HIV binding to its receptor creates specific epitopes for the CD4/gp120 complex

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ABSTRACT **Effective vaccines against the human immunodeficiency virus (HIV) must cope with the genetic variation of the viral envelope (gp120) to combat or prevent acquired immunodeficiency syndrome (AIDS). Here we describe novel epitopes that are accentuated when gp120 complexes with its receptor (CD4). The presentation of these epitopes results through conformational rearrangements in the CD4/gp120 complex. Monoclonal antibodies directed to these epitopes inhibit syncytium formation, thus indicating the potential use of these epitopes as subunit vaccines.—Gershoni, J. M., Denisova, G., Raviv, D., Smorodinsky, N. I., Buyaner, D. HIV binding to its receptor creates specific epitopes for the CD4/gp120 complex. *FASEB J.* 7: 1185–1187; 1993.**

Key Words: AIDS • HIV • receptor/ligand interactions • vaccine

VIRUSES CHARACTERISTICALLY ATTACK SPECIFIC tissues or cell types, a phenomenon that is the result of a selective recognition process between the components of the viral envelope and cellular receptors on the surface of the target organ. In the particular case of acquired immunodeficiency syndrome (AIDS),[2] the first step in HIV infection of T4 lymphocytes is the binding of the viral particle to its corresponding receptor (1). In molecular terms this equates to the association of the viral envelope-protein gp120 to the lymphocyte membrane glycoprotein CD4 (2). This recognition event has been identified as a target for the development of novel therapeutics. Most notable, for example, are molecular decoys that have been suggested based on the concept that a derivative of a natural receptor can intercept a pathogen before it reaches its target, thus neutralizing its effect (3). In fact, soluble CD4 (sCD4) and its modifications have been tested for their therapeutic potential and much work is being conducted in this field (2, 3; see also ref 4). The main attribute of this approach lies in the fact that the aspects of the viral envelope responsible for receptor recognition are not permitted to alter, and by definition must remain "conserved." Clearly, therefore, development of an immune response directed to these conserved domains of gp120 should act as a functional protective vaccine. Unfortunately, the areas of gp120 responsible for the recognition do not appear to be very immunogenic and thus generation of engineered decoys is an attractive alternative to simply relying on a natural immune response against these selected epitopes.

Another set of epitopes that should prove to have "vaccine potential" would be additional conserved domains of the viral envelope, which when complexed with corresponding antibodies prevent viral pathogenesis.

Such an alternative direction is described in this study where focus is placed on a different aspect of the CD4/gp120 interaction that has recently been recognized; after binding, postbinding phenomena associated with the complex that are crucial for viral entry exist, and thus provide a new target for drug design (5, 6). Specifically, once gp120 binds to CD4 two events occur; dissociation of gp120 from its envelope anchor, gp41, leading to shedding of the former; and exposure of a hydrophobic region of gp41, which enables membrane fusion (7). Proof for the involvement of CD4 in these processes lies in the ability to produce monoclonal antibodies toward CD4 that prevent infection without interfering with CD4/gp120 complex formation (8, 9).

In view of these findings it has been proposed (8) that upon CD4/gp120 complex formation, conformational rearrangements of the proteins could occur that might reveal otherwise cryptic epitopes. These novel epitopes could be either exclusively complex-dependent or at least markedly enhanced in the complex and thus would be favored candidates for subunit vaccines were antibodies directed against them found to be neutralizing and of broad cross-reactivity.

RESULTS

In order to test these ideas we immunized mice with a preparation of the CD4/gp120 complex. Monoclonal antibodies derived from such mice were screened first against the CD4/gp120 complex itself and subsequently against sCD4 and gp120 individually. In this manner mAbs directed against complex related epitopes could be identified. **Table 1** summarizes the characteristics of five mAbs derived from such an experiment. As can be seen, three of the mAbs have preferred affinity for the complex over the individual proteins. Notable is mAb CG-10 that appears to be exclusively specific for the complex, a fact that is also demonstrated in **Fig. 1.** In this experiment protein blots of either gp120 or sCD4 were prepared and probed with the mAbs. Alternatively, the blots were first incubated with sCD4 or gp120, respectively, to generate complexes in situ on the blot and then probed with the mAbs. As is illustrated, CG-10 bound only to the blots that contained complex and therefore no direct evidence exists indicating which of these proteins harbors the CG-10 epitope. Alternatively, one cannot exclude the possibility that the epitope of CG-10 might be comprised of residues contributed by both components of the complex. Each of the five mAbs were then biotinylated and competitive ELISA experiments allowed the conclusion that the mAbs represent four distinct epitopes (see **Table 2**).

