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Characterization of human IgG repertoires in an acute HIV-1 infection

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ABSTRACT

All known broadly neutralizing antibodies (bnAbs) are highly somatically mutated and therefore significantly differ from their germline predecessors. Thus although the mature bnAbs bind to conserved epitopes of the HIV-1 envelope glycoprotein (Env) with high affinity their germline predecessors do not or weakly bind Envs failing to initiate an effective immune response. The identification of less somatically mutated bnAbs and/or antibody maturation intermediates that are clonally related to bnAbs may be useful to circumvent the major problem of initiating immune responses leading to elicitation of bnAbs. Here, we describe the identification of IgG antibodies from an acutely HIV-1-infected patient using a combination of phage display and high-throughput sequencing. We found two antibodies with only a single point mutation in the V region of their heavy chain variable domains compared to their putative germline predecessors which bound with high affinity to several Envs. They targeted the Env gp41 and did not neutralize HIV-1. Using high-throughput sequencing, we identified several highly abundant CDR3s, germline-like as well as somatically mutated V genes in the VH/VL repertoires of the patient which may provide antibody intermediates corresponding to known bnAbs as templates for design of novel HIV-1 vaccine immunogens.

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Introduction

Advancement in high-throughput screening technologies has led to the recent discoveries of several potent broadly neutralizing antibodies (bnAbs) against HIV-1 recovered from peripheral blood mononuclear cells (PBMCs) of HIV-1-seropositive donors (Pietzsch et al., 2010; Scheid et al., 2009; Walker et al., 2011; Wu et al., 2010, 2011). Elicitation of such bnAbs remains a major challenge attributing to virus evasion strategies and host immune regulatory mechanisms. We found that germline-like predecessors of bnAbs bind weakly or undetectably to all tested HIV-1 envelope glycoproteins (Envs) (Xiao et al., 2009). This finding suggests that HIV-1 could have evolved a strategy to reduce or eliminate the immunogenicity of the highly conserved epitopes of bnAbs by using holes in the human germline B cell receptor repertoires — absence of or reduced binding of germline antibodies to the conserved epitopes that is not sufficient to initiate and/or maintain an effective immune response. To overcome this fundamental issue, we resorted to explore large naïve IgM repertoires for identifying antibody maturation intermediates that are clonally related to HIV-1 bnAbs (Prabakaran et al., 2012b). We also found that several human monoclonal antibodies (mAbs) selected from another large naive IgM phage-displayed library were able to bind with high affinity to recombinant Envs of HIV-1 isolates from different clades (Chen et al., 2010). Although those antibodies enhanced or did not neutralize infection by some of the HIV-1 primary isolates, they could have implications for the B-cell-lineage vaccine design strategy (Dimitrov, 2010; Haynes et al., 2012).

For this study, we constructed two IgG antigen-binding fragment (Fab) phage display libraries from an acutely HIV-1-infected patient, which were panned against the Env to identify HIV-1 specific binders. We analyzed the genetic origin, diversity and level of maturation of the selected antibody binders; in parallel, we used high-throughput sequencing to analyze the extent of germline diversity, complementarity determining region 3 (CDR3) lengths, somatic mutation and most frequently expressed clones in the two libraries. We found germline-lineaged antibodies exhibiting cross-reactivity against the Envs, in contrast to bnAbs and other antibodies capable of binding to Env which respective germline-versions are unable to bind (Chen et al., 2010; Xiao et al., 2009). This may have significant bearing on the development of predecessor antibodies useful in the new vaccine design approach. Further, combined phage display and high-throughput sequencing methods helped identify several long, highly abundant CDR3s and highly mutated V-genes in V_H/V_L chains of the HIV-1-infected patient

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which may be potential candidates useful in the development of effective HIV-1 vaccines.

Materials and methods

Library construction and selection of antibodies against HIV-1 Envs

We isolated mRNA from frozen PBMCs that had been derived from an HIV-1 patient at two different time points in the period of about 40 days and 8 months post infection, converted them into cDNA, and constructed two separate Fab libraries encoding IgG heavy and light chains as briefly described previously (Chen et al., 2008). The two libraries were panned against Envs, a homologous gp140 and a consensus gp140, respectively. Procedures followed in this study were in accordance with the ethical standards of concerned institutional policies and the Research Donor Program of Frederick National Laboratory.

Expression, purification and binding of antibodies

Soluble Fabs of antibodies were expressed in *E. coli* and purified by affinity chromatography methods. To analyze the genetic origin and properties of the selected antibodies, heavy and light chain variable domains of these binders were sequenced. Further, binding and competition ELISAs were performed as described previously (Chen et al., 2010).

