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Vaccine focusing to cross-subtype HIV-1 gp120 variable loop epitopes

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ABSTRACT

We designed synthetic, epitope-focused immunogens that preferentially display individual neutralization epitopes targeted by cross-subtype anti-HIV V3 loop neutralizing monoclonal antibodies (mAbs). Vaccination of rabbits with these immunogens resulted in the elicitation of distinct polyclonal serum Abs that exhibit cross-subtype neutralization specificities mimicking the mAbs that guided the design. Our results prove the principle that a predictable range of epitope-specific polyclonal cross-subtype HIV-1 neutralizing Abs can be intentionally elicited in mammals by vaccination. The precise boundaries of the epitopes and conformational flexibility in the presentation of the epitopes in the immunogen appeared to be important for successful elicitation. This work may serve as a starting point for translating the activities of human broadly neutralizing anti-HIV-1 monoclonal antibodies (bNAbs) into matched immunogens that can contribute to an efficacious HIV-1 vaccine.

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1. Introduction

The envelope spike of HIV (gp120, gp41) is highly antigenically variable and is the primary target of human antibody-mediated HIV neutralization [1–4]. Clinical and preclinical studies have demonstrated that most HIV-specific antibodies (Abs) elicited via gp120 immunization are strain-specific or narrowly reactive and cannot protect against HIV acquisition [5–12]. Nevertheless, a few Abs raised by immunization can protect against infection by HIV or related viruses in animal models [13–25] and in humans [26–29]. In addition, some monoclonal antibodies isolated from HIV-infected subjects cross-react broadly with HIV-1 viruses [30–35]. Focused presentation of the epitopes targeted by these Abs on non-HIV scaffolds has been proposed as a way to specifically elicit desirable Abs in mammalian hosts by vaccination, while avoiding elicitation of the type-specific or narrowly reactive Abs that dominate the response to immunization with whole gp120. "Epitope-focusing", which can be viewed as a form of "reverse vaccinology", has long been proposed as a method to achieve focused presentation of epitopes on synthetic immunogens [36,37]. These methods start from a high-resolution crystal structure of the complex of a neutralizing monoclonal antibody (mAb) isolated from HIV-infected human subjects and seek to mimic, by protein design, the 3D structure of the epitope within a non-HIV scaffold protein. To this point, immunogens designed to elicit specific anti-HIV bNabs in mammalian serum via vaccination using epitope-focused immunogens have uniformly failed to elicit cross-strain HIV neutralizing serum Abs [38–41].

We sought to design immunogens to specifically and preferentially elicit polyclonal cross-subtype HIV neutralizing Abs targeting cross-strain neutralizing epitopes also targeted by mAbs 3074 and 2219, which occur in the third sequence variable (V3) loop of gp120 in up to 80% of circulating HIV viruses [42–47]. We designed these immunogens using the novel epitope-focusing strategy described in this report, and tested them for immunogenicity along with native constructs in a parent study [48]. Here, we analyze in detail the functional and structural properties of these constructs with regard to whether we preferentially elicited polyclonal serum antibodies mimicking the specificity of the mAb, based on which the immunogen used for elicitation was designed.

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2. Methods

2.1. Immunogen design and production

Antigens, consisting of sequence-modified V3 loops, for both the protein immunogens and for their mirror chimeric SF162-V3 loop pseudoviruses (psVs) were designed as described in Fig. 1.

V3 loop sequences designed in this manner were inserted into a cholera toxin subunit B (CTB) scaffold at an appropriate fusion point, and tested by ELISA for reactivity with specific mAbs as previously described [49]. Genes for CTB with the indicated inserts consisting of full-length V3 loops with the V3 crown sequence modified as in Fig. 1 were chemically synthesized and cloned into pSUMO plasmids, followed by expression in *E. coli* and purification by affinity chromatography. The final sequences for the designed immunogens were:

2219- MTPQNITDLC AEYHNTQIHT LNDKIFSYTE SLAGKREMAI ITF**CTRPSNN TRKSI*NF*GPG QTFYATGEIIGDIRQAHC**AT FQVEVPGSQH IDSQKKAIER MKDTLRIAYL TEAKVEKLCV WNNKTPRAIA AISMAN

3074- MTPQNITDLC AEYHNTQIHT LNDKIFSYTE SLAGKREMAI ITF**CTRPSNN T*TE*SI*NIGPG QTFYATGEIIGDIRQAHC**AT FQVEVPGSQH IDSQKKAIER MKDTLRIAYL TEAKVEKLCV WNNKTPRAIA AISMAN

The inserted V3 sequence is in bold and the mutations are italics; Fig. 1.

2.2. Binding of the immunogen constructs to anti-V3 mAbs

Immunogen-Ab binding was evaluated by ELISA as previously described [49].

