Acta virol. 34 : 164—170, 1990

NEUTRALIZING ANTIBODIES AND SERUM INTERFERON LEVELS IN THE DIFFERENT STAGES OF HIV INFECTION

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Received February 23, 1989

Summary. — The sera of patients infected with HIV were investigated for neutralizing antibodies (NA) and interferons. All samples from asymptomatic HIV carriers contained NA in high titres. In the sera of patients with AIDS related complex and AIDS the antibodies were found rarely and in lower titres. An early peak of acid-labile interferon (IFN)-α was observed in asymptomatic HIV-infected persons, and a late peak was found in AIDS patients. The data suggest that HIV NA may have beneficial effect in the asymptomatic phase. The presence of acid-labile IFN-α may indicate stimulation of IFN system by HIV-infected cells.

Key words: *human immunodeficiency virus*; *neutralizing antibodies*; *acid-labile interferon-α*; *stages of HIV infection*

Introduction

Human immunodeficiency virus (Barré-Sinoussi *et al.*, 1983; Popovic *et al.*, 1984) has been clearly established as the primary aetiologic agent in the pathogenesis of the acquired immune deficiency syndrome (AIDS) and related disorders. The epidemiologic studies show that a high percentage of infected persons will develop AIDS or AIDS related complex (ARC) (Biggar, 1987), but latency varies from one individual to another. The natural defenses of an individual after HIV infection have not been completely defined yet.

Previous reports have confirmed the existence of virus NA in HIV-infected persons, however, data concerning the role of these antibodies are still controversal. Faulkner-Valle *et al.* (1987) have reported a significant correlation between the presence of HIV NA and the asymptomatic phase of the disease. On the other hand, little or no correlation can be observed between the NA levels and clinical status of HIV-infected persons (Prince *et al.*, 1987).

Interferons (IFNs) may also play role in the defense mechanisms against HIV-induced destruction of the immune system. IFN-α has been found in

most serum samples of asymptomatic HIV-carriers (Levin *et al.*, 1985). On the contrary, Abb (1987) could frequently demonstrate IFN-α in patients with AIDS or ARC but rarely in asymptomatic HIV-infected persons.

On the basis of these controversal findings we searched for correlation between the presence of virus NA, serum IFN level, and the clinical as well as immunological status of HIV-infected persons.

Materials and Methods

Serum samples were obtained from HIV-positive asymptomatic homosexual males and haemophiliacs, from patients suffering from ARC and AIDS as well as from uninfected healthy controls. The sera were stored at — 70 °C until use. All assays were performed with encoded serum samples, and data of persons were identified after the evaluation of tests.

Cell cultures. The HTLV-III$_B$ infected H9 (H9/HTLV-III$_B$) cells as well as uninfected H9 cells were grown in suspension culture in RPMI-1640 medium supplemented with 15% foetal calf serum (Gibco Bio-Cult LTD, Paisley, Scotland), glutamine (2 mmol/l) and gentamycin sulphate (50 μg/ml). The interferon-sensitive WISH human amnion cells and Madin-Darby bovine kidney (MDBK) cells were grown in Dulbecco's modified Eagle's medium in the presence of 5% foetal calf serum (Gibco Bio-Cult LTD, Paisley, Scotland).

HIV-neutralization assay. A stock of the HTLV-III$_B$ isolate was prepared and the tissue culture infective dose (TCID$_{50}$) for the half of the inoculated H9 cultures was determined. The extent of CPE was quantified by indirect immunofluorescence assay (IFA) by measuring the percentage of infected cells. IFA was performed according to standard procedures using human immune serum as primary antibody (Ho *et al.*, 1984). For neutralization assay 100 TCID$_{50}$ of HIV in 100 μl was mixed with 100 μl of heat inactivated (56 °C, 30 min) human serum at twofold dilutions started from 1 : 4, and incubated at 4 °C for 2 hr, followed by 15 min at room temperature. The virus was then absorbed onto 10^6 H9 cells [pretreated in 2 μg/ml polybrene (Sigma Chemical Co., St. Louis, MD. U.S.A.) at 37 °C for 30 min] for one hr at 37 °C. Then the mixture was diluted with completed RPMI-1640 to a cell concentration of 10^5/ml in plastic flasks (Greiner und Söhne, GmbH, Nürtingen, F.R.G.) and incubated for 7 days at 37 °C. On day 7 the cultures were harvested and viral infectivity was measured by cytoplasmic IFA.

