Acta virol. **36:** 392-400, 1992

PREVALENCE AND SPECIFICITY OF LYMPHOCYTOTOXIC ANTIBODIES IN DIFFERENT STAGES OF HIV INFECTION

B. SZABÓ[1], F. D. TÓTH[1]*, J. KISS[1], G. FÜST[2], E. ÚJHELYI[2], D. BÁNHEGYI[3], A. HORVÁTH[4], S. R. HOLLÁN[2]

[1]Institute of Microbiology, Medical University, Debrecen; [2]National Institute of Hematology and Blood Transfusion, Budapest; [3]László Hospital of Infectious Diseases, Budapest; and [4]National Institute of Dermatology and Venereal Diseases, Budapest, Hungary

Received May 20, 1991

Summary. – Sera obtained from 27 HIV-infected persons were investigated for complement-dependent humoral cytotoxicity. Uninfected as well as HTLV-IIIB-infected H9 cells were used as cellular targets either before or after stimulation by phytohemagglutinin (PHA) or concavalin A (Con-A). The degree of cytotoxicity was determined by ^{51}Cr-release assay. Two different antibodies could be found in sera of HIV-infected persons, one being directed against HIV-induced cell surface component(s) and the other reacting with structure(s) present on activated T4 cells. Asymptomatic HIV-carriers were found to have antibodies exerting complement-dependent cytotoxicity to HIV-infected T4 cells. These antibodies were reactive mainly after stimulation of HIV-infected target cells by Con-A. Sera of ARC and AIDS patients contained autoantibodies reactive with PHA-stimulated or HIV-infected T4 lymphocytes. These data suggest that HIV-specific antibodies represent an anti-viral immune defense, while autoantibodies may be important in destruction of the immune system in AIDS.

Key words: HIV-infection; lymphocytotoxic antibodies; antiviral defense; autoimmune phenomenon; stage-specific distribution

Introduction

The human immunodeficiency virus (HIV; Barré-Sinoussi *et al.*, 1983; Popovic *et al.*, 1984) has been established as the primary etiologic agent in the pathogenesis of the acquired immune deficiency syndrome (AIDS) and related disorders. The evolution of the disease towards AIDS results mainly from a progressive destruction of the T4 lymphocyte pool. However, there is a strong

*Whom requests for reprints should be addressed to corresponding author: Institute of Microbiology, Medical University of Debrecen, H-4012 Debrecen, P.O.B. 17, Hungary

evidence that the direct cytopathic effect of the virus cannot, by itself, account for the T4 cell depletion, because the virus infects less than 1 per 10 000 T4 cells at any time (Harper *et al.,* 1985).

A cluster of investigations supports an alternative view regarding AIDS as a viral induced autoimmune disorder. Many patients with AIDS or AIDS-related conditions (ARC) have serum antilymphocyte antibodies (Kloster *et al.,* 1984; Tomar *et al.,* 1988). The molecular mimicry between the viral envelope protein and the major histocompatibility complex class II (MHC II) antigens with both interact specifically with the T4 molecule could be the main factor giving rise to autoimmune phenomena (Shearer and Levy, 1984). According to this view, T4 cell activation whether triggered by the virus or any other stimulus, elicits expression of MHC II antigens in T4 cells, rendering them the targets of the autoimmune response. Stricker *et al.* (1987) described an AIDS-related serum autoantibody that reacts with an antigen 18K restricted to phytohemagglutinin-stimulated or HIV-infected T4 cells.

In a previous study (Tóth *et al.,* 1989) we demonstrated that the sera of asymptomatic HIV-carriers contain complement-dependent cytotoxic antibodies reactive with HIV-infected T4 lymphocytes. This cytotoxic activity could be enhanced by pretreatment of HIV-infected target cells with con-A but not with PHA. None of the sera obtained from HIV-infected asymptomatic persons showed cytotoxic activity against uninfected T4 cells before or after stimulation with lectins. In the present study, searching for the pathogenetical role of antilymphocyte antibodies, we have investigated their distribution and specificity in different phases of HIV-infection.

Materials and Methods

Serum samples. Sera obtained from asymptomatic HIV-carrying persons, patients with ARC or AIDS and healthy subjects were inactivated at 56 °C for 30 min and stored at −40 °C until used. All ARC and AIDS patients met the criteria for the disease established by the Centers for Disease Control (Selik *et al.,* 1984).

