CYTOMEGALOVIRUS INFECTION AND PROGRESSION TOWARDS AIDS IN HAEMOPHILIACS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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Summary To examine whether cytomegalovirus (CMV) infection could accelerate progression of human immunodeficiency virus (HIV) infection to AIDS, serological studies were done on 108 HIV-infected haemophiliacs. In the 1·3–9 years from time of first recognised HIV seroconversion, the age-adjusted risk of CDC group IV disease in CMV-seropositive patients was 2·5 times that in CMV-seronegative patients. CMV-seropositive patients were also more likely to have detectable p24 antigenaemia. Survival analysis showed that CMV-seropositive patients were at greater risk of HIV disease than CMV-seronegative patients from about 2 years after HIV seroconversion. Thus CMV infection is associated with a more rapid progression to HIV disease.

Introduction

MANIFESTATIONS of disease due to human immunodeficiency virus (HIV) may not occur until several years after initial infection, which suggests either that HIV infection is chronic or that it first establishes latency and produces disease only after reactivation. The latter possibility seems the more likely because active production of viral p24 antigen ceases after initial infection in some individuals, but may be detected many months or years later, when it may indicate a poor prognosis.[1] If factors other than HIV itself are required for activation of the latent HIV genome or the progression of chronic disease, the identification of such cofactors is important—it might help understanding of the pathogenesis of AIDS, and treatment aimed at these cofactors might delay the onset of symptoms of immunodeficiency.

Herpesviruses may be one such cofactor.[2-5] These viruses are common among AIDS patients, and they interact at a molecular level with HIV. Cytomegalovirus (CMV) has been reported to infect virtually all homosexual AIDS patients[6] but since both viruses may be acquired sexually, this association may merely reflect a certain lifestyle. CMV, but not herpes simplex virus (HSV), infection is associated with a homosexual lifestyle, and homosexual men have raised anti-CMV, but not anti-HSV, antibody levels.[7] These observations are unlikely to have resulted from polyclonal B cell activation (or non-specific immunosuppression). Hence homosexual men are likely to have chronic CMV infection, or be subject to CMV reinfections, which could indicate a possible role for this virus in the progression of disease in HIV-infected individuals.

CMV is frequently isolated from the semen of healthy homosexual men.[8] To avoid the confounding effect of CMV and HIV being acquired by the same route, we chose for our investigation of whether CMV could be a cofactor, haemophiliacs whose HIV infection had been acquired through contaminated clotting factor concentrates. The prevalence of CMV among these patients would be expected to be similar to that of the general population. Haemophilia was unlikely to have significantly increased their exposure to CMV (which is cell-associated) since haemophiliacs generally receive non-cellular blood products.

Patients and Methods

Patients

108 HIV seropositive patients with haemophilia A, haemophilia B, or von Willebrand's disease regularly attending the Royal Free Hospital Haemophilia Centre acquired HIV infection between November, 1979, and July, 1985, from contaminated clotting factor concentrates. The group was of mean age 35 years; 5 were children aged under 14 years. Patients are reviewed regularly and assessed for the presence of signs and symptoms attributable to HIV infection. In this report, HIV disease and AIDS refer to those symptoms and signs classifiable as CDC group IV disease.[9]

Serum Samples

As part of the routine management of these patients, serum samples were obtained regularly and stored at −20°C. These were tested for HIV antibodies, and the time of seroconversion—the mid-point between the dates of last HIV antibody negative and first HIV antibody positive serum—was identified for 59 patients. In the remaining 49 patients the first available serum sample contained HIV antibodies. To assess whether CMV infection was associated with activation of HIV infection serum samples were taken and tested for p24 antigen at each clinic visit.

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TABLE I—ASSOCIATION BETWEEN CDC GROUP IV HIV INFECTION AND SEROPOSITIVITY FOR CMV OR HSV

Serological status	n	% with AIDS	Relative risk of AIDS	
			Unadjusted	Adjusted for age
CMV negative	46	13	1·0	1·0
CMV positive	58	41	3·2 (p = 0·003)	2·5 (p = 0·02)
HSV negative	40	32	1·0	1·0
HSV positive	63	25	0·8 (p = 0·44)	0·7 (p = 0·32)

TABLE II—PREVALENCE OF AIDS AND ANTIBODIES TO CMV AND HSV BY AGE-GROUPS

Age group	n	AIDS (%)	CMV (%)	HSV (%)
< 25	33	9	26	65
25–35	41	32	60	58
> 35	34	50	79	61

The most recent serum sample was used for determining seropositivity for CMV and HSV. In addition, the earliest available serum sample from those CMV-seropositive patients was also tested for CMV antibodies to determine if this infection had been acquired recently.

