British Journal of Haematology, 1989, 72, 387–390

Human immunodeficiency virus detection: correlation with clinical progression in the Edinburgh haemophiliac cohort

R. J. G. CUTHBERT, C. A. LUDLAM, SELMA REBUS,* J. F. PEUTHERER,* D. W. J. AW,* DIANNE BEATSON,† C. M. STEEL† AND BILLIE REYNOLDS *Department of Haematology, Royal Infirmary of Edinburgh, *Department of Bacteriology, University of Edinburgh, and †MRC Clinical and Population Cytogenetics Unit, Edinburgh*

Received 19 December 1988; accepted for publication 13 February 1989

Summary. HIV p24 antigenaemia and virus detection in cultures of peripheral blood lymphocytes were examined in 16 of 18 haemophiliacs infected with HIV by a single batch of Scottish National Blood Transfusion Service factor VIII concentrate. Six (38%) had p24 antigenaemia and 11 (69%) had positive lymphocyte cultures. All seven patients with serious HIV disease (CDC group IV) had positive lymphocyte cultures whereas four (57%) had p24 antigenaemia. Four of nine (44%) patients with asymptomatic HIV disease (CDC groups II and III) had positive cultures and two (22%) had p24 antigenaemia. Twenty-eight of 36 samples from the symptomatic group were HIV culture positive compared with nine of 30 samples from the asymptomatic group ($P<0{\cdot}001$). None of 14 antibody negative haemophiliacs who also received the implicated batch of factor VIII had p24 antigenaemia or positive HIV cultures.

The ability to detect HIV in cultured lymphocytes correlates with the clinical severity of HIV disease in this cohort.

Before the introduction of heat treatment procedures to inactivate HIV in lyophilized blood products factor VIII concentrates carried a high risk of transmission of the human immunodeficiency virus (HIV). In most centres at least 60% of severe haemophiliacs have developed antibodies to HIV. This relates to the predominant use of commercial factor VIII concentrates (Melbye *et al.* 1984; Cheinsong-Popov *et al.* 1986). The clinical consequences have tended to vary from centre to centre with some reporting high rates of morbidity and mortality from the complications of HIV infection, while others have a relatively low rate of such complications. In all these situations the donor sources of plasma (and therefore HIV) have been heterogeneous and it has been difficult to trace precisely the specific exposure events leading to seroconversion as a consequence of HIV transmission in factor VIII concentrates.

We have previously described a cohort of haemophiliacs treated exclusively with locally produced (Scottish National Blood Transfusion Service) factor VIII concentrate who seroconverted as a consequence of exposure to a single batch of HIV contaminated concentrate (Ludlam *et al.* 1985). This batch of factor VIII was transfused between March and May 1984. It is possible that this batch was contaminated by a single HIV positive plasma donation, since the prevalence of HIV infection in the donor population was low in 1983 when the plasma for this batch was collected. Thus the whole cohort may have been exposed to a single strain of HIV (Simmonds *et al.* 1988). This could be of relevance in studying the natural history of HIV infection if differences in virulence and consequent clinical complications are demonstrated for different strains of HIV.

In this report we describe the detection of HIV from patients in the Edinburgh haemophiliac cohort. The study demonstrates a positive correlation between the detection of virus in cultured lymphocytes and progression to serious clinical complications of HIV infection.

Correspondence: Dr R. J. G. Cuthbert, Department of Haematology, Royal Infirmary of Edinburgh, Lauriston Place, Edinburgh EH3 9YW.

METHODS

Patients. Eighteen haemophiliacs were found to have developed antibodies to HIV between April and November 1984. They had received a common batch of unheated Scottish National Blood Transfusion Service factor VIII concentrate between March and May 1984. Fourteen other patients who have remained HIV seronegative also received factor VIII from this batch. These 32 patients form the study group. All were HIV seronegative before exposure to this batch, and there was no evidence of subsequent HIV

exposure. Thirty of the 32 patients in this cohort have been studied two to six times during the fourth year (February 1987 to August 1988) following exposure to HIV.

