Human immunodeficiency virus type 1 neutralization by plasma from B or F genotype infected individuals

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Anti-human immunodeficiency virus type 1 (HIV-1) "binding antibodies" (antibodies capable of binding to synthetic peptides or proteins) occur throughout HIV-1 infection, are high-titered and highly cross-reactive, as confirmed in this study by analyzing plasma from B and F genotype HIV-1 infected individuals. Plasma from individuals infected with clade F HIV-1 displayed the most frequent cross-reactivity, in high titers, while Bbr plasma showed much higher specificity.

Similarly, neutralization of a reference HIV-1 isolate (HIV-1 MN) was more frequently observed by plasma from F than B genotype infected individuals. No significant difference was seen in neutralization susceptibility of primary B, Bbr or F clade HIV-1 by plasma from individuals infected with the classical B (GPGR) or F HIV-1, but Bbr (GWGR) plasma were less likely to neutralize the F genotype primary HIV-1 isolates.

The data indicate that both B and F genotype derived vaccines would be equally effective against B and F HIV-1 infection, with a slightly more probable effectiveness for F than B genotype. Although the Bbr variant appears to induce a much more specific humoral immune response, the susceptibility in neutralizing the Brazilian HIV-1 B genotype Bbr variant is similar to that observed with the classical B genotype HIV-1.

Key words: human immunodeficiency virus type 1 - neutralization - genotype - Brazil

Humoral immune response against human immunodeficiency virus type 1 (HIV-1) is extensive but is detectable only fairly late in infection, and may be of little importance in the control of the acute infection. Attempts to "immunotype", "serotype" or "neutrotype" HIV-1 have shown that, although "reactivity clusters" can be identified (Bradac & Ho 1992, McKnight et al. 1992, Mascola et al. 1994, 1996, Cheingsong-Popov et al. 1994, Weber et al. 1996, Kostrikis et al. 1996, Zolla-Pazner et al. 1999, Zhang et al. 2002, Zolla-Pazner 2004), these have little correlation to the clades identified by genotyping HIV-1. In Southeastern Brazil, although infection by the so-called Brazilian variant of the classical B clade of HIV-1, Bbr, can mostly be distinguished by a more specific reactivity pattern towards homologous synthetic peptides, no distinction of plasma of individuals infected with the prevalent B or the F clades of HIV-1 could be observed using synthetic envelope peptides (Bongertz et al. 2003).

The importance of the anti-HIV-1 humoral immune response has been a highly controversial subject since antibodies capable of neutralizing the virus were first described (Ho et al. 1985. Robert-Guroff et al. 1985, Weiss et al. 1985). In the course of the last three years, however, the high protection obtained in monkeys infused with anti-HIV-1 monoclonal antibodies has increased interest in HIV-1 neutralization (Baba et al. 2000, Mascola et al. 2000, Nishimura et al. 2002, Ferrantelli et al. 2004). Studies in animal models have confirmed the importance of HIV-1 neutralizing antibodies (NAb) in HIV-1 infection and efforts to analyze and to induce such antibodies in humans have been increased.

In the Southeast region of Brazil, genotypes B and F are the prevalent circulating HIV-1 subtypes. Little is known about cross-neutralization in Brazil, and the present analysis attempts to compare the neutralization potency of antibodies in plasma from individuals infected with B (comprising the classical B genotype displaying the amino acid sequence GPGR at the tip of the V3 loop (B-GPG)and the Bbr or B" variant, with GWGR at the tip of the V3 loop) or F genotype HIV-1, as well as an attempt to analyze the comparative neutralization susceptibility of B (GPG and GWG) and F HIV-1 primary isolates. This information is of fundamental importance for a rational choice of anti-HIV/AIDS vaccination agents.

