NOTES

Antibody Binding in Proximity to the Receptor/Glycoprotein Complex Leads to a Basal Level of Virus Neutralization[▽]

Xinzhen Yang,^{1,2,3,4*} Inna Lipchina,⁴ Michelle Lifton,¹ Liping Wang,⁴ and Joseph Sodroski^{3,4,5}

Division of Viral Pathogenesis, Beth Israel Deaconess Medical Center,¹ Department of Medicine² and Department of Pathology,³ Division of AIDS, Harvard Medical School, Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute,⁴ and Department of Immunology and Infectious Diseases, Harvard School of Public Health,⁵ Boston, Massachusetts 02215

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Hypothetically, antibodies may neutralize enveloped viruses by diverse mechanisms, such as disruption of receptor binding, interference with conformational changes required for virus entry, steric hindrance, or virus aggregation. Here, we demonstrate that retroviral infection mediated by the avian sarcoma-leukosis virus (ASLV-A) envelope glycoproteins can be neutralized by an antibody directed against a functionally unimportant component of a chimeric receptor protein. Thus, the binding of an antibody in proximity to the retroviral envelope glycoprotein-receptor complex, without binding to the entry machinery itself, results in neutralization. This finding provides additional support for the hypothesis that steric hindrance is sufficient for antibody-mediated neutralization of retroviruses.

The generation of virus-neutralizing antibodies contributes to the success of prophylactic vaccines against viral infections. In vitro, neutralization can be defined as the disruption of the viral life cycle prior to viral gene expression due to the direct binding of antibody molecules to viral surface antigens, without the necessary participation of other factors (2). Once bound to the envelope glycoprotein (Env) spike of a virus, an antibody can hypothetically effect neutralization by steric hindrance, direct receptor competition, prevention of necessary conformational changes or induction of deleterious changes in the viral Env, causing virion aggregation, or occupation of a large fraction of the virion surface (11, 12).

Studies of the stoichiometries of neutralization of different strains of human immunodeficiency virus type 1 (HIV-1) by nine different representative antibodies revealed that the binding of one antibody molecule is sufficient to neutralize the function of the whole Env trimer (23). As the nine antibodies tested bind to very different structural and functional elements on the HIV-1 gp120 and gp41 envelope glycoproteins, the shared stoichiometry implies that a generic mechanism underlies HIV-1 neutralization by antibodies. One such mechanism is steric hindrance, in which the bulk of the antibody molecule interferes with the virus entry process. This hypothesis is supported by experiments demonstrating that an unrelated antibody, the M2 anti-FLAG antibody, can effectively neutralize HIV-1 virions that carry an exogenous FLAG epitope in the functionally unimportant V4 variable region of gp120 (14). Importantly, M2 antibody binding to the FLAG-tagged gp120 does not compete for binding to the CD4/CCR5 receptors and

does not inhibit CD4-induced conformational changes within gp120. As these results suggest the hypothesis that steric hindrance is sufficient for antibody-mediated neutralization of HIV-1, we sought to test this hypothesis using a novel experimental design. We investigated whether a model antibody can achieve neutralization when targeted to the vicinity of the viral Env spike and its cognate receptor without actually binding to the entry machinery per se.

Avian sarcoma-leukosis virus (ASLV-A) Env was selected for this study because of the extensive knowledge available regarding its entry process. In natural ASLV-A entry, the viral Env binds to the receptor, Tva, on the cell surface (1). Receptor binding and endocytosis, with an accompanying drop in pH, initiate conformational changes in the Env trimer that lead to viral-cell membrane fusion (3, 5). The N-terminal 48 amino acids of Tva form an independent motif that can support virus entry either as a soluble protein or fused with the N terminus of the epidermal growth factor receptor (15, 17a). We constructed a Tva-CCR5 fusion protein (Tva-R5) to serve as a functional receptor for ASLV-A. To express the Tva-R5 fusion protein, a three-fragment, PCR-based technique was used to produce a gene that encodes, from the N to the C terminus, the N-terminal 104 amino acids of Tva (including the signal sequence), a glycine-glycine (GG) linker, human CCR5 with a deletion of 15 amino acid residues from its N terminus, a GGG linker, and a C9 tag. This fragment was then inserted into the pcDNA3.1(Zeo/–) vector (Invitrogen) between the HindIII and XbaI sites. The coding sequences in the final constructs were sequenced fully to verify the integrity of the construction. The Tva-R5 protein was designed so that the Tva moiety can bind to the ASLV-A Env to support entry, while the CCR5 moiety anchors the chimeric protein and can be recognized by the 2D7 anti-CCR5 antibody. The use of Tva-R5 allowed us to test whether the binding of the 2D7 antibody to the CCR5

* Corresponding author. Mailing address: R.E 213A, 330 Brookline Avenue, Boston, MA 02215. Phone: (617) 667-2052. Fax: (617) 667-8210. E-mail: xyang1@bidmc.harvard.edu.

