DOI: 10.1002/anie.200603274

Peptidomimetics

Synthetic Mimetics of the CD4 Binding Site of HIV-1 gp120 for the Design of Immunogens**

*Raimo Franke, Tatjana Hirsch, Heike Overwin, and Jutta Eichler**

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 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 (coreceptor 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] Thus far, only a few human monoclonal antibodies have been identified that efficiently neutralize a broad range of primary HIV-1 isolates in vitro and protect against viral challenges 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). This has been 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 contact residues of gp120 (D368, E370, W427, and D457) for its interaction with CD4 could be identified. These residues are located in three sequentially distant regions of gp120 (Figure 1). Mutation of these residues abrogates binding of gp120 to CD4.[11] By using previously established strategies for the generation of scaffolded peptides as synthetic mimetics 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,[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 generation of antibodies that recognize the CD4bs within the structural context of viral gp120, and thus neutralize the virus.[15]

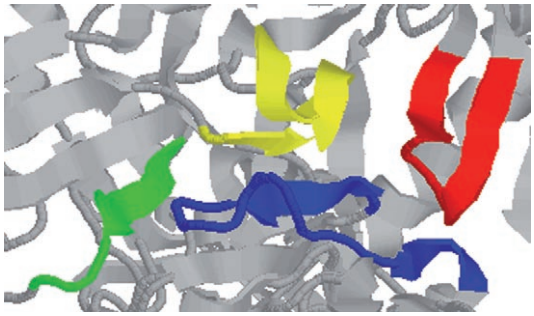
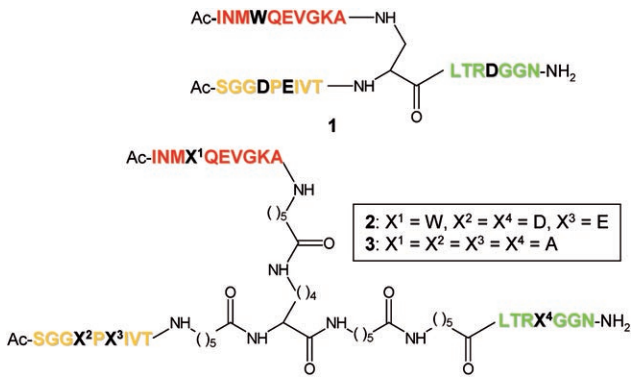


Figure 1. Section of the crystal structure of the complex of core gp120 with D1D2–CD4 (PDB entry 1RZJ).[10] The figure shows the interface of gp120 and CD4, which involves gp120 fragments 424–433 (red), 365–373 (yellow), and 454–460 (green) as well as the CDR2-like loop of CD4 (blue).

Peptides **1** and **2**, which present three gp120 fragments that make up the primary binding site for CD4, that is, 424INMWQEVGKA433 (Fragment A), 365SGGDPEIVT-373 (Fragment B), and 454LTRDGGN460 (Fragment C), through conformationally flexible scaffolds, were generated by solid-phase peptide synthesis. The peptides were obtained with high purity after purification by preparative HPLC (see the Supporting Information).

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



[*] R. Franke, T. Hirsch, H. Overwin, Priv.-Doz. Dr. J. Eichler
Helmholtz Centre for Infection Research
Inhoffenstrasse 7, 38124 Braunschweig (Germany)
Fax: (+49) 531-6181-3499
E-mail: jutta.eichler@helmholtz-hzi.de

[**] This work was supported by the BioFuture Program (grant no. 0311882) of the German Federal Department of Education and Research (BMBF). 2D-sCD4 (cat. no. 7356) and mAb b12 (cat. no. 2640) were obtained through the NIH AIDS Research and Reference Reagent Program. We thank Christian Erck for preparing the immunogen conjugate.

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

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

[a] A gp120 peroxidase conjugate was used as the ligand.

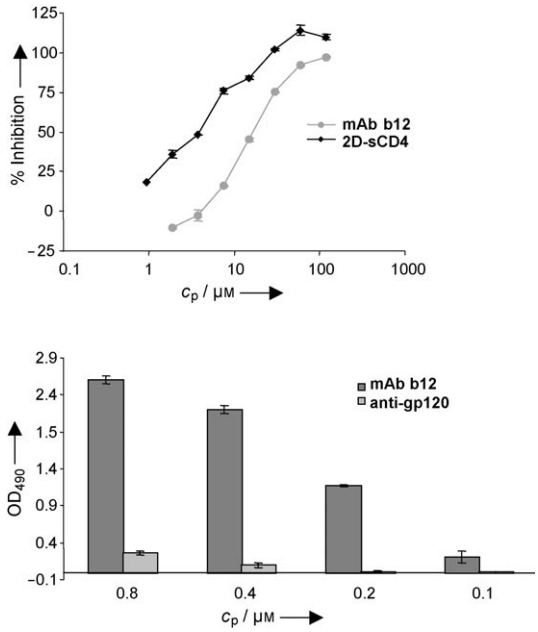


Figure 2. Top: Competition of **2** with gp120(IIIB) for binding to immobilized mAb b12 and 2D-sCD4. Bottom: Direct binding of biotinylated **2** to immobolized mAb b12 and to an antibody that recognizes the V3 loop of gp120 (anti-gp120). c_p = peptide concentration, OD_{490} = the optical density at 490 nm.

