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THE DEFINITION OF EPSTEIN BARR VIRUS (EBV)'S ROLE IN HTLV-III INFECTED USAF PERSONNEL AS RELATED TO DISEASE PROGRESSION

FINAL REPORT

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The main objective of this study was to define the role of EBV in the progressive evolution of AIDS by means of a prospective evaluation of persons with HIV infections. Detailed EBV antibody responses and virologic studies to determine the EBV load in body fluids and EBV strains were performed serially. The possible concurrent presence of HIV in EBV cell cultures established from body fluids from HIV infected persons also was examined. The HIV infected participants had an enhanced antibody response to a broad spectrum of EBV antigens. In addition, an increased amount of EBV was detected in oropharyngeal secretions as well as an increased proliferative ability of peripheral blood mononuclear cells compared to normal, healthy adults. The consequences of this highly increased burden of EBV in HIV patients are not well understood but could support the possibility that EBV may be a stimulus to produce tumors or other lymphoproliferative manifestations. The subgroup of patients with oral hairy leukoplakia (OHL) had an overall high body burden of EBV, including a high density of

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replicating virus in the oral lesions, and more abnormal EBV antibody responses than HIV-infected personnel lacking OHL. This finding further strengthened the speculation that EBV is not well immunoregulated in HIV-infected patients and can lead to various lesions, some as lymphomas (reported by others) being quite deleterious. No correlation was noted between specific EBV genotypes and progression of the HIV infection, but the numbers tested (and the changes in disease progression) were small at this point in the study.

Foreword

For the protection of human subjects the investigators have adhered to policies of applicable Federal Law 45CFR46.

The investigators have abided by the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (April 1982) and the Administration Practices Supplements.

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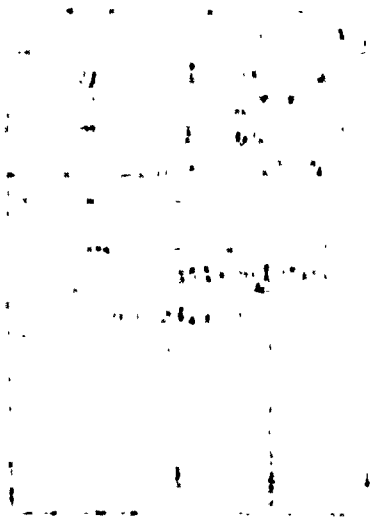

Statement of Problem

Infection with human immunodeficiency virus type 1 (HIV-1), the cause of acquired immunodeficiency syndrome (AIDS), may remain quiescent for long periods, including years, without producing any clinical signs of disease. The present study hypothesized that repeated reactivation of latent infection by strains of Epstein-Barr virus (EBV), a member of the herpesvirus group, and possibly other forms of *in vivo* interaction between HIV-1 and EBV provokes clinical progression of the HIV-1 infection. It also was speculated that abnormalities in immunoregulation of the latent EBV infection in patients with HIV-1 infections would result in an increased burden of EBV, that in turn could be responsible for the production of severe lymphoproliferative lesions, lymphomas, or other as yet ill-defined manifestations.

Background

At the start of this study, it had already been demonstrated that HIV-1 was essential to the development of AIDS. However, there is some evidence suggesting that EBV could possibly play a role in the clinical progression of AIDS or, at a minimum, inducing manifestations and characteristic pathologic lesions that may be severe and fatal (1). A prevalence study performed by this investigator and collaborators showed that patients with AIDS-related complex (ARC) and AIDS had an exaggerated antibody response to EBV antigens and a highly increased load of EBV in oropharyngeal secretions and peripheral mononuclear cells (2). It was speculated that these findings were evidence of recurrent intense (re)activation of the latent EBV infection in patients with ARC and AIDS. B cell lymphomas containing EBV-DNA were also reported in increased frequency in homosexuals and AIDS patients (3). This latter finding is being noted moreso, possibly as a result of the increased longevity of HIV-infected patients through the administration of antiretroviral chemotherapy. Oral hairy leukoplakia was found to contain EBV markers and also appeared to indicate a poor prognosis for the HIV-infected patient (4). Moreover, a lymphocytic interstitial pneumonia that produced significant morbidity and mortality in children with HIV-1 infections was found to be associated with EBV (5).

