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HIV antibodies: further questions and a plea for clarification

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Summary

The existence of specific antibody/protein reactions is the crucial assumption underlying proof of HIV isolation, proof of HIV infection and the causative role of HIV in AIDS. However, since

1. *antibodies which react with the 'HIV' proteins arise following allogenic stimuli in non-HIV-infected animals and humans, as well as in mice and humans with autoimmune disorders; antibodies to antigens from both mycobacteria and yeasts cross-react with HIV* env *and* gag *proteins;*
2. *individuals belonging to the AIDS risk groups are subjected to allogenic stimuli and have high levels of autoimmune antibodies, while the vast majority of patients in the AIDS risk groups are infected with either or both mycobacteria or yeasts;*

the evidence for the existence of HIV and its putative role in AIDS must be reappraised.

Key words: AIDS – Antibodies – HIV – HL23V – Isolation – Infection – Mannans mycobacteria – Retroviruses – Specificity – Yeasts

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Introduction

In the 1986 commentary on his experiments reported in *Science* in 1984, Gallo wrote, 'Since we considered the mere detection of virus particles in cultures from AIDS and ARC patients to be insufficient to confirm scientifically our hypothesis that such particles were implicated in the aetiology of the disease, we decided to obtain specific reagents'.[1] Furthermore, in the absence of 'specific reagents' the 'reverse transcriptase assay and electron microscopic examination' are not sufficient to prove the existence of a unique retrovirus. In fact, according to Gallo and his colleagues, unlike their evidence, the 1983 data of Montagnier did not prove 'true isolation'[2] of HIV. However, the only salient difference between the two groups is that Gallo's group used a leukaemic cell line from which they obtained greater amounts of 'specific reagents' and thus were able to perform many more antibody tests.

Critical analysis of the specificity of the tests

The only way to obtain 'specific reagents' is to isolate the virus, that is, obtain viral particles separate from everything else. If this is not done it is impossible to say which reagents (proteins) originate from the virus and which are contaminants. Only then can the viral proteins be used as 'specific reagents' with which to perform antibody tests. Even then, because a given antigen can react with antibodies directed against other antigens (cross-reactions), the specificity of the reactions must be determined by using viral isolation as a gold standard. However, instead of using this procedure, the only one scientifically valid, Gallo and his colleagues cultured a leukaemic cell line (HT) with tissues derived from AIDS patients. Proteins derived from the culture supernatants (but without proof of origin from a retrovirus or even particles, viral or non-viral, or even from the patients), were incubated with sera from AIDS patients or those at risk. These experiments showed that some proteins reacted sometimes with some of the sera. From these reactions, not only did Gallo and his associates conclude that the antibodies present in the patient sera were directed against the proteins, but also that these were the HIV proteins. In other words, the reaction between some proteins and some antibodies, when there was no evidence that the proteins originated from a particle or against which antigens the antibodies were directed, was considered proof for the existence of a unique retrovirus, HIV, and even for its 'true isolation'. However, one cannot argue a case requiring a premise (the existence of virus-specific proteins and antibodies) contingent upon proof of the argument (virus isolation). Apart from being illogical, to do so invites grave risk of failure of the argument should there arise independent evidence that antibodies are not virus specific. In fact, even at the beginning of the AIDS era, the retrovirological literature already embraced such a caveat.

In the mid-1970s, Gallo and his colleagues reported the isolation of the first human retrovirus, HL23V. In fact, the evidence for the 'isolation' of HL23V surpassed that of HTLV-I and HIV in at least two aspects. Unlike HIV, Gallo's group: (a) reported the detection of reverse transcriptase activity in fresh, uncultured leucocytes;[3] and (b) published an electron micrograph of virus-like particles banding at a sucrose density of 1.16 g/ml, the density which defines retroviral particles.[4] Following the discovery of HL23V, some researchers attempted to determine its prevalence utilising antibody tests[5] while others were interested in determining the specificity of the antibody reactions. The latter included one group from the Laboratory of Cellular and Molecular Biology, National Cancer Institute, and another from the Laboratory of Viral Oncology, Memorial Sloan-Kettering Cancer Center. Using the 'viral glycoproteins', these groups[6–7] found that the antibodies present in human sera which reacted with these proteins were 'directed against carbohydrate structures' and concluded that 'The results are consistent with the idea that the antibodies in question are elicited as a result of exposure to many natural substances possessing widely cross-reacting antigens and are not a result of widespread infection of man with replication competent oncoviruses' (retroviruses). In 1981 Gallo accepted the evidence that the antibodies which reacted with the presumed viral proteins of HL23V were not so directed 'but against the carbohydrate moieties on the molecule that are introduced by the host cell as a post-transcriptional event, and which are therefore cell-specific and not virus-specific'.[8] This discovery was of such significance that today nobody, not even Gallo, considers HL23V as being the first human retrovirus, or even a retrovirus.

