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Can repeated plasma donation by asymptomatic HIV-infected individuals delay the onset of AIDS?

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SUMMARY

Healthy HIV-positive regular donors of plasma in a programme of passive immunotherapy for AIDS patients were studied over a period of about two years. None developed symptoms of clinical progression; most seemed to make substantial gains of CD4 cells by comparison with asymptomatic individuals who were not donating. The effects of donation did not seem to diminish with repetition, and donor CD4 counts tended towards stabilizing within normal limits. Asymptomatic HIV-positive individuals were compared immunologically with 'normals' and people with AIDS, using a battery of 25 measurements on peripheral blood. The immunological profiles of donor and non-donor asymptomatics, indistinguishable at the start, became dissimilar: donors' profiles resembled AIDS less, non-donors became less like 'normal' and a few non-donor results could not be distinguished from AIDS. Improvement in the CD4 counts and amelioration of the immunological profile in donors provide prima facie evidence that plasmapheresis may be therapeutic for asymptomatic HIV-positive people. Further studies are justified.

1. INTRODUCTION

Despite advances in preventing opportunistic infections in AIDS, dealing with HIV infection itself or its effects on the immune system has made little headway. Recently, trials of anti-HIV plasma from healthy HIV-positive people (passive immunotherapy (PIT), originally introduced by Karpas *et al*. (1985, 1988, 1990*a*)), seem to have achieved palliation of the disease, showing significantly improved frequency and duration of opportunistic infections in AIDS patients receiving PIT (Vittecoq *et al*. 1992, 1995), and possibly reduction in the death rate in those starting with CD4 counts of 50–200 (Levy *et al*. 1994).

The feasibility of this therapy depends upon healthy, HIV-positive donors; so the consequences of repeated donation for them are important. Cummins *et al*. (1991) observed that their levels of anti-HIV antibody remained stable or increased, while their CD4 counts 'had actual cell counts significantly higher than the predicted value'.

The Cambridge–London Passive Immunotherapy (PIT) Programme, which has offered PIT on a compassionate basis to AIDS and ARC patients, has been monitoring healthy HIV-positive plasma donors regularly. The peripheral blood lymphocytes of donors attending the Royal London Hospital provide intriguing evidence that plasmapheresis may be therapeutic in early stages of HIV disease.

2. PARTICIPANTS AND METHODS

(*a*) *Participants*

Ethical committee approval was obtained for the programme. Prospective donors were fully informed of potential risks before giving written consent. Most were recruited as partners or friends of the plasma recipients. Donors were: (i) HIV-positive, (ii) willing to undertake a regular, long-term commitment to donating plasma, (iii) healthy and certified fit to undergo plasmapheresis by their own physician, (iv) without previous HIV-related illnesses, (v) not on immunomodulating or anti-HIV drugs, (vi) tested with anti-HIV titres of 1 in 160 or better (in the Karpas cell test, which has been shown to correlate with the level of anti-HIV neutralizing antibody (Karpas *et al*. 1988)), (vii) with CD4 counts of 500 mm^{-3} or better during the previous three months, (viii) older than 20, and (ix) not pregnant.

Donation was suspended, temporarily or permanently, if a donor's anti-HIV antibody decreased by two dilution steps or more in the Karpas test, or the CD4 count fell below 400 mm^{-3}.

(*b*) *Plasmapheresis*

Each month 830 ml plasma was taken (two female donors donated 500 and 600 ml, only) with the Autopheresis C (Baxter Health Care), without fluid re-

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placement. Donors were instructed to eat a few hours beforehand and to drink before and during plasmapheresis.

(*c*) *Monitoring*

At each donation, blood was taken for anti-HIV antibody and lymphocyte subset determination. In a few cases, the first CD4 counts were found below 500 cells mm^{-3}. These donors were withdrawn. One donor donated twice as a result of laboratory delays. Blood and serum were stored. Anti-HIV titres were measured in Cambridge using the Karpas cell test (Karpas 1990*b*). Lymphocyte subsets were determined with a Becton Dickinson FACScan cytometer and CD3, CD4, HLA-DR, CD57, CD16, CD19 reagents in the Clinical Laboratory of the Department of Immunology at the Royal London Hospital. The laboratory participated in and organized national quality assurance schemes in clinical flow cytometry.

