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A broad range of mutations in HIV-1 neutralizing human monoclonal antibodies specific for V2, V3, and the CD4 binding site

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ABSTRACT

The HIV vaccine-induced neutralizing antibodies (Abs) display low rates of mutation in their variable regions. To determine the range of neutralization mediated by similar human monoclonal Abs (mAbs) but derived from unselected chronically HIV-1 infected subjects, we tested a panel of 66 mAbs specific to V3, CD4 binding site (CD4bs) and V2 regions. The mAbs were tested against 41 pseudoviruses, including 15 tier 1 and 26 tier 2, 3 viruses, showing that the neutralization potency and breadth of anti-V3 mAbs were significantly higher than those of the anti-CD4bs and anti-V2 mAbs, and only anti-V3 mAbs were able to neutralize some tier 2, 3 viruses. The percentage of mutations in the variable regions of the heavy (VH) and light (VL) chains varied broadly in a range from 2% to 18% and correlated moderately with the neutralization breadth of tier 2, 3 viruses. There was no correlation with neutralization of tier 1 viruses as some mAbs with low and high percentages of mutations neutralized the same number of viruses. The electrostatic interactions between anti-V3 mAbs and the charged V3 region may contribute to their neutralization because the isoelectric points of the VH CDR3 of 48 anti-V3 mAbs were inversely correlated with the neutralization breadth of tier 2, 3 viruses. The results demonstrate that infection-induced antibodies to CD4bs, V3 and V2 regions can mediate cross-clade neutralization despite low levels of mutations which can be achieved by HIV-1 vaccine-induced antibodies.

1. Introduction

The identification of anti-HIV-1 broadly neutralizing antibodies (bnAbs) suggests the possibility of designing immunogens that can induce potent and cross-reactive antibodies (Abs) in HIV vaccinees. Although this approach is very attractive, it faces several major challenges including immunogen design, an increased level of somatic mutations (15–36%) in bnAbs, and the fact that the induction of bnAbs by a HIV vaccine has not been achieved in any animal model (reviewed in van Gils and Sanders, 2013; West et al., 2014).

In contrast to the concept that bnAbs need to be induced to reduce infection by HIV-1, are the results of the recent RV144 clinical vaccine efficacy trial, which showed a reduction in HIV-1 infection of 31.2% in vaccinees (Haynes et al., 2012). This vaccine used a prime and boost regimen with a recombinant HIV-avian pox virus and two different recombinant gp120 proteins which induced a broad range of anti-gp120 Abs, including three types of neutralizing Abs against CD4-binding site (CD4bs), V3 and V2 regions; however, bnAbs were not detected (Gottardo et al., 2013; Haynes et al., 2012). Data analysis showed that reduced infection was inversely correlated with levels of anti-V2 plasma Abs (Haynes et al., 2012; Zolla-Pazner et al., 2013). The anti-V3 Abs were also correlated with infection risk but only in vaccinees with lower levels of gp120-specific plasma IgA Abs (Gottardo et al., 2013).

The plasma Abs from recipients of the RV144 neutralized tier 1 pseudoviruses and presence of neutralizing anti-V3 Abs was determined based on peptide blocking assays which does

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not exclude that other, conformation-dependent neutralizing Abs, were involved (Haynes et al., 2012; Montefiori et al., 2012). In addition, two anti-V3 mAbs – CH22 and CH23 – derived from recipients of the vaccine displayed weak neutralizing activity which could be related to a low level of mutations, 3.7% and 4.5%, respectively, in their variable regions (Montefiori et al., 2012). This is comparable with the low percentage of mutations observed in other vaccine-induced anti-V2 and anti-gp120 mAbs (Liao et al., 2013; Moody et al., 2012). It is possible that during several months of vaccination, responding Abs are characterized by a limited percentage of mutations, but the range of their neutralization potency and breadth is unknown due to the existence of only several such mAbs (Liao et al., 2013; Montefiori et al., 2012).

To address this issue we analyzed the neutralization potency and breadth as well as the percentage of mutations in 66 human mAbs against CD4bs, V3 and V2 regions of HIV-1 gp120 which were derived from chronically infected individuals. These three types of neutralizing Abs, anti-CD4bs, anti-V3 and anti-V2, are commonly present in the plasma of HIV-1 infected individuals (Kayman et al., 1994; Lynch et al., 2012; Vogel et al., 1994) and corresponds to HIV vaccine induced neutralizing Abs which can be classified as conventional Abs in contrast to bnAbs (Zolla-Pazner, 2014).

This study showed that anti-V3 mAbs neutralized tier 1 and some tier 2, 3 viruses from diverse HIV-1 subtypes, while anti-CD4bs and anti-V2 mAbs neutralized only tier 1 viruses. The percentage of mutations was not related to neutralization breadth of tier 1 viruses but moderately correlated with the neutralization breadth of tier 2, 3 viruses which was mediated by anti-V3 mAbs. The electrostatic interactions between anti-V3 mAbs and V3 may contribute to neutralization of tier 2, 3 viruses which inversely correlated with low isoelectric points (pI) of the CDR domain in the heavy chains.