HIV antibody and HIV p24 antigen detection. Serum HIV antibody was detected by an ELISA technique and results confirmed by immunoblotting (Simmonds *et al*, 1988). Serum HIV core protein p24 was detected by an ELISA antigen capture assay (Dupont). All positive samples were confirmed by neutralization using the reagent supplied by Dupont.

HIV detection in lymphocyte cultures (modified from Levy & Shimanbukuro, 1985). Peripheral blood lymphocytes were harvested from whole blood on a Ficol-Hypaque gradient, and washed twice in RPMI 1640 culture medium (GIBCO). Cultures of $2-3 \times 10^6$ cells/ml were set up in 10 ml of RPMI 1640 medium containing 2% penicillin and 2% streptomycin and enriched with 10% fetal calf serum (Northumbria Biochemicals) and 80 units/ml human recombinant interleukin-2 (Dupont). Mitogenesis was stimulated by the addition of 3 μg/ml phytohaemagglutinin (Wellcome). Cultures were incubated in 25 ml flat-bottomed flasks at 37°C in an atmosphere of 5% CO_2 and air. Culture supernates were harvested every 7 d and stored at −20°C. They were subsequently tested in batches for the presence of p24 antigen by the ELISA antigen capture assay (Dupont). Positive results were confirmed by neutralization, and by a hybridization technique.

Hybridization with a biotinylated probe. A biotinylated nick-translated probe (Hahn *et al*, 1984) was used in the confirmatory dot-blot hybridizations. The probe consisted of the HIV cDNA clone BH10 inserted 3′–5′ in the transcription vector pSP64. The amplified clone was nick-translated with DNA polymerase I in the presence of deoxyribonucleotide and biotin-11-dUTP. Culture supernates were spotted on to nitrocellulose filters. They were then probed under high stringency conditions. The reaction was completed by incubation with streptavidin and biotin-conjugated alkaline phosphate and a solution of nitro-blue tetrazolium and 5-bromo-4-chloro-3-indoly I phosphate.

T-cell subsets and HLA typing. Peripheral blood CD4 lymphocyte counts were estimated by indirect immunofluorescence, and HLA phenotyping was conducted by two-stage complement-dependant lymphocytotoxicity as previously described (Steel *et al*, 1988).

Fig 1. HIV p24 antigen detected in culture supernates from PHA-stimulated peripheral blood lymphocytes grown in interleukin-2 enriched RPMI 1640 medium.

Table 1. Distribution of clinical complications of HIV infection and correlation with ability to detect HIV by isolation from PBL or presence of p24 antigenaemia in 16 HIV antibody positive haemophiliacs

Patient	CDC group	HIV detection in culture (No. tests positive/ No. examinations)	p24 antigen
1	IVD	6/6 (100%)	+
2	IVC-1	2/2 (100%)	+
3	IVA	5/6 (83%)	+
4	IVA	3/5 (60%)	–
5	IVA	4/5 (80%)	–
6	IVA	5/6 (83%)	+
7	IVC-2	3/6 (50%)	–
8	III	2/2 (100%)	+
9	III	4/4 (100%)	–
10	II	0/4	–
11	II	1/3 (33%)	–
12	II	2/2 (100%)	–
13	II	0/2	+
14	II	0/3	–
15	II	0/6	–
16	II	0/4	–

RESULTS

Clinical follow up. The study period was from early 1987 to mid 1988. None of the 14 HIV seronegative patients have developed any clinical sequelae of HIV infection. Seven of the 18 HIV seropositive patients remain asymptomatic (CDC group II), and two others who are asymptomatic have persistent generalized lymphadenopathy (CDC group III) (CDC, 1986). Six patients have developed constitutional symptoms (CDC group IVA) and four of these have been treated with zidovudine. During the study period one patient developed oral candidiasis (CDC group IVC-2), one patient who died from Hodgkin's disease had opportunistic infection (CDC group IVC-1) (CDC group IVD), and one patient died from high grade immunoblastic-type non-Hodgkin's lymphoma (CDC group IVD).

