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Traits of the immune system—Enhancing antibodies in HIV infection: Their possible implication in HIV vaccine development

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Summary Considering recent HIV vaccine failures, the authors believe that it would be most important to find new targets for vaccine-induced immunity, and to analyze the data from previous trials, using an innovative approach. In their review article, the authors briefly summarize the significance of the antibody-dependent enhancement of infection in different viral diseases and discuss role of these types of antibodies as the obstacles for vaccine development. Findings which indicate that complement-mediated antibody-dependent enhancement (C-ADE) is present also in HIV-infected patients, are summarized. Previous results of the authors, suggesting that C-ADE plays a very important role in the progression of HIV infection are described. Data reflecting that enhancing antibodies may develop even in vaccinated animals and human volunteers, and may be responsible for the paradoxical results obtained in some subgroups of vaccinees are discussed. Finally, based on their hypothesis, the authors offer some suggestions for the future development of vaccines.

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Introduction

The end of 2007 is a difficult period for HIV/AIDS vaccine development. Both phase III trials of gp120 vaccines [1,2] aiming to raise sterilizing, antibody-mediated immunity, failed in 2003. There was no difference in the frequency of the breakthrough infection between the vaccine and placebo arms. A further trial of an other type of vaccine (V520 of Merck), using a different strategy, was prematurely stopped in September 2007 [3]. The Merck vaccine relies exclusively on the stimulation of cellular immune response. Although, it is well known that antibodies are important to prevent infections and eventually all successful vaccines raise antibody-mediated protection, T-cell vaccines are hoped to control the level of HIV in the body of vaccinees for a long time and thereby prevent progression to
AIDS [4]. Unfortunately, this was not the case in any of the trials finished until now. Not only the vaccines did not provide any protection against HIV-1 infection, but also there was no difference between the vaccine and placebo arms as regards the progression of HIV disease in breakthrough infection [3,5,6].

In our belief, it is a time to draw lessons from these failures, to find new targets for vaccine-induced immunity, and to analyze the data from the trials performed so far, using an innovative approach.

Many experimental and clinical data, as well as an experience obtained in other viral infections indicate that a proportion of the antibodies — which develop early and persist throughout the course of HIV infection — are "traitors" of the immune system. That is, instead of containing or eliminating the virus, these antibodies facilitate its production in the body of infected persons. These enhancing antibodies counteract the effect of the neutralizing antibodies and the relative powers of the "good guys" versus "bad guys", that is, the balance between enhancing and neutralizing antibodies, are decisive for the progression of HIV disease. Further, we assume that all types of HIV vaccines may raise enhancing antibodies as well, and these antibodies can reduce the efficacy of the vaccines. At least in HIV infection, enhancing antibodies mostly exert their effect through activation of the complement system. Therefore, the balance of enhancing/neutralizing antibodies can be correctly measured only in the presence of fresh HIV seronegative human serum containing human complement.

The role of antibody-dependent enhancement in viral infection and disease

In general, virus-specific antibodies play an important role in the control of viral infections in several ways. In certain instances, however, the presence of specific antibodies can be beneficial to the virus. This paradoxical process is known as the antibody-dependent enhancement (ADE) of viral infection. In ADE, virus-specific antibodies enhance the entry of the virus into, and in some cases, its replication within T cells, monocytes/macrophages and granulocytic cells, through interactions with Fc and/or complement receptors. A significant, 4 log-fold or even greater increase in the in vitro production of several viruses was observed following exposure to low-affinity antibodies [7]. ADE of infection has been described for numerous viruses belonging to different families and orders as reviewed [8,9]. Common properties of these viruses include (a) replication (partially or exclusively) in macrophages; (b) induction of the abundant production of antibodies that poorly neutralize even the homologous virus; and (c) they cause persistent infection commonly characterized by prolonged viremia.

Antigenic diversity of the isolates is another common feature, which renders these viruses partially resistant to neutralization by antibodies raised against heterologous isolates. ADE of virus infection has been suggested as a disease-enhancing factor in several human and animal viral diseases [9].

