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ORIGINAL ARTICLES

Determinants of HIV disease progression: six-year longitudinal study in the Edinburgh haemophilia/HIV cohort

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Markers of immune function present before infection may determine the subsequent course of disease in HIV-infected individuals. In 1983, we measured immune function in a group of haemophiliacs in Edinburgh. In 1984, 18 of these patients became infected with HIV-1 from contaminated factor VIII. We have followed-up these patients since their seroconversion. The rate of disease progression, as assessed by the appearance or not of AIDS symptoms or signs within five years of seroconversion, was related both to the concentration of total plasma IgM before exposure to infection and to the pattern of specific IgM and IgA anti-HIV response around the time of IgG seroconversion. Disease progression also correlated with concentrations of plasma interleukin-2 receptor (a marker of lymphocyte activation) and with the number and percentage of circulating DR+ve (activated) T cells. Our findings show that the extent of host immune reactivity, which may be genetically determined, is a powerful factor in the pathogenesis of HIV-associated disease.

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Introduction

We have reported previously on changes in clinical and laboratory measures of disease progression in a cohort of 18 haemophilia A patients infected with human immunodeficiency virus-1 (HIV-1) from a common source in 1984.[1,2] An association between the HLA haplotype A1 B8 DR3, rapid decline in circulating CD4 cells, and early onset of HIV-related symptoms was noted in this cohort[3] and subsequently confirmed by other studies.[4,5] We now report a more detailed analysis of response to HIV infection in these patients, showing that measures of immune function before exposure and early in the course of infection (around the period of seroconversion) identify a subgroup who progress rapidly to symptomatic disease.

Patients and methods

Patients

The 18 HIV-infected patients are a subgroup of 32 previously HIV-seronegative haemophiliacs from Edinburgh exposed in 1984 to a batch of factor VIII contaminated with infectious HIV-1.[1,6] All 32 had been studied as part of an assessment of immunological changes in haemophilia during the two years before use of the contaminated factor VIII,[7] and all but 1 (who now lives abroad) have since been followed-up regularly at the Edinburgh Haemophilia Centre. Phylogenetic analysis of HIV proviral sequences has produced strong evidence for a common source of infection in at least 13 of the infected patients.[8,9]

For the analysis of total plasma immunoglobin and activation markers, seropositive patients have been subdivided according to rate of clinical progression of HIV-related disease. The 10 patients in whom Centers for Disease Control (CDC) stage IV disease developed within five years of seroconversion (ie, by the end of 1989) form the symptomatic subgroup, and the remaining 8 patients the symptom-free subgroup. Disease progression in these subgroups was correlated with decline in circulating CD4 cells and rise in plasma β_2 microglobulin.[2] The two subgroups are comparable in age distribution, severity of haemophilia, and severity of liver disease (as judged by plasma alanine aminotransferase concentrations). The average infecting dose of HIV-1 may have been higher for the symptomatic patients than for

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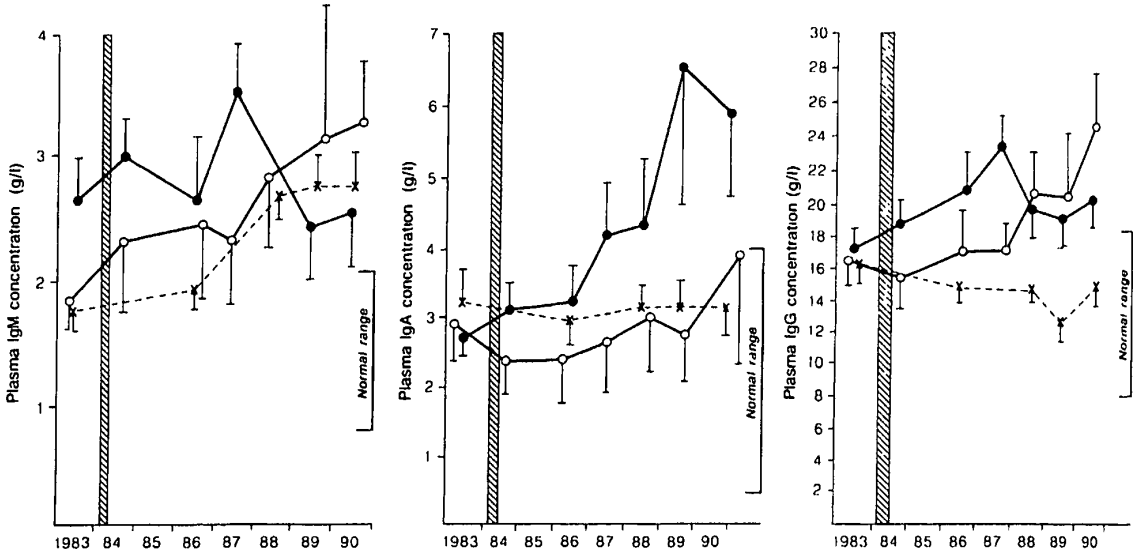

Fig 1—Change in mean (SE) total plasma IgM, IgA, and IgG concentrations.

