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VIRUS NEUTRALIZING ANTIBODIES AT DIFFERENT STAGES OF THE HIV DISEASE: INCREASED LEVELS AFTER AZIDOTHYMIDINE TREATMENT

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Summary. - Specific HIV-1 neutralizing activity was measured in single serum samples obtained from 52 individuals suffering from different stage of HIV disease, as well as in serum samples collected during a four years follow up of other 13 HIV-1 seropositive persons, from whose seven developed AIDS. Three of these persons were treated with azidothymidine. In the former group of single serum specimens, the specific neutralizing antibody positivity rate was 81 per cent in symptomless persons, 92 per cent in patients with ARC and 43 per cent in patients with AIDS. From 13 HIV-1 infected individuals, prospectively investigated from 1986 to 1990, six remained asymptomatic and no significant fluctuation of specific virus neutralizing antibody levels was noted. During this time period, remaining seven patients developed AIDS. In the sera of AIDS patients, specific neutralizing activity was either not detected or its titres were rather low before the appearance of clinical disease. Three AIDS patients were administered azidothymidine. Specific neutralizing antibody titres increased significantly one month after the beginning of azidothymidine administration and persisted at relatively high levels over several months of follow up.

Key words: neutralizing antibody; HIV-1; ARC; AIDS; azidothymidine

Introduction

HIV-1 (HIV) has been identified as the primary causative agent of AIDS (Barré-Sinoussi *et al.*, 1983), the terminal stage of HIV disease. It develops mostly after a latency period of several years, with the rate of progression

varying among infected individuals. The presence of specific virus neutralizing antibodies (NA) is supposed to play a role in the natural history of HIV disease, especially in the later phases. The occurence of NA in the sera of HIV-infected persons was first described by Robert-Guroff *et al.* (1985) and by Weiss *et al.* (1985). Nevertheless, the importance of NA in the pathogenesis of the HIV disease was not defined in appropriate details as yet. The published observations are rather discordant. Some investigators have demonstrated a certain relatedness between the levels of NA and clinical status in persons studied (Faulkner-Valle *et al.*, 1986; Ranki *et al.*, 1987; Robert-Guroff *et al.*, 1987). The other data did not support such correlations (Groopman *et al.*, 1987; Prince *et al.*, 1987; Böttiger *et al.*, 1988). Recently, observations from longitudinal NA studies have been reported (Robert-Guroff *et al.*, 1988; Alesi *et al.*, 1989). These studies revealed certain predictive, prognostic value of NA levels, especially if their continuous decrease was noted. The high NA titres were associated with more stabilized clinical state, whereas low or decreasing NA titres usually signaled worsening of the disease, although exceptions from both these situations were observed.

We present here results of NA examinations of single sera obtained from individuals suffering from HIV disease at different stages of progression and then of sera obtained sequentially from HIV seropositive asymptomatic individuals, patients with ARC, AIDS and from AIDS patients being under the treatment with azidothymidine (AZT).

Patients, Materials and Methods

Patients. In sera of all persons investigated, the presence of specific HIV antibodies has previously been shown by an enzyme immunoassay (Wellcozyme) and confirmed by immunoblot (Western) (DuPont) analysis. The first study group consisted of 40 haemophiliacs and 12 homosexual men (52 single serum specimens). In 32 persons the infection had still asymptomatic character, ARC was diagnosed in 13 of them and 7 suffered from AIDS.

The second study group consisted of 13 HIV seropositive persons from which 72 serum samples were consecutively collected between 1986 and 1990. During this four years period 6 persons remained without clinical symptoms but seven patients developed AIDS. In one of them generalized lymphadenopathy was observed at the time of diagnosis in 1987. The lymphadenopathy disappeared but p24 antigenaemia (Abbott HIV antigen EIA) persisted until the sudden onset of AIDS in 1990. Three of the AIDS patients have been treated with AZT (Retrovir, Burroughs Wellcome Co.).

