CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Mar. 1996, p. 230–232 Vol. 3, No. 2
1071-412X/96/$04.00+0

Antibody against Human Immunodeficiency Virus Type 1 (HIV-1) Tat Protein May Have Influenced the Progression of AIDS in HIV-1-Infected Hemophiliac Patients

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Received 28 July 1995/Returned for modification 4 October 1995/Accepted 5 December 1995

Retrospective analysis of serum samples from a group of hemophiliac patients who became infected with human immunodeficiency virus type 1 (HIV-1) between 1984 and 1985 has shown that, at variance with other HIV-1-infected patients, at the onset, or at least at a very early phase of HIV-1 infection, they constantly have elevated levels of antibodies against HIV-1-transactivating Tat protein and an absent or barely detectable p24 antigenemia. Anti-Tat antibodies in initial serum samples from hemophiliac patients were probably the consequence of the passive administration of immunoglobulins present in low- or intermediate-purity clotting factor concentrates prepared from HIV-1-infected blood. Furthermore, the analysis of serial serum samples obtained during the course of the disease, in which passively acquired anti-Tat antibodies were substituted by actively produced antibodies, demonstrated an inverse relationship between anti-Tat antibody and p24 antigenemia levels throughout the observation period. These data seem to suggest that anti-Tat antibody may have some influence on the course of HIV-1 infection.

The human immunodeficiency virus type 1 (HIV-1) genome codes for a variety of viral regulatory and accessory proteins that can affect virus replication. One regulatory protein is Tat, a transactivating protein which interacts with a transactivation response element formed by nascent viral mRNAs. Tat facilitates the recruitment of cellular transcription factors and other still incompletely characterized cellular proteins in the transcription complex to enhance the rate of transcription initiation and/or elongation (2).

Tat is crucial to virus replication. Tat's potential effects on HIV-1 pathogenesis, however, go well beyond its role in the virus life cycle. Current data indicate that biologically active Tat is released from HIV-1-infected cells and is readily internalized by nearby, or perhaps distant, cells, on which it may exert a series of pleiotropic effects. The paracrine action of Tat has been extensively studied in vitro, and depending on the amount of exogenously added Tat, its effects may go from the suppression of immunocompetent cells to transactivation of cellular genes and stimulation of endothelial and Kaposi's sarcoma spindle cells (3).

Recently, we have reported experimental evidence suggesting that Tat may autocrinously influence both cellular physiology and HIV-1 long terminal repeat-directed gene expression in Tat-producing cells (13). We (9) and others (12) further demonstrated that anti-Tat monoclonal antibody is an efficient inhibitor of HIV-1 replication both in cells experimentally infected with HIV-1 and in cocultures of peripheral blood mononuclear cells from HIV-1-infected patients.

Antibodies against Tat protein have been described in HIV-1-infected patients (1, 4), but at a lower prevalence than antibodies to structural HIV-1 proteins (5, 10).

Former observations did not show any correlation between anti-Tat antibody prevalence and clinical status (1, 4). More recently, low-level antibody reactivity to Tat protein and other accessory gene products, although without predictive value in patients who are HIV-1 p24 antigen negative and who have normal $CD4^+$ lymphocyte counts (11), has been found to be associated with progression to AIDS relatively rapidly after infection with HIV-1 (5, 10, 11).

Together, these data prompted us to investigate the prevalence of anti-Tat antibody and HIV-1 p24 core antigen levels in initial serum samples obtained from three groups of HIV-1-infected patients and respectively represented by the following: (i) Serum samples were obtained at the putative time of infection, or at the closest possible time of infection, from 11 hemophiliac patients (five patients between the ages of 5 and 10 years and six patients between the ages of 20 and 31 years at the time of their seroconversion) who, by the retrospective analysis of available serum samples, became infected between 1984 and 1985. Hemophiliac patients were chosen because we speculated that if they had been treated with low- or intermediate-purity clotting factor concentrates prepared from infected blood, they would also have received a large amount of specific immunoglobulins (7, 8), including antibodies against various HIV-1 soluble products. (ii) Serum samples were obtained at birth from 16 HIV-1-infected infants born to seropositive mothers; these patients were considered possible passive recipients of anti-Tat antibody of maternal origin. (iii) Serum samples were obtained at the time of seroconversion from 58 HIV-1-infected intravenous drug (IVD) abusers and/or homosexual men (mean age, 27 ± 6.1 years).