Most data on the pathological significance of ADE were accumulated in dengue virus (DV) infection. In the majority of cases, DV infection is usually manifested as a mild febrile disease, referred to as "dengue fever"; afflicted patients recover in 7–10 days without complications. Less frequently, however, it progresses to a severe and life-threatening syndrome, the dengue hemorrhagic fever (DHF), which is always accompanied by thrombocytopenia, sometimes by frank hemorrhage, and leaking of plasma into interstitial spaces, which result in hypovolemia and, sometimes, circulatory collapse. The most severe clinical form of DHF, in which profound plasma leakage leads to shock, is referred to as the dengue shock syndrome (DSS).

The association between ADE of infection and the severity of disease has been extensively studied. Some evidence indicates a strong correlation. It was demonstrated experimentally, in Rhesus monkeys, that anti-DV maternal antibodies enhanced DV infection [10]. In the clinical setting, a greater risk to develop DSS was demonstrated in children with specific maternal antibodies or in patients who had previously been infected with either of the four DV serotypes and were subsequently exposed to the DV-2 strain of the virus [11]. Accordingly, it was found that DV produced more severe clinical manifestations in older individuals with subneutralizing levels of antibodies (induced by previous DV infections), than in individuals who had never been exposed to the virus [12]. Additionally, case-control studies demonstrated that the presence of DV antibodies is a significant risk factor for increased severity of disease and mortality in DHF/DSS [13,14].

Several important, prospective sero-epidemiological studies conducted in areas endemic for DHF/DSS revealed immunopathological events leading to development of this disease [15]. Following reinfection with a dengue virus of a different serotype, severe disease is associated with high levels of antibody-enhanced viral replication early in the course of the disease, followed by a cascade of memory T-cell activation, and a 'storm' of inflammatory cytokines and other chemical mediators. These compounds, mainly released from T cells, monocytes/macrophages and endothelial cells, eventually increase vascular permeability.

As for the mechanism of ADE, evidence from in vitro and in vivo studies strongly suggests that viral replication in Fc-receptor bearing cells, especially monocytes and macrophages, is enhanced by pre-existing, subneutralizing, and non-protective concentration of dengue IgG antibodies [16]. Enhanced replication of DV in these target cells leading to high levels of viremia at the early stage of illness is correlated with DHF/DSS incidence [17,18]. In addition of IgG, IgM type antibodies and enhancement of DV infection via complement receptors were also implicated [19].

Besides dengue virus infection, ADE was found to affect the course of other life-threatening infectious disorders in humans. The severity of respiratory syncitial virus (RSV) infections in young children was found to be associated with the presence of maternal antibodies [20]. These observations are supported also by animal experiments. Monkeys were immunized with an inactivated RSV vaccine and then, infected with the virus. The extent of infection, as well as pathological scores were higher in the immunized, than in the control animals [21].

According to recent data, ADE may have an important role in the pathogenesis of Ebola-virus infection as well. Takada et al. [22] showed that in humans, Ebola Zaire virus...
infection induces antibodies that enhance the in vitro infectivity of the Zaire Ebola virus species.

Interestingly, enhancement was mediated by antibodies to the viral glycoprotein and by the complement component C1q. The authors suggest that ADE may be responsible for the extreme virulence of the Zaire virus among the four Ebola virus species.

In addition, ADE of infection has also been implicated as a major obstacle to the development of vaccines against viruses, such as dengue viruses, bluetongue virus, influenza virus, lentiviruses and RSV (reviewed in [9]). In all cases, the presence of antibodies induced by vaccination increased the susceptibility to subsequent viral infections and/or enhanced the severity of clinical disease caused by virus challenge, in vaccinated individuals. Recently, ADE was implicated in the pathogenesis of atypical morbilli virus infection observed after immunization with formalin-inactivated vaccine [23], and was suggested as a concern in developing a vaccine for severe acute respiratory syndrome (SARS) as well [24].