(*d*) *Database and statistical methods*

Databases were set up for: (i) PIT donors, (ii) Ambrose King Centre (AKC) clients, and (iii) 82 adult individuals from a normal database described in detail elsewhere (Hulstaert *et al*. 1994; Erkeller-Yuksel *et al*. 1992). Two subsets were created from AKC data; (i) AKC5—consisting of sequences where the first record in 1992–1993 had a CD4 count $> = 500\ mm^{-3}$, and (ii) AKC-A—the first entries classifiable as AIDS results for 78 patients in 1992–1993 (on attendance or conversion to CDC category C, Centers for Disease Control, 1993). CSS/3:Statistica was used for all statistical methods. During estimation or analysis, non-significant or redundant terms or variables were progressively eliminated.

Censoring of the modelling data was carried out by identifying outliers from normal probability plots of residuals and eliminating those inconsistent in the original data ('rogues') with other results for the particular individual. In a few AKC5 cases, where the 'rogue' was first in a sequence, the rest no longer met the entry criteria and were omitted. Approximately 1% of the PIT and 3% of the AKC5 CD4 data were removed. Fifty-two PIT donors with 354 results, and 51 AKC5 clients with 246 results remained.

Crude estimates of serial correlation were made from Durbin–Watson statistics on model residuals of the ordered databases. Tests were significant at 5% for the PIT data and significant at 1% for the AKC5 data for changes in CD4; but insignificant for proportional rates of change.

For the discriminant analysis, outliers were identified from pooled within-group correlation scatterplots for each pair of variables. One normal and one donor outlier were censored. Logarithmic transforms of absolute counts (except for total white cell and non-lymphocyte count) and logarithms of the percentages of $CD4^+HLA\text{-}DR^+$, CD8, $CD8^+HLA\text{-}DR^+$, $CD8^+CD57^+$, $CD8^+CD57^-$, NK and B cells were taken; records with zero values for the untransformed variables were omitted. Missing items were deleted casewise.

3. RESULTS AND DISCUSSION

(*a*) *Donor outcomes*

Twenty-six donors were studied during a period of nearly three years while the mean number of donations was 6.8 per donor (range 1–25). The donors' levels of anti-HIV antibody varied by one, or infrequently two, dilution steps throughout the programme, some donors experiencing sustained increases, others a decrease. Overall, it did not change in the first six months (mean dilution change 0.092, s.e. 0.052, $p = 0.08$, $N = 152$), but decreased significantly after 12 months (mean dilution change -0.339, s.e. 0.129, $N = 59$). The six month/12–24 month difference was $t = 3.610$, $p = 4.7 \times 10^{-4}$. No donor developed symptoms indicating clinical progression of disease while participating in the programme.

(*b*) *CD4 counts*

CD4 T helper cell counts in HIV-infected individuals fall substantially with time: different studies report rates varying between about 70 and 160 cells $mm^{-3}\ yr^{-1}$ (Phillips *et al*. 1989; De Wolf *et al*. 1988; Sheppard *et al*. 1993). Some of the differences may reflect different starting CD4 counts in the study groups: for both homosexuals (Munoz *et al*. 1988), and haemophiliacs (Aledort *et al*. 1992), the actual CD4 count at the outset is an important determinant of the rate of loss.

Our concern was whether the CD4 counts of some donors, e.g. individuals starting with CD4 counts close to 500 cells mm^{-3}, would be adversely affected by donation. To examine this the changes in CD4 counts after the first count were plotted against different numbers of donations and different starting CD4 counts. Figure 1*a* shows this, together with a locally-fitted spline best-fit 3D surface to the data; in figure 1*b* the graph is rotated to show the high end of the starting CD4 counts. Somewhat unexpectedly, the CD4 counts tended to increase with plasma donation for initial CD4 counts in the range 500–900 cells mm^{-3} (figure 1*a*), but above this range they decreased (figure 1*b*).

Since our donors were not a single cohort of individuals these trends might be purely artefactual, produced by chance or the PIT programme, acting to select participants in the long-term who happened to have had suitable CD4 counts at the beginning. If so, the trends should disappear on examining long-term and short-term donors separately. The donors were divided into long-term and short-term groups at ten donations, when the trends were apparent and the long-term group not too small. Figure 2 shows the changes over the first ten donations: in figure 2*a* the long-term donors, and in figure 2*b* the short-term donors. The subgroups exhibit the same behaviour as the overall group of donors during this period, with only minor differences. Short-term donors appeared to respond with greater increases at low starting CD4 counts and smaller decreases at high starting counts.