Approach to Problem (AIMS)

The aims of the current protocol were to determine if changes in EBV serologic findings and the quantity or type of EBV strains in oropharyngeal secretions and peripheral blood mononuclear cells correlated with clinical and immunologic

progression of the underlying HIV-1 infection. A positive correlation would be compatible, albeit not specific, with the concept that EBV contributes in some form to the progression of the HIV-1 infection. Specific changes in EBV parameters that correlate with specific changes or manifestations related to the HIV-1 infection would provide further evidence of a more intimate, perhaps causative or contributory, association. As patients in this prospective study evolved into an increasingly more progressive HIV infection state, it was to be determined if EBV markers (or unusual strains of EBV) were present in selected pathologic lesions and/or abnormalities in T cell immunoregulation of EBV coincided with the progression of event(s). The results from this part of the investigation also could indicate that EBV was responsible, directly or indirectly, in the manifestations or evolution of the HIV-1 infection.

Materials and Methods

Study group. Individuals eligible to enroll in this study were required to have a documented HIV-1 infection, as determined by the screening HIV-1 antibody program instituted by the Armed Services. Those individuals that were Air Force personnel were then evaluated for their HIV-1 infection at Wilford Hall Medical Center, San Antonio, Texas, and asked to sign the consent form for entry into the collaborative prospective study directed by Dr. (Col.) R. Neal Boswell and which includes this protocol. With the on-site evaluation, the HIV-1 infection of the participants was then staged according to the Armed Services (Walter Reed or WR) Disease Classification (Table 1) (6). Detailed EBV serologic and virologic studies for the present protocol were to be performed serially every 6-12 months routinely, and, if possible, at the time of clinical changes.

EBV Serologic Testing. Specific EBV serologic testing included antibody determinations to EBV capsid antigen (to include IgM, IgG, and IgA antibodies), diffuse and restricted components of EBV early antigen, and to EBV nuclear antigen. This testing involves standard, indirect immunofluorescent techniques that have been described by the principal investigator and other workers (2, 7).

EBV Virologic Testing. This testing included the following procedures in oropharyngeal secretions, peripheral blood lymphocytes, and selected pathologic tissues: prevalence and titer of infectious EBV according to a transformation assay (8, 9), prevalence and quantity of EBV-DNA by spot hybridization (10), and prevalence of EBV antigens utilizing reference sera or monoclonal EBV antibodies.

Isolates of EBV were examined for strain differences by performing blot hybridization with cloned probes on agarose gel-separated products of extracted DNA that has undergone restriction enzyme digestions (11).

In addition, the concomitant presence of in vitro HIV-1 antigen and infectious virus was searched for in established EBV cell cultures that were derived from specimens obtained from individuals infected with HIV-1.

EBV Immunologic Testing. The regression assay to be used to determine the efficiency of the patient's T cells in inhibiting the transformation of EBV infected B cells has been described by Crawford et al. (12).

Results

Serologic Findings. Individuals with HIV-1 infection almost uniformly were found to have antibodies to EBV, indicating an exposure to this virus (Table 2). (Control rates are shown in Table 4.) The intensity of the IgG antibody response to EBV antigens of the replicative cycle, viral capsid antigen and early antigen, was at increased levels in early Walter Reed (WR) classes. The level of the IgG antibody response to EBV capsid antigen was even higher in the later WR classes 4-6, in comparison to 1-3. A similar rise in later WR classes was not as apparent in the IgG antibody response to EBV early antigen. However a shift in the direction of the antibody response to components of EBV early antigen occurred with increased WR classification, i.e. emergence of an increasing amount of antibody responses to diffuse (D) component or D plus restricted (R) components with higher WR classification.

Serum IgM antibodies to EBV capsid antigen, indicating an acute, usually primary infection, were not found to any significant degree in HIV-1 infected patients regardless of WR classification. However, the study group was not comprised of a large group of individuals with advanced WR classes for adequate comparison with early WR classes. While the prevalence of antibodies to EBV nuclear antigen was similar among the WR classes (and controls), there was a decline in the GMT of this antibody response with advanced WR stages 4-6 (Table 2).

Virologic Findings. The high positive rates of EBV in oropharyngeal secretions of patients with HIV-1 infections were much higher compared to healthy controls and approached rates seen in acute, EBV induced IM (Table 3). The high positive rates of EBV in the HIV-1 infected patients were relatively similar throughout the WR classes. (Control rates are shown in Table 5.)