Questions regarding proof of viral identity

As mentioned above, the specificity of HIV antibody/HIV protein reactions can be determined only by using HIV isolation as a gold standard. This has not been done and would seem impossible at the moment because to date nobody has fulfilled even the first step in the only scientifically valid method for retroviral isolation, that is, electron micrographic demonstration of virus-like particles at the sucrose density gradient of 1.16 g/ml. The detection of virus-like particles in non-banded culture fluids and phenomena such as reverse transcriptase activity and antigen/antibody reactions are not scientifically valid proof for the isolation of a unique retrovirus. In fact, nowhere in the HIV/AIDS literature is there proof of the existence of cell-free particles possessing all the morphological features required of a retroviral particle, that is, particles 'with a diameter of 100–120 nm' AND 'studded with projections (spikes, knobs)'.[9] In addition, 'HIV' cannot be 'isolated' from all AIDS patients and the methods used for 'HIV isolation' are not specific.[10–11] Furthermore, as in the case of HL23V, there is abundant evidence that antibodies present in human sera which react with 'HIV proteins' are also non-specific:

1. 'One half of the molecular weight of gp120 is represented by oligomannosidic oligosaccharides.... Polyclonal antibodies to mannan from yeast also recognise the carbohydrate structure of gp120 of the AIDS virus';[12]
2. 'The immunochemical determinants of the antigenic factors of *Candida albicans* display a high identity with the glycoprotein (gp) 120 of HIV-1: they contain $\alpha(1 \rightarrow 2)$- and $\alpha(1 \rightarrow 3)$-linked mannose terminal residues';[13]
3. Antibodies to the mannans of *Candida albicans* 'block infection of H9 cells by HIV-1' as well as the binding of lectins to gp120;[13]
4. Recognition of gp120 by antibodies to a synthetic peptide of the same antigen was 'partially abolished if it was absorbed with the total polysaccharide fraction of *C. albicans*' while the antigen recognition by antibodies to 'gp120 from human T-cell lymphotropic virus type IIIB ... was totally blocked'. From these data the authors concluded: 'These results indicate that mannan residues of *C. albicans* can serve as antigens to raise neutralising antibodies against HIV infection';[13]
5. 'Normal human serum contains antibodies capable of recognising the carbohydrate moiety of HIV envelope glycoproteins ... from 100 ml of human serum approximately 200 μg of MBIgG was recovered [MBIgG = mannan-binding IgG] ... MBIgG bound to HIV envelope glycoproteins gp160, gp120 and gp41';[14]
6. Kashala, Essex and their colleagues have shown that antibodies to carbohydrate-containing antigens such as lipoarabinomannan and phenolic glycolipid that constitute the cell wall of *Mycobacterium leprae*, a bacterium which 'shares several antigenic determinants with other mycobacterial species' cause 'significant cross-reactivities with HIV-1 *pol* and *gag* proteins'. This led the authors to warn that among leprosy patients and their contacts there is a 'very high rate of HIV-1 false-positive ELISA and WB results', that 'ELISA and WB results should be interpreted with caution when screening individuals infected with *M. tuberculosis* or other mycobacterial species', and furthermore that 'ELISA and WB may not be sufficient for HIV diagnosis in AIDS-endemic areas of central Africa where the prevalence of mycobacterial diseases is quite high';[15]
7. Not only mycobacteria (*M. leprae, M. tuberculosis*, *M. avium-intracellulare*) but also the walls of all fungi (*Candida albicans*, *Cryptococcus neoformans*, *Coccidioides immitis*, *Histoplasma capsulatum*, including *Pneumocystis carinii)*,[16–18] contain carbohydrate (mannans). One hundred per cent of AIDS patients (even those with 'no Candida clinically') have *C. albicans* antibodies, leading researchers from St. Bartholomew's and St. Stephen's Hospitals to state: 'It is possible that Candida may act as a cofactor in the development of overt AIDS in HIV-infected individuals'.[19] It may also be of interest to note that in gay men the only sexual act which is a risk factor for seroconversion is passive anal intercourse (exposure to semen)[20] and that

mannose is present in both sperm and seminal plasma.[21]

