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Sequential serological studies of homosexual men with and without HIV infection. Epstein–Barr virus activation preceding and following HIV seroconversion

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SUMMARY

Viral cofactors may be important in the pathogenesis of HIV infection and the development of AIDS, but their role is still imperfectly understood. Sequential serological studies were performed in a cohort of 100 homosexual men and 70 matched healthy controls over a mean period of 4 years. Of the patients, 18 were found to be HIV^+ on admission to the study and 15 seroconverted to HIV^+ during the follow up (seroconversion group). Serum antibodies of both IgG and IgA isotypes against Epstein–Barr virus (EBV) and cytomegalovirus (CMV) were determined. IgG antibodies indicate past infection, while a marked increase in IgG titre or a positive IgA titre were taken to indicate active infection or reactivated latent infection. EBV and CMV infections were about two to four times more prevalent in the homosexual men both HIV^- and HIV^+, compared with controls. Active infections were increased in the homosexual men and particularly in the HIV^+ patients. The seroconversion group revealed activation of both EBV and CMV following HIV infection. When the antibody profile of seroconverting patients at the time preceding seroconversion was compared with a matched group of 39 homosexual men who remained HIV^-, no change was found in CMV antibodies, but four out of 15 (26·6%) of the patients had high titres of anti-EBV IgA preceding seroconversion, as compared with only one out of 39 (2·6%) of HIV^- homosexual men ($P < 0·05$). This suggests a role for EBV reactivation in the pathogenesis of HIV infection in some patients.

Keywords HIV Epstein–Barr virus cytomegalovirus immunosuppression, viral

INTRODUCTION

Associated viral and other infections in patients infected with HIV are well known, and pose complex and intriguing problems. Theoretically, several main interactions may occur. First, associated infections may be endemic in the population at risk for contracting HIV, yet have no bearing on the propensity for HIV seroconversion, nor show any significant change following this event. Second, some associated infections may lead to an immune impairment which may itself increase the risk of HIV infection/propagation in a susceptible population. Third, preceding associated infections and HIV infection may occur independently, yet the former may become activated following HIV-induced immune impairment. Although many studies have been devoted to the interaction between HIV and other infectious agents, some aspects of the above problems have remained controversial and incompletely resolved (Hirsch *et al.*, 1984; Laurence, 1990).

We have been following a large cohort of homosexual men since 1983 (Handzel *et al.*, 1984; Bentwich *et al.*, 1987, 1988). This cohort is of particular interest since it is virtually free of other factors such as alcoholism and drug abuse which are often prevalent among similar populations in other countries and may independently contribute to immune derangements and co-infections. In this study we analyse Epstein–Barr virus (EBV) and cytomegalovirus (CMV) serologies in 100 homosexual men followed sequentially, with particular reference to 15 patients who became HIV^+ during the study period and to the events preceding seroconversion.

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SUBJECTS AND METHODS

Patients

A follow up of healthy homosexual men in the Tel Aviv metropolitan area was initiated by us in 1983 and presently

includes over 800 subjects. The analysis of data from 100 of these patients constitutes this report. All had a detailed initial interview and repeated and extensive examinations and blood tests to evaluate their immune status, as previously described (Bentwich *et al.*, 1988). Mean follow-up time was 4 years, and serial studies were performed at varying intervals but at least once a year in most cases. The control population consisted of 70 age- and sex-matched hospital and laboratory personnel with no evidence of disease, who were tested concomitantly with the study group.

Serology

Serum antibodies to HIV were measured by ELISA as described (Saxinger & Gallo, 1983), and positive results were confirmed in each case by Western blots (Towbin, Staehelin & Gordon, 1979). Serial serum specimens were tested for IgG and IgA antibodies to CMV antigens by the immunoperoxidase assay (Sarov & Haikin, 1983). Antibodies to EBV viral capsid antigen of the IgG and IGA type were detected by an indirect immunofluorescence assay (Gotleib-Stematsky *et al.*, 1983). All tests were performed blindly.

Statistical analysis

Statistical analyses of the data were carried out between the groups by χ^2 test, Student's *t*-test and the Wilcoxon two-sample rank test, using the CLINSTAT computer program.

