Respiratory Medicine (1992) **86**, 49–52

Short Report

High titers of Epstein-Barr virus antibodies in adult patients with lymphocytic interstitial pneumonitis associated with AIDS

M. R. KRAMER*§, M. J. SALDANA†, M. RAMOS* AND A. E. PITCHENIK*‡

**Pulmonary Division and the †Pathology Department, University of Miami School of Medicine and ‡The Veterans Administration Medical Center, Miami, Florida, U.S.A.*

Introduction

Lymphocytic interstitial pneumonitis (LIP) was classically described by Carrington and Leibow in 1966 in non-AIDS patients (1). This pathological description of diffuse lymphocytic infiltration of the interstitium was later associated with various autoimmune disorders such as Sjogren's syndrome, thyroiditis and others (2). Since the beginning of the AIDS epidemic several reports described the common finding of LIP in children infected with human immume deficiency virus (HIV) (3). Less commonly, LIP was reported in adults, mostly Haitians, with AIDS or AIDS-related complex (4–6). While the pathological and radiological manifestations are well described, little is known about the pathogenesis of this disorder. There is some evidence in children with AIDS that Epstein-Barr virus (EBV) is associated with LIP. Feckler and associates (7) reported the detection of EBV-DNA genome in saliva and lung tissue of a child with LIP and Adinman and colleagues (8) reported similar findings in eight additional AIDS-children with LIP. In order to explore the possible etiologic role of EBV in HIV-seropositive adults with LIP we measured the EBV serology in the peripheral blood of five patients with LIP and compared them to 19 HIV-positive patients without LIP.

Patients and Methods

Five HIV-seropositive patients with pathological diagnosis of LIP were studied. Diagnosis was made by transbronchial biopsy in four patients and open lung biopsy in one. Bronchoscopy specimens were negative for bacterial, mycobacterial, fungal, *Pneumocystis carinii* or Cytomegalovirus. Ninteen HIV-seropositive patients with biopsy proven AIDS-related (non-LIP) pulmonary disorders served as controls. All patients had diagnostic fiberoptic bronchoscopy with transbronchial biopsies and bronchoalveolar lavage to evaluate abnormal chest film associated with respiratory symptomatology. HIV antibodies were initially measured by ELISA and then confirmed by the Western Blot test. EBV serology was performed with the standard immunofluorescent technique (Organon Teknika, Durham, North Carolina). We measured antibodies against viral capsid antigen (VCA), early antigen R and D (Early), nuclear antigen (EBNA) and IgM. Titers of antibodies were given as the reciprocals of the end-point dilution of serum with positive fluorescence. Total T-helper cell numbers and T-helper/T-suppressor cell ratios were measured in the peripheral blood of LIP and control patients.

STATISTICAL ANALYSIS

Group geometric mean titers were calculated for antibody of EBV by taking the $\log_{10}$-transformed value of the individual titers. Comparison between groups was carried out by analysis of variance and Fisher's exact test.

Results

EBV SEROLOGY IN LIP VERSUS NON-LIP PATIENTS

All patients in both groups had detectable antibodies against EBV capsid antigen. The patients with LIP had significantly higher antibody titers for capsid antigen with a geometric mean of 2229 versus 166 in the non-LIP group ($P<0{\cdot}005$) (Fig. 1). When corrected for total globulin levels, i.e. geometric mean/total globulin, titers were still significantly higher in the

Received 11 January 1991 and accepted in revised form 12 June 1991.

§To whom correspondence should be addressed at: Pulmonary Department, Hadassah University Hospital, Kiryat Hadassah, POB 12000, Jerusalem, Israel.

Supported in part by Veterans Administration Merit program.

