Enhancing antibodies in HIV infection

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SUMMARY

The author has summarized the history of discovery, the mechanism and the clinical significance of antibody-dependent enhancement (ADE) of HIV infection. ADE has two major forms: (*a*) complement-mediated antibody-dependent enhancement (C-ADE) and (*b*) complement-independent Fc receptor-dependent ADE (FcR-ADE). The most important epitope responsible for the development of C-ADE-mediating antibodies is present in the immunodominant region of gp41 while antibodies mediating FcR-ADE react mainly with V3 loop of gp120. There are at least three fundamentally different hypotheses for the explanation of ADE *in vitro*: (*a*) increased adhesion of HIV-antibody-(complement) complexes to FcR or complement receptor carrying cells; (*b*) facilitation of HIV-target cell fusion by complement fragment deposited on the HIV-virions and (*c*) complement activation products may have a non-specific stimulatory effect on target cells resulting in enhanced virus production. FcR-ADE and C-ADE have been measured *in vitro* mostly by using FcR-carrying and complement receptor-carrying cell lines, respectively; no efforts have been made to standardize these methods. Several data support the possible clinical significance of FcR-ADE and C-ADE: (*a*) Cross-sectional and longitudinal studies indicate a correlation between the amounts of FcR-ADE and C-ADE-mediating antibodies and clinical, immunological and virological progression of the HIV-disease; (*b*) ADE may facilitate maternal–infant HIV-1 transmission; (*c*) According to experiments in animal models, ADE are present and may modify the course of SIV (simian immunodeficiency) infection as well. The author raises a new hypothesis on the mechanism of the *in vivo* effect of C-ADE. According to the hypothesis, C-ADE-mediating antibodies exert their effect through enhancement of HIV propagation and consequent facilitation of the progression of HIV disease. Finally, according to observations from animal experiments and human clinical trials it cannot be excluded that ADE-mediating antibodies may develop, diminish the beneficial effect or may be harmful in volunteers vaccinated with HIV-1 candidate vaccines.

Key words: AIDS, HIV, HIV infection, SIV, enhancement, C-ADE, complement, neutralization vaccines.

INTRODUCTION

Antibody-dependent enhancement (ADE) of infection with parasites is a phenomenon by which uptake of the complexes of microorganism–antibody (and complement proteins or fragments) is facilitated by interactions of antibodies with the Fc receptors (FcR) and/or of complement proteins or fragments with the complement receptors (CR) on the target cells. The first type of antibody-dependent enhancement is designated as complement-independent or FcR-dependent. In the following, the term FcR-ADE will be used for the effect of that type of infection-enhancing antibodies. The effect of the second type of antibodies is mediated by the activation of the complement system and it is dependent on the presence of CR on the target cells. The term C-ADE (complement-mediated, antibody-dependent enhancement) will be applied for this type of infection enhancement.

A significant, 4 log-fold or even greater increase in the *in vitro* production of several viruses (alpha, pox, bunya, reo and herpes) was observed following exposure to low-affinity antibodies (Porterfield, 1986). ADE may be clinically important especially in the case of flaviviruses. For example, dengue haemorrhagic fever is usually more severe in individuals or experimentally infected monkeys with low levels of anti-dengue antibodies than in seronegative individuals (Halstead, Shortwell & Casals, 1979; Halstead, 1988). The presence of infection-enhancing antibodies was found to be a significant (relative risk = 6·2) risk factor for severe dengue haemorrhagic fever among children in Bangkok, a dengue haemorrhagic fever endemic region (Kliks *et al.* 1989).

Antibodies that may enhance HIV infection *in vitro* were described shortly after the first isolation of HIV. Their presence in the blood of HIV-infected patients as well as in HIV- or SIV-infected experimental animals was confirmed by several groups. The data on the mechanism and clinical significance of FcR-ADE and C-ADE, are however, highly controversial.

The purpose of the present article is to review the literature on the antibody-mediated enhancement in HIV infection. The paper is based partly on the critical analysis of literature data and partly on the

Parasitology (1997) **115**, S127–S140. Printed in the United Kingdom
https://doi.org/10.1017/S0031182097001819 Published online by Cambridge University Press

results of our own group. This group consists of researchers from different Hungarian, Austrian, French, Swiss and German institutions and started to study the mechanism and clinical significance of C-ADE in 1990; our first paper was published in 1991 (Tóth *et al*. 1991).

DIFFERENT FORMS OF ANTIBODY-DEPENDENT ENHANCEMENT IN HIV INFECTION

Two types of enhancing antibodies were described approximately at the same time in the late eighties. Robinson and colleagues (Robinson, Montefiori & Mitchell, 1987) found that sera from HIV-infected individuals enhance *in vitro* HIV infection of the CR2 (complement receptor type2)-bearing T lymphoblastoid cell line MT2. The same authors demonstrated this enhancement to be dependent on antibodies and mediated by complement (Robinson, Montefiori & Mitchell, 1988). Gras, Strub & Dormont (1988) reproduced the same phenomenon with peripheral blood lymphocytes. According to further studies (Robinson *et al*. 1989*a*) C-ADE of HIV-1 infection is characterized by > 1 $\log_{10}$ increases in infectious virus release.

FcR-ADE, that is the ability of heat-inactivated sera from HIV-seropositive patients or IgG purified from such sera to accelerate and/or enhance production of HIV by cells infected with mixtures containing these antibodies, was first described by Homsy, Tateno & Levy (1988) and by Takeda, Tuazon & Ennis (1988). Shortly thereafter, FcR-ADE of HIV-1 infection was observed *in vitro* with many different HIV-1 isolates of both T cell- and monocyte-tropic phenotypes, using different target cells (Homsy *et al*. 1989; Jouault *et al*. 1989; Laurence *et al*. 1990; Perno *et al*. 1990; Matsuda *et al*. 1989; Takeda, Sweet & Ennis, 1990). The extent of *in vitro* enhancement of HIV infection by C-ADE is one or two orders of magnitude greater than that by FcR-ADE (Mascola *et al*. 1993).

