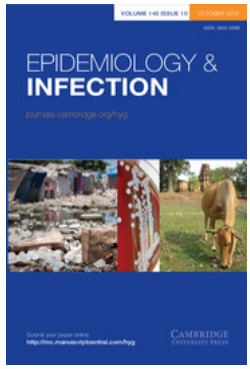

The effect of CMV infection on progression of human immunodeficiency virus disease in a cohort of haemophilic men followed for up to 13 years from seroconversion

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The effect of CMV infection on progression of human immunodeficiency virus disease in a cohort of haemophilic men followed for up to 13 years from seroconversion

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SUMMARY

The effect of prior infection with cytomegalovirus (CMV) on progression of HIV disease in a cohort of 111 men with haemophilia was studied after 13 years follow-up. The relative hazards associated with CMV positivity on progression to AIDS, death and a CD4 count of $0{\cdot}05 \times 10^9$/l were 2·28, 2·42 and 2·34, respectively. CMV seropositive patients were significantly older than the seronegative and this was controlled for by using a Cox proportional hazards model. The relative hazards for the three endpoints decreased to 1·89, 1·82 and 1·93 respectively and were marginally non-significant ($P = 0{\cdot}05$, 0·08 and 0·08 for the three endpoints respectively). We conclude that this cohort continues to show evidence of a 'co-factor' effect associated with prior infection with CMV which is confounded by age but not completely explained by age differences. The potential biological significance of these results is discussed in the context of recent controlled clinical trials which show a survival benefit from long-term high-dose acyclovir, a drug with activity *in vivo* against CMV and other herpesviruses.

INTRODUCTION

Although the human immunodeficiency virus (HIV) is the known cause of the acquired immune deficiency syndrome (AIDS), the incubation period from infection with HIV to the development of AIDS is long and variable [1–4]. This wide variation in the incubation period is far greater than that found for most other acute viral diseases, suggesting that other factors may contribute to disease progression. Among these, infectious agents, termed co-factors, could interact with HIV and increase its pathogenicity.

Different viruses could interact with HIV by a variety of mechanisms [5]. For example, some viruses, including herpes simplex virus (HSV), cytomegalovirus (CMV), hepatitis B virus and Epstein–Barr virus have all been shown to

transactivate HIV *in vitro* [6–9]. However, there are only a few studies which assess the relative importance of these viral co-factors *in vivo*. It is well documented that older individuals experience more rapid progression of HIV disease than younger individuals, perhaps due to a more rapidly declining immune system in older individuals [10–12]. As the prevalence of antibodies to HSV and CMV increases with age [13], any assessment of the potential effect of these viruses on progression of HIV disease must take account of the possible confounding effect of age.

We have chosen to study whether CMV could act as a co-factor for HIV disease in a cohort of men with haemophilia in whom the prevalence of CMV infection is 60%, similar to that seen in young blood donors and attenders at antenatal clinics [14]. We have previously reported that in this cohort prior CMV infection is associated with more rapid progression to AIDS [15] but this has not been confirmed by others [16–19]. Here we extend follow-up to 13 years to assess whether this association remains after adjustment for age differences. Furthermore, sufficient follow-up is now available on the patients to analyse both survival and immune depletion as endpoints.

MATERIALS AND METHODS

Patients and their clinical management

A total of 111 men with haemophilia registered at the Royal Free Hospital Haemophilia Centre, London, became infected with HIV between 1979 and 1985 following treatment with unsterilized blood clotting factor concentrates [20, 21]. All patients are seen at the Centre approximately every 3–6 months when they undergo a clinical and laboratory review. Serum samples are regularly taken and stored at −20 °C. It has been possible retrospectively to test these serum samples for HIV seropositivity and hence dates of seroconversion have been estimated for all patients [20].

