ANTIBODIES

NIH / ANTIBODIES

This document was submitted by the Perth Group (www.theperthgroup.com) as part of the Internet debate that took place as a preamble to the Presidential AIDS Advisory Panel meeting held in Johannesburg in July 2000.

In "The Evidence That HIV Causes AIDS" (http://www.niaid.nih.gov/factsheets/evidhiv.htm) one reads: "Nearly everybody with AIDS has antibodies to HIV...numerous studies from around the world show that virtually all AIDS patients are HIV-seropositive; that is they carry antibodies that indicate HIV infection". The relationship between a positive antibody test and AIDS is said to prove that HIV is the cause of AIDS.

There is no doubt that many, if not all, AIDS patients, at least in the USA, Europe and Australia, have a positive antibody test. However, there is no agreement as to whether these tests "indicate HIV infection". For example, the packet insert for the Axsym system (HIV-1/HIV-2) manufactured by Abbott Laboratories includes the words "At present there is no recognized standard for establishing the presence or absence of HIV-1 antibody in human blood".[1] This contradicts the above-mentioned NIH document which reads:

"MYTH: *HIV antibody testing is unreliable.*

FACT: Diagnosis of infection using antibody testing is one of the best-established concepts in medicine. HIV antibody tests exceed the performance of most other infectious disease tests... Current HIV antibody tests have sensitivity and specificity in excess of 98% and are therefore extremely reliable".[2, 3]

COMMENTARY

It is incomprehensible how a body of scientists at the National Institutes for Health in the US could present both sides of a scientific debate as a series of "MYTHS" and "FACTS". Especially without providing the names of scientists who hold the opposing view or any citations to enable the reader to investigate the matter himself. The only conclusion one can make from this behaviour is that the NIH does not want their readers to learn the full story.

Here we examine one very important "FACT" and leave it up to the reader to make his own judgement as to whether or not it is a "MYTH".

FACT: THERE IS NO EVIDENCE A RETROVIRUS HAS BEEN ISOLATED FROM THE TISSUES OF AIDS PATIENTS. HENCE THERE IS NO GOLD STANDARD FOR ANTIBODY TESTING FOR "HIV" INFECTION AND NO PROOF A RETROVIRUS CAUSES AIDS

To prove the specificity of an antibody test or any antibody antigen reaction, one must:

(i) perform the test in hundreds, if not thousands, of individuals who are assumed to be infected;

(ii) perform the test in a control group consisting of at least an equal number of individuals who are thought not to be infected, but who are sick;

(iii) using the same samples prove the existence of HIV by a test independent of the antigen-antibody reaction, that is by using a gold standard for the reaction.

The only gold standard for the HIV antibody test is HIV itself, that is HIV isolation (purification). At present no such proof exists.[4, 5] Nowhere in the cited WHO 1998 reference can one find a gold

standard being used to prove the specificity of the antibody test. All one can find there, as the title indicates, "Comparative Evaluation of the Operational Characteristics of Commercially Available Assays", is a comparison between 34 HIV test kits against "a panel of 595 human sera (prevalence 33.6% for HIV-1 and 10 % for HIV-2), of which 192 were from Africa, 99 from Asia, 206 from Europe and 98 from South America. The panel included 332 HIV negative specimens and 203 sera positive for HIV-1 and 60 positive for HIV-2 specimens. In addition the sensitivity of the HIV test kits is assessed on 8 seroconversion panels from Boston Biomedica (BBI)". In fact, they did not even use as a gold standard what is at present considered to represent HIV isolation.