It has been clear since the first description of FcR-ADE and C-ADE that different types of FcR and CR, respectively, are indispensable for these phenomena to occur. The contribution of the CD4 receptors in antibody-dependent HIV-1 infection enhancement especially in FcR-ADE has been, however, highly controversial until now. In principle, both FcR-ADE and C-ADE may have two forms, a CD4-dependent and a CD4-independent one. Probably the co-receptors (chemokine receptors), like CCR5 an essential cofactor for the infection of CD4-carrying cells by the macrophage-tropic HIV-strains (Alkhatib *et al*. 1996; Deng *et al*. 1996) or CXCR4 an essential cofactor for the T-cell adapted HIV-1 strains (Feng *et al*. 1996) are also involved in the HIV-entry These forms of ADE are summarized schematically in Fig. 1.

CD4-dependent and CD4-independent forms of FcR-ADE

According to the experiments of Takeda, Sweet & Ennis (1990) two receptors, FcγR and CD4 are required for antibody-dependent enhancement of HIV-1 infection. Pretreatment of human macrophages and monocytic cell lines with anti-CD4 or soluble recombinant CD4 was found to markedly inhibit FcR-ADE. Similar results were obtained by Perno *et al*. (1990) by using human peripheral blood monocytes/macrophages, and by Zeira, Byrn & Groopman (1990) in the case of the infection of U937 monocytoid cells with the $HTLV_{IIIB}$ HIV-1 strain. Connor *et al*. (1991) demonstrated that at lower levels of antibody opsonization, there are enough interactions with FcγR to stabilize the virus at the cell surface allowing antibody-dependent enhancement of HIV-1 infection of monocytes and monocyte-derived macrophages through high-affinity CD4 interaction. Nottet *et al*. (1992) were also able to abrogate antibody-dependent enhancement of HIV-1 infection of an EBV-transformed B cell line with a monoclonal antibody against CD4. By contrast, other authors like Homsy *et al*. (1989) or more recently Trischmann, Davis & Lachmann (1995) failed to inhibit FcR-ADE of human macrophages by anti-CD4 antibodies. Use of different strains, experimental conditions or target cells may be responsible for these discrepancies.

CD4-dependency of C-ADE, non-antibody-dependent complement-mediated enhancement of HIV infection

It was demonstrated very early that C-ADE of HIV-1 infection of MT-2 cells requires CD4 and CR type 2 (CR2) (Robinson, Montefiori & Mitchell, 1990). Similar findings were obtained by Tremblay *et al*. (1990) and Gras & Dormont (1991) with the CR2-dependent C-ADE of Epstein–Barr virus-transformed B-lymphocytic cell lines. The CD4 dependency of C-ADE was confirmed more recently by Lund *et al*. (1995) and by our group (Prohászka *et al*. 1997) as well.

A special form of the HIV-1 infection enhancement is the complement-mediated non-antibody-dependent one. Although HIV-1 can bind and activate purified complement components (Dierich *et al*. 1993), non-antibody dependent complement-mediated HIV infection is usually studied by mixing HIV-1 stocks with HIV-seronegative serum samples. Under these conditions enhancement may (Gras & Dormont, 1991) or may not (Boyer *et al*. 1992) require the CD4 receptors to occur. According to the most recent studies of Saarloos, Lint & Spear (1995) 'antibody-independent' mechanisms of complement-activation by HIV-infected cells are at least partly due to a cross-reactive IgM type antibody

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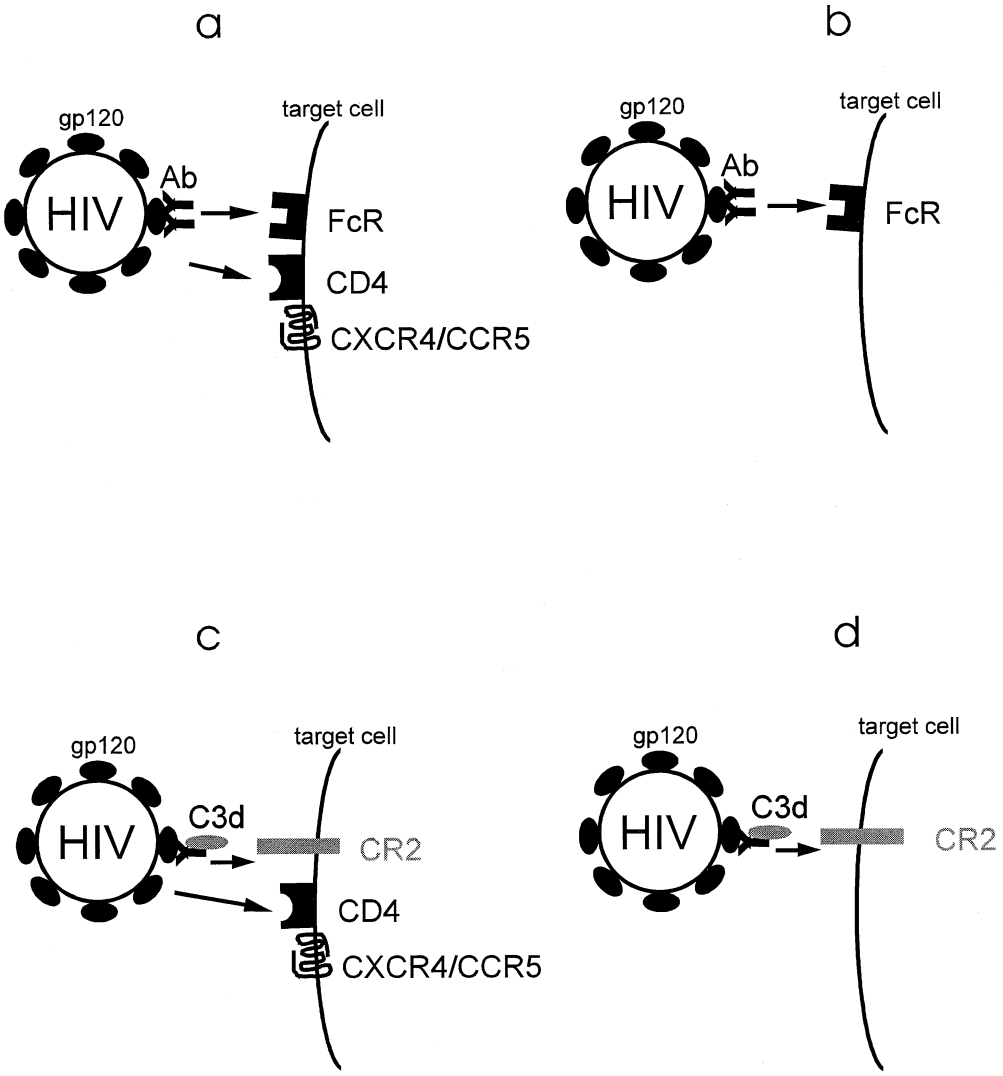