RESULTS

Sixty-seven homosexual men who were HIV$^-$ were selected randomly for inclusion in the present study (HIV$^-$ group) in addition to 18 who were found to have serum antibodies to HIV on presentation (HIV$^+$ group) and 15 cases who became HIV$^+$ during the observation period (seroconversion group). The latter patients had no HIV-related symptoms or signs over the time of the study. The duration of follow up for the seroconversion group varied, but was at least 4 months prior to seroconversion (range 4–12 months), and 9–30 months after it occurred. Except for the seroconversion group, whose serologic profiles were analysed separately, results given are those of the last follow up available, and for the HIV$^-$ group remained essentially the same during the time of the study.

As shown in Fig. 1, only 40% of controls had high titres of anti-EBV IgG antibody ($\geq$1/256) as compared with 78% and 89% among the HIV$^-$ and HIV$^+$ subjects, respectively. Higher titres of IgG were more prevalent among the HIV$^-$ group, and even more so in HIV$^+$ subjects. Analysis of anti-EBV IgA is also revealing in that only 3% of controls had high titres ($\geq$32; reciprocals of titres were used throughout) *versus* 9% of HIV$^-$ and no less than 45% of HIV$^+$ cases. Evidence of past CMV infection was less common: only 20% of controls showed IgG antibodies as opposed to about 80% of either HIV$^-$ or HIV$^+$ subjects, who also showed the higher titres (but no great difference between the groups). Anti-CMV IgA was also more frequent among HIV$^-$ and HIV$^+$, yet the prevalence of high titres did not exceed 21% in the seropositives and was even less striking in the HIV$^-$ group.

We next analysed the sequential changes in titres in the 15 subjects who became HIV$^+$, and compared them with those of 39 out of 67 HIV$^-$ subjects, who constituted a matched group, also tested sequentially, over the same period as the 'seroconverters.' Titres of EBV and CMV antibodies increased after seroconversion (Table 1). This was particularly striking when all positive IgA titres were compared (26·5% pre-conversion *versus* 55% post-conversion for EBV, and 7% *versus* 50% for CMV), but was also prominent for anti-EBV IgG, and less evident for anti-CMV IgG (some increase when titres of $\geq$64 are considered). Thus, an increase often occurred following seroconversion; however, only anti-EBV IgG and anti-CMV IgA were found to be statistically significant, due to the small number of patients in this group.

When the question of possible increase in antibody titres which preceded seroconversion was addressed, the only signifi-

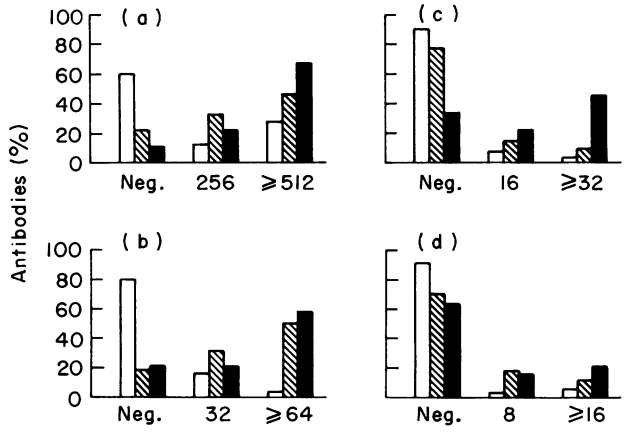

Fig. 1. Prevalence of serum antibodies to EBV (a, c) or CMV (b, d) among healthy controls ($n=70$) (□); HIV$^-$ homosexual men ($n=67$) (▨), and HIV$^+$ homosexual men ($n=18$) (■) Percentages of subjects showing IgG antibodies (a, b) or IgA antibodies (c, d) of negative, intermediate or high titres are shown. Titres are given as the reciprocals and 'negative' EBV titres include very low titres of $\leq$32.

