Acquired immune deficiency syndrome

Retroviruses linked with AIDS

from Robin Weiss

THE publication of four papers[1-4] in the 4 May issue of *Science* by R. C. Gallo and colleagues on a link between acquired immune deficiency syndrome (AIDS) and a retrovirus related to human T-cell leukaemia virus (HTLV) was preceded by much comment which has generated considerable confusion. The main causes of this sorry state of affairs are the intense interest in, and fear of, AIDS and the decision that the US Secretary of State for Health should herself announce 'the identification of the cause of AIDS' and hence the 'means of preyention'. Moreover, rivalry and lack of cooperation between different US governmental agencies — the Center for Disease Control, the National Institute of Allergy and Infectious Diseases and the National Cancer Institute (NCI) — led to uncoordinated press statements. These have exacerbated the already contentious matter of deciding precedence between L. Montagnier's group at the Institut Pasteur in France, which published first[5,6] but with skimpy data, and Gallo's group at NCI, which delayed submission until a thorough characterization of their virus and repeated isolations from different patients had been accomplished. Furthermore, the two groups are using three different names for what I believe will turn out to be the same virus.

Why look for a virus?

There no longer seems to be any doubt that AIDS is caused by an infectious agent. The view that it might instead result from repeated exposure to different human antigens, while plausible for homosexuals with multiple sexual partners and for haemophiliacs receiving factor VIII pooled from thousands of blood donors, cannot apply to AIDS developing after a single blood transfusion or in infants of affected mothers. What kind of infectious agent, then, might give rise to this disease? Again, the discovery of the transmission of AIDS by blood or by semi-purified blood products helped narrow the field because it is unlikely that a fungus or a bacterium (perhaps secreting an immunosuppressive toxin comparable to cyclosporin A) would be transmitted by such routes. In any case, no sooner had AIDS been categorized as a new, epidemic and usually fatal syndrome in 1981, than the search for its causative agent was directed mainly towards viruses.

AIDS patients, and those with extended lymphadenopathy syndrome (ELAS) which may be either a milder form or early phase of AIDS, are infected with many viruses. Cytomegalovirus, Epstein–Barr virus and hepatitis B virus are examples of ubiquitous persistent infections in the population at risk as well as many other individuals. They may appear in more virulent form in AIDS and ELAS patients but that is probably a result rather than a cause of the disease. In turn, they may play a part in exacerbating immune deficiency once it sets in, but these well known widespread viruses did not seem likely to be the initiating agent of AIDS. Instead the pointers were towards a new virus, or a new satellite agent which might increase the pathogenicity of a common virus, just as the delta agent causes hepatitis B to be more severe.

Montagnier's viruses (LAV and IDAV)

Last May, Montagnier and colleagues reported the transient production of cell-free reverse transcriptase activity and virus particles when leukocytes from an ELAS patient were co-cultivated with fresh T cells from normal subjects[5]. The virus showed no cross-reaction with the core antigens of HTLV-1, the virus that is frequently associated with human T-cell leukaemia, though there was slight cross-reaction with antigens on the membranes of HTLV-1-infected cells. Montagnier named the virus lymphadenopathy virus one (LAV-1). Scepticism of the French group's observations grew because other laboratories had difficulty in confirming them and because of a widely held opinion among electron microscopists that the particles depicted in the paper resembled an arenavirus more than a retrovirus.

At the Cold Spring Harbor Meeting on HTLVs last September, Montagnier presented further data, including electron micrographs of particles that resembled D-type retroviruses with truncated (cylindrical) cores and long stalks where budding of the virus from the plasma membrane of infected cells was incomplete[7]. The major core antigen, p25, of LAV-1 was found to be antigenically related to equine infectious anaemia virus (EIAV), a little-studied retrovirus that causes haemolytic anaemia and fever in horses. In its ultrastructure, EIAV is similar to LAV-1 as seen in the more recent electron micrographs[7,8]. At Cold Spring Harbor, Montagnier also described further isolates of retroviruses from two AIDS patients[7], one of them a haemophiliac. The virus from the haemophiliac and a virus isolated from his brother, who is also a haemophiliac but does not have AIDS, have recently been described in *Lancet*[6] and have been named immune deficiency associated viruses (IDAVs). IDAV is indistinguishable from LAV.