Anti-Tat antibody levels were studied by an enzyme-linked immunosorbent assay performed as described previously (9). HIV-1 p24 core antigen levels were assayed by a commercial solid-phase sandwich-type immunoassay (Organon Teknika, Durham, N.C.) after immunocomplex disaggregation by a sample basification procedure.

In initial serum samples obtained from hemophiliac patients, the occurrence and levels of HIV-1 neutralizing antibodies were also investigated. For this purpose, 3×10^3 50%

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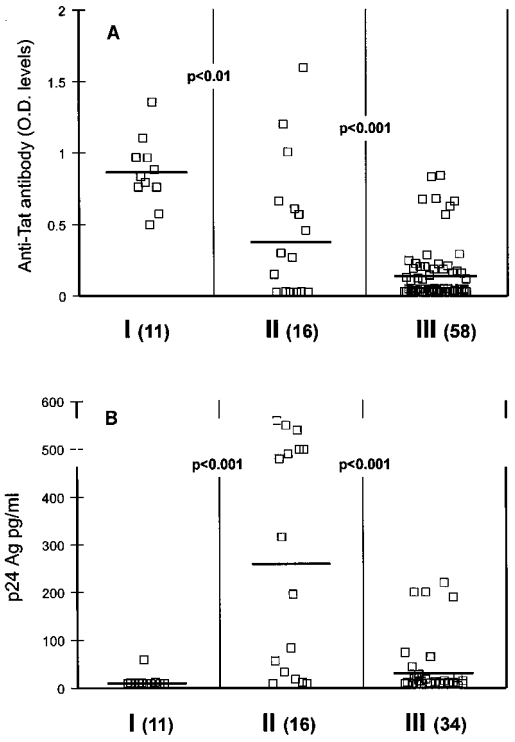

FIG. 1. Anti-Tat antibody levels (A) and p24 antigenemia levels (B) in initial serum samples from hemophiliac patients (group I), infants born to HIV-1-seropositive mothers (group II), and IVD abusers or homosexual male patients (group III) infected with HIV-1. The number of subjects examined is given in parentheses. Bars indicate mean values. O.D., optical density; Ag, antigen.

tissue culture infective doses of HIV-$1_{(IIIB)}$ in a volume of 40 μl were incubated with 10 μl of serum diluted 1:10 and 1:100 for 1 h at 37°C. The virus-antibody mixture was then assayed for residual infectivity by inoculation into C8166 cell cultures (2×10^5 cells in 100 μl of RPMI plus 10% fetal calf serum per well of a 96-well microtiter plate) in quadruplicate. After 5 days of incubation the wells were scored for the presence of syncytia, and a mean of 90 to 95% reduction of the number of syncytia in the indicator cells was considered a positive neutralization.

Anti-Tat antibody levels in the initial serum samples from the various patient groups are reported in Fig. 1A. High levels of anti-Tat antibodies were present in all HIV-1-infected hemophiliac patients, in only 10 of 16 (62.5%) HIV-1-infected infants born to seropositive mothers, with significantly ($P < 0.01$) lower mean optical density levels, and in a still smaller fraction (24 of 58; 41.37%) of HIV-1-infected IVD abuser or homosexual male subjects, in whom anti-Tat antibodies were present at very low levels, in most cases being only barely detectable and with a mean value significantly ($P < 0.001$) lower than that observed in HIV-1-infected newborn infants. Antigenemia (p24) levels in initial serum samples available from the various patient groups are shown in Fig. 1B. Antigenemia was undetectable or was only barely appreciable in hemophiliac patients, was present at elevated levels in most HIV-1-infected newborn infants, and was present, although at significantly lower levels, in some IVD abuser or homosexual male subjects.

A major shortcoming of the data reported in Fig. 1 is the lack of certainty that the serum samples available from the various groups of HIV-1-infected patients were in fact all obtained at exactly corresponding points during the evolution of HIV-1 infection. Nevertheless, the results are suggestive enough to admit the existence at the onset or at least at a very early phase of HIV-1 infection of a decreasing gradient of anti-Tat antibody prevalence and levels in HIV-1-infected hemophiliac patients, HIV-1-infected infants born to seropositive mothers, and HIV-1-infected IVD abuser or homosexual male subjects.