To deal with the systematic errors that can arise in measuring absolute CD4 counts, the PIT group was

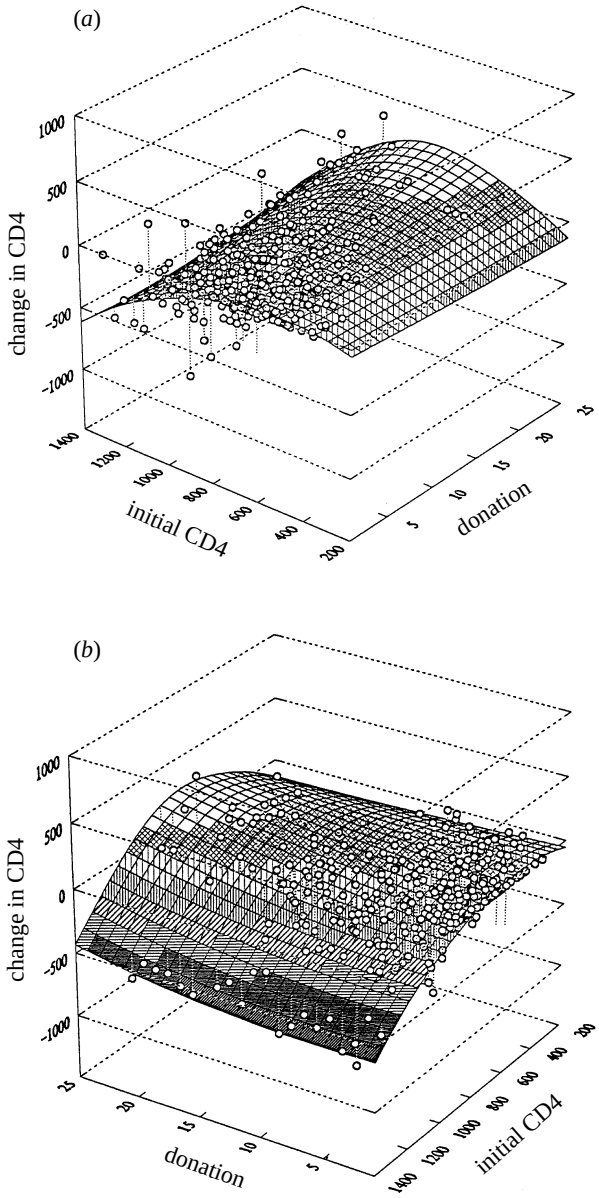

Figure 1. Changes in CD4 count in plasma donors at different times after recruitment. Axes show the change in CD4 count (cells mm^{-3}, z-axis) for different numbers of donations and for different starting CD4 counts (cells mm^{-3}). (*a*) Direct view: numbers of donations on the x-axis, initial CD4 counts on the y-axis; (*b*) rotated view to show high initial CD4 counts: numbers of donations on the y-axis, initial CD4 counts on the x-axis. Increasing initial counts run from right to left.

