The Edinburgh Haemophiliac Cohort – MRC news, No 48 – September 1990

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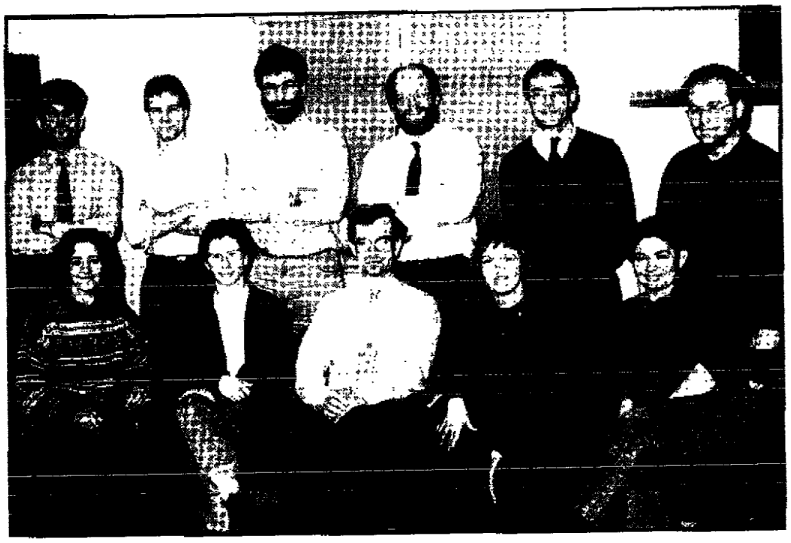
The Edinburgh Haemophiliac Cohort

IN EDINBURGH we have had the opportunity to study a unique group of haemophiliacs who became infected in the Spring of 1984 by transfusion of a single batch of factor VIII concentrate. Whereas most haemophiliacs treated with commercial factor VIII concentrate were infected between 1979 and 1983, those in Scotland were spared infection at that time, because they were treated with factor VIII manufactured from plasma collected locally by the Transfusion Service from donors who were free of HIV infection. That a batch of factor VIII infected with HIV had subsequently been transfused only emerged later when anti-HIV tests became available in late 1984, and the batch was identified by inspecting the detailed patient's transfusion records.



Front row (left to right): Ms H Cameron, Ms A Batchelor, Dr C Ludlam, Dr D Beatson, Dr S Rebus

Back row (left to right): Dr H Watson, Dr A J Leigh Brown, Dr P Simmonds, Dr J Peutherer, Dr M Steel, Dr P Balfe

Of the 32 patients who received the HIV infected batch, only 18 developed antibodies to HIV, while the remaining individuals appear to be free of HIV. These haemophiliacs have been very carefully followed up, with close monitoring for the appearance of clinical manifestations of HIV infection as well as frequent measurements of immune and virological parameters. While all the 14 individuals who were exposed to HIV but who remained anti-HIV negative are well, 10 of the 18 HIV antibody positive patients have progressed to CDC Group IV disease in five years. Detailed monitoring has allowed us to identify some factors which are predictive of clinical deterioration and others that reflect decline of the patients' condition.

Immunological studies

This cohort of haemophiliacs provides a unique opportunity to investigate host factors that determine both the risk of seroconversion for HIV and the rate of progression to symptomatic disease, after infection with HIV.

We have shown that the risk of seroconversion is related to the amount of the contaminated batch of Factor VIII used, but there is no clearcut association with age, HLA type, severity of haemophilia or pre-exposure circulating T cell subset numbers.