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[2]Abbreviations: AIDS, acquired immunodeficiency syndrome; sCD4, soluble CD4; HIV, human immunodeficiency virus.

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TABLE 1. *Binding of mAbs to CD4/gp120 and its components*[a]

	mAb	CD4/gp120	gp120	CD4
1	CG-4	0.63	0.64	0.11
2	CG-9	1.03	0.21	0.62
3	CG-10	0.94	0.20	0.15
4	CG-25	1.04	0.23	0.50
5	CG-76	1.04	0.15	1.31

[a]A BALB/c mouse was inoculated with CD4/gp120 complex and its splenocytes were used to fuse with NS-0 myeloma cells (kindly provided by C. Milstein, Cambridge, England). These five clones were all found to be IgG1 and were analyzed for their ability to bind CD4/gp120 complex or each protein individually by standard ELISA assay (OD_{405nm} values are given). Soluble CD4 (DuPont Biotechnology Systems, Mass.) and recombinant gp120 (ABT, MA) were mixed at a molar ratio 1:1 in Tris-buffered saline and used to immunize BALB/c mice in complete Freund's adjuvant. Monoclonal antibodies were produced using standard procedures. ELISA was performed by first producing the complex in solution and using it or the separate proteins to coat wells of EIA plates (Costar, Mass.). Detection of IgG was with alkaline phosphatase conjugated goat anti-mouse antibody (Sigma, Mo.).

TABLE 2. *Epitope definition by competitive ELISA*[a]

	mAb	CG-4	CG-9	CG-10	CG-25	CG-76
1	CG-4	79	10	0	4	13
2	CG-9	20	90	26	88	0
3	CG-10	7	5	93	8	32
4	CG-25	34	96	32	94	23
5	CG-76	ND	ND	ND	ND	96

[a]The mAbs were biotinylated with *d*-biotin-N-hydroxy succinnimide ester (Sigma, Mo.) and each of the biotinylated mAbs was used against all the others in competitive ELISA in which CD4/gp120 complex was used to coat the wells. Figures represent % inhibition taking the value in the absence of any competing antibody as 100%. ND, not determined.

The conformational nature of the epitopes defined by the mAbs CG-9, CG-25, and CG-76 was evaluated as is demonstrated in **Fig. 2** where protein blots of CD4, denatured to various degrees, were analyzed. It appears that the epitope for CG-76 is particularly sensitive to reduction of CD4. Note also its affinity for the dimer of CD4 (indicated by the arrow in the figure). Although monoclonals CG-9 and CG-25 map to the same region on CD4 (Table 2), they are not identical, as can be illustrated by the differential sensitivity of the two toward β-mercaptoethanol.

If the epitopes defined by these mAbs are to be considered as subunit vaccine candidates, it is necessary to evaluate their neutralizing potential. For this each antibody was tested for its inhibitory effects in the syncytium assay described by Ashorn et al. (10). **Figure 3** presents examples of such assays under various conditions. Whereas mAb CG-4 had no effect (data not shown), all the other mAbs were found inhibitory to different degrees, the strongest being CG-9 and the weakest being CG-10.