These viruses are promising candidates as aetiological agents of AIDS and ELAS because 60–75 per cent of AIDS patients have serum antibodies that react with the p25 antigen of LAV-1, and because they are cytotoxic *in vitro* to the OKT4$^+$ subgroup of T cells which contains the majority of helper T cells. To date, LAV-1, IDAV-1 and IDAV-2 have not been thoroughly characterized biochemically, and permanent cell lines producing the viruses are not yet available, although nearly a year has passed since the first report of LAV appeared in print[5].

Gallo's virus (HTLV-3)

The virus now described by Gallo *et al.* has been designated HTLV-3 because it is considered to be a new member of the HTLV family (now called lymphotropic, instead of leukaemia, viruses). Some 50 HTLV-3 isolates have been obtained from different AIDS and ELAS patients with the ingenious use of a T-cell leukaemia line (HT) that is highly permissive to HTLV-3 replication[1]. The infection of HT by HTLV-3 is easily detected by the formation of syncytia similar to those we described for HTLV-1 and HTLV-2 (ref. 9). The continuous production of high titres of HTLV-3 in HT allowed Gallo's group to characterize the viral proteins and prepare antibodies to them. Schüpback *et al.*[3] report a cross-reaction of HTLV-3 p24 with other HTLV strains, particularly HTLV-2. Genome analysis, yet to be published[10], indicates that HTLV-3 is significantly related to HTLV-1 and HTLV-2.

More than 80 per cent of ELAS and AIDS patients have serum antibodies that are related to specific HTLV-3 antigens, especially the envelope glycoprotein, gp41. A relatively high proportion of a small sample of homosexuals and drug addicts without AIDS also have HTLV-3 antibodies.

Are HTLV-3 and LAV/IDAV the same virus?

The NCI group place their AIDS virus firmly in the HTLV family whereas the French group have emphasized that their virus has antigens that cross-react with EIAV and that it lacks relationship to HTLV-1. Nonetheless, in my view the similarities between HTLV-3 and LAV are more remarkable than the discrepancies. Disregarding the original electron micrographs of Barré-Sinoussi *et al.*[5], the ultrastructure of both viruses is very similar (compare Fig. 2 of Vilmer *et al.*[6] with Fig. 1 of Gallo *et al.*[2]). While HTLV-1 and HTLV-2 endow OKT4$^+$ cells with indefinite growth potential, neither HTLV-3 nor LAV has this capacity for 'immortalization'. Both HTLV-3 and LAV can be passaged as free virus, whereas HTLV-1 is much less stable and unlikely to survive the process of factor VIII preparation — although we have reported cell-free transmission of HTLV-1 (ref. 11).

The apparent differences in immunological cross-reactions could be explained by the different tests and reagents used by

the two groups, particularly as HTLV-3 is more distant from HTLV-1 than HTLV-2 and most AIDS and ELAS patients lack antibodies recognizing p24 of HTLV-1. Given the relationship claimed between LAV and EIAV it will be interesting to ascertain whether the latter belongs to the HTLV family (which includes bovine leukosis virus). The relationship of HTLV-3 and LAV could be rapidly resolved by an exchange of reagents between the two laboratories.