2.3. Rabbit immunizations

The DNA-prime, protein-boost immunizations and serum harvesting were performed as previously described [50]. A codon-optimized *env* gene from HIV clade C primary isolate 92BR025.9 for the DNA prime was prepared where the V3 sequence is: CTRPNNNTRKSIRIGPGQAFYATGEIIGDIRQAHC. Five animals of each group received the DNA prime 3 times via Gene Gun followed by two boosts with either $V3_{2219}$-CTB, $V3_{3074}$-CTB or $V3_{447}$-CTB (V3 sequence is identical with clade B consensus) at weeks 10 and 14. A total of 100 μg/per injection of each V3-CTB was administered intramuscularly with incomplete Freund's adjuvant (IFA). Blood samples were collected prior to immunization and two weeks after each immunization.

2.4. Virus construction

Chimeric pseudoviruses (psVs) were constructed and produced by standard methods that have been previously described [51]. SF162 Env variants containing modified V3 sequences were generated by sequentially introducing the necessary modifications by site-directed mutagenesis using the QuikChange kit, as described by the manufacturer (Stratagene, Inc.). The sequences of all mutant Envs were confirmed by sequencing the complete gene (Genewiz, Inc.).

The sequences of the V3 loops of the chimeric psVs used in neutralization experiments in Fig. 2 were:

psV-SF162-$V3^{2219}$: CTRPSNNTRKSINFGPGQAFYATGDIIGDIRQAHC
psV-SF162-$V3^{3074}$: CTRPSNNTRESIRIGPGQTFYATGDIIGDIRQAHC

These have the same distribution of 2219, 3074 and 447 epitopes as the V3 loop sequences inserted into the scaffold to produce $V3_{2219}$-CTB or $V3_{3074}$-CTB, but they differ in the minor underlined positions from the immunogen V3 loop sequences (as they were constructed for testing before the immunogen designs were finalized). These minor non-epitope amino acid differences are believed not to have a significant structural influence on the V3 loop crown due to the Ab specific behavior of similarly altered psV. The sequences of the chimeric psV's bearing consensus subtype V3 loop sequence shown in Fig. 3 were previously published [50]. The sequences of the V3 loops of the chimeric psVs with specific epitopes perturbed (Fig. 5B) are:

Consensus B: CTRPNNNTRKSIHIGPGRAFYTTGEIIGDIRQAHC
"−447, +2219, +3074": CTRPNNNTRKSIHIGPG**Q**AFYTTGEIIGDIRQAHC
"−3074, +2219": CTRPNNNTRKSIH**M**GPGRAFYTTGEIIGDIRQAHC
"−2219, +3074": CTRPNNNTR**E**SIHIGPGRAFYTTGEIIGDIRQAHC
"−3074, −2219": CTRPNNNTR**E**SIH**M**GPGRAFYTTGEIIGDIRQAHC

where the bolded underlined residues are the mutations perturbing the respective epitopes.

2.5. Neutralization assays

Neutralization assays using chimeric psVs were performed as described previously [47,51]. Standard NAb and serum neutralization assays performed by the Vaccine Immune Monitoring Center of the Center for AIDS Vaccine Collaboration were also performed as previously described [52,53]. Briefly, 8 pseudoviruses from the clade B and 6 from the clade C standard Tier 2 psV panels [52,54] were used along with an additional four Tier 1A and Tier 1B psVs from clades AG, B and C. Two-fold serial dilutions of heat-inactivated sera were prepared starting at a dilution of 1:10. The serum/psV mixtures were then incubated with the TZM.bl target cells and luciferase activity was measured at 48 h. Pools of pre-bleed sera were tested as negative controls against each psV, and all sera were also tested against a negative control psV carrying the envelope of murine leukemia virus. The percent neutralization was calculated relative to the effect of the pre-immune serum from the same rabbit at the same dilution. All sera were assayed in duplicate in at least two experiments against each virus. The 50% neutralizing titers (NT_{50}) were determined using the method of Least Squares.

3. Results

Three structural classes of V3 loop-targeted cross-subtype neutralizing mAbs have been defined: those similar to mAb 3074, those similar to mAb 2219 and those similar to mAb 447-52D [44,56–58]. We designed antigenic sequences to mimic the flexible structure of the crown of the V3 loop with amino acid mutations incorporated to disrupt the neutralization epitopes targeted by 447-52D but preserve the neutralization epitopes targeted by 3074 or 2219 respectively (Fig. 1). The same approach was applied to the other two V3 mAbs (3074 and 2219). The designed sequences consisted of the consensus subtype C V3 loop sequence with point mutations introduced to "knock out" the neutralization epitopes targeted by 447-52D and either 2219 or 3074 (see Section 2). Briefly, we reasoned that the peptide segment bearing the desired epitope needed to be present in a flexible loop and that this could be achieved by starting with a known V3 loop (consensus subtype C sequence) and introducing point mutations to "knock out" all but one desired neutralization epitope. The identity of the specific amino acid

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