Serum of an AIDS patient, who was found HIV-positive by ELISA and IFA served as primary antibody. Neutralizing titre was defined as the greatest dilution which blocked 90% of virus CPE.

IFN assays. The serum samples were tested for their IFN activity in cytopathogenic effect inhibition assays as previously described (Epstein and McManus, 1980). In brief, 2×10^4 WISH cells were incubated with twofold serial dilutions of plasma samples (started from 1 : 2.5) in 96-well microtitre plates and then challenged with vesicular stomatitis virus. Virus-induced cytopathology was evaluated microscopically at 24—30 hr after infection. The assays included a reference IFN-α which was standardized to NIH-4-023-901-527 (National Institute of Health, Bethesda, MD. U.S.A.). The titres of triplicated plasma samples are expressed in international units (IU) per ml.

For characterization of IFN, assays were performed in duplicate on WISH and MDBK cells. Further characterization of IFN was done by neutralization using anti-human IFN-α (anti-Hu-IFN-α) serum, (a generous gift from Dr. I. Mécs, Institute of Microbiology, University Medical School, Szeged, Hungary). The polyclonal goat IFN-α-specific antiserum was diluted to neutralize 300 IU/ml of IFN-α. After an incubation for 1 hr at 37 °C the samples were assayed for the residual IFN activity. For determination of stability at pH 2.0 IFN containing samples and standards were dialyzed against glycine-HCl buffer pH 2.0 at 4 °C for 18 hr. They were neutralized to pH 7.4 by further dialysis in three changes in TRIS buffer pH 7.6. The residual activity was measured in antiviral assay.

T-cell parameters. The absolute number of T4 and T8 lymphocyte subsets was determined by OKT monoclonal antibodies (Ortho-mune OKT$^+$4 and OKT$^+$8, Ortho Diagnostic Systems Inc., Raritan, N. J. U.S.A.). Murine monoclonal antisera specific for subsets of human T lymphocytes were used to label live cells as previously described (Markham *et al.*, 1984).

Results

The mean T4-cell number of the asymptomatic HIV-infected persons was 767 (range 485—1322) and the mean T8-cell number of them was 1031 (range 478—2073). The mean T4-cell number of the patients with ARC was 378 (range 176—589), whereas the mean T8-cell number was found to be 939 (range 441—1359). The AIDS patients had a mean T4-cell number of 118 (range 2—374) and a mean T8-cell number of 531 (range 58—1290).

Table 1 shows the HIV-neutralizing and IFN titres of sera from asymptomatic HIV-carriers. Persons from 1 to 13 were homosexual males, while patients from 14 to 20 suffered from haemophilia. HIV-neutralizing antibodies were found in all serum samples of asymptomatic virus carriers, and with some exception, in high titres (from 128 to 512). Such antibodies could be detected in 8 of 11 patients with ARC (Table 2) and only in 3 of 8 patients suffering from manifested AIDS (Table 3). HIV-antibody negative sera from 40 healthy control persons did not show any HIV-neutralizing activity.

Serum IFN was found in 6 of 13 asymptomatic HIV-infected homosexuals (20—80 IU), whereas only two of 7 asymptomatic haemophiliacs had slightly elevated (10 and 40 IU) IFN levels (Table 1). All ARC patients were found to be negative for the presence of circulating IFN (Table 2), in contrast to the manifest AIDS patients, in 6 of whom elevated serum IFN level could be detected (Table 3).

Table 1. HIV and IFN titres in serum samples from asymptomatic HIV-infected persons

Patient No.	Neutralizing titre	IFN titre*
1	8	0
2	8	0
3	16	0
4	128	0
5	32	20
6	128	0
7	128	0
8	256	20
9	512	80
10	128	20
11	128	20
12	256	40
13	128	0
14	128	0
15	512	0
16	512	0
17	128	0
18	128	20
19	64	40
20	16	0

* on WISH cells

Table 2. HIV NA and IFN titres in sera from patients with AIDS-related complex

Patient no.	Neutralizing titre	IFN titre*
21	16	0
22	64	0
23	0	0
24	0	0
25	32	0
26	128	0
27	256	0
28	512	0
29	256	0
30	512	0
31	0	0

* on WISH cells

On the basis of parallel titration on WISH and MDBK cells, based on neutralization with anti-Hu-IFN-α immune serum as well as on treatment at pH 2.0, in the vast majority of cases the IFN activity proved to be acid-labile IFN-α except of one AIDS patient (No. 39, Table 4). Serum sample of this patient contained both acid-labile and acid stable IFN-α. Out of the 40 control sera, 8 samples contained acid stable IFN-α in titres below 1 : 10. We failed to detect acid labile IFN-α in any of the control serum samples.

