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A CRITICAL ANALYSIS OF MONTAGNIER'S 1983 "SEMINAL" PAPER

"Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS)". *Science* 1983; 220:868-71

A detailed analysis of Montagnier's and other experts' evidence in regard to the existence of HIV can also be viewed at

<http://thepertgroup.com/montagniernobel.html>

<http://thepertgroup.com/HIV/TPGVirusLikeNoOther.pdf>

<http://thepertgroup.com/hivexist.html>

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The first experiment: Montagnier's evidence and his interpretation

Evidence

Cells obtained from the enlarged lymph nodes of a gay man (BRU) were cultured with PHA, IL-2 and antiserum to human α -interferon. In the culture supernatant reverse transcription of the synthetic template-primer An.dT₁₂₋₁₈ using Mg²⁺ as the divalent cation was found.

Interpretation

Proof for the isolation and production of a retrovirus.

The second experiment: Montagnier's evidence and his interpretation

Evidence

T lymphocytes from a healthy blood donor were cultured for three days. Then half of this culture was co-cultured with lymphocytes from BRU's lymph nodes. Reverse transcriptase (RT) activity was detected in the co-culture but not in the cultures containing T lymphocytes from the healthy blood donor.

Interpretation

Transmission of the virus from BRU's cells to the healthy donor cells.

COMMENTS

1. Neither experiment included controls. A control is an "Essential part of a scientifically valid experiment, designed to show that the factor being tested is actually responsible for the effect observed. In the control experiment all factors, apart from the one under test, are exactly the same as in the test experiments, and all the same measurements are carried out".
<https://web.archive.org/web/20021022123834/http://www.tiscali.co.uk/reference/encyclopaedia/hutchinson/m0024903.html>
2. Montagnier's first experiment should have included a culture which tested cells obtained from patients with clinical and biochemical abnormalities similar to BRU but not at risk of AIDS. In the second experiment a control should have consisted of cells from similar sick individuals co-cultured with healthy blood donor cells.
3. To avoid bias, both experiments (test and control) must be performed blindly.
4. The main property of viruses is that they are microscopic particles of particular morphologies. No evidence for the existence of particles was reported from either Montagnier's first or second experiment.
5. Since the co-culture contained not only lymphocytes from the healthy blood donor but also lymphocytes from BRU's lymph nodes, the detection of RT activity cannot be considered proof of viral transmission. The activity may have been solely due to the BRU cells.
6. According to the dictionary, "isolation" comes from the Latin word "*insulatis*" meaning "made into an island". Isolation means to place apart or alone, or to separate a substance in a mixture from everything else in that mixture. Hence isolation = purification. The detection of reverse transcription is not isolation of anything, let alone a virus. For example, when a doctor orders a blood test on a patient with chest pain he is looking for evidence of enzymes that leak out of injured heart muscle. If such enzymes are present no one would consider calling this "isolation of the heart". Detection of RT activity could be considered proof of detection of a retrovirus but if and only if it is specific to retroviruses. This is not the case.
7. Enzymes which cause reverse transcription were first discovered in 1970 in retrovirus particles independently by Temin/Mituzani and Baltimore. Some HIV experts believe this enzymatic activity is specific to retroviruses and even "absolutely unique to the lentivirus group",¹ that is, only to the group of retroviruses which Montagnier now claim "HIV" belongs. This is not the case.
8. Temin was one of the first to claim and prove that reverse transcription is not specific to retroviruses.²⁻⁴ In 1972, at a meeting held at the Pasteur Institute with Jean Claude Chermann as the secretary, Barre-Sinoussi and Chermann were fully aware that reverse transcription is not specific to retroviruses. "This enzymatic activity can be explained by the presence of some virus particles in these regions [sucrose density bands other than 1.16 g/ml], and since similar

polymerase activity has been found in normal cells, may be mainly ascribed to the cellular enzyme".⁵

