thus have not been "purified and characterized" by the Perth group's criteria".

Regarding "the Perth group's criteria", let us repeat what we wrote in our rapid response "A plea for the references on HIV purification" (3 July 2003) which reads: "Let us be very clear on this matter. These "requirements" and "restrictions" which are really a common sense approach were formulated by virologists such as Barre-Sinoussi, Chermann, (2) Crawford, (3) Toplin (4) and are consequences of the very nature of retroviruses. Since retrovirus-like particles are ubiquitous in nature it follows that you must prove that the particles in question: (i) are indeed viral particles, that is, that they are infectious; (ii) have reverse transcriptase and RNA but not DNA; (iii) if they represent a unique retrovirus, they must have unique RNA and proteins". The first and absolutely necessary step to prove this is to purify the particles. These are the criteria which Montagnier's group and Gallo's group claimed to have fulfilled in proving the existence of HIV.

The aim of Sinoussi et al and Crawford et al was not to characterize the viruses (which were well known at that time) but to use the density gradient method to purify the viruses. Again, let us repeat what we wrote in our rapid response "A plea for the references on HIV purification (3 July 2003) which reads: "We would be grateful to Brian Foley if he can provide us with references showing HIV-1 preparations in which "No apparent differences in physical appearance could be discerned among the viral particles in these regions" as Sinoussi et al. did and with "a high degree of homogeneity" and "virtual absence of DNA" as Crawford et al did. Then and only then can we proceed and characterize the viral particles." We are still waiting for a reply.

We are truly amazed by Brian Foley's logic in his statement: "If it is indeed a "fact" that a virus must be purified before it can be cloned, then the dozens of infectious molecular clones of HIV-1 that have been cloned, sequenced and studied would be de facto evidence that HIV has indeed been purified".

Where is the published evidence of HIV purification and where is the evidence that the clones are indeed those of HIV?

Brian Foley wrote: "Most of the early infectious molecular clones of HIV-1 were created from the proviral DNA stage of the viral life cycle, and thus infected cells and not free viral particles were the source of the material...Isolation of 100% pure viral particles by centrifugation or any other method is NOT a necessary step in cloning a retrovirus or any other virus".

As we have repeatedly said, to claim proof for molecular cloning of HIV it is absolutely necessary to have proof that the molecule originated from an HIV particle. Without the proof that the genome originated from a HIV particle(s) it is impossible to prove the existence of HIV "proviral DNA".

Let us remind Brian Foley how the first and still some of the best known "HIV" clones (pLAV13; pLAV75; pLAV82; IHXB-2; IHXB-3; IBH5; IBH8 and IBH10) were obtained.(1) (2) (3) (4) All were obtained in a similar way:

"To clone the HIV genome, we isolated unintegrated viral DNA [in fact, all the extra-chromosomal DNA present in the cells

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or in other cases the DNA present in cells] after acute infection of H9 cells with concentrated HTLV-III [after culturing the H9 cells with supernatant from co-cultures containing tissues derived from AIDS patients] and cloned this DNA into a  $\lambda$  phage library to be screened with viral cDNA...extra-chromosomal DNA was extracted according to a procedure of Hirt and assayed for its content of unintegrated viral DNA using HTLV-III cDNA as a probe. The synthesis of this cDNA was primed with oligo(dT) and reverse-transcribed from poly(A)-containing RNA of virions that had been banded twice on sucrose density gradients".(3) In other words:

- (i) Montagnier and Gallo obtained the "HIV" genome by "purifying" the "virus" in sucrose density gradients;
- (ii) The poly(A)-RNA which banded at the density of 1.16g/ml, (the "purified" virus) was defined as "HIV" genome. All the "HIV" clones derived from this poly(A)-RNA.