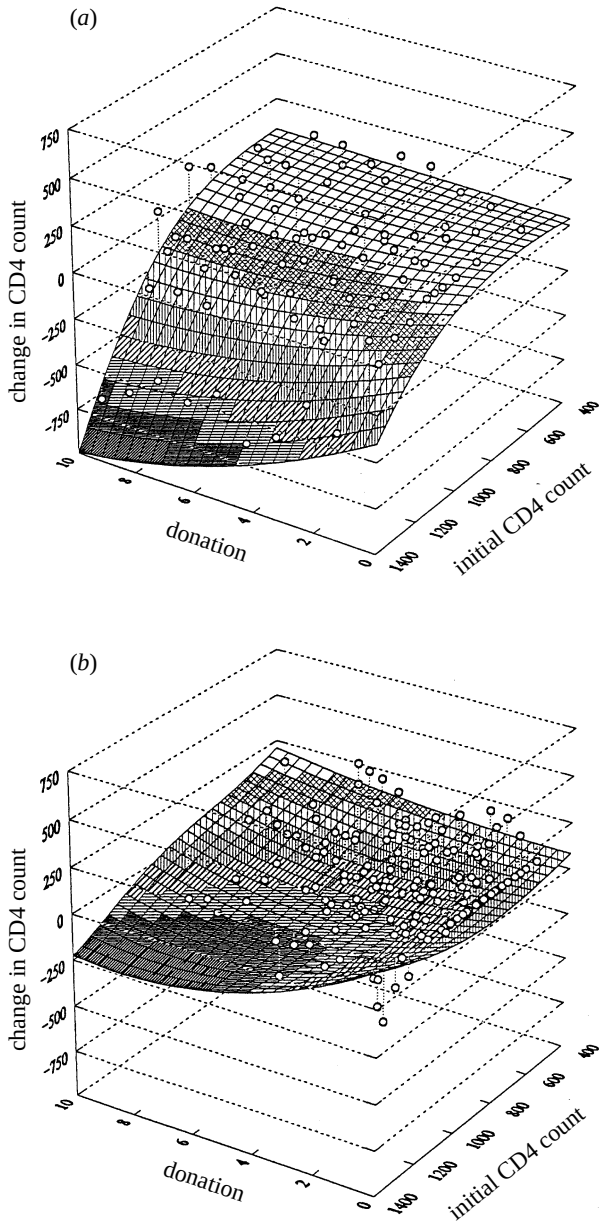

Figure 2. Changes in CD4 count in short-term (up to ten donations) and long-term (more than ten donations) plasma donors over the first ten donations. Axes show the change in CD4 count (cells mm^{-3}, z-axis) for different numbers of donations and for different starting CD4 counts (cells mm^{-3}). (*a*) Long-term donors, rotated view (to show high initial CD4 counts): numbers of donations on the y-axis, initial CD4 counts on the x-axis. Increasing initial counts run from right to left. (*b*) Short-term donors, rotated view.

compared with AKC5, a group of HIV-positive individuals—not being recruited for donors—submitting specimens through the same clinic facilities as PIT during the same period. AKC5 comprised individuals with two or more results, whose first CD4 count in 1992–1993 was 500 cells mm^{-3} or greater. The range and mean of the initial CD4 counts for AKC5 were very similar to PIT (AKC5: mean 695, range 508–1411; PIT: mean 719, range 410–1287).

Changes of CD4 counts were calculated for AKC5 as above and examined, using best-fit 3D surfaces, for possible discrepancies between long-term and short-term subgroups. The changes for PIT and AKC5 groups were then related to the initial CD4 counts and the number of months (for AKC5) or donations (for PIT) using empirical linear, quadratic and cubic polynomial models. Cubic models were significantly better (F-test for loss functions linear or cubic: PIT, $F = 1.265$, d.f.1 352, d.f.2 349, $p = 1.39 \times 10^{-2}$; AKC5, $F = 1.653$, d.f.1 243, d.f.2 238, $p = 5.5 \times 10^{-5}$), the final models accounting for 41.7% (PIT) and 54.3% (AKC5) of the variance in CD4 changes. Since there is some degree of serial correlation in the data, the significance figures cannot be taken too literally. However, table 1*b* (PIT) and 1*c* (AKC5) shows that CD4 changes for both of these were strongly influenced by the starting CD4 count and the time after

Table 1. *Cubic model for changes in CD4 counts in PIT donors and AKC5 clients*

($\Delta = a_1 + a_2N + a_3t + a_4N^2 + a_5t^2 + a_6Nt + a_7N^2t + a_8Nt^2 + a_9N^3 + a_{10}t^3$, where Δ = change in CD4 count, N = donations or months, t = initial CD4 count. (*a*) Coefficients in the full model and their differences. (*b*) Model for PIT CD4 changes after elimination of redundant coefficients. (*c*) Model for AKC5 CD4 changes after elimination of redundant coefficients.)

