JOURNAL OF CLINICAL MICROBIOLOGY, June 1991, p. 1215–1220
0095-1137/91/061215-06$02.00/0

Vol. 29, No. 6

Reactivation of Epstein-Barr Virus during Early Infection with Human Immunodeficiency Virus

M. ABID RAHMAN,[1†] LAWRENCE A. KINGSLEY,[1,2] ROBERT W. ATCHISON,[2] STEVEN BELLE,[1] MARY C. BREINIG,[2] MONTO HO,[2,3,4] AND CHARLES R. RINALDO, JR.[2,4*]

Departments of Epidemiology,[1] Infectious Diseases and Microbiology,[2] Medicine,[3] and Pathology,[4] Graduate School of Public Health and School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15261

Received 7 November 1990/Accepted 18 March 1991

Reactivation of Epstein-Barr virus (EBV) in early human immunodeficiency virus (HIV) infection was investigated in 49 homosexual men who seroconverted to HIV (cases) as compared with 49 matched controls who remained seronegative to HIV during a longitudinal study. EBV infection was reactivated in cases 6 months, but not 12 months, prior to HIV seroconversion as compared with controls and remained reactivated during 18 months of follow-up after HIV seroconversion, as shown by increases in immunoglobulin (Ig) G antibody titers to EBV early antigen. Antibody titers to EBV viral capsid antigen did not differ between cases and controls prior to the time of seroconversion to HIV but were significantly increased among cases by the first seropositive study visit and remained elevated during the 18 months after HIV seroconversion. Total serum IgG levels were increased in cases at the visit of seroconversion, and during 18 months of follow-up, but did not correlate with enhanced IgG production specific for EBV antigens. Significant decreases in numbers of $CD4^+$ cells and increases in numbers of $CD8^+$ cells during this early phase of HIV infection were not associated with changes in patterns of EBV antibody responses. Reactivation of EBV beginning 6 months before HIV seroconversion may have implications regarding the role of this herpesvirus in the pathogenesis of HIV.

Epstein-Barr virus (EBV) is a ubiquitous human herpesvirus known to be the causative agent of acute infectious mononucleosis (15). EBV has also been linked with lymphoproliferative disorders such as Burkitt's lymphoma, nasopharyngeal carcinoma, and X-linked lymphoproliferative syndrome (16, 23). EBV is widespread among different populations, and infection occurs during early childhood in most parts of the world. After primary infection, EBV remains latent for life.

Evidence suggests that EBV is expressed in unusual patterns among individuals with AIDS or who are at risk for developing AIDS. These patterns include elevated titers of antibodies directed against EBV antigens (27, 29, 31), increased oropharyngeal excretion of EBV (2, 10a, 25, 31), increased numbers of circulating EBV-infected B cells (4, 26), and isolation of EBV from the plasma of AIDS patients (1). It appears that EBV, which exists in a latent state in immunocompetent hosts, is reactivated by human immunodeficiency virus (HIV)-induced immunosuppression. Such EBV infections may be related to the development of oral "hairy" leukoplakia (11) and lymphoproliferative disorders (3, 9, 12) described in AIDS patients.

There is little known concerning the role of EBV in the longitudinal progression of initial HIV infection to the development of AIDS. We have therefore examined the relationship between HIV and EBV during early HIV infection. Homosexual men with documented seroconversion to HIV were compared with nonseroconverters in a nested case-control design. The main objectives of this study were to determine when reactivation of EBV occurred with respect to HIV infection and the possible role for EBV in the immunological alterations and clinical disorders observed during early HIV infection.

(This investigation was part of a thesis submitted by M. A. Rahman in partial fulfillment of the requirements for a Ph.D. degree from the University of Pittsburgh, Pittsburgh, Pa.)

MATERIALS AND METHODS

Study design. This was a nested case-control study (21), utilizing a specific subgroup of homosexual and bisexual men from the Pitt Men's Study, the Pittsburgh portion of the Multicenter AIDS Cohort Study (18). Informed consent was obtained from all participants in accordance with the guidelines of the Institutional Review Boards of the University of Pittsburgh and the National Institutes of Health. Each study participant completed an interviewer-administered clinical-epidemiologic questionnaire and a physical examination and donated blood for laboratory testing at biyearly visits.

At the end of a 2.5-year follow-up, 49 HIV seroconverters were matched with 49 men who remained seronegative to HIV for the same study period. A matched study design was used to maximize efficiency and to minimize potential bias (8). The matching criteria used to pair HIV seroconverters (termed cases) with nonseroconverters (termed controls) included (i) the visit at which seroconversion of the case was documented, (ii) the number of partners with whom receptive anal intercourse was reported during the preceding 6 months, and (iii) the number of study visits, so that the control had at least as many study visits as the matched case.

