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COMPLEMENT-DEPENDENT CYTOTOXICITY OF ANTIBODIES REACTIVE WITH HIV-INDUCED CELL SURFACE ANTIGENS IN HIV-CARRYING HAEMOPHILIACS

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Summary. — Sera obtained from HIV-infected as well as uninfected haemophiliacs and from healthy subjects were investigated for the presence of lymphocytotoxic antibodies. Using the ^{51}Cr-release test, HIV-infected haemophiliacs were found to produce serum antibodies exerting complement-dependent cytotoxic effect on HIV-infected T4 cells. The antibodies were reactive mainly when HIV-infected target cells were stimulated with concanavalin-A. Results of complement-dependent antibody cytotoxicity and indirect membrane immunofluorescence tests suggest that envelope antigen(s) of HIV may be the target(s) for cytotoxic antibodies.

Key words: *human immunodeficiency virus*; *lymphocytotoxic antibodies*; *^{51}Cr-release technique*

Introduction

Acquired immune deficiency syndrome (AIDS) and related disorders result from infection of T helper-inducer lymphocytes (T4) with human immune deficiency virus (HIV) (Barré-Sinoussi *et al.*, 1983; Popovic *et al.*, 1984). The mechanism by which it exerts its cytotoxic effect on T4 cells is not known. HIV replication alone in infected cells is not sufficient for cytotoxicity (Sodroski *et al.*, 1986). An alternative view regarding the pathogenesis of AIDS is based on the fact that many patients with AIDS or lymphadenopathy syndrome have serum antilymphocyte antibodies (Kloster *et al.*, 1984; Tomar *et al.*, 1985). A number of autoimmune phenomena suggest the role of molecular mimicry between the viral envelope protein and the major histocompatibility complex class II antigens which both interact specifically with the T4 molecule and could be the main factor giving rise to autoimmune phenomena (Shearer and Levy, 1984). An autoantibody reactive with lectin-stimulated or HIV-infected T4 cells was found in patients with AIDS or AIDS-related conditions (Stricker *et al.*, 1987). The autoantibody reacted with an antigen of relative molecular weight 18 000 and induced cytotoxicity

of lectin-stimulated or HIV-infected T4 cells in the presence of complement. The cytotoxic antibodies may also react with viral proteins expressed in the cell membrane of infected cells by fixing complement and determine cytolysis, however, evidence in favour of this hypothesis is lacking.

Although these important discoveries contributed to our understanding of AIDS, little is known regarding the sequence of events that leads from a viral infection to the profound deregulation of immune system. In the present study, searching such a mechanism, we investigated the presence of cytotoxic antibodies in HIV-infected haemophiliacs. Here we report cytotoxic activity of antibodies directed against HIV-infected but not against uninfected lectin-stimulated T4 cells in asymptomatic HIV-carriers.

Materials and Methods

Serum samples. Sera obtained from HIV-infected as well as from uninfected haemophiliacs and from healthy subjects were inactivated at 56 °C for 30 min and stored at −40 °C until used.

Cell cultures. The H9 and HTLV-IIIB-producing H9 cells were kindly supplied by Dr. R. C. Gallo (National Cancer Institute, Bethesda, Md.); they were maintained in RPMI-1640 medium containing 20 % foetal calf serum (Gibco, Paisley, Scotland) and the T-cell growth factor (Sigma, St. Louis, Mo.).

Stimulation of H9 and HTLV-IIIB-producing H9 cells. Phytohaemagglutinin (PHA; Difco, Detroit, Mi.) or concanavalin-A (Con-A; Sigma, St. Louis, Mo.) were added to cells at concentration of 20 μg/ml or 1 μg/ml, respectively; the stimulation proceeded for 72 hours (Oppenheim and Schecter, 1976).

ELISA. The Vironostika anti-HTLV-III Microelisa System was the product of Organon Teknika (Turnhout, Belgium). Sera were reacted at a 1 : 100 dilution with anti-HTLV-III negative human serum pool. Titres were expressed in optical density (O.D.) values.