than that of **1**. This indicates an arrangement of fragments and spacer residues in **2** that better presents the CD4bs fragments and resembles the orientation and distances in the structure of the gp120-CD4 complex more than that of **1**[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, as substitution of the four primary contact residues (D368, E370, W427, and D454) in **2** with alanine (peptide **3**) resulted in a 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, analogues of **2**, in which Fragments A and B were deleted (**4** and **5**, respectively), had no affinity to CD4, whereas the affinity of an analogue lacking Fragment C (**6**) was only slightly decreased (Table 1 and the Supporting Information for peptide structures). This suggests a lesser importance of Fragment C for the interaction with CD4 which is in agreement with the known weaker contribution of D457 to the interaction of gp120 with CD4, as compared with the other three primary contact residues.[2]

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; IC_{50} = half maximal inhibitory concentration). 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 antipeptide antisera. Rabbits were immunized with conjugate **7** (see the Supporting Information), in which **2** was covalently linked to the carrier protein keyhole limpet hemocyanin (KLH) through an additional cysteine residue attached to **2**. The resulting polyclonal antisera specifically recognized gp120; that is, 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 **7** (preimmune serum) did not recognize gp120. The anti-**2**-antisera were also found to compete with mAb b12 for binding to gp120 (Figure 4),

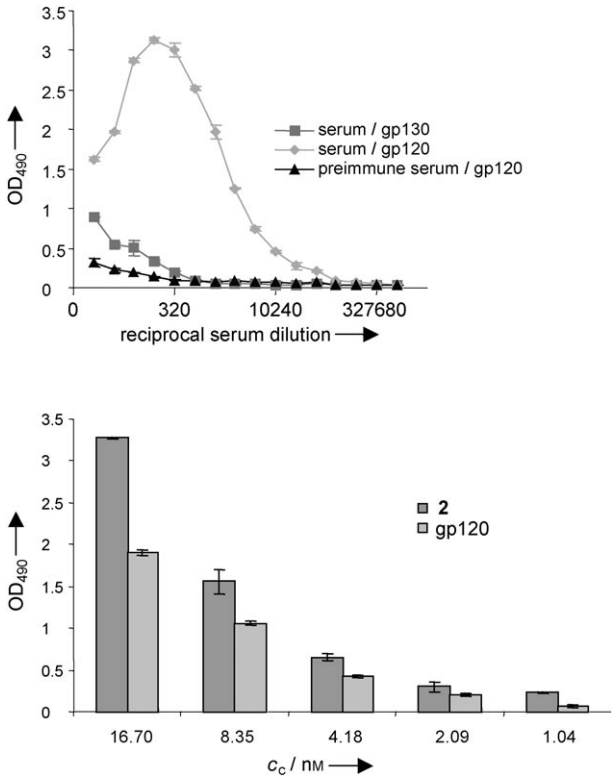


Figure 3. Recognition of gp120(IIIB) by anti-**2**-antiserum. Top: concentration-dependent binding of anti-**2**-antiserum and preimmune serum to immobolized gp120(IIIB) and gp130. Bottom: Direct binding of anti-**2**-antiserum (dilution: 1:4000) to immobolized **2** and gp120(IIIB). c_c = coating concentration.

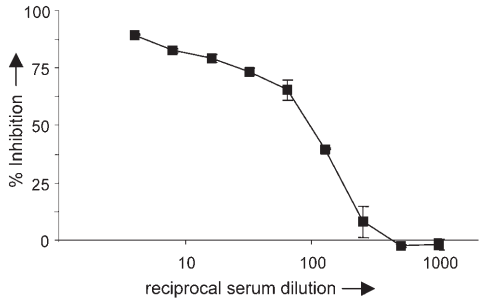


Figure 4. Competition of anti-**2**-antiserum with immobolized mAb b12 for binding to gp120(IIIB).

indicating the presence of antibodies with binding specificities related to mAb b12, in the anti-**2**-antisera.

