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Synthetic Mimicry of the CD4 Binding Site of HIV-1 gp120 for the Design of Novel Immunogens[**]

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Despite enormous efforts in basic and clinical research, HIV-1 vaccine development is greatly hampered by the difficulty in eliciting a virus-neutralizing antibody response.[1] The virus evades the host immune response by exposing variable and glycosylated regions, respectively, on its exterior envelope, while the conserved binding sites for its cellular receptors are either buried, (CD4 binding site),[2] or exposed only upon binding to CD4 (co-receptor binding sites).[3] Innovative strategies in immunogen design are needed to address the neutralizing antibody issue, which remains one of the most difficult problems to be solved in the development of a successful HIV-1 vaccine.[4] Only a few human monoclonal antibodies have been identified thus far, which efficiently neutralize a broad range of primary HIV-1 isolates in vitro, and protect against viral challenge in vivo.[5] The CDR H3 loop of one of these broadly neutralizing antibodies, mAb b12,[6,7] closely resembles the gp120-binding CDR2-like loop of CD4, and fits into a recessed pocket of gp120 that constitutes its binding site for CD4 (CD4bs), as shown by crystal structure analysis and molecular modeling studies,[5] as well as by site-directed mutagenesis of gp120.[8] The epitope for mAb b12 has therefore been postulated to overlap the CD4bs, which represents a conserved region in this otherwise highly variable protein. Consequently, synthetic mimetics of the CD4bs are promising immunogen candidates for the elicitation of virus-neutralizing antibodies.[9]

Based on the crystal structure of core gp120 complexed with an extracellular two-domain fragment of CD4,[2,10] the primary gp120 contact residues (D368, E370, W427 and D457) for its interaction with CD4 could be identified, which are located in three sequentially distant regions of gp120 (Figure 1). Mutation of these residues abrogates binding of gp120 to CD4.[11] Using previously established strategies for the generation of scaffolded peptides for the synthetic mimicry of discontinuous protein binding sites,[12,13] we have recently generated scaffolded peptides presenting three gp120 fragments that comprise its primary contact residues for CD4. These peptides were found to compete with gp120 for binding to CD4 and mAb b12, respectively,[14] and now serve as a template for rational vaccine design. This approach is based on the premise that the correct presentation of the CD4bs in synthetic mimetics will enable the elicidation of antibodies that recognize the CD4bs also within the structural context of viral gp120, and thus neutralize the virus.[15]

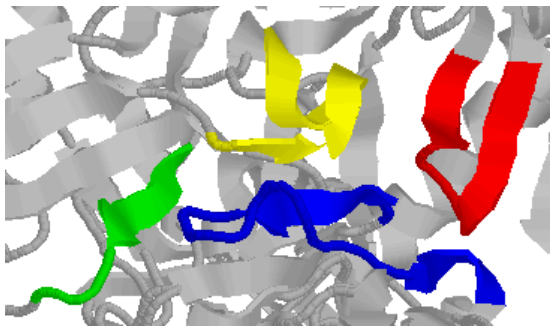
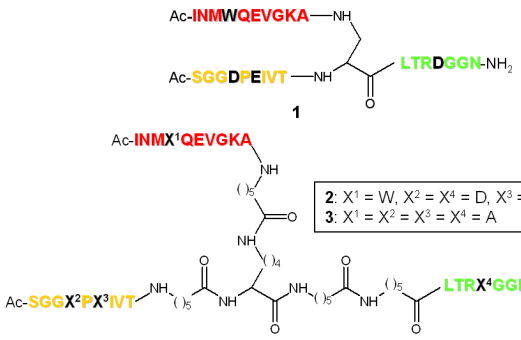


Figure 1. Section of the crystal structure of the complex of core gp120 with D1D2-CD4 (pdb entry 1RZJ),[10] showing the interface of gp120 and CD4, which involves gp120 fragments 424-433 (red), 365-373 (yellow), and 454-460 (green), as well as the CDR2-like loop of CD4 (blue).

Peptides **1** and **2**, presenting three gp120 fragments that make up its primary binding site for CD4, i.e. 424INMWQEVGKA433 (Fragment A), 365SGGDPEIVT373 (Fragment B), and 454LTRDGGN460 (Fragment C), through conformationally flexible scaffolds, were generated by solid-phase peptide synthesis, and obtained in high purity after purification by preparative HPLC (see Supporting Information for analytical data).