Fig. 1. Four possible forms of the antibody-dependent enhancement of HIV-1 infection (ADE). (*a*) FcR- and CD4-dependent ADE. Out of FcRs, FcγRIII seems to be the most important. Probably the co-receptors (chemokine receptors), like CCR5 for the macrophage-tropic or CXCR4 for the T-cell adapted HIV-1 strains are also involved in the HIV-entry. (*b*) ADE dependent only on FcR. (*c*) Complement-mediated, CD4-dependent ADE: virus-antibody complexes activate complement, complement activation products, like, C4b, C3b, C3bi, C3dg covalently bind to the complexes. Out of complement receptors, CR2 seems to be the most important. Interaction between CR2 and its ligand, C3dg facilitates ADE. Probably the co-receptors (chemokine receptors), like CCR5 for the macrophage-tropic or CXCR4 for the T-cell adapted HIV-1 strains are also involved in the HIV entry. (*d*) Complement-mediated, non-CD4-dependent ADE. Existence of this form of ADE is doubtful.

present in normal human serum. It cannot be excluded that similar mechanisms are responsible for the complement-mediated non-antibody dependent HIV-1 infection enhancement, too.

MAPPING OF EPITOPES ON HIV ENVELOPE PROTEINS WHICH BIND ENHANCING ANTIBODIES

According to the studies of Robinson and his colleagues, the most important epitope responsible for the development of C-ADE-mediating antibodies seems to be the immunodominant epitope (amino acids 586 to 620) on gp41. Three of 16 human monoclonal antibodies (mAbs) were found to enhance HIV-1 infection of the MT-2 target cells. Using analyses by radioimmunoprecipitation, Western blot and ELISA, it was demonstrated that antigen specificity of all the 3 mAbs can be localized to the *N*-terminal end of gp41 containing the so-called immunodominant domain of the protein (Robinson *et al.* 1990*a*, 1990*b*, 1991). This epitope on gp41 is recognized by sera from essentially all HIV-infected subjects. Similar results were obtained with 2 other mAbs. The same group characterized further the antigen specificity of C-ADE-mediating antibodies. They found 4 different anti-gp41 mAbs to mediate C-ADE. These mAbs were mapped to 2 distinct domains on the gp41 by using synthetic

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peptides. The first domain (amino acids 579 to 613) was recognized by 3/4 of the mAbs, while the 4th one bound to a 2nd gp41 domain (amino acids 644 to 663). C-ADE of MT-2 cells mediated by sera from HIV patients was blocked only by the mAbs to the first domain. We have found 3 mAbs against this domain to mediate C-ADE of another CR2-carrying cell line, MT-4 (Füst *et al*. 1994*b*). Interestingly enough one of these mAbs, 181-D, was previously found to be non-enhancing by Robinson *et al*. (1991).

The 2 domains on gp41 that can bind enhancing antibodies are conserved between HIV-1 isolates as well as between HIV-2 and SIV isolates. These data suggest that 2 conserved regions within the HIV-1 gp41 are most important in the development of C-ADE-mediating antibodies during HIV infection. In addition to anti-gp41 antibodies, however, antibodies against the V3 loop of gp120 can also mediate C-ADE at least according to the experiments of Jiang, Lin & Neurath (1991) performed with heterologous (rabbit) antisera.

In contrast to the findings on C-ADE, it seems that the antibodies mediating FcR-ADE react mainly with the gp120, although Eaton *et al*. (1994) described an anti-gp41 human monoclonal antibody which enhanced HIV-1 infection the absence of complement. Murine mAbs directed against a domain (amino acids 724 to 752) on gp41 were also found to enhance HIV-1 infection *in vitro*. No antibodies reactive with this region were, however, found in the sera of 100 HIV-infected individuals, indicating that this domain is not immunogenic in humans (Niedrig *et al*. 1992). One human anti-gp120 mAb which was able markedly to enhance FcR mediated infection on monocytic cells was found to bind to a conformational site of non-variable sequences in the C-terminal part (amino acids 272 to 509) of gp120 (Takeda *et al*. 1992). Interestingly, deletions in both the N and C terminus of gp120 significantly decreased the binding of this antibody to the gp120 (Lee *et al*. 1994). Kliks *et al*. (1993) studied the neutralizing *vs*. FcR-ADE-mediating effect of different human mAbs against the V3 loop of gp120. These mAbs show different effects on 3 distinct HIV-1 strains: neutralization, enhancement or no effect. Only one amino acid in the mAb-binding epitopes proximal to the crown of V3 loop was different among these 3 strains. According to the recent study of Trischmann *et al*. (1995), the extent of enhancing effect markedly depends on the antigen structure of the virus strain: while rat antisera against the 5 variable regions (V1–V5) of gp120 and conserved parts of gp120 and gp41 facilitated infection of primary human macrophages with the homologous virus HIV-SF2mc, infection of the same cell with a heterologous strain, HTLVIIIB, was enhanced only by antisera to V4 and V5. Recently Auewarakul *et al*. (1996) also found FcR-ADE activity in individuals infected with subtype B and E viruses in Thailand to be mostly isolate-specific and independent of genetic subtypes.