9. In 1973 Gallo reported the finding in leukemic cells of a protein with reverse transcriptase properties "closely related to the enzyme of primate retroviruses...but it must be emphasized that this result do not indicate that the enzyme is specifically found only in leukemic cells...it will be important to determine whether this activity is only found in neoplastic cells or if it is generally present in rapidly proliferating cells".⁶ In the same year Gallo acknowledged that "Many laboratories subsequently reported the detection of reverse transcriptase in extracts from normal cells". Gallo and his colleagues themselves reported: "An endogenous and completely RNA-dependent...DNA polymerase [reverse transcriptase] activity was obtained from leukemic blood lymphocytes (and myeloblasts) and from PHA stimulated (but not in unstimulated) normal human blood lymphocytes".⁷ In 1976 Gallo stressed that to prove a reverse transcribing enzyme is retroviral one has to (a) first purify the retroviral particles—

"A. PURIFICATION OF VIRUS

For detection and analysis of virus-associated enzyme reactions, it is essential to use virus preparations as free of cellular contaminants as possible", by banding in "sucrose density gradients"; (b) "1. The enzyme should be present in a particulate fraction and catalyze an endogenous synthesis of DNA...2. It is essential to demonstrate that, in the endogenous DNA synthesis, the DNA product should at least in part be an RNA.DNA hybrid...that the DNA product should hybridize back to RNA in the particle. These will demonstrate that the endogenous synthesis is RNA directed. 3. Purified enzyme should show a preference for (dT)₋₁₅.(A)_n over (dT)₋₁₅.(dA)_n as a primer-template (with Mg²⁺ or Mn²⁺)".⁸

In the same paper Gallo also wrote "Reverse transcriptases from different mammalian type C viruses are in general 4.5 S in size, show much more activity in the presence of Mn²⁺ than with Mg²⁺ (when synthetic primer-templates are used), and are related by immunological properties, although in general they can be distinguished from one another by the same assays".⁸

10. By 1975 it became clear that reverse transcription can be catalysed not only by reverse transcriptases but also by the cellular DNA polymerases. In fact, in 1975 an International Conference on Eukaryotic DNA polymerases defined DNA polymerase γ as the cellular enzyme which "copies An.dT₁₅ with high efficiency but does not copy DNA well".⁹ Thus, the copying of the template-primer An.dT₁₅ cannot be considered synonymous with the presence of a reverse transcriptase, retroviral or cellular. And certainly cannot be considered proof for retroviral detection, production and isolation.
11. In 1984 Rey and Montagnier published a paper entitled "Characterization of the RNA dependent DNA polymerase of a new human T-lymphotropic retrovirus (lymphadenopathy associated virus)". This paper has been analysed in detail in several postings at the BMJ Online debate,¹⁰ (search for "Montagnier's reverse

transcriptase activity”). Here it is sufficient to say that in Rey paper one cannot find any evidence for the existence of a retroviral reverse transcriptase based on the evidence enumerated (above) by Gallo. For example, Montagnier claims that because his enzyme preferred Mg^{2+} to Mn^{2+} it was an enzyme of a mammalian retrovirus. Yet according to Gallo (above), “Reverse transcriptases from different mammalian type C viruses...show much more activity in the presence of Mn^{2+} than with Mg^{2+} (when synthetic primer-templates are used)”. On the other hand, it has been known for more than forty years that cellular DNA polymerases use Mg^{2+} as the bivalent cation. In fact Montagnier does not satisfy his own rules. In July 1997 Montagnier was interviewed *en camera* at the Pasteur Institute by the French journalist Djamel Tahi. Montagnier was asked “*But there comes a point when one must do the characterisation of the virus. This means: what are the proteins of which it's composed?*” He replied “...*analysis of the proteins of the virus demands mass production and purification. It is necessary to do that*”.¹¹ However in the Rey paper Montagnier states “this enzyme can be distinguished from other cellular DNA polymerases activities and from terminal deoxynucleotidyl transferase (TdT) by purification from LAV infected T lymphocytes using phosphocellulose column”. In other words, although Montagnier agreed that characterisation of viral proteins requires purification of the virus particles, in 1984 he claimed to have characterised the HIV reverse transcriptase by purifying a protein from cells cultures, not retroviral particles. In fact, in this paper Montagnier did not present any evidence that retroviral particles even existed in his cell cultures, let alone purification of particles. Even if we assume the protein he purified from the “LAV infected lymphocytes” was a reverse transcriptase how did he know it was an HIV RT and not a cellular RT? (The videotape of this interview is the property of Djamel Tahi [email dtahi@terraincognita.fr]).