Table 1. Changes in EBV and CMV antibodies following HIV seroconversion

	IgG Antibodies			IgA Antibodies		
EBV ($n=15$)						
Titre:	None*	256	≥512	None	16	≥32
Pre-	8 (53)	2 (13·5)	5 (33·5)	11 (73·5)	—	4 (26·5)
Post-	2 (13)	4 (27)	9 (60)	7 (47)	3 (20)	5 (33)
P†		0·02			NS (>0·2)	
CMV ($n=14$)						
Titre:	None	32	≥64	None	8	≥16
Pre-	2 (14)	6 (43)	6 (43)	13 (93)	—	1 (7)
Post-	1 (7)	4 (29)	9 (64)	7 (50)	3 (21)	4 (29)
P†		NS			<0·02	

Numbers of patients for each reciprocal of titre are given; percentage is shown in parentheses.

* Including very low titres of $\leq$32.

† *P* value of all subjects with positive titres *versus* 'none'.

NS, not significant.

Pre- and post-, i.e. HIV seropositivity.

Table 2. Prevalence of high titres of IgG and IgA antibodies to EBV and CMV in the pre-seroconversion period of HIV$^+$ subjects ($n=15$) and a comparable period of observation of HIV$^-$ homosexual men ($n=39$)

	IgG		IgA	
	n	%	*n*	%
EBV				
High titre*				
pre-seroconversion	4/15	26·6	4/15	26·6
High titre in				
matched HIV$^-$ men	7/39	17·9	1/39	2·6
P	NS		<0·05	
CMV				
High-titre†				
pre-seroconversion	5/14	35·7	1/14	7·1
High titre in				
matched HIV$^-$ men	15/35	42·8	2/35	5·7
P	NS		NS	

* A reciprocal titre of ≥512 for IgG of ≥32 for IgA antibodies which persisted after seroconversion or in a comparable follow up of the HIV$^-$ men.

† A reciprocal titre of ≥64 for IgG or ≥16 for IgA antibodies which persisted after seroconversion or in a comparable follow up of the HIV$^-$ men.

cant change in comparison with our control group of matched HIV$^-$ men was noted in anti-EBV IgA. As shown in Table 2, four of 15 (26·6%) of the seroconversion group showed a high titre of anti-EBV IgA before seroconversion (≥32), which remained elevated in the year following it, while a similar occurrence was found in only one of 39 HIV$^-$ men (2·6%) ($P<0·05$).

No correlation of antibody titres to EBV with those to CMV was found in any of the groups.

DISCUSSION

We found increased prevalence and higher titres of IgG antibodies to CMV and EBV in the HIV$^-$ group as compared with controls, indicating past infection (Berry *et al.*, 1988), and very likely sexual transmission, as previously noted (Rinaldo, Kingsley & Lyter, 1986; Buimovici-Klein *et al.*, 1988; Rinaldo, 1990). We then used the sequential serologies as probes to examine the interactions between these viral cofactors and HIV, with particular reference to the effect of prior herpes virus infection or reactivation on the acquisition/course of HIV infection. Such interactions are of special interest since both EBV and CMV are immunosuppressive (Ho, 1982; Rouse & Horohov, 1986; Specter, Bendinelli & Friedman, 1989), and have also been shown to activate HIV *in vitro* (Pagano *et al.*, 1988; Skolnik, Kosloff & Hirsch, 1988; Albrecht *et al.*, 1989). An active viral infection can be diagnosed by finding specific IgA antibodies in the serum (Brunell, Gershon & Uduman, 1975; Sarov *et al.*, 1984; Partanen *et al.*, 1985; Porath *et al.*, 1987; Weiblen *et al.*, 1990), or by demonstrating a new appearance of high titres of IgG antibodies or a rise greater than four-fold in titre (White & Fenner, 1986). Using these criteria, and focusing on the 15 men who seroconverted to HIV$^+$, we observed marked changes in both EBV and CMV antibodies post-seroconversion. Although only the increase in anti-EBV IgG and anti-CMV IgA were statistically significant, they are supported by the complementary increase in the other antibodies (Table 1), and by the finding of high titres of IgA antibodies in the HIV$^+$ subjects as compared with healthy HIV$^-$ men (45% *versus* 9% for anti-EBV IgA, and 21% *versus* 12% for anti-CMV IgA; Fig. 1). However, increased IgA responses after seroconversion may be part of HIV-related B cell changes and thus should be interpreted with caution. Activation of both EBV and CMV following HIV infection has already been noted by others and may be due to both direct effects (Lai, Li & Volsky, 1989) and to the HIV-related profound defect in cellular immunity which is important in controlling latent herpes virus infections (Birx, Redfield & Tosato, 1986; Rahman *et al.*, 1989; Rinaldo, 1990). There is strong experimental evidence (Gendelman *et al.*, 1986; Pagano *et al.*, 1988; Skolnik *et al.*, 1988; Albrecht *et al.*, 1989; Clouse *et al.*, 1989) and some clinical observations suggesting that activated EBV or CMV infections may be associated with enhanced replication of pre-existing HIV and accelerated progression to AIDS, but this cannot be inferred from our series. As for possible interactions between EBV and CMV, antibody titres were independent of each other in our cohort and no evidence for coactivation of these viruses was found.