POSSIBLE MECHANISMS OF *IN VITRO* EFFECT OF ENHANCING ANTIBODIES

According to Lund *et al*. (1995) there are at least three fundamentally different hypotheses for the explanation of ADE *in vitro*: (*a*) increased adhesion of HIV-antibody-(complement) complexes to FcR or CR carrying cells; (*b*) facilitation of HIV-target cell fusion by complement fragment deposited on the HIV-virions and (*c*) complement activation products may have non-specific stimulatory effect on target cells resulting in enhanced virus production. Recent experiments of Mouhoub *et al*. (1996) demonstrated that ligation of complement receptors type 1 (CR1) on $CD4^+$ T lymphocytes enhances viral replication in HIV-infected cells. This observation lends credence to the third hypothesis. The most probable explanation for the mechanism of the FcR-ADE and C-ADE, however, is the increase of adhesion of virus particles to the target cells. This may facilitate entry of the virus particle via the usual route. Alternatively, binding can be followed by a receptor-mediated endocytosis of the FcR or CR-bound HIV which may lead to the fusion of viral envelope with the membrane of an endosomal vesicle then allowing the insertion of viral core to the cytoplasm before it is degraded in a lysosome (Kozlowski *et al*. 1995).

The increased adhesion is mediated by the interaction to the virus-bound antibody and FcR, and the interaction of the virus or virus-antibody complex-bound complement fragment to the CR. It seems that although all 3 types of FcγR can mediate FcR-ADE, the FcγRIII (CD16) is the most important in the FcR-ADE of primary human macrophages (Trischmann *et al*. 1995) and U937 cells (Laurence *et al*. 1990). According to the studies of Zoellner, Feucht & Laufs (1992), proteases produced by macrophages may significantly increase FcR-ADE.

As for C-ADE, June *et al*. demonstrated first in 1991 that it is mediated by an increased virus binding to the MT-2 target cells. This increased adhesion strongly correlated to higher level of provirus formation 8–28 h after infection. Bakker *et al*. (1992) found that antibody and complement enhance binding and uptake of HIV-1 by human monocytes.

In recent experiments (Prohászka *et al*. 1997), using 3 different anti-gp41 mAbs, we have demonstrated the existence of an alternative route of the C-ADE. Addition of purified C1q to mixtures of mAbs and $HTLV_{IIIB}$ used for infection of MT-4 target cells significantly increased HIV production of the infected cells as compared to the cell cultures

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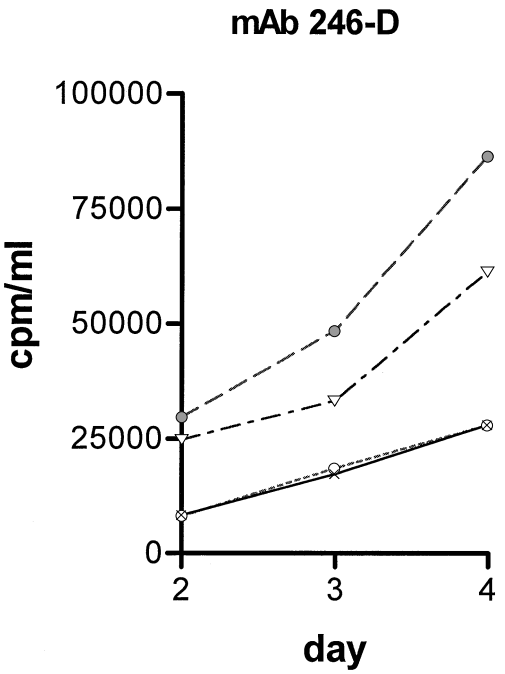

Fig. 2. C-ADE with purified C1q. Human monoclonal anti-gp41 antibody 246-D (alone: ○) was mixed with normal human serum (●), purified C1q (▽) or with buffer (×) and thereafter with a stock of HIV-1_{IIIB}. MT-4 cells were infected with the mixtures and virus production was measured on days 2, 3, and 4 by the reverse transcriptase assay. For methodical details, see text. (Adapted from Prohászka *et al.* 1997.)

infected with the mAb-virus mixtures (Fig. 2). We assume that this enhancement is due to an additional bridge between the virus particles and target cells though interaction of C1q bound to the virus-antibody complexes and the C1q (collectin) receptors on the target cells. Experiments studying this hypothesis are in progress in our laboratories.

SIGNIFICANCE OF THE ADE *IN VIVO*

Data on the presence of ADE-mediating antibodies in vivo

Measurement of FcR-ADE and C-ADE in the serum and other bodily fluids of humans and experimental animals. The extent of enhancement and the ratio of neutralization/enhancement measured in a given sample depend in several factors specific for the assay system employed. Variables such as target cells, the density of FcRs or CRs on the target cells, serum dilutions tested and the strains of HIV-1 used can markedly influence the final outcome of the measurement. Usually each laboratory applies a different method for the measurement of the titre of enhancing antibodies, and to my best knowledge no efforts have been made for standardization of the procedures. For the measurement of FcR-ADE, primary human monocytes/macrophages or the U937 cell line are used while lymphoblastoid cell lines MT-2 or MT-4 are mostly applied for the measurement of C-ADE. Although in some studies primary isolates were used, laboratory strains – most frequently HTLVIIIB – are applied in the clinical studies.

ADE in the blood of HIV patients and infected experimental animals. Using sensitive methods C-ADE were detected from 95% of HIV-infected individuals by Robinson *et al.* (1989*a*) but they found that the titres of such antibodies varied greatly between patients. FcR-ADE was found in different frequencies in the sera of HIV patients: while Takeda *et al.* (1988) found FcR-ADE in almost all serum samples tested, Laurence *et al.* (1990) and Homsy, Meyer & Levy (1990) found FcR-ADE-mediating antibodies in only 5/16 and 7/16 patients, respectively. ADE could be detected also in sera of HIV-infected chimpanzees and SIV-infected rhesus macaques (Robinson *et al.* 1989*b*, Montefiori *et al.* 1990).

Cross-sectional and longitudinal studies of FcR-ADE and C-ADE-mediating and related antibodies in HIV patients. Results concerning the clinical relevance of the enhancing antibodies are contradictory. Homsy *et al.* (1990) were the first to demonstrate that the appearance of the Fc-receptor-dependent enhancing type antibodies are associated with the progression of HIV disease. FcR-ADE with homotypic isolates was primarily observed in AIDS patients and in longitudinal studies clinical progression was found to be associated with the appearance of FcR-ADE-mediating antibodies.