The time points were designated as 6-month intervals relative to the time of seroconversion to HIV, where T_{-3} is approximately 1.5 years prior to HIV seroconversion, T_{-2} is 1 year prior to HIV seroconversion, T_{-1} is 6 months prior to HIV seroconversion, T_0 is the visit at which HIV seroconversion was documented, T_1 is 6 months post-HIV seroconversion, T_2 is 1 year post-HIV seroconversion, and T_3 is 1.5 years of follow-up after seroconversion. Fewer than the

* Corresponding author.
† Present address: AIDS Activities Coordinating Office/Epidemiology Unit, Department of Public Health, City of Philadelphia, Philadelphia, PA 19107.

TABLE 1. EBV antibody titers and levels of serum IgG and rubella virus antibody pre- and postseroconversion to HIV

Serologic assay	GMT									
	HIV seroconverters (cases)					HIV seronegatives (controls)				
	T_{-1}[a] (49)[b]	T_0 (49)	T_1 (41)	T_2 (34)	T_3 (27)	T_{-1} (49)	T_0 (49)	T_1 (41)	T_2 (34)	T_3 (27)
EBV antibody										
EBV-VCA IgG	94	106[c]	155[c]	224[c]	311[c]	83	66	52	66	55
EBV-EA IgG	35[c]	33[c]	37[c]	53[c]	72[c]	7	7	5	6	5
EBNA IgG	12	11	13	13	12	10	8	9	9	9
Total serum IgG	1,333	1,324[d]	1,524[e]	1,746[e]	1,854[e]	1,416	1,156	1,037	962	1,049
Rubella virus IgG	50	47	64	60[d]	68	51	77	87	93	100

[a] Time of study designated as 6-month intervals relative to the time of seroconversion to HIV.
[b] Number of matched case-control pairs.
[c] $P < 0.05$ as compared with matched controls.
[d] $P < 0.01$ as compared with matched controls.
[e] $P < 0.005$ as compared with matched controls.

seven study visits were available for certain case-control pairs because of attrition, as is noted in the text.

Determination of HIV serostatus. Serum samples were tested for antibodies to HIV by enzyme-linked immunosorbent assay (ELISA) (LAV-EIA; Genetic Systems, Seattle, Wash.). ELISA-positive specimens were confirmed by a Western blot assay (Novapath Immunoblot; Bio-Rad Laboratories, Hercules, Calif.). A positive Western blot reaction was defined as the presence of a band representing products from at least two of the three major HIV open-reading frames: *gag* (p15, p24, p55), *pol* (p32, p65), and *env* (gp41, gp120, gp160). Antibody to recombinant HIV envelope protein (ENV9 ELISA; DuPont Co., Wilmington, Del.) and serum HIV p24 *gag* protein (DuPont) were assayed as previously described (30).

Quantitative determination of antibodies to EBV antigens. Indirect immunofluorescence tests were used to detect immunoglobulin (Ig) G antibodies to EBV viral capsid antigen (EBV-VCA) and EBV early antigen (EBV-EA) as previously described (13, 14). Briefly, titers of IgG antibodies to EBV-VCA were determined on acetone-fixed HR1-K cells by using fluorescein isothiocyanate-labeled goat antibody to human IgG. Antibody titers to EBV-EA were determined by using Raji cells treated with 5'-2-iododeoxyuridine as a source of cells containing early antigen. Anticomplement immunofluorescence methods were used to determine the titers of antibodies to EBV nuclear antigen (EBNA). Human C3 was used as a source of the complement, and fluorescein isothiocyanate-labeled antibody to human C3 was used to bind with the complex (28). Raji cells expressing EBNA were used as a source of antigen.

Serial twofold dilutions of blind-coded test sera were made beginning at a dilution of 1:5. Titers of antibodies to EBV antigens were reported as the reciprocals of the end-point dilution of serum samples showing positive fluorescence. Each assay included known positive and negative control sera.

Subjects with EBV-VCA IgG titers of ≥5 were considered seropositive to EBV. Reactivation of EBV was defined as a fourfold or greater rise in antibody titers to EBV-VCA in sequential specimens. The presence of antibody to EBV-EA (titer, ≥5) was also used as evidence of reactivation of EBV infection.

Quantitative determination of IgG and rubella virus antibodies. Total serum IgG levels were measured quantitatively by indirect immunofluorescence in milligrams per deciliter (FIAX; M. A. Bioproducts, Walkersville, Md.). IgG antibody to rubella virus was measured by indirect immunofluorescence using automated fluorometry (FIAX).

T-cell subsets. The percentages and absolute numbers of lymphocyte subsets were determined by monoclonal antibodies (Becton-Dickinson, Mountainview, Calif.) with standard methods (30).