Rapid progression to CDC Group IV disease is related to HLA type in this cohort (the haplotype A1 B8 DR3 is a marker of high risk). This is reflected in the progressive fall of circulating CD4 cells, the rate of which distinguishes the A1 B8 DR3 group from the others. Further parameters that track the progress of disease are elevated plasma β-2 microglobulin (β-2-m), Neopterin and IgA. Of these, changes in the β-2-m level appear to be the best predictor of impending symptomatic disease, but a small number of currently asymptomatic patients have shown rising IgA and

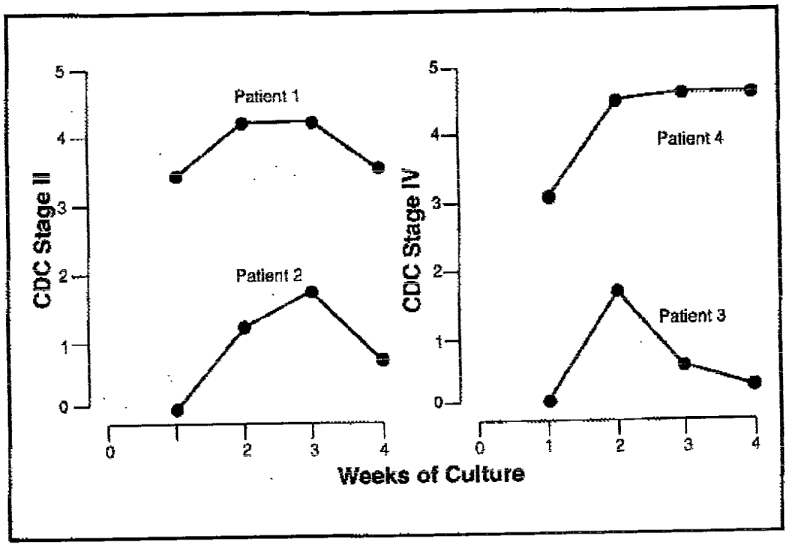


Figure 1. Variation in in vitro culture properties of HIV-1 infected peripheral mononuclear cells from symptomatic and asymptomatic patients.
Mean p24 antigen concentration (Log_e pg/ml) of 4 different culture procedures is given for the first 4 weeks of culture

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neopterin levels within the past year so that further longitudinal study of the cohort is required before definitive statements can be made. A rise in circulating CD8 cells has been seen three years after infection in the antibody positive group but this parameter does not distinguish symptomatic from asymptomatic patients. Similarly, a decline to almost zero levels of delayed-type hypersensitivity skin test responses has been noted in all antibody positive patients.

We are currently studying, both retrospectively and prospectively, changes in plasma levels of soluble Interleukin-2 receptor which promises to be a particularly valuable measure of disease progression. If confirmed, this will further support the hypothesis that immunological activation is a major contributor to the pathogenesis of HIV-related disease.

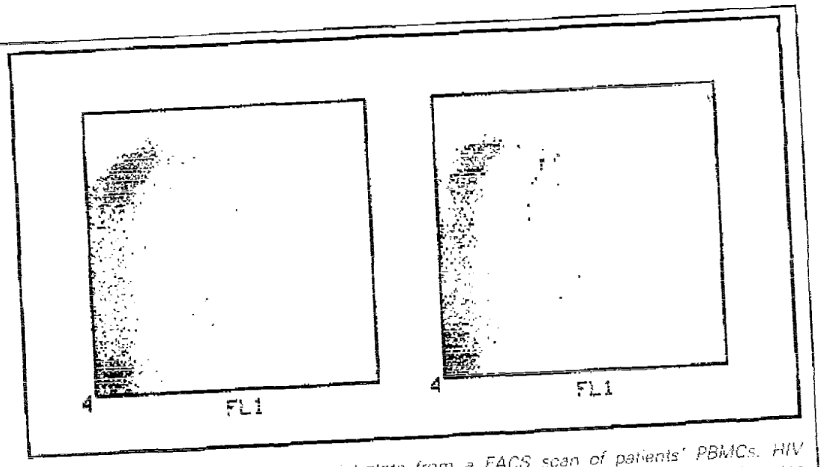

Figure 3. Two dimensional colour dot-plots from a FACS scan of patients' PBMCs. HIV positive patient on the left, control HIV negative subject on the right. T_4 (Thelper) lymphocytes are identified as yellow, T_8 (suppressor/cytotoxic) cells as blue. The relative proportions of yellow to blue are clearly different in the two plots. In fact the T_4/T_8 ratios were 0.14 for the patient and 1.3 for the control subject. (Normal ratio > 1)

Virological studies

We have monitored the progress of HIV infection in the cohort by HIV antigen and antibody assays, culture of peripheral blood lymphocytes and PCR. Once the patients have developed symptoms, it becomes easier to isolate HIV from lymphocytes, and this is a more sensitive indicator of disease progression than the detection of antigens in plasma. We have been able to detect HIV by PCR in all anti-HIV antibody positive individuals. Of the patients who received the infected batch of factor VIII and remained HIV antibody negative, all who have been investigated with PCR have tested negative. This result confirms other virological studies which indicated that the 14 antibody negative recipients of the HIV contaminated factor VIII concentrate have not become infected.