Methods

Antibodies to HIV were measured by enzyme immunoassay ('Wellcozyme', Wellcome, Beckenham). All sera screening positive for HIV antibodies were sent to a reference laboratory for confirmation. All were confirmed as positive, on the basis of the strong concordance between the results of testing sera by gelatin particle agglutination (Fujirebio, Tokyo, Japan), competitive enzyme immunoassay (Wellcome), and antiglobulin immunoassay containing recombinant antigens (Abbott, Maidenhead). p24 antigen was measured by enzyme immunoassay (Abbott). Antibodies to CMV or HSV were measured by radioimmunoassays described in detail elsewhere.[9,11]

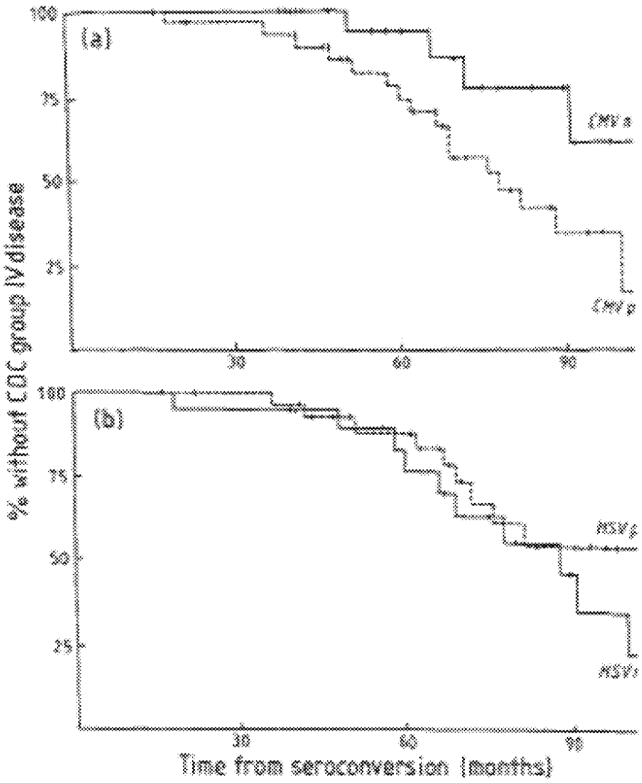
Statistical Analyses

Logistic regression[12] was used to analyse the relation between AIDS and CMV status, allowing for age. The results of these analyses are expressed as the relative risk of AIDS developing in the CMV-seropositive group compared with that in the CMV seronegative group. For those patients in whom the date of seroconversion was known, Kaplan-Meier survival curves were calculated and the confounding effect of age was investigated by use of a Cox proportional hazards model.[13] Again, the results are expressed in terms of relative risk of AIDS developing.

The results of the logistic regression are expressed as relative risks rather than relative odds so as to be comparable with the results of the survival analysis.

Results

By November 1988, 31 (29%) of the 108 HIV-seropositive patients had had manifestations of HIV disease classifiable as CDC group IV, and 9 of these had died. 5 other patients had died from unrelated causes. None of the patients were classified as having CDC group IV disease on the basis of severe CMV infection. 19 (18%) patients had detectable levels of p24 antigen in serum on at least two occasions during follow-up. 58/104 (56%) patients were seropositive for CMV, and 63/103 (61%) were seropositive for HSV. The CMV and HSV serostatus could not be determined for 4 and 5 of the patients, respectively, because of non-specific binding in the assays used.



Kaplan-Meier curves showing temporal development of CI group IV disease in (a) CMV seropositive and seronegat patients, and (b) HSV seropositive and seronegative patients.

Times at which subjects are lost to follow-up are indicated by vert dashes.

relative risk of AIDS for CMV-positive patients was 3 (95% CI 1·4–7·2) (table I). Older patients were more lik to be both CMV positive and to have AIDS (table I Controlling for age reduced the estimated relative risk AIDS to 2·5 in CMV-positive patients compared w CMV-negative patients, but the difference remain statistically significant (p = 0·02). No significant differe was observed between patients seropositive or seronegat for HSV (table I).

Kaplan-Meier survival curves for the 59 patients wh date of HIV seroconversion could be identified showed t CMV-seropositive patients progressed to CDC group disease more rapidly than did those seronegative for virus (fig, a). The risk of AIDS for CMV-positive patie was 3·6 times that for CMV-negative patients (95% 1·2–10·8, p = 0·02), or, after allowing for the confound effect of age, 2·6 (95% CI 0·8–8·3, p = 0·11). The lack statistical significance at the usual 5% level could be du the small sample rather than to an absence of a t difference. The relative risks estimated from the survi analysis agree well with those based on the larger data where information on date of seroconversion was available. The Kaplan-Meier curves showed no differe between patients seropositive and those seronegative HSV in progression to AIDS (fig, b).

