BACK

The Perth Group
June 8th 2013

ELECTRON MICROSCOPIC STUDIES OF "HIV" IN PLASMA

Over the past few months we have been trying to convince Andrew Maniotis that electron microscopic (EM) studies will provide no useful information if conducted on cells, even if the cells are lymphocytes and much less red blood cells. We have also advised him of the following EM techniques.

Negative staining

1. Non-HIV infected plasma is spiked with different concentrations of latex particles whose dimensions are similar to retroviruses. A grid is then placed on a droplet of the above then removed and negatively stained with uranyl sulphate or phosphotungstic acid solution. It can then be directly examined by transmission electron microscopy (TEM). This will determine at what concentration particles of size similar to retroviruses will be visible by TEM. Once these data are obtained they can be used in subsequent experiments. LINK

2. Repeat 1 using "infected" sera (HIV positive). Then latex particle concentration and proposed viral concentration are compared.

Concentrated pellets fixed with glutaraldehyde

1. Again spike non-HIV infected plasma with different concentrations of latex particles whose dimensions are similar to retroviruses. Spin at 30,000 rpm in an ultracentrifuge for 2 hours. The pellet can then be fixed with glutaraldehyde then sectioned and embedded in epoxy resin for ultrathin sectioning and TEM.

2. Repeat 1 using "infected" sera (HIV positive). Then latex particle concentration and proposed viral concentration are compared.

3. Alternatively the pellet can be resuspended in a small volume of fluid and the negative staining technique applied.

Note: At all times the latex particles will act as a positive control for the method and determine its sensitivity. Once the latter is measured it can be used on any number of samples.

INTERPRETATION OF THE EM STUDIES

The meaning of the viral load test

Almost all individuals will think the term "viral load" refers to measurements of a concentration of viral particles. Indeed this assertion is frequently asserted *ex cathedra*. In 1995 Wei and Ho wrote "virtually all HIV-1 infected individuals, regardless of clinical stage, exhibit persistent plasma viraemia in the range of 10^2 to 10^7 virions per ml".[1] ("*The virion* = intact, fully assembled, infective virus particle"[2]).

These authors were citing Pitiak *et al* who reported "Quantative competitive polymerase chain reaction methods were used to quantify virion-associated human immunodeficiency virus type-1 (HIV-1) RNA in plasma from 66 patients…HIV-1 RNA, ranging from 100 to nearly 22,000,000 copies per milliliter of plasma (corresponding to 50 to 11,000,000 virions per milliliter), was readily quantified in all subjects".[3] However, none of these authors nor any other scientists have published data correlating plasma "HIV" RNA copy numbers with retroviral-like particle counts. Hence no one knows if a patient with a viral load of one million has many, some or no lentiviral-like particles in his plasma.

If:

(i) the EM findings in the non-HIV latex preparation show it is possible to visualise latex particles when the concentration is, for example, higher than 100,000/ml particles but;

(ii) no retroviral-like particles are seen in "HIV" infected individuals with "HIV" RNA copy numbers of, for example, 200,000/ml;

it means that whatever the viral load means, it is not "HIV" viremia. And whatever the RNA, it is not "HIV" RNA.

This does not mean that the viral load test cannot be used to monitor disease progression if such a correlation has been proven beforehand.

<u>de Harven's viral load</u>

de Harven states: "The concept of "Viral load" implies the presence of retroviral particles in the blood plasma, i.e. viremia". If this is correct then given that retroviral nucleic acid is RNA one will expect that the viral load measures RNA. That this is the fact is known by everybody, from those who introduced the viral load test to the technician who performs the test. de Harven is the exception. All along, including in his 2010 JAPS paper, he claims that the viral load test measures DNA. If this is the case, then given that the viral particles contain RNA and not DNA, it is nonsensical to try to correlate viral load with plasma particles and to claim that "Viral load implies" viremia.

We have repeatedly pointed out to de Harven, the latest in our "The HIV Puzzle – what is being measured?" LINK that his claims, including those in his JAPS paper, contradict the presently available facts, and make no scientific sense.

