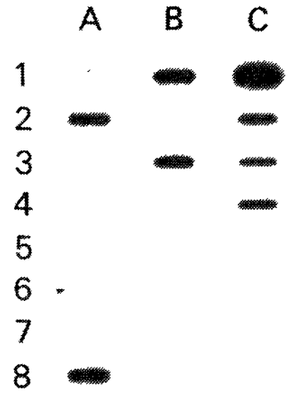
HIV infection in Manchester, 1959

SIR,—HIV may have arisen from primate progenitor strains 40–50 years ago,[1,2] but the earliest recorded cases of AIDS, identified retrospectively, were in the 1960s.[3] The earliest human serum which has been accepted as anti-HIV-1 positive is a sample taken in 1959 in Zaire.[4] We have evidence to show that HIV proviral DNA sequences were present in tissues of a patient who died in Manchester, UK, in 1959. This fatal case of cytomegalovirus and pneumocystis infection in a 25-year-old former naval seaman, published in 1960,[5] was looked at again in 1983, in the AIDS era.[6] Since paraffin blocks were still available we decided to review the case again, using DNA amplification[7] to search for very small quantities of HIV proviral DNA in target cells.

Two cases were investigated, the other being an age and sex matched control who had died in a road-traffic accident in 1959. Sections were cut with separate knives for case and control and with careful cleaning, with alcohol-soaked swabs, of knives between blocks. Sections, in sterile Eppendorf tubes, were coded and sent to the virology unit. Two or three sections were dewaxed by adding 1 ml octane, mixing gently for 30 min, centrifuging at 10 000 *g*, and discarding the supernatant. This process was repeated, followed by two further extractions with ethanol instead of octane. The pellet was dried under vacuum for 30 min. Tissue was resuspended in 200 µl buffer (50 µmol/l "tris" [pH 8·5] with 1 mmol/l EDTA, and 0·5% 'Tween 20') to which proteinase K had been added to a final concentration of 250 µg/ml. After gentle mixing the tubes were left overnight at 37°C, after which a brief spin at 10 000 *g* was used to pellet the residual tissue, the supernatant being retained for DNA amplification.

Amplification was done in a 100 µl reaction volume containing ammonium sulphate 16·6 mmol/l, magnesium chloride hexahydrate 4 mmol/l, sodium EDTA dihydrate 6·7 µmol/l, tris/HCl at pH 8·8 (67 mmol/l), bovine serum albumin 0·017%, 750 µmol/l of each deoxynucleotide triphosphate, and 10 µmol/l of primers. 10 µl cellular DNA and 1 unit of *Taq* polymerase (Boehringer) were added. After denaturation at 94°C for 7 min the reaction mixes were subjected to fifty amplification cycles (55°C for 2 min for reannealing, 75°C for 1·5 min for primer extension, 90°C for 1 min for denaturation). Primers used were SK38/39 specific for the HIV *gag* region.[8] Reaction products (25 µl) were made single-stranded and slot-blotted onto nitrocellulose for probing with the specific oligonucleotide SK19 labelled with ^{32}P.

Only four of the coded samples were HIV positive, and this was confirmed with the use of subsequent extraction products. A positive control (DNA from HIV-infected CEM cells) consistently gave the expected result, as did DNA from normal peripheral blood lymphocytes. When the code was broken the four positive samples were all from the patient who had died with symptoms suggestive of AIDS (figure). The tissues were kidney, bone marrow, spleen, and pharyngeal mucosa. Brain and liver (and all six samples from the control) were negative.



Autoradiograph following slot-blot hybridisation of PCR products and probing with ^{32}P-labelled SK19.

Control case tissues are A1, 4, 6, and 7 and B2 and B4.
Immunodeficient case tissues are A2, 3, 5, and 8, and B1 and 3.
Negative control cells are B5–8
Positive-control cells (DNA extracted from 100 000, 1000, 100, and 10 HIV-infected CEM cells, respectively) are C1–4

We conclude that the patient who died in Manchester in 1959 with an unexplained immunodeficiency and overwhelming pneumocystis and cytomegalovirus co-infection of the lung had HIV infection.

We thank Mrs J. S. Mosley for technical help and Mrs M. Pike for secretarial help.

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HIV and conjunctival malignancies

SIR,—At our outpatient clinic we have noted a striking increase in patients with conjunctival neoplasms. At the same time the frequency of HIV infection in Rwanda has been increasing.[1] To test the hypothesis that HIV is a risk factor for conjunctival malignancy we have done a case-control study.

Between May, 1989, and May, 1990, all patients with clinical evidence of conjunctival dysplasia or malignancy were tested for HIV antibodies by ELISA, with confirmation by immunofluorescence or western blot. Excisional biopsy specimens were sent for pathological examination, and all patients with a diagnosis of conjunctival intraepithelial neoplasia or squamous cell carcinoma (invasive neoplasia) were retained as cases since these lesions seem to be part of a single disease entity.[2] 11 patients met the case definition. To select controls from the same outpatient department we took blood from all patients on two consecutive days at the end of the study period. This could only have underestimated a possible relation between HIV and conjunctival malignancy, since the seroprevalence of HIV in Rwanda is still rising. Patients referred from elsewhere and patients with ocular malignancies were excluded from the control group. We randomly selected two controls, matched on sex and age within five years, for each case and tested their blood for HIV antibody.

The cases consisted of 4 women and 7 men of average age 37 years (range 26–51). The interval between onset of disease and removal of the lesion averaged 6 months (range 1–24). 7 patients were symptom-free, 1 was being treated for tuberculosis, 1 had lymphadenopathy, 1 had a history of herpes zoster, but only 1 had AIDS. 5 patients had conjunctival intraepithelial neoplasia (conjunctival dysplasia 2, intraepithelial epithelioma 3) and 6 had conjunctival squamous cell carcinoma. 9 cases (82%) were HIV seropositive. Of the 22 controls (mean age 36, range 21–49) only 6 (27%) were positive. A Mantel-Haenszel estimate of the odds ratio for multiple matched controls was 13 ($p = 0·005$; 95% confidence interval 2·2–76·9).

Squamous cell carcinomas of the skin, oral cavity, epiglottis, oesophagus, lung, anorectum, and cervix have been reported in HIV-infected patients.[3–5] Although two case-reports of conjunctival squamous cell carcinoma in seropositive patients[6,7] suggested a possible relation between conjunctival neoplasms and HIV seropositivity, firm epidemiological data were lacking until now. Two striking clinical features in our series were the young age of the