Zidovudine has been available for individuals with AIDS and AIDS-related complex (ARC) since 1987, and from October 1988 it has been available as part of the MRC/Agence Nationale de Recherches sur le SIDA (ANRS) Concorde trial of early versus deferred zidovudine. Secondary prophylaxis for *Pneumocystis carinii* pneumonia (PCP) with pentamidine or co-trimoxazole has been available since March 1988, and primary prophylaxis since February 1989. Secondary prophylaxis for candidiasis with fluconazole has been available since March 1988 and primary prophylaxis since April 1990. All patients are advised to start zidovudine and primary prophylaxis once their CD4 count is $< 0{\cdot}2 \times 10^9$/l. Patients developing either PCP or candidiasis are offered secondary prophylaxis regardless of their CD4 count. To date 51 patients have received zidovudine, 35 PCP prophylaxis (26 primary, 9 secondary) and 39 prophylaxis for candidiasis (13 primary, 26 secondary). Currently, whilst acyclovir may be given to patients experiencing herpes zoster repeatedly, it is not routinely prescribed for patients in the cohort.

Laboratory methods

Between 1982 and 1986 absolute CD4 counts were calculated from the lymphocyte count and CD4 percent values [20]. A whole blood lysis method has

been used since 1986, and the percentage of CD4 lymphocytes analysed by flow cytometry, using a FACScan (Becton Dickinson, Crawley, UK) [22]. A monoclonal CD4 antibody (RFT4) to the p55 CD4 antigen was used with a monoclonal CD3 antibody (UCHT1) as described previously [22]. More recently absolute CD4 counts have been directly obtained on an ORTHO Cytoron-Absolute (ORTHO Diagnostics, High Wycombe, UK). Quality control of flow cytometry was monitored as part of the UK National External Quality Assurance Scheme. We have compared CD4 counts in these patients from before and after the change in methods in 1986 and have seen no consistent difference. Antibodies to CMV were measured on early stored serum samples by radioimmunoassay as described elsewhere [23].

Statistical methods

Comparisons of patients with and without antibody to CMV were done using standard non-parametric methods (Mann–Whitney U test [24]). Analyses which assessed the effect of CMV on HIV disease progression were performed using standard survival methods. Plots of survival from seroconversion to endpoints of AIDS and death were estimated using Kaplan–Meier methods [25] and the univariate effect of CMV status on these were tested for significance using the log-rank test. Cox proportional hazards models [26] were used to assess the independent effect of CMV status on disease progression after adjusting for the patient's age and CD4 count in multivariate models using the procedure 'PROC PHREG' in the Statistical Analysis System (SAS) package [27]. For this analysis, progression to an endpoint of a CD4 count of $0{\cdot}05 \times 10^9$/l was also used. The low CD4 count of $0{\cdot}05 \times 10^9$/l was chosen as an endpoint due to the rapidly increasing risk of death once the CD4 count has fallen to this level [28]. The approximate date on which the CD4 count fell to $0{\cdot}05 \times 10^9$/l was estimated by linear interpolation. When studying progression to AIDS or a low CD4 count, patient follow-up was right-censored at death or at the cut-off date for the analysis (31 December 1992), if the patient had not developed AIDS or reached a low CD4 count by that time. When studying progression to death, patient follow-up was right-censored at December 1992 if still alive on that date. CMV status and age at seroconversion were considered to remain fixed throughout follow-up, and the patient's CD4 count was modelled as a time-dependent covariate. In order to assess the validity of the proportional hazards assumption, an interaction term between the logarithm of time and CMV status was added to the model and tested for significance.

In order to estimate the potential effect of CMV positivity on death at certain CD4 levels, the dates on which the CD4 count was estimated to fall below certain levels ($0{\cdot}2$, $0{\cdot}3$, $0{\cdot}4$, $0{\cdot}5$, $0{\cdot}6 \times 10^9$/l) were calculated using linear interpolation. Survival methods were used to assess the prognosis associated with CMV seropositivity, after adjustment for patient age at each CD4 baseline date.