X=14 patients who remain seronegative for HIV; ●=10 patients (reduced to 6 by 1990) in whom HIV-related disease developed within five years of seroconversion; ○=8 seropositive patients free of HIV-related symptoms up to Dec 1989. Vertical hatched bars represent the period when contaminated factor VIII was in use.

the symptom-free (51 bottles of the contaminated factor VIII used compared with 30), but the difference is not statistically significant, since there was great individual variation of use within each subgroup (ranges 9–109 and 10–60 bottles, respectively). The 14 patients who used the suspect batch of factor VIII, but who did not seroconvert, and who are negative for HIV provirus by a sensitive polymerase chain reaction assay,[6] form the seronegative subgroup in this study.

Assays

In all assays a normal range of values was established by testing of samples from healthy volunteers. Analyses were done either on fresh blood or on plasma that had been stored at −20°C. Soluble interleukin-2 receptors (IL2R) were measured in plasma by a two-site enzyme-linked immunosorbent assay (T-Cell Sciences, Cambridge, USA), immunoglobulins were measured by radial immunodiffusion on Mancini plates (Behring, Hounslow, UK), and activated T cells by surface staining of fresh whole blood with directly conjugated monoclonal antibodies CD3-FITC and DR-PE ('Simultest', Becton-Dickinson, San Jose, USA) and subsequent scoring on an FACScan (Becton-Dickinson) cytopherometer.

Multiple blood samples from around the time of IgG seroconversion were available from 16 of the infected haemophiliacs (the same 16 from whom HIV proviral sequence data have been obtained). These samples were tested by antibody-capture radioimmunoassays for HIV-specific IgM, IgG, and IgA.[9,10] Results are expressed as the ratio of the count obtained with the test plasma divided by the mean count for four replicate negative controls (T/N ratio). Negative samples had T/N ratios of around 1, and samples with ratios greater than 2 were considered reactive. All samples from haemophilia patients were coded and tested blind in duplicate. T/N ratios of paired tests showed discrepancies of less than 10%, (usually less than 5%). Pre-exposure samples tested by capture radioimmunoassay, including samples from 10 haemophiliacs who subsequently seroconverted, were negative for HIV antibody of all immunoglobulin classes (T/N ratios less than 1·4). HIV-specific IgG was quantified by indirect enzyme immunoassay (Du Pont, Wilmington, USA).[11] Immunoblotting,

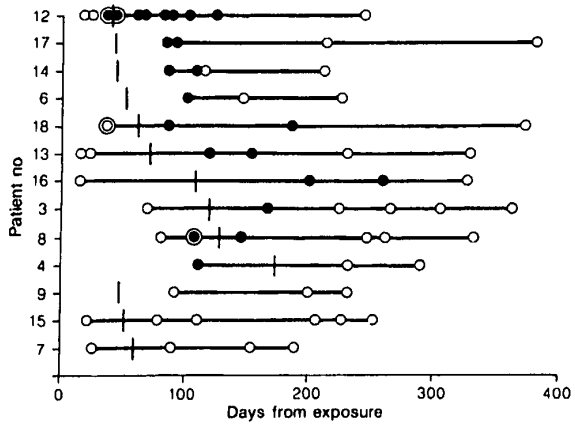

Fig 2—Detection of HIV-specific IgM after exposure to HIV in 13 patients known to have been infected with the same variant of virus.

◉=IgM-positive, p24 antigen-positive; ●=IgM-positive, p24 antigen-negative; ◎=IgM-negative, p24 antigen-positive; ○=IgM-negative, p24 antigen-negative. Time of IgG seroconversion is indicated by vertical bars. Patients are identified by the case numbers assigned in ref 11. Patients 9, 15, and 7 were persistently anti-HIV IgM negative.