However:

- (i) Neither Montagnier nor Gallo published electron micrographic proof for purification. Furthermore, according to Montagnier, neither he nor Gallo has purified HIV, as we have repeatedly mentioned,. The only electron micrographs published to date are those of Bess et al (5) and Gluschkof et al (6). Although they spared no effort their "purified" HIV consisted mainly of cellular fragments and some particles which they called "HIV" but not one of which had all the morphological characteristics attributed to it;
- (ii) In 1972 Gallo and his colleagues showed that retrovirus genomes contain poly(A) regions and concluded that poly(A)-RNA may be a diagnostic property of retroviruses.(7) However, at present as well as in 1972, evidence existed (of which Gallo was aware, (7)) which shows that poly(A) sequences are present in all cells as well as viruses other than retroviruses and in fungi.(8)
- (iii) Neither Montagnier nor Gallo had proper controls, that is banded material originating from cultures, which, with one exception (instead of containing material derived from AIDS patients they should have material derived from non-AIDS patients) were treated in exactly the same manner as the cultures from which they obtained the "HIV" poly(A)-RNA (the "HIV genome"). In fact they had no controls at all. Bess et al (5) did have controls and showed that the banded cellular microvesicles also had poly(A)-RNA.

Given the above data, any scientist, including Brian Foley and us, must conclude that at present there is no proof for the existence of the "HIV" genome and thus of "HIV" clones and "HIV" sequences.

Brian Foley wrote: "When Dolly the lamb was cloned, her genome was never "isolated" it was always mixed together with chromosomal proteins nuclear membrane proteins, and many other macromolecules as well as nucleotides and water and hundreds of other smaller molecules. The "proof" of her cloning cannot be made by any claims of 100% pure sheep DNA or anything like that". In fact, when Dolly the sheep was cloned, there was absolute evidence that the DNA came exclusively from the parent sheep and not from any other animal or even any other sheep. There was no other DNA but the DNA originating from the parent sheep. Put simply, DNA from a sheep is sheep DNA. DNA from a retrovirus is retroviral DNA.

Brian Foley wrote: "Anyone can make such claims. The proof needs to come from examining the DNA of the allegedly cloned sheep and showing that it is identical (or very nearly identical) to the alleged parental clone". We are extremely pleased with this statement and totally agree. This is what we have been saying all along regarding HIV cloning. The question is why does Brian Foley consider this absolutely necessary for sheep but not for HIV?

Brian Foley wrote: "A molecular clone of a virus, whether it is a complete genome or only a fraction of one, can be sequenced to tell us exactly what virus the clone is derived from. An infectious molecular clone tells us not only what virus it is derived from, but also that the clone is viable and can produce progeny virions". You can sequence any fragment of nucleic acid and you can clone them. But how do you know what virus the clone is derived from if you don't know

beforehand what the viral genome is, that is, that the genome originated from the virus particles?

Brian Foley wrote: "In the case of human viruses it is not considered ethical to inject the progeny virions nor the cloned infectious DNA into a group of humans to prove that the clone produces disease in these medical "volunteers", so we can only test them in cell cultures to show that they produce similar pathologies in the cells (some strains of HIV-1 M group viruses induce syncytia in T-cell cultures, others do not, for example) and that the progeny virions have the properties of the parent (for example antibodies harvested from the infected patient from which the virus was derived, and from other patients infected with similar strains of HIV-1 M group virus bind to the progeny virus)".

The causative role of an infectious agent cannot be determined by the use of cell cultures but by the use of animal models. In fact this was the initial condition put by Montagnier to prove the causative role of HIV in AIDS. Unless you have proof that the antibodies are specific (which we have been requesting from the beginning), you cannot talk about HIV antibodies and you cannot use these antibodies to support any argument. Most importantly, before you can talk about the diseases which an agent can cause it is absolutely necessary to have proof the agent exists.

Brian Foley wrote: "The sequences of the genomes (or fractions thereof) of any organism from viruses to bacteria to mammals, can tell us not only about the organism the DNA was directly derived from, but also about the relationship of that parent to other organisms". Sequences in a fragment of RNA or DNA can tell you no such things unless you first have proof of the existence of the genome of the given organism, that is, the genome and thus the sequences originated from that organism.

Brian Foley wrote: "If we look at a gorilla, a human and a chimpanzee for example we cannot tell if the human is more closely related to the chimpanzee or the gorilla. However if we sequence almost any small region (say 10 kilobases) of the genomes of the 3 organisms we can see that the chimpanzee and human DNA are a bit more similar to each other than the gorilla is to either the chimp or the human". We agree that if you have the genomes then by sequencing you can tell the relationship between them.