8. Since antibodies to mannans react with the 'HIV proteins' then, as Essex and his colleagues[15] have pointed out, for mycobacterial infection in Africa, one would expect the sera of all people infected with fungi and mycobacteria to cross-react with the 'HIV-1 glycoproteins' as well as to cause 'significant cross-reactivities with HIV-1 *pol* and *gag* proteins'.
9. Researchers from the University of Rome injected healthy mice with an *E. coli* lipopolysaccharide (LPS) and reacted their sera with two synthetic peptides, one encompassing gp120 V3 loop of 'HIV-1 MN' and the other 'representing a gp41 immunodominant epitope'. The 'LPS-treated mice showed a significant antibody reactivity' with the two peptides. (V. Colizzi *et al.*, personal communication.)
10. In the same study, the authors reported data from the sera of HIV-negative subjects with autoimmune disorders. Recombinant gp120 and a panel of synthetic peptides derived from the amino acid consensus sequences of the HIV gp120, gp41, p24 or several unrelated proteins were tested by specific ELISA. 'The first set of experiments performed on four patients with Sjorgern syndrome (SjS) and four patients with systemic lupus erythematosus (SLE) revealed a significant anti-gp120 reactivity compared to healthy HIV-negative controls. Moreover, such binding could be almost completely inhibited by preincubation with free gp120. A significant anti-p24 reactivity was observed in 18 out of 29 [62%] sera from SjS patients and in 13 out of 25 [52%] from SLE patients, while anti-gp41 was observed only in 3 out of 14 [21%] SjS and in 2 out of 20 [10%] SLE affected patients. Similar analyses were performed in the murine model of autoimmunity, showing that sera from MRL/lpr mice were able to bind all HIV related peptides in age-dependent manner. The analysis of a panel of HIV unrelated peptides showed that SLE as MRL/lpr sera bind both HIV related and unrelated peptides while SjS sera failed to do so.' In other words, sera which contain autoantibodies react with the principal 'HIV proteins' gp120, gp41 and p24.[22]
11. The same authors also reported similar results from (i) experiments where 'Two month old male CBA mice were immunised for 6 weeks with 50×10^6 allogenic lymphoid cells obtained from either BALB/c or B6 male mice'; (ii) 'Sera from 62 polytransfused (at least 10 transfusions/year) patients with thalassemia'.
12. Similarly, in 1991, Kion and Hoffman reported 'Mice of the autoimmune strains MRL-lpr/lpr and MRL-+/+ made antibodies against gp120'. Mice that have been exposed to T-lymphocytes from another murine strain were shown to make antibodies against gp120 and p24 of HIV.[23]

Questions arising

1. Given the fact that individuals with fungal and mycobacterial infections have antibodies which may react with 'HIV proteins' in the absence of 'HIV', and that *E. coli* is an intestinal commensal and a potential bacterium in all of us, how can one assert that:
   (a) reactions between antibodies in the sera of AIDS patients and proteins present in cultures derived from the tissues of AIDS patients is proof that the reacting proteins are constituents of a unique retrovirus HIV and the antibodies are specific to these proteins?;
   (b) PCP, candidiasis, cryptococcosis, coccidioidomycosis, histoplamosis, tuberculosis or *Mycobacterium avium-intracellulare* disease, that is, the vast majority of the opportunistic infections (88% of AIDS cases diagnosed between 1988 and 1992 had one or more fungal or mycobacterial infections[24]) which signify AIDS are caused by HIV on the basis of a positive antibody test?
   (c) a positive antibody test in individuals with fungal and mycobacterial infections proves HIV infection?
2. Since:
   (a) mice and patients with autoimmune diseases (SjS and SLE) and AIDS patients share many clinical and immunological (autoantibodies) manifestations[25,26];
   (b) patients polytransfused with allogenic blood and mice injected with foreign cells and foreign proteins develop 'HIV antibodies' but are not infected with HIV;

   why should gay men, i.v. drug users and haemophiliacs, who are all exposed to foreign cells and/or foreign proteins, not also develop 'HIV antibodies' and not be infected with HIV?