0954-6111/92/010049+04 $03.00/0

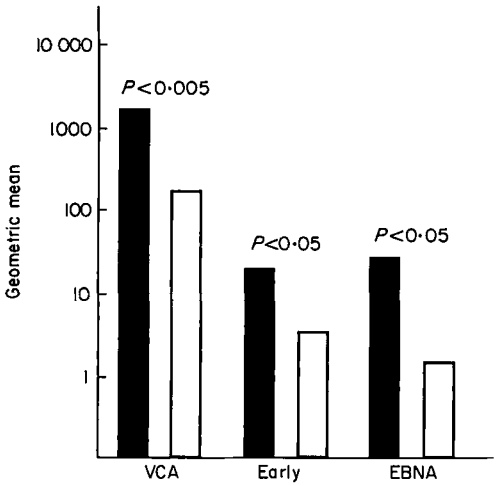

Fig. 1 Geometric means of EBV antibody titers of LIP (■) and non-LIP (□) patients. For abbreviations see Patients and Methods section.

LIP group (331 versus 47, $P<0.05$). Titers higher than 1:640 were present in five out of the five LIP patients versus two of the nineteen non-LIP patients ($P<0.001$). The geometric mean of the early antibody was 29 in the LIP group versus 2 in the non-LIP group ($P<0.05$). Antibody to EBV early antigen were present in all patients with LIP in whom it was tested and was undetected in 14 of 19 patients without LIP ($P<0.05$). IgM antibodies were not detected in both groups. Both groups had similarly low total T-helper cell count ($P>0.2$) and low T-helper/T-suppressor cell ratios ($P>0.2$).

CLINICAL FEATURES (TABLE 1)

All LIP patients had shortness of breath, tachypnea and dry cough. Chest radiograph showed bilateral, diffuse reticulonodular infiltration more prominent at the bases in all cases. Marked hyperglobulinemia (four had polyclonal and one monoclonal pattern) was noted with mean globulin of 6·7 g l^{-1} as compared to 3·5 g l^{-1} in the non-LIP group ($P<0.02$). The non-LIP patients had various pulmonary pathologies which are shown in Table 1. The histology of the lung biopsies in the LIP patients showed diffuse lymphocytic infiltration of the interstitium as well as peribronchial infiltration as demonstrated in the open lung biopsy from patient No. 2 (Plate 1).

Discussion

LIP is characterized by diffuse interstitial infiltration of the lungs with lymphocytes and plasma cells (4–6). It is a common finding in children with AIDS and is occasionally found in adults, commonly

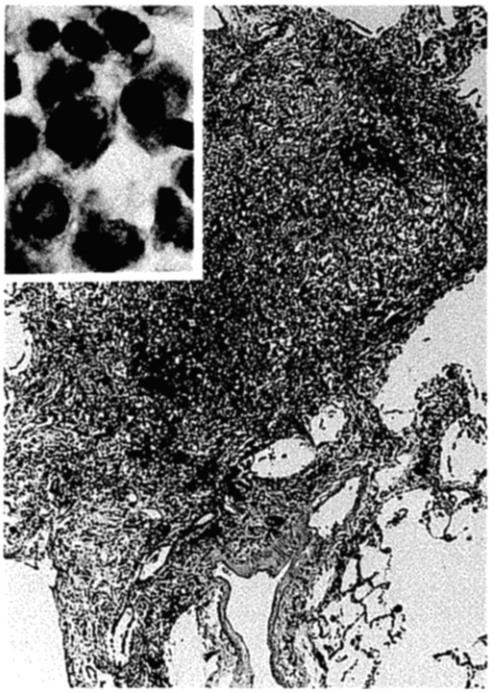

Plate 1 Open lung biopsy in patient No. 2 of the LIP group. The lung tissue is replaced by a dense interstitial infiltrate composed of lymphoid cells in a delicate fibrous stroma. The airway at bottom is compressed by the lymphoid infiltrate. The corresponding pulmonary artery shows no vasculitis. The inset shows a detail of the cell population composed of lymphocytes and plasma cells. Hematoxylin-eosin stain: × 350; inset × 1200.