Brian Foley wrote: "Likewise with viral DNA or cDNA sequences, we can tell the relationships of the viruses of the HIV-1 M group to each other, to the viruses of the HIV-1 O and N groups, and to the various SIVs that have been sequenced to date."

No such thing can be done unless you first have proof that the RNAs from which the cDNAs were derived originated from HIV particles. The difference between the sequences of the chimpanzee genome and the human genome is less than 2%, while the genomes of RNA viruses do not differ by more than 1% and even this 1% sequence differences are considered to represent "extreme variability". (9) For example, "The type 3 Sabin poliovirus vaccine differed from its neurovirulent progenitor at only 10 nucleotide positions after 53 *in vitro* and 21 *in vivo* passages in monkey tissues. In 1977, H1N1 influenza A virus reappeared in the human population after 27 years of dormancy with sequences mainly identical to those of the 1950s virus". How then does Brian Foley explain that differences of up to 40% as is the case in the "HIV" sequences (10) (11) represent the genome of one and the same object? In other words, if a mere difference of less than 2% leads to the appearance of two totally different objects (namely, humans and chimpanzees), how then can differences of up to 40% lead to the appearance of the same object (namely "HIV")? Indeed, if the sequence variation was the only fact one knew about these nucleic acids sequences the only conclusion one could come to is they are not the nucleic acids of a retrovirus or indeed any virus. Or, as Peter Duesberg has so aptly stated, "there is a range, a small range, in which you can mutate around without too much penalty, but as soon as you exceed it you are gone, and you are not HIV any longer, or a human any longer...then you are either dead or you are a monkey, or what have you".

Brian Foley wrote: "If the Perth group thinks that there is some problem with the theory that HIV-1 and HIV-2 cause immune deficiency in humans, perhaps they should try to explain why the detection and sequencing of HIV-1 M group genomes in humans so very well correlates with the detection of immune deficiency diseases in only infected and not non-infected people."

If the HIV genome has been proven to exist and if AIDS is caused by HIV and if in AIDS patients there is a very high concentration of HIV (as it is claimed) then it should be very easy to detect the HIV genome by standard hybridization techniques. For some unknown reason Montagnier's group did not report such data. Gallo's group did and they found the hybridization bands to be very faint. (4) Their explanation was either the HIV concentration was very low or that the results may be due to non-specific hybridization with nucleic acids of other retroviruses such as HTLV-I or HTLV-II. However, by 1994 Gallo admitted "We have never found HIV DNA in the tumour cells of KS...In fact we have never found HIV DNA in T-cells".(12). The results of other researchers was similar to that of Gallo.

There have been many reports of detection of the "HIV" genome using PCR. There are numerous problems with this technique. For example, the specificity of PCR has never been determined using a proper gold standard. Even when a totally unsuitable gold standard such as the antibody test is used, the specificity of PCR varies from 0 to 100% leading researchers from different institutes in the USA to conclude "Our investigation produced two main findings. First, the false-positive and false-negative rates of PCR that we determined are too high to warrant a broader role for PCR in either routine screening or in the confirmation of diagnosis of HIV infection. This conclusion is true even for the results reported from more recent, high-quality studies that used commercially available, standardized PCR assays...We did not find evidence that the performance of PCR improved over time". (13) This means that Brian Foley's claim that "the detection and sequencing of HIV-1 M group genomes in humans so very well correlates with the detection of immune deficiency diseases in only infected and not non-infected people" is totally unsubstantiated.

Brian Foley wrote: "Not only does the pattern of epidemic spread of these viral genomes precisely coincide with the pattern of immune deficiency on a global scale (for example sequences of HIV-1 M group CRF01\_AE virus appeared in Thailand coincident with the AIDS epidemic in Thailand) but it also coincides on local scales (for example the people infected with nef gene defective virus from transfusion in Australia survived longer than most people infected with viruses that have all genes functional)".