High positive rates of peripheral blood spontaneous lymphoproliferation induced by EBV were found in all WR classes compared to healthy controls (Table 3). There appeared to be a trend for this high positivity rate to decline somewhat in those with more advanced WR classes 5 and 6, but this was difficult to ascertain because of the relatively smaller number of evaluable patients with more advanced WR classification. The estimated mean number of EBV-infected lymphocytes (virocyte count) from peripheral blood specimens also was increased among all WR classes compared to adults. Again, the relatively smaller number of patients with more advanced WR classification, in addition to the broad standard deviations for virocyte counts, restricted the ability to make adequate comparisons among the WR classes. As noted in Table 5, healthy controls were found to have a prevalence of <15% for spontaneous proliferation of peripheral blood lymphocytes and a mean count of <2 of EBV infected cells per 10^7 mononuclear cells. The high rates of levels of EBV burden in peripheral blood mononuclear cells in HIV-1 infected patients approached the values seen in acute EBV induced IM (Table 5).

Immunologic (EBV-specific) Findings. Preliminary findings from a regression assay examining predominantly the host's T8 cell immune capacity to restrict transformation revealed that a significant proportion of patients in early WR classes tested to date had abnormal responses compared to healthy controls (Figure 1). The few patients tested at this time preclude the determination of interclass differences.

Patients with Oral Hairy Leukoplakia (OHL+). Fifty HIV-1 infected males (almost all declared homosexuals or bisexuals) and one HIV-1 infected female were identified as having OHL. Thirty-three of these patients were in early WR classes 1 and 2. With rare exceptions, all OHL+ lesions contained herpesvirus-like particles visualized by electron microscopy performed at Wilford Hall Medical Center.

As a group the OHL+ patients had significantly greater geometric mean titers (GMT) of IgG antibodies to EBV antigens of the replicative cycle, capsid antigen and early antigen, more depressed titers of antibodies to EBV nuclear antigen and a greater prevalence of IgG antibodies to early antigen complex compared to healthy controls (Table 4). For the same antibody responses, the OHL- group were either intermediate between the OHL+ group and healthy controls or else tended to be closer to the latter's results. The OHL+ group had a greater GMT of IgG antibodies to capsid antigen, a similar rate of IgG antibody responses to early antigen complex, while a lower prevalence and GMT of IgM to capsid antigen, lower GMT of IgG antibody to early antigen complex and greater prevalence and GMT of antibodies to nuclear

antigen than the acute IM group. The antibody response to early antigen complex was directed to D component, anti-D or anti-DR, in 21 of 46 (45.7%) OHL+ patients. This rate was greater than that found in the non-OHL matched control group, 24 of 90 (26.7%) ($p<.05$), but not as high as in the IM group, 27/27 (100%) ($p<.0005$).

The prevalence and content of EBV in oropharyngeal secretions and peripheral blood mononuclear cells was uniformly greater in the OHL+ group than in the healthy controls and, in some cases, than in the OHL- matched control group (Table 5). The titers of transforming EBV in oropharyngeal secretions from OHL+ patients were similar to OHL- matched controls and, in both cases, greater than that noted in the acute IM group ($p<.05$) and in healthy controls that are found to be excreting virus in these secretions ($p<.005$). Aside from the titer of oropharyngeal EBV, other values for the OHL+ group were either similar to that of the acute IM group or approached it to a greater degree than the OHL- matched control group.

Cryostat sections of 10 tongue biopsies from OHL+ patients were examined for the presence of viral capsid antigen and early antigen by an indirect immunofluorescent technique using monoclonal antibodies to these specific antigens. Dense concentrations of EBV capsid antigen and early antigens were detected in 8 biopsies, while viral capsid antigen alone was detected in one. Transforming EBV was grown from 2 of 10 OHL+ biopsy specimens cultivated in vitro with umbilical cord mononuclear cells.

EBV DNA Analysis. EBV DNA content in oropharyngeal secretions from HIV-1 infected patients was determined by a DNA hybridization method using a virus specific probe. In this analysis, the patients were placed in three groups according to symptomatology: asymptomatic, ARC (mild to moderate symptomatology), and frank AIDS (severe opportunistic infection or AIDS-related cancer). Controls included healthy individuals and patients with acute EBV IM. Of 124 oropharyngeal specimens from 111 patients, 33% were positive for EBV DNA with concentrations from 10^5 to $>10^8$ genome equivalents/ml (Figure 1). Ten (40%) of 25 specimens from patients with AIDS and 24 (43%) of 56 specimens from patients with ARC were positive for EBV DNA. In contrast, only 4 (23%) of 30 specimens from HIV-1 antibody positive, asymptomatic patients were positive. A positive correlation existed between the concentration of EBV DNA detected and the severity of the diagnosis (Table 6). The sensitivity and specificity of the hybridization assay compared to a qualitative bioassay (lymphocyte transformation) was 48% and 95% respectively. EBV DNA was not detected in healthy controls and persons with acute EBV IM, including those with

oropharyngeal EBV detected by the bioassay. Although patients with AIDS tested had higher levels of transforming EBV in oropharyngeal secretions compared to patients with acute IM (Table 6), these levels still could not explain or account for the extremely high EBV DNA present in these secretions.