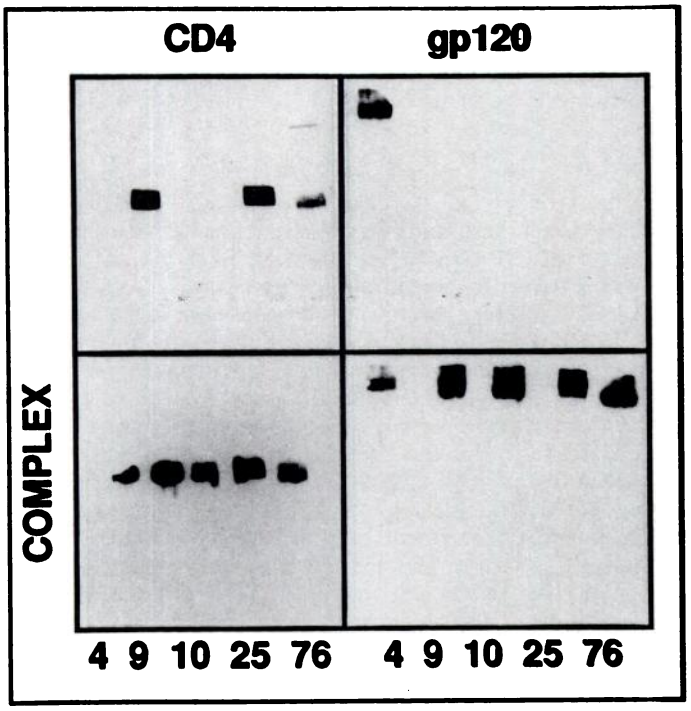

Figure 1. Reaction of mAbs with antigens (CD4, gp120, or in situ produced complex) by protein blot analysis. 1 μg of gp120 or CD4 was run on 10% SDS-polyacrylamide gels and then transferred to nitrocellulose membranes (for details of the blotting procedures, see ref 14). The membranes were quenched with 5% milk in TBS and incubated directly with the mAbs CG-4, CG-9, CG-10, CG-25, and CG-76 (designated 4, 9, 10, 25, and 76, respectively, in the top two panels) or first preincubated overnight +4°C with a solution of gp120 (10 μg/ml, bottom left) or CD4 (10 μg/ml, bottom right) followed by the mAbs. The secondary probe was a goat-anti-mouse horseradish peroxidase conjugate and the signals were generated by the ECL reaction (Amersham Co., Ill.).

DISCUSSION

The major obstacle for developing an AIDS vaccine has been the fact that the virus exhibits extensive genetic drift. Thus, for example, the primary neutralizing determinant of the virus (V3 loop) can generate highly effective neutralizing antibodies; however, these are sometimes extremely strain-specific (11; compare also refs 12 and 13). Therefore, there is a constant search for novel epitopes that are less prone to variation. In this study we have described three epitopes that appear to have neutralizing potential and thus might qualify as potential vaccine candidates.

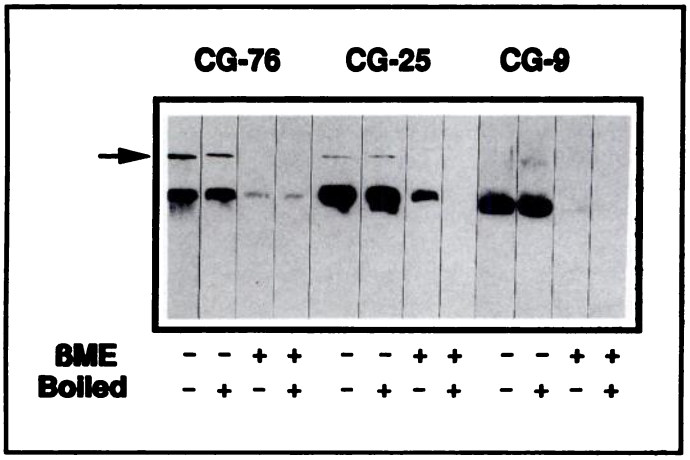

Figure 2. Protein blot analysis of epitope stability. CD4 (1 μg/sample) was either not treated or treated with 5% β-mercaptoethanol, boiled or not in 1% SDS, and then run on 10% SDS-polyacrylamide gels, transferred to nitrocellulose membranes, and probed with mAbs CG-76, CG-25, and CG-9. The secondary probe was a goat-anti-mouse horseradish peroxidase conjugate and the signals were generated by the ECL reaction (Amersham Co., Ill.).

