CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, May 1996, p. 363–364 Vol. 3, No. 3
1071-412X/96/$04.00+0

Antibody Responses to Defined Epitopes in the Epstein-Barr Virus BZLF1-Encoded Transactivator Protein among Human Immunodeficiency Virus-Infected Patients

R. TEDESCHI,[1] J. DILLNER,[2] AND P. DE PAOLI[1]*

Microbiology-Immunology and Virology Division, Centro di Riferimento Oncologico, Istituto Nazionale di Ricovero e Cura a Carattere Scientifico, Aviano, Italy,[1] and The Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Sweden[2]

Received 5 October 1995/Returned for modification 2 January 1996/Accepted 9 February 1996

The Epstein-Barr virus BZLF1-encoded replication activator (ZEBRA) is a key mediator of reactivation from latency to the viral productive cycle. In the present study, the serum antibody responses against three defined ZEBRA epitopes (designated ZEBRA-1, -19, and -22) were determined for 50 human immunodeficiency virus (HIV)-seropositive patients and 100 matched healthy control subjects. The anti-ZEBRA responses were more commonly found among HIV-seropositive patients than among healthy controls for all the three ZEBRA epitopes tested ($P < 0.0003$, $P < 0.003$, and $P < 0.001$, respectively). Comparison of ZEBRA antibody levels with the degree of immunodeficiency (CD4 cell counts), CDC grouping, and HIV p24 antigen positivity showed little association, suggesting that induction of ZEBRA antibodies is an early event after HIV infection.

Patients with human immunodeficiency virus (HIV) infection show signs of Epstein-Barr virus (EBV) reactivation, including elevation of antibody titers to several EBV antigens and an increased EBV shedding in saliva (19). EBV reactivation, i.e., the switch between latency and EBV replication, is mediated by the EBV BZLF1 gene which encodes the *Bam*Z-encoded EBV reactivator protein (ZEBRA) (1, 10, 15, 18). Each EBV-associated disease has its own typical pattern of antibody response to various EBV proteins and antigenic complexes (12). The disease specificity of the EBV antibody responses can vary even for different epitopes in the same protein (2, 20). The presence of an antibody response to ZEBRA has been demonstrated among patients with nasopharyngeal carcinoma, in a high proportion of asymptomatic HIV carriers, and in AIDS patients (8, 9, 13).

The present study was designed to investigate whether the epitope-specific antibody response to three previously described, serologically reactive ZEBRA peptides (designed 1, 19, and 22) is increased among HIV-infected patients.

Serum samples from 50 HIV-seropositive subjects seen at the Division of AIDS & Medical Oncology at Centro di Riferimento Oncologico in Aviano were studied. As controls, 100 healthy blood donors, matched with the HIV-seropositive subjects for age and sex, were used. Peptides were synthesized by the multiple simultaneous peptide synthesis method (3, 7), and their homogeneities were assayed by reversed-phase high-performance liquid chromatography on a C_8 column. Peptides at 20 μg/ml were coated in 0.1 M Tris HCl onto half-area enzyme-linked immunosorbent assay (ELISA) plates (Costar, Cambridge, Mass.) overnight at room temperature. Sera were tested by an indirect two-step ELISA with monoclonal antibody against immunoglobulin G (IgG; 1:5,000; Sigma) and a horseradish peroxidase-conjugated goat antibody to mouse IgG (1:2,000; Southern Biotechnology, Birmingham, Ala.) as previously described in detail (20). Reactivity was expressed as the difference in optical density (dOD) by subtracting the absorbances obtained with antigen-coated wells from that obtained when the same serum sample was incubated on wells coated only with buffer. The cutoff level for determination of positivity was calculated for each antigen as the mean plus 4 standard deviations of the reactivity of EBV viral capsidic antigen (VCA)-negative sera. The IgG titer to the EBV early antigen (EA) was measured with a recombinant EA IgG ELISA kit (Biotest, Frankfurt, Germany) and that to HIV type 1 p24 antigen was measured with an HIV Ag-1 monoclonal EIA kit (Abbott, North Chicago, Ill.). CD4 cell counts were determined by standard methods (17).

The data were analyzed by using exact methods from contingency tables, the chi-square test, or the Mann-Whitney nonparametric ranking test. The anti-ZEBRA responses of the HIV-seropositive patients compared with those of the healthy controls were statistically elevated for all three epitopes (Table 1). Although ZEBRA-1 and ZEBRA-19 were regularly immu-

TABLE 1. ZEBRA-1, -19, and -22 epitope reactivities in HIV-positive patients

Serum specimen source (no.)[a]	% Positive	Mean dOD (1,000)[b]	Odds ratio[c]	95% Confidence interval	*P* value[c]	ZEBRA epitope
HIV+ (50)	50	976.64	3.76	1.81–7.84	0.00023	1
HD (100)	21	425.40				
HIV+ (50)	42	427.45	3.09	1.46–6.55	0.00249	19
HD (100)	19	233.56				
HIV+ (50)	14	140.54	16.12	1.94–733.4	0.00073	22
HD (100)	1	45.57				

[a] HIV +, HIV positive; HD, healthy donor.
[b] The cutoff levels for seropositivity were the means + 4 standard deviations of EBV VCA-negative sera and were 0.307, 0.167, and 0.141 for the three epitopes, respectively.
[c] The odds ratios and the *P* values for significant differences compared with the results for healthy donors were calculated by using exact methods from contingency tables.