Our group demonstrated first a similar association between C-ADE and the clinical progression of HIV-disease. In our first study (Tóth *et al.* 1991) we found C-ADE only in 4/20 (20%) of asymptomatic HIV-patients but in 12/19 (63%) of patients in the symptomatic (ARC or AIDS) stage of the disease. In a more recent study (Füst *et al.* 1994*a*) these findings were confirmed. At longitudinal measurements a strong negative correlation ($R = -0{\cdot}458$, $P = 0{\cdot}00044$) was found between the extent of C-ADE and the $CD4^+$ cell counts. Moreover, high titre C-ADE predicted rapid decline of the $CD4^+$ cell counts and an increased probability of the AIDS development. In a 5-year follow up period AIDS developed in 33 and 83% of patients with missing or low titre C-ADE and in those with high titre C-ADE, respectively.

Very recently (F. D. Tóth *et al.* unpublished) similar results were obtained at a relatively short-term longitudinal study performed in 125 serum and plasma samples from 25 HIV patients untreated or treated according to different antiviral protocols.

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The most important results of this study can be summarized as follows. (1) Addition of complement abrogated neutralization in a part of the serum samples tested. (2) High extent of C-ADE predicted immunological progression and, to a lesser extent, clinical progression in the patients tested. (3) A significant positive correlation was found between the levels of C-ADE and the amounts of plasma HIV-1 RNA in samples obtained from the patients at the same time. These findings, which confirm and extend our previous results, indicate that C-ADE measured in serum samples from HIV-1-infected individuals, correlate with immunosuppression and disease not only in the early and intermediate but in advanced-stage patients as well. Besides these observations, some indirect evidence seems to support the proposition that C-ADE-mediating antibodies are associated with immunosuppression and disease in HIV infection. In a collaborative study (Tóth *et al*. 1994) we have found a strong positive correlation ($R = 0{\cdot}408$, $P = 0{\cdot}003$) between C-ADE and the IgA type anti-Fab antibodies measured in the same serum samples. Anti-Fab autoantibodies are known to be strongly associated with the progression of HIV disease (Süsal, Daniel & Opelz, 1996). It is most probable (see above) that C-ADE mediating antibodies are mainly directed against the immunodominant epitope of gp41. In this context, it is interesting that Monell *et al*. (1993) found the antibody response of HIV-infected individuals for the immunodominant (amino acids 598 to 609) sequence to continue to increase over time in HIV patients. Similarly, Radkowski *et al*. (1993) and Thomas *et al*. (1996) observed increased avidity of the anti-gp41 antibodies in the late symptomatic stage of HIV disease. According to the studies of Zwart *et al*. (1994) a high level of antibodies against the gp41 immunodominant peptide correlated with rapid progression of disease. Lallemant *et al*. (1994) also noted a correlation between higher levels of antibodies to this peptide and increased risk of maternal–fetal transmission.

In contrast to these findings, Montefiori *et al*. (1991) did not find any correlation between the clinical course of the HIV disease and the C-ADE. More recently they were unable to detect any difference in the amounts of C-ADE-mediating antibodies between long-term non-progressors and progressor patients (Montefiori *et al*. 1996). Two main methodological differences exist between the procedures used for the measurement of C-ADE between Montefiori's group and ours. While we use a near-physiological (25%) NHS concentration as complement source, the other group always applied a strongly (1:20–1:27) diluted NHS. It seems reasonable to suppose that the closer the complement concentration is in the mixtures used for infecting the MT-4 cells to that in the blood, the higher the physiological relevance of the measurement. The mathematical model of Lund *et al*. (1995) also suggests that the enhancing effect of complement is dose-dependent. On the other hand, when we compared the main titres in HIV-seropositive sera reported by Montefiori *et al*. (1995) and our group (Füst *et al*. 1994*a*) the assay of the other group seems to be about 10 times more sensitive, which might decrease the discriminative power of the measurement.

We are aware of the fact, however, that the test system we applied for the measurement of neutralization/enhancement is far from the physiological conditions. The target cell, MT-4, like MT-2 used in other studies, an HTLV-1 infected cell-line and $HIV1_{IIIB}$ is a laboratory strain passaged many times through cell lines. In spite of these draw-backs, the measurement seems to have clinical relevance. This apparently paradoxical observation can be best explained by assuming that complement-dependent enhancement is due to broadly reacting antibodies which can bind and activate complement not only with $HIV\text{-}I_{IIIB}$ but most HIV-1 strains present in the body of infected persons (Berman, Eastman & Wilkes, 1994; Nkengasong *et al*. 1994).

Possible role of ADE in the maternal–infant HIV-1 transmission. As mentioned previously, Kliks *et al*. (1993) demonstrated that anti-V3 loop mAbs that neutralize one HIV strain enhance infection of another and have no effect on a third strain. In a group of North American mothers they have observed that transmission mainly occurs to infants infected with viruses that are either resistant to neutralization by the mother's serum or enhanced by this serum sample (Kliks & Levy, 1994). Similar findings were reported by Lallemant *et al*. (1994) and Markham *et al*. (1994). Both groups observed that mothers who transmitted infection to their offspring had a significantly higher mean concentration of IgG1 antibodies to the V3 loop of the gp120 than the non-transmitters. In the studies of Markham *et al*. (1994), the geometric mean of IgG was 16·8, and 9·4 μg/ml in the 23 transmitter and 103 non-transmitter Haitian mothers, respectively. Moreover, the experiments of Lallemant *et al*. (1994) demonstrated that higher antibody titres to the immunodominant domain of gp41 were correlated to a higher risk of perinatal transmission. Finally, Tóth *et al*. (1994*b*) demonstrated that antibodies mediating both FcR-ADE and C-ADE are able to increase the HIV infection of human syncytiotrophoblast cells. This could be one of the mechanisms of the facilitating effect of ADE on the spread of HIV-1 from mother to the fetus.