Both peptides **1** and **2** were found to compete with recombinant monomeric gp120(IIIB) for binding to recombinant two-domain CD4 (2D-sCD4) (Table 1 and Figure 2, top). The affinity to CD4 of **2**, however, was substantially higher than that of **1**, indicating an arrangement of fragments and spacer residues in **2** that better provides for

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

a presentation of CD4bs fragments that resembles their orientation and distances in the structure of the gp120-CD4 complex[10] (T373-A433: 18 Å, T373-L454: 13 Å, L454-A433: 26 Å. Although the affinity of **2** to 2D-sCD4 was approximately 170-fold lower than that of gp120(IIIB) (Table 1), the binding specificity of **2** is similar to gp120, since substitution of the four primary contact residues (D368, E370, W427 and D454) in **2** with alanine (peptide **3**) resulted in loss of affinity to CD4 (Table 1). Peptide **2** can thus be considered a functional mimetic of the CD4bs of gp120.

The affinities of the three individual fragments (A: Ac-INMWQEVGKA-NH_2, B: Ac-SGGDPEIVT-NH_2, and C: Ac-LTRDGGN-NH_2) were largely abolished (Table 1), indicating a synergistic effect of combining all three fragments in one molecule. Furthermore, analogs of **2**, in which Fragments A and B, respectively, were deleted (**4** and **5**), had no affinity to CD4, while the affinity of an analog lacking Fragment C (**6**) was only slightly decreased (Table 1, see Supporting Information for peptide structures), pointing to a lesser importance of Fragment C for the interaction with CD4. This result is in agreement with the known weaker contribution of D457 to the interaction of gp120 with CD4, as compared to the other three primary contact residues.[2]

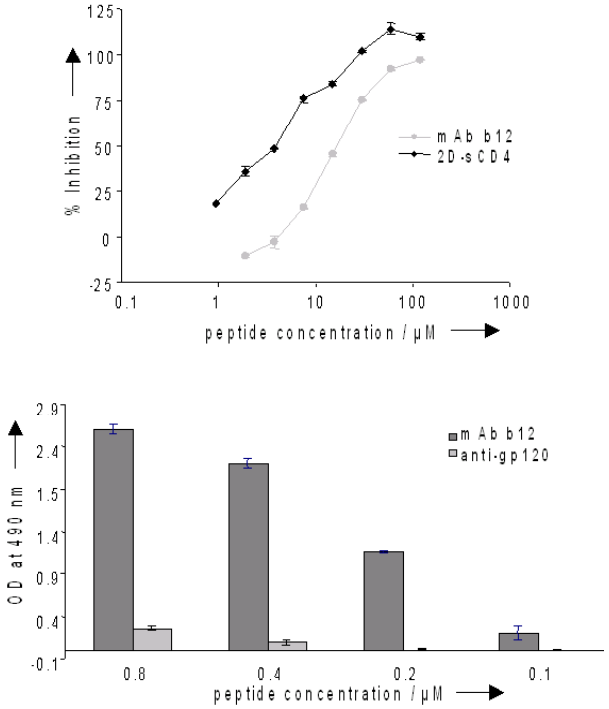

Figure 2. Top: Competition of **2** with gp120(IIIB) for binding to plate-bound mAb b12, and 2D-sCD4, respectively. Bottom: Direct binding of biotinylated **2** to plate-bound mAb b12, and an antibody that recognizes the V3 loop of gp120 (anti-gp120), respectively.

In addition to its affinity to CD4, **2** was also specifically recognized by mAb b12, but not by an antibody whose epitope has been mapped to the V3 loop of gp120 (Figure 2, bottom). Furthermore, **2** was shown to compete with gp120 for binding to mAb b12 (Figure 2, top, IC_{50} = 17 µM). Taken together, these results reconfirm the postulated overlap of the CD4bs of gp120 with the epitope for mAb b12, rendering **2** a promising starting point for vaccine design.

Consequently, **2** was used as a synthetic immunogen to raise anti-peptide antisera. Rabbits were immunized with conjugate **7** (see Supporting Information for structure), in which **2** was covalently linked to the carrier protein KLH via an additional cysteine residue attached to **2**. The resulting polyclonal antisera specifically recognized gp120, as they did not bind to the extracellular domain of another receptor glycoprotein, gp130 (Figure 3). Furthermore, serum taken from the same rabbit prior to immunization with the **2**-KLH conjugate (pre-immune serum), did not recognize gp120. The anti-**2**-antisera were also found to compete with mAb b12 for binding to gp120 (Figure 4), indicating the presence of antibodies having binding specificities related to mAb b12, in the anti-**2**-antisera.

Table 1. IC_{50} values of peptides, gp120(IIIB) and mAb b12 in the competitive gp120(IIIB) – 2D-sCD4 binding assay.

Peptide	IC_{50} [µM]
1	28
2	6
3	>120
4	>120
5	>120
6	24
Fragment A	91
Fragment B	>120
Fragment C	>120
gp120(IIIB)[a]	0.035
mAb b12	0.033

[a]gp120-peroxidase conjugate was used as ligand.