Data analysis. Group geometric mean titers (GMT) were calculated for antibody to EBV-specific antigens by taking the antilog of the mean of the $\log_{10}$-transformed values of the individual titers. Statistical procedures for this matched-paired study included odds ratios and McNemar's matched-pair test to identify odds ratios significantly greater than unity, paired Student's *t* test, and Wilcoxon's matched-pair procedure. Spearman rank order correlation was used to estimate the associations between total serum IgG levels and the numbers of T-cell subsets and to estimate antibody titers to EBV.

RESULTS

Reactivation of EBV associated with HIV seroconversion. All (49 of 49) seroconverters (cases) and 96% (47 of 49) of HIV nonseroconverters (controls) were seropositive for EBV at the time of entry into the study on the basis of an EBV-VCA IgG titer of ≥5. The subjects were grouped by EBV-VCA IgG titers of ≥160 or <160 at T_{-1}, 6 months prior to HIV seroconversion. No significant differences between cases and controls were observed for elevated titers of EBV-VCA IgG 6 months prior to HIV seroconversion (chi-square = 0.64; odds ratio = 1.5; $P = 0.42$). A similar analysis of EBV-EA IgG, however, showed that anti-EBV-EA titers were significantly elevated in cases as compared with controls, in that 38 of 49 (78%) of the cases had EBV-EA IgG titers of ≥20, whereas 11 of 49 (23%) of the controls had elevated anti-EA titers. Matched-pair analysis showed that 31 of the cases had elevated titers such as these, while only 4 of the matched controls had EBV-EA IgG titers of ≥20 (odds ratio = 7.75; chi-square = 19.3; $P < 0.0001$).

Table 1 compares the GMT of antibodies to EBV of cases with those of controls before and after seroconversion to HIV. EBV-VCA IgG titers were not found to be significantly different between cases and controls at T_{-1} (6 months prior to HIV seroconversion). There were, however, significantly elevated titers of anti-EBV-EA in the cases as compared with the matched controls 6 months before HIV seroconver-

sion, with further rises occurring during the 18-month follow-up period.

At the visit of documented HIV seroconversion (T_0), the GMT of antibodies to EBV-VCA and EBV-EA IgG were found to be significantly higher in cases than in controls (Table 1). The GMT of EBV-VCA IgG in the cases were found to be consistently elevated compared with those of controls from T_1 through T_3, with the highest titers occurring at T_3. EBV-EA IgG titers in cases remained at the pre-T_0 elevated levels from T_1 through T_2, with a rise occurring at T_3. Antibody titers to EBV-VCA and EBV-EA did not show significant changes for controls from T_1 through T_3. EBNA IgG titers did not show significant differences between cases and controls at any time before or after HIV seroconversion.

Levels of serum IgG were determined quantitatively to investigate whether there was an association between IgG directed against EBV antigens and total serum IgG. Table 1 indicates that levels of total serum IgG were significantly elevated in the cases at T_0 and remained elevated from T_1 through T_3 as compared with matched controls. Further analysis showed, however, that there was no significant correlation between EBV-VCA or EBV-EA IgG titers and total serum IgG levels in cases or controls ($P > 0.05$; Spearman rank correlation). Moreover, anti-rubella virus IgG levels in the cases were comparable to, or significantly lower than, the levels the in controls (Table 1). There was also no association between anti-rubella virus IgG levels and quantitative levels of serum IgG in the cases or controls ($P > 0.05$; Spearman rank correlation).

A fourfold or greater rise in antibody titers to EBV-VCA was used to provide further evidence of a role for EBV reactivation in HIV infection. On the basis of this parameter, reactivation of EBV was strongly associated with HIV seroconversion (odds ratio = 3.0; confidence limits = 1.1 to 8.8; McNemar's chi-square = 6.0; $P < 0.05$). The data were also analyzed separately for the 27 matched pairs for the entire 1.5 years of follow-up after seroconversion, i.e., through T_3. Results were consistent with those reported for the 49 case-control pairs, with evidence that EBV reactivation evident 6 months prior to seroconversion remained through T_3 (data not shown).

We further studied the 16 cases that had serum samples available prior to T_{-1}. GMT for antibodies to EBV-VCA and EBV-EA did not differ significantly between cases and their matched controls at T_{-3} or T_{-2} (Fig. 1). Furthermore, both EBV-VCA and EBV-EA titers for this subgroup of 16 case-control pairs were comparable to those of the larger study group (Table 1), with complete data from T_{-3} through T_3.

We considered that inapparent HIV infection might exist in a portion of the seroconverters prior to the first Western blot-confirmed, ELISA-positive visit. Of 38 seroconverters with EBV-EA IgG titers of ≥20, 36 were tested for the presence of an early indicator of HIV infection at visits T_{-3} through T_{-1} (Table 2). Fifteen (42%) of these 36 cases with elevated EBV-EA IgG titers were positive for at least one of the following HIV serologic markers at a visit prior to T_0: HIV p24 antigen, antibody to ENV9, and at least one HIV antigen-reactive band in the Western blot. This was in contrast to cases with low EBV-EA IgG titers (<20), where only 1 of 11 (9%) had evidence of HIV infection by these tests prior to T_0 ($P < 0.05$; Fisher's exact probability test).