<u>The absence of particles</u>

If a correlation between viral load and particles does exist but such particles cannot be found at the concentrations higher than those predetermined by the latex particle data, it suggests the patient is not infected with "HIV".

If there is no correlation between viral load and particles, or if the viral load suggests the viral particle concentration is lower than that which can be detected (as determined by the latex particle data) then no particle detection by EM does not mean no infection. The particles may be present but the RNA is not viral or the EM test is not sufficiently sensitive to detect the particles.

The presence of particles

Finding particles, even with all the morphological characteristics of lentiviruses is not proof the person is infected with a virus. This is because one of the two main characteristics of viruses is infectivity. That is, their ability to enter cells in which they replicate producing identical particles.

"Virus: one of a group of minute infectious agents, with certain exceptions (e.g. poxviruses) not resolved in the light microscope, and characterised by a lack of independent metabolism and by the ability to replicate only within living host cells. Like living organisms, they are able to reproduce with genetic continuity and the possibility of mutation. They range from 200-300nm to 15nm in size and are morphologically heterogeneous, occurring as rod-shaped, spherical, or polyhedral, and tadpole-shaped forms; masses of the spherical or polyhedral forms may be made up of orderly arrays, to give a crystalline structure. The individual particle, or virion, consists of nucleic acid (the nucleoid), DNA or RNA (but not both) and a protein shell, or capsid, which contains and protects the nucleic acid". (Dorland's Illustrated Medical Dictionary 26th Edition).

"A virus is a small infectious agent". Wikipedia.

EM examination does not prove infectivity. Further experiments have to be done to prove the particles are infectious and hence a virus.

de Harven appears to be the only person who has his own definition of a virus. According to him any particle seen with the EM with morphological characteristics of retroviruses is a virus. In an email to Martin Barnes he wrote: "False [that the particles have to be infectious]: To call something a virus it has to be infectious. I remember very well, and actually took some participation, in a major conference at the NY Academy of Sciences in the 1960s, conference that was under the title "Viruses in search of Diseases", during which many viruses, undisputedly demonstrated by electron microscopy, had no known infectivity". LINK

Everybody, including the HIV experts, some of them in sworn testimony in court (see addendum), agree that to prove a particle is a virus, i.e. infectious, one must purify the particles. Peter Duesberg is the exception.

Duesberg: "Since infectious HIV DNA has been isolated from infected human cells that is free of HIV's own proteins and RNA as well as from all cellular macromolecules, HIV isolation has passed the most rigorous standards available today". LINK

However:

(i) It is not possible to know that a cell is infected with a virus unless you first prove the virus exists, i.e. purify the particles and prove they are infectious.

(ii) The only way to obtain the viral DNA from an infected cell is to hybridise the cellular DNA with the viral DNA. Everybody, including the "HIV" experts agree this is not possible unless one first obtains the viral DNA

from purified particles (see addendum). This is also the view of Dr Vincent Racaniello, Professor of Microbiology and Immunology at Columbia University NYC who has a passion to "educate the world about viruses". His 24 lecture Virology course "Biology W3310/4310" is available at the Academic Earth website and is well worth watching. LINK Dr Racaniello tells his students "it's really important that people understand how they [viruses] work because 99.99% of the world doesn't get it…The public for the most part is afraid of viruses because they don't understand them and what they do know about them they get from the popular press and the movies and it's mostly wrong". Dr. Racaniello repeatedly emphasises that viruses are particles. Not DNA. And "If you want to do genetics [to obtain the viral DNA from infected cells] of animal viruses you have to be able to isolate pure populations".

(iii) Neither Duesberg nor Brian Foley, who repeated Duesberg's claims, have ever been able to come up with evidence of an infectious molecular clone. That is, proof that when artificially inserted "HIV" DNA into the cells they start to produce virus particles. When, after the lengthy debate in the BMJ, Foley failed to come up with such proof, he, John Moore and Wain-Hobson put pressure on the BMJ and the debate was stopped. LINK

Since, to date, no proof exists for purification of virus-like particles from cultures containing tissue(s) from patients assumed to be infected, detection of such particles in plasma, irrespective of their number, cannot be considered proof for infection with a virus.