Molecular analysis of HIV

HIV isolates show extensive genetic variability especially in some regions of the genome, and gp120 is perhaps the most variable protein known in its amino acid sequence. This may allow the virus to escape the host immune system. We have been studying the relationships between HIV sequences obtained from each of the cohort patients. The common source of virus and known time of infection allows us to determine accurately the rate and nature of changes in the HIV sequence over time, and to follow the direction of change taken in each of the patients.

We have developed an ultra-sensitive procedure, based on the PCR, which has allowed us simultaneously to titre the number of provirus molecules in the peripheral circulation and to obtain the sequence of selected regions of gp120 from each. In the lymphocytes of each of the patients studied there is approximately one HIV provirus per infected cell but there is wide variation between patients in the abundance of infected cells. In general, higher numbers are seen in symptomatic patients, although the greatest provirus abundance we observed occurred in an asymptomatic individual.

Over 100 nucleotide sequences have now been obtained from eight patients. HIV sequences from several patients show strong similarities, reflecting the common source of infection of this cohort, although two patients show much wider divergence in their HIV sequences. The average rate of nucleotide sequence change among the cohort was about 0.8% per year in two of the immunologically important regions of gp120. The interaction between the virus and the immune response is extremely complex, but the samples donated by these patients will permit a much more accurate assessment than would be possible from work on unrelated virus samples.

This cohort of haemophiliacs has become one of the most extensively studied groups of HIV infected individuals in the world. The success of the project is due, in a large part, to the close collaboration between a group of investigators with a variety of medical and scientific skills. Many are employed full time on MRC project grants awarded by both the Strategic and the Directed Programmes.

A great deal has been learnt from the careful study of these unfortunate individuals. If past results are any predictor of future findings further insights into host virus interaction will emerge with our continuing endeavours.

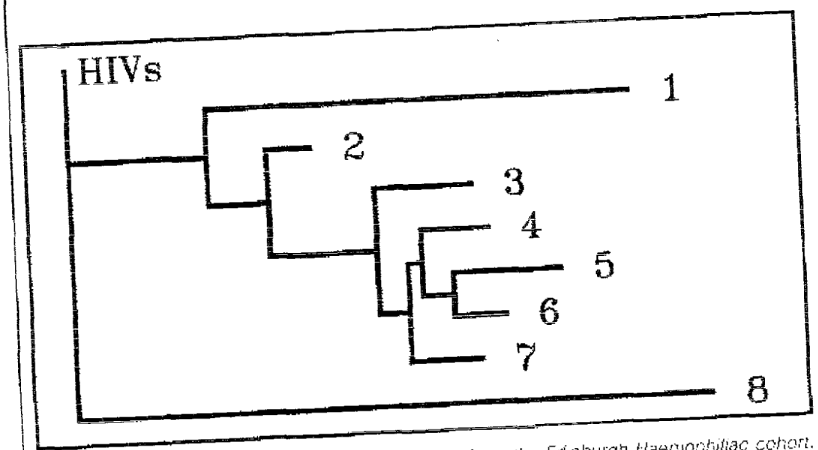

Figure 2. Relationships among HIV env sequences from the Edinburgh Haemophiliac cohort. Sequences from the CD4-binding region from 7 cohort (# 1–# 7) and one non-cohort (# 8) patient were compared. The diagram shows the pattern of relationships. 5 cohort patients (# 3–# 7) have very similar sequences; the non-cohort patient is very different. 'HIVs' indicates the average degree of similarity of several published isolates to the cohort sequences

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