In the UK, prophylaxis for PCP and candidiasis and antiretroviral therapy (zidovudine) for patients without AIDS became available from 1987 onwards. The direct modelling in this cohort of treatment effects is limited as few data are available on individual treatment usage in the cohort. Further, most treatment is instigated once the CD4 count falls below $0{\cdot}2 \times 10^9$/l, resulting in treatment effects

Table 1. *Comparison of CMV-positive and CMV-negative patients in the cohort*

Patient characteristics		CMV-positive	CMV-negative
Number of patients		59	50
Date of seroconversion*	Median	May 1982	April 1982
	Range	Oct 1979–Mar 1985	Nov 1979–Jul 1985
Age at seroconversion (years)†	Median	25·6	18·7
	Range	4·0–77·8	2·1–73·0
Number developing AIDS		30 (50·8%)	14 (28·0%)
Number of deaths		30 (50·8%)	12 (24·0%)

* P-value = 0·77.
† P-value = 0·03.

which are confounded with those of the CD4 count. Consequently, in order to assess whether these results were independent of the treatment, all analyses were repeated with the inclusion of an additional covariate to represent simply the availability of pre-AIDS prophylaxis and antiretroviral therapies. This was included in the model as a time-dependent covariate taking the value of zero before November 1987 and one after. This then allows for the fact that patients who have survived at least to November 1987 have the added survival advantage of any therapy available to them, whether or not they actually receive it.

RESULTS

Antibodies to CMV were measured on early blood samples from patients in the cohort. 59/109 (54%) were found to have antibody to CMV. CMV status is unknown for two patients. A comparison of the patients known to be seropositive and seronegative for CMV is shown in Table 1. CMV positive patients were older at seroconversion to HIV, but in general they did not seroconvert any earlier or later than those who were CMV negative.

By the end of 1992, a total of 44 of the patients had developed AIDS and 42 had died, with Kaplan–Meier progression rates of 47·1 and 48·3% by 13 years, respectively. CD4 counts had fallen below $0{\cdot}05 \times 10^9$/l on at least one occasion in 35 patients, a progression rate of 43·3% by 13 years after seroconversion. Unadjusted for age, CMV status was significantly associated with a faster progression to AIDS ($P = 0{\cdot}009$, log-rank test) and to death ($P = 0{\cdot}008$, log-rank test). Progression rates to AIDS and death, stratified by CMV status are shown in Figs 1 and 2. The effect of CMV status on progression to AIDS and death remains apparent, although reduced slightly, in both older and younger individuals.

Cox proportional hazards models were used to quantitate the effects of CMV seropositivity on the hazards of developing AIDS, of dying or of reaching a low CD4 count, after adjusting for age at HIV seroconversion (Table 2). Using this approach, before adjusting for age, patients known to be CMV positive were over twice as likely to develop AIDS, die or reach a low CD4 count as those known to be CMV negative. After adjusting for the patient's age at seroconversion, the effect of CMV positivity on progression to all three end points decreased slightly and became marginally non-significant ($P = 0{\cdot}05$, 0·08 and 0·08 for progression to AIDS, death and a CD4 count $< 0{\cdot}05 \times 10^9$/l respectively). In order to assess whether the effect of CMV status acts through the CD4 count, the relative hazards