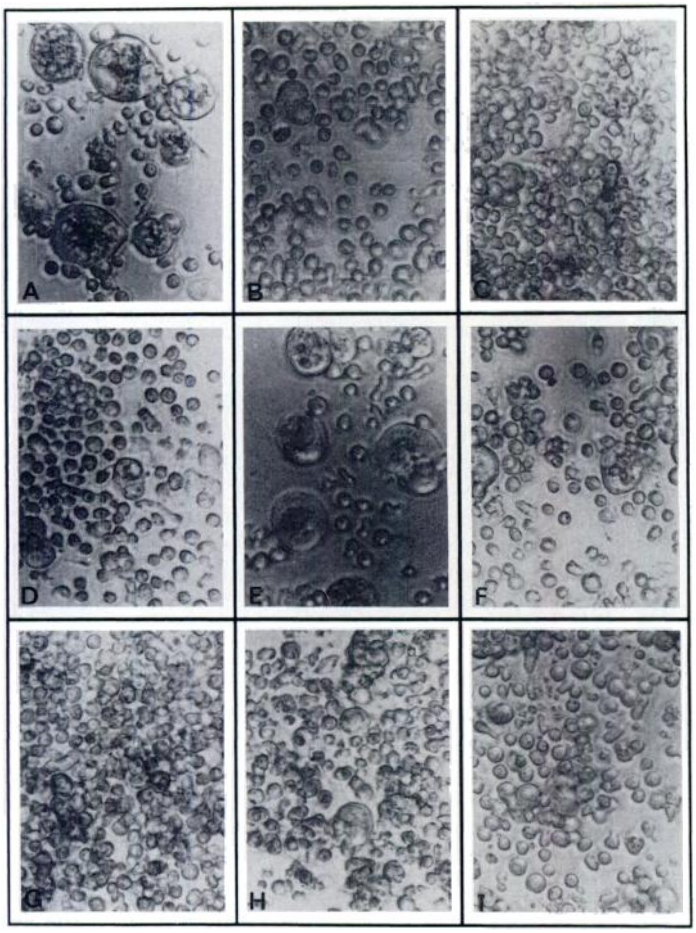

Figure 3. Inhibition of syncitium formation by the mAbs. Syncytium formation in tissue culture between BS-C-1 cells infected with recombinant vaccinia virus (VPE-16, a kind gift from Dr. B. Moss, NIH), which express gp120 on the cell surface and CEM cells (CD4+ cells). The mAbs were added to the cells (10 μg/ml) and incubated overnight at 37°C (*B, D, E, F*) or preincubated with CEM cells for 12 h before mixing at concentration 1 μg/ml (*C, G, H, I*). CG-25 (*B, C*); CG-9 (*D, G*); CG-10 (*E, H*); CG-76 (*F, I*). *A)* The control where no mAb is added.

CG-76 might represent an epitope similar to that previously described by Celada et al. [8; mAb 55]. This class of mAbs recognizes CD4 but has only marginal enhancement subsequent to complexation of the latter with gp120. Thus CG-9, CG-25, and CG-10 represent unique, thus far not yet described epitopes in their marked preference or complete requirement for CD4/gp120 complex. The fact that preincubation of the CEM cells with CG-10 increased this mAb's inhibitory capacity might hint that the corresponding epitope (or parts of the epitope) could lie within CD4, but is only rarely exhibited in the isolated protein. It is suggested that complexation with gp120 reveals such epitopes and locks CD4 into a configuration not frequently presented otherwise. Such behavior allows the formulation of the following strategy for vaccine development.