A serological test devised in Essex's laboratory[12] (the 'HTLV-MA' assay) appears to correlate closely with AIDS and ELAS in homosexuals, haemophiliacs and other recipients of blood transfusions[12-14]. The HTLV-MA test is an immunofluorescence assay of the binding of serum to the surface of HTLV-1-infected cells, in particular to the gp61 envelope precursor of HTLV-1 that has been defined by Hattori *et al.*[15]. I have been frankly dubious about HTLV-MA as a specific test of HTLV-1 antibodies because of its lack of correlation with our more specific env antigen assays[9,16] and because faint fluorescence at 1 in 4 dilutions of serum were counted as positive. It now seems much more likely that the HTLV-MA weakly cross-reacts with HTLV-3 or LAV. No doubt Essex *et al.* will re-analyse their sera using a more appropriate antigen. Indeed, as mentioned earlier, a weak cross-reaction by live-cell immunofluorescence between sera of LAV-positive patients and HTLV-1-infected cells has been observed[5].

Immunodeficiency caused by other retroviruses

One of the reasons for suspecting that a retrovirus might be the cause of AIDS is that several animal viruses (including some strains of avian and feline leukaemia viruses) are known to cause various kinds of immunodeficiency, anaemia and aplasia. Indeed, it has not been lost on opportunistic retrovirologists that the US AIDS budget is a lucrative source of research funds. For years, the immunodeficiencies caused by some retroviruses were regarded as a nuisance because infected animals died of 'laboratory' infections before they could develop leukaemia. The focus of pathogenesis has curiously changed in recent months with an eruption of vintage viruses carrying new appellations such as FAIDS (feline AIDS) and SAIDS (simian AIDS).

The prototype D-type retrovirus, the Mason-Pfizer monkey virus (MPMV), was first isolated from a rhesus monkey in 1970 (ref. 17). Much effort was expended in attempts to demonstrate oncogenic properties of MPMV but as Fine and Schochetman's excellent 1978 review[18] relates, the only pathogenic effect of MPMV was runting and immunodeficiency. Recently, outbreaks of simian 'AIDS' in the New England and California regional primate centres have been closely associated with a new strain of D-type virus related to MPMV[19,20]; experimental inoculation of this virus into young monkeys induces the disease [20]. Simian AIDS differs from human AIDS in affecting a much wider spectrum of blood cells than OKT4+ T cells; nonetheless the identification of a retrovirus with this disease is of considerable interest. The human AIDS viruses are not antigenically related to MPMV.

HTLV-1 in AIDS/ELAS patients

HTLV-1, until recently known as ATLV in Japan[21], is most closely associated with adult T-cell leukaemia–lymphoma[22], a malignancy of OKT4+ T cells. This clearly influenced Gallo's search for a virus in AIDS patients as it seemed reasonable to surmise from a knowledge of animal retroviruses that the same virus that initiated clonal malignant transformation in one situation, or a variant, might destroy the same target cell population in another situation. Another hint came from the fact that opportunistic infections, as characterize AIDS, frequently accompany the appearance of HTLV-1-induced malignancy.

Last year, Gallo's laboratory reported the isolation of HTLV-1 and proviral DNA from homosexual AIDS and ELAS patients[23,24], and the same virus has been identified in Haitian infants with AIDS[25]. Using serological tests which do not cross-react with HTLV-3/LAV we have also found that about 5 per cent of ELAS patients in London have antibodies specific to HTLV-1 (ref. 26), a far higher incidence than in healthy homosexuals and random blood donors. The significance of HTLV-1 infection in subjects at risk of AIDS is not clear, but from its low prevalence it seems more likely to be a blood-borne infection reflecting the life style of the patients than an alternative aetiological agent of AIDS.

Prospects for controlling AIDS

If HTLV-3/LAV really is the cause of AIDS, and I find the evidence convincing, then several important developments should occur quickly. First, we need to know what proportion of patients at risk actually have evidence of infection. The preliminary report [4] that a substantial proportion of clinically normal homosexuals have HTLV-3 antibodies is worrying, if it is a forewarning of future illness; it is clear, however, that cofactors play an essential part in precipitating AIDS. Second, reliable ELISA screening tests for blood banks are urgently needed, and might have to be used as routinely as tests for hepatitis B virus. Third, there is the prospect of developing prophylactic immunization and perhaps intervention for infected individuals too. As Groopman pointed out in these columns last week[27], the economic and logistical impact of the identification of the cause of AIDS is formidable, and the seropositive individuals will face severe psychological and behavioural problems.