Currently, the reaction between an antibody directed against Montagnier's p24 and antigens in cultures is considered proof for HIV isolation. Firstly, a reaction between an antibody and an antigen cannot be considered proof for isolation of a retrovirus. Secondly, the reaction is totally non specific. In 1992, Jorg Shupbach, the principle author of one of the first four 1984 papers published by Gallo's group on HIV isolation, reported that the whole blood cultures of 49/60 (82%) of "presumably uninfected but serologically indeterminate individuals and 5/5 seronegative blood donors were found positive for p24".[6] The non-specificity of the p24 antigen test is so obvious that it is accepted by no less an authority on HIV testing than Philip Mortimer and his colleagues from the UK Public Health Laboratory Service, "Experience has shown that neither HIV culture nor tests for p24 antigen are of much value in diagnostic testing. They may be insensitive and/or non-specific".[7] Thirdly, since this reaction is an antibody-antigen reaction itself, it cannot be used as a gold standard for the antibody test. Even if one uses this reaction as a gold standard for the antibody test, then the WHO data shows the specificity of the antibody test to be very low indeed. In a large WHO study published in 1994, between 1992-93, 224 specimens were collected in Brazil, Rwanda, Thailand and Uganda from asymptomatic "HIV positive" individuals. Serostatus was first confirmed in the country of origin and then at the "centralized laboratories responsible for confirming serology, virus isolation, virus expression, and distribution of reagents (George-Speyer-Hans Chemotherapentisches Forschunginstitut (GSH) in Frankfurt, Germany; National Institute for Biological Standards and Control (NIBSC) in London, United Kingdom,; and DAIDS/NIAID in Bethesda, Maryland, United States". In this WHO study, "of a total of 224 virus cultures, 83 were positive (Isolation rate=37%)".[8]

As in the WHO reference, in Sloand *et al*[3] no data is presented to prove the specificity of the HIV antibody tests. It is only stated: "Antibodies to HIV-1 proteins, which develop during the course of infection, include antibodies to viral core antigen (p24) and antibodies to viral envelope proteins (gp120 and gp41). Antibodies to HIV-1 polymerase (p55) develop later, if at all. The most widely used test, the enzyme-linked immunosorbent assay (ELISA), is used in conjunction with a confirmatory test, the Western blot…Although there is variability depending on the test kit used, up to 70% of the initially positive ELISA results are not confirmed by the second ELISA. Samples that are repeatedly reactive by ELISA must then be confirmed positive by a Western blot or equivalent test. This procedure enables separation by electrophoresis of individual viral proteins, such as viral core (p24, p55 and p17) and envelope (gp120, gp160 and gp41) proteins, into well-defined bands for use as HIV-1 antigen standards. The separated bands are transferred or "blotted" onto a nitrocellulose membrane that is cut into strips and exposed to the serum sample. Serum antibodies to the antigen standards are detected and characterised as discrete coloured bands by use of antihuman antibody in conjugation with an enzyme, as shown in Fig. 1. The diagnostic pattern of bands identified in the Western blot is more specific than the ELISA for viral antibodies".

An antibody test, Western blot (WB), cannot be used as a gold standard for another antibody test, ELISA. Just because in the WB the "viral" antigens are separate, this is not proof that the WB is more specific than ELISA. Neither can the specificity of an antibody test be determined by repeating the test, no matter how many times. Furthermore, at present there is no proof that the "viral core (p24, p55 and p17) and envelope (gp120, gp160 and gp41) proteins" or any other protein used in the ELISA or WB are HIV proteins.[9, 10]

According to Luc Montagnier the characterisation of proteins as HIV proteins "demands mass production and purification [of the virus]. It is necessary to do that. And there I should say that that partially failed".[11] In fact since the material which Montagnier *et al* used to characterise the

"viral core" protein, p24, did not even have retrovirus-like particles, much less "purified" HIV, then one has no choice but to conclude that Montagnier and his associates did not prove that p24 is an HIV protein. Neither has anybody else since.

When Djamel Tahi asked Montagnier if Robert Gallo purified HIV, he replied: "Gallo ?...I don't know if he really purified. I don't believe so". Like Montagnier, Robert Gallo and his colleagues did not publish electron micrographs to show that their "purified" virus contained retrovirus-like particles. Unlike Montagnier el al who considered the protein of molecular weight 24,000 (p24) as being the characteristic HIV proteins, Gallo *et al* considered a protein of molecular weight 41,000 (p41), which is the molecular weight of actin, as the most specific HIV protein. The only proof they gave for this was its banding at the density of 1.16gm/ml and reaction with the sera of AIDS patients.