Relationship of number of T lymphocytes to EBV reactivation. An evaluation of T-lymphocyte subsets demonstrated differences between cases and controls. The cases had significantly lower numbers of $CD4^+$ cells than did controls by 6 months postseroconversion (Table 3). The mean number of $CD8^+$ cells was found to be significantly increased in HIV seroconverters. This elevation was noted at the visit of documented seroconversion (T_0) and at visits T_1 through T_3. Nonetheless, EBV-VCA and EBV-EA IgG titers were not found to be significantly associated with $CD4^+$ or $CD8^+$ T-lymphocyte counts for either cases or their matched controls ($P > 0.05$; Spearman rank correlation). There were also no significant relationships found between mean $CD4^+$ or $CD8^+$ cell numbers and EBV activation, as defined as a fourfold or greater increase in anti-VCA IgG titers occurring at consecutive study visits ($P > 0.05$; paired t test).

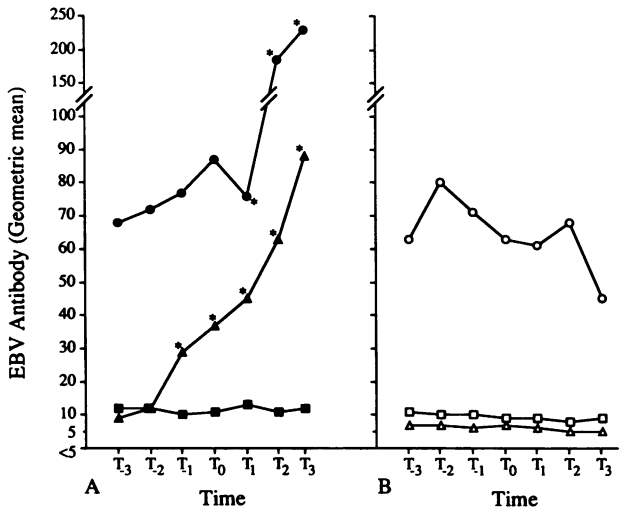

FIG. 1. EBV antibody titers in 16 cases and 16 matched controls tested 18 months prior to and after seroconversion to HIV. (A) HIV seroconverters; (B) HIV seronegatives. Symbols: ● and ○, EBV-VCA IgG; ▲ and △, EBV-EA IgG; ■ and □, EBNA IgG; *, $P < 0.05$ as compared to matched HIV seronegative controls.

Association of clinical symptoms with EBV reactivation. The role of EBV in symptoms associated with lymphadenopathy (LAD) was evaluated. Diagnosis of LAD was defined as the presence of lymph nodes more than 1 cm in diameter in two or more noncontiguous, extrainguinal sites, with any number and site of cervical nodes counted only once. Increased rates of LAD were observed in HIV seroconverters, in that 70% (34 of 49) of HIV seroconverters had

TABLE 2. Association between elevated EBV-EA IgG titer (≥20) and evidence of HIV infection in seroconverters prior to T_0

HIV serologic assay	No. of serum samples positive (% of total tested) for:	
	EBV-EA IgG titer ≥ 20 (n = 36)	EBV-EA IgG titer < 20 (n = 11)
ENV9 alone	2 (6)	1 (9)
Western blot alone[a]	6 (17)	0 (0)
p24 antigen alone	3 (8)	0 (0)
ENV9 and Western blot[a]	2 (6)	0 (0)
ENV9 and p24 antigen	1 (3)	0 (0)
Western blot[a] and p24 antigen	1 (3)	0 (0)
ENV9 and Western blot[a] and p24 antigen	0 (0)	0 (0)

[a] Positive bands in Western blot at one or two of the three major HIV gene regions.

TABLE 3. Numbers of T lymphocytes found pre- and postseroconversion to HIV

Type of T lymphocyte	No. of T lymphocytes (SE)[a] in:									
	HIV seroconverters (cases)					HIV seronegative (controls)				
	T_{-1}[b] (49)[c]	T_0 (49)	T_1 (41)	T_2 (34)	T_3 (27)	T_{-1} (49)	T_0 (49)	T_1 (41)	T_2 (34)	T_3 (27)
$CD4^+$ cells	1,033 (71)	937 (78)	726 (45)[d]	706 (56)[d]	694 (53)[d]	942 (62)	978 (45)	1,072 (66)	1,080 (72)	1,075 (58)
$CD8^+$ cells	688 (59)	715 (42)[d]	728 (49)[d]	790 (61)[d]	772 (57)[d]	547 (38)	538 (42)	543 (39)	590 (57)	483 (35)
$CD4^+/CD8^+$ ratio	1.9 (0.18)	1.6 (0.18)[e]	1.2 (0.10)[d]	1.1 (0.11)[d]	1.0 (0.09)[d]	2.1 (0.16)	2.2 (0.16)	2.4 (0.24)	2.2 (0.18)	2.4 (0.13)

[a] Arithmetic mean.
[b] Time of study designated as 6-month intervals relative to the time of seroconversion to HIV.
[c] Number of matched case-control pairs.
[d] $P < 0.005$ as compared with matched controls.
[e] $P < 0.05$ as compared with matched controls.