TABLE I—CORRELATION BETWEEN DETECTION OF IgM ANTI-HIV AT SEROCONVERSION AND DISEASE STATUS ASSESSED AT END OF 1990

	13 haemophiliacs with sequence-identical virus		All 16 haemophiliacs who used the same batch of contaminated factor VIII	
Diseases status	IgM anti-HIV positive (n=10)	IgM anti-HIV negative (n=3)	IgM anti-HIV positive (n=11)	IgM anti-HIV negative (n=5)
Symptomatic	9	0	10	0
Symptom-free	1	3	1	5
p (Fisher's exact test)	0·014		0·002	

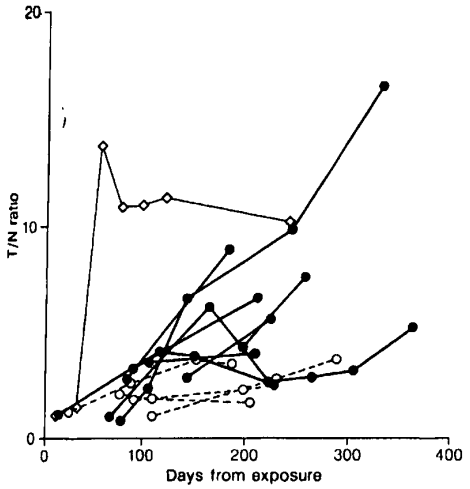

Fig 3—HIV-specific IgA response after exposure to HIV and clinical status after 6 years.

●=Symptomatic patients, ○=symptom-free patients, ◇=patient symptomatic at seroconversion (CDC I) and after 2 years (CDC IV).

anticore protein (Abbott, Abbott Park, USA), and p24 antigen capture (Du Pont) assays were done as described previously.[11]

Results

Total immunoglobulins

The mean concentration of total plasma IgM in pre-exposure (1983) samples was significantly higher for patients in whom rapidly progressive HIV-related disease subsequently developed than for the symptom-free seroconverter and the seronegative subgroups (symptomatic *vs* seronegative, $p<0.02$; symptomatic *vs* symptom-free, $p<0.05$; two-tailed *t* test). Differences among subgroups have gradually been eliminated as the mean concentration of IgM in the plasma of seronegative patients has risen (fig 1). Mean IgG and IgA concentrations were at the upper end of the normal range in pre-exposure samples but there were no differences among patient subgroups (fig 1). For the HIV-seronegative haemophiliacs, total plasma IgG has fallen slightly since 1984, whereas total IgM has risen. However, among the seropositive patients, plasma IgG concentration has risen since infection. During the first three years after infection the rise was greatest in the symptomatic subgroup, but since 1988 the difference between symptomatic and symptom-free subgroups has disappeared. Changes in IgA have been reported previously;[2] the major finding is a steep rise in mean concentration in the seropositive patients, which is almost completely restricted to the symptomatic subgroup.

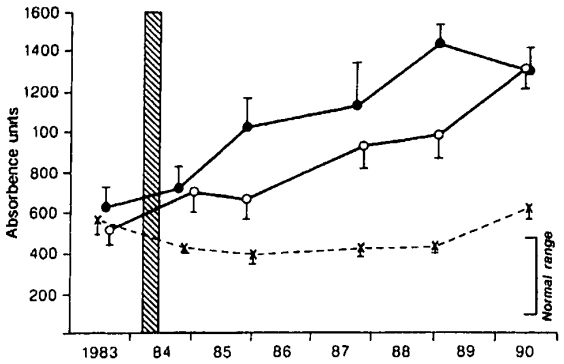

Fig 4—Mean (SE) plasma soluble IL2R concentrations.

See legend to fig 1 for explanation of symbols.

Specific humoral responses to HIV

A detectable specific IgM response to primary infection with HIV developed in 10 of the 13 haemophiliacs known to have been infected with the same virus variant from the contaminated batch of factor VIII (fig 2). This response lasted a mean of 105 days (SD 25 days). Despite frequent sampling in the 100 days after seroconversion, no specific IgM was detected in the other 3 individuals (fig 2). There was a striking association between detection of specific IgM at seroconversion and the development of disease within six years of infection. This association is strengthened when results from the 3 haemophiliacs evidently infected with different HIV variants are included (table I).

In all patients, IgA seroconversion occurred at about the same time as the development of specific IgG and IgM responses. Concentrations of specific IgA rose gradually over the first year after exposure in most individuals; however, the IgA response was much smaller in those patients who remained symptom-free up to the end of 1990 (fig 3). Furthermore, the patient who had symptomatic disease at the time of seroconversion (patient 12) showed a much greater and more prolonged IgA response than the others. Mean specific IgA concentrations at 150 days were 2·3 (range 1·6-3·95) g/l for the symptom-free patients and 5·6 (3·1–11·0) g/l for those who progressed to CDC stage IV disease ($p<0.01$, *t* test).

