VIRUSMYTH HOMEPAGE

INTERVIEW LUC MONTAGNIER

Did Luc Montagnier Discover HIV?

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Text of a videotape interview performed at the Pasteur Institute, July 1997. Please note: The answers by Luc Montagnier have been numbered for easier reference to the analyses in the reply by Papadopulos-Eleopulos et al.

DT: A group of scientists from Australia argues that nobody up till now has isolated the AIDS virus, HIV. For them the rules of retrovirus isolation have not been carefully respected for HIV. These rules are: culture, purification of the material by ultracentrifugation, Electron Microscopic (EM) photographs of the material which bands at the retrovirus density, characterisation of these particles, proof of the infectivity of the particles.

LM: No, that is not isolation. We did isolation because we "passed on" the virus, we made a culture of the virus. For example Gallo said : "They have not isolated the virus...and we (Gallo et al.), we have made it emerge in abundance in an immortal cell line." But before making it emerge in immortal cell lines, we made it emerge in cultures of normal lymphocytes from a blood donor. That is the principal criterion. One had something one could pass on serially, that one could maintain. And characterised as a retrovirus not only by its visual properties, but also biochemically, RT [reverse transcriptase] activity which is truly specific of retroviruses. We also had the reactions of antibodies against some proteins, probably the internal proteins. I say probably by analogy with knowledge of other retroviruses. One could not have isolated this retrovirus without knowledge of other retroviruses, that's obvious. But I believe we have answered the criteria of isolation. Totally. (1)

DT: Let me come back on the rules of retrovirus isolation which are : culture, purification at the density of retroviruses, EM photographs of the material at the retrovirus density, characterisation of the particles, proof of the infectivity of the particles. Have all these steps been done for the isolation of HIV? I'd like to add, according to several published references cited by the Australian group, RT is not specific to retroviruses and, moreover, your work to detect RT was not done on the purified material?

LM: I believe we published in Science (May 1983) a gradient which showed that the RT had exactly the density of 1.16. So one had a peak which was RT. So one has fulfiled this criterion for purification. But to pass it on serially is difficult because when you put the material in purification, into a gradient, retroviruses are very fragile, so they break each other and greatly lose their infectivity. But I think even so we were able to keep a little of their infectivity. But it was not as easy as one does it today, because the quantities of virus were nonetheless very weak. At the beginning we stumbled on a virus which did not kill cells. The virus came from an asymptomatic patient and so was classified amongst the non-syncythia-forming, non-cytopathogenic viruses using the co-receptor ccr5. It was the first BRU virus. One had very little of it, and one could not pass it on in an immortal cell line. We tried for some months, we didn't succeed. We succeeded very easily with the second strain. But there lies the quite mysterious problem of the contamination of that second strain by the first. That was LAI. (2)

DT: Why do the EM photographs published by you, come from the culture and not from the purification?

LM: There was so little production of virus it was impossible to see what might be in a concentrate of virus from a gradient. There was not enough virus to do that. Of course one looked for it, one looked for it in the tissues at the start, likewise in the biopsy. We saw some particles but they did not have the morphology typical of retroviruses. They were very different. Relatively different. So with the culture it took many hours to find the first pictures. It was a Roman effort! It's easy to criticise after the event. What we did not have, and I have always recognised it, was that it was truly the cause of AIDS. (3)

DT: How is it possible without EM pictures from the purification, to know whether these particles are viral and appertain to a retrovirus, moreover a specific retrovirus?

LM: Well, there were the pictures of the budding. We published images of budding which are characteristic of retroviruses. Having said that, on the morphology alone one could not say it was truly a retrovirus. For example, a French specialist of EMs of retroviruses publicly attacked me saying: "This is not a retrovirus, it is an arenavirus". Because there are other families of virus which bud and have spikes on the surface, etc. (4)

DT: Why this confusion? The EM pictures did not show clearly a retrovirus?

LM: At this period the best known retroviruses were those of type C, which were very typical. This retrovirus wasn't a type C and lentiviruses were little known. I myself recognised it by looking at pictures of Equine infectious anaemia virus at the library, and later of the visna virus. But I repeat, it was not only the morphology and the budding, there was RT...it was the assemblage of these properties which made me say it was a retrovirus. (5)

DT: About the RT, it is detected in the culture. Then there is purification where one finds retroviral particles. But at this density there are a lot of others elements, among others those which one calls "virus-like".