HIV detection

Typical p24 antigen levels from culture supernates are presented in Fig 1. Concentrations of greater than 1000 pg/ml have been observed in some patients. Hybridization with the biotinylated probe confirmed all the positive results.

Thirty-eight samples were examined from the 14 HIV seronegative patients; none were positive. Sixteen of the 18

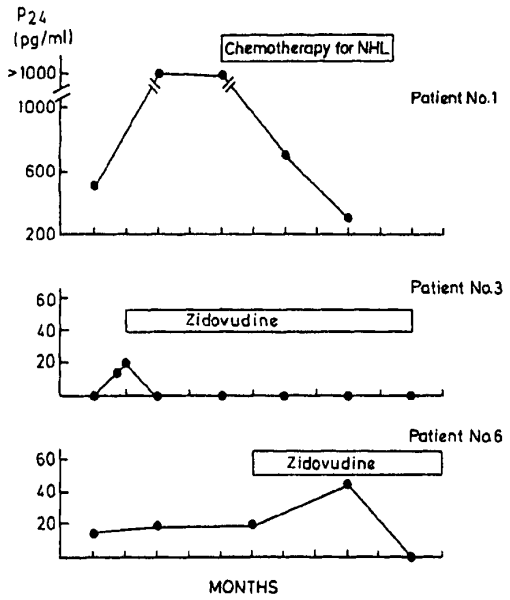

Fig 2. HIV p24 antigenaemia and relationship to antiviral or cytotoxic chemotherapy in one HIV seropositive haemophiliac with NHL, and two HIV seropositive haemophiliacs treated with the antiretroviral drug zidovudine.

HIV seropositive patients have been studied. One patient died from a non-HIV cause, and one patient moved abroad before the study period. Both had constitutional symptoms (CDC group IVA). Table I shows the distribution of HIV detection in culture and p24 antigenaemia comparing symptomatic (CDC group IV) with asymptomatic patients (CDC groups II and III). HIV was detected in cultures from 11 of the 16 (69%) HIV antibody positive patients including all seven patients with evidence of clinical progression to serious complications (CDC group IV) of HIV infection. In contrast less than half (4/9; 44%) of the asymptomatic HIV antibody positive patients were positive. Once a virus could be detected in lymphocyte cultures from any one patient, all subsequent specimens from that patient were positive. In total 28 of 36 samples from the seven CDC group IV patients were positive for HIV in culture compared with nine of 30 samples from the CDC groups II and III patients ($\chi^2 = 13{\cdot}2866$; $P < 0{\cdot}001$).

HIV p24 antigenaemia was less frequent than detection of HIV in cultured lymphocytes, i.e. six of 16 (38%) HIV antibody positive patients (Table I). However, p24 antigenaemia was present in four of seven (57%) symptomatic patients but only in two of nine (22%) asymptomatic patients. There was no detectable p24 antigenaemia in any of the 14 HIV antibody negative patients.

HIV could be detected in cultures from three of the four patients treated with zidovudine before initiation of treatment (patients 3, 6 and 7); they remained consistently positive subsequently. HIV was detected from the fourth (patient 4) within 6 months of beginning treatment. Two of this treatment group were p24 antigen negative throughout the study (patients 4 and 7). The other 2 (patients 3 and 6) were p24 antigen positive before treatment (Fig 2). Patient 3 had a good clinical response to treatment. Patient 6, however, had a poor clinical response with persistence of constitutional symptoms, and was subsequently found to have poor compliance with treatment. Patient 1 had very high concentrations of p24 antigen which fell significantly during a course of combination chemotherapy for NHL (Fig 2).