	a_1	a_2	a_3	a_4	a_5	a_6	a_7	a_8	a_9	a_{10}
(*a*)										
PIT	$8.731_{10}{}^{+2}$	$-3.876_{10}{}^{+1}$	$-3.148_{10}{}^{+0}$	$3.478_{10}{}^{+0}$	$3.946_{10}{}^{-1}$	$6.504_{10}{}^{-2}$	$4.200_{10}{}^{-3}$	$-9.950_{10}{}^{-6}$	$-2.406_{10}{}^{+0}$	$-1.630_{10}{}^{-6}$
s.e.	$(3.628_{10}{}^{+2})$	$(2.942_{10}{}^{+1})$	$(1.385_{10}{}^{+0})$	$(1.559_{10}{}^{+0})$	$(1.722_{10}{}^{-3})$	$(5.546_{10}{}^{-2})$	$(1.467_{10}{}^{-3})$	$(3.095_{10}{}^{-5})$	$(4.236_{10}{}^{-2})$	$(6.902_{10}{}^{-7})$
AKC	$-3.498_{10}{}^{+3}$	$1.084_{10}{}^{+2}$	$1.351_{10}{}^{+1}$	$-9.545_{10}{}^{-1}$	$-1.640_{10}{}^{-2}$	$-2.739_{10}{}^{-1}$	$4.768_{10}{}^{-3}$	$1.029_{10}{}^{-4}$	$-6.440_{10}{}^{-2}$	$6.196_{10}{}^{-6}$
s.e.	$(4.706_{10}{}^{+2})$	$(3.644_{10}{}^{+1})$	$(1.653_{10}{}^{+0})$	$(2.327_{10}{}^{+0})$	$(1.866_{10}{}^{-3})$	$(7.319_{10}{}^{-2})$	$(1.953_{10}{}^{-3})$	$(3.732_{10}{}^{-5})$	$(7.454_{10}{}^{-2})$	$(6.784_{10}{}^{-7})$
p(diff.)	$< 5.00_{10}{}^{-7}$	$1.844_{10}{}^{-3}$	$< 5.00_{10}{}^{-7}$	$1.146_{10}{}^{-1}$	$< 5.00_{10}{}^{-7}$	$2.660_{10}{}^{-4}$	$8.163_{10}{}^{-1}$	$4.000_{10}{}^{-5}$	$4.075_{10}{}^{-2}$	$< 5.00_{10}{}^{-7}$
(*b*)										
estimate	–	–	–	$3.764_{10}{}^{+0}$	–	–	$4.116_{10}{}^{-3}$	$-7.340_{10}{}^{-5}$	$-2.186_{10}{}^{-1}$	–
s.e.	–	–	–	$(4.493_{10}{}^{-2})$	–	–	$(7.647_{10}{}^{-4})$	$(6.531_{10}{}^{-6})$	$(2.734_{10}{}^{-2})$	–
p	–	–	–	$1.320_{10}{}^{-15}$	–	–	$9.650_{10}{}^{-8}$	$3.330_{10}{}^{-25}$	$1.910_{10}{}^{-14}$	–
(*c*)										
estimate	$-3.372_{10}{}^{+3}$	$7.553_{10}{}^{+1}$	$1.319_{10}{}^{+1}$	–	$-1.620_{10}{}^{-2}$	$-2.112_{10}{}^{-1}$	$1.299_{10}{}^{-3}$	$9.654_{10}{}^{-5}$	–	$6.104_{10}{}^{-6}$
s.e.	$(2.896_{10}{}^{+2})$	$(2.233_{10}{}^{+1})$	$(1.102_{10}{}^{+0})$	–	$(1.366_{10}{}^{-3})$	$(5.735_{10}{}^{-2})$	$(5.098_{10}{}^{-4})$	$(3.329_{10}{}^{-5})$	–	$(5.412_{10}{}^{-7})$
p	$4.290_{10}{}^{-25}$	$8.410_{10}{}^{-4}$	$3.870_{10}{}^{-26}$	–	$1.040_{10}{}^{-25}$	$2.869_{10}{}^{-4}$	$1.150_{10}{}^{-2}$	$4.080_{10}{}^{-3}$	–	$6.450_{10}{}^{-24}$

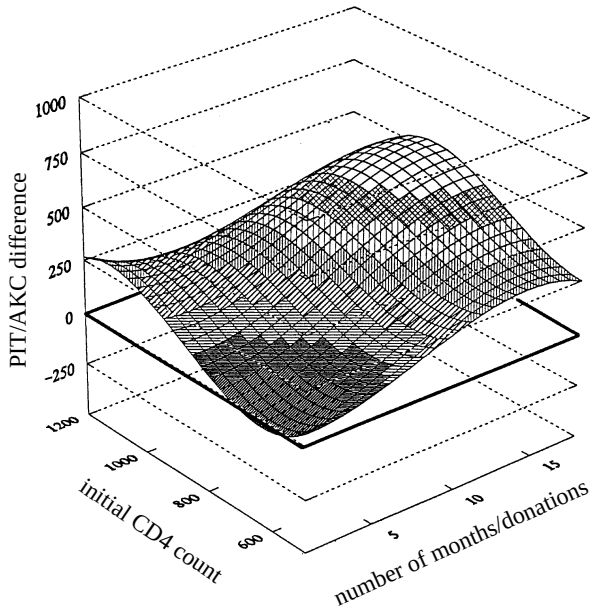

Figure 3. Trend differences for CD4 counts between plasma donors (PIT), and untreated Ambrose King Centre (AKC) patients 1992–1993 whose initial CD4 counts were 500 cells mm^{-3} or greater (AKC). Axes show gains or losses in CD4 (PIT/AKC difference, cells mm^{-3}, z-axis), time after starting (number of months or donations, x-axis), and CD4 count on entry (initial CD4 count, y-axis).