LM: Exactly, exactly. If you like, it is not one property but the assemblage of the properties which made us say it was a retrovirus of the family of lentiviruses. Taken in isolation, each of the properties isn't truly specific. It is the assemblage of them. So we had: the density, RT, pictures of budding and the analogy with the visna virus. Those are the four characteristics. (6)

DT: But how do all these elements allow proof that it is a new retrovirus? Some of these elements could appertain to other things, "virus-like"...?

LM: Yes, and what's more we have endogenous retroviruses which sometimes express particles - but of endogenous origin, and which therefore don't have pathological roles, in any case not in AIDS. (7)

DT: But then how can one make out the difference?

LM: Because we could "pass on" the virus. We passed on the RT activity in new lymphocytes. H. We got a peak of replication. We kept track of the virus. It is the assembly of properties which made us say it was a retrovirus. And why new? The first question put to us by Nature was: "Is it not a laboratory contamination? Is it perhaps a mouse retrovirus or an animal retrovirus?". To that one could say no! Because we had shown that the patient had antibodies against a protein of his own virus. The assemblage has a perfect logic! But it is important to take it as an assemblage. If you take each property separately, they are not specific. It is the assemblage which gives the specificity. (8)

DT: But at the density of retroviruses, did you observe particles which seemed to be retroviruses? A new retrovirus?

LM: At the density of 1.15, 1.16, we had a peak of RT activity, which is the enzyme characteristic of retroviruses. (9)

DT: But could that be something else?

LM: No..in my opinion it was very clear. It could not be anything but a retrovirus in this way. Because the enzyme that F. Barre-Sinoussi characterised biochemically needed magnesium, a little like HTLV elsewhere. It required the matrix, the template, the primer also which was completely characteristic of an RT. That was not open for discussion. At Cold Spring Harbour in September 1983, Gallo asked me whether I was sure it was an RT. I knew it, F. Barre-Sinoussi had done all the controls for that. It was not merely a cellular polymerase, it was an RT. It worked only with RNA primers, it made DNA. That one was sure of. (10)

DT: With the other retroviruses you have met in your career did you follow the same process and did you meet the same difficulties?

LM: I would say that for HIV it is an easy process. Compared with the obstacles one finds for the others...because the virus does not emerge, or indeed because isolation is sporadic - you manage it one time in five. I am talking about current research into others illnesses. One can cite the virus of Multiple Sclerosis of Prof. Peron. He showed me his work a decade ago and it took him around ten years to finally find a gene sequence which is very close to an endogenous virus. You see...it is very difficult. Because he could not "pass on" the virus, he could not make it emerge in culture. Whereas HIV emerges like couch grass. The LAI strain for example emerges like couchgrass. That's why it contaminated the others. (11)

DT: With what did you culture the lymphocytes of your patient? With the H9 cell line?

LM: No, because it didn't work at all with the H9. We used a lot of cell lines and the only one which could produce it was the Tambon Iymphocytes. (12)

DT: But using these kinds of elements it is possible to introduce other things capable of inducing an RT and proteins, etc..

LM: Agreed completely. That's why finally we were not very ardent about using immortal cell lines. To cultivate the virus en masse - OK. But not to characterise it, because we knew we were going to bring in other things. There are MT cell lines which have been found by the Japanese (MT2, MT4) which replicate HIV very well and which at the same time are transformed by HTLV. So, you have a mix of HIV and HTLV. It is a real soup. (13)

DT: What's more it's not impossible that patients may be infected by other infectious agents?

LM: There could be mycoplasmas...there could be a stack of things. But fortunately we had the negative experience with viruses associated with cancers and that helped us, because we had encountered all these problems. For example, one day I had a very fine peak of RT, which F. Barre-Sinoussi gave me, with a density a little bit higher, 1.19. And I checked! It was a mycoplasma, not a retrovirus. (14)

DT: With the material purified at the retrovirus density, how is it possible to make out the difference between what is viral and what is not? Because at this density there's a stack of other things, including "virus-like" particles, cellular fragments...