12. According to Varmus, “reverse transcription is hardly unique to retroviruses; it is now recognized as a widespread phenomenon in eukaryotic cells”.¹² Yet in the Tahi interview, in response to the first question, Montagnier said that reverse transcriptase is “truly specific for retroviruses”. However, eight questions later Montagnier conceded that reverse transcriptase is only a characteristic of retroviruses. The non-specificity of reverse transcription has even appeared in the popular press. In 2001 the Australian magazine *Shares* published an article about investing in biotechnology stocks which pointed out that reverse transcription is not specific to retroviruses.¹³

The third experiment: Montagnier’s evidence and his interpretation.

In this experiment umbilical cord lymphocytes were cultured with cell free supernatants obtained from the co-culture of the BRU and healthy blood donor cells. The evidence can be divided in two, electron microscopy and purification.

A. Electron microscopy

An electron micrograph was published showing budding and cell free retrovirus-like particles. In the text one reads “Electron microscopy of the infected umbilical cord lymphocytes showed characteristic immature particles

with dense crescent (C-type) budding at the plasma membrane". In the abstract on reads "This virus is a typical type-C RNA tumor virus".

Interpretation

The virus isolated from BRU is an Oncovirus type-C particle.

COMMENTS

1. The *Retroviridae* Family is divided into sub-families known as Oncovirus, Lentivirus and Spumavirus. Oncovirus is divided into three genera called Oncovirus type B, type C and type D. Lentivirus has a single genus known as Lentiviruses.
2. The morphological appearances of retroviral particles are not specific. In the 1970s this was accepted by many eminent retrovirologists including Temin. In 1976 Robert Gallo wrote "Release of virus-like particles morphologically and biochemically [which reverse transcribe] resembling type-c virus but apparently lacking the ability to replicate have been frequently observed from leukaemic tissue".¹⁴ Because of this, to claim that a retroviral-like particle is a retrovirus, several steps must be undertaken, as enumerated at the 1972 Pasteur Institute meeting.^{5 15 16} Montagnier has never published any evidence for even the first step, that is, EM of two consecutive cultures showing retroviral-like particles with identical morphological characteristics.
3. All retrovirologists including Temin, Todaro, Duesberg, Weiss and Gallo have pointed out that cultured cells in general, and in particular, chemically stimulated cell cultures or cells co-cultured with other cells (the types of cultures practically ubiquitous in AIDS research), may release retrovirus-like particles even when not infected with a retrovirus. One reason for this apparently strange and unexpected phenomenon is the presence in cells of what is known as endogenous retroviruses. (Endogenous means "from within", the opposite of "exogenous, "from without"). Unlike all other viruses, whose presence means acquisition from without, retrovirus-like particles can arise *de novo*. This is because animals including humans are said to be born with retroviral DNA which they inherit from their parents. It is estimated that about 10% of the human genome contains such endogenous retroviral genetic sequences. However, to date there is no evidence that these retrovirus-like particles are transmissible, that is, they are viruses. Gallo agrees with us. Responding to a question put to him in an Australian court case Gallo stated: "...endogenous retroviruses aren't viruses as your first witness [E.P-E] properly said, they are particles, they have never been transmitted. A virus is something that infects, that you prove goes from person. A to B. Short of that they are particles. Where a virus at least has to be transmitted in vitro in the laboratory, it goes from one cell to another, it's never been demonstrated for endogenous retrovirus" (T1298).¹⁷
4. Type-C particles are ubiquitous and their existence largely a mystery. In the 1970s there were many reports of type C particles in human leukaemia patients, in embryonic cells and in the majority of human placentas.¹⁸
5. In 1993 Dourmashin *et al* published evidence of cell-free and budding retrovirus-like particles in cultures of uninfected, umbilical cord lymphocytes. The cell-free

particles have a diameter smaller than that attributed to HIV by most but not all HIV experts.^{19 20}