Data from an animal model. According to the studies of Montefiori *et al*. (1990) SIV infection enhancing

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Table 1. Possible mechanisms of the antibody-dependent enhancement of HIV-1 infection *in vivo*

1. Abrogation of the effect of neutralizing antibodies
2. Enhancement of the HIV production by different cells *in vivo*
   - monocytes/macrophages (mainly FcR-ADE)
   - $CR2^+$ $CD4^+$ T lymphocytes (mainly C-ADE)
   - short-living $CD4^+$ lymphocytes responsible for $> 99\%$ of HIV production in the body of infected persons (see hypothesis in Fig. 3)
3. Effect of the trapping of virus-(antibody)-complement complexes on FDC in germinal centres of lymph nodes

complement-dependent antibodies appeared in rhesus macaques soon (28 days) after experimental inoculation of SIV. C-ADE titre increased over time and peaked just prior to the death of macaques from opportunistic infections or lymphoma. According to the experiments of Gardner *et al*. (1994, 1995), passive immunization of rhesus macaques with pooled plasma or purified immunoglobulin from healthy SIVmac251-infected animals prior to or after challenge with the same virus did not confer protection or even enhanced infection and facilitated the development of disease, although plasma and immunoglobulin preparations contained a high level of SIV-binding and neutralizing antibodies. Another finding that seems to be important was reported by Montefiori *et al*. (1995). These authors found that C-ADE activity in sera during acute primary infection in macaques inoculated with the SIVmac251 strain appeared before neutralizing antibodies and coincided with the initial peak and decline of plasma antigenaemia. As the neutralizing antibodies developed and their titre increased in the infected animals, the extent of C-ADE activity gradually decreased.

Possible mechanisms of ADE in vivo

In principle, enhancing antibodies may exert their effect through different routes (Table 1).

Abrogation of the effect of neutralizing antibodies. Sera of HIV-infected subjects may contain different types of antibodies in parallel. When several human monoclonal antibodies derived from lymphocytes of HIV patients were studied, 10/20 mAbs were found to mediate exclusively neutralization, 5 exclusively mediated antibody-dependent cellular cytotoxicity (ADCC) while 2 mAbs had both ADCC-mediating and C-ADE effect (Forthal *et al*. 1995). Kostrikis *et al*. (1996), using quantitative analysis of serum neutralization of HIV-1 from different subtypes, found enhancement in more than one-quarter (28 %) of the 1213 combinations of sera and HIV-1 isolates. The extent of neutralization and enhancement was comparable. Therefore, it is conceivable that during natural HIV-1 infection the balance of the 'good', that is ADCC-mediating or neutralizing, and the 'bad', that is FcR-ADE- or C-ADE-mediating-antibodies, determines the effect of antibodies on *in vitro* HIV production. In addition, complement may have a dual effect on the fate of HIV virions. According to the studies of Spear *et al*. (1990), HIV-1 can be neutralized by viral lysis with subneutralizing doses of antibody from HIV-infected persons in the presence of complement. This finding indicates that complement may stimulate neutralizing capacity of specific antibodies. Recently Sullivan *et al*. (1996) proved the role of complement-mediated lysis of the antibody-bound HIV-1 plasma virions in the clearance of virus *in vivo*. On the other hand, according to the early study of Robinson *et al*. (1988), addition of complement of some sera may decrease or fully abrogate the neutralizing effect of the human antibodies or even may induce neutralizing antibodies to mediate C-ADE. Similar results were obtained with rabbit antibodies against the V3 loop of gp120 by Jiang *et al*. (1991): antisera to V3 of 21 distinct HIV-1 isolates were tested for their neutralizing and enhancing effect in the presence or absence of human complement. In the absence of complement, each antibody was virus-neutralizing whereas addition of complement enhanced virus infection by 10 strains. In our most recent study (F. D. Tóth *et al*. unpublished), we also found that addition of complement to heat-treated serum samples of some HIV-infected patients did not affect neutralization while in the case of other serum samples neutralizing sera became strongly enhancing. These observation, taken together, also suggest that a mixture of various antibodies is present in the sera of HIV-infected individuals. Sera of some subjects contain, while those of others do not contain C-ADE-mediating antibodies. Therefore when neutralization is measured in the presence of complement and by using a CR-carrying target cell, antibodies in the serum samples tested may neutralize, enhance or do not affect *in vitro* virus production. A similar situation may exist *in vivo* (see above).

Another factor which may result in ADE could be the emergence of strains in the HIV-infected patients in which growth is enhanced *in vitro* and possibly *in vivo* by the antibodies which effectively neutralize T-cell adapted laboratory isolates. For example, in the recent studies of Sullivan *et al*. (1995), an antibody reacting with the CD4-binding site of gp120 was found markedly to enhance infection of PBMC by two primary HIV-1 isolates.

Enhancement of HIV-1 production by different cells in vivo. According to *in vitro* studies, HIV production by cells of the monocyte–macrophage lineage may be

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increased by FcR-ADE *in vivo*. These cells carry FcγRIII and therefore complexes of HIV and FcR-ADE-mediating antibodies may attach to these cells and increase HIV production by them. Although Schadduck *et al*. (1991) failed to detect enhancement of HIV-1 infection of human blood monocytes and peritoneal macrophages, most papers published both before (reviewed in Mascola *et al*. (1993)) and after publication of this paper (Trischmann *et al*. 1995; Kostrikis *et al*. 1996) demonstrated the ability of the sera of HIV patients to mediate FcR-ADE. As for the C-ADE, most *in vitro* studies have demonstrated the ability of this type of ADE to enhance HIV-1 production of B lymphocytes and lymphoblastoid T cell lines. B cells are usually not infected in the patients, therefore the increase of their infection may not be pathologically important. Recently it was demonstrated (Fischer, Delibrias & Kazatchkine, 1991; June *et al*. 1992) that a large proportion, at least 30 %, of the peripheral T cells both $CD4^+$ and $CD8^+$ subsets carry CR2, the receptor which is used for the C-ADE of the T lymphoblastoid cell lines. According to June *et al*. (1992) the percentage of $CD4^+$ cells carrying CR2 is significantly lower in the blood of both asymptomatic and symptomatic HIV patients compared with the uninfected control donors. This finding indicates that the $CD4^+CR2^+$ cells are eliminated early from the blood of HIV-infected individuals. Therefore it is probable that if C-ADE has a biological significance *in vivo*, its effect is exerted at least partly by the increase of HIV-1 production and facilitation of destruction of $CD4^+$ T cells.