LM: Yes, that's why it is easier with the cell culture because one sees the phases of virus production. You have the budding. Charles Dauget (an EM specialist) looked rather at the cells. Of course he looked at the plasma, the concentrate, etc...he saw nothing major. Because if you make a concentrate it's necessary to make thinly sliced section [to see a virus with the EM], and to make a thin section it is necessary to have a concentrate at least the size of the head of a pin. So enormous amounts of virus are necessary. By contrast, you make a thin section of cells very easily and it's in these thin sections that Charles Dauget found the retrovirus, with different phases of budding. (15)

DT: When one looks at the published electron microscope photographs, for you as a retrovirologist it is clear it's a retrovirus, a new retrovirus?

LM: No, at that point one cannot say. With the first budding pictures it could be a type C virus. One cannot distinguish. (16)

DT: Could it be anything else than a retrovirus?

LM: No.. well, after all, yes .. it could be another budding virus. But there's a ... we have an atlas. One knows a little bit from familiarity, what is a retrovirus and what is not. With the morphology one can distinguish but it takes a certain familiarity. (17)

DT: Why no purification?

LM: I repeat we did not purify. We purified to characterise the density of the RT, which was soundly that of a retrovirus. But we didn't take the peak...or it didn't work...because if you purify, you damage. So for infectious particles it is better to not touch them too much. So you take simply the supernatant from the culture of lymphocytes which have produced the virus and you put it in a small quantity on some new cultures of lymphocytes. And it follows, you pass on the retrovirus serially and you always get the same characteristics and you increase the production each time you pass it on. (18)

DT: So the stage of purification is not necessary?

LM: No, no, it's not necessary. What is essential is to pass on the virus. The problem Peron had with the multiple sclerosis virus was that he could not pass on the virus from one culture to another. That is the problem. He managed it a very little, not enough to characterise it. And these days to characterise means above all at the molecular standard. If you will, the procedure goes more quickly. So to do it : a DNA, clone this DNA, amplify it, sequence it, etc..So you have the DNA, the sequence of the DNA which tells you if it is truly a retrovirus. One knows the familiar structure of retroviruses, all the retroviruses have a familiar genomic structure with such and such a gene which is characteristic. (19)

DT: So, for isolation of retroviruses the stage of purification is not obligatory? One can isolate retroviruses without purifying?

LM: Yes .. one is not obliged to transmit pure material. It would be better, but there is the problem that one damages it and diminishes the infectivity of the retrovirus. (20)

DT: Without going through this stage of purification, isn't there a risk of confusion over the proteins that one identifies and also over the RT which could come from something else?

LM: No .. after all, I repeat if we have a peak of RT at the density of 1.15, 1.16, there are 999 chances out of 1,000 that it is a retrovirus. But it could be a retrovirus of different origin. I repeat, there are some endogenous retroviruses, pseudo-particles which can be emitted by cells, but even so, from the part of the genome that provides retroviruses. And which one acquires through heredity, in the cells for a very long time. But finally I think for the proof - because things evolve like molecular biology permitting even easier characterisation these days - it's necessary to move on very quickly to cloning. And that was done very quickly, as well by Gallo as by ourselves. Cloning and sequencing, and there one has the complete characterisation. But I repeat, the first characterisation is the belonging to the lentivirus family, the density, the budding, etc.. the biological properties, the association with the T4 cells. All these things are part of the characterisation, and it was us who did it. (21)

DT: 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?

LM: That's it. So then, analysis of the proteins of the virus demands mass production and purification. It is necessary to do that. And there I should say that that partially failed. J.C. Chermann was in charge of that, at least for the internal proteins. And he had difficulties producing the virus and it didn't work. But this was one possible way, the other way was to have the nucleic acid, cloning, etc. It's this way which worked very quickly. The other way didn't work because we had at that time a system of production which wasn't robust enough. One had not enough particles produced to purify and characterise the viral proteins. It couldn't be done. One couldn't produce a lot of virus at that time because this virus didn't emerge in the immortal cell line. We could do it with the LAI virus, but at that time we did not know that. (22)

DT: Gallo did it?

LM: Gallo? .. I don't know if he really purified. I don't believe so. I believe he launched very quickly into the molecular part, that's to say cloning . What he did do is the Western Blot. We used the RIPA technique, so what they did that was new was they showed some proteins which one had not seen well with the other technique. Here is another aspect of characterising the virus. You cannot purify it but if you know somebody who has antibodies against the proteins of the virus, you can purify the antibody/antigen complex. That's what one did. And thus one had a visible band, radioactively labelled, which one called protein 25, p25. And Gallo saw others. There was the p25 which he called p24, there was p41 which we saw... (23)

DT: About the antibodies, numerous studies have shown that these antibodies react with other proteins or elements which are not part of HIV. And that they can not be sufficient to characterise the proteins of HIV.