Antibody-dependent enhancement of HIV infection

Antibodies that may enhance HIV infection in vitro were described shortly after HIV has been first isolated. Their presence in the blood of HIV-infected patients, as well as in HIV- or SIV-infected experimental animals was confirmed by several groups. Two types of enhancing antibodies were described approximately at the same time, in the late eighties. Robinson et al. [25] found that sera from HIV-infected individuals enhance in vitro HIV infection of the CR2 (complement receptor type 2)-bearing T lymphoblastoid cell line MT2. The same authors demonstrated this enhancement to be dependent on antibodies and mediated by complement, and coined its name 'complement-mediated antibody-dependent enhancement (C-ADE)' [26]. Gras and Dormont [27] could reproduce the same phenomenon in peripheral blood lymphocytes. According to further studies [28], C-ADE of HIV-1 infection is characterized by increased synthesis of protein and RNA, as well as enhanced release of infective viruses.

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 mixture containing these antibodies was first described by Takeda et al. [29] and Homsy et al. [30]. Shortly thereafter, FcR-ADE of HIV-1 infection has been observed in vitro with many different HIV-1 isolates of both X4 and R5 phenotypes, using different target cells (reviewed in [8]).

Except for early work with FcR-ADE [31], most data on the clinical significance were reported on C-ADE. Therefore, the following discussion will be largely restricted to the latter type of infection-enhancement. In the light of a recent observation indicating that antibody binding to the Fc receptor, but not complement, can mediate antibody-dependent protection against HIV [32], the dominant role of C-ADE in enhancement does not seem surprising.

C-ADE is mediated mostly by antibodies against the N-terminal end of gp41, containing the so-called immunodominant domain of the protein [33, 34], although the role of anti-gp120 cannot be excluded either [35]. On gp41, two domains (amino acids 579 to 613, and 644 to 663) were mapped by means of monoclonal antibodies as the main binding site for C-ADE antibodies [36], which was further characterized with use of site-directed mutagenesis by Mitchell et al. [37].

The mechanism of C-ADE was investigated by several studies during the last two decades. As summarized recently by Robinson [38], binding of the antibody to gp41 initiates the complement cascade, leading to deposition of the complement component C3d,g on the virion. Opsonized virions subsequently bind to complement receptor type 2 (CD21). Ultimately, the engagement of CD21 and CD4 receptors by opsonized virions leads to an increased rate of HIV spread through the tissue culture and to a ten-fold increase in the release of reverse transcriptase (RT) into the culture medium, as well as to an increase of the level of HIV genomic RNA. Most importantly, C-ADE leads to an almost 100-fold increase in the number of the infective progeny virus synthesized, compared with infections by HIV-1 alone. C-ADE of HIV-1 infection requires CD4, but the binding of C1q to C1q-R of virions may also increase C-ADE [39].

According to recent studies by Robinson [38], the CXCR4 coreceptor is also required — in addition to CD4 and complement receptors — for enhanced entry of HIV into MT2 cells.

The significance of C-ADE in the progression of HIV disease

Findings in both experimental animal- and human studies indicate that C-ADE may be strongly associated with the progression of SIV or HIV disease.

Previous studies have shown that passive immunization of Rhesus macaques with antibodies from chronically infected monkeys could lead to enhanced pathogenesis after inoculation with SIVmac251 [40]. Moreover, disease progression after immunization was correlated to the titer of antibodies directed against an enhancing domain in both passive [41] and active immunization studies [42]. In other words, the higher the titer of antibody to primary enhancing domain the faster the animals proceeded to AIDS.

Several findings of our group and others [43] indicate that C-ADE is correlated to the progression of HIV disease as well. We used a method described first in [44] in all of our studies to measure C-ADE. Briefly, serum samples from patients were heat-treated (56 °C, 30 min) and diluted at 1:64 with culture medium. Test serum dilutions were mixed with identical volumes of fresh pooled sera from HIV-seronegative healthy persons; HIV-1mp (100 TCID50) was added and incubated at 37 °C for 1 h. Then, the mixture was added to and incubated together with 5 × 10^5 MT-4 cells without a change for 4 days at 37 °C. Growth of HIV in the cultures was monitored on days 3 and 4 with reverse transcriptase (RT) assay. The results were measured as count per minute, and expressed finally as an index (enhancement/neutralization index = E/N I) value. This E/N index is a ratio between virus growth in the presence and in the absence of test serum. Samples with an E/N I below 0.5 (i.e. two-fold decrease in virus production) were considered neutralizing, whereas samples with an E/N I over 2.0 (i.e. two-fold increase in virus production) were considered enhancing. The method for the extreme virulence of the Zaire virus among the four Ebola virus species.
has two major benefits: (1) it uses almost physiological complement concentration (25% in the infecting mixture) and (2) virus production is measured by RT assay, which measures only infective viruses.