Cells and virus. The MT4 cells were used for NA examinations. Cells were cultivated in RPMI 1640 nutrient medium (Seromed) supplemented with 10 per cent heat-inactivated foetal calf serum (Seromed) and antibiotics. All virus-neutralization assays were carried out using the BRU strain of HIV type 1 (Barré-Sinoussi *et al.*, 1983). Stocks of infectious virus were prepared from clarified supernatants of the CEM cell line persistently infected with BRU and cultivated in suspension. Supernatants were filtrated (FlowPore; 0.45 μm), aliquots frozen at -70 °C and titrated for cytopathic effect on MT4 cells. The titrations were made on 24-well plates (NUNC Co.). Each dilution of the viral suspension was tested in quadruplicate as follows: 0.1 ml of 10-fold dilutions of viral suspension were mixed with 3×10^5 MT4 cells and incubated at 37 °C in an

atmosphere of 5 per cent CO_2. After three days the grown up cells suspension was diluted to a concentration of $3x10^5$ cell per ml. On the 7th day cells were stained with trypan blue and their viability evaluated. The endpoint dilution at which viral cytopathic effect developed within seven days in all cell cultures infected was used for neutralization assay.

Virus-neutralization assay. We used the method described earlier (Rey *et al.*, 1987). Briefly, heat-inactivated sera were diluted two-fold in the 24-well plates to a final volume of 0.8 ml and mixed with 0.1 ml of stock virus dilution (100 per cent cytopathic dose). After incubation for one hour at 37°C, MT4 cells were added. Then, the assay was carried on as described for virus titration on MT4 cells. The sera that failed to neutralize HIV infectivity at dilution 1:50 were considered as not containing virus neutralizing activity.

Results

Virus-neutralizing antibodies in single serum samples

Totally 52 individual sera were divided into three groups according to the clinical status of their donors: infected asymptomatic persons (n=32), patients with ARC (n=13) and patients with AIDS (n=7). The relatively highest percentage of sera with NA (92 per cent) and with highest NA titres were observed in the group of patients with ARC. On the other hand only 43 per cent of sera from patients with AIDS contained measurable NA (Table 1).

Virus-neutralizing antibody levels in serum samples collected during a prospective study lasting for five years

Together 72 serum samples serially collected from 13 HIV-seropositive subjects were tested for NA. Fig. 1 depicts the NA profile of six subjects remaining asymptomatic (1986-1990). Over the whole observation period, five of them were positive for NA without significant fluctuation of specific antibody titres. No neutralizing activity was found in the serum samples of the subject No. 6 until 1988. However, in serum specimens collected in 1989 the NA arised. The NA profile of patient No. 7, in whom the lymphadenopathy was observed at the time of HIV-1 infection diagnosis is shown on Fig. 2. NA was found in his serum samples collected from 1987 to the end of 1989, together always with antigenaemia (HIV, p24; ABBOTT HIV antigen EIA). At the end of 1988 the

Table 1. Specific virus neutralizing antibodies in HIV-1 seropositive asymptomatic persons, patients with ARC and with AIDS

HIV disease	No. of tested	No. of positives (%)	Range of titres*
asymptomatic	32	26 (81)	50 - 3200
ARC	13	12 (92)	50 - 12 800
AIDS	7	3 (43)	50 - 200

*antibody titre is expressed as the reciprocal value of the highest serum dilution inhibiting the HIV 1 cytopathic effect

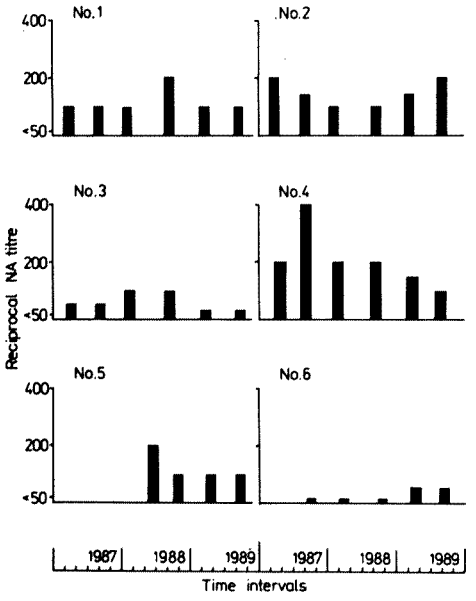


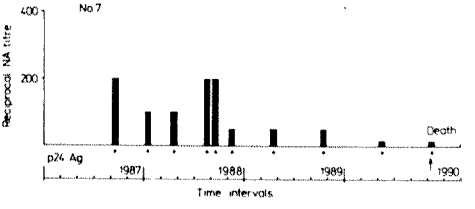
Fig. 1
Specific virus neutralizing antibody levels prospectively investigated in 6 asymptomatic HIV-1 seropositive persons

NA level dropped from the titre 1:200 to 1:50 with no increase until the end of 1990, when the patient suddenly developed AIDS and died. Before the onset of the peracute AIDS he had no clinical problems.