It is not possible to claim that the "pattern of epidemic spread of these viral genomes precisely coincide with the pattern of immune deficiency on a global scale" including Thailand unless there is proof from blind control studies that the "HIV" genome is present only in AIDS patients and not in other patients now or prior to the "epidemic" era. But since the specificity of the tests for the "HIV" genome varies from 0 to 100%, it means that it is unlikely that such correlation exists.

The Sydney cohort consist of AIDS patients who are said to have been infected from transfusion with platelets (one patient) or erythrocytes (7 patients) from an "infected" gay man between January 1981 and July 1984. The donor and six patients were reported in 1992 (14) and the other two patients in a 1999 update. (15) The many problems associated with this cohort include the following:

- (i) One of the patients had systemic lupus erythematosus diagnosed in 1982 (before the transfusion). In April 1987, nine days before death, "She was positive to antibodies to HIV". Since this patient: (a) was treated with cyclophosphamide, azathioprine, hydrocortisone and continuously with prednisone; (b) had lupus, salmonella bacteraemia, extensive herpes simplex virus lesions, staphylococcal pneumonitis and pneumococcal pneumonia; (c) these drugs and diseases are all immunosuppressive and the diseases may also result in high levels of potentially cross-reactive antibodies; she could have had a positive antibody test and developed AIDS (*Pneumocystis carinii* pneumonia) in the absence of "HIV".
- (ii) The "first HIV serological result" in one of the "infected" patients "on February 2, 1996, was weakly positive according to enzyme-linked immunosorbent assay with an indeterminate Western blot, and subsequent results have been similar."
- (iii) Up to 1992, "Virus was isolated from PBMC obtained by Ficol/Hypaque separation from heparinised blood. PBMC ( $3 \times 10^6$ /ml) were mixed with an equal number of phytohaemagglutinin-stimulated PBMC (3  $\mu$ g/ml) from HIV-1 seronegative donors. The cultures were maintained in...and interleukin-2...and the release of p24 core antigen into

the supernatants was monitored every 3 days by a standard assay". Positive results were obtained only from one patient in December 1990. By 1998 the isolation procedure was modified as follows: "peripheral-blood mononuclear cells from selected donors were phytohemagglutinin-activated and then cocultured with fresh peripheral-blood mononuclear cells from the Sydney Blood Bank Cohort...and 20 percent of the cell population was treated on day 0 with ultraviolet irradiation. Viral replication was quantified by extracellular soluble p24 production". They reported that "HIV-1 has been isolated from cultures of peripheral-blood mononuclear cells from five members of the Sydney Blood Bank Cohort". Since a reaction between antibodies to the "HIV" p24 and antigens present in the cell culture are totally non-specific (16) and since controls were not used, similar success of "HIV" "isolation " may have been obtained in a randomly selected cohort of 9 healthy blood donors.

- (iv) "The *nef*-LTR region of the HIV-1 provirus was amplified by triple-nested or booster polymerase chain reaction (PCR) from genomic DNA extracted from peripheral-blood mononuclear cells". Since there is no proof that the primers and probes used originated from an HIV particle and since the PCR is non-specific and no controls were used, similar success of *nef* detection may have been obtained in a randomly selected cohort of 9 healthy blood donors.
- (v) According to the authors of the Sydney Cohort study "Since the *nef* gene has been clearly established as a cause of slowly progressive SIV infection in macaques, it seems likely that the *nef* gene deletion is the principal cause of the attenuation of the Sydney Blood Bank Cohort HIV". (Only three of the nine patients in the cohort have died, two of causes unrelated to "HIV infection" and one, the patient with lupus, "of causes possibly related to HIV".) In the early 1990s two papers were published on the importance of the SIV *nef* gene in pathogenesis including one by Kestler et al (17) who concluded: "Our results indicate that *nef* is a critical component of the virus ability to induce AIDS". However, this claim was not confirmed by other researchers including Baba et al (18) who concluded that because *nef* deleted "constructs have retained their pathogenic potential, they should not be used as candidate live, attenuated virus vaccines against human AIDS". In fact, according to Gallo (19) HIV can induce AIDS even if it has no genome at all.

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