Discussion

High titered virus NA could be detected in asymptomatic HIV-carriers. It is reasonable to assume that during the asymptomatic phase of HIV infection high-titred antibodies have a beneficial effect by preventing infection of a large amount of susceptible target cells. HIV-neutralizing anti-

Table 3. HIV NA and IFN titres in sera from AIDS patients

Patient no.	Neutralizing titre	IFN titre*
32	0	10
33	0	20
34	0	20
35	0	0
36	16	0
37	0	20
38	64	40
39	256	20

* on WISH cells

Table 4. Characterization of IFN detected in the serum samples of HIV-infected persons

Patient No.	Diagnosis	IFN activity on cells*					
		WISH			MDBK		
		before	after	after	before	after	after
		anti-Hu-IFN-α treatment		pH 2.0 treatment	anti-Hu-IFN-α treatment		pH 2.0 treatment
5		20	0	0	40	0	0
8		20	0	0	20	0	0
9		80	0	80	80	0	0
10	Asympto-	20	0	0	40	0	0
11	matic	20	0	0	20	0	0
12		40	0	0	40	0	0
18		20	0	0	20	0	0
19		40	0	0	40	0	0
32		10	0	0	20	0	0
33		20	0	0	20	0	0
34		20	0	0	20	0	0
37	AIDS	20	0	0	40	0	0
38		40	0	0	40	0	0
39		20	0	10	20	0	10

* Expressed in IU/ml

bodies were found with less frequency in patients with ARC and AIDS as well. It is known that HIV can infect target cells not only from the blood stream, but also by cell fusion (Gallo *et al.*, 1987). By this route HIV can avoid neutralization. This mode of virus transmission could be prevented by destroying HIV-infected cells mediated by HIV-specific T-effector cells (Zarling *et al.*, 1986) or by antibody-dependent cellular cytotoxicity (Lyerly *et al.*, 1987). Moreover, HIV-sensitized T8 cells can suppress HIV-replication in T4 lymphocytes, without killing them (Walker *et al.*, 1986). The development of HIV-induced pathogenesis is characterized by gradual decrease of T4- and T8-cells. Thus, it is plausible that neutralizing antibodies alone cannot efficiently involve new target cells in advanced stages of HIV infection. There are at least three mechanisms which may antagonize or switch off the effect of NA. First, the antigen variants emerged from *in vivo* mutation of HIV genome might result in failure of neutralization in the late phases of the infection (Fisher *et al.*, 1988). Second, enhancement of virus infection by antibodies (Robinson *et al.*, 1987) may interfere with the neutralization and reduce its effect. Third, one set of recently identified autoantibodies, cytotoxic for HIV-infected as well as uninfected T4 cells (Stricker *et al.*, 1988) may contribute to ongoing immunosuppression.

It has been described that sera of patients with HIV infection contain an unusual acid-labile form of IFN-α (Levin *et al.*, 1985; Abb, 1987). We have

demonstrated an early peak of serum acid-labile IFN-α during HIV infection mainly in the homosexual males, but rarely in the haemophiliacs. This difference can be explained by repeated allogeneic stimuli of homosexual males. Most AIDS patients had relatively high serum IFN titres. Surprisingly, we have failed to detect IFN in serum samples from patients with ARC. Capobianchi *et al.* (1987) found that the production of acid-labile IFN-α could be induced by HIV-infected cells rather than by the HIV alone. Hence, the late peak of serum acid-labile IFN-α may be the consequence of expansion of the HIV-infected cell population.

Acknowledgements. The authors are thankful to Dr. R. C. Gallo for providing H9 and HTLV-$-III_B$-infected H9 cell lines. We are also indebted to Marianna Lelesz and Erika Markovics for technical assistance and to Erika Pap for typing the manuscript.

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