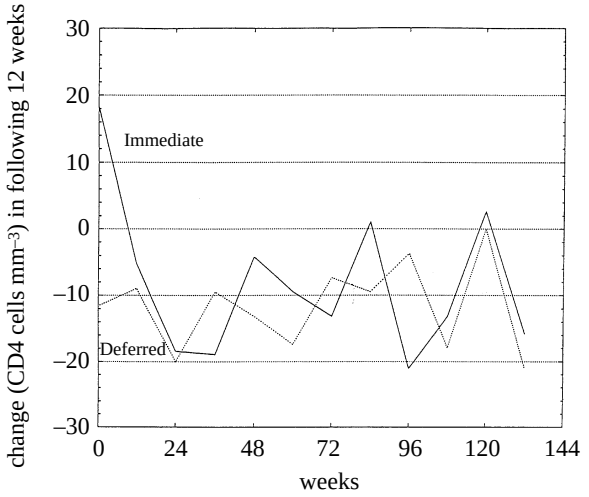

Figure 4. Rate of change in median CD4 counts with immediate or deferred administration of zidovudine in asymptomatic HIV patients. Replotted from figure 2 (Concorde Coordinating Committee 1994). Axes show the change in CD4 counts in each 12-week period (cells mm^{-3} per 12weeks, y-axis) for different times from baseline (weeks, x-axis).

recruitment, but in each group the trend was quite specific. They differ substantially, even comparing the same set of cubic coefficients (table 1*a*). Clearly, PIT had a highly distinctive effect. In figure 3 the difference between the PIT and AKC5 trends is plotted to show effective gains and losses in CD4 counts arising from plasma donation. After the first few months, PIT donors with starting counts of 600–900 cells mm^{-3} began showing substantial effective gains of CD4. At low starting CD4 counts the gain may not have been sustained. However, the cubic model for PIT fits the data less well at high numbers of donations, deviating from the locally-fitted surface above 15 donations, so the long-term outcome is uncertain.

(*c*) *Clinical significance of CD4 counts*

The prognostic value of the CD4 count is generally recognized (Choi *et al.* 1993; Ellenberg 1993; Moss *et al.* 1988; Graham *et al.* 1993), although some doubt about its use as a surrogate marker for clinical outcome in therapeutic trials has been raised, particularly by the recent Concorde trial of zidovudine in asymptomatic HIV: persistent, though minor, differences in median CD4 counts between immediate (Imm) and deferred (Def) users of zidovudine occurred without

Table 2. *Classification of 'normal' and HIV-infected individuals by discriminant analysis of 25 lymphocyte and white cell measurements*

(Post hoc classification; *a priori* probabilities proportional to group sizes.)

		classification categories assigned to group members			
group	percent correct	$p = 0.51938$ 'normals'	$p = 0.22481$ donors	$p = 0.10853$ AKC5	$p = 0.14729$ AIDS
'normals'	100.0000	70	0	0	0
donors	90.0000	1	27	1	1
AKC5	21.4286	0	11	3	0
AIDS	84.2105	0	3	0	16
total	87.2181	71	41	4	17

significant differences in the three-year survival or progression to AIDS or death (Concorde Coordinating Committee 1994). However, this is misleading. Figure 4—in which Concorde's figure 4 is replotted as rates of change in median counts—demonstrates that zidovudine acted simply to interrupt briefly the process of CD4 loss in the Imm group in the first three months; otherwise, it had no effect. According to the mean rates, the loss may have accelerated in the subsequent six months. Such a transient CD4 effect might plausibly delay various ARC- or AIDS-related symptoms slightly, but is hardly to be expected to produce cumulative long-term benefit. In fact, the study reports an early, transient, initially significant, improvement at one year in the progression to ARC, AIDS or death in the Imm group, which disappeared by three years.