Cell cultures. The H9 and HTLV-IIIB-producing H9 cells were kindly supplied by Dr. R. C. Gallo (National Cancer Institute, Bethesda, MD) and maintained in RPMI-1640 medium containing 20 % foetal calf serum (Gibco).

Stimulation of H9 and HTLV-IIIB-producing H9 cells. The cell cultures received PHA (20 μg/ml, Difco) or Con-A (1 μg/ml, Sigma) for 72 hr (Oppenheim and Schecter, 1976).

ELISA test. The Vironostika anti-HTLV-III Microelisa System was the product of Organon Teknika (Turnhout, Belgium). Sera were reacted at a 1:100 dilution. Titers were expressed in absorbancy (A) values.

Indirect membrane immunofluorescence assay (IFA). Immunofluorescence was performed as described elsewhere (Tóth *et al.,* 1989). Briefly, 100 μl sample of diluted serum was incubated with 10^6 target cells in the first phase of reaction. After washing, the cells were incubated with 100 μl of goat anti-human IgG conjugated with FITC (Hyland). IFA antibody titers reflect the serum dilution at which 50 % of the target cells fluoresced markedly.

Radioimmunoprecipation assay (RIPA). The experimental conditions described by Kurth *et al.* (1977) were utilized throughout the study. The HTLV-III p24 antigen was kindly supplied by Dr.

S. Oroszlan (Frederick Cancer Research Center, Frederick, MD) and labelled with ^{125}I by the chloramine-T method (Greenwood *et al.*, 1963). Anti-p24 titers are defined as ng of viral protein precipitated by 10 μl of serum diluted 1:10.

Cytotoxic antibody assay. Complement-dependent antibody cytotoxicity was detected by ^{51}Cr-release technique. Details of the method used were described elsewhere (Szabó *et al.*, 1983). H9 and HTLV-IIIB-infected H9 cells without stimulation and those stimulated by PHA or Con-A were used as targets. For the study, $5x10^4$ ^{51}Cr-labelled target cells were added to 100 μl of heat-inactivated (56 °C, 30 min) serum samples diluted in PBS. After incubation at 37 °C for 30 min, 100 μl of non-toxic guinea pig serum (Human Institute, Budapest, Hungary) were added as a source of complement, and the incubation was continued for additional 30 min. After centrifugation the supernatant fluid was assayed for released radioactivity. Target cells incubated in the presence of complement served as background controls. Titers are given as the highest serum dilution at which 50 % of ^{51}Cr isotope was released by the labelled cells.

Determination of total T4 lymphocytes and helper/suppressor T-cell ratios. The enumeration of T4 lymphocytes and the determination of helper/suppressor T-cell ratios were performed according to standard methods (Ioachim *et al.*, 1983). Cells were stained for surface markers by OKT4 and OKT8 monoclonal antibodies (Ortho-mune, Ortho-Diagnostics, Raritan, N. J.).

Results

Serum antibody against HIV

Serum samples obtained from asymptomatic HIV-carrying persons as well as from patients with ARC or AIDS were investigated for HIV-specific antibodies by ELISA, IFA and RIPA. High A values (>1.4) were found in 7 samples from asymptomatic HIV-carriers and in 9 samples from patients with ARC or AIDS by the ELISA test, and these results were in correlation with those obtained in IFA experiments (Table 1 and 2). Anti-p24 antibodies were found in all asymptomatic persons and in 2 patients with ARC. No correlation could be observed between the results of ELISA and RIPA experiments.