An apparent drawback of the measurements performed in our system seems to be that a single, T cell-adapted strain, HIV-1LIIIb is used for experiments with clinical samples, whereas in most other systems, the use of primary HIV-1 isolates and assays with primary cells is essential for in vivo relevance. Recent, elegant studies by Mitchell et al. [37] and Robinson [38] help to resolve this apparently paradoxical situation. According to Mitchell et al. [37] and Robinson [38] found anti-HIV immunoglobulin-enhanced replication of HIV strains belonging to different clades, indicating that antibodies mediating C-ADE are broadly reactive.

With this method, we obtained evidence indicating that C-ADE occurs early in the course of HIV infection and it is strongly associated with and is predictive of the progression of HIV disease.

Using four different seroconversion plasma panels (from Boston Biomedica) on cultures infected with plasma—virus—human complement mixtures, a marked rise of E/Ni was observed in all four panels, with values reaching an index value of 3—4 (corresponding to 3—4 times enhancement in virus production, concomitantly with or just before seroconversion, approximately day 35—40 post infection. Furthermore, this was associated, in general, with an increase in p24 antigenemia and with the appearance of HIV-1 RNA (Fig. 1, result in one seroconversion panel).

By contrast, in cultures infected with virus—serum mixtures without complement, an E/Ni value of 1 or lower was observed at all time-points and in all 4 seroconversion panels [45].

In a study performed in 98 patients with advanced HIV disease [58 patients in Centers for Disease Control and Prevention (CDC) stage III or IV, 40 patients in CDC stage IV or IV; or IV] we found a very strong correlation between E/Ni (that is, the extent of enhancement over neutralization) and viral load on the other. Spearman’s rank correlation coefficients were calculated when viral load was measured by the Amplicor and NASBA methods (A: 0.709; R < 0.0001 and R = 0.627; p < 0.0001, respectively). By contrast, much weaker correlation (A: 0.276; p = 0.0059 and R = 0.207; p = 0.041, respectively) was found in cultures infected with mixtures containing heat-inactivated serum (that is, no active complement). When viral load in samples which neutralized (E/Ni index < 0.5), did not neutralize or enhance (E/Ni index 0.5—2.0) or enhanced (E/Ni index > 2) HIV production in the presence of complement were compared, a parallel significant (Kruskall—Wallis test p = 0.0014) increase was observed (Fig. 2). We found a negative correlation between the E/Ni indices and CD4 cell count (Spearman’s rank correlation coefficient —0.279, p = 0.0058.) [45].

Twelve serum samples from 20 AIDS patients, whereas only 3 samples from 20 asymptomatic cases had an E/Ni index over 2. Conversely, none of the AIDS patients’ samples, but 8/20 samples of asymptomatic patients neutralized HIV in the presence of complement [44]. Recently, Subbramanian et al. [43] reported quite similar findings. They found C-ADE mediated antibodies in 72% of patients with HIV disease and the titer of these antibodies was higher in patients with AIDS, than in those with asymptomatic infection. By contrast, neutralization in the presence of complement could be detected in the sera of asymptomatic patients, predominantly.

Most importantly, the neutralization assay performed in the presence of complement predicted the development of AIDS in 21 asymptomatic HIV patients during a median follow-up period of 61 (55—64) months (Fig. 3), while the same test performed in the absence of complement had no predictive value. Similar results were obtained when the
decline of CD4 cell count was compared in patients with or without neutralization in the presence of complement. In addition, both E/N I values and HIV-1 RNA levels increased significantly during a 17-month observation period in 18 HIV-infected patients who remained untreated or were on AZT-monotherapy [46].