6. In sucrose density gradients Montagnier's particles did not band at the density of 1.16 g/ml, the density characteristic of retroviral particles (see below).
7. Both in his book *Virus*²¹ (published in 2000) and his *Nature Medicine* letter of October 2003 on "The historical accuracy of HIV isolation",²² Montagnier stated that by June 1983 it was "clear" to him that HIV was a Lentivirus. In a paper published in *Science*, July 6th,²³ Montagnier claimed transmission from a blood donor to a recipient. Lymphocytes from both patients were cultured and stimulated (including with PHA). "On day 2 one lymphocyte culture from each patient was cocultivated with fresh human fetal cord lymphocyte cells in 5 percent interleukin-2. Additional fresh fetal cord lymphocytes were added to the culture on days 10 and 17. Four days after the last addition of fetal cord lymphocytes, cultures were prepared by standard methods for thin-section electron microscopy". The authors claimed "These virus particles were indistinguishable from those depicted in the original characterization of LAV (2, 6) but were different from the typical morphology of HTLV-I and -II". Reference 2 is Montagnier's 1983 *Science* paper where "HIV" was reported to be a "typical" type-C particle, that is, a particle with morphology identical to HTLV-I and HTLV-II. More importantly, looking at the three EM published in this paper, the particles do not possess the morphological features of any retroviruses. Incredibly, none of EMs even has a size bar.
8. In a paper published in 1984²⁴ (*Lancet* April 7th) Montagnier describes experiments conducted in July, August and September 1983 on the isolation of HIV from two siblings with haemophilia B. In the published EM the particles are reported as Oncovirus type-C particles – "The morphology of these particles was similar to that seen in preparations of T lymphocytes infected with LAV⁵". Reference 5 is Montagnier's 1983 *Science* paper where the particles are reported as "typical type-C".²⁵ Also in 1984 Montagnier and his colleagues published yet another EM of HIV, this time from a culture containing T4 lymphocytes of a health donor infected with HIV isolated from one of the haemophilia siblings. The caption to the EM reads – "These particles are morphologically similar to D particles such as those found in Mason-Pfizer virus or the virus recently isolated from simian AIDS".²⁶ This means that one month after he claimed to have discovered a new retrovirus in BRU, he knew that HIV is a Lentivirus yet did not publish a correction to his 1983 paper. Furthermore, in 1984 he published evidence which contradicted what was "clear" to him in June 1983. In other words, if HIV is indeed a Lentivirus, then what Montagnier discovered in 1983 and 1984 is not HIV. In a 1988 joint article where Montagnier and Gallo describe the discovery of HIV by Montagnier in 1983, they wrote "Electron micrographs of the new virus were different of those of HTLV-I [type-C particles] and resembled those of a retrovirus of horses [Lentivirus]". Yet, in 1983 both agreed that what Montagnier discovered was a "typical type-C" retrovirus. This is no different for claiming that one and the same object is a human, a chimpanzee and a gorilla.

9. Even if Montagnier had proof that the particles in the umbilical cord lymphocytes culture were viral, their origin could not have been BRU's lymphocytes. Every published diagram of the "HIV" particle shows it studded with spikes (knobs). In his book *Virus*, published in 2000, Montagnier wrote "Particles of HIV are shaped like little spheres each with roughly 80 rounded projections shaped like pegs", made of the "HIV" protein gp120. According to all HIV experts, including Montagnier, the pegs, spikes or knobs, are absolutely critical for infectivity. In other words, if a particle does not have knobs it cannot be transmitted and hence it cannot be a virus. To date, nobody, not even Gelderblom, has published evidence that proves that cell-free "HIV" particles have knobs. According to Gelderblom and his colleagues, immediately after being released from the cell membrane "HIV particles" possess an average of 0.5 knob per particle which are rapidly lost, but also pointed out "it was possible that structures resembling knobs might be observed even when there was no gp120 [knobs] present, i.e. false positives".²⁷ In a paper published in 2003 by researchers using atomic absorption spectrometry, Kuznetsov and his colleagues contradicted what virtually all HIV experts claim. They reported "The clusters of gp120 do not form spikes on the surface of the HIV as is commonly described in the literature. The clusters are hardly protrusions at all. We suggest that spikes, knobs, observed by negative-staining electron microscopy may be an artifact of the penetration of heavy metal stain between envelope proteins. Indeed, the term "spike" appears to have assumed a rather imprecise, possibly misleading definition, and might best be used with caution...That is, some of the protein tufts [clusters] we observed may represent cellular proteins".²⁸ Since the umbilical cord lymphocytes were cultured with "cell-free supernatant of the infected culture", even if the supernatant contained retroviral particles, they could not have been infectious. Similarly, the two haemophilia siblings could not have been infected by contaminated factor IX as Montagnier claims. In fact no haemophilia patient could be infected by contaminated factor VIII or IX because these therapeutic agents are made from plasma which is cell-free. Since the "HIV" in plasma must be cell-free, the particle will be devoid of knobs and thus non-infectious.²⁹ In his *Science* 1984 paper Montagnier claimed to have infected the healthy blood donor cells with the HIV from one of the haemophilia B siblings. However, the haemophilia patient's HIV was reported as type-C while that of the "infected" healthy donor cells as another retroviral species, type-D. Whatever the explanation for the "virus" in the healthy blood donor culture it cannot be the "virus" from the sibling.