NA profile of other six subjects who progressed to AIDS are shown on the Figs 3 and 4. The NA was either not recovered, or the neutralizing antibody titres were low (1:50) in all these subjects before the appearance of the final phase of the disease. This NA level pattern was observed already 12 or 18 months before AIDS diagnosis in two of them, similarly, as it was seen in the patient No. 7. In four patients a temporary increase of the NA titres was noted after the development of AIDS. In one patient NA level raised from negativity to a titre of 1:400.

Three patients with AIDS were treated with AZT. The NA levels increased significantly in all of them one month after beginning the standard full dose AZT treatment (Fig. 4). This unexpected finding was reproducibly monitored during several months of the follow-up.

Fig. 2
Specific virus neutralizing antibody levels prospectively investigated in a patient developing AIDS
p24 antigenaemia was assayd by ABBOTT HIV antigen EIA. The arrow indicates the time of AIDS diagnosis.



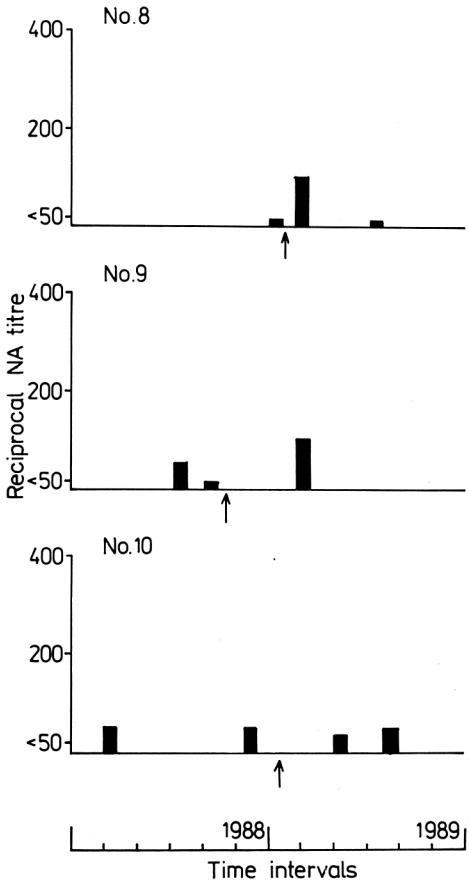


Fig. 3
Specific virus neutralizing antibody levels prospectively investigated in 3 patients with AIDS
The arrow indicates the time of AIDS diagnosis.

Discussion

The present study yields data allowing interpretations also in more general terms. First, marked differences in specific NA positivity rate were seen between the patients with developed AIDS and patients with earlier stages of the HIV disease. Further, in the sera of persons which did not develope ultimate clinical manifestations of HIV infection during the 4 years observation period, the NA levels were relatively high and no significant fluctuations in their titres were seen. On the other hand, no detectable NA or in extremely low titres only were present in patients with the downhill course of HIV disease. These observations suggest that certain temporal relationships may exist

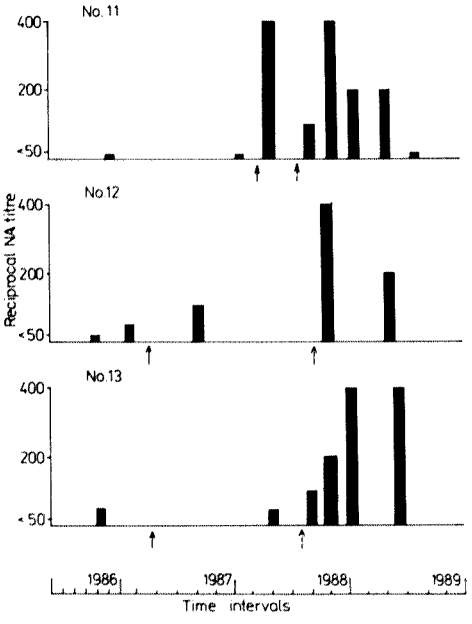


Fig. 4
Specific virus neutralizing antibody levels prospectively investigated in 3 patients with AIDS treated with azidothymidine (AZT)
The first arrow (↑) indicates the time of AIDS diagnosis and second one (↑) the beginning of AZT treatment.