More recently, we measured the changes of E/N indices in 28 patients undergoing HAART [47]. While a substantial proportion of the samples obtained at the beginning of HAART enhanced virus production in the presence of complement and neutralization was rare, sera obtained from 20/28 patients after 19.0 (10.5—24.0) months of HAART treatment neutralized HIV even in the presence of complement (Fig. 4). Median (25th—75th percentile) value of E/N I dropped significantly (p < 0.0001) from 1.32 (0.79—2.29) E/N I to 0.37 (0.19—0.57) during the follow-up period under HAART. The comparison of cultures inoculated with mixtures of HIV and purified IgG (prepared from serum pools taken before and during HAART) also revealed markedly decreased E/NI values [47].

In sharp contrast to these findings, clinical usefulness of measuring CEs was challenged by Montefiori’s group [48,49]. They did not find any difference in the frequency of neutralizing or enhancing antibodies (measured in the presence of complement) in patients in different stages of HIV disease. Additionally, long-term non-progressor state was similarly not associated with the lack of enhancing antibodies. The main difference between their method and our procedure was the final concentration of human complement in the mixtures added to target cells. While complement concentration was 25% in our experiments, Montefiori’s group [48,49] used a concentration of 2.5%. It seems reasonable to suppose that the closer are the experimental conditions to in vivo conditions, the higher is the pathological relevance of the measurement.

Figure 3 Development of AIDS during a 61 (55—64) months median (IQ range) follow-up period in 21 asymptomatic HIV patients with different baseline neutralization in the presence of complement. P value for Fisher’s exact test is indicated. Adapted from [46].

Figure 4 Distribution of samples with neutralization (E/N index < 0.5), neither neutralization nor enhancement (E/N index 0.5—2.0), or enhancement (E/N index > 2.0) measured in the presence of complement in the same 28 AIDS patients at the beginning of and after the administration of HAART for 19.0 (10.5—24.0) months. P value for χ² test is indicated. Adapted from [47].

Possible influence of enhancing antibodies on the efficacy of HIV vaccines

Thus, many data indicate that during the natural course of HIV infection, a mixture of different antibodies is present in the blood of patients and some types of antibodies may antagonize other antibody types. As it was detailed above, enhancing antibodies appear earlier after HIV infection than neutralizing antibodies. Furthermore, the balance of ‘‘good’’ and ‘‘bad’’ antibodies changes during the progression of HIV disease, and ‘‘bad’’, enhancing antibodies become predominant in the advanced stage. This issue was addressed by the elegant study of Subbramanian et al. [43]. These authors measured in parallel the concentrations of virus-neutralizing antibodies (NAs), NA in the presence of complement (C-NA), as well as those of antibody-dependent cellular cytotoxicity-mediating (ADCC) antibodies and infection-enhancing antibodies in the presence or absence of complement (C-ADE versus FcR-ADE). Antibodies that may control HIV progression (NA, C-NA or ADCC) were found mainly in asymptomatic patients, whereas in AIDS stage, the balance shifted towards infection-enhancing (FcR-ADE or C-ADE) antibodies. These findings raise the possibility that infection-enhancing antibodies can be developed in experimental animals or volunteers immunized with candidate HIV vaccines. Similarly, it seems plausible that, similarly to natural infections, they may abrogate the beneficial effect of neutralizing or ADCC-mediating antibodies. Certain data from the SIV-monkey model and HIV vaccine trials indicate that this may indeed occur. Antibodies to the putative SIV infection-enhancing domain diminish the beneficial effects of an SIV gp160 vaccine in Rhesus macaques [42]. Verrier et al. [50] demonstrated that in hyperimmunized Rhesus macaques, whole virus particle-based HIV-1 vaccines can play a facilitator role in the transmission of the virus and/or the evolution of the disease. In a recent passive immunization study, SIV immunoglobulin decreased plasma viremia and delayed disease progression in four monkeys out of six; however, the remaining two animals in the group had higher viral loads in their peripheral blood cells than any other of the 12 control animals, and progressed more rapidly than the controls [51]. In another, recent experiment conducted on Rhesus macaques immunized with a recombinant Varicella–Zoster Virus vaccine expressing HIV gp160, those
that received the vaccine raised high amounts of non-neutralizing antibodies and manifested increased levels of SIV replication, more rapid CD4 depletion, and accelerated progression to AIDS, compared with controls. This observation indicates that candidate AIDS vaccines may not simply be either efficacious or neutral; they may also have the potential to be harmful [52].