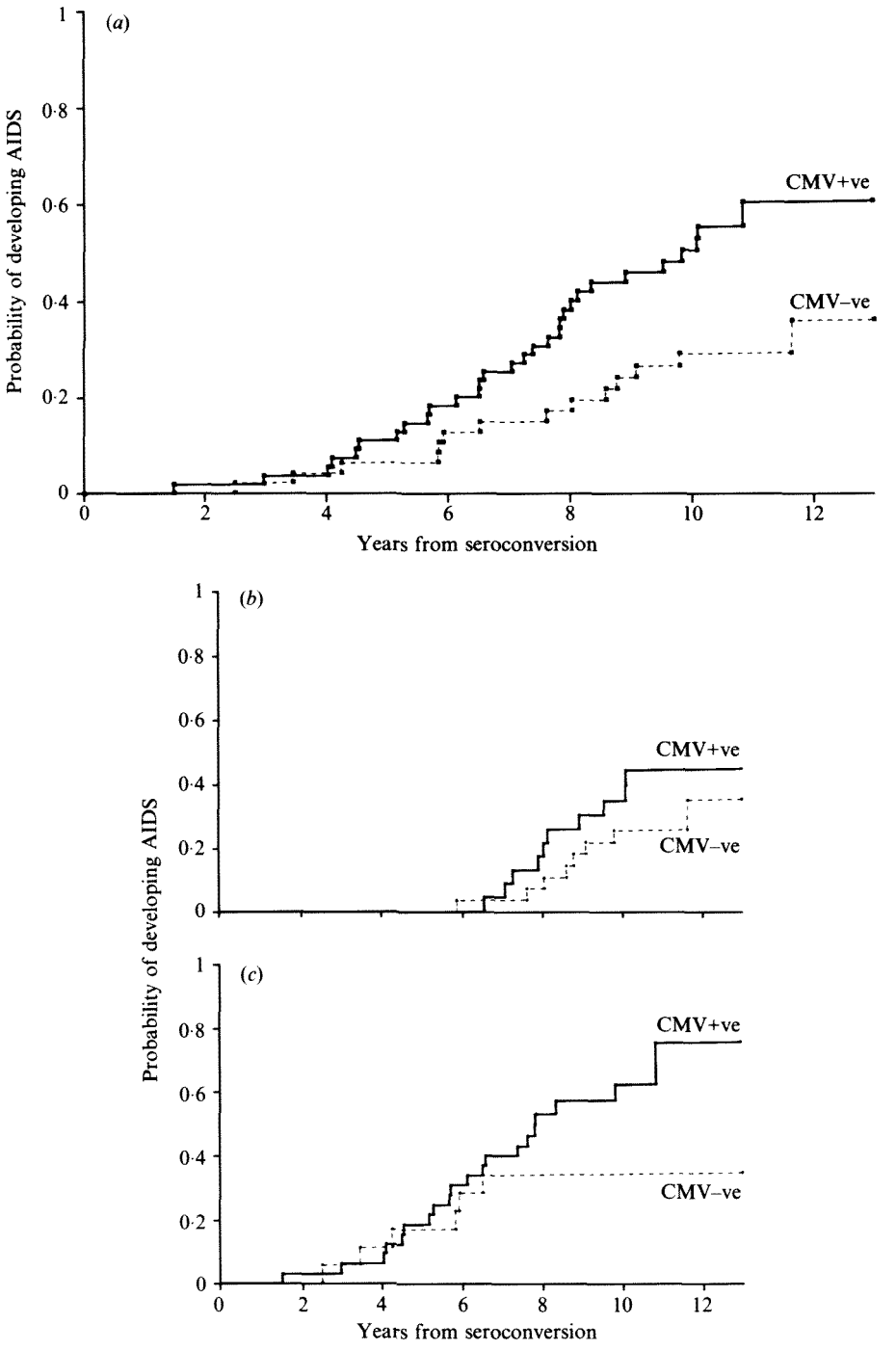

Fig. 1. Kaplan–Meier curves showing progression from HIV seroconversion to the development of AIDS, stratified by CMV status at seroconversion for (i) all patients, (ii) patients $\leqslant 35$ years at seroconversion, and (iii) patients > 35 years at seroconversion.