An inverse correlation between prevalence and levels of passively acquired anti-Tat antibodies and initial p24 antigenemia values was evident for initial serum samples from the first two groups of patients. The presence of HIV-1-neutralizing antibody was observed in 6 of 11 initial serum samples from hemophiliac patients tested at a 1:10 dilution and in only 3 of 11 initial serum samples tested at a 1:100 dilution (data not shown).

Furthermore, because low- or intermediate-purity clotting factor concentrates also contain appreciable amounts of albumin (7), which could have acted as a carrier for low-molecular-weight protein, as is the case for extracellularly released Tat protein, we hypothesized that hemophiliac patients could have probably also received variable amounts of soluble HIV-1-specific antigenic products. In our opinion, therefore, they could represent a natural experiment on the possible influence not only of passively administered anti-Tat antibody but also of anti-Tat active immunization on the course of a concurrent HIV-1 infection.

Therefore, to gain a deeper insight into the possible inverse correlation between anti-Tat antibody and p24 antigenemia, serial serum samples from HIV-1-infected hemophiliac patients, for whom retrospective serum samples were available over a period of at least 5 years and obtained at intervals not exceeding 6 months (10 of 11 HIV-1-infected hemophiliac patients), were analyzed for anti-Tat antibody and p24 antigenemia levels, and the results were correlated with $CD4^+$ peripheral blood lymphocyte counts and the clinical outcome of infection.

The mean values of anti-Tat antibody and p24 antigenemia levels observed in serum samples obtained at similar or very close time points from HIV-1-infected hemophiliac patients and mean $CD4^+$ peripheral blood lymphocyte counts, together with their computer-assisted interpolated behaviors, are represented in Fig. 2. As shown in Fig. 2, in serial serum samples obtained during the course of the disease, in which passively acquired anti-Tat antibodies were progressively substituted by actively produced antibodies, an inverse relationship between the anti-Tat antibody level in serum and p24 antigenemia was also clearly present. Throughout the observation period, the mean $CD4^+$ lymphocyte count was declining (473 ± 300 to $134 \pm 128/\mu l$), and 9 of the 11 patients showed the onset of clinical symptoms of AIDS 37 to 70 months (56.75 ± 11.63 months) after seroconversion. In particular, three pediatric hemophiliac patients and two adult hemophiliac patients died 9 to 10 years and 6 to 7 years after seroconversion, respectively. The two patients who at the moment do not show clinical symptoms of full-blown AIDS were 7 and 10 years old, respectively, at the time of seroconversion.

These data, together with the constant presence in initial serum samples from hemophiliac patients of high levels of anti-Tat antibody associated with an absent or barely detectable p24 antigenemia apparently not related to the variable presence and low levels of neutralizing antibodies, suggest a possible influence of anti-Tat antibodies on the progression of AIDS and, in particular, a possible influence of passively acquired anti-Tat antibody on the initial course of concurrent HIV-1 infection.

Our data were obtained from a relatively small number of patients, and the pathogenesis of HIV-1 infection is a complex,