* Corresponding author. Mailing address: Microbiology-Immunology and Virology Division, Centro Regionale Di Riferimento Oncologico, Istituto Nazionale di Ricovero e Cura a Carattere Scientifico, via Pedemontana Occidentale, Aviano I-33081, Italy. Phone: 434 659421 or 434 659404. Fax: 434 659402.

TABLE 2. Serological reactivities of three ZEBRA epitopes among HIV-positive patients belonging to different CDC groups and among healthy donors

Subject	No. of patients	ZEBRA-1		ZEBRA-19		ZEBRA-22	
		Mean dOD (1,000)	% Positive	Mean dOD (1,000)	% Positive	Mean dOD (1,000)	% Positive
CDC group							
II	22	830.36	50[a]	364.18	41[a]	241.91	27[a]
III	6	626.67	33	344.83	50	54.67	0
IV	22	932.91	55[a]	339.36	41[a]	61.04	5
HD[b]	100	425.40	21	233.56	19	45.57	1

[a] $P \leq 0.05$ (P values for significant differences compared with the results for healthy donors were calculated by the chi-square test).
[b] HD, healthy donor.

noreactive with HIV-positive sera (50 and 42% of the serum samples were positive for ZEBRA-1 and ZEBRA-19, respectively), sera from several healthy donors were also reactive.

The presence of anti-ZEBRA antibodies was not significantly associated with CD4 cell counts (data not shown) or with CDC grouping (Table 2).

ZEBRA reactivity and p24 antigenemia were compared by using the most reactive peptides (ZEBRA-1 and ZEBRA-19). The responses to ZEBRA-1 and ZEBRA-19 were similar between HIV p24-positive and HIV p24-negative HIV-positive patients. ZEBRA-1 antibody levels were, however, slightly elevated among p24 antigen-positive patients (Table 3).

For 31 of the HIV-positive serum samples, we compared the ZEBRA reactivities with a traditional EBV reactivation marker, IgG anti-EA antibodies, but no relation was found.

The characterization of the antibody response to defined EBV antigens is important for the diagnosis of EBV-associated diseases (4, 12, 16). Most diagnostic serological assays measure the antibody response against antigenic complexes or whole proteins, while only a few studies have investigated the epitope specificity of the antibody response (5, 6, 14).

In the present study we found that HIV-positive patients have elevated antibody levels to all three ZEBRA epitopes tested. This is clearly different from the case for patients with nasopharyngeal carcinoma, in whom ZEBRA-22 was found to be a disease-specific epitope (20). In fact, the reactivity of sera from HIV-positive patients with the three ZEBRA epitopes closely resembles the ZEBRA response pattern seen in the sera of patients with primary EBV infection (20).

The fact that the ZEBRA response in HIV-positive patients was independent of the degree of immunodeficiency, as defined by the CD4 cell count, suggests that EBV reactivation and the production of ZEBRA are early events after HIV infection and are not primarily dependent on the immunodeficiency. A direct interaction between EBV and HIV has been suggested (11).

Our data suggest that an unrestricted antibody response of the ZEBRA epitope is an early event in the course of HIV infection. Assessment of whether EBV ZEBRA serology might have clinical uses will require further work.

TABLE 3. Serological reactivities to the ZEBRA-1 and ZEBRA-19 epitopes in HIV p24-positive and HIV p24-negative patients

Patient group	ZEBRA-1		ZEBRA-19	
	% Positive	Mean dOD (1,000)	% Positive	Mean dOD (1,000)
HIV positive, p24 negative	43 (15/35)[a]	1,235.7	40 (14/35)	806.3
HIV positive, p24 positive	67 (10/15)[b]	1,599.0	47 (7/15)[c]	679.7

[a] Values in parentheses are number positive/total number of patients tested.
[b] $P = 0.03$ (P values for significant differences in antibody levels were calculated by the Mann-Whitney test).
[c] $P = 0.81$.

This study was supported by the Swedish Cancer Society.
We are indebted to Ettore Bidoli for the statistical analysis.

