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A critique of the Montagnier evidence for the HIV/AIDS hypothesis

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Summary In 1983 Luc Montagnier and his colleagues claimed to have discovered a novel retrovirus presently known as human immunodeficiency virus (HIV). By 1984 HIV was almost universally accepted to be the cause of AIDS. However, 20 years later, HIV cannot account for the phenomena for which the retroviral hypothesis was proposed, namely, Kaposi's sarcoma, decrease in T4 lymphocytes and thus the opportunistic infections in AIDS patients which were assumed to be the direct results of this decrease. Agents other than HIV to which patients belonging to the AIDS risk groups are exposed cause decrease in T4 cells. Neither have the main predictions of the HIV hypothesis been fulfilled. HIV seropositivity in the developed countries still remains restricted to the original high risk groups, no HIV vaccine exists, and no successful animal model has been developed. In this communication, we critically analyse the evidence which in 1983 was claimed to prove the existence of HIV. The phenomena which Montagnier and his colleagues considered proof for the existence of HIV are detection of reverse transcriptase activity; the presence of retrovirus-like particles in the culture; immunological reactivity between proteins from the culture supernatant which, in sucrose density gradients, banded at the density of 1.16 g/ml ("purified virus") and antibodies in a patient's (BRU) serum. Reverse transcriptase activity can be found in viruses other than retroviruses and in all normal cells. Reverse transcription can be brought about not only by the enzyme reverse transcriptase but also by normal, cellular DNA polymerases. Retrovirus-like particles are ubiquitous in cultures not infected with retroviruses, especially in conditions employed by Montagnier et al. From the reaction between proteins in the "purified virus" and antibodies in the patient serum Montagnier concluded that the proteins were HIV proteins and the antibodies were HIV antibodies. Since all

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antibodies are polyspecific, from such a reaction it is not possible to define the origin of even one reactant let alone both. Even if this were possible, since Montagnier's "purified virus" did not contain particles with the "morphology typical of retroviruses", the proteins cannot be retroviral. We conclude that, these phenomena are non-specific to retroviruses and thus cannot be considered proof for the existence of a unique retrovirus HIV.

At the beginning of the AIDS era a small number of researchers hypothesised that the cause of the syndrome may be a retrovirus. In a recent commentary Luc Montagnier and Robert Gallo stated that "a clear-cut isolate" was obtained by Luc Montagnier and his colleagues in early 1983 [1]. By 1984, this "isolate" which later became known as human immunodeficiency virus (HIV), was accepted as the causative agent of AIDS. It is also accepted that HIV is a retrovirus, that is, a virus which has an enzyme, reverse transcriptase [RT].

The whole purpose of a hypothesis is to explain observations and to make predictions. The observations the HIV hypothesis was proposed to explain were threefold. The high frequency of a malignancy, Kaposi's sarcoma (KS), a few opportunistic infections (OI) and a decrease in a specific cell type, T4 lymphocytes, whose numbers are determined by the binding of antibodies. Because, no other infectious agent causes such a diverse number of diseases, it was hypothesised that HIV causes the syndrome indirectly. That is, HIV kills the T4 cells and the decrease in T4 cells (immune deficiency) leads to the appearance of the diseases which constitute the clinical syndrome. The decrease in T4 cells was considered the "hallmark" of HIV infection and AIDS [2,3].

However, in regard to haemophilia patients, as far back as 1985 some of the best known British researchers including Robin Weiss concluded: "It is commonly assumed that the reduction in T-helper-cell numbers [T4 cells] is a result of the HTLV-III virus [HIV] being tropic for T-helper-cells. Our finding in this study that T-helper-cell numbers and the helper/suppressor ratio did not change after infection supports our previous conclusion that the abnormal T-lymphocyte subsets are a result of the intravenous infusion of factor VIII concentrates per se, not HTLV-III infection" [4]. One year later researchers from the CDC wrote: "Haemophiliacs with immune abnormalities may not necessarily be infected with HTLV-III/LAV, since factor concentrate itself may be immunosuppressive even when produced from a population of donors not at risk for AIDS" [5].

Gay men are exposed to many immunosuppressive agents [6—8] including semen and drugs. Semen is immunosuppressive, induces programmed cell death, is mitogenic and carcinogenic [9—15].