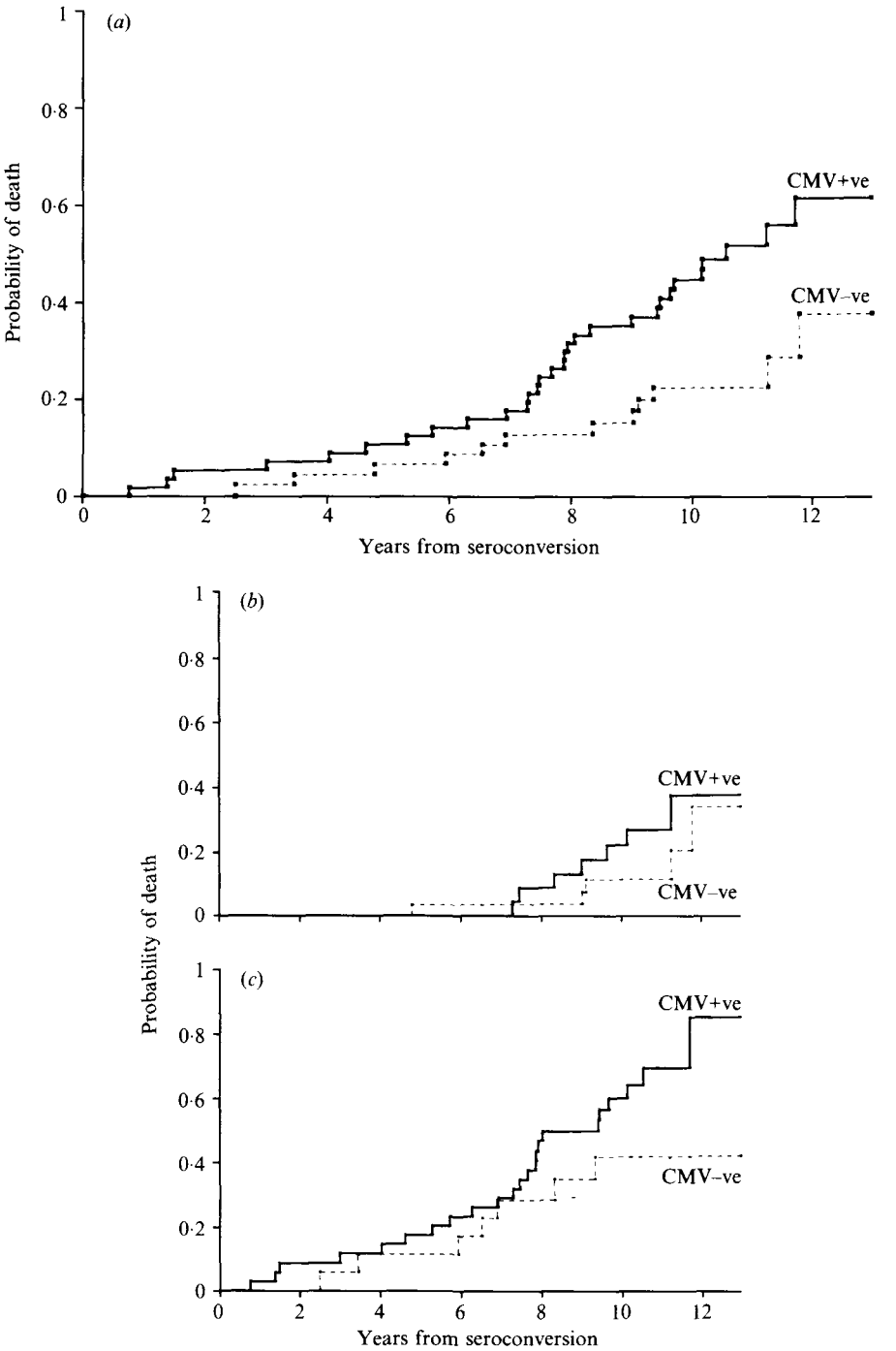

Fig. 2. Kaplan–Meier curves showing progression from HIV seroconversion to death, stratified by CMV status at seroconversion for (i) all patients, (ii) patients $\leqslant$ 35 years at seroconversion, and (iii) patients $>$ 35 years at seroconversion.

Table 2. *Relative hazards (and 95% confidence intervals) of progression to AIDS, death or a CD4 count of 0·05 × 10⁹/l associated with CMV positivity unadjusted for other factors, and with CMV positivity after adjustment for patients' age at seroconversion and most recent CD4 count*

	Progression to		
	AIDS	Death	CD4 0·05 × 10⁹/l
Unadjusted	2·28 (1·20–4·31)	2·42 (1·24–4·73)	2·34 (1·14–4·79)
Adjusted for age	1·89 (0·99–3·60)	1·82 (0·93–3·58)	1·93 (0·93–4·01)
Adjusted for age and CD4 count*	2·17 (1·13–4·16)	1·73 (0·88–3·39)	— —

* Progression to CD4 count of 0·05 × 10⁹/l is not adjusted for most recent CD4 count.