REFERENCES

1. **Carey, M., J. Kolman, D. A. Katz, L. Gradoville, L. Barberis, and G. Miller.** 1992. Transcriptional synergy by Epstein-Barr virus transactivator ZEBRA. J. Virol. **66:**4803–4813.
2. **Cheng, H. M., Y. T. Foong, C. K. Sam, U. Prasad, and J. Dillner.** 1991. Epstein-Barr virus nuclear antigen 1 linear epitopes that are reactive with immunoglobulin A (IgA) or IgG in sera from nasopharyngeal carcinoma patients or from healthy donors. J. Clin. Microbiol. **29:**2180–2186.
3. **Dillner, J., L. Dillner, G. Utter, C. Eklund, A. Rotola, S. Costa, and D. Diluca.** 1990. Mapping of linear epitopes of human papilloma virus type 16: the L1 and L2 open reading frames. Int. J. Cancer **45:**529–535.
4. **Dillner, J., and B. Kallin.** 1988. The Epstein-Barr virus proteins. Adv. Cancer Res. **50:**95–151.
5. **Dillner, J., R. Szigeti, W. Henle, G. Henle, R. A. Lerner, and G. Klein.** 1987. Cellular and humoral immune responses to synthetic peptides deduced from the amino-acid sequences of Epstein-Barr virus encoded proteins in EBV-transformed cells. Int. J. Cancer **40:**455–460.
6. **Foong, Y. T., H. M. Cheng, C. K. Sam, J. Dillner, W. Hinderer, and U. Prasad.** 1990. Serum and salivary IgA antibodies against a defined epitope of the Epstein Barr virus nuclear antigen (EBNA) are elevated in nasopharyngeal carcinoma. Int. J. Cancer **45:**529–535.
7. **Houghten, R. A.** 1985. General method for the rapid solid phase synthesis of large number of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. Proc. Natl. Acad. Sci. USA **82:**5131–5153.
8. **Joab, I., J. C. Nicolas, G. Schwaab, G. de The, B. Clausse, M. Perricaudet, and Y. Zeng.** 1991. Detection of anti Epstein-Barr virus transactivator (ZEBRA) antibodies in sera from patients with nasopharyngeal carcinoma. Int. J. Cancer **48:**647–649.
9. **Joab, I., H. Triki, J. de Saint Martin, M. Perricaudet, and J. C. Nicolas.** 1991. Detection of anti Epstein-Barr virus trans-activator (ZEBRA) antibodies in sera from patients with human immunodeficiency virus. J. Infect. Dis. **163:**53–56.
10. **Klein, G.** 1989. Latency and transformation: the strategy of EBV. Cell **58:**5–8.
11. **Lai, P. K., X. Li, and D. J. Volski.** 1989. Induction of EBV in lymphoblastoid cells by HIV1. Int. J. Cancer **43:**1104–1111.
12. **Lennette, E. T.** 1991. The Epstein-Barr virus, p. 847–852. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology. American Society for Microbiology, Washington, D.C.
13. **Mathew, A., H. M. Cheng, C. K. Sam, I. Joab, U. Prasad, and C. A. Cochet.** 1994. High incidence of serum IgG antibodies to the Epstein-Barr virus replication activator protein in nasopharyngeal carcinoma. Cancer Immunol. Immunother. **38:**68–70.
14. **Middledorp, J. M., and R. H. Meloen.** 1988. Epitope mapping of EBV major capsidic protein using systematic synthesis of overlapping oligopeptides. J. Virol. Methods **21:**147–159.
15. **Miller, G.** 1990. The switch between latency and replication of Epstein-Barr virus. J. Infect. Dis. **161:**833–844.
16. **Pearson, G. R.** 1988. Elisa tests and monoclonal antibodies for EBV. J. Virol. Methods **21:**97–104.
17. **Rahman, A., L. A. Kingsley, and M. Breinig.** 1989. Enhanced antibody response to Epstein-Barr virus in HIV-infected homosexual men. J. Infect. Dis. **159:**472–479.
18. **Rooney, C. M., D. T. Rowe, T. Ragot, and P. J. Farrell.** 1989. The spliced BZLF1 gene of Epstein-Barr virus (EBV) transactivates an early EBV promoter and induces the virus productive cycle. J. Virol. **63:**3109–3116.
19. **Sumaya, C. V., R. N. Boswel, Y. Ench, D. L. Kisner, E. M. Hersh, J. M. Reuban, and P. W. A. Mansell.** 1986. Enhanced serological and virological findings of EBV in patients with AIDS and ARC. J. Infect. Dis. **154:**864–870.
20. **Tedeschi, R., Y. T. Foong, H. M. Cheng, P. De Paoli, T. Lethinen, T. Elfborg, and J. Dillner.** 1995. The disease associations of the antibody response against the Epstein-Barr virus transactivator protein ZEBRA can be separated into different epitopes. J. Gen. Virol. **76:**1393–1400.