LAD at least once during T_{-1} through T_3 as compared with only 27% (13 of 49) of nonseroconverters. Furthermore, matched-pair analysis demonstrated a significant association between diagnosis of LAD and HIV seroconversion (odds ratio = 11.5; McNemar's chi-square = 16.0; $P < 0.0001$). The majority of the HIV seroconverters with LAD had evidence of reactivation of EBV infection (68% [23 of 34]). Among these 23 cases, reactivation of EBV infection as shown by a fourfold or greater rise in EBV-VCA IgG titer occurred more often at a study visit after a diagnosis of LAD. Seven of the 13 HIV seronegative controls with LAD also had elevated antibody titers to EBV. Six of these seven HIV-seronegative men with LAD exhibited enhanced EBV-VCA IgG titers subsequent to LAD. There was a significant association observed between the probability of EBV reactivation and evidence of LAD in the HIV seroconverters. Binomial distribution using normal approximation for LAD in this group did not indicate random occurrence of these data (95% confidence intervals, 0.68 to 0.98).

Seven of the 49 HIV seroconverters developed AIDS during 3 to 4 years of follow-up examination. Reactivation of EBV infection was noted in four of these seven HIV seroconverters prior to a diagnosis of AIDS as evidenced by elevated titers of anti-EBV-VCA (≥160) or anti-EBV-EA (≥20) at T_0.

DISCUSSION

Homosexual men had evidence of reactivated EBV infection as shown by elevated mean titers of EBV-EA IgG antibodies 6 months prior to the study visit at which HIV seroconversion was first confirmed (T_0). Titers of both EBV-EA and EBV-VCA IgG were significantly elevated by study visit T_0 and progressively increased during the 18 months of follow-up after seroconversion. Such serologic data are classic evidence for reactivation of EBV infection (7, 13). These results are corroborated by our findings of an increased rate of oropharyngeal excretion of EBV 6 months prior to HIV seroconversion in a subgroup of these homosexual men (10a). The chronic nature of this reactivation is supported by cross-sectional studies showing increased EBV-EA and EBV-VCA IgG titers (27, 29, 31) and oropharyngeal excretion of EBV (2, 25, 31) in HIV-seropositive subjects. These data are also supported by a recent study showing elevated anti-EBV IgA titers in homosexual men with relatively early HIV infection (20).

Increased IgG antibody titers to EBV-EA and EBV-VCA observed in this investigation are likely due to activation of EBV during very early HIV infection. Indeed, it is probable that some of the HIV seroconverters were infected with HIV prior to the detection of HIV antibodies by conventional ELISA and Western blot assay. There are increasing reports of such silent HIV infections as indicated by partial antibody reactivity, p24 antigenemia, virus isolation, and viral DNA amplification (polymerase chain reaction) (17, 30, 33). In the present study, we were able to assess all of these parameters except HIV DNA by the polymerase chain reaction. The data show that there was a strong association between elevated antibody titers to EBV-EA and the presence of one or more of these markers of HIV infection at a study visit prior to T_0. Such an association was not noted at visits prior to T_0 in HIV seroconverters who did not have evidence of EBV reactivation.

HIV seroconverters had increased levels of total serum IgG very early during HIV infection. Matched controls did not show such increases in IgG levels during the course of follow-up. These findings confirm and extend reports of hypergammaglobulinemia among HIV-infected men, especially in those with clinical symptoms associated with AIDS (22, 34). In the present study, however, we did not find significant, direct correlations between increases in serum IgG levels and increases in EBV antibody titers in the same individuals. There was also no increase in anti-rubella virus IgG levels after seroconversion to HIV. These findings imply that enhanced antibody production against EBV antigens is not due to nonspecific B lymphoproliferation but is a specific phenomenon, as shown in our previous cross-sectional studies (27, 29).