2. Materials and methods

2.1. Human monoclonal antibodies

Sixty-six human mAbs specific to gp120 HIV-1 were studied, including 48 anti-V3, 12 anti-CD4bs and six anti-V2 mAbs (Table 1, Supplementary Table 1). All mAbs were produced in our laboratory and those which displayed the ability to neutralize HIV-1 pseudoviruses were selected for this study (Table 1). One human mAb, 1418 against parvovirus B19, was used as negative control (Gigler et al., 1999). The anti-HIV-1 mAbs were derived from 58 unselected and chronically HIV-1 infected individuals and except for two clonal anti-V2 mAbs (1393A and 1361), each mAb is unique with different VH CDR3 sequences (Supplementary Table 1). Forty mAbs were derived from individuals living primarily in the New York City area whom were presumably infected with subtype B viruses (Table 1). Twenty-six mAbs originated from individuals infected with non-clade B viruses and living in Cameroon, India, Ivory Coast and Thailand; the blood donors were infected with subtypes C, G, H, F2, CRF01_AE, CRF02_AG, CRF09_cpx, CRF13_cpx viruses or undetermined (Table 1). The subtypes of plasma viruses were determined by sequencing the envelope proteins in our lab as previously described (Zhong et al., 2002). Immunoglobulin (IG) gene usage (IGHV, IGKV and IGLV) and alleles evaluated by IMGT/V-QUEST are shown in Supplementary Table 1.

All but four mAbs were generated using the hybridoma technology based on fusion of Epstein-Barr virus (EBV)-transformed lymphocytes with the heteromyeloma cell line SHM-D33 (Gorny et al., 1991). Four mAbs were produced using the single B cell method (Li et al., 2012). Sixty-two mAbs have been described in various papers (see references in Table 1), while four mAbs (4415, 4591, 4647 and 4682) were produced for this study using the hybridoma method. Briefly, these latter mAbs were generated from peripheral blood mononuclear cells (PBMC) which were transformed by EBV and screened by ELISA using V3-cholera toxin B (CTB)-fusion proteins containing the V3 consensus sequences from clades A and C (Totrov et al., 2010). The V3-CTB reactive cells were fused with SHM-D33 heteromyeloma, and the resulting hybridoma cells were cloned by limiting dilutions until monoclonality was achieved.

Blood samples were obtained from infected donors who signed informed consent forms, which were approved by the New York University (NYU) and Veteran Affairs (VA) Institutional Review Boards, the Ethics Committee of All India Institute of Medical Sciences, New Delhi, and the National Ethical Committee of Cameroon.

All sequences of the VH and VL regions were deposited in GenBank and the accession numbers are shown in Supplementary Table 1.

2.2. Amplification and nucleotide sequencing of the mAb variable domains

The nucleotide sequences of the heavy (VH) and light (VL) chain genes of the variable domains of mAbs produced by hybridoma technology were determined as described (Gorny et al., 2011, 2009). Briefly, messenger RNA was extracted from the hybridoma cell lines producing anti-HIV-1 Env mAbs and reverse transcribed into cDNA using oligo dT primer. A homopolymeric tail was added to the 3′ end of cDNA by the terminal deoxynucleotidyl transferase (TdT). The IG genes coding for the VH and VL were amplified from poly-C tailed cDNA by PCR using the forward primer containing anchored tail (Invitrogen) and the reverse primer specific for the constant region encoded by IGHG, IGKC or IGLC genes. PCR amplification was performed using a cycling program, and ethidium bromide-stained 0.8% agarose gels were used to visualize the PCR products. The bands of appropriate size were excised, purified and cloned into the 2.1-TOPO TA cloning vector (Invitrogen); plasmids were transformed into Top10 competent cells. For each heavy and/or light chain, 6 to 12 independent clones were screened. The plasmids with the appropriate inserts were sequenced in both directions using M13 primers. All sequencing reactions were performed at Macrogen, Rockville, MD.

2.3. Analysis of immunoglobulin gene sequences

The nucleotide sequence data of the 66 mAbs were analyzed using Pregap4 and BioEdit software. The percentage of mutations was determined by two methods: (a) in the variable (V) regions and (b) in the V plus CDR3 (V + CDR3) using IMGT/V-QUEST (Brochet et al., 2008; Giudicelli et al., 2011) and IMGT/JunctionAnalysis (Giudicelli and Lefranc, 2011; Yousfi Monod et al., 2004) from the international ImMunoGeneTics information system® (http://www.imgt.org) (Lefranc et al., 2015). The V region includes nucleotides up to 2nd-CYS 104 codon which delimits the end of FR3-IMGT of all functional IG V genes of the VH and VL (V-KAPPA and V-LAMBDA) (Lefranc, 2014). This system provides the data about percentage of identical nucleotides in the V region of antibody which allows calculating the percentage of mutations. In addition, we calculated the percentage of mutations in the CDR3 domains which was added to mutations in the V region (V + CDR3); this analysis included the regions of CDR3 which have the corresponding germ line sequences, 5′V-REGION, D-REGION and 3′J-REGION, but not the palindromic (P) and non-templated (N) nucleotides regions.

2.4. Neutralization assay

All 66 anti-HIV-1 Env-specific mAbs and one negative control mAb 1418 were tested in the same laboratory (M. S. Seaman) for neutralizing activities against a standard panel of 41