Southern analysis using EBV Eco RI A probe was performed to determine the EBV genotype in oropharyngeal specimens and/or peripheral blood lymphocytes from HIV-infected patients. The DNA patterns were classified as "standard", the pattern being identical to the non-permissive EBV-infected Burkitt tumor-derived cell line denoted Raji, or otherwise as "unique."

Results are summarized according to EBV DNA patterns and patient classification in Table 7. A unique, variant EBV isolate was present in the positive oropharyngeal washing of 8 of 21 patients. No differences by WR classification were noted with the numbers of specimens examined to date. In patients with matched oropharyngeal and blood lymphocyte specimens, the EBV DNA patterns were identical in both specimens. A representative Southern analysis of oropharyngeal washing and peripheral blood lymphocytes from HIV-1 infected patients is depicted in Figure 2. Taking into account the relatively few specimens tested, a similar degree of genomic variability was detected in various nonHIV-1 infected control groups as patients with acute or "chronic" IM, healthy family contacts of acute IM patients, and healthy individuals with an old quiescent EBV infection. In 70% of cases with unique DNA patterns, there was at least an alteration in the Bam HI H region of the EBV genome. There was one case, though, of an HIV-infected patient (WR2) that had a suggestion of a change in genotype of his EBV isolate collected at 2 points in time, a phenomenon that has not been documented before. Southern analysis of isolates and stored tongue tissues from OHL+ patients currently is being performed.

Surveillance for HIV-1 Infection in EBV Cell Cultures In Vitro. All 224 EBV cell cultures established from oropharyngeal washing and 54 peripheral blood mononuclear (lymphocytic) cell cultures that developed spontaneous proliferation, i.e. were EBV infected, collected from HIV-infected individuals, were negative for HIV-1 DNA, as determined by the P^{32} labeled DNA probe (DuPont de Nemours & Company, Boston Massachusetts). None of 22 supernates from EBV cell cultures established from oropharyngeal washings or from proliferating transformed peripheral blood lymphocytes collected from HIV-1 infected individuals yielded infectious HIV-1 isolates, as determined by cytopathogenic effect or reverse transcriptase activity in inoculated Alec cells.

Discussion

The presence of an intense IgG antibody response to EBV antigens of the replicative cycle throughout the WR classes suggest that there is an increased carrier state of EBV in patients with HIV-1 infections. This type of antibody response is known to occur in patients with immunodeficiencies of a variety of causes and in chemically immunosuppressed patients. However, the titers found in this HIV-infected population even exceeded those of the latter immunocompromised groups. Furthermore, antibodies to EBV nuclear antigen were found in unexpectedly normal to high levels in the early WR classes, as is classically seen in patients with nasopharyngeal carcinoma. This finding is not commonly in other forms of immunodeficiencies, the latter usually exhibiting normal to depressed titers of antibody to this EBV antigen. The intense antibody responses to antigens for the replicative cycle reflected those of a reactivated type EBV infection, i.e. activation of a previously quiescent latent EBV infection, since antibodies and EBV nuclear antigen were almost uniformly present.

An interesting correlation was noted between a significant decrease of an antibody response to EBV nuclear antigen along with a shift of antibody response to EBV early antigen complex directed to D component (instead of R component) and advanced WR classification. These serologic changes were associated with a general increase in symptomatology in HIV-1 infected patients, although not to any specific manifestation.

The IgM antibody response to EBV capsid antigen was not increased in this HIV-1 infected patient group, but the number of patients tested that were in the more advanced WR classes was relatively small. In a large concurrent evaluation of AIDS and ARC patients' sera from the M.D. Anderson Cancer Institute, Houston, it was noted that there was a trend for an increase in IgM responses in the ARC patients. It also was demonstrated that in a few instances, 3 of 11 episodes, the patient's EBV specific IgM antibody response was associated temporally with a febrile/hepatitis episode that heralded the onset of ARC. Again, as with the present study group, the IgM antibody response was associated with a reactivated type infection and not a primary infection.