A clinical entity much like LAD has been described in infectious mononucleosis patients during primary infection with EBV (15). Most of the individuals in the present study were found to be seropositive to EBV at entry and thus were not candidates for a primary infection with EBV. LAD was noted in a majority (70%) of the men who seroconverted to HIV and in a smaller proportion of the HIV-seronegative homosexual men (27%). Elevated titers of EBV antibodies, however, were not associated with a concurrent diagnosis of LAD. Of interest is that serologic evidence of reactivation of EBV was noted significantly more often in both HIV-seropositive and -seronegative men at a study visit subsequent to a diagnosis of LAD. Nonetheless, it should be emphasized that numerous confounders unrelated to EBV could be associated with development of LAD in homosexual men. In fact, examination of lymphoid tissue from HIV-seropositive patients has not shown an association between EBV cellular expression and persistent LAD (32). We were also unable to clearly assess the potential role of EBV infection with respect to the development of clinical

criteria of AIDS because only seven of the HIV seroconverters developed AIDS during this early postinfection period.

HIV seroconverters had elevated levels of $CD8^+$ cells 6 months prior to seroconversion and throughout the remainder of the study period as compared with controls. Active EBV infection is normally controlled by complex immunoregulatory responses that include elaboration of activated EBV-specific cytotoxic-suppressor T cells ($CD8^+$ cells) (5, 6). Therefore, the elevated numbers of $CD8^+$ cells in HIV seroconverters could partially be explained by increased EBV activity. We found no evidence, however, that EBV contributed to the overall increase in $CD8^+$ cells in HIV seroconverters on the basis of antibody titers to EBV-VCA, EBV-EA, or both.

$CD4^+$ cell deficits were noted in cases by 6 months after seroconversion. These deficits may lead to further reactivation of EBV as evidenced by the increased levels of circulating antibodies to this herpesvirus. There was no direct, causal relationship discernible, however, between EBV activity as detected by these serologic methods and loss of $CD4^+$ cells in HIV seroconverters.

Our study suggests that the significant decline in the levels of $CD4^+$ cells in HIV seroconverters may have a profound impact on the control of EBV beginning very early in HIV infection. These data extend other studies that show disregulation in the control of EBV latency in late-term patients with AIDS and AIDS-related complex (1, 2, 4, 25–27, 31, 34). For example, elevated levels of cell-associated EBV DNA and increased titers of transforming virus have been reported in patients with AIDS and, to a lesser extent, in persons with AIDS-related complex and in asymptomatic subjects with indeterminant duration of HIV infection. Thus, it appears that reactivation of EBV is an early, possibly indirect, result of the HIV-induced immunodeficient state. This also could be related to direct reactivation of EBV in B cells by infection with HIV (19). The herpesvirus in turn may enhance HIV infection by transactivating HIV DNA replication (24). Of interest is a recent study that has noted that there are elevated levels of EBV DNA excreted in the oropharynges of HIV-seropositive homosexual men who progressed to AIDS as compared with those who were HIV seronegative (10).

The present study provides strong support for persistent reactivation of EBV in a majority of homosexual men beginning very early during HIV infection. Our findings suggest that HIV-infected individuals should be observed longitudinally for changes in EBV status and for the role of EBV in the pathogenesis of HIV infection. Further follow-up is required to investigate the role of the EBV in the pathogenesis of longer-duration HIV infection.

ACKNOWLEDGMENTS

This study was supported in part by Public Health Service contracts NO1-AI-32513 and NO1-AI-72632 from the National Institutes of Health and by the Pathology Education and Research Foundation of the University of Pittsburgh.

We thank Susan Zhou for assistance in statistical analysis, Kathie Grovit, Judy Fossati, and Debbie Laurie for assistance in preparation of the manuscript, Jeanne Neumann of DuPont Laboratories for ENV9 and p24 antigen assays, and the Pitt Men's Study staff for technical assistance.