Indirect membrane immunofluorescence assay (IFA). Uninfected and HTLV-IIIB-infected H9 cells were used as targets. For the assay, 100 μl of diluted serum sample were incubated with 10^6 target cells at 37 °C for 20 min. After washing with PBS, 100 μl of goat anti-human-IgG serum conjugated with FITC (Hyland, Costa Mesa, Ca.) and appropriately diluted in PBS were added. The samples were incubated at 37 °C for 20 min and washed again with PBS. Titres reflect the serum dilution at which 50 % of target cells showed marked fluorescence.

Radioimmunoprecipitation assay (RIPA). The experimental conditions described by Kurth *et al.* (1977) were utilized throughout. The HTLV-III p24 antigen was kindly supplied by Dr. S. Oroszlan (Frederick Cancer Research Center, Frederick, Md.); it was labelled with ^{125}I by the chloramine-T method (Greenwood *et al.*, 1963). Anti-p24 titres are defined as ng of viral protein.

Cytotoxic antibody assay. Complement-dependent antibody cytotoxicity was detected by the ^{51}Cr-release technique. Details of the method used by us 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. The target cells suspended in Eagle's minimal essential medium (Flow Laboratories, Inc., Rockville, Md.) were incubated with ^{51}Cr (as sodium chromate; Isotope Institute of Hungarian Academy of Sciences, Budapest, Hungary). For the study, 5×10^4 ^{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, 1000 μl of non-toxic guinea pig serum (Human Institute, Budapest, Hungary) were added as a source of complement, and incubation was continued for an 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. Titres were determined as the highest serum dilutions at which 50 % of the isotope was released by the labelled cells.

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

Results

Detection of HIV-specific antibodies in the sera obtained from haemophiliacs and healthy donors

Serum samples obtained from 35 haemophiliacs and 50 healthy donors were investigated for the presence of HIV-specific antibodies by ELISA, IFA, and RIPA. HIV-specific antibodies could be detected in 11 of the 35 haemophiliacs, but in none of the 50 healthy donors (Table 1).

Positive O.D. values (>1.4) were found in 7 haemophiliacs which was in correlation with results of IFA (≥ 20). In contrast, no correlation could be observed between ELISA and RIPA results. Hence, the ELISA results reflected mainly the presence of antibodies reactive with HIV-induced cell surface antigens, i.e. with virus envelope glycoprotein(s).

Correlation between T4 cell count, T4/T8 ratios and lymphocytotoxic antibodies in HIV-infected haemophiliacs

Complement-dependent cytotoxic activity of serum samples to uninfected as well as to HTLV-IIIB-infected H9 cells was investigated by the ^{51}Cr-release assay. Antibody-mediated cytotoxicity to normal H9 cells before or after their stimulation by PHA or Con-A was not found in any of the serum samples obtained from haemophiliacs or healthy donors. Similarly, sera from uninfected haemophiliacs and healthy donors were not reactive with HTLV-IIIB-infected H9 cells regardless whether stimulated or not with PHA or Con-A (data not shown).

In contrast, 5 out of 11 sera from HIV-infected haemophiliacs exerted complement-dependent cytotoxicity on HTLV-IIIB-infected H9 cells (Table 2). Two out of 5 sera (nos 2 and 3) reacted both with unstimulated and Con-A stimulated targets. Three samples (nos 1, 6, and 10) were reactive only with Con-A-stimulated HTLV-IIIB-H9 cells. No definite correlation could be

Table 1. Detection of HIV-specific antibodies in the sera of haemophiliacs

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

Table 2. The number of T4 lymphocytes, helper/suppressor ratios and complement-dependent antibody cytotoxicity against HTLV-IIIB-H9 cells in HIV-infected haemophiliacs

