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Neutralizing antibodies in relation to antibody-dependent cellular cytotoxicity-inducing antibodies against human immunodeficiency virus type I

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SUMMARY

The presence of neutralizing antibodies against human immunodeficiency virus type 1 (HIV-1) was investigated in sera from 73 HIV-1 seropositive subjects at different clinical stages. Virus neutralization was measured as survival of MT-4 cells in a 6–7 day microassay. Sixty-three sera were also tested for antibodies inducing virus-specific antibody-dependent cellular cytotoxicity (ADCC). Neutralizing antibodies were found in 59% of sera tested, the positivity rate being 50% (9/17) in asymptomatic subjects, 67% (12/18) in patients with persistent generalized lymphadenopathy (PGL) and 54% (14/26) in AIDS patients (not significant differences). ADCC antibodies were present in 43% of the sera. Neutralizing antibodies and ADCC-inducing antibodies were found simultaneously in 35% (22/63) of the sera. Neutralizing antibodies alone were found in 22% (14/63) and ADCC antibodies alone in 6% (4/63) of the sera tested. Thirty-seven per cent (23/63) of the sera were negative for both types of antibodies, 62% of the sera with neutralizing antibodies also had ADCC inducing antibodies and 85% of the sera with ADCC antibodies had neutralizing antibodies. The titres of ADCC antibodies were higher than those of neutralizing antibodies. Thus, the presence of ADCC antibodies was related to the presence of neutralizing antibodies, but no correlation was found between the titres of these antibodies in sera positive for both activities.

Keywords HIV-1 Neutralizing antibody ADCC

INTRODUCTION

Subjects infected with immunodeficiency virus type 1 (HIV-1), the etiological agent of AIDS, develop antibodies to various HIV viral proteins demonstrable by a variety of methods. Antibodies able to neutralize the infectivity of HIV-1 *in vitro* have been demonstrated, usually at low titres, in a fairly high proportion of HIV-1-infected individuals (Robert-Guroff, Brown & Gallo, 1985; Weiss *et al.*, 1985; Harada *et al.*, 1986; Ranki *et al.*, 1987). We have found earlier that approximately 40% of HIV-1-infected individuals have virus-specific antibody-dependent cellular cytotoxicity (ADCC)-inducing antibodies when tested against HTLV-IIIB-infected cells (Ljunggren *et al.*, 1987a). Neutralizing antibodies as well as antibodies active in ADCC appear to be directed against the envelope glycoproteins of HIV-1 (Lasky *et al.*, 1986; Putney *et al.*, 1986; Ho *et al.*, 1987; Ljunggren *et al.*, 1988). However, there are no reported studies on the relation between these two types of antibody.

In this work we have studied the presence and titres of neutralizing antibodies in relation to specific ADCC antibodies in HIV-1-infected individuals at different clinical stages.

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MATERIALS AND METHODS

Subjects

Sera from 73 HIV-1 seropositive subjects were tested. HIV-1 antibodies were determined by enzyme-linked immunosorbent assay (ELISA) (Organon Teknica) and confirmed by Western blotting (WB) as described previously (Albert *et al.*, 1987). Only sera reacting with at least one of the glycoproteins 41 or 120 were considered positive. The HIV-1-seropositive subjects were divided into three groups according to their clinical status. 1. Asymptomatic subjects ($n = 18$) 2. Subjects with persistent generalized lymphadenopathy (PGL) ($n = 18$) 3. Patients with acquired immunodeficiency syndrome (AIDS) ($n = 26$). In 11 subjects the clinical status was unknown. Sera from 13 HIV-1-seronegative blood donors served as controls.

Neutralizing assay

The HTLV-1-infected T cell line MT-4 (Harada *et al.*, 1986) was used as target cells in a microassay. Neutralization was measured as the ability of serum to inhibit the cytopathic effect of HIV-1 on MT-4 cells. The virus used for infection was purified HTLV-III B received as a gift from Dr R.C. Gallo, NCI, Bethesda. The virus dilution used was the highest dilution giving maximum cell death in the cell cultures without any protective sera added. The experiments were performed in 96-well U-bottom miocrotitre plates in RPMI 1640 with 15% fetal calf serum. Equal amounts (50 μl) of a serum dilution (fourfold dilutions starting from 1:20) and a virus dilution were mixed and incubated at room temperature for one hour. Then 5×10^3 MT-4 cells were added to each well and the cultures incubated at 37° C with 5% CO_2. After 5–6 days the cultures were visually inspected and the size of the cell pellet measured, whereafter the cultures were pulsed with ^{3}H-thymidine for 18 hours, harvested and counted. The neutralization titre was defined as the reciprocal of the highest serum dilution giving the same size of cell pellet, and the same amount of thymidine incorporation as the control cultures without virus. All samples were tested in duplicate.