Studies conducted in drug users show that the decrease in T4 cells precedes a positive antibody test ("HIV infection") and not vice versa, that is, the effect precedes the cause. In one study in drug users "The relative risk for seroconversion among subjects with one or more CD4 count <500 cells/μl compared with HIV-negative subjects with all counts >500 cells/μl was 4.53" [16]. In another study, "low number of T4 cells was the highest risk factor for HIV infection" [17].

That the supposed effect, immunosuppression, precedes the cause, that is, HIV infection, was recognised by Montagnier as long ago as 1985: "This syndrome [KS and OI] occurs in a minority of infected persons, who generally have in common a past of antigenic stimulation and of immune depression before LAV [HIV] infection" [18]. Most importantly, although no effort has been spared, to date nobody has proved that HIV kills the T4 cells either directly or indirectly or that it decreases their numbers by any other means such as "down regulation" of the CD4 receptor [19]. Since, according to the HIV hypothesis of AIDS the OI are the direct result of the T4 cell killing by HIV and, since such proof does not exist, the HIV hypothesis cannot account either for the decrease in T4 cells or the OIs.

At present it is accepted that HIV plays no role, either directly or indirectly, in the causation of KS [20,21].

The HIV theory predicted that HIV was sexually transmitted and therefore AIDS would spread throughout the heterosexual population. This has not occured. In fact data from the largest, longest, best designed and executed studies available conducted in the USA and Africa show that HIV is not heterosexually transmitted [22—25].

The prediction by proponents of the HIV hypothesis that a vaccine would be developed by 1986 also has not been fulfilled [26]. In 1984 Montagnier said that the only way to prove HIV is the cause of AIDS is to have an animal model [27]. Although no effort has been spared no model of a retrovirus causing AIDS has been forthcoming.

Since after 20 years the HIV hypothesis cannot explain the three phenomena for which it was put forward and its main predictions have not been fulfilled then it should be abandoned or at least reappraised. In our view the reappraisal should begin with HIV.

In a 1983 paper [28] published in *Science* Montagnier and his colleagues presented three lines of evidence which, according to them, proved the existence of a new human exogenous retrovirus presently known as human immunodeficiency virus, HIV. These were detection of: (i) reverse transcriptase activity; (ii) retrovirus-like particles; (iii) immunological reactivity between proteins from the culture supernatant which in sucrose density gradients banded at the density of 1.16 g/ml ("purified virus") and antibodies in a patient's (BRU) serum.

The detection of RT activity (reverse transcription of the synthetic template-primer $An \cdot dT_{15}$) in the stimulated cell culture of BRU was considered proof for virus isolation. Detection of the same activity in a co-culture of the cells from BRU with T-lymphocytes from a healthy donor was considered proof for virus transmission. However, 10 years earlier Francois Barré-Sinoussi and Jean Claude Chermann [29], the principal and second authors of the *Science* paper, were aware that RT is not specific to retroviruses and can be found even in normal cells. In the early 1970s Gallo and his associates proved that cultures of leukaemic cells transcribe the $An \cdot dT_{15}$ template-primer as does material banding at 1.16 g/ml originating from "PHA stimulated (but not unstimulated) normal human blood lymphocytes" [30]. In 1975 an International Conference on eukaryotic DNA polymerases defined DNA polymerase γ as the cellular enzyme which "copies $An \cdot dT_{15}$ with high efficiency but does not copy DNA well" [31]. By 1984 Montagnier and his colleagues were aware that in the late 1970s there was evidence that "Among a number of template primers, $(rA)n \cdot (dT)_{12-18}$ has been most frequently employed since RT shows high activity with this template primer. However, the problem is that the cellular DNA polymerases (pol, β and γ) also effectively utilise the same template primer" [32,33]. In 1984 Montagnier and his associates themselves showed that the DNA polymerases of normal "non infected cells" transcribe $An \cdot dT_{15}$ [33]. Nowadays the non-specificity of RT is known even to the general public in the form of reports in share market magazines concerning biotechnology stocks [34]. Since RT is not specific to retroviruses and since $An \cdot dT_{15}$ can be transcribed by other cellular enzymes (β and γ), transcription of the template-primer $An \cdot dT_{15}$ cannot be considered proof for the detection of a retrovirus. Neither can its transcription in two consecutive cultures be considered proof for transmission and isolation of a retrovirus [35].