Thus the rate of change in CD4 counts, a strong prognostic indicator (Phillips *et al*. 1991; Easterbrook *et al*. 1993), may also be a more useful test than the absolute CD4 count for deciding whether a treatment is actually doing something at a particular time. And the rate may also vary with the starting count (Sheppard *et al*. 1993). The CD4 data for the PIT group were therefore re-examined as proportional rates of change. The counts were expressed as logarithms, and successive changes determined as differences in the $\log_{10}$ CD4 counts, these changes being divided by the interval between successive dates to give monthly rates. The rates were related to the current CD4 counts and the numbers of donations by modelling. After reduction of cubic models, the final model was quadratic, eliminating the number of donations as redundant, so:

$$rate = a_1 + a_2(CD4\ count) + a_3(CD4\ count)^2,$$

where $a_1 = 2.432 \times 10^{-1}$ (s.e. 5.217×10^{-2}, $p = 4.78 \times 10^{-6}$), $a_2 = -4.580 \times 10^{-4}$ (s.e. 1.284×10^{-4}, $p = 4.20 \times 10^{-4}$), and $a_3 = 1.635 \times 10^{-7}$ (s.e. 7.556×10^{-8}, $p = 3.12 \times 10^{-2}$).

Calculating proportional rates reduces serial correlation in the data but also incurs larger errors than straight CD4 changes: the model accounts for less of the variance, 18.6%, compared to 41.7% (for straight CD4 changes). With some caution, therefore, one may infer that (i) the proportional rates of change were strongly influenced by the current CD4 count, but not by the number of donations; (ii) PIT effects are therefore not obviously transient in the short-term, contrasting with the effect of zidovudine at a similar stage of HIV infection; (iii) the proportional rate of change decreases, at a decelerating rate, with higher CD4 counts; and (iv) for low counts, the rate is positive (i.e. CD4 increases), and for high counts, it is negative (i.e. they decrease). The CD4 counts are thus tending to stabilize around some particular level, regardless of the starting count. This should be about 700 cells mm^{-3}, in the middle third of the normal range. A second zone of stability around 2100 cells mm^{-3} is also implied by the equation. It is outside the range of the data and probably artefactual; but, interestingly, a recent study has found two distinct groups of long-term survivors of HIV infection: (i) 'asymptomatic long-term survivors', who have CD4 counts around 600; and (ii) 'non-progressors', with CD4 counts well above normal. These two groups are different from one another in a number of respects (Ferbas *et al*. 1995).

(*d*) *Other lymphocyte subsets*

HIV has profound effects on the immune system apart from destroying CD4 helper cells, e.g. hypergammaglobulinaemia, potent cytotoxic T-cell anti-HIV responses (Kundu & Merrigan 1991; Mihailov *et al*. 1993; Quint *et al*. 1992), and suppressor cells producing soluble inhibitory factors for HIV replication (Walker *et al*. 1986, 1989; Chen *et al*. 1993; Mackiewicz *et al*. 1994). So the percentages and absolute counts, obtained directly or derived by calculation, were recorded for the following: total white blood cells, total lymphocytes, non-lymphoid white cells, T cells, activated T cells ($CD3^+HLA\text{-}DR^+$), CD4, activated CD4 ($CD4^+HLA\text{-}DR^+$), CD8, activated CD8 ($CD8^+HLA\text{-}DR^+$), CD8 'suppressors' ($CD8^+CD57^+$), CD8 'cytotoxic' cells ($CD8^+CD57^-$), NK ($CD16^+$) and B cells ($CD19^+$). The data were obtained for PIT and other relevant groups: 'normal' individuals, AIDS patients (AKC-A), and non-donor asymptomatics (AKC5).

A discriminant analysis—a technique which allows for the intercorrelations of measurements—was carried out in order to distinguish between 'normal', AKC-A, and the starting state of PIT and AKC5 individuals. The technique combines measurements in a number of independent 'roots', summarizing group differences. Only six of the 25 listed measurements contributed significantly: $\log_{10}$ CD4 count, percentage of T cells,

Table 3. *Classification of asymptomatic results with 'normal' and HIV-infected individuals not used in the discriminant analysis, at different times after recruitment*

(*A priori* classification; probabilities same for all groups. Number of individuals misclassified shown in brackets.)