The serologic findings in the HIV-1 infected group apparently were reflective of an abnormally elevated burden of transforming EBV present in oropharyngeal secretions and peripheral blood lymphocytes of patients with HIV-1 infections of all WR classes. In general, the HIV-1 infected group had an increased burden of EBV in these secretions or

body fluids that were well above healthy controls, but not usually at the levels seen in acute EBV induced IM. However, it was also noted that in these HIV-1 infected patients with detectable virus in oropharyngeal secretions, the titers of the transforming virus was greater than that seen in individuals with acute EBV IM. In addition, the HIV-1 infected group, particularly those with advanced disease, as ARC and moreso AIDS, had much higher levels of EBV DNA in oropharyngeal secretions than individuals with acute EBV IM, and that was greater than expected from the corresponding level of transforming virus. The reason for the latter finding is not clear, but could mean that HIV-1 infected patients contained EBV in salival secretions that was defective or nontransforming, in addition to transforming virus. Alternatively, it could be speculated that the results were due to the presence of neutralizing antibody in oropharyngeal secretions of HIV-1 infected patients which prevented or decreased the efficiency of transformation. Increased numbers of study patients in the more advanced WR classes are needed to determine if there truly are increases in the EBV burden with increasing WR classification. Nonetheless, the excessively high levels of EBV demonstrated in HIV-1 infected patients as a whole posed significant concern because of the increased potential for malignant trans- formation of a clone of EBV that could emerge and lead to serious lymphoproliferative lesions and lymphomas.

The subset of HIV-1 infected patients with OHL+ can provide important information on the potentially deleterious effects that may be produced by an overly large burden of EBV permitted by the host's abnormal immunoregulation of EBV. The OHL+ patients appear to have a most intense level of EBV in peripheral blood lymphocytes and possibly oropharyngeal secretions in comparison to those HIV-1 infected patients of the same WR class. It has been shown clearly that large numbers of replicating virus are present in these tongue lesions. However, it remains to be explained why the tongue should be so susceptible to a rampant growth of invading EBV. Perhaps the EBV present in these lesions consists of a variant, i.e. a defective or unusual DNA fragment pattern--a task that deserves to be performed with continuance of this project. It still is not incontrovertibly known if the presence of OHL+ indicates a poor prognosis for HIV-1 infected patients.

This point needs to be crit. ..ly evaluated, along with detailed EBV studies of other possible EBV-related manifestations, during a sufficiently long surveillance period. Moreover, it also would be important to determine if the use of an anti-EBV chemotherapeutic agent, as acyclovir, in patients with OHL+, or even as a result of the currently tested acyclovir-AZT combination therapy for patients with

progressing HIV infection (regardless of the presence or absence of OHL+), produces any long-term improvement of the general HIV-1 infection status.

The number of HIV-1 infected patients with serial specimens and also with documented progression of their WR classification in the current evaluation is much too small to uncover a correlation between EBV findings and clinical/ immunologic progression of the HIV-1 infection. As a corollary, it was not possible to determine if any EBV findings, such as an early anti-D response, recurrent anti-VCA IgM responses, low initial anti-EBV nuclear antigen responses, very high initial anti-VCA IgG responses, and highly elevated EBV burden in oropharyngeal secretions or peripheral blood mononuclear cells, have any prognostic significance. The data accumulated thus far provides a prevalence and cross-sectional evaluation of EBV findings in the study population. The continuation of this prospective study will allow for a better interpretation and extension of the present findings and correlation of EBV with progression of the HIV-1 infection and EBV-related pathologic lesions. It would be important to expand the study by examining patients with acute hepatitis, chronic recurrent parotiditis (Sjorgren's syndrome), pneumonias, and central nervous system manifestations, including pathologic tissue examination, for a potential association with EBV.

Table 1

Armed Forces (Walter Reed or WR)
Disease Classification of Infection
by Human Immunodeficiency Virus (HIV)

Class	HIV ANTIBODY and/or VIRUS ISOLATION	CHRONIC LYMPH-ADENOPATHY	T HELPER CELLS/mm3	DELAYED HYPER SENSITIVITY	THRUSH	OPPORTUNISTIC INFECTION
1	+	-	>400	Normal	-	-
2	+	-	>400	Normal	-	-
3	+	+/-	<400	Normal	-	-
4	+	+/-	<400	Partial anergy	-	-
5	+	+/-	<400	Partial/ complete anergy	+	-
6	+	+/-	<400	Partial/ complete anergy	+/-	+

Table 2

The prevalence and geometric-mean titer (GMT) of serum antibodies to Epstein-Barr Virus (EBV) according to the Walter Reed (WR) classification.