Concluding comments

Since 1983, antigen/antibody reactions between some proteins present in cell cultures derived from tissues of AIDS patients and antibodies in the sera of AIDS patients have been considered proof that the antigens are constituent proteins of a new, unique, exogenously acquired retrovirus, HIV and that antibodies are specific to this virus. The same antigen/antibody reactions are routinely used to diagnose HIV infection and the correlation between such reactivity and the presence or the development of AIDS is considered proof that HIV is the cause of AIDS. However, by an antigen/antibody reaction it is not possible to prove that the origin of certain proteins is a retrovirus or that antibodies are specifically directed against the retrovirus. The only scientifically valid method of proving an antigen is a retroviral protein is to isolate the retroviral particle. For many

decades prior to the AIDS era, the method used for this purpose was banding cultures supernatants in density gradients.[27–28] The only scientifically valid method of determining the specificity of antibodies present in sera of both healthy individuals and AIDS patients is the use of HIV isolation as a gold standard. Since there is no such data proving the origin of either 'HIV' proteins or antibodies, and since there is ample evidence that reactions between 'HIV' proteins and 'HIV' antibodies are non-specific, including reactivity caused by organisms and agents to which AIDS patients and those at risk are subjected, it is vital for the scientific community to utilise valid methods in order to prove whether the 'HIV' proteins and antibodies arise as the result of a new, unique, exogenously acquired retrovirus. Until these data are forthcoming, a positive 'HIV' antibody test may be used only as a marker for the presence or the development of AIDS. At present, there is no scientific basis for using these tests to prove HIV infection.

References

1. Gallo, R. C., Sarin, P. S., Kramarsky, B., Salahuddin, Z., Markham, P., and Popovic, M., (1986). First isolation of HTLV-III. *Nature*, **321**, 119.
2. Popovic, M., Sarngadharan, M. G., Read, E., and Gallo, R. C., (1984). Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science*, **224**, 497–500.
3. Gallo, R. C., Wong-Staal, F., Reitz, M., Gallagher, R. E., Miller, N., and Gillepsie, D. H., (1976). Some evidence for infectious type-C virus in humans. In: Baltimore, D., Huang, A. S., and Fox, C. F. (Eds), *Animal Virology*, Academic Press, New York, pp. 385–405.
4. Gallagher, R. E., and Gallo, R. C., (1975). Type C RNA tumor virus isolated from cultured human acute myelogenous leukemia cells. *Science*, **187**, 350–353.
5. Teich, N. M., Weiss, R. A., Salahuddin, S. Z., Gallagher, R. E., Gillepsie, D. H., and Gallo, R. C., (1975). Infective transmission and characterisation of a C-type virus released by cultured human myeloid leukaemia cells. *Nature*, **256**, 551–555.
6. Snyder, H. W., and Fleissner, E., (1980). Specificity of human antibodies to oncovirus glycoproteins: Recognition of antigen by natural antibodies directed against carbohydrate structures. *Proc. Natl Acad. Sci. USA*, **77**, 1622–1626.
7. Barbacid, M., Bolognesi, D., and Aaronson, S. A., (1980). Humans have antibodies capable of recognizing oncoviral glycoproteins: Demonstration that these antibodies are formed in response to cellular modification of glycoproteins rather than as consequence of exposure to virus. *Proc. Natl Acad. Sci. USA*, **77**, 1617–1621.
8. Kalyanaraman, V. S., Sarngadharan, M. G., Bunn, P. A., and Gallo, R. C., (1981). Antibodies in human sera reactive against an internal structural protein of human T-cell lymphoma virus. *Nature*, **294**, 271–273.
9. Gelderblom, H. R., Özel, M., Hausmann, E. H. S., Winkel, T., Pauli, G., and Koch, M. A., (1988). Fine structure of human immunodeficiency virus (HIV), immunolocalization of structural proteins and virus–cell relation. *Micron Microscopica*, **19**, 41–60.
10. Papadopulos-Eleopulos, E., Turner, V. F., and Papdimitriou, J. M., (1993). Is a positive Western Blot proof of HIV infection? *Bio/Technology*, **11** (June), 696–707.
11. Mortimer, P., Codd, A., Connolly, J., *et al.*, (1992). Towards error free HIV diagnosis: notes on laboratory practice. *Pub. Health Lab. Service Micrbiol. Digest*, **9**, 61–64.
12. Muller, W. E. G., Schroder, H. C., Reuter, P., Maidhof, A., Uhlenbruck, G., and Winkler, I., (1990). Polyclonal antibodies to mannan from yeast also recognize the carbohydrate