Lymphocyte activation

Mean soluble IL2R concentrations (fig 4) were slightly higher than normal in pre-exposure samples but with no differences among subgroups. Within a few months of seroconversion, a progressive rise in concentrations in seropositive patients was apparent which, from 1985 to 1989, was more pronounced for the symptomatic subgroup.

TABLE II—MEAN (RANGE) NUMBER ACTIVATED (DR+ve) T CELLS IN BLOOD AND % OF CIRCULATING T CELLS IN BLOOD THAT ARE ACTIVATED, BY PATIENT GROUP

Patient group	July–Dec 1988	Jan–June 1989	July–Dec 1989	Jan–June 1990	July–Dec 1990
Seronegative					
No ($\times 10^9$/l)	1·2 (0·6–2·3)	0·9 (0·6–2·0)	1·8 (0·5–5·5)	0·9 (0·3–2·0)	1·6 (0·7–2·7)
%	7·5 (4–11)	9·0 (4–15)	10·5 (4–32)	7·0 (3–12)	10·0 (4·5–22)
Symptom-free					
No ($\times 10^9$/l)	2·3 (0·5–3·8)	3·0 (1·4–6·3)	4·3 (1·1–100)	4·0 (0·9–8·9)	3·2 (1·3–6·8)
%	25·5 (15–34)	21·0 (13–26)	27·2 (10·0–48)	30·0 (10·5–43)	35·3 (27–48)
Symptomatic					
No ($\times 10^9$/l)	3·7 (2·7–5·4)	4·4 (2·3–6·4)	4·4 (3·2–6·1)	4·4 (1·2–5·7)	3·5 (0·3–5·7)
%	30·0 (20–40)	36·8 (28–43)	45·0 (33–56)	42·5 (27–52)	44·0 (19–64)

Soluble IL2R in plasma are almost certainly shed from activated lymphocytes.[12] Accurate numbers and percentages of activated (DR+ve) T cells have been available only since mid-1988. There is, however, evidence of a progression over the next 30 months (table II). Seropositive patients have had more activated T cells (both as absolute counts and as a proportion of total T cells) in blood than seronegative patients at all time points, and, until lately, the difference has been growing. In terms of absolute numbers, the symptomatic and symptom-free seropositive patients have had similar mean values for most periods, but because symptomatic patients tend to have lower total lymphocyte counts, activated T cells have formed a higher percentage of the total in this subgroup, reaching 50% or more at times. Although activated CD4 and CD8 cells were not counted separately, activated T cells greatly exceeded total circulating CD4 cells in number in many cases; thus it is clear that most of the DR + ve T cells belonged to the CD8 subset.

Discussion

Within the Edinburgh haemophilia/HIV cohort, which is uniquely homogeneous with respect to time and source of infection, we have now shown that the course of HIV-associated disease is related to at least two patient characteristics recognisable before exposure to the virus—namely, HLA type[3] and total plasma IgM concentration. Furthermore, the pattern of specific IgM and IgA responses in the very early stages of infection distinguishes individuals in whom the disease is likely to run a rapidly progressive course from those who remain symptom-free for a long time. Although we could not show a corresponding association with specific IgG response in our cohort, one study, which used very frequent sampling, found a correlation between the titre of the first positive IgG anti-gp120 env and the subsequent rate of disease progression.[13]

Others have noted that, although the clinical manifestations of AIDS are attributable to immune deficiency, they are accompanied by laboratory evidence of immune activation (hypergammaglobulinaemia, activated T cells, high concentrations of circulating cytokines, and autoimmune phenomena).[14-17] Our findings show a very clear correlation between markers of immune activation and disease progression within a homogeneous patient group.

Many patients with haemophilia A show some degree of immunological abnormalities of the above type, even in the absence of HIV infection, probably as a consequence of repeated infusions of foreign protein and/or of past and current infection with hepatitis viruses.[3,18,19] However, these can be no more than contributory factors to the gross and progressive changes seen especially in the symptomatic members of the Edinburgh cohort, since the patient subgroups were comparable in terms of age, severity of haemophilia, and degree of liver damage. Past infection with hepatitis B was universal, although no patients were carriers of hepatitis B surface antigen during the study. All patients were infected with hepatitis C virus and had abnormal liver function[20] (unpublished). Some changes with time were seen in seronegative patients, notably a decline in total plasma IgG and a rise in IgM. The introduction of heat-treated factor VIII from early 1985 may have had some bearing on these changes.