In a Franco-German study, published in 1997, the authors, which included Hans Gelderblom, pointed out that although the 1.16gm/ml band, which is used for "biochemical and serological analyses", is "considered to contain a population of relatively pure virus particles,...in none of the studies has the purity of the virus preparation been verified".[5] However, by 1997, ample evidence existed which showed that the 1.16gm/ml band contains many cellular proteins including actin and myosin, the latter also an ubiquitous protein which has two light chains of molecular weight 24,000 and 18,000. Evidence also exists that AIDS patients have antibodies to both actin and myosin.[12]

Before 1987 the p120 and p160 bands could not be visualised in WB strips. This was not unexpected since according to the HIV experts p160 is present only in infected cells, not in virus particles, and p120 to be present only in the particles' knobs (spikes), which are rapidly lost when the particles are released. Since the protein on the WB strips are obtained from purified HIV particles which do not possess knobs[13 210] (p120) then neither p120 nor p160 should be present. Nevertheless, in 1987, by modifying "blot preparation", proteins of molecular weights of 120,000 and 160,000 were found which reacted with patients sera.[14 306] However, no amount of "blot preparation" modification can create what is not already present. The explanation for the presence of these bands was found in 1989 by researchers who showed that in the Western blot strip, "the components visualised in the 120-160 kDa region do not correspond to gp120 or its precursor but rather represent oligomers of gp41".[15 248] It was also shown that the WB pattern obtained is dependent on many factors including temperature and the concentration of sodium dodecyl sulphate used to disrupt the "pure virus". "Confusion over the identification of these bands has resulted in incorrect conclusions in experimental studies. Similarly, some clinical specimens may have been identified erroneously as seropositive, on the assumption that these bands reflected specific reactivity against two distinct viral components and fulfilled a criterion for true or probable positivity. The correct identification of these bands will affect the standards to be established for Western blot positivity: it may necessitate the reinterpretation of published results".[16 773] No notice was taken of these findings and recommendations.

Definite proof that what is considered "purified" HIV, the 1.16 gm/ml band contains neither retroviral proteins nor HIV was published in 1997. In that year, two papers were published in *Virology* with the first electron micrographs of "purified HIV" obtained by banding the supernatant of "infected" cultures in sucrose density gradients. One of the studies was by researchers from the AIDS Vaccine Program SAIL, National Cancer Institute–Frederick Cancer Research and Development, Frederick, Maryland, USA and the other by researchers from France and Germany.[4, 5] The authors of both studies claimed their "purified" material contain retrovirus-like particles and in fact that they were HIV particles. But they admitted that their material predominantly contained particles which were not viruses but "mock virus", that is "budding membrane particles frequently called microvesicles". Indeed, the caption to one of the electron micrographs of the "purified" HIV reads: "Purified vesicles from infected H9 cells (a) and activated PBMC (b) supernatants". It does not read "purified HIV". In further experiments the supernatants from non-infected cultures were also banded in sucrose gradients. They claimed that the banded material from these cultures contained only microvesicles, "mock virus" particles, but no particles with the morphology of HIV.

No reason(s) is given, other than morphological, for why some of the particles in the fractions from the "infected" cells are virus particles and the others "mock virus". As far as morphology is concerned, none of the particles have all the morphological characteristics attributed to HIV, or even retroviruses.

The minimum absolutely necessary but not sufficient condition to claim that what are called "HIV-1 particles" are a retrovirus and not cellular microvesicles is to show that the sucrose density fractions obtained from the "infected" cells contain proteins which are not present in the same fractions obtained from non-infected cells that is in the "mock virus". However, the researchers from the USA have shown this is not the case. The only difference one can see in their SDS-polyacrylamide gel electrophoresis strips of "purified virus" and "mock virus" is quantitative, not qualitative. This quantitative difference may be due to many reasons including the fact that there were significant differences in the history and the mode of preparation of the non-infected and "infected" H9 cell cultures, in addition to the "infection". A similar finding was reported by the same authors a few years earlier.[17] However, while in both studies the proteins of molecular weight "near 42 kDa" (42,000) are labelled as "Actin" and "in the 30- to 40-kDa range" as "HLA DR", all the proteins with molecular weight higher than approximately 42,000 and lower than approximately 30,000 are left unlabelled in the earlier paper.[17] In the 1997 study, three proteins of molecular weight lower than 30.000 are labelled as $p24^{CA}$, $P17^{MA}$, and $p6/p7^{NC}$ and are said to represent "major bands of viral proteins". However, according to the authors, "these labels were added when one of the reviewers asked for them. He felt if would help orient readers when looking at the figure–the reviewer is correct. We did not determine the identities of the bands in the particular gel".(Bess, personal communication).