MATERIALS AND METHODS

A global analysis of several different projects, performed with approval of the Fiocruz Ethical Committee, is presented, mainly of a recent study carried out with plasma from injection drug users (IDU) (Bongertz et al. 2003). Plasma were obtained from 105 patients diagnosed as HIV-1 positive according to the norms of the Brazilian Ministry of Health, who were in all stages of HIV-1 infection (47.6% being asymptomatic individuals). Only a small number of the patients (10.5%) were under antiretroviral therapy, as blood collected for diagnosis of HIV infection was used. The majority of the samples were obtained from male individuals (73.3%).

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Seroreactivity to synthetic V3 envelope peptides was carried out using reagents and methods described earlier (Bongertz et al. 1999). Briefly, biotinylated decapentamer synthetic peptides corresponding to HIV-1 consensus amino acid sequences of HIV-1 genotypes A, B (including variant Bbr), C, D, E, and F (including an empirically chosen Brazilian sequence Fbr) were bound onto avidin-coated immunoplates, incubated with duplicates of serially diluted sample and control plasma. Bound antibodies were evaluated after washing with 8M, adding horse radish peroxidase labeled anti-human IgG (Fc specific) and the peroxide/TMB substrate/chromogen mixture color intensity was read at 450 nm.

The methods used for HIV-1 neutralization in peripheral blood mononuclear cells (PBMC) (Bongertz et al. 2001) and the CEM T lymphocytic cell line (Bongertz et al. 2002) have been described previously. Briefly, serially diluted plasma specimens were incubated for 1 h at 37°C with 10 to 50 ID (infectious doses, previously determined, at least two dilutions used in each assay) of viral stock. Incubation with host cells was carried out for seven days (with two changes of culture medium after 24 and 48 h). When primary HIV-1 isolates were employed, pre-activated normal human PBMC were prepared at a concentration of 10^6/ml. For analysis of the T-cell line adapted HIV-1 isolate MN, both PBMC and CEM cells (10^5/ml) were used. Neutralization was considered positive when a reduction of at least 75% of viral input was detected as measured by p24 concentration (Beckman-Coulter Co, San Diego, CA). Neutralization was evaluated at 50-89% and at ≥ 90% reduction of viral input as measured in positive control wells. The number of assays run for each isolate and the number of plasma tested varied, and are specified in the text for the different groups analyzed.

Determination of the genetic subtype of the HIV-1 isolates was carried out using the heteroduplex mobility assay (HMA) with the primers and technique described by Delwart et al. (1993). Identification of the Brazilian B subtype variant Bbr was carried out using Fok I restriction fragment length polymorphism determination (Morgado et al. 1998).

The Mann-Whitney two-tailed unpaired test was used for comparative evaluation of frequencies, and statistical significance established at < 5% (Instat Program, GraphPad, San Diego, CA).

RESULTS

Seroreactivity showed the very high extent of cross-reactivity of the plasma samples with the synthetic peptides tested. Fig. 1 shows that plasma specimens from individuals infected with clade F HIV-1 display the most frequent reactivity not only with the homologous V3F peptide, but also with the heterologous V3 peptides used, in high titers, much higher than those displayed by B-GPG clade plasma specimens. Indeed, all F plasma samples recognized, for example, the V3B peptide, which is recognized by only 85% of B-GPG plasma specimens. As indicated before (Bongertz et al. 1999), the Bbr plasma specimens showed much lower cross-reactivity with the different V3 peptides, and most high-titered reactions were observed against the homologous Bbr synthetic V3 peptide.

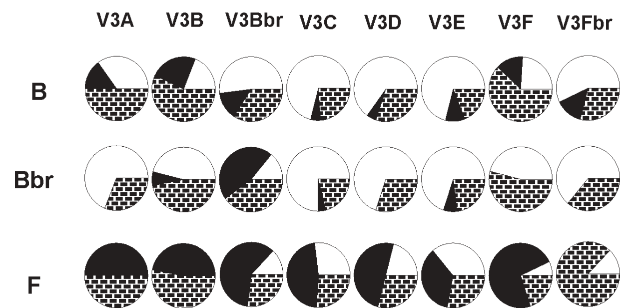

Fig. 1: percentage of plasma from individuals infected with B, Bbr or F genotype human immunodeficiency virus type 1 (HIV-1) recognizing individual synthetic HIV-1 envelope V3 peptides corresponding to different HIV-1 genotypes/variant consensus sequences. Black areas indicate plasma with reactivities at 1:1000 or higher dilutions while chequered areas indicate plasmas with reactivities at dilutions below 1:1000.