REFERENCES

1. **Ablashi, D. V., A. Fladager, P. D. Markham, and S. Z. Salahuddin.** 1987. Isolation of transforming Epstein-Barr virus from plasma of HTLV-III/LAV-infected patients. Intervirology **27**: 25–31.
2. **Alsip, G., Y. Ench, C. V. Sumaya, and R. N. Boswell.** 1988. Increased Epstein-Barr virus DNA in oropharyngeal secretions from patients with AIDS, AIDS-related complex, or asymptomatic human immondeficiency virus infection. J. Infect. Dis. **157**:1072–1076.
3. **Andiman, W. A., R. Eastman, K. Martin, B. Z. Katz, A. Rubinstein, J. Pitt, S. Pahwa, and G. Miller.** 1985. Opportunistic lymphoproliferations associated with Epstein-Barr viral DNA in infants and children with AIDS. Lancet **ii**:1390–1393.
4. **Birx, D. L., R. R. Redfield, and G. Tosato.** 1986. Defective regulation of Epstein-Barr virus infection in patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related disorders. N. Engl. J. Med. **314**:874–879.
5. **Blumberg, R. S., T. Paradis, R. Byington, W. Henle, M. S. Hirsch, and R. T. Schooley.** 1987. Effects of human immunodeficiency virus on the cellular immune response to Epstein-Barr virus in homosexual men: characterization of the cytotoxic response and lymphokine production. J. Infect. Dis. **155**:877–890.
6. **Blumberg, R. S., T. J. Paradis, D. Crawford, R. E. Byington, M. S. Hirsch, and R. T. Schooley.** 1987. Effects of human immunodeficiency virus (HIV) on the cytotoxic response to Epstein-Barr virus (EBV) transformed B lymphocytes. AIDS Res. Hum. Retroviruses **3**:303–315.
7. **Breinig, M. K., B. Zitelli, T. E. Starzl, and M. Ho.** 1987. Epstein-Barr virus, cytomegalovirus, and other viral infections in children after liver transplantation. J. Infect. Dis. **156**:273–279.
8. **Breslow, N. E., and N. E. Day.** 1980. Statistical methods in cancer research, vol. 1. The analysis of case-control studies. IARC Scientific Publications no. 32. International Agency for Research on Cancer, Lyon, France.
9. **Ciobanu, N., and P. H. Wiernik.** 1986. Malignant lymphomas, AIDS, and the pathogenic role of Epstein-Barr virus. Mt. Sinai J. Med. **53**:627–638.
10. **Diaz-Mitoma, F., A. Ruiz, G. Flowerdew, S. Houston, B. Romanowski, T. Kovithavongs, J. Preiksaitis, and D. L. Tyrrell.** 1990. High levels of Epstein-Barr virus in the oropharynx: a predictor of disease progression in human immunodeficiency virus infection. J. Med. Virol. **31**:69–75.

10a. **Ferbas, J., M. Rahman, L. Kingsley, M. Ho, J. Armstrong, and C. Rinaldo.** Unpublished data.

11. **Greenspan, J. S., D. Greenspan, E. T. Lennette, D. I. Abrams, M. A. Conant, V. Petersen, and U. K. Freese.** 1985. Replication of Epstein-Barr virus within the epithelial cells of oral "hairy" leukoplakia, an AIDS-associated lesion. N. Engl. J. Med. **313**:1564–1571.
12. **Groopman, J. E., J. L. Sullivan, C. Mulder, D. Ginsburg, S. H. Orkin, C. J. O'Hara, K. Falchuk, F. Wong-Staal, and R. C. Gallo.** 1986. Pathogenesis of B cell lymphoma in a patient with AIDS. Blood **67**:612–615.
13. **Henle, W., and G. Henle.** 1981. Epstein-Barr virus specific serology in immunologically compromised individuals. Cancer Res. **41**:4222–4225.
14. **Henle, W., and G. Henle.** 1982. Immunology of Epstein-Barr virus, p. 209–252. *In* B. Roizman (ed.), The herpesviruses, vol. 1. Plenum Press, New York.
15. **Henle, W., G. Henle, and C. A. Horowitz.** 1979. Infectious mononucleosis and Epstein-Barr virus associated malignancies, p. 441–475. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral, rickettsial, and chlamydial infections, 5th ed. American Public Health Association, Washington, D.C.
16. **Ho, M., G. Miller, R. W. Atchison, M. K. Breinig, J. S. Dummer, W. Andiman, T. E. Starzl, R. Eastman, B. P. Griffith, R. L. Hardesty, H. T. Bahnson, T. R. Hakala, and J. T. Rosenthal.** 1985. Epstein-Barr virus infections and DNA hybridization studies in posttransplantation lymphoma and lymphoproliferative lesions. J. Infect. Dis. **152**:876–886.
17. **Imagawa, D. T., M. H. Lee, S. M. Wolinsky, K. Sano, F. Morales, S. Kwok, J. J. Sninsky, P. G. Nishanian, J. Giorgi, J. L. Fahey, J. Dudley, B. P. Visscher, and R. Detels.** 1989. Human immunodeficiency virus type 1 infection in homosexual men

who remain seronegative for prolonged periods. N. Engl. J. Med. **320**:1458–1462.