ADCC assay

The ADCC assay was performed as described previously (Ljunggren *et al.*, 1987a, b). Briefly, the monocytoid cell line U937 clone 2 (U937-2) was infected with cell-free supernatants from Molt 3 cells infected with the HTLV-IIIB strain of HIV-1 for one hour at 37°C and kept in continuous passage.

Peripheral blood lymphocytes were used as effector cells. They were derived from normal healthy donors and collected by density centrifugation on Ficoll-Isopaque and adherent cells were removed by the scrubbed nylon wool technique. The effector/target cell ratio was 20:1.

Fifty-one Cr-labelled target cells (1×10^4) were mixed with serum dilutions. Supernatant was harvested after 3 h and released radioactivity was calculated (Merrill, Ullberg & Jondall, 1981). The spontaneous release never exceeded 10%. No ADCC activity could be detected in any sera against uninfected U937-2 target cells or, in any HIV antibody negative sera.

Statistical analysis

When comparing differences in neutralization titres between the groups, Student's *t*-test combined with a Mann–Whitney *U*-test was used. Fischer's exact test was used when comparing frequencies of neutralizing and ADCC antibodies.

RESULTS

Neutralizing antibodies against HIV-1 in titres of 20 or more were found in 59% (43/73) of all HIV-1 antibody positive sera tested (Table 1). The number of sera with neutralizing antibodies in relation to clinical status are shown in Table 1. The highest proportion of sera with neutralizing antibodies (67%) was found in the subjects with PGL but the differences between the groups of subjects at different clinical stages were not significant. The mean neutralizing antibody titre was lowest in the group of AIDS patients. None of the 13 HIV-1 seronegative sera examined had neutralizing antibodies against HIV-1.

ADCC antibodies were present in 43% of the 63 HIV-1 antibody positive sera tested. The relation between the presence

Table 1. Presence and titres of neutralizing antibodies to HIV-1 in relation to clinical status

Clinical status	No positive/ No tested	Neutralizing anti-HIV-1 titres (geom mean and 95% conf int)
Asymptomatic	9/18 (50%)	80 (32–202)
PGL	12/18 (67%)	50 (30–85)
AIDS	14/26 (54%)	33 (21–52)
Unknown	8/11 (73%)	34 (16–70)
Total	43/73 (59%)	

Table 2. Presence of neutralizing antibodies in relation to ADCC inducing antibodies against HIV

	ADCC antibody positive	ADCC antibody negative	Total
Neutralizing antibody positive	23	14	37
Neutralizing antibody negative	4	22	26
Total	27	36	63

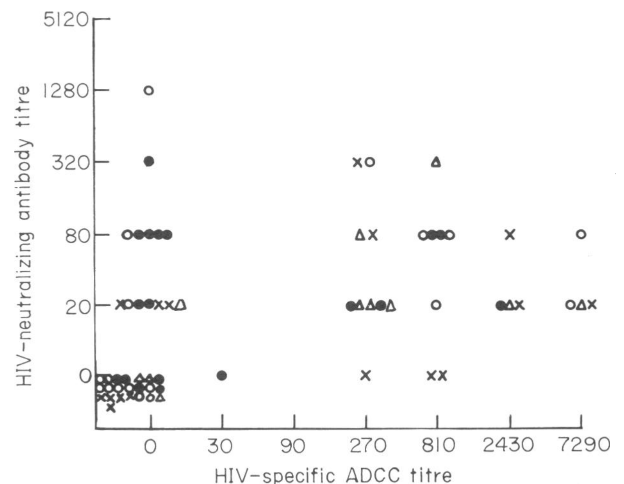

Fig. 1. Correlation of titres of neutralizing antibodies to titres of ADCC-inducing antibodies against HIV-1 in asymptomatic subjects (○), patients with PGL (●), AIDS-patients (×) and in HIV-1-infected subjects with unknown clinical status (△).