It seems highly probable that enhancing antibodies may develop in human volunteers immunized with candidate HIV vaccines. For example, complement-dependent enhancing antibodies were detected in two trials: in 11 of 19 volunteers [53], and in 6/24 subjects [54] vaccinated with high dose (640 μg) of a HIV-1 gp160 candidate vaccine. Indirect evidence indicates that enhancing type antibodies could have developed during recent phase III vaccine trials as well. Gilbert et al. [55] have recently reported on the correlation between immunological responses to the recombinant gp120 vaccine and the incidence of HIV-1 infection in a phase 3 vaccine trial. They found that vaccinees who developed low antibody responses had a slightly, but significantly higher chance of becoming infected, than placebo recipients. In Caucasian recipients, this odds ratio was as high as 2.20 (1.29–3.74; p = 0.004), and 2.00 (1.22–3.29; p = 0.006) for MN CD4 blocking and for GNE8 V2-binding antibodies, respectively. On the other hand, even the high antibody response appeared to fail to protect vaccinees against HIV-1 infection. The authors put forward two alternative explanations of their findings: (a) the vaccine induced an immune response that enhanced susceptibility to HIV-1 infection in low responders and (b) differing antibody responses to rgp120 merely identified the diverse capabilities of vaccinees to resist HIV-1 infection [55]. In the discussion of their paper, they weighed the relative plausibility of the two explanations, but could not decide. In accordance with the Editorial Comment by Graham and Mascola [56], we would like to discuss the possibility, corroborated by the abundance of literature data summarized above, that antibody-mediated enhancement may develop and can diminish or abrogate the beneficial effects of the vaccine or can even be harmful for vaccinated volunteers. We believe that this risk should be seriously considered during the future development of vaccines.

Proposals

Findings summarized above indicate that the balance of "good" (neutralizing or ADCC mediating) and "bad" (infection-enhancing) antibodies strongly correlates with, and possibly it is one of the driving forces behind, the progression of HIV disease. We suppose that these findings obtained in HIV-infected persons may have relevance for HIV-1 vaccination trials as well. Based on these assumptions, we would like to put forward four proposals.

(1) Since it cannot be excluded that HIV vaccines increase the likelihood of infection, we propose that serum samples obtained from vaccinated and placebo-treated volunteers with breakthrough infections in major vaccine trials be tested for C-ADE and FcR-ADE.

(2) In accordance with the proposals of Robinson [38], we also suggest to avoid the inclusion of the whole gp41 or at least the major epitopes for C-ADE on gp41 into HIV vaccines, and to reinvestigate the epitopes responsible for the development of different types of enhancing antibodies. With this approach, it may be possible to develop preventive vaccines with strong neutralizing and weak ADE inducing characteristics.

(3) Based on literature data referred to above—indicating that a neutralization assay performed in the presence of complement exhibit much stronger correlations to disease progression than the traditional test, we propose using an alternative to the traditional method for measuring neutralizing antibodies, in order to find an effective vaccine. It is reasonable to assume that measurement of the balance between neutralizing and enhancing antibodies in the presence of complement may reflect also the proportion of protective and anti-protective antibodies present in vaccinees. This method can be easily adapted to animal experiments. Therefore, it may prove useful as early as during the selection of candidate vaccines by eliminating potentially harmful vaccines.

(4) We also believe that measurement of the virus production by cells infected with HIV-1 in the presence of antibodies and complement may provide a better immune correlate than we have currently.

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