18. **Kaslow, R. A., D. G. Ostrow, R. Detels, J. P. Phair, B. F. Polk, and C. R. Rinaldo.** 1987. The multicenter AIDS Cohort Study: rationale, organization, and selected characteristics of the participants. Am. J. Epidemiol. **126**:310–318.
19. **Lai, P. K., X. Li, and D. J. Volsky.** 1989. Induction of Epstein-Barr virus in B-lymphoblastoid cells by human immunodeficiency virus type 1. Int. J. Cancer **43**:1104–1111.
20. **Margalith, M., B. Sarov, I. Sarov, C. Rinaldo, R. Detels, J. Phair, R. Kaslow, H. Ginzburg, and A. Saah.** 1990. Serum IgG and IgA antibodies specific to Epstein-Barr virus capsid antigen in a longitudinal study of human immunodeficiency virus infection and disease progression in homosexual men. AIDS Res. Hum. Retroviruses **6**:607–616.
21. **Miettinen, O. S.** 1982. Principles of epidemiologic research, p. 62–95. *In* D. G. Kleinbaum, L. L. Kupper, and H. Morgenstern (ed.), Epidemiologic research: principles and quantitative methods. Wadsworth Publishing Co., Belmont, Calif.
22. **Mizuma, H., S. Litwin, and S. Zolla-Pazner.** 1988. B-cell activation in HIV infection: relationship of spontaneous immunoglobulin secretion to various immunological parameters. Clin. Exp. Immunol. **71**:410–416.
23. **Purtilo, D. T.** 1987. Epstein-Barr virus: the spectrum of its manifestation in humans. South. Med. J. **80**:943–947.
24. **Quinlivan, E. B., E. Holley-Guthrie, E.-C. Mar, M. S. Smith, and S. Kenney.** 1990. The Epstein-Barr virus BRLF1 immediate-early gene product transactivates the human immunodeficiency virus type 1 long terminal repeat by a mechanism which is enhancer independent. J. Virol. **64**:1817–1820.
25. **Quinnan, G. V., H. Masur, A. H. Rook, G. Armstrong, W. R. Frederick, J. Epstein, J. F. Manischewitz, A. M. Macher, L. Jackson, J. Ames, H. A. Smith, M. Parker, G. R. Pearson, J. Parillo, C. Mitchell, and S. E. Straus.** 1984. Herpesvirus infections in the acquired immune deficiency syndrome. JAMA **252**:72–77.
26. **Ragona, G., M. C. Sirianni, S. Soddu, B. Vercelli, G. Sebastiani, M. Piccoli, and F. Aiuti.** 1986. Evidence for disregulation in the control of Epstein-Barr virus latency in patients with AIDS-related complex. Clin. Exp. Immunol. **66**:17–24.
27. **Rahman, M. A., L. A. Kingsley, M. K. Breinig, M. Ho, J. A. Armstrong, R. W. Atchison, D. W. Lyter, and C. R. Rinaldo, Jr.** 1989. Enhanced antibody responses to Epstein-Barr virus in HIV-infected homosexual men. J. Infect. Dis. **159**:472–479.
28. **Reedman, B. N., and G. Klein.** 1973. Cellular localization of an Epstein-Barr virus (EBV) associated complement fixing antigen in producer and non-producer lymphoblastoid cell lines. Int. J. Cancer **11**:499–552.
29. **Rinaldo, C. R., L. A. Kingsley, D. W. Lyter, B. S. Rabin, R. W. Atchison, A. J. Bodner, S. H. Weiss, and W. C. Saxinger.** 1986. Association of HTLV-III with Epstein-Barr virus infection and abnormalities of T lymphocytes in homosexual men. J. Infect. Dis. **154**:556–561.
30. **Rinaldo, C., L. Kingsley, J. Neumann, D. Reed, P. Gupta, and D. Lyter.** 1989. Association of human immunodeficiency virus (HIV) p24 antigenemia with decrease in CD4+ lymphocytes and onset of acquired immunodeficiency syndrome during the early phase of HIV infection. J. Clin. Microbiol. **27**:880–884.
31. **Sumaya, C. V., R. N. Boswell, Y. Ench, D. L. Kisner, E. M. Hersh, J. M. Reuben, and P. W. A. Mansell.** 1986. Enhanced serological and virological findings of Epstein-Barr virus in patients with AIDS and AIDS-related complex. J. Infect. Dis. **154**:864–870.
32. **Uccini, S., D. Vitolo, F. Monardo, A. Faggioni, B. Monarca, A. Vitale, L. P. Ruco, and C. D. Baroni.** 1989. Interaction of HIV and EBV at lymphoid tissue level: immunohistochemistry and in situ hybridization. Acta Pathol. Microbiol. Immunol. Scand. Suppl. **8**:28–32.
33. **Wolinsky, S. M., C. R. Rinaldo, S. Kwok, J. J. Sninsky, P. Gupta, D. Imagawa, H. Farzadegan, L. P. Jacobson, K. S. Grovit, M. H. Lee, J. S. Chmiel, H. Ginzburg, R. A. Kaslow, and J. P. Phair.** 1989. Human immunodeficiency virus type 1 (HIV-1) infection a median of 18 months before a diagnostic Western blot. Evidence from a cohort of homosexual men. Ann. Int. Med. **111**:961–972.
34. **Yarchoan, R., R. R. Redfield, and S. Broder.** 1986. Mechanism of B cell activation in patients with acquired immunodeficiency syndrome and related disorders. J. Clin. Invest. **78**:439–447.