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. Polyclonal antisera raised against this peptide were found to recognize gp120 with a specificity related to that of mAb b12. Ongoing studies that address the issue of structural mimicry of the CD4bs of gp120 by synthetic peptides through structural analysis of complexes of peptides with CD4 and mAb b12 are expected to provide insight into these interactions at the atomic level. This will in turn 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 (Fmoc = 9-fluorenylmethoxycarbonyl). To prevent aspartimide formation within the sequence of Fragment C (LTRDGGN), the dipeptide DG was introduced by coupling of Fmoc-Asp(O*t*Bu)-(Hmb)Gly-OH (Hmb = 2-hdroxy-4-methoxybenzyl). Excess of *N*-hydroxybenzotriazole (HOBt) was used to reopen the lactone, which may result from reaction of the phenolic hydroxy group with the carboxy group of the protected dipeptide, to subsequently form the OBt ester. After the sequence of Fragment C was completed, the scaffold sequence was assembled, which included Lys(ivDde) (**2**, **3**) and Dap(ivDde) (**1**; Dap = 2,3-diaminopropionic acid, ivDde = 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl) residues followed by the sequence of Fragment B (Ac-SGGDPEIVT). After removing the ivDde group from the Lys and Dap side chains, the resulting free amino group was acylated with Ahx (6-aminohexanoic acid) (**2**, **3**), and Fragment A (Ac-INMWQEVGKA) was assembled. Peptides were cleaved from the resin by using a mixture of CF_3COOH/CH_2Cl_2/triisopropylsilane/water and purified by preparative HPLC.

Binding assays were performed in 96-well microtiter plates by using a peroxidase-based colorimetric readout (see the Supporting Information for experimental details).

Received: August 10, 2006
Revised: November 1, 2006
Published online: January 9, 2007

Keywords: biomimetic synthesis · HIV-1 gp120 · peptides · protein design · synthetic vaccines

[1] D. R. Burton, *Proc. Natl. Acad. Sci. USA* **2005**, *94*, 10018.
[2] P. D. Kwong, R. Wyatt, J. Robinson, R. W. Sweet, J. Sodroski, W. A. Hendrickson, *Nature* **1998**, *398*, 648.
[3] L. Wu, N. P. Gerard, J. R. Wyatt, H. Choe, C. Parolin, N. Ruffing, A. Borsetti, A. A. Cardoso, E. Desjardin, W. Newman, C. Gerard, J. Sodroski, *Nature* **1996**, *384*, 179.
[4] D. R. Burton, R. C. Desrosiers, R. W. Doms, C. W. Koff, P. D. Kwong, J. P. Moore, G. J. Nabel, J. Sodroski, I. A. Wilson, R. Wyatt, *Nat. Immunol.* **2004**, *5*, 233.
[5] E. O. Saphire, P. W. H. I. Parren, R. Pantophlet, M. B. Zwick, G. E. Morris, P. M. Rudd, R. A. Dwek, R. L. Stanfield, D. R. Burton, I. A. Wilson, *Science* **2001**, *293*, 1155.
[6] C. F. Barbas III, E. Bjorling, F. Chiodi, N. Dunlop, D. Cababa, T. M. Jones, S. L. Zebedee, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9339.
[7] D. R. Burton, J. Pyati, R. Koduri, S. J. Sharp, G. B. Thornton, P. W. H. I. Parren, L. S. W. Sawyer, R. M. Hendry, N. Dunlop, P. L. Nara, M. Lamacchia, E. Garratty, E. R. Stiehm, Y. J. Bryson, Y. J. Cao, J. P. Moore, D. D. Ho, C. F. Barbas III, *Science* **1994**, *266*, 1024.
[8] R. Pantophlet, E. E. Saphire, P. Poignard, P. W. H. I. Parren, I. A. Wilson, D. R. Burton, *J. Virol.* **2003**, *77*, 642.
[9] S. E. M. Howie, G. J. Cotton, I. Heslop, N. J. Martin, D. J. Harrison, R. Ramage, *FASEB J.* **1998**, *12*, 991.
[10] C. Huang, M. Venturi, S. Majeed, M. J. Moore, S. Phogat, M. Y. Zhang, D. S. Dimitrov, W. A. Hendrickson, J. Robinson, J. Sodroski, R. Wyatt, H. Choe, M. Farzan, P. D. Kwong, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 2706.
[11] U. Olshevsky, E. Helseth, C. Furman, J. Li, W. Haseltine, J. Sodroski, *J. Virol.* **1990**, 64, 5701.
[12] R. Franke, C. Doll, V. Wray, J. Eichler, *Protein Pept. Lett.* **2003**, *10*, 531.
[13] R. Franke, C. Doll, V. Wray, J. Eichler, *Org. Biomol. Chem.* **2004**, *2*, 2847.
[14] R. Franke, T. Hirsch, J. Eichler, *J. Recept. Signal Transduct.* **2006**, 26, 453.
[15] D. C. Douek, P. D. Kwong, G. J. Nabel, *Cell* **2006**, *124*, 677.