Patient no.	T4 cells per μl	T4/T8 ratio	Titre of cytotoxic antibodies		
			No stimulation	After stimulation with	
				PHA	Con-A
1	840	0.89	—	—	40
2	450	0.57	10	—	20
3	796	0.57	10	—	40
4	326	0.39	—	—	—
5	403	0.87	—	—	—
6	636	0.81	—	—	20
7	1056	1.33	—	—	—
8	1204	1.48	—	—	—
9	1203	2.0	—	—	—
10	441	0.76	—	—	80
11	524	0.69	—	—	—

observed between the number of T4 lymphocytes and the cytotoxic activity of the serum samples. On the contrary, complement-dependent antibody cytotoxicity was found only in patients with T4/T8 ratios below 1.0 but not in those who had T4/T8 ratios higher than 1.0. Comparison of data listed in Tables 1 and 2 shows that the presence or absence of complement-dependent cytotoxicity antibodies correlated with antibody titres detected in IFA. Sera positive by IFA in 1 : 40 or higher dilutions had cytotoxic activity as compared to those with lower IFA titres.

The seropositive haemophiliacs showed no symptoms of AIDS or AIDS-related disease.

Discussion

Using the ^{51}Cr-release technique, we found that HIV-infected haemophiliacs produce serum antibodies which exert complement-dependent cytotoxicity to HIV-infected T4 cells mainly after their stimulation with Con-A. The antibodies could not be detected in the sera from uninfected haemophiliacs and healthy subjects. The target molecule was not found on the uninfected H9 cells even after stimulation by PHA or Con-A. Thus the cellular target seemed distinct from the M_r 18 kD protein described by Stricker *et al.* (1987) to be shared by HIV-infected as well as normal lectin-stimulated T4 cells. The target antigen of the complement-dependent antibody cytotoxicity in our experiments, appeared to be specifically induced by HIV infection. Expression of the cellular target may be increased by Con-A stimulation. This assumption was strengthened by the fact that the lymphocytotoxic activity could be enhanced by Con-A pretreatment of HTLV-

IIIB-infected T4 cells in two cases (Table 2). The presence of cytotoxic activity was associated with high titre values of antibodies reactive with HIV-induced cell surface antigens as detected in IFA. In contrast, there was no correlation between the cytotoxic activity and the amount of antibodies reactive with the p24 HIV antigen as measured by RIPA. It is likely, therefore, that the cytotoxic antibodies reacted with the envelope HIV antigens. However, the precise nature of the antigen(s) reactive in complement-dependent antibody cytolysis should be determined.

Out of 11 HIV-infected haemophiliacs, eight had T4/T8 ratios below 1.0 but only five of these had cytotoxic antibodies. One can assume that free cytolytic antibodies were absent or that they were present in the form of immune complexes. Our results suggest that HIV-carrying cells may not express enough target antigen(s) for immune destruction and therefore, would not be effectively eliminated even by such a host response. In the bovine leukaemia retrovirus system, provirus-containing cells do not turn on antigen expression prior to being allogenically stimulated (Onuma *et al.*, 1976). To determine whether the differential effect of PHA and Con-A on antibody-mediated cytotoxicity was reflected in the expression of viral antigens or virus-induced cytolysis, we have to monitor these events in HTLV-IIIB-infected H9 cells after stimulation by PHA or Con-A.

What is the significance of the antibody directed against HIV-infected cells? Complement-dependent antibody-mediated cytotoxicity was found in clinically asymptomatic virus carriers. Thus, the appearance of the cytotoxic antibody preceded the onset of AIDS or AIDS-related disease. It is notable that the lymphocytotoxic antibody detected by us is different from that found by Stricker *et al.* (1987). The cytotoxic antibodies may have a dual character: they could participate in the ongoing immunosuppression or could represent an anti-viral immune response to destroy HIV-infected lymphocytes. Long-term follow up studies are presently underway to show whether patients with cytotoxic antibodies are able to control their immune deficiency and remain healthy.

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