Table 1. HIV-specific antibodies in sera of asymptomatic HIV-carrying persons

Patient No.	Titer of antibodies to HTLV-IIIB		
	ELISA (A)	IFA	RIPA (ng)
1	>2.000	1:80	1.1
2	>2.000	1:40	3.1
3	>2.000	1:80	2.6
4	0.946	1:10	3.8
5	1.417	1:20	5.0
6	1.415	1:40	4.3
7	0.953	1:10	5.2
8	1.058	1:10	2.2
9	1.870	1:20	1.2
10	>2.000	1:80	2.2
11	1.049	1:10	2.2

Table 2. HIV-specific antibodies in sera of patients with ARC and AIDS

Patient No.	Diagnosis	Titer of antibodies to HTLV-IIIB		
		ELISA (A)	IFA	RIPA (ng)
12	ARC	>2.000	1:40	-
13		>2.000	1:40	2.1
14		>2.000	1:80	1.1
15		>2.000	1:40	-
16		>2.000	1:40	-
17		0.850	1:20	-
18		0.680	1:20	-
19		0.910	1:20	-
20	AIDS	0.883	1:10	-
21		>2.000	1:40	-
22		1.537	1:20	-
23		1.309	1:10	-
24		1.789	1:20	-
25		1.201	1:20	-
26		1.850	1:40	-
27		0.601	1:10	-

Lymphocytotoxic antibodies in different stages of HIV infection

Complement-dependent antibody-mediated cytotoxicity to uninfected H9 cells before or after their stimulation by PHA or Con-A was not found in any of the serum samples obtained from asymptomatic HIV-carrying persons (Table 3). Two of the 11 sera from asymptomatic HIV-carriers exerted cytotoxicity to HTLV-IIIB infected cells. After stimulation of virus-infected target cells by Con-A, 3 additional sera were also positive. Moreover, the Con-A stimulation of the cellular targets resulted in the increase of the cytotoxic effect of sera No. 2 and No. 3 against HTLV-IIIB infected H9 cells.

In contrast, all sera obtained from ARC or AIDS patients exerted complement-dependent cytotoxicity to PHA-stimulated H9 cells (Table 4). One sample (No. 12) was reactive with Con-A-stimulated H9 cells, too. Sera of all ARC and AIDS patients were also reactive with HTLV-IIIB infected H9 cells. Stimulation of HTLV-IIIB infected cellular targets by PHA led to the increase of the cytotoxicity of 7 serum samples (No. 16, 19, 21, 22, 24, 25 and 26). This phenomenon could be demonstrated mainly with the sera obtained from AIDS patients. On the contrary, the Con-A stimulation of virus-infected H9 cells did not cause any change in the reactivity of ARC and AIDS sera.

Sera obtained from 20 healthy, uninfected persons did not react with uninfected or HTLV-IIIB-infected H9 cells either before or after stimulation by mitogens.

Table 3. The number of T4 lymphocytes, T4/T8 ratios and complement-dependent lymphocytotoxic activity in asymptomatic HIV-carriers

Patient No.	T4 cells per μl	T4/T8 ratio	Titer of cytotoxic antibodies to					
			H9 cells			HTLV-IIIB-H9 cells		
			without stimulation	after stimulation by		without stimulation	after stimulation by	
				PHA	Con-A		PHA	Con-A
1	840	0.89	-	-	-	-	-	1:40
2	450	0.57	-	-	-	1:10	-	1:20
3	796	0.57	-	-	-	1:10	-	1:40
4	326	0.39	-	-	-	-	-	-
5	403	0.39	-	-	-	-	-	-
6	636	0.81	-	-	-	-	-	1:20
7	1056	1.33	-	-	-	-	-	-
8	1204	1.48	-	-	-	-	-	-
9	1203	2.0	-	-	-	-	-	-
10	441	0.76	-	-	-	-	-	1:80
11	524	0.69	-	-	-	-	-	-

Table 4. The number of T4 lymphocytes, T4/T8 ratios and complement-dependent lymphocytotoxic activity in patients with ARC and AIDS

Patient No.	T4 cells per μl	T4/T8 ratio	Titer of cytotoxic antibodies to					
			H9 cells			HTLV-IIIB-H9 cells		
			without stimulation	after stimulation by		without stimulation	after stimulation by	
				PHA	Con-A		PHA	Con-A
12	247	0.31	-	1:20	1:10	1:40	1:40	1:40
13	589	0.49	-	1:10	-	1:10	1:10	1:10
14	579	0.35	-	1:10	-	1:10	1:10	1:10
15	352	0.36	-	1:10	-	1:10	1:10	1:10
16	224	0.38	-	1:40	-	1:20	1:40	1:20
17	482	0.45	-	1:20	-	1:20	1:20	1:20
18	364	0.38	-	1:10	-	1:10	1:20	1:10
19	288	0.30	-	1:40	-	1:20	1:40	1:20
20	10	0.03	-	1:10	-	1:10	1:10	1:10
21	24	0.04	-	1:40	-	1:10	1:40	1:10
22	74	0.07	-	1:20	-	1:10	1:20	1:10
23	145	0.05	-	1:10	-	1:10	1:10	1:10
24	86	0.07	-	1:40	-	1:10	1:40	1:10
25	50	0.07	-	1:20	-	1:10	1:20	1:10
26	125	0.08	-	1:40	-	1:20	1:40	1:20
27	45	0.04	-	1:10	-	1:10	1:10	1:10