LM: No! Because we had controls. We had people who didn't have AIDS and had no antibodies against these proteins. And the techniques we used were techniques I had refined myself some years previously, to detect the src gene. You see the src gene was detected by immunoprecipitation too. It was the p60 [protein 60]. I was very dexterous, and my technician also, with the RIPA technique. If one gets a specific reaction, it's specific. (24)

DT: But we know AIDS patients are infected with a multitude of other infectious agents which are susceptible to ...

LM: Ah yes, but antibodies are very specific. They know how to distinguish one molecule in one million. There is a very great affinity. When antibodies have sufficient affinity, you fish out something really very specific. With monoclonal antibodies you fish out really ONE protein. All of that is used for diagnostic antigen detection. (25)

DT: For you the p41 was not of viral origin and so didn't belong to HIV. For Gallo it was the most specific protein of the HIV. Why this contradiction?

LM: We were both reasonably right. That's to say that I in my RIPA technique...in effect there are cellular proteins that one meets everywhere - there's a non-specific "background noise", and amongst these proteins one is very abundant in cells, which is actin. And this protein has a molecular weight 43000kd. So, it was there. So I was reasonably right, but what Gallo saw on the other hand was the gp41 of HIV, because he was using the Western Blot. And that I have recognised. (26)

DT: For you p24 was the most specific protein of HIV, for Gallo not at all. One recognises thanks to other studies that the antibodies directed against p24 were often found in patients who were not infected with HIV, and even in certain animals. In fact today, an antibody reaction with p24 is considered non specific.

LM: It is not sufficient for diagnosing HIV infection. (27)

DT: No protein is sufficient?

LM: No protein is sufficient anyway. But at the time the problem didn't reveal itself like that. The problem was to know whether it was an HTLV or not. The only human retrovirus known was HTLV. And we showed clearly that it was not an HTLV, that Gallo's monoclonal antibodies against the p24 of HTLV did not recognise the p25 of HIV. (28)

DT: At the density of retroviruses, 1.16, there are a lot of particles, but only 20% of them appertain to HIV. Why are 80% of the proteins not viral and the others are? How can one make out the difference?

LM: There are two explanations. For the one part, at this density you have what one calls microvesicles of cellular origin, which have approximately the same size as the virus, and then the virus itself, in budding, brings cellular proteins. So effectively these proteins are not viral, they are cellular in origin. So, how to make out the difference?! Frankly with this technique one can't do it precisely . What we can do is to purify the virus to the maximum with successive gradients, and you always stumble on the same proteins. (29)

DT: The others disappear?

LM: Let's say the others reduce a little bit. You take off the microvesicles, but each time you lose a lot of virus, so it's necessary to have a lot of virus to start off in order to keep a little bit when you arrive at the end. And then again it's the molecular analysis, it's the sequence of these proteins which is going allow one to say whether they are of viral origin or not. That's what we began for p25, that failed ...and the other technique is to do the cloning, and so then you have the DNA and from the DNA you get the proteins. You deduce the sequence of the proteins and their size and, you stumble again on what you've already observed with immunoprecipitation or with gel electrophoresis. And one knows by analogy with the sizes of the proteins of other retroviruses, one can deduce quite closely these proteins. So you have the p25 which was close to the p24 of HTLV, you have the p18..in the end you have the others. On the other hand the one which was very different was the very large protein, p120. (30)

DT: Today, are the problems about mass production of the virus, purification, EM pictures at 1.16, resolved?

LM: Yes, of course. (31)

DT: Do EM pictures of HIV from the purification exist?

LM: Yes. of course. (32)

DT: Have they been published?

LM: I couldn't tell you...we have some somewhere .. but it is not of interest, not of any interest. (33)

DT: Today, with mass production of the virus, is it possible to see an EM, after purification, of a large number of viruses?

LM: Yes, yes. Absolutely. One can see them, one even sees visible bands. (34)

DT: So for you HIV exists?

LM: Oh, it is clear. I have seen it and I have encountered it. (35) *

Notes: Go here for the reply by the Perth Group.

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