Haitians, with AIDS or ARC (4–6). The etiology of this syndrome is not known. High levels of HIV-specific IgG were found in bronchoalveolar lavage from one patient with LIP but not in AIDS patients without LIP suggesting a direct role of HIV in the pathogenesis of LIP (9). Other studies in children implicated EBV as a possible etiology (7,8). *In yitro* studies showed that there is interaction between HIV and EBV; Birz *et al.* (10) have shown that there is an abnormally high number of EBV infected B-cells in the circulation of adults with AIDS, and *in vitro* EBV-infected B-cells are susceptible to infection by HIV (11).

Reactivation of a latent EBV infection is characterized by elevated titers to the IgG capsid antigen as well as elevation of the early antigen antibody titers. This is while the IgM is not increased and the anti-nuclear antibodies are detected from the previous infection (12). All our LIP patients demonstrated a pattern of recent recrudescent EBV infection while most non-LIP patients showed a pattern of past EBV infection. Andinam *et al.* (8) have found EBV-DNA genome in eight out of ten children with LIP. These children also

Table 1 Clinical features of patients with and without LIP

Patient No.	Age (years) sex	Risk factor	Pulmonary pathology	T4/T8 ratio	Total T4	Serum albumin	Serum globulin
1	33/M	Haitian	LIP	0·2	169	3·3	5·3
2	28/F	Haitian	LIP	0·2	84	3·5	11·2
3	36/F	IVDA	LIP	ND	ND	3·2	4·6
4	35/M	IVDA	LIP	0·2	365	2·7	6·3
5	48/F	IVDA	LIP	0·3	235	3·3	6·4
1	32/M	Homosex	Pneumocystosis	0·6	253	3·1	3·7
2	46/M	Transfus	Pneumocystosis	0·3	82	2·4	3·7
3	48/M	Homosex	Pneumocystosis	ND	ND	2·8	3·2
4	32/M	Homosex	Cytomegalovirus, KS	ND	ND	2·7	3·8
5	34/M	Homosex	Nocardiosis	0·3	101	1·9	2·6
6	33/M	Homosex	Pneumocystosis	0·2	236	1·9	2·6
7	28/M	Homosex	Pneumocystosis, KS	0·3	23	3·4	4·9
8	30/M	IVDA	Pulmonary hemorrhage	1·1	172	3·9	4·2
9	40/M	Haitian	Tuberculosis	0·7	124	3·1	3·8
10	34/M	IVDA	Non-spec pneumonitis	ND	ND	2·8	3·8
11	27/M	Homosex	Cryptococcosis	0·2	10	2·1	2·7
12	34/M	IVDA	MAC	0·7	99	2·3	3·4
13	40/M	Homosex	Pneumocystosis	0·5	167	2·7	3·0
14	32/M	Haitian	MAC-disseminated	1·8	216	2·4	3·8
15	33/F	Haitian	Pneumocystosis	0·1	8	2·9	3·7
16	47/F	Heteros	Pneumocystosis	0·1	51	3·1	4·1
17	51/M	Homosex	Pneumocystosis	ND	ND	3·1	3·1
18	27/M	IVDA	MAC-disseminated	0·2	287	2·4	3·3
19	48/M	Heteros	Pneumocystosis	0·1	84	2·3	4·1

Abbreviations: IVDA, intravenous drug abuse; KS, pulmonary Kaposi sarcoma; MAC, *Mycobacterium avium* complex; ND, not determined.

had an EBV reactivation pattern with extremely high titers (higher than 1:1280). Our findings in adults with LIP are in concordance with their findings.

EBV infection is common among HIV-seropositive patients. EBV seropositivity was found to be as high as 94–99% in active homosexual men and was 100% positive in HIV-positive homosexual men (13,14). EBV titers were found to be significantly higher in HIV-positive than in HIV-negative homosexuals and it was suggested by Rahman *et al.* that the HIV infection might cause loss of control of a latent EBV infection (14). EBV-DNA genome was found in oropharyngeal secretion from patients with positive HIV testing (15). Moreover, certain cases of non-Hodgkin's lymphoma in patients with AIDS were associated with the finding of EBV genome sequence (16). This was also the case in two patients with Burkitt's-like lymphoma (17) and in AIDS-related CNS lymphoma (8).