Very recent findings of Sullivan *et al*. (1996) demonstrated that the effect of antibodies and complement on HIV-1 replication *in vivo* could be more complex than was claimed so far. The authors isolated HIV-1 virions from the plasma of HIV-infected individuals and demonstrated that exogenously added complement caused lysis of a fraction of the virions whereas another fraction of the virions was opsonized by complement without being lysed. They also proved that isolated virions already had bound specific antibodies *in vivo*, complement activation leading to lysis and opsonization was initiated by the virus–antibody complexes via the classical pathway. It is conceivable that lysis observed by the authors *in vitro* occurs *in vivo* as well and has a significant role in the rapid clearance of the virus (according to Perelson *et al*. (1996), the half-life of the virus is 6 h). In principle, ADE-mediating antibodies may exert their effect to enhance virus production *in vivo* in 2 different ways (Fig. 3). (*a*) They can inhibit complement-mediated lysis of plasma viruses induced by non-ADE mediating antibodies. (*b*) The non-lysed virus particles that are coated by antibody and complement fragments can be eliminated and killed by the phagocytic cells. On the other hand, it cannot be excluded that a part of the antibody on the non-lysed particles can mediate FcR-ADE or C-ADE. If it is really the case, ADE triggered by these antibodies may result in an increased rate of infection of the short-living $CD4^+$ cells which produce more than 99 % of the virus load in the body of the HIV-infected individuals (Perelson *et al*. 1996). Viral load measured by the concentration of HIV-1 RNA in plasma is the most important predictive marker of HIV disease. The higher the plasma HIV-RNA concentration the quicker progression can be expected (Mellors *et al*. 1996; O'Brien *et al*. 1996).

If our hypothesis, summarized in Fig. 3, is correct, a correlation should exist between the HIV-RNA concentration and the amount of ADE-mediating antibodies. To the best of our knowledge, no data on a parallel measurement of viral load and FcR-ADE or C-ADE have been reported until now. Findings of Montefiori *et al*. (1995) on the very high C-ADE activity in acute primary infection of SIV-inoculated macaques and the gradual but finally highly significant decrease of C-ADE in parallel to the drop of p24 antigenaemia at the switch from the acute primary infection to the asymptomatic phase seems to support our assumption. Similarly, it is well known that viral load increases during the symptomatic stage of HIV disease as compared to the asymptomatic stage. Our findings on the more frequent occurrence of C-ADE in the ARC/AIDS patients as compared to the asymptomatic ones (Tóth *et al*. 1991), and on a gradual increase in the C-ADE titres with the progression of HIV disease (Füst *et al*. 1994*a*; Tóth *et al*. unpublished) also seem to support the hypothesis summarized in Fig. 3. This hypothesis can be experimentally tested as soon as the HIV-propagating short-lived $CD4^+$ cell population is better characterized.

Effect on the trapping of virus-antibody-complement complexes on follicular dendritic cells (*FDC*) *in germinal centres of lymph nodes*. Lymphoid germinal centres are most important reservoirs of human immunodeficiency virus type 1 particles (Tenner-Racz *et al*. 1988; Fox *et al*. 1991). Trapping of HIV-1 on the surface of the cells may be one of the main protective mechanisms against increase of the virus load. On the other hand, CD4 cells can be infected by HIV-1 present on the FDC. According to the *in vitro* studies of Jolling *et al*. (1993), binding of human immunodeficiency virus type 1 particles to FDC is mediated by complement. It is tempting to speculate that *in vivo* the enhancing antibodies may influence the bonds between virus-complement complexes and FDC during the early phase of lymph node infection, and contribute by this way the sharp increase of viral load during the symptomatic stages of HIV disease.

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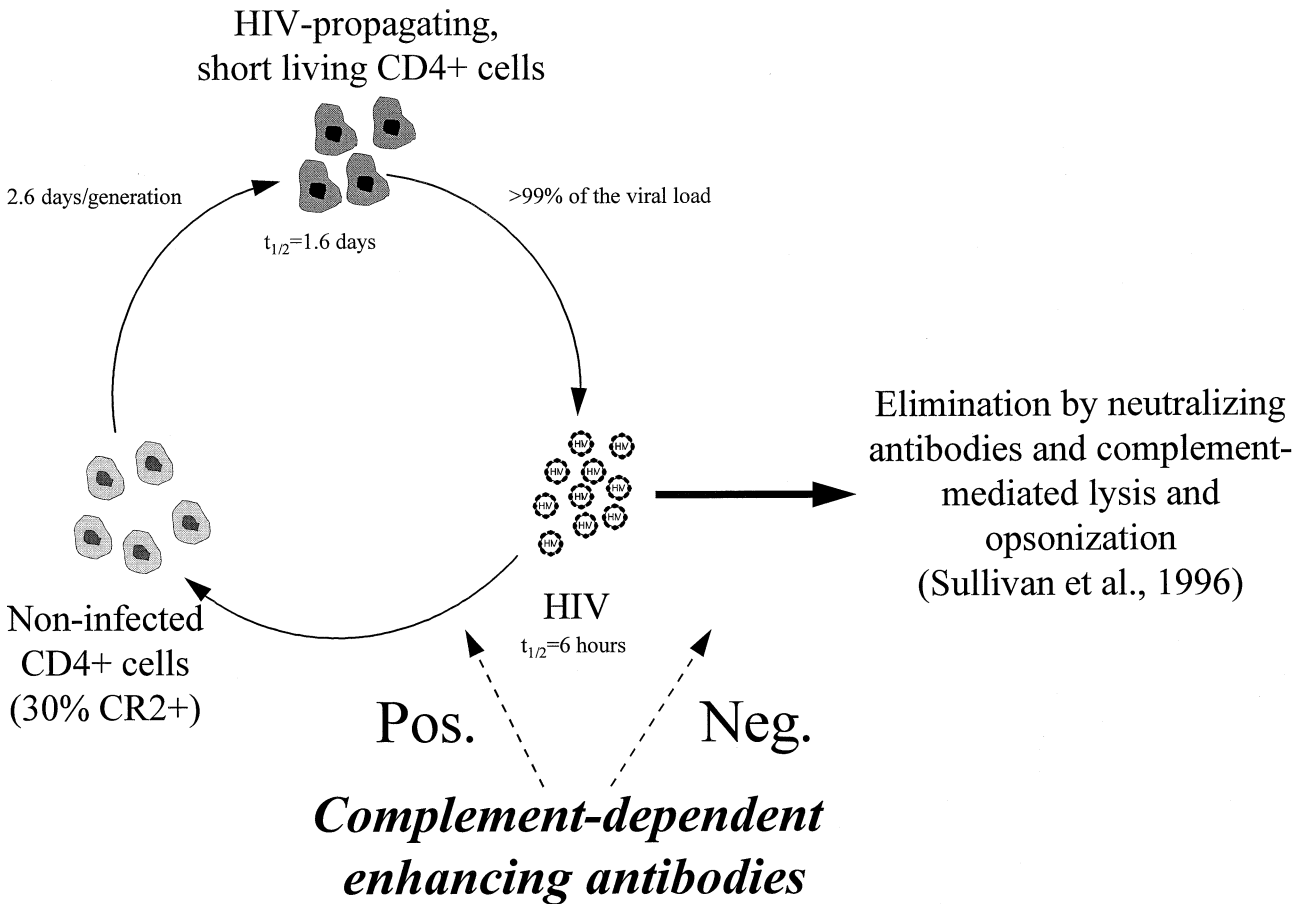