structure of gp120 of the AIDS virus: an approach to raise neutralizing antibodies to HIV-1 infection in vitro. *AIDS*, **4**, 159–162.
13. Muller, W. E. G., Bachmann, M., Weiler, B. E., *et al.*, (1991). Antibodies against defined carbohydrate structures of *Candida albicans* protect H9 cells against infection with human immunodeficiency virus-1 in vitro. *J. Acquir. Immun. Defic. Syndr.*, **4**, 694–703.
14. Tomiyama, T., Lake, D., Masuho, Y., and Hersh, E. M., (1991). Recognition of human immunodeficiency virus glycoproteins by natural anti-carbohydrate antibodies in human serum. *Biochem. Biophys. Res. Commun.*, **177**, 279–285.
15. Kashala, O., Marlink, R., Ilunga, M., *et al.*, (1994). Infection with human immunodeficiency virus type 1 (HIV-1) and human T cell lymphotropic viruses among leprosy patients and contacts: correlation between HIV-1 cross-reactivity and antibodies to lipoarabinomannan. *J. Infect. Dis.*, **169**, 296–304.
16. Ezekowitz, R. A., Williams, D. J., Koziel, H., *et al.*, (1991). Uptake of *Pneumocystis carinni* mediated by the macrophage mannose receptor. *Nature*, **351**, 155–158.
17. Hoffman, O. A., Standing, J. E., and Limper, A. H., (1993). *Pneumocystis carinni* stimulates tumor necrosis factor-alpha release from alveolar macrophages through a beta-glucan-mediated mechanism. *J. Immunol.*, **150**, 3932–3940.
18. O'Riordan, D. M., Standing, J. E., and Limper, A. H., (1995). *Pneumocystis carinni* glycoprotein A binds macrophage mannose receptors. *Infect-Immun*, **63**, 779–784.
19. Matthews, R., Smith, D., Midgley, J., *et al.*, (1988). Candida and AIDS: Evidence for protective antibody. *Lancet*, **ii**, 263–266.
20. Caceres, C. F., and van Griensven, G. J. P., (1994). Male homosexual transmission of HIV-1. *AIDS*, **8**, 1051–1061.
21. Mann, T., and Lutwak-Mann, C., (1981). *Male Reproductive Function and Semen*, Springer-Verlag, New York.
22. Fraziano, M., Montesano, C., and Lombardi, V. R., *et al.*, (1996). Epitope specificity of anti-HIV antibodies in human and murine autoimmune diseases. *AIDS Res. Hum. Retroviruses*, **12**, 491–496.
23. Kion, T. A., and Hoffman, G. W., (1991). Anti-HIV and anti-anti-MHC antibodies in alloimmune and autoimmue mice. *Science*, **253**, 1138–1140.
24. Hu, D. J., Fleming, P. L., Castro, K. G., *et al.*, (1995). How important is race/ethnicity as an indicator of risk for specific AIDS-defining conditions? *J. Acquir. Immun. Def. Syndr. Hum. Retrovirol.*, **10**, 374–380.
25. Calabrese, L. H., (1989). The rheumatic manifestations of infection with the human immunodeficiency virus. *Semin. Arthritis Rheum.*, **18**, 225–239.
26. Nakamara, M., Burastero, S. E., Ueki, Y., Larrie, J. W., Notkj, A. L., and Casali, P., (1988). Probing the normal and autoimmune B cell repertoire with Epstein-Barr virus: Frequency of B cells producing monoreactive high affinity autoantibodies in patients with Hashimoto's disease and systemic lupus erythematosus. *J. Immunol.*, **141**, 4165–4172.
27. Sinoussi, F., Mendiola, L., Chermann, J. C., (1973). Purification and partial differentiation of the particles of murine sarcoma virus (M. MSV) according to their sedimentation rates in sucrose density gradients. *Spectra*, **4**, 237–243.
28. Toplin, I., (1973). Tumor virus purification using zonal rotors. *Spectra*, **No. 4**, 225–235.