Relationship between T4 cell count, T4/T8 ratios and lymphocytotoxic antibodies

Complement-dependent cytotoxic activity to normal, stimulated H9 cells was found only in patients with ARC or AIDS, but not in healthy HIV-carriers. The T4 cell count of ARC patients varied between 224 and 589. The number of T4 cells in AIDS patients ranged from 10 to 145. The ARC patients had T4/T8 ratios below 0.5 and the T4/T8 ratio in AIDS was lower than 0.1. The T4 cell count of asymptomatic HIV-carriers varied between 326 and 1204. The T4/T8 ratios in asymptomatic persons ranged from 0.39 to 2.0. Antibodies exerting cytotoxic effect on HTLV-IIIB infected H9 cells were found in "healthy" HIV-carriers with T4/T8 ratios below 1.0.

Discussion

The lymphocytotoxic antibodies reacting only with HIV-infected T4 cells seem to be distinct from those reacting equally with uninfected, stimulated T4 lymphocytes and HIV-infected T4 targets. While the destruction of infected T4 cell represents a normal immune cytotoxic response, the destruction of noninfected T4 cells would represent an autoimmune phenomenon.

The cellular target of cytotoxic antibodies in asymptomatic carriers seems to be HIV-specific and its expression may be increased by Con-A stimulation. It is possible that provirus-containing cells do not turn on antigen expression enough high for immune destruction prior to being allogenically stimulated. A similar phenomenon was observed in the bovine leukosis virus system (Onuma *et al.*, 1976).

The expression of MHC II antigens in human T cells activated *in vitro* by plant mitogens have been documented (Yu *et al.*, 1980). Gazit *et al.* (1980) described alloantigens expressed in mitogen activated T cells different from MHC I and MHC II antigens. It is known that T4 lymphocytes are highly responsive to PHA, whereas they respond to Con-A in a lesser degree (Buckley, 1980). The difference in the antigen-inducing capability of PHA and Con-A treatment suggests that antibodies reacting with PHA-stimulated uninfected H9 cells are directed against alloantigens but not against MHC II determinants. This assumption corresponds with the findings of Stricker *et al.*, (1987). The antibodies reacting with Con-A-stimulated cells showed HIV-specificity, with the exception of one serum sample (No. 12). Experiments are in progress in our laboratory to identify the molecular target(s) present on Con-A-stimulated cells.

The autoantibody reacting with both PHA-stimulated, uninfected and HIV-infected T4 cells was associated with clinical disease rather than simple exposure to HIV. The autoantibody is found in ARC. Thus, its appearance preceds the onset of AIDS. The reactivity of sera from asymptomatic HIV-carriers was enhanced by Con-A stimulation of HIV-infected target cells, whereas ARC and

AIDS sera showed increased cytotoxicity against PHA-stimulated T4 targets. Hence, the ratio of anti-HIV and antilymphocytic cytotoxicity seems to change in favour of the latter during the HIV-induced pathogenesis. These findings indicate that the anti-T4-cell autoantibody may be important in destruction of the immune system in AIDS.

In conclusion, our results allow us to assume that at least two different antibodies can be found in HIV-infected persons, one being directed against HIV-induced cell surface component(s) and the other against structures present in activated T4 cells. The nature of the target antigens is currently under investigation.

Acknowledgements. The authors are thankful to Dr. R. C. Gallo for providing H9 and HTLV-IIIB-infected H9 cell lines, and appreciate very much the help of Dr. S. Oroszlan afforded by the preparation of the p24 polypeptide of HTLV-III. We are also indebted to Miss M. Lelesz and Miss E. Markovics for the technical assistance.

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