TURNOVER OF HIV-1 AND CD4 LYMPHOCYTES

In the studies by Ho et al[1] and Wei et al[2] where they claim to have determined the concentration of HIV particles and the dynamics of particle and T4 cell production and destruction, as well as in the accompanying commentary by Wain-Hobson, the authors themselves acknowledge that they have made many assumptions, extrapolations and inferences, which, if proven wrong, may or may not significantly affect their conclusions. Moreover, since many of their premises, including the following have not been proven their studies need to be properly assessed before they can be accepted as an "important landmark in the process of understanding the disease called AIDS".

1. Wei and his colleagues studied "Twenty-two HIV-1 infected subjects with $CD4^+$ lymphocyte counts between 18 and 251 per mm^3", and Ho and his colleagues "twenty infected patients" whose pretreatment $CD4^+$ lymphocytes "ranged from 36 to 490 per mm^3". Neither group studied non "HIV-1 infected subjects", with similar lymphocyte counts and clinical symptoms, that is, they have ignored one of the most fundamental requirements of basic experimental research, controls. Undoubtedly they, like everybody else, by "HIV-1 infections" mean a positive antibody test. Yet to date nobody has proven that a positive antibody test is proof of HIV infection[4], a fact accepted by both Blattner[5] and Mortimer[6].

2. Both studies as well as Wain-Hobson assume that "CD4 T-cell loss is a consequence of viral [HIV] infection". Yet in the vast HIV/AIDS literature there is not one single paper, either from in vitro or in vivo studies, which proves such a claim. In fact, there is no

evidence that in AIDS patients there is a preferential destruction of the T4 cells by any agent. All the evidence suggest a post-translational loss of CD4 surface markers and acquisition of CD8 surface markers (as determined by antibody reactions) induced by factors other than HIV.[7] As far back as 1984 Klatzmann, Montagnier and their colleagues accepted that the decrease in T4 cells may be "due to either modulation of T4 molecules at the cell membrane or steric hindrance of antibody-binding sites", and not to their destruction by HIV[8].

3. Both groups used molecular techniques to quantify HIV. Yet as far back as 1989 Wain-Hobson and his colleagues concluded that "the task of defining HIV in molecular terms will be difficult". The basis for their conclusion was the fluctuation in the quasispecies in vivo, the high frequency of defective viruses and the "evident differences between quasispecies in vivo and in vitro"[9]. Since then nobody has proven them wrong. Indeed, according to Wain-Hobson "an asymptomatic patient can harbour at least 10^6 genetically distinct variants of HIV, and for an AIDS patient the figure is more than 10^8" and to, Wei et al "major changes in the HIV-1 quasispecies occur quickly and continuously".

4. A positive PCR signal is considered unambiguous evidence for the detection of the HIV genome. Yet the specificity of the PCR, any form of PCR, for the HIV genome, has not been determined. Correspondence between different forms of PCR or PCR and other techniques does not prove specificity. If the PCR detects the HIV genome and there is massive HIV infection, Southern hybridisation should be more than sufficient to detect it. Yet, as Gallo at

present admits, in 1984 Shaw, Gallo and their colleagues had negative results, although they studied many tissues from AIDS patients, including lymph nodes and used a southern hybridisation technique which could "detect less than one viral DNA copy per ten cells"[10].

5. Ho and his colleagues do not give details of the method they have used. They only state: "plasma samples were tested with the branched DNA signal-amplification assay as previously described[12,13]". Both these references are "in the press". According to Wei et al "Viral RNA was determined by QC-PCR assay[6]", or was "confirmed by QC-PCR[6]". Ref 6 is a paper published in 1993 by Piatak and his colleagues[11] including 4 co-authors of the Wei study, which according to Wain-Hobson constitutes the background to the latest two studies. In that paper they used QC-PCR and "targeted a highly conserved sequence in HIV-1 gag".

However:-

(a) The gag sequences have been found in people known not to be HIV infected[4].

(b) The human genome contains endogenous retroviral genomic sequences[4]. The gag gene is a group specific gene, because of this, the gag gene even if specific to a retrovirus, cannot be considered HIV specific, a fact accepted by Blattner.[5] Even if the gag gene was HIV specific, because most of the genomes are defective, finding it is no proof of the existence of the whole HIV genome.

6. Even if Wei et al and Ho et al had used a method which detected nothing else but the HIV genome, the whole HIV genome, such evidence cannot be used to quantify the HIV particles as they have done. As Piatak and his colleagues, including Shaw, admitted in their 1993 paper, to quantify the HIV particles one must have prior evidence that the RNA actually belongs to a HIV particle. No such evidence was presented by either of the two groups In their 1993 paper Shaw and his colleagues stated:

(A) that they have determined the total virion levels "by measurement of viral RNA in virus preparations that had been quantified directly by electron microscopic particle counts (25)". However they did not publish any electron microscopy data. No such method has been used in the three publications in ref. 25. In the first there is an electron micrograph[12]. However the electron micrograph is not from plasma or fresh tissue but from an H9 culture supernatant "clarified by centrifugation". Although some of the particles have morphological characteristics similar to retroviruses many do not. Furthermore, no relationship has been established between the RNA and the particles in the "viral stock". The other two publications, which actually are letters to Nature, do not even have EM data. The author of the first letter[13] expresses his frustration in not being able to find any valid data regarding "the relationship of the number of HIV particles" and p24 in plasma or culture and proceeds to calculate it by making many assumptions. The authors of the second letter[14] doubt the validity of such a calculation and state "....measurement of the total amount of viral protein (p24 or gp 120) in HIV

cultures or in the plasma of HIV infected individuals are of very limited value for estimation of their number of infectious particles present".