Table 3. *Relative hazards (and 95% confidence intervals) of death associated with CMV status. Baseline dates are defined as the date on which the CD4 count first fell below certain levels. Relative hazards for CMV status are adjusted for age at baseline*

Baseline CD4 count (× 10⁹/l)	No. of patients	No. of events	Relative hazard	95% CI
0·2	60	30	1·79	0·80–4·04
0·3	66	26	2·83	1·12–7·14
0·4	69	23	2·40	0·92–6·27
0·5	59	21	2·71	0·95–7·77
0·6	54	19	1·74	0·61–4·92

of developing AIDS and dying associated with CMV status are also shown in Table 2 after adjustment for the patients' most recent CD4 count, updated as a time-dependent covariate. After this additional adjustment the effect of CMV on survival was further reduced, whilst the effect of CMV on progression to AIDS returned towards its original value and attained statistical significance. Because of its obvious relationship with the most recent CD4 count, the relative hazard associated with CMV positivity on progression to a CD4 count of 0·05 × 10⁹/l was not adjusted for the patients' CD4 counts during follow up.

The addition of an interaction term between CMV status and log(time) to the model did not significantly improve the fit of the model ($P = 0·71$), suggesting that the proportional hazards assumption was reasonable.

Examination of the Kaplan–Meier plots suggested that the CMV effect may be smaller in younger patients than in older patients. However, the addition of an interaction term between CMV status and age was not significant for any of the three endpoints ($P = 0·85$, 0·78 and 0·88 for progression to AIDS, death and a CD4 count of 0·05 × 10⁹/l respectively).

In order to assess whether the hazard of death associated with CMV seropositivity was dependent on the CD4 count of the patient, the proportional hazards model was fitted with baseline dates defined as the first time a patients CD4 count fell to a certain level (0·2, 0·3, 0·4, 0·5, 0·6 × 10⁹/l). Results from this

analysis are shown in Table 3 and indicate that the size of effect of CMV status remains reasonably constant, after adjustment for age at CD4 baseline at all CD4 baselines.

The analyses were repeated with the addition of a term representing the availability of pre-AIDS prophylaxis and antiretroviral therapies. With the inclusion of this term the results were essentially unchanged.

DISCUSSION

The results presented here show that there is an association between CMV seropositivity and HIV disease progression measured by the time to AIDS, and also time to a low CD4 count and time to death. Whilst some of these effects may be explained by differences in age at seroconversion in the two groups, there remains a residual effect of CMV status on progression to AIDS which is unexplained by adjustment for this factor. We will discuss our findings and those of others for each of the three endpoints assessed.

Time to AIDS

For the time to AIDS we find an effect of CMV status (relative hazard 2·28) which is somewhat lower (relative hazard 3·2) than we reported in 1989 [15]. After controlling for age the relative hazard has also declined from 2·5 to 1·9. The results depicted in Figs 1 and 2 show that CMV seropositive patients progress to AIDS more rapidly than seronegative patients, even when considering younger and older individuals separately. After adjustment for patient age in the Cox proportional hazard model, the relative hazard fails to reach the conventional 5% level of significance (Table 2). However, the fact that the lower limit of the confidence interval is only marginally below 1 suggests that strict adherence to this convention is not sensible; a slight change in either the length of follow-up or an extra AIDS case could esily lead to a relative hazard which was the same but statistically significant. We conclude that prior CMV infection approximately doubles the risk of progression to AIDS. Comparisons of the Kaplan–Meier plots within the two age groups with those generated from all patients show that age differences do not completely explain the CMV effect. Unfortunately we do not have information on when patients seroconverted to CMV. There is a suggestion that the CMV effect is smaller in younger individuals than in older individuals. If any effect of CMV status on progression of HIV disease exists, then those who have been infected with CMV for longest and who are also likely to be those who were older at HIV seroconversion, may experience the greatest effect of CMV on the progression of their disease. However, there is no evidence of an interaction between age and CMV status. This suggests that this is unlikely to be the case so that the differences which are apparent from the Kaplan–Meier plots are more likely to be the result of random variation due to the small numbers of individuals who are younger and who have antibody to CMV. Two of the AIDS-defining conditions, CMV retinitis and other CMV disease, are CMV-related [29] and so this might be one possible explanation for our findings. However, in our cohort only one patient developed either of these two conditions as his initial AIDS-defining condition, showing that this relationship cannot expain our results.