Although we have previously reported[1] that factor VIII requirements throughout 1984 were significantly higher for the seropositive than the seronegative patients, this difference was not detectable before infection. The mean factor VIII usage figures for 1983 were 46 300 units (range 0–99 000), 43 800 units (10 000–68 200), and 38 000 units (0–133 700) for the subgroups later designated symptomatic, symptom-free, and seronegative, respectively. There are no statistically significant differences between any of these values.

When the number of patients studied is small and when several (possibly) independent indices are measured, the risk of a chance deviation from a normal distribution for any one of these indices (a type 2 statistical error) is correspondingly greater. However, the validity of our findings is supported by two observations: first, the mean plasma IgM concentration was higher for symptomatic patients not only in the preinfection sample but continuously over the next four years; and second, the specific IgM anti-HIV response in the early seroconversion phase is probably not independent of total plasma IgM. Our data suggest that underlying the variable rates of disease progression among patients infected with an identical virus are inherent differences in immune responsiveness. The HIV envelope glycoprotein includes several potent antigenic epitopes capable of eliciting a proliferative response from T cells even on primary exposure.[21] The higher concentrations of total IgM, before HIV infection, in the subgroup with subsequent rapid disease progression, probably reflect a general tendency to mount a prolonged and intense IgM response to many antigens—precisely the pattern of specific antibody response to HIV that subsequently developed in these patients.

In an extreme form, this pattern of immune response is seen in the syndrome of immune deficiency with elevated IgM, T-cell activation, and autoimmune manifestations, which often has a genetic basis.[22,23] Although the differences in immune responsiveness among the members of our cohort, for which total plasma IgM concentration serves as a crude indicator, were probably within the normal population range and, in most circumstances, would be clinically insignificant, we suggest that they made an important contribution to the course of HIV infection. The European Collaborative Study[24] of childen born to HIV-infected mothers also recorded a direct relation between total plasma immunoglobulin (specifically a high IgM) and subsequent rapid disease progression.

The underlying basis of immunological "hyper-responsiveness" is uncertain. Although it is tempting to invoke immune-response genes within the major histocompatibility complex, these have yet to be demonstrated convincingly in man. As an alternative, it may be argued that enhanced activity of specific cellular and humoral immune systems reflects a relative failure of non-specific immunity and that this defect accounts for the success of HIV as a pathogen. Whatever the precise mechanisms, much evidence has accumulated that the pathogenesis of HIV disease is related directly to the nature and degree of the host immune response. We have now shown that individuals at risk of rapid disease progression can be identified in the earliest stages of infection and even before exposure. Our findings lend further support to the proposition[3] that modulation of host immune function should be examined further in therapeutic trials among HIV-infected patients.

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Dynamic graciloplasty for treatment of faecal incontinence

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Serious faecal incontinence due to anal sphincter damage should be treated by surgery. Graciloplasty has had limited success because the gracilis is a fast-twitch muscle and fatigues quickly. A favourable outcome in a patient who had dynamic (electrically stimulated) graciloplasty encouraged us to further assess this procedure.

Gracilis muscle transposition was done in ten patients with complete anal incontinence due to anal atresia, sphincter damage, or neurogenic causes, and who had had several other unsuccessful treatments. 6 weeks after muscle transposition, intramuscular leads were implanted and connected to an implantable electric stimulator. Eight patients became continent, one patient still has a diverting colostomy, and a fistula developed in the other patient. Anal sphincter pressure improved from 35 mm Hg without stimulation to 62 mm Hg with stimulation at 8 weeks (mean increase 28 mm Hg [95% confidence interval 18, 36], $p<0.01$). Retention time of a phosphate enema increased from 22 to 281 s (mean increase 259 s [82, 436], $p<0.01$). Defaecography showed that the new sphincter was functioning. Defaecation was possible when the stimulator was turned "off" with a magnet.

Dynamic graciloplasty can restore continence and it improves quality of life in faecally incontinent patients for whom other treatments have been unsuccessful.

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Introduction

Faecal incontinence is a distressing disorder and its prevalence is substantial but difficult to estimate.[1] Serious incontinence due to anal sphincter damage should be treated by surgery. When no functional sphincter remnant is

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