	Class					
	1	2	3	4	5	6
IgM-CA						
Prevalence*	2/219(0.9)	1/124(0.8)	0/52(0)	0/27(0)	0/36(0)	1/33(3.3)
GMT	2.5	2.5	2.5	2.5	2.5	2.7
IgG-CA						
Prevalence	230/230(100)	123/124(99.2)	52/52(100)	26/27(96.3)	36/36(100)	33/33(100)
GMT	340.8	353.8	395.9	446.6	435.3	447.7
IgG-EA						
Prevalence+	156/201(77.6)	87/117(74.4)	41/52(78.9)	23/27(85.2)	25/36(69.4)	29/33(87.9)
Anti-D¥	26/156(16.7)	10/87(11.5)	19/41(46.4)	8/23(34.8)	7/25(28%)	13/29(44.8)
Anti-R	124/156(79.5)	61/87(70.1)	18/41(43.9)	12/23(52.2)	13/25(52)	14/29(48.3)
Anti-DR	6/156(3.8)	16/87(18.4)	4/41(9.8)	3/23(13.0)	5/25(20)	2/29(6.9)
GMT+	22.0	24.5	26.7	35.2	23.3	24.0
Anti-EBNA						
Prevalence	190/192(99.0)	104/109(95.4)	51/52(98.1)	24/27(88.9)	35/36(97.2)	32/33(96.9)
GMT	26.1	26.5	23.5	15.1	19.3	11.6

* Number of positive specimens/total number of specimens (%)

\+ The prevalence and GMT of total positive reactions to EA components.

¥ The positive reactions were divided into these directed to the diffuse (D) component or restricted (R) component of EBV early antigen complex or an undifferentiated (DR) response. % of response to the individual complex are based on those of the total positive anti-EA IgG reaction group.

Table 3

The prevalence and titers of EBV in oropharyngeal washings and peripheral blood mononuclear cells of HIV infected patients according to WR class.

	1	2	3	4	5	6
Oropharyngeal washings						
Prevalence*	186/322	64/108	23/38	15/28	26/40	20/36
%	57.8	59.3	60.5	53.6	65.0	55.6
Peripheral mononuclear cells						
Spontaneous proliferation	207/467	140/227	23/53	15/30	14/45	11/36
Prevalence						
%	44.3	61.7	43.4	50.0	31.1	30.6
Virocyte count						
Mean estimate+	40.6	169.6	56.2	94.1	174.5	35.9
(±1 SD)	(165.9)	(934.3)	(206.7)	(358.7)	(497.4)	(99.8)
Number	393	190	46	24	33	28

* no. of positive specimens/total no. of specimens. All the oropharyngeal washings have not yet been processed.

\+ no. of EBV infected cells per 10^7 mononuclear cells as per limiting dilution assay on a feeder layer of umbilical cord cells (±1 SD)

Table 4

Comparison of Antibodies to EBV Antigens in Patients with OHL (OHL+) and Control Groups

Antibodies	HIV-Infected OHL+ (WR 1-6) (n=51)	HIV-Infected OHL-# (WR 1-6) (n=93)	Healthy Controls (n=31)	Controls with Infectious Mononucleosis (n=27)
IgM to EBV capsid antigen				
Prevalence (%)	0 (0.0)	1 (1.1)	0 (0.0)	26 (96.4)
GMT	2.5	2.5	2.5	111.4***
IgG to EBV capsid antigen				
Prevalence (%)	51 (100)	93 (100)	30 (96.8)	27 (100.0)
GMT	500.9	330.8*	136.4**	288.9*
IgG to EBV early antigen				
Prevalence (%)	46 (90.2)	69* (74.2)	7***(22.6)	27 (100.0)
GMT	30.5	21.0	6.5**	86.5**
To EBV nuclear antigen				
Prevalence (%)	46 (90.2)	92 (98.9)	30 (96.8)	10***(37.0)+
GMT	14.0	25.2*	26.8*	1.7**

\# Age and sex matched. There are 2 controls for each OHL+ patient, except for 9 instances.

\+ The sera of these 10 patients had only low titers of antibody, 2.5-5.

*, **, *** are p values <.05, <.005, and <.0005, respectively, in comparison to the OHL+ group.

NOTE: Prevalence data are no. of patients (%). GMT = geometric mean titer. To figure this mean, we defined nondetectable titers for antibodies to EBV nuclear antigen (<2.5) and for all others (<10) as 1.25 and 5, respectively.

