BACK

Gallo's HIV Isolation: Unanswered Questions and a Plea for Clarification

The enquiries on some of Gallo's scientific contributions (Science, July 15, 1994) have clarified the ethical issues regarding his isolation of HIV, however some basic scientific questions still remain unanswered.

For the isolation and continuous production of HIV, Popovic, Gallo and their colleagues used a T-cell line, HT. By culturing this lymphoid cell line with tissue derived from AIDS patients they claimed to have isolated and continuously produced HIV. This "parental" HT cell line "was tested for HTLV before being infected in vitro and was negative by all criteria including lack of proviral sequences". Irrespective of what the term "HTLV" includes, "all criteria" cannot be more than reverse transcriptase (RT), retroviral particles as seen with the electron microscope, immunofluorescence reactions with patients' sera and proviral nucleic acid detection by hybridisation.

While the HT cultures/cocultures used to isolate HIV were subjected to a number of stimulants including polybrene and radiation, they were compared only to the "parental" HT cell line which was merely "left alone" to serve as control. Furthermore, unlike the "parental" HT culture, all the HT cultures/cocultures, including the cultures infected with "pure" HIV, contained, in addition to HIV, non-HIV material (including cellular and non cellular products), which originated from the AIDS patients. These are all significant variables and affect interpretation.

As far back as 1972, Gallo himself was the first to show that RT which in sucrose density gradients bands at 1.16g/ml can be found in "PHA stimulated (but not unstimulated) normal human blood lymphocytes". Elsewhere he states: "that human leukaemia carried type C virus-related information. An examination of blood samples from normal donors showed the absence of any specific R-MuLV-related information although this clearly depended on the metabolic state or the type of cells used as control since these sequences were found in phytohemagglutinin-treated human blood lymphocytes". (MuLV = Murine leukaemic virus, a retrovirus).
In the proper evaluation of a scientific experiment, appropriate controls are a basic requirement. In the case of retrovirus detection, controls are an essential element for the reasons listed below:

1. The human genome contains DNA sequences, sometimes hundreds to thousands, of many retroviral families including HIV sequences. [1,2,3]
2. New retroviruses may arise by combination between retroviral DNAs, DNAs of defective retroviruses or even by the rearrangement of cellular DNA.[4]
3. Cells in culture, sooner or later, spontaneously start to release retroviruses. The appearance can be accelerated and the yield increased a million-fold by stimulating the cultures with a number of agents, including those used in the HT AIDS cultures/cocultures.[5] In fact, as far back as 1976, George Todaro, a well known retrovirologist, and his colleagues at the laboratory of Viral Carcinogenesis, National Cancer Institute, emphasised "that the failure to isolate endogenous viruses from certain species may reflect the limitations of in vitro cocultivation techniques".[6]
4. Gallo and Montagnier agree that no HIV (RT, retrovirus-like particles, antigen antibody reaction, HIV genomic sequences) can be detected in T-cell

cultures/cocultures with tissue from AIDS patients, unless the cultures are stimulated.

5. Monocytes from HIV^+ patients in which no HIV DNA can be detected, even by the use of polymerase chain reaction, become positive for HIV RNA after cocultivation with normal ConA activated T cells, leading the authors to conclude that "HIV expression can be activated in monocytes which lack detectable HIV DNA".[7]
6. An antibody found in AIDS patient sera binds to an 18kD antigen in: HIV infected peripheral blood T cells and HIV infected HUT78 cells and because of this, the antigen is considered to be an HIV protein. However, PHA stimulated normal, uninfected peripheral blood T cells and PHA stimulated non-infected HUT78 cells, but not unstimulated peripheral blood T cells and HUT78 also have a protein of the same molecular weight which react with AIDS patient sera.[8]
7. Mixed lymphocyte cultures (cocultures) lead to retroviral expression.[9]
8. Extracts, even from normal unstimulated cells, when added to the cultures may increase retroviral expression.[10]
9. All the cultures/cocultures from tissues from AIDS patients contain non HIV material including cellular derivatives.

Regarding the HT cell line and thus its clone (H9) used by Gallo and his colleagues for the "isolation" and "continuous" production of HIV, one must also keep in mind that:

1. As far back as 1972, Gallo and Todara, presented evidence "that human leukaemia cells contain reverse transcriptase with antigenic properties of the polymerase of type C virus". Before the AIDS era many researchers detected "retroviral" particles in several malignant tissues. HT is a leukaemic cell line. Gallo, Popovic and their colleagues reported virus-like particles with morphological characteristics which they ascribed to HIV in the HIV infected HT cell line and no particles in the "parental" HT line. However, many authors have observed that the HIV infected HT (H9) cell line contains, in addition to the particles with morphological characteristics ascribed to HIV, several other viral-like particles with morphological appearances unlike those of HIV and whose nature is unknown.[11,12,13] Moreover, British and American researchers presented evidence that non-HIV infected H9 (HT) cultures contain budding virus-like particles.[14] These two omissions cast doubt on the reliability and significance of Gallo's electron microscopy studies and his conclusion that the detection of virus-like particles with the morphological characteristics ascribed to HIV is proof of an externally derived infectious agent.
2. Thanks to the "Gallo probe", it became known that the HT line is in fact HUT78, a cell line established from a patient with mature T4-cell leukaemia, a disease which Gallo claims is caused by HTLV-I.[15] Although Gallo and Popovic claimed to have tested the "parental" (HUT78) cell culture for HTLV before being infected in vitro and it "was negative by all criteria including lack of proviral sequences", one year earlier, in 1983, Gallo claimed to have shown that HUT78 "contained HTLV proviral sequences".[16]

Gallo and his colleagues "isolated" HIV by density gradient banding, a method which cannot distinguish between retroviruses. Since the H9 (HUT78) cultures contain a retrovirus, HTLV-I, even when not infected with HIV (that is, cultured with HIV infected tissues from AIDS patients or contaminated with LAV), how can one be confident that:

1. The retrovirus isolated from H9 cultures/cocultures is HIV and not HTLV-I or even a combination of the two?

2. The antibody tests based on antigens derived from H9 cultures represent reactions with HIV antigens and not antigens of HTLV-I?
3. The retroviral genetic sequences derived from the H9 cell line which are used to synthesise both the probes and primers for the hybridisation/PCR studies are indeed genomic sequences of HIV and not those of HTLV-I or a combination of the two?
4. Could both:
   (a) Gallo's failure to detect the HIV genome in the T cells of AIDS patients[17],
   (b) and his claim that the nucleic acid sequences of "the genome of HTLV-III are homologous to the structural genes (*gag, pol* and *env*)" of HTLV-I.[18]
   be explained by the inadvertent use of the HTLV-I genome (or a combination of the HIV and HTLV-I genome), instead of HIV in his hybridisation studies?

The above questions must be answered not only because they address basic scientific problems but, and most importantly, because they have profound diagnostic and clinical implications.

Eleni Papadopulos-Eleopulos
Bruce Hedland Thomas
David A Causer

Department of Medical Physics
Royal Perth Hospital
Perth, Western Australia 6001

Valendar F Turner

Department of Emergency Medicine
Royal Perth Hospital

John M Papadimitriou

Department of Pathology
University of Western Australia
Stirling Highway, Crawley
Western Australia 6009

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