Of interest, a high infection rate with EBV was found in a serosurvey of 93 HIV-negative Haitians residing in Haiti in which 92 (99%) had EBV anti-capsid antibodies detected mostly in low titers (Pitchenik, unpubl. obs.). This perhaps may explain a higher incidence of LIP in Haitians.

These data suggest that patients with AIDS are susceptible to EBV reactivation which may progress into LIP or lymphoma, or even both as was noted in one case (8). Our serology study (though limited in size) shows that EBV recrudescence is common in LIP associated with AIDS. Further studies are necessary to elucidate the exact role of EBV perhaps with interaction with HIV that might cause this unusual pulmonary manifestation.

References

1. Carrington CB, Liebow AA. Lymohocytic interstitial pneumonitis. *Am J Pathol* 1966; **48:** 36a (abstr.).
2. Gibbs AR, Seal RME. Primary lymphoproliferative conditions of the lung. *Thorax* 1978; **33:** 140–152.
3. Scott GB, Buck BE, Leterman JG, Bloom FL, Parks WP. AIDS in infants. *N Engl J Med* 1984; **310:** 76–81.
4. Solal-Celigny P, Coudrec LJ, Herman V *et al.* Lymphoid interstitial pneumonitis in AIDS related complex. *Am Rev Respir Dis* 1987; **131:** 956–960.
5. Oldham SAA, Castillo M, Jacobson FL, Mones JM, Saldana MJ. HIV-associated lymphocytic interstitial pneumonitis: radiologic manifestation and pathological correlation. *Radiology* 1989; **170:** 83–87.
6. Tierstien AS, Rosen MJ. Lymphocytic interstitial pneumonia. *Clin Chest Med* 1988; **9:** 467–471.

7. Fackler JC, Nagel JE, Adler WH, Mildvan PT, Ambinder RF. EBV infection in a child with AIDS. *Am J Dis Child* 1985; **139:** 1000–1004.
8. Andinam WA, Eastman R, Martin K *et al.* Opportunistic lymphoproliferation associated with EBV-DNA in infants and children with AIDS. *Lancet* 1985; **2:** 1390–1393.
9. Resnick L, Pitchenick AE, Fisher E, Croney R. Detection of HTLV-3/LAV-specific IgG and antigen in broncho-alveolar lavage fluid from two patients with lymphocytic interstitial pneumonitis associated with ARC. *Am J Med* 1987; **82:** 553–556.
10. Birz DL, Redfield RR, Tosato G. Defect regulation of EBV infection in patients with AIDS or ARC. *N Engl J Med* 1986; **314:** 874–879.
11. Montagnier L, Gruest J, Chamaret S *et al.* Adaptation of lymphadenopathy associated virus (LAV) to replication in EBV-transformed B-lymphoblastoid cell lines. *Science* 1985; **225:** 63–66.
12. Sumaya CV. Serologic testing for EBV: developments in interpretation. *J Infect Dis* 1985; **151:** 984–987.
13. Detels R, Visscher BR, Fahey JL *et al.* The relation of CMV and EBV antibodies to T cell subsets in homosexually active men. *J Am Med Assoc* 1984; **251:** 1719–1722.
14. Rahman MA, Kingsley LA, Breining MK *et al.* Enhanced response to EBV in HIV-infected homosexual men. *J Infect Dis* 1989; **159:** 472–479.
15. Alsip GR, Ench Y, Sumaya CV, Boswell RN. Increased EBV DNA in oropharyngeal secretions from patients with AIDS, ARC, or asymptomatic HIV infection. *J Infect Dis* 1988; **157:** 1072–1076.
16. Hochberg FH, Miller G, Schooly RT, Hirsh MS, Feorino P, Heule W. CNS lymphoma related to EBV. *N Engl J Med* 1983; **309:** 745–749.
17. Ziegler JL, Beckstead JA, Voldenbring PA *et al.* Non-Hodgkin's lymphoma in 90 homosexual men. *N Engl J Med* 1984; **311:** 565–570.