Table 5

The Prevalence and Content of EBV in Saliva and Peripheral Blood Mononuclear Cells of Patients with OHL (OHL+) and Controls

Parameter	HIV-infected OHL+ (UR 1 - 6)	HIV-infected OHL- # (UR 1 - 6)	Healthy Controls	Controls with Infectious Mononucleosis
Oropharyngeal EBV				
Prevalence (%)	34/49(77.6)	55/93(59.1)	5/23***(21.7)	17/19 (89.5)
Titer $\log_{10}$ TD*	2.5	2.4	0.1***	0.4**
Number titered	20	12	5	8
Peripheral blood lymphocyte spontaneous proliferation				
Prevalence (%)	30/51(58.8)	36/93*(38.7)	3/23***(12.0)	10/15 (66.7)
Estimate of EBV-infected cells	80.0	19.6*	1.9**	244.7*
(±1 SD)	(248.5)	(50.9)	(3.9)	(611.7)
Number examined	50	90	7	9

Age and sex matched

* Transforming doses

Note: Results are no. positive/no. tested (%), except for EBV-infected cells, which shows the no. of cells/10^7 mononuclear cells (± 1 SD).

*, **, *** are p values <.05, <.005, <.0005, respectively, in comparison to OHL+ group.

Table 4

Hybridization of EBV DNA Probe to Oropharyngeal Specimens of Patients with AIDS, ARC or Asymptomatic HIV Antibody Positivity

Signal Intensity	Strong	Moderate		Negative
EBV Genome Equiv/ml	10^7- $>10^8$	10^6-10^7	10^5-10^6	$<10^5$
Clinical Diagnosis			n = # specimens	
AIDS	5	3	2	17
ARC	1	9	14	43
Asymptomatic HIV Antibody Positive	0	2	5	23

Note: The strength of each hybridization signal was assigned relative to all specimens in the assay. EBV genome equivalents/ml were estimated by visual comparison to the serial Raji cell dilutions.

Table 7

Variation in DNA Patterns of EBV Isolates in Oropharyngeal Washings or Peripheral Blood Mononuclear Cells According to WR Class

	Class						
	1	2	3	4	5	6	Total
Oropharyngeal Washings							
No. with							
Prototype (Raji-like isolate)	7	5	-	1	0	0	13
Variant isolate	5	0	-	0	2	1	8
Peripheral Blood Mononuclear Cells							
No. with Cells							
Prototype	8	4	-	1	1	0	14
Variant isolate	4	0	-	0	1	1	6