I am most grateful to R. C. Gallo and L. Montagnier for permission to utilize information in papers in press for this article. □

1. Popovic, M. *et al. Science* **224**, 497 (1984).
2. Gallo, R. C. *et al. Science* **224**, 500 (1984).
3. Schüpbach, J. *et al. Science* **224**, 503 (1984).
4. Sarngadharan, M. G. *et al. Science* **224**, 506 (1984).
5. Barré-Sinoussi, F. *et al. Science* **220**, 868 (1983).
6. Vilmer, E. *et al. Lancet* **i**, 753 (1983).
7. Montagnier, L. *et al.* in *Human T Cell Leukaemia Virus* (eds Gallo, R. C., Essex, M. & Gross, L.) (Cold Spring Harbor Laboratory, New York, in the press).
8. Montagnier, L. *et al. Ann. Virol.* (in the press).
9. Nagy, K. *et al. Int. J. Cancer* **32**, 321 (1983).
10. Wong-Staal, F. Personal communication.
11. Clapham, P. *et al. Science* **222**, 1125 (1983).
12. Essex, M. *et al. Science* **220**, 859 (1983).
13. Essex, M. *et al. Science* **221**, 1061 (1983).
14. Jaffe, H. W. *et al. Science* **223**, 1309 (1984).
15. Hattori, S. *et al. Gann* **74**, 790 (1983).
16. Clapham, P. *et al. Proc. natn. Acad. Sci. U.S.A.* **81**, 3083 (1984).
17. Chopra, H. C. & Mason, M. M. *Cancer Res.* **30**, 2081 (1970).
18. Fine, D. & Schochetman, G. *Cancer Res.* **38**, 3123 (1978).
19. Daniel, M. D. *et al. Science* **223**, 602 (1984).
20. Marx, P. A. *et al. Science* **223**, 1083 (1984).
21. Watanable, T. *et al. Science* **222**, 1178 (1983).
22. Hanaoka, M. *et al.* (eds) *Gann Monogr.*, **28** (1982).
23. Gallo, R. C. *et al. Science* **220**, 865 (1983).
24. Gelmann, E. P. *et al. Science* **220**, 862 (1983).
25. Parks, W. in *Human T-cell Tumor Virus* (eds Gallo, R. C., Essex, M. & Gross, L.) (in the press).
26. Tedder, R. *et al.* Unpublished observations
27. Groopman, J. E. *Nature* **308**, 769 (1984).

Robin Weiss is at the Institute of Cancer Research, Chester Beatty Laboratories, Fulham Road, London SW3 6JB.

100 years ago

In a crowded house on Tuesday last the Convocation of the University of Oxford passed the much-debated statute allowing women to enter for "certain of the honour examinations of the University." The statute has been opposed on very different grounds. The old Conservative Oxford School (fast becoming extinct among the resident teachers) of course objected to any change in favour of the higher education of women; with them went a portion for the High Church party, who look with disfavour on any proposal tending to bring women into intellectual competition with men. Others, again, opposed the statute on the ground that it was unfair to men, who have to keep certain terms and pass certain examinations within a specified time if they wish to enter for an honour school, whereas the statute allows women to enter for honours without the same preliminary examinations, and without restrictions as to time and residence. Others again feared an influx of young ladies into Oxford, as likely to destroy the manliness of the undergraduates and spoil the natural modesty of the lady students. To these arguments the success which the present halls for ladies in Oxford have met with is the best answer. Their presence has not revolutionised the University; they have not been a stumbling-block to discipline nor a rock of offence to the Church. The women's examinations were on the same subjects, and the papers were set by the same men, as in the men's honour examinations before this statute passed. Now the same papers will serve for both, trouble will be saved, and the women who obtain honours will win a certificate universally recognised throughout the country. From *Nature* **30**, 19, 1 May 1884.