of neutralizing antibodies and ADCC-inducing antibodies is shown in Table 2 and Figure 1. Neutralizing antibodies were found in a higher proportion of the sera than ADCC-inducing antibodies. Both these types of antibodies were found simultaneously in 37% (23/63) of the sera. Neutralizing antibodies alone were found in 22% (14/63) and ADCC antibodies alone in 6% (4/63) of the sera. In 35% (22/63) of the sera tested neither of these two types of antibodies were found. The presence of neutralizing antibodies and the presence of ADCC-inducing antibodies were clearly related to each other ($P<0{\cdot}0009$).

As shown in Fig. 1 the ADCC antibody titres were higher than the neutralizing antibody titres. The highest titre for ADCC antibodies was above 7290 whereas the highest neutralization titre (seen in only one patient) was 1280. This serum lacked ADCC capacity. No correlation was found between the titres of ADCC and neutralizing antibodies among the positive sera. Four sera positive for ADCC lacked neutralizing antibodies. Three of these sera with high ADCC-titres were from AIDS patients, and one serum, with the lowest ADCC-titre in this study, came from a subject with PGL.

DISCUSSION

The method used for measuring neutralizing antibodies in this study is a modification of methods described earlier (Harada *et al.*, 1986; Ranki *et al.*, 1987). Ranki *et al.*, have developed a microassay using the interleukin 2 (IL-2) dependent T-helper cell line, ATH-8, as target for HIV-1 infection. Using this system it is easy to examine several sera or serum dilutions simultaneously and the results can be obtained both by visual inspection and by ^{3}H-thymidine incorporation. Instead of using ATH-8 cells we have used the MT-4 cell line described by Harada. This cell line is easier to culture since it does not require interleukin-2. The MT-4 cell line was also more susceptible to HIV-1 infection than the ATH-8 cells in our hands. Starting with as few as 5000 cells per well, the MT-4 cell cultures do not need any change of culture medium during the 7 days of the neutralization experiment. Another similar assay for determining the neutralizing antibodies has recently been described (Rey *et al.*, 1987). In that system the cytopathic effect of HIV on MT-4 cells was observed under the microscope after trypanblue staining.

In the present study we found more sera with neutralizing antibodies than with ADCC-inducing antibodies against HIV-1. On the other hand, the neutralization titres were, in general, lower than ADCC titres. We have shown earlier that the proportion of sera with ADCC was evenly distributed among subjects at different clinical stages of HIV-1 infection, whereas the titres of ADCC antibodies tended to be lower in AIDS patients as compared to PGL patients and asymptomatic subjects (Ljunggren *et al.*, 1987a). In this study neutralizing antibodies were somewhat more frequent in PGL patients than in asymptomatic subjects or AIDS patients, and the titres of these antibodies were slightly lower in the AIDS patients as compared to the other groups. However, the differences did not reach significance. There are previous reports of the finding of a higher neutralizing capacity in sera of patients with PGL as compared to those with AIDS (Harada *et al.*, 1986; Ranki *et al.*, 1987), whereas in some of the other studies no correlation between neutralizing titres and stage of the disease was observed (Groopman *et al.*, 1987; Wendler, Bienzle & Hunsman, 1987; Prince *et al.*, 1987).

We found that the presence of ADCC antibodies was related to the presence of neutralizing antibodies, but there was no correlation between the titres of these antibodies in sera positive for both types of antibody. A few neutralizing epitopes and at least one epitope for ADCC antibodies, different from the neutralizing epitopes, have been identified on the envelope glycoprotein of HIV (Chanh *et al.*, 1986; Lasky *et al.*, 1986; Putney *et al.*, 1986; Weiss *et al.*, 1986; Ho *et al.*, 1987; Lyerly *et al.*, 1987).

The role of neutralizing antibodies and ADCC antibodies for protection against HIV infection *in vivo* is not clear. It has recently been reported that chimpanzees immunized with HIV-1 were not protected against infection upon challenge with live HIV-1 in spite of the presence of neutralizing antibodies in their sera (Hu *et al.*, 1987). No such study of ADCC antibodies has been reported. Further studies of both these types of antibody in association with HIV vaccine trials in animals and man are needed.

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