between the NA levels and the asymptomatic stage of HIV infection or eventual clinical deterioration. In our study we used the BRU strain of the HIV-1, epidemiologically prevalent at present, with sensitivity to the neutralization comparable with other strains of this lentivirus (Vujcic *et al.*, 1990). Thus, our data are consistent with earlier observations (e.g. Alesi *et al.*, 1989; Robert-Guroff *et al.*, 1988) ascribing to the NA levels in HIV-infected persons a predictive, prognostic value concerning e.g. an imminent clinical progression of their disease. Nevertheless, in such a complex situation as the development of the AIDS is, the post hoc/propter hoc considerations dealing only with NA may appear rather simplistic.

AIDS patients are positive for the presence of specific HIV antibodies, sometimes reduced, as demonstrable by EIA or immunoblot, but neutralizing activity against HIV is not always detected in proportionally significant amounts. Similarly, a considerable variability among individual patients in the rate of progression to clinically apparent disease is known. Factors decisively contributing to the development of the full-blown AIDS are not precisely defined as yet. The decline of several immune functions (e.g. Stevens *et al.*, 1987; Polk *et al.*, 1987) appears to be associated with an increased risk of progression to this final stage of the HIV disease. NA are also discussed in this respect but the association of low NA levels or their absence with the shorter life-span of infected persons is not equivocal (e.g. Sei *et al.*, 1988, 1989), although observed

in a majority of AIDS cases. On the other hand, it cannot be excluded that an association of higher NA levels with earlier and clinically milder stages of the HIV disease may also reflect the relatively less damaged immunological functions in such individuals. Further studies are warranted in order to define more specifically in above situations the protective relevance of NA - mostly studies *in vitro* - or their eventual role in slowing the course of infection.

Our notion of absent or low NA levels in AIDS patients fits, at least partially, the findings of high concentrations of an infection-enhancing activity (cell surface Fc receptor mediated specific IgG antibody enhancement) in sera with low or undetectable neutralizing activity (Robinson *et al.,* 1988; Homsy *et al.,* 1988). If this enhancing activity will be identified as a different category of antibody from neutralizing antibody (Takeda and Ennis, 1990), the character of their mutual interactions for the outcomes of HIV infection may add another complexity into the understanding of mechanism underlying the HIV disease progression.

An unexpected and according our knowledge until now not described observation, was the 4-8-fold increment of the NA titre values following and during several months lasting AZT treatment in three from three patients studied (Fig. 4). At present we do not have a plausible explanation for this interesting finding. Two situations are considered, however. The AZT administration results in a significant, although temporal and not complete inhibition of HIV replication, thus decreasing the viral load in the patient's organism, demonstrable also by a reduction of the HIV p24 antigenaemia. During the AZT administration the p24 levels may drop to about ten per cent of pretreatment values (Jackson *et al.,* 1988). The relative lack of HIV antigen could possibly contribute to a decreased formation of virus-antibody complexes and the NA may eventually become detectable. The improved immune response regulations due to an increase of T CD4-cells number, due to the AZT antiviral effect cannot also be ruled out completely.

The enhancing effect of specific NA on the inhibition of HIV replication by AZT was described recently (Nakashima *et al.,* 1987). The mechanism(s) of an increased specific NA synthesis in AZT treated AIDS patients - an event, recognized by this study, most probably of a secondary nature - is not elucidated at present. Nevertheless, if confirmed by further investigations, the higher NA levels may contribute to a more efficient limitation of the virus spread in AZT administered patients suffering from advanced HIV disease, in which a better therapeutic effect could be then expected.

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