In the same experiment, stimulated umbilical cord lymphocytes were cultured with supernatant from the co-culture. Montagnier and his colleagues reported that electron microscopy of the "umbilical cord lymphocytes showed characteristic immature particles with dense crescent (C-type) budding at the plasma membrane... This virus is a typical type-C RNA tumor virus". However, in 1984 they reported HIV to be a type-D retrovirus [36] and later claimed that HIV is a lentivirus. These taxonomical differences imply that if HIV were a newly discovered mammal it could be either human, a gorilla or an orang-utan. Before the AIDS era it was known that retrovirus-like particles are ubiquitous [37,38] including "in the majority, if not all, human placentas" [39]. Since, as Gallo pointed out in 1976 they do not replicate, the majority of retrovirus-like particles are not retroviruses [40]. In 1976 George Todaro wrote: "We emphasise that the failure to isolate endogenous viruses from certain species may reflect the limitations of in vitro cocultivation techniques" [41]. Retrovirus-like particles have been seen in many non-infected cell lines used for "HIV isolation" including cord blood lymphocytes [42]. In the only electron microscopy study, either in vivo or in vitro in which suitable controls were used and in which extensive blind examination of controls and test material was performed, virus particles indistinguishable from "HIV" were found in 18/20 (90%) of AIDS as well as in 13/15 (88%) of non-AIDS related lymph node enlargements [43].

In the *Science* 1983 paper Montagnier and his associates wrote: "That this new isolate was a retrovirus was further indicated by its density in a sucrose gradient, which was 1.16". They claimed that the 1.16 g/ml band represented "purified, labelled virus", but did not publish electron micrographs to prove this or that the particles seen in the culture banded at 1.16 g/ml and were present even in an impure form. In a 1997 interview Montagnier gave to the French journalist Djamel Tahi he said no electron micrographs of the 1.16 g/ml band, the "purified" virus, were published because even after "a Roman effort" they could not find particles with "the morphology typical of retroviruses" [44].

Since: (a) the finding of retrovirus-like particles especially in cultures and under the conditions used by Montagnier and his associates is not unusual; (b) the particles published in the 1983 paper: (i) did

not have the morphological characteristics presently attributed to lentiviruses (HIV), that is, "relatively homogenous diameter of about 110 nm, the dense cone-shaped core and the "lateral bodies", and in fact they were classified as "typical type-C" particles; (ii) did not have the principle physical characteristic of retroviruses, that is, in sucrose density gradients they did not band at the density of 1.16 g/ml; (iii) were not proven infectious. (The finding of RT activity even in unlimited numbers of cultures cannot be considered proof for infectivity); (c) Montagnier et al. had no controls and the experiment was not blind; — it is difficult to accept the claim that the particles seen in the 1983 study were a unique human lentivirus HIV [35] or even retroviral.

"When purified, labelled virus" was incubated with the patient's serum Montagnier and his colleagues found three proteins in the 1.16 g/ml band — p80, p45 (now called p41) and p25 (now called p24) that reacted with antibodies present in the serum. They concluded that p25 (p24) was an HIV protein and the antibodies which reacted with it, HIV antibodies. However, (i) if such a conclusion can be drawn from this reaction then p41 and p80 should also be HIV proteins (not cellular proteins as they stated) and the antibodies which reacted with them should also be HIV antibodies; (ii) from an antibody-antigen reaction it is not possible to determine the origin even of one reactant, much less both. For example, even if proof existed that p24 was an HIV protein, because (a) AIDS patients and those at risk have a plethora of antibodies; (b) all antibodies including monoclonal antibodies cross-react [45]; it is not possible to claim that the patient's antibodies which reacted with p24 were HIV antibodies.

In the 1997 interview with Djamel Tahi Montagnier admitted the only way to prove a protein is viral is to purify the virus: "... analysis of the proteins of the virus demands mass production and purification. It is necessary to do that". To further questioning he stated: "I repeat we did not purify" which means that they could not have proven p24 to be an HIV protein. To the contrary, the fact that in the "purified" virus they did not have particles with "the morphology typical of retroviruses. They were very different" — proves beyond reasonable doubt that p24 is not an HIV protein.

Is it possible that in 1983, in a rush to find the cause of AIDS, Montagnier and his colleagues misjudged their data and concluded it proved the existence of a new retrovirus (presently known as HIV or, as Barre Sinoussi calls it, the "AIDS virus" [46]) while alternative explanations were discarded?

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