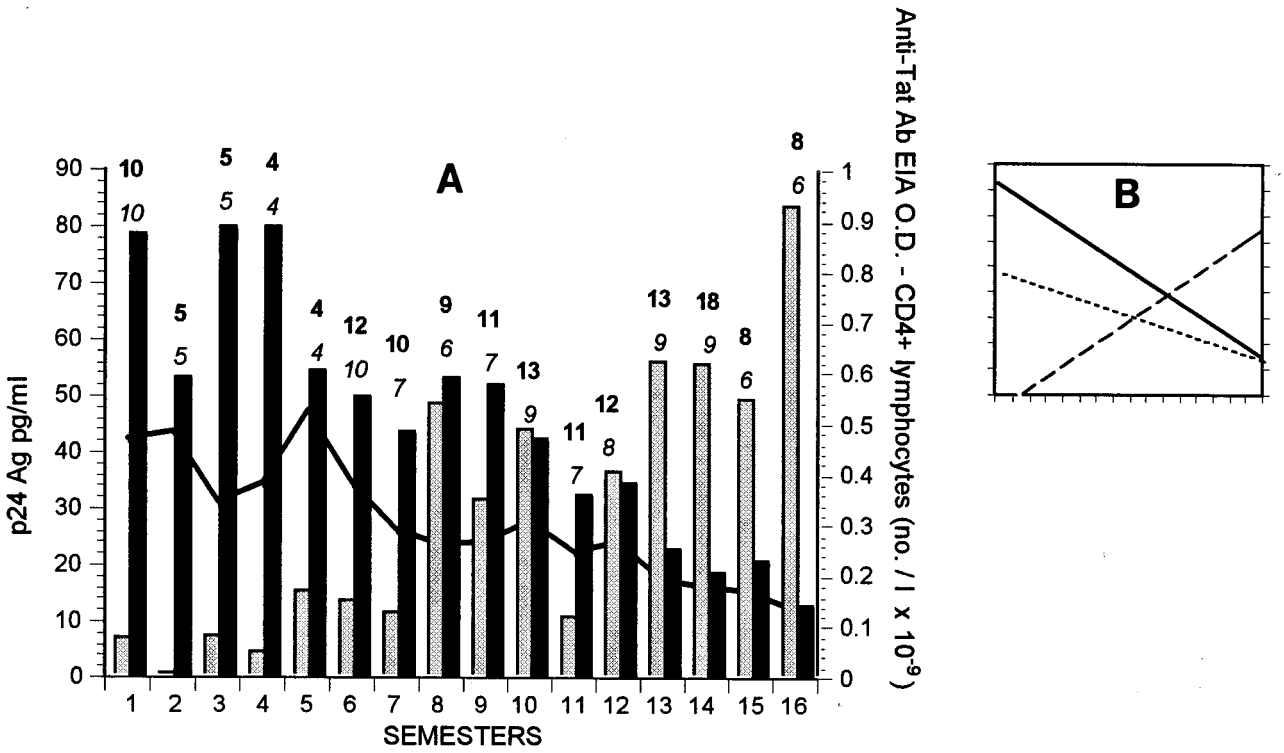

FIG. 2. (A) Mean anti-Tat antibody levels (black columns) and p24 antigenemia levels (shaded columns) in serum samples obtained from hemophiliac patients at similar time points (within one semester) during an 8-year observation period. Continuous line, mean number of peripheral blood $CD4^+$ lymphocytes (number of cells per 10^{-9}). The numbers of serum samples (boldface numbers) and the numbers of patients (italic numbers) examined at each point are indicated at the tops of the histograms. (B) Computer-assisted interpolation of mean anti-Tat antibody (——), p24 antigenemia (– – –), and $CD4^+$ lymphocytes (. . .) levels throughout the observation period. Ag, antigen; Ab, antibody; EIA, enzyme immunoassay; O.D., optical density.

multifactorial process with many features that still remain mysterious (6). It may therefore be too simplistic to focus on the results obtained by analyzing only two parameters, namely, anti-Tat antibody levels in serum and the p24 antigenemia level, whose inverse relationship may be merely incidental or only indirectly influenced. However, if one takes into consideration the fact that at least some of the effects of Tat protein seem to be the consequence of an autocrine loop (13) and that anti-Tat antibody is an efficient inhibitor of HIV-1 replication both in cells experimentally infected with HIV-1 and in cocultures of peripheral blood mononuclear cells from HIV-1-infected patients (9, 12), then it could be a reasonable case that extracellular Tat protein may be a functionally relevant species in HIV-1 infection and that anti-Tat antibody may interfere with possible Tat-driven pathogenesis.

In our opinion, the possibility that anti-Tat antibody may exert some influence on the progression of HIV-1 infection in vivo deserves more attention, and further research is necessary to definitely establish whether extracellular Tat protein may eventually be considered a possible target for specific immune treatments.

This research was supported by the AIDS Research Project of the Italian Ministry of Health and by grants from the Italian Research Council (CNR-FAT.MA Target Project), Regione Emilia-Romagna, and Ministry of University and Scientific Research (60%).

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