Summary

1. A negative EM study does not disprove infection with "HIV".
2. If a retrovirus "HIV" does exist, and the RNA of this particle is detected and measured in the "viral load" test, we can predict (a) the presence in plasma of retroviral particles; (b) a high correlation between the RNA copy number and the concentration of particles. If the appropriate EM studies are done but do not confirm either (a) or (b) or both then the existence of HIV and the HIV theory of AIDS warrants urgent revision. If (b) is confirmed this suggests the RNA is a constituent of the particles but it does not prove the particles are virions.

REFERENCES

1. Wei X, Ghosh SK, Taylor M, et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature.* 1995;373:117-122.

2. Nermut M, Steven A. *Animal virus structure.* Vol 3: Elsevier Science; 1987.

3. Piatak M, Jr., Saag MS, Yang LC, et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science.* 1993;259(5102):1749-1754.

ADDENDUM ON PURIFICATION (from ENV Commentary) LINK

QUESTION: IS PURIFICATION NECESSARY TO PROVE THE EXISTENCE OF A NEW RETROVIRUS?

White and Fenner: "It's an essential pre-requisite".

Montagnier: "It is necessary".

Gallo: "You have to purify". (T1257)

Barré-Sinoussi: "...you have to purify the virus from all this mess".

JC Chermann: "Yes, of course…Absolutely".

Prof. David Gordon: "It's a natural step from obtaining the virus in cell culture to then obtain purified virus". (T1034)

Prof. Dominic Dwyer: "The purification, as far as one can go, is important in analysis of any virus or bacteria, for that matter well". (T1199)

ANSWER: Yes, absolutely

QUESTION: WHY IS PURIFICATION NECESSARY?

White and Fenner: "…for the chemical analysis of viruses". To prove that the virus particles have unique proteins and RNA.

Montagnier: "…analysis of the proteins of the virus [obviously this also applies to the viral RNA, the genome] demands mass production and purification. It is necessary to do that".

Montagnier: "To prove that you have a real virus".

Barré-Sinoussi: "Because we wanted these diagnostic kits [the antibody tests] to be as specific as possible. If you use a preparation of virus which is not purified of course you will detect antibody to everything not only against the virus but also to all the proteins that are produced in the supernatant".

JC Chermann: To identify the HIV proteins and RNA they had to extract them "from the virus which we had concentrated and purified".

Gallo: "Conclusive serological testing, in our view, required finer, more specific assays based on using purified virus particles of [*sic*: or] proteins obtained from the virus instead of whole cells infected with virus".

Gelderblom: "...because this house [the Robert Koch Institute in Berlin] in '85 already established ELISA antigen material ["HIV" proteins]...for testing people...we had to look at the material that was used for the ELISA".

Prof. David Cooper: “Once the virus is purified, it’s then genetically sequenced and those sequences are unique [must be unique] just like every organism on the planet has unique sequences and markers”. (T673).

Prof. David Gordon: “…because purification of virus is then very useful for further studies for the nature of the virus and the nature of the immune response against the virus”. (T1032)

Prof. Dominic Dwyer: “Well, in the diagnostic sort of situation what that really is looking for is looking for presence of those conserved bits of genetic material that you know to be the pathogen, be it HIV or flu or whatever, you then use that technology to see whether those sequences or those bits are present in something else, in another clinical sample, for example. And that really now has become, you know, the main method of diagnosis of many pathogens in a laboratory now…I mean with genetic testing – I guess the upside of course is you can do it on everybody, it’s pretty cheap, it’s extremely reliable and robust, the downside is that you have to know the genetic structure to begin with, you have to have the genetic sequence of what you are after. So when a new virus emerges, like SARS, you can’t necessarily use, reliably, nucleic acid testing until you get the sequence of that new virus for the first time. So then in fact you are in a first identifier, you are required to use these more traditional methods of virus culture and microscopy and so on”. (T963)