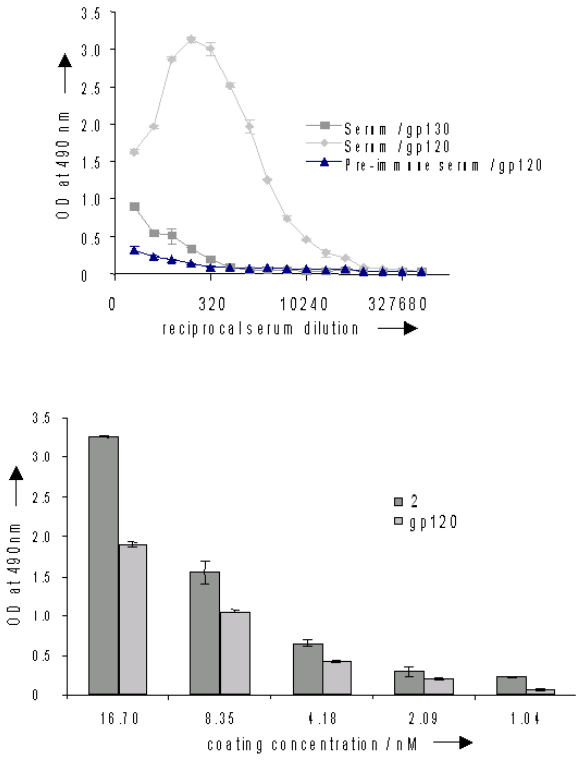

Figure 3. Recognition of gp120(IIIB) by anti-**2**-antiserum. Bottom: Direct binding of anti-**2**-antiserum (dilution: 1: 4000) to plate-bound **2** and gp120(IIIB), respectively. Top: Concentration-dependent binding of anti-**2**-antiserum and pre-immune serum, respectively, to plate-bound gp120(IIIB) and gp130, respectively.

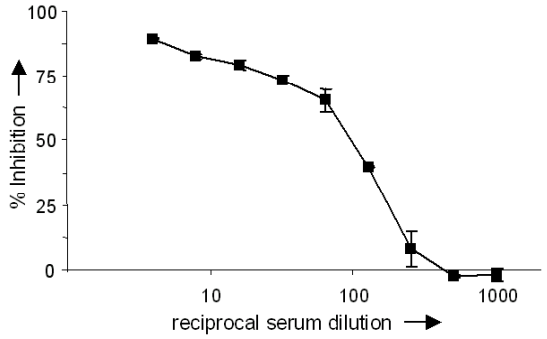

Figure 4. Competition of anti-**2**-antiserum with plate-bound mAb b12 for binding to gp120(IIIB).

In conclusion, structure-based design was used to generate a synthetic peptide that functionally mimics the CD4 binding site of HIV-1 gp120, as it competes with gp120 for binding to CD4 and the broadly neutralizing mAb b12, respectively. Polyclonal antisera raised against this peptide were found to recognize gp120 with a specificity related to that of mAb b12. Ongoing studies addressing the issue of structural mimicry of the CD4bs of gp120 by synthetic peptides, through structural analysis of complexes of such peptides with CD4 and mAb b12, respectively, are expected to provide insight into these interactions at the atomic level, which will guide the design of improved synthetic mimetics of the CD4 binding site of gp120 as immunogen candidates for the elicitation of broadly neutralizing anti-HIV-1 antibodies.

Experimental Section

Peptides were synthesized as C-terminal amides by automated Fmoc/t-Bu-based solid-phase synthesis. In order to prevent aspartimide formation within the sequence of Fragment C (LTRDGGN), the dipeptide DG was introduced by coupling of Fmoc-Asp(OtBu)-(Hmb)Gly-OH. Excess of HOBt was used to re-open the lactone that may result from reaction of the phenolic hydroxyl with the carboxyl group of the protected dipeptide, and to subsequently form the OBt ester. After completing the sequence of Fragment C, the scaffold sequence was assembled, which included Lys(ivDde) (**2**, **3**) and Dap(ivDde) (**1**) residues, respectively, followed by the sequence of Fragment B (Ac-SGGDPEIVT). After removing the ivDde group from the Lys and Dap side chains, respectively, the resulting free amino group was acylated with Ahx (**2**, **3**), and Fragment A (Ac-INMWQEVGKA) was assembled. Peptides were cleaved from the resin using a mixture of TFA/DCM/triisopropylsilane/water, and purified by preparative HPLC.

Binding assays were performed in 96-well microtiter plates using a peroxidase-based colorimetric readout.

See Supporting Information for experimental detail.

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