Since both the "purified HIV" and the "mock" virus contain the same proteins, one has no choice but to conclude that the 1.16 g/ml band, the "purified HIV":

(i) has no HIV proteins and thus no HIV;

(ii) the proteins used as antigens in both the ELISA and WB antibody tests are non HIV;

(iii) since the only evidence which is said to prove that the antibodies present in the AIDS patients sera are HIV antibodies is their reaction with the proteins which band at 1.16 gm/ml and the assumption that they are HIV; and since no HIV proteins are present at this band; it follows that the AIDS patients do not have HIV antibodies.

In conclusion, although evidence exists for a correlation between the antibody tests and AIDS, no evidence exists which proves that a positive antibody test means HIV infection.

REFERENCES

1. Abbott Laboratories Diagnostics Division. 100 Abbott Park Rd. Abbott Park. Illinois: USA. 1988, 1998. Packet Insert Axsym system (HIV-1/HIV-2).

http://aids-kritik.de/aids/diverses/abbott-hiv-test.htm

2. WHO. HIV Test Kits.

3. Sloand EM, Pitt E, Chiarello RJ, Nemo GJ. HIV Testing State of the Art. Journal of the American Medical Association 1991; 266:2861-2866.

4. Bess JW, Gorelick RJ, Bosche WJ, Henderson LE, Arthur LO. Microvesicles are a source of contaminating cellular proteins found in purified HIV-1 preparations. Virology 1997; 230:134-144.

5. Gluschankof P, Mondor I, Gelderblom HR, Sattentau QJ. Cell membrane vesicles are a major contaminant of gradient-enriched human immunodeficiency virus type-1 preparations. Virology 1997; 230:125-133.

6. Schupbach J, Jendis JB, Bron C, Boni J, Tomasik Z. False-positive HIV-1 virus cultures

using whole blood. AIDS 1992; 6:1545-6.

7. Mortimer P, Codd A, Connolly J, et al. Towards error free HIV diagnosis: notes on laboratory practice. Public Health Laboratory Service Microbiology Digest 1992; 9:61-64.

8. WHO. HIV type 1 variation in World Health Organization-sponsored vaccine evaluation sites: genetic screening, sequence analysis, and preliminary biological characterization of selected viral strains. AIDS Research and Human Retroviruses 1994; 10:1327-1343.

9. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM, Causer D. The Isolation of HIV: Has it really been achieved? Continuum 1996; 4:1s-24s.

10. Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM. Has Gallo proven the role of HIV in AIDS? Emergency Medicine [Australia] 1993; 5:113-123.

11. Tahi D. Did Luc Montagnier discover HIV? Text of video interview with Professor Luc Montagnier at the Pasteur Institute July 18th 1997. Continuum 1998; 5:30-34.

12. Matsiota P, Chamaret S, Montagnier L. Detection of Natural Autoantibodies in the serum of Anti-HIV Positive-Individuals. Annales de l'Institut Pasteur Immunologie 1987; 138:223-233.

13. Layne SP, Merges MJ, Dembo M, et al. Factors underlying spontaneous inactivation and susceptibility to neutralization of human immunodeficiency virus. Virology 1992; 189:695-714.

14. Burke DS. Laboratory diagnosis of human immunodeficiency virus infection. Clinical and Laboratory Medicine 1989; 9:369-392.

15. Pinter A, Honnen WJ, Tilley SA, et al. Oligomeric structure of gp41, the transmembrane protein of human immunodeficiency virus type 1. Journal of Virology 1989; 63:2674-9.

16. Zolla-Pazner S, Gorny MK, Honnen WJ. Reinterpretation of human immunodeficiency virus Western blot patterns. New England Journal of Medicine 1989; 320:1280-1281.

17. Arthur LO, Bess JW, Jr., Urban RG, et al. Macaques immunized with HLA-DR are protected from challenge with simian immunodeficiency virus. Journal of Virology 1995; 69:3117-24.