B. PURIFICATION

Montagnier needed to determine if his virus was HTLV-I, HTLV-II or a new retrovirus. To do this he had to compare the protein of his "isolate" with those of the former two. To do this he firstly had to characterise the proteins of his "isolate". All retrovirologists, including Montagnier, agree that the only way to characterise the viral proteins (and RNA) is to purify the viral particles. That is, one must obtain the viral particles separated, isolated everything else that is not viral particles. Or at the very least, from everything else that contains proteins (and RNA).^{16 30 31} As Gallo pointed out in his 1976 paper (above), the method of

choice for purifying retroviral particles is banding in density gradients. In sucrose density gradients retroviruses band at the density of 1.16 g/ml. In the second part of his third experiment Montagnier took the supernatant from the “infected umbilical cord lymphocyte culture” and banded it in a sucrose density gradient. At the 1.16 g/ml he found RT activity and claimed this band was “purified” virus. The proteins from the 1.16 g/ml band were reacted with different sera and “electrophoresed on 12.5 percent polyacrylamide-SDS slab gel”. Montagnier found three proteins which reacted with antibodies present in the BRU serum, p25, p45 and p80. The p25 did not react with antiserum to HTLV-I p24. Montagnier made no comments in regard to p80 or the antibodies which reacted with it. He said that p45 “may be due to contamination of the virus by cellular actin” (the molecular weight of actin is 41K). He claimed that p25 (p24) was “a major” protein of his virus. In a later paper, published in *Science*, October 1984, Montagnier wrote “The 43-kD band and the 84-kD band are cellular contaminants”.³² In another paper published in 1984 Montagnier wrote “Sera from some AIDS patients bound a lot of cellular protein. In ELISA this problem was overcome by comparing the serum binding to the viral antigen with binding to a lysate of uninfected lymphocytes. This binding was apparent in the RIPA and only sera which specifically precipitated the p25 [p24] were regarded as positive” [RIPA=radioimmune precipitation assay].³³ Montagnier’s evidence raises a highly critical question: On what basis could Montagnier possibly claim only the p24 protein and the antibodies that reacted with it were HIV, but not any other protein or antibody was HIV? Especially given that p24 was found in material in which there was cellular debris but no retroviral particles.

COMMENTS

1. Like the first and second experiment this experiment was not conducted blindly.
2. Even if one accepts the detection of RT activity as proof of the presence of a retrovirus at the 1.16 g/ml band, it is not possible to claim the virus was purified.
3. How can one claim the virus was purified when two out of three proteins were non-viral?³² If two were non-viral, why not the third as well?
4. There is no precedent for the existence of a one protein retrovirus.
5. Given the nature of the antibody/antigen reaction, including cross-reactivity,³⁴⁻³⁸ it not possible to determine the origin of one reactant much less of both, as Montagnier claimed.
6. Although banding in density gradients is the method of choice for retroviral purification, long before the AIDS era retrovirologists knew that material other than retroviruses including cellular fragments, may also band at the same density.³⁹⁻⁴¹ This is why EM of the 1.16 g/ml band is mandatory. Yet although Montagnier claimed his 1.16 g/ml band material was “purified” virus, he did not publish even one EM to show that this material contained particles of any kind, viral, non-viral, pure or impure. The failure to publish an EM is even more enigmatic given that in 1972 the principal and second authors of the Montagnier “Isolation” paper asserted that the first step in claiming