		classification categories assigned to group members				
months	groups	'normals'	asymptomatic	AIDS	total	(error)
0–6	donors	7 (5)	174	3 (3)	184	(8)
	ACK5	2 (2)	35	0 (0)	39	(2)
6–12	donors	2 (2)	68	0 (0)	70	(2)
	AKC5	1 (1)	35	9 (0)	36	(1)
> 12	donors	3 (1)	38	0 (0)	41	(1)
	AKC5	0 (0)	44	3 (3)	47	(3)
	(errors)	(11)		(6)		(17)

and logs of the percentage of activated T, activated CD4, CD8 and activated CD8 cells. The first significant root ($p < 5.0 \times 10^{-7}$) discriminated 'normal' individuals from HIV-infected individuals: 'normals' characteristically possessed higher CD4 counts and lower percentages of CD8, activated CD8 and activated CD4 cells than HIV-infected individuals. The second significant root ($p < 5.0 \times 10^{-7}$) differentiated AIDS patients from asymptomatic HIV individuals: AIDS patients characteristically had lower levels of CD4 cells and lower percentages of CD8 and T cells than the two asymptomatic groups. It was possible to classify individuals as 'normal', AIDS or asymptomatic with considerable accuracy, but not as donor or non-donor (table 2). 'Normal', AIDS and asymptomatics differed markedly from one another (p-levels $< 10^{-18}$); however, the PIT and AKC5 groups were indistinguishable ($p = 0.744$). AKC5 was slightly closer to 'normal' and more distant from AIDS than PIT (squared Mahalonobis distances: AKC5–'normal' 13.89; AKC5–AIDS 22.09; PIT–'normal' 15.02; PIT–AIDS 21.95; 'normal'–AIDS 46.41).

The classificatory functions obtained using the initial PIT and AKC5 results were applied to later PIT and AKC5 results. Marginal changes occurred with time. As table 3 shows, AKC5 tended to have fewer results that could be misclassifed as 'normal' and more as AIDS; PIT had fewer AIDS and a higher proportion of 'normal' misclassifications. The contrasting trend of the AKC5 and PIT groups for individuals misclassified as 'normal' or AIDS was significant ($p = 4.52 \times 10^{-3}$, exact probability from Freeman & Halton (1951)). A second set of discriminant functions was calculated using the PIT and AKC5 data after 12 months. The individuals then were few in number; and with some of them contributing several readings, the data were not entirely independent. Consequently, this analysis may be sensitive to the changing composition of the groups. With that reservation, the PIT group were closer to 'normals' and further from AIDS, and the AKC5 group was equidistant between AIDS and 'normals' (squared Mahalonobis distances: PIT–'normal' 12.26; PIT–AIDS 30.22; AKC5–'normal' 18.83; AKC5–AIDS 17.51; 'normal'–AIDS 47.53). The PIT and AKC5 groups have become distinct ($p = 5.1 \times 10^{-12}$), discriminated by a third 'root' ($p = 2.0 \times 10^{-6}$) characterizing AKC5 by higher percentages of T, activated T and activated CD8 cells, and lower percentages of CD4 cells. This recalls the increased expression of activation markers (Sheppard *et al.* 1993), especially CD38 on CD8 cells, which have been found to indicate poor prognosis in HIV infection (Levacher *et al.* 1992). It suggests the possibility that CD8 responses and CD4 cell loss have progressed in AKC5 but have ameliorated to some degree in PIT donors.

(*e*) *Can donating plasma delay the onset of AIDS?*

Healthy HIV-positive individuals donating plasma apparently gained CD4 cells over a similar group of 51 non-donors attending the same clinic during a two-year period. The effect of plasmapheresis did not obviously diminish with repeated donation, and appeared to be acting to stabilize CD4 counts within normal limits. Changes in CD4 count have prognostic value, attested by several studies, including Concorde, strongly implying that donors should benefit clinically. The immune status of donor and non-donor asymptomatics also tended to diverge with time, donors seeming to remain at the same distance from 'normals' while distancing themselves further from the state of individuals with AIDS, whereas non-donors approached the state of AIDS more closely.

4. CONCLUDING REMARKS

Our study of asymptomatic HIV-infected individuals who have been donating plasma regularly offers prima facie evidence that plasmapheresis in itself could be therapeutic. This prospect deserves serious study and clinical trial. It supports the conclusion of earlier and also recent studies (Stricker *et al.* 1995) that repeated monthly plasma donation is unlikely to be detrimental to healthy HIV-infected people.

This was a study for the Cambridge–London Passive Immunotherapy (PIT) Programme. The participants were R. Hillman, G. Forster, B. Goh (Ambrose King Centre, the Royal London Hospital, Whitechapel, London E1), S. Ash (Pasteur Suite, Ealing General Hospital, Uxbridge Road, Southall, Middlesex UB1 3HW), K. Smith (FACTS Medical Centre, 23–25 Weston Park, Crouch End, London NW8 9SY). This programme is now organized from Ealing General Hospital by Dr S. Ash.

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