The issue of whether prior herpes virus reactivation occurring in some subjects may predispose them to acquire HIV infection or facilitate it by immunosuppressive effects, is a much more complex one. Although the immunosuppressive effects of both EBV and CMV in homosexual men are clearly established (Drew *et al.*, 1985; Rinaldo *et al.*, 1986; Lai *et al.*, 1989), and could increase susceptibility to HIV infection (Pagano *et al.*, 1988; Rinaldo, 1990), there are very limited data actually supporting such a possibility. We have analysed the 'pre-conversion' period serologies of 15 homosexual men who later were found to be HIV$^+$, and compared them with 39 matched homosexual men who remained HIV$^-$, tested under parallel circumstances. While the prevalence of high titres of CMV antibodies was similar in the two groups and high anti-EBV IgG was only slightly more common pre-seroconversion (Table 2), the difference in anti-EBV IgA was striking: four out of 15 (26·6%) of the subjects who later became HIV$^+$ had a high titre of anti-EBV IgA, but similarly high titres were found in only one out of 39 (2·6%) of the men who remained HIV$^-$ ($\chi^2=3·88$; $P<0·05$). This suggests a significant association between HIV infection and preceding EBV activation.

The implications of this finding are presently unclear. We believe it should first be examined in a larger group of seroconverting patients, and also correlated to changes in anti-EBV IgM. Then, our observation could be interpreted either as an epiphenomenon or as a cause and effect relation. According to one opinion, seroconversion after HIV infection may be delayed (Ranki *et al.*, 1987) so that the change in EBV IgA may be secondary to an early, occult HIV infection and immunosuppression. However, PCR data do not generally suggest that this is a general phenomenon (Hart *et al.*, 1988). It is also possible that in infection via sexual contact, the partner who transmitted the HIV may have transmitted EBV as well, or provided some stimulus for activating a previously quiescent EBV infection. Another attractive interpretation favours the view that homo-

sexual men with marked EBV reactivation are more prone to contract HIV infection, or activate latent HIV. This can be related to several mechanisms. First, virus-induced tumour necrosis factor (Beutler, 1988) may enhance HIV replication and expression (Folks *et al.*, 1989). Second, polyclonal activation by EBV can induce HIV replication in latently infected cells (Folks *et al.*, 1986). Third, HIV expression could be up-regulated in cells following transfection with heterologous viral genes (Fauci, 1988). EBV activation may therefore play a role in the activation of HIV and progression to full-blown AIDS, a phenomenon which is still ill understood (Rosenberg & Fauci, 1989). In this context, it is intriguing that the anti-HIV agent zidovudine (AZT) also effectively inhibits EBV replication (Lin *et al.*, 1988). Thus, some of the drug's beneficial effects in early HIV infection (Friedland, 1990) may be due to this combined effect.

Taken together, we feel very strongly that reactivation of EBV infection may play an important role in the sequence of events from infection with HIV to AIDS. Although our data are by no means conclusive, they do suggest that such reactivation may also be a cofactor for HIV infection. Clearly, this is based on small numbers and more study is indicated to elucidate these problems.

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