(B) "To demonstrate conclusively that the HIV-1 RNA quantified by QC-PCR was virion associated", Piatak, Shaw and their colleagues stated to have "fractioned samples of HIV-1 containing culture supernatant and plasma from infected patients by using buoyant density centrifugation on continuous (20 to 60%) sucrose gradients. The HIV-1 RNA peaks corresponded precisely to the peaks of HIV-1 p24 antigen, both of which localised to fractions of the expected specific gravity for HIV-1 particles[26]", but published no data.

Ref. 26 refers to the 1983 Barré-Sinoussi et al paper and to the 1984 Popovic et al and Levy et al Science papers on HIV isolation. None of these authors presented evidence of the presence of HIV particles, or any particles at the 1.16g/ml density, the retroviral density or anywhere else in the sucrose gradient. The finding by these authors (and claimed by Piatak et al), of proteins including p24, which react with AIDS patients sera and subsequently (but not in the references cited) at the density of 1.16 g/ml of Adenylic acid rich RNA is not proof that the RNA or the proteins belonged to an HIV particle or any particle, viral or non viral, or of the existence of a direct relationship between the RNA and the proteins. Indeed, Piatak and his colleagues themselves did not find a relationship between "HIV RNA" and "immune complex-dissociated HIV p24 antigens". As Barré-Sinoussi, Chairman,

and other retrovirologists pointed out in 1973, the first necessary, but by no means sufficient step for proving that an RNA belongs to a retroviral particles is to have electron microscopy evidence that the material which bands at 1.16 gm/ml contains nothing else but particles with "no apparent differences in physical appearances"[15]. If Pantaleo et al[16] have demonstrated that the "lymphoreticular tissues serve as the primary reservoir and site of replication for HIV-1", and if Piatak et al have demonstrated "plasma viraemia in the range of 10^2 to 10^7 virions per ml", as Wei et al claim, at present ample electron microscopy data should exist to confirm it. Yet to date nobody has presented evidence of the existence of HIV particles in plasma. In the electron-micrograph published by Pantaleo et al to demonstrate massive HIV infection of lymph nodes only very few extracellular (and no budding) particles are seen and these have the same morphology as particles reported in 13/15 (87%) of patients with "non-HIV" lymphadenopathies[17].

Science advances by critical assessment of data and thorough testing of hypotheses. These principles will serve the cause of basic AIDS research and eventually lead to an understanding of the disease. If one view or another is consequently proven to be erroneous then there is no alternative but for the champions of that view to recant. Until then, let scientific enquiry take precedent over personal polemics.

REFERENCES

1. Ho, D.D., Neumann, A.U., Perelson, A.S., Chen, W., Leonard, J.M. & Markowitz, M. Nature 373, 123-126 (1995). "Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection".

2. Wei, X., Ghosh, S.K., Taylor, M.E., Johnson, V.A., Emini, E.A. et al. Nature 373, 117-122 (1995). Viral dynamics in human immunodeficiency virus type 1 infection".

3. Wain-Hobson, S. Nature 373, 102. "Virological mayhem".

4. Papadopulos-Eleopulos, E., Turner, V.F. & Papadimitriou, J.M. Bio/Technology, 11, 696-707 (1993). "Is a Positive Western Blot Proof of HIV Infection?"

5. Blattner, W.A. Viral Infections of Humans, in: "Retroviruses" 3rd Edition, edited by A.S. Evans, Plenum Medical Book Company, New York, 545-592 (1989).

6 Mortimer, P.P. Med. Internat. 56, 2334-2339 (1989). "The AIDS virus and the AIDS test".

7. Papadopulos-Eleopulos, E., Turner, V.F., Papadimitriou, J.M., Causer, D., Hedland-Thomas, B. et al. Genetica. In Press. "A critical analysis of the HIV-T4-cell-AIDS hypothesis".

8. Klatzmann, D., Barre-Sinoussi, F. & Nugeyre, M.T. Science 225, 59-63 (1984). "Selective tropism of Lymphadenopathy Associated Virus (LAV) for helper-inducer T-lymphocytes".

9. Meyerhans, A., Chevnier, R., Albert, J., Seth, M., Kwok, S. et al. Cell 58, 901-910 (1989). "Temporal Fluctuations in HIV Quasispecies In Vivo Are Not Reflected by Sequential HIV Isolations".

10 Shaw, G.M., Hahn, B.H., Arya, S.K., Groopman, J.E., Gallo, R.C. et al. Science 226, 1165-1171 (1984).

11. Piatak, M., Saag, M.S., Yang, L.C., Clark, S.J., Kappes, J.C. et al. Science 259, 1749-1754 (1993). "High Levels of HIV-1 in Plasma

During All Stages of Infection Determined by Competitive PCR".

12. Layne, S.P., Merges, M.J., Dembo, M., Spuge, J.L., Conley, S.R. et al. Virology 189, 695-714 (1992). "Factors Underlying Spontaneous Inactivation and Susceptibility to Neutralisation of Human Immunodeficiency Virus".

13. Bourinbaiar, A.S. Nature 349, 111 (1991). "HIV and gag".

14. McKeating, J.A., Moore, J.P. Nature 349, 660 (1991). "HIV infectivity".

15. Sinoussi, F., Mendiola, L., Chermann, J.C., et al. Spectra No. 4, 237-243 (1973). "Purification and partial differentiation of the particles of murine sarcoma virus (M. MSC) according to their sedimentation rates in sucrose density gradients".

16. Pantaleo, G. et al. Nature 362, 355-358 (1993)

17. O'Hara, C.J., Groopman, J.E. & Federman, M. Hum Pathol 19, 545-549 (1988).