A possible AIDS vaccine could be directed toward unique cryptic epitopes of the CD4/gp120 complex, including those epitopes that are harbored within the CD4 molecule. Such epitopes would not be subject to genetic drift and thus allow broad spectrum efficacy. As they are not routinely presented in the healthy individual and become targets for the neutralizing antibodies only in the instance of the pathological state of viral infection, it would be expected that vaccination would have only mild or no deleterious effects in healthy recipients. Thus the concept of cryptic CD4/gp120 complex dependent epitopes as targets for AIDS vaccines and immunotherapeutics are currently being developed. FJ

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REFERENCES

1. Capon, D. J., and Ward, R. H. R. (1991) The CD4-gp120 interaction and AIDS pathogenesis. *Annu. Rev. Immunol.* **9**, 649–678
2. Sweet, R. W., Truneh, A., and Hendrickson, W. A. (1991) CD4: its structure, role in immune function and AIDS pathogenesis, and potential as a pharmacological target. *Curr. Opin. Biotechnol.* **2**, 622–633
3. Gershoni, J. M., and Aronheim, A. (1988) Molecular decoys: ligand-binding recombinant proteins protect mice from curarimimetic neurotoxins. *Proc. Natl. Acad. Sci. USA* **85**, 4087–4089
4. White, J. M., and Littman, D. R. (1989) Viral receptors of the immunoglobulin superfamily. *Cell* **56**, 725–728
5. Camerini, D., and Seed, B. (1990) A CD4 domain important for HIV-mediated syncytium formation lies outside the virus binding site. *Cell* **60**, 747–754
6. Hillman, K., Shapira-Nahor, O., Gruber, M. F., Hooley, J., Manischewitz, J., Seeman, R., Vujcic, L., Geyer, S. J., and Golding, H. (1990) Chemically induced CD4 mutants of a human T cell line. Evidence for dissociation between binding of HIV I envelope and susceptibility to HIV I infection and syncytia formation. *J. Immunol.* **144**, 2131–2139
7. Bosch, M. L., Earl, P. L., Fargnoli, K., Picciafuoco, S., Giombini, F., Wong-Staal, F., and Franchini, G. (1989) Identification of the fusion peptide of primate immunodeficiency viruses. *Science* **244**, 694–697
8. Celada, F., Cambiaggi, C., Maccari, J., Burastero, S., Gregory, T., Patzer, E., Porter, J., McDanal, C., and Matthews, T. (1990) Antibody raised against soluble CD4-rgp120 complex recognizes the CD4 moiety and blocks membrane fusion without inhibiting CD4-gp120 binding. *J. Exp. Med.* **172**, 1143–1150
9. Healey, D., Dianda, L., Moore, J. P., McDougal, J. S., Moore, M. J., Estess, P., Buck, D., Kwong, P. D., Beverley, P. C. L., and Sattentau, Q. J. (1990) Novel anti-CD4 monoclonal antibodies separate human immunodeficiency virus infection and fusion of $CD4^+$ cells from virus binding. *J. Exp. Med.* **172**, 1233–1242
10. Ashorn, P. A., Berger, E. A., and Moss, B. (1990) Human immunodeficiency virus envelope glycoprotein/CD4-mediated fusion of nonprimate cells with human cells. *J. Virol.* **64**, 2149–2156
11. Starcich, B. R., Hahn, B. H., Shaw, G. M., McNeely, P. D., Modrow, S., Wolf, H., Parks, E. S., Parks, W. P., Josephs, S. F., Gallo, R. C., and Wong-Staal, F. (1986) Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. *Cell* **45**, 637–648
12. Profy, A. T., Salinas, P. A., Eckler, L. I., Dunlop, N. M., Nara, P. L., and Putney, S. D. (1990) Epitopes recognized by the neutralizing antibodies of an HIV-1 infected individual. *J. Immunol.* **144**, 4641–4647
13. Ohno, T., Terada, M., Yoneda, Y., Shea, K. W., Chambers, R. F., Stroka, D. M., Nakamura, M., and Kufe, D. W. (1991) A broadly neutralizing monoclonal antibody that recognizes the V3 region of human immunodeficiency virus type 1 glycoprotein gp120. *Proc. Natl. Acad. Sci. USA* **88**, 10726–10729
14. Gershoni, J. M. (1988) Protein blotting: a manual. *Methods Biochem. Anal.* **33**, 1–58

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