Of greater interest would be if this extensive cross-binding activity was mirrored by extensive cross-neutralization, as this would be indicative of cross-protection, important for anti-HIV/AIDS vaccine research. Therefore, neutralization of primary HIV-1 isolates and of a T cell line adapted HIV-1 isolate by plasma specimens from individuals infected with the different HIV-1 genotypes/variant prevalent in southeastern Brazil was evaluated.

Neutralization of HIV-1 MN was more frequently observed by plasma specimens from the HIV-1 F genotype than B genotype infected individuals, however, due to the smaller number of F plasma specimens available for testing, no statistical analysis could be carried out.

Interestingly, a more frequent and more effective neutralization of HIV-1 MN replication in pre-activated human donor peripheral blood mononuclear cells (PBMC) ($p = 0.0290$) and in a T-lymphocytic cell line (CEM) ($p = 0.0247$) was observed by Bbr variant plasma samples than by classical B genotype HIV-1 plasma specimens (Fig. 2).

No difference in neutralization of primary B clade HIV-1 ($n = 33$) by B ($n = 151$), Bbr ($n = 66$) or F ($n = 35$) plasma or of the HIV-1 Bbr isolates ($n = 20$) by B ($n = 52$), Bbr ($n = 70$) or F ($n = 18$) plasma samples at low dilutions (1:10) could be observed (Fig. 3). At higher dilutions (1:100,

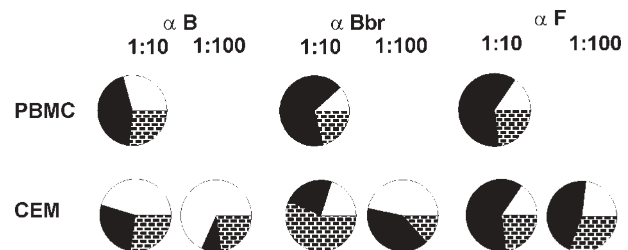

Fig. 2: neutralization of human immunodeficiency virus type 1 (HIV-1) MN replication in pre-activated peripheral blood mononuclear cells (PBMC) or a T lymphocytic cell line (CEM): Preincubation of HIV-1 MN with classical B-GPG (αB, n = 72), the Brazilian variant Bbr (αBbr, n = 43) or F genotype plasma (αF, n = 13) at the dilutions of 1:10 or 1:100, as indicated. Black areas indicate neutralization of at least 90% of viral input, while chequered areas indicate neutralization of 50 to 89.9% of viral input.

data not shown), F plasma samples were not as frequently effective against B clade HIV-1 isolates, but no statistical significance was observed.

Although only a limited number of primary HIV-1 F genotype isolates (n = 4) was available at the high activities needed for neutralization assays, they were also equally susceptible to B or F plasma specimens (n = 25 and 14, respectively). However, plasma samples from individuals infected with the Brazilian Bbr variant of HIV-1 (n = 14) were less frequently able to neutralize the F genotype primary HIV-1 isolates (p = 0.0409) (Fig. 3).

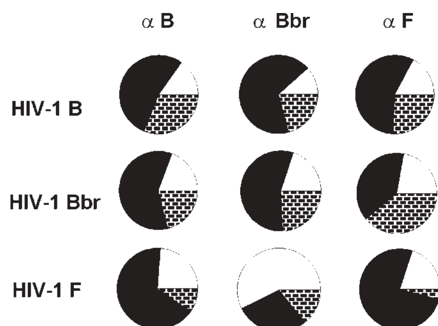

Fig. 3: neutralization of primary human immunodeficiency virus type 1 (HIV-1) isolates of clade B (n = 33), variant Bbr of clade B (n = 20) or of clade F (n = 4) by plasma from individuals infected with clade B (αB), Bbr variant of clade B (αBbr) or HIV-1 clade F (αF). Areas in brick pattern indicate 50-89% neutralization of viral input, and areas in black indicate neutralization of ≥ 90% of viral input.