Analysis of antibody sequence diversity from the libraries and statistical calculations

The heavy (V_H) and light (V_L) chain variable domains of antibodies from the two libraries were sequenced using the high-throughput 454 sequencing pipeline method. The complete set of primers used in the PCR amplification of heavy and light chains, which also included adaptor sequences along with target amplification sequence, were described in detail elsewhere (Prabakaran et al., 2012a). For quality control of antibody sequences, we trimmed the sequence data and retained only sequences of length more than 300 nucleotides (nt), covering the entire antibody variable domains consisting of the three CDRs along with framework regions (FRs). We used the IMGT/HighV-QUEST analysis tool for genetic diversity analysis of antibody sequences (Alamyar et al., 2010). The output results from the IMGT/HighV-QUEST analysis in CSV files were stored at PostgreSQL database, and Structured Query Language (SQL) was effectively used to retrieve the data for the sequence analysis. Statistical calculations involving germline usage, distribution of antibody CDR3 lengths and somatic mutations were carried out using SAS JMP10® statistical software (SAS Institute, Cary, NC).

Results

Construction of antibody libraries from an HIV-1 patient and selection of antibodies against HIV-1 Envs

HIV-1 patient blood obtained from two time points at approximately 40 days and 8 months post infection was used to construct two Fab phage display libraries. To identify human IgG-derived antibodies specific for HIV-1 Envs, we panned the libraries against the homologous gp140 (CH12.0544.2 gp140) isolated from the patient and a consensus gp140 (Cons gp140) designed by aligning >1,000 sequences of group M. Upon panning of the library generated using serum collected at the first time point, we were able to select six unique specific binders (Fig. 1). Selection of antibodies were performed and screened by ELISA for specific binding to the targets and not to unrelated bovine serum

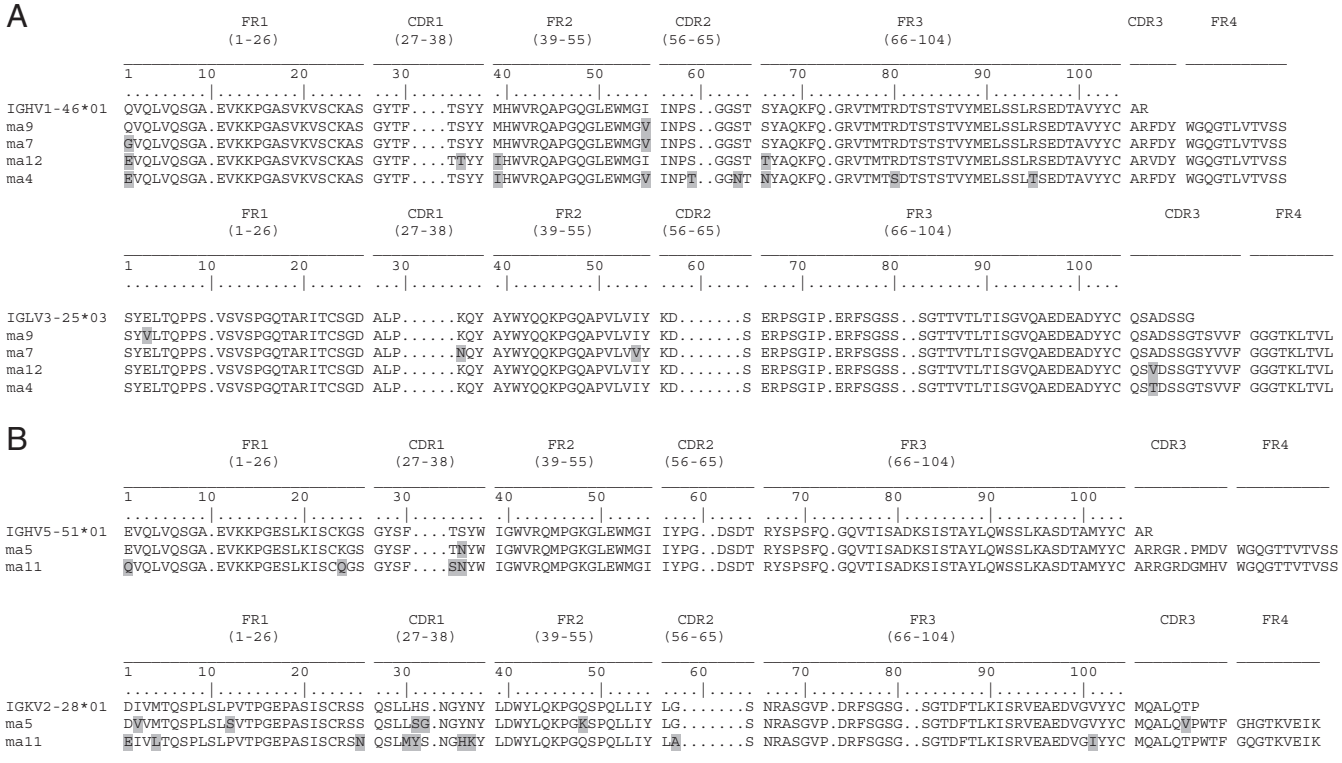

Fig. 1. The amino acid sequences of heavy and light chains of the selected group 1 (A) and group 2 (B) mAbs in alignment with the corresponding germlines of human antibody V genes. The CDRs and FRs are indicated according to the ImMunoGeneTics annotation (http://imgt.cines.fr/). The somatic mutations in the V genes of the selected antibodies are highlighted with gray color.