There was no significant difference in factor VIII concentrate usage between the symptomatic (CDC group IV): mean annual factor VIII $65{\cdot}2 \times 10^3$ units, SD = 47·00; and the asymptomatic (CDC groups II and III): mean annual factor VIII $50{\cdot}67 \times 10^3$ units, SD = 27·37. In addition there was no significant difference between the subgroups who were repeatedly HIV culture positive: mean annual factor VIII $58{\cdot}74 \times 10^3$ units, SD = 40·67, and repeatedly culture negative: mean annual factor VIII $58{\cdot}14 \times 10^3$ units, SD = 24·19. Thus the amount of factor VIII transfused did not influence clinical progression or the ability to isolate HIV.

Absolute CD4 counts were significantly lower in the CDC group IV patients (mean CD4 = $0{\cdot}18 \times 10^9$/l, SD = 0·16) compared with the CDC group II and III patients (mean CD4 = $0{\cdot}52 \times 10^9$/l, SD = 0·12). $t = 4{\cdot}86$, $P < 0{\cdot}001$. It is not possible to assess whether the lower CD4 counts in symptomatic patients independently influences ability to detect HIV in culture, since falling CD4 values are usually found as disease progresses. However, within the asymptomatic group no significant difference in CD4 values was demonstrated between the four patients who were HIV positive on culture (mean CD4 = $0{\cdot}60 \times 10^9$/l, SD = 0·11) and the five who were negative (mean CD4 = $0{\cdot}45 \times 10^9$/l, SD = 0·10).

We have recently demonstrated that expression of the HLA haplotype A1, B8, DR3 is associated with a relatively rapid rate of clinical progression to serious HIV disease (Steel *et al*, 1988). There was a significantly higher rate of successful HIV detection in culture from the six patients with this haplotype (24 of 28 samples) compared with the 10 patients not expressing this haplotype (13 of the 38 samples). However, it is not possible to establish whether HLA A1, B8, DR3 expression is independently associated with improved ability to detect HIV, since the study group is small.

DISCUSSION

The inadvertent exposure of 32 haemophiliacs to HIV due to transfusion of a single HIV-contaminated batch of factor VIII concentrate has allowed us to study the natural history of HIV infection in a well-defined group. The cohort may have been exposed to a single viral strain, over a relatively short period of 1–3 months. There have been no new HIV seroconversions at the Edinburgh Haemophilia Centre since this transfusion accident, although two heat-treated batches of factor VIII were recently proven to contain HIV positive plasma donations (Cuthbert *et al*, 1988). Thus it seems likely that this cohort has had no further exposure to HIV since

1984, and consequently any clinical complications that develop relate directly to HIV infection from this batch of factor VIII. Compared with other cohorts, the rate of morbidity and mortality in this cohort is relatively high with half the HIV seropositive patients having developed serious clinical complications within 4 years (Goedert *et al*, 1986; Eyster *et al*, 1987). Thus in clinical terms the implicated viral strain appears to be particularly virulent.

We have demonstrated that the ability to detect HIV in lymphocyte culture correlates with the presence of advanced clinical complications of HIV infection better than p24 antigenaemia. This may reflect the reduced sensitivity of the direct p24 antigen assay due to formation of immune complexes. Removal of lymphocytes from the plasma will avoid this problem. The greater success of detection in cultures will result from enhanced expression of viral proteins, and transfer and expression of virus in fresh T-cells. It is probable that the virus is no longer controlled when the immune system becomes severely deranged in the more advanced stages of disease, and this allows widespread dissemination of virus throughout the body. This is reflected in ease of detection of virus in culture, and increased antigen expression leading eventually to p24 antigenaemia.

All evidence at the time of writing suggests that the 14 HIV seronegative patients have escaped HIV infection following exposure to the implicated HIV-contaminated batch of factor VIII. Previously we have demonstrated that they received significantly lower doses of this batch of factor VIII (Ludlam *et al*, 1985), and therefore may have received no virus or only very low doses which were unable to establish infection. Alternatively small amounts of HIV genomic material may have been incorporated into host DNA without stimulating an immune response and without causing T-cell destruction.

ACKNOWLEDGMENTS

We thank Ms Audrey Trotter for typing the manuscript. This work was supported by the Medical Research Council and the Scottish Home and Health Department.

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