A number of other studies have reported findings on the potential effect of CMV positivity on HIV disease progression. Three studies [16–18] found no statistically significant effect of CMV on progression to symptomatic HIV infection or AIDS. However, these studies had flaws either in design or conduct [30]. Results from a recent study from the United States of men with haemophilia with known dates of HIV seroconversion [19] did not find a significantly raised risk of AIDS associated with CMV seropositivity after adjustment for age, and offered the strongest evidence against the hypothesis that CMV status is associated with rapid disease progression. The patients included in that study were of a similar age range to those in our study and the proportion who were CMV positive was also similar. Serum samples were tested for CMV antibody under code in London using the same radioimmunoassay, thus removing the effect of laboratory differences. Because both studies are carried out in haemophilic patients, the effects of other possible co-factors (e.g. gender, HIV exposure category, intravenous drug use, etc.) on progression rates between the two studies are unlikely to explain these differences. However, it may be possible that differences in covariates other than those currently known to be associated with progression of HIV disease could explain the contrasting results, and we are continuing to collaborate with the USA investigators to study this with the aim of identifying other previously unrecognized factors which may explain the differences. These may include differences in patient selection methods or in the background prevalence rates of antibodies to other viruses in the patient populations.

Survival

Sufficient follow-up has now occurred in our cohort to allow an analysis of survival which shows a raised risk of death associated with CMV seropositivity. The association of CMV status with death is not attributable to clinically recognized CMV disease. Thus there is no evidence that CMV is causing opportunistic disease and we have argued elsewhere that this supports the concept of CMV as a 'co-factor', increasing the burden of disease without declaring itself clinically [5]. This concept is supported by autopsy findings that CMV is present in at least one tissue in 66% of AIDS patients [31].

It is interesting to speculate on the clinical effects of anti-CMV therapy administered to AIDS patients. Recently, two controlled trials used acyclovir in homosexual men with advanced HIV disease [32, 33]. In both trials an approximately 40% reduction in the number of deaths was seen in patients receiving acyclovir compared to those in the control group. As acyclovir is known to inhibit the DNA polymerase enzyme of several herpesviruses, such a virus could be involved in the pathogenesis of death in AIDS patients. CMV is a strong candidate in this setting because CMV has been found by cell culture in the majority of AIDS patients coming to autopsy [31] and because controlled trials in transplant patients have shown that high-dose acyclovir can decrease CMV replication and disease [34–36]. All patients in these trials were CMV positive and had low CD4 counts. Results from Table 3 suggest that at low CD4 counts a relative hazard value < 2 associated with death is reasonable and consistent with the approximate halving of the death rate in these two trials, which again suggests that the inhibition of CMV replication is a plausible reason for the reduced

mortality seen. Further studies of quantitative CMV virology during such trials will be required to determine if suppression of CMV, and/or other herpesviruses, correlates with improved survival. This work is in progress under the auspices of the AIDS Clinical Trials Group.

Low CD4 count

The hypothesis that the CMV effect might be mediated through loss of CD4 cells was investigated. The results presented here suggest that CMV status has a significant effect on the rate of CD4 decline, as shown by a faster progression to a CD4 count of $0{\cdot}05 \times 10^9$/l in CMV seropositive individuals than in seronegative. It has previously been shown that there is an effect of age on progression to AIDS which is not completely explained by more rapidly declining CD4 counts [12]. Certainly, the effects of CMV, age and CD4 count do not act totally independently of each other. However, results from Table 2 suggest that whilst CMV positives do indeed progress to a CD4 count of $0{\cdot}05 \times 10^9$/l more rapidly than CMV negatives, largely independently of their age at seroconversion, this rapid drop in CD4 cells does not fully explain the CMV effect on progression to AIDS. Further functional studies of activated CD8+ T lymphocytes are required to answer these questions.

In summary, our results show a continued association between CMV and progression of HIV disease which cannot entirely be explained by age. These results have implications for both our understanding of the pathogenesis of HIV and for therapy, which are being actively pursued in clinical trials of anti-herpes drugs.

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