CMV and p24 Antigen

p24 antigen was detected on at least two occasions dur

TABLE III—POSSIBLE INTERACTIONS BETWEEN CMV AND HIV

Mechanism	Level of interaction	Effect
Transactivation of HIV genome	Single cell	Activation of one latent virus by the other virus
Presentation of CMV antigens to HIV-infected cells	Cell–cell	
Lymphokines from CMV infection drive productive HIV infection	Cell–cell	
CMV infection recruits HIV-infected macrophages	Single organ	Increased cell–cell transmission and/or uptake of virus
Fc receptor on CMV-infected cells facilitates uptake of antibody-coated HIV	Single cell	
Incorporation of CMV glycoproteins into HIV envelope (pseudotype formation) broadens cellular tropism	Single cell	
Productive HIV infection increases lymphocyte turnover and β_2 microglobulin release, increasing CMV infectivity	Cell–cell	
HIV-induced syncytia increase cell–cell transmission of CMV	Cell–cell	

42/58 patients who were CMV seropositive had sera taken and stored an average of 65 months previously for testing for CMV IgG antibodies. 2 of these were initially seronegative, and so had acquired CMV infection recently. 1 of these had HIV disease.

Discussion

Our study shows that CMV infection was strongly associated with the development of HIV disease in HIV-infected haemophiliacs. The results were obtainable because a substantial proportion of the haemophilia population studied had not been infected with CMV—the proportion of CMV seropositive individuals (57%) was virtually identical to that reported for young adult blood donors or antenatal patients[13] in Britain. A smaller study of haemophiliac patients in the USA revealed a similar rate of CMV seropositivity, measured by latex agglutination, but showed no association between symptomatic HIV disease and CMV serostatus.[14]

Our findings suggest an adverse effect of age on prognosis in HIV disease. Although CMV seroprevalence also increases with age,[7] our results show CMV infection to be an independent and additional risk factor in the development of AIDS.

Comparable studies of homosexual men who are at high risk of various sexually transmitted infections have been confounded by the rarity of individuals without CMV infection.[8,15,16] Nevertheless, evidence from studies of these populations supports the hypothesis that CMV is a major cofactor for expression of HIV disease. CMV infection is almost universal amongst homosexual patients with AIDS,[5] and a high titre of CMV IgG antibodies has been reported to be a significant risk factor for the development of AIDS in these patients.[17] Our previous studies have suggested that high titres of CMV IgG antibodies in homosexual men result from chronic CMV infection or reinfection rather but previous work in our laboratory has shown that CMV is closely associated with β_2m and that concentrations of this protein in urine may be raised by CMV infection.[18] We suggest, therefore, that the association between β_2m and progression of HIV disease may be linked through CMV infection and plan to investigate this possibility in detail.

There are many levels at which CMV might interact with HIV (table III). It has been shown in vitro that, at the cellular level, the major immediate early protein of CMV can activate the HIV long terminal repeat, although the specific site of activation, and the gene product that mediates this activation have not been identified.[20] Not only does CMV enhance HIV replication in vitro, but superinfection of CMV-infected cells with HIV can enhance CMV replication.[21] A recent report shows that both HIV and CMV can co-infect brain cells[22] so that this in vitro mechanism might be operative in vivo. Indeed, each virus has characteristics that could facilitate the entry of the second virus into the same cell (table III), and viral or cellular proteins or lymphokines produced by infection with one virus could facilitate replication or uptake of the second virus.

Finally, CMV and HIV are both viruses that induce immunosuppression,[23,24] and so loss of immune surveillance and production of both viruses might be greater with a combined infection than with single infections. All of these possible mechanisms can be investigated in vitro and in vivo, and all are potentially amenable to control by an effective CMV vaccine. However, for the many individuals at risk of AIDS who have already been infected with CMV a drug that inhibits CMV replication might be expected to reduce interaction of that virus with HIV. Unfortunately, serious side-effects of existing anti-CMV drugs and/or their poor bioavailability by the oral route may make the necessary controlled trials very difficult. Even so, we would suggest that placebo-controlled trials of antiviral agents with activity against CMV could now be justified in CMV seropositive HIV-infected individuals.

We thank the scientific staff of the virology and haemophilia laboratories for doing the assays, and the members of the virology section, University College and Middlesex School of Medicine, Riding House Street, London, for the reference serology.

This study was supported solely by departmental funds.

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