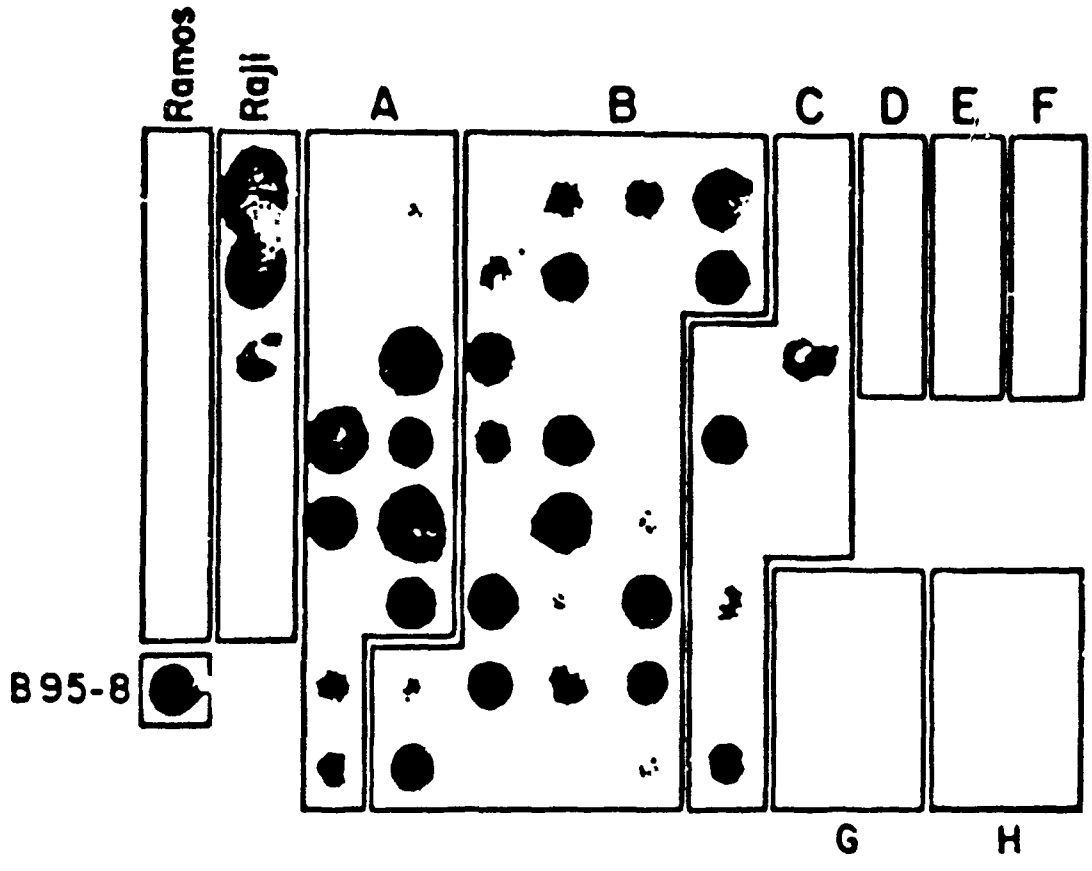

Figure 1. 124 oropharyngeal specimens were assayed for the presence of EBV DNA in an initial screen. All presumptive positive and representative negative specimens from each group were rescreened on the filter shown above. Ramos (negative control) and Raji (positive control) cells were spotted in six, serial 10-fold dilutions (10^6 to 10^1 cells per spot, from top to bottom). 300 µl of each mouthwash and the B95-8 (positive control) cell supernatant were spotted as indicated. Sections A through C indicate EBV DNA-positive oropharyngeal specimens from patients with AIDS (A), patients with ARC (B) and asymptomatic persons positive for HIV antibody (C). Sections D through F are EBV DNA-negative oropharyngeal specimens from patients with AIDS (D), patients with ARC (E), and asymptomatic persons for HIV antibody (F). G and H show oropharyngeal specimens, all EBV DNA-negative, from patients with infectious mononucleosis and from normal adults, respectively.

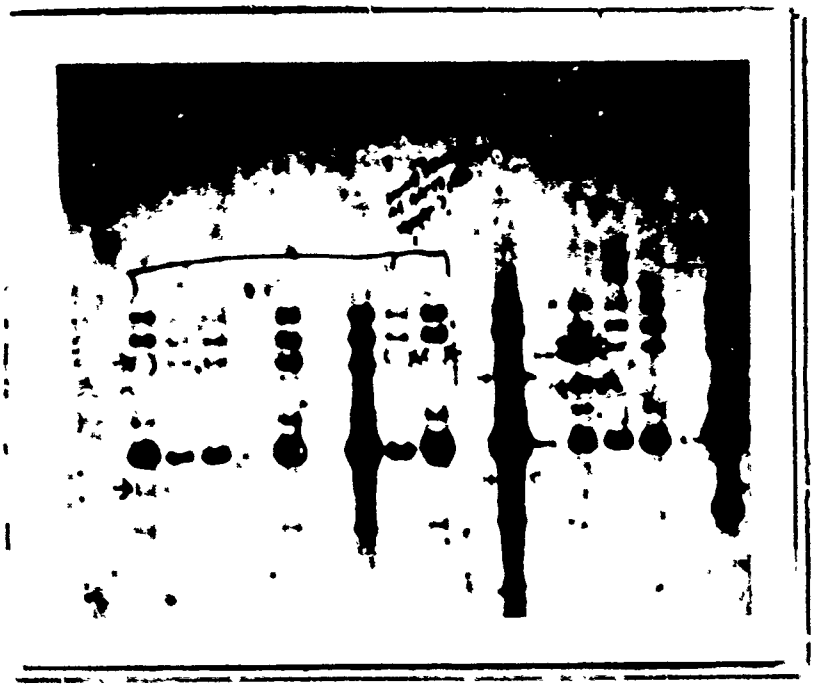

Figure 2. Southern analysis using EBV Eco RI "A" probe to determine the EBV genotype in oropharyngeal (throat) washings (TW) and peripheral blood lymphocytes (PBL) in HIV infected individuals of different WR classes. The genotypes were classified as standard if the pattern was identical to the non-permissively EBV infected Burkitt's tumor derived cell line, Raji (right edge). Arrows (→) point to new DNA bands and with parenthesis (()) signify loss of bands in comparison to the Raji pattern.

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