DISCUSSION

HIV-1 neutralizing antibodies have come again to the forefront of research since the recent demonstrations of their protective capacities (Baba et al. 2000, Mascola et al. 2000, Hofmann-Lehmann et al. 2001, Parren et al. 2001, Ruprecht et al. 2001, Nishimura et al. 2002, Ferrantelli & Ruprecht 2002, Xu et al. 2002, Mascola 2002, Xiao et al. 2002, Ferrantelli et al. 2004). Moreover, their importance in the early control of HIV-1 infection is implied by the finding of immune complexes containing HIV-1 RNA (Dianzani et al. 2002), which could be an explanation for the disparity between quantification of viral RNA and of infectious HIV virions (Igarashi et al. 1999). Also, the increase in neutralization susceptibility of cultured HIV-1 and its decrease after inoculation indirectly confirms the control displayed by HIV-1 neutralizing antibodies in infection (Pugach et al. 2004). Another factor has been the growing conviction that the same kind of obstacles experienced in efforts to develop anti-HIV/AIDS vaccines dependent on neutralizing antibody induction are also true for vaccines dependent on stimulation of anti-HIV cellular immune response (i.e., HIV-1 variability, viral escape - Mascola et al. 2003). Therefore, nowadays the consensus is that the immune response to be induced by an anti-HIV/AIDS vaccine must include both humoral and cellular immune response, with specificities that are only now in the process of being defined.

One of the objectives of an effective anti-HIV/AIDS vaccine is that it should confer protection against all HIV-1 genotypes prevalent in the areas where the vaccine is to be applied. In Brazil, B and F are the prevalent genotypes found, with an increasingly important occurrence of genotype C HIV-1 in the Southern region of the country. Therefore, the cross-protection among these genotypes has to be analyzed.

The data presented show that plasma from individuals infected with genotype F HIV-1 more frequently possess neutralizing antibodies able to neutralize not only the homologous F but also the heterologous B genotype HIV-1 primary isolates, similar to the more frequent cross-binding activity observed in plasma samples from F in comparison to B genotype HIV-1 infected individuals. This appears to be more significant when lower concentrations of antibodies are compared by using higher dilutions of plasma samples, both for binding and for neutralization activity of the antibodies, as exemplified by the very high neutralization potency of the majority of the plasma specimens from individuals infected with HIV-1 genotype F against the reference isolate HIV-1 MN.

Attempts to correlate extent of seroreactivity or neutralization with individual progression of HIV-1 infection were made but statistical analyses indicated that no difference in extent of clinical disease progression between individuals belonging to the group infected with clade F or B HIV-1 could be detected.

Of interest are the results observed with plasma from individuals infected with the Brazilian variant Bbr of B genotype HIV-1. Seroreactivity assays show a high specificity of binding by homologous plasma samples; but this specificity is a characteristic rather of the antibodies than of the epitopes recognized, as the synthetic V3 peptide correspondent to this variant is well recognized by the heterologous F plasma specimens. This might be interpreted as an indication that this viral variant is less able to induce an antibody response than are F genotype viruses, as also indicated by the less frequent neutralization of HIV-1 F genotype isolates by Bbr plasma samples.

HIV-1 F genotype appears to be specially antigenic and able to induce a more potent humoral immune response than that induced by the B genotype viruses. This may be linked to the exceptionally high capacity of F genotype HIV-1 to recombine with HIV-1 from other genotypes (Vicente et al. 2000), reflected by the low number of pure F genotype HIV-1 (Morgado et al. 2002), and the high percentage of B/F recombinants described in Brazil (Guimarães et al. 2002). Therefore, further studies on the specific anti-HIV-1 genotype F immune response are indicated.

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