purification is to have EM of the 1.16 g/ml showing nothing else but “particles with no apparent differences in physical appearances”.⁵ The reason for the lack of such EM proof became clear in the 1997 Tahi interview when Montagnier was asked why he did not publish an EM of his “purified” virus. He responded that even after a “Roman effort” *“We saw some particles but they did not have the morphology typical of retroviruses. They were very different. Relatively different”*. When he was asked *“Why no purification?”* he replied *“I repeat, we did not purify”*. When he was asked if Gallo had managed to purify HIV he replied *“I don’t know if really purified. I don’t believe so”*. The interview concluded with the question *“Do EM pictures from the purification exist?”* to which Montagnier replied *“Yes. Of course”*. He was then asked if such pictures have been published. He responded *“I couldn’t tell you...we have some somewhere but it is not of interest, not of any interest”*. In 2005, Djamel Tahi also interviewed Charles Dautet, the Pasteur Institute electron microscopist and one of the co-authors of the 1983 Montagnier paper. Dautet was also asked why no electron micrographs of purified HIV were published. His response was *“We have never seen virus particles in the purified virus. What we have seen all the time was cellular debris, no virus particles”* (personal communication, D.Tahi).

7. On page 869 of his paper Montagnier and his colleagues wrote “That this new isolate was a retrovirus was further indicated by its density in a sucrose gradient, which was 1.16, and by its labeling with [³H] uridine (Fig. 1)”. Since viruses are particles one would have thought that Fig. 1 would be an EM showing retroviral particles in the 1.16 g/ml band. Instead Fig. 1 is a graph showing measurements of RT activity at various densities in a sucrose gradient which are maximum at the 1.16 g/ml band. The facts that (a) in the 1.16g/ml band there were no particles with the morphology of retroviruses much less a unique virus; (b) all that was present was cellular debris; is as good a proof as any that the RT activity, the particles in the culture and the p24 have no relationship whatsoever to a new or any retrovirus.

CONCLUSION

The evidence in Montagnier’s 1983 *Science* paper does not prove the discovery of a new retrovirus or even the detection of a retrovirus, old or new.

EPILOGUE

Montagnier’s claim as well as that of other “HIV” experts regarding the isolation of “HIV” was questioned by us from the very beginning.^{42 43} When neither Montagnier nor anyone else responded to our critique, in 1991 we personally made him aware of it by sending him some of our published papers. He responded “Thank you for your letter of October 7th and enclosed papers. I will certainly return to you after reading them”. His letter is reproduced in this reference.⁴⁴ He did not return. In 1992, at a Symposium on HIV/AIDS held in Amsterdam, one of us (EPE), questioned Montagnier in regard to the isolation of HIV. Montagnier made it clear that the only evidence for the existence of HIV is p24. All the other phenomena are non-specific. When it was pointed that p24

was also non-specific, Montagnier expressed surprise and responded that he was not aware of such evidence. EPE promised to send him the evidence, which she did, but Montagnier did not respond.⁴⁵ In 1993 we published a paper in which the Montagnier 1983 and four Gallo *Science* 1984 papers are critically analysed in detail.³⁴ Neither Montagnier nor Gallo nor anyone else responded. In 2004 we published a detailed critique of Montagnier's 1983 seminal paper. Neither Montagnier nor any of the other authors responded.⁴⁶

NOTE

In all the HIV literature detection of reverse transcription using the synthetic template-primer An.dT₁₂₋₁₅ is considered proof for "HIV" detection, production, and even isolation and HIV quantification. Since 1987 the detection a reaction between an antibody to Montagnier's "HIV" p24 protein and the plethora of antigens in cell cultures/co-cultures is considered proof for "HIV" isolation.

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