Fig. 3. A new hypothesis on the mechanism of the effect of complement-mediated enhancing antibodies on the facilitation of HIV propagation and consequently progression of HIV disease. According to Sullivan *et al.* (1996), antibody-dependent, complement-mediated lysis and opsonization have a major role in the rapid elimination of plasma virions. Enhancing antibodies may down-regulate elimination by inhibiting lysis of plasma virions or by facilitating the HIV-1 infection of not infected $CD4^+$ cells and their entry in the rapid-turnover cycle which is responsible for the production of more than 99% of HIV-1 virions in the body (Perelson *et al.* 1996). Since high levels of plasma HIV-1 predicts a quick progression of HIV-disease (Mellors *et al.* 1996; O'Brien *et al.* 1996) any factor interfering with the elimination of plasma virions may contribute to the progression of HIV disease.

POTENTIAL RISKS OF ADE IN HUMAN HIV VACCINE TRIALS

A report on a workshop of this topic, held in December, 1992 was given by Mascola *et al.* (1993). Based on early data from SIV model (Montefiori *et al.* 1990) and volunteers vaccinated with candidate subunit HIV vaccines (Bernard *et al.* 1990; Dolin *et al.* 1991; Haubrich *et al.* 1992), authors of the report concluded that although ADE can be detected in a part of vaccinated people, no evidence is available which indicates that ADE occurs in these subjects when they are infected with HIV-1. They stressed that determination of *in vivo* correlates of protection/enhancement as well as development of an HIV animal model is necessary to evaluate the ADE risk accurately. Unfortunately, controversial results were obtained in recent studies performed in SIV infected macaques, too. Montefiori *et al.* (1995) failed to find any correlation between the extent of C-ADE measured on the day of challenge with wild virus and the protection by vaccination in macaques immunized with live attenuated SIVmac239/nef deletion strain or primed with recombinant SIVmne gp120 vaccinia virus and boosted with SIVmne rgp160. Some protected animals had a C-ADE activity similar to that measured in unprotected macaques. According to the authors, however, 'it remains to be determined whether C-ADE-mediating antibodies interfere with vaccine efficacy after heterologous virus challenge or when animals are challenged with homologous or heterologous virus after vaccine-induced immune responses are waned'. In another study (Mitchell *et al.* 1995), it was found that the beneficial effect of a recombinant SIV gp160 vaccine was markedly diminished when the animals were boosted with a synthetic peptide corresponding to the immunodominant gp41 epitope of SIV gp41 (amino acids 603–622) and challenged finally with SIVmac251. Animals immunized with this protocol had detectable p27 antigen longer and died of simian AIDS before the animals of the control groups immunized with the gp160 vaccine but not boosted at all or boosted with an irrelevant peptide. En-

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hancement of infection in animals vaccinated with recombinant subunit vaccines after challenge with the homologous virus was observed in two other animal retrovirus models: in feline immunodeficiency virus (FIV) infection (Hosie *et al*. 1992; Siebelink *et al*. 1995) and in equine infectious anaemia virus (EIAV) infection (Wang *et al*. 1994).

In a human vaccine trial performed with a gp160 candidate vaccine (VaxSyn) Keefer *et al*. (1994) reported on the detection of C-ADE in 11/19 subjects immunized with the higher dose of 640 μg.

CONCLUSIONS

ADE was first described 10 years ago. Since that time several important details of the 2 types (FcR-dependent and complement-mediated) ADE and mechanisms of *in vitro* effects of the ADE-mediating antibodies were revealed. FcR-ADE and C-ADE were detected in HIV-infected patients and in experimental animals infected with HIV and SIV, several findings seem to demonstrate the role of ADE in the progression of HIV disease; these observations, however, need further support. Standardization of the methods used for the measurement of FcR-ADE and C-ADE is crucial for comparative data from different labs. Development of C-ADE was observed in macaques immunized with SIV vaccines and in phase I/II trials of human subunit gp160 vaccines. Nevertheless, no studies on the *in vivo* relevance of ADE in vaccinated people have been published. It would be most interesting to study if 'breakthrough' infections which sometimes occur in volunteers immunized with the currently used candidate subunit vaccines were associated with the development of ADE-mediating antibodies when HIV-1 infection occurred. Independently of the result of these studies, however, efforts to eliminate the epitopes which are responsible for induction of ADE-mediating antibodies (Lee *et al*. 1994) such as epitopes corresponding to the immunodominant epitopes of gp41 or the V3 loop of gp120 in the development of new vaccine candidates seems to be reasonable.

ACKNOWLEDGEMENT

I am highly indebted to Dr Zoltán Prohászka for his kind help in preparing the Figures.

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https://doi.org/10.1017/S0031182097001819 Published online by Cambridge University Press