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FIG. 1. Cell surface expression of the Tva-R5 receptor. 293T cells were transfected with 10 μ g of plasmids expressing Tva or Tva-R5, using the Lipofectamine reagent (Invitrogen) according to the manufacturer's recommendations. At 24 h after transfection, the cells were removed from culture flasks with 10 mM EDTA in 1 $\times$ PBS. Approximately 1 $\times$ 10⁶ cells in 100 μ l of 1 $\times$ PBS containing 1% BSA were then incubated with 1 μ g of the 2D7 anti-CCR5 antibody (PharMingen), 2 μ l of anti-Tva antiserum (clone 12D1.4-3, a gift from Christina Ocshenbauer-Jambor, University of Alabama at Birmingham), or the M2 anti-FLAG antibody (Sigma-Aldrich) for 1 h on ice. After three washes, the cells were incubated with a 1:200 dilution of fluorescein isothiocyanate-conjugated anti-mouse Fc antibody (Sigma-Aldrich) and then analyzed by FACS. The results from FACS are shown (red, 2D7 anti-CCR5 antibody; green, 8C5 anti-Tva antibody; gray, M2 anti-FLAG antibody, which is used here as a negative control). Results from a typical experiment are shown.

moiety in the Tva-R5 receptor could block ASLV-A entry mediated by the Tva motif of Tva-R5. We also constructed a similar vector to express the wild-type Tva with a C9 tag to be used as a control.

To evaluate the cell surface expression of Tva and Tva-R5, 10 μ g of the Tva- or Tva-R5-expressing plasmids was transfected into 293T cells in 10-cm dishes using the Lipofectamine reagent. At 24 h after transfection, the cells were stained with the M2 anti-FLAG antibody (Sigma) as a control, anti-Tva ascites fluid, or the 2D7 anti-CCR5 monoclonal antibody and analyzed by fluorescence-activated cell sorting (FACS) (Fig. 1) (22). Cells expressing the control wild-type Tva were stained only by the anti-Tva antibody and not by the anti-CCR5 antibody. Importantly, cells transfected with plasmids expressing Tva and Tva-R5 were stained by the anti-Tva antibody at comparable levels, indicating that the Tva-R5 receptor was efficiently expressed in this context. The Tva-R5-expressing cells were stained by the 2D7

anti-CCR5 antibody, suggesting that the CCR5 part of the molecule preserves its structural integrity.

To test whether the Tva-R5 protein could support virus entry mediated by ASLV-A Env, Tva-R5 or Tva was transiently expressed in 293T cells, which were used as target cells for the single-round infection of a lentiviral vector pseudotyped with ASLV-A Env. To pseudotype ASLV-A Env onto HIV-1 luciferase reporter viruses [HIV-1(ASLV-A)], 3 μ g of the pCB6/WTa vector expressing wild-type ASLV-A Env (A gift from Judy White, University of Virginia) (5) was cotransfected with 1.5 μ g of the pCMV Δ P1 Δ envpA plasmid, 4.5 μ g of the pHIV-1Luc vector, and 1 μ g of the pc-Rev plasmid into the 293T cells by following a standard protocol (see the Fig. 2 legend for details). HIV-1(ASLV-A), when used at higher concentrations, infected Tva-expressing target cells with an efficiency 3 to 4 logs above the background luciferase signal detected in the mock-transfected target cells (Table 1). At lower multiplicities of infection, Tva-R5 supported HIV-1(ASLV-A) infection at five- to sevenfold-higher levels than the wild-type Tva receptor. At larger amounts of input viruses, these differences were diminished. Thus, the Tva motif within the Tva-R5 protein is functionally competent as an ASLV-A receptor.

The 2D7 anti-CCR5 monoclonal antibody can potently block CCR5-tropic HIV-1 strains (22). Its binding motif has been mapped to the second extracellular loop (ECL-2) of CCR5 by mutagenic analysis and by its ability to compete for chemokine binding (16, 21). To determine whether the 2D7 anti-CCR5 antibody could inhibit entry of HIV-1(ASLV-A) mediated through the Tva-R5 protein, 293T cells transiently expressing Tva-R5 were incubated for 1 hour at 37°C with culture medium containing different concentrations of the 2D7 antibody. The HIV-1(ASLV-A) virus in 2 μ M Polybrene was then added. Incubation with 4 nM or higher concentrations of the 2D7 antibody resulted in strong inhibition of HIV-1(ASLV-A) infection supported by the Tva-R5 receptor (Fig. 2). This inhibition was specific for the interaction between the 2D7 antibody and Tva-R5, because in parallel experiments, 2D7 incubation with the target cells expressing wild-type Tva did not result in a reduction of HIV-1(ASLV-A) infection. We also tested whether compound A, a small-molecule ligand of CCR5 that can block HIV-1 infection (4, 7), could interfere with HIV-1(ASLV-A) infection mediated by Tva-R5. HIV-1(ASLV-A) infection of cells expressing the Tva-R5 receptor was not disrupted by incubation with up to 1000 nM of compound A (compound 60 in reference 7) (Fig. 2). By contrast, 1,000 nM compound A inhibited HIV-1_{YU2} infection of CCR5-expressing cells by over 90% (data not shown). Thus,

TABLE 1. Entry of HIV-1(ASLV-A) supported by Tva and Tva-R5

Expt	Input virus (μ l)	Arbitrary luciferase units from transfected cells		
		Tva	Tva-R5	Mock
1	25	5,368,331	7,807,094	458
	2.5	162,189	823,561	
2	25	3,837,186	8,238,837	654
	2.5	92,691	691,644

FIG. 2. Specific inhibition of HIV-1(ASLV-A) infection of Tva-R5-expressing cells by the 2D7 anti-CCR5 monoclonal antibody. To create target cells for the single-round infection assay, 293T cells were transiently transfected using the Lipofectamine reagent with 10 μ g of the pc-Tva plasmid, expressing the wild-type Tva protein, or the pc-Tva-R5 plasmid, expressing the Tva-R5 fusion protein. At 24 h after transfection, the cells were removed from the plates with 10 mM EDTA-1 $\times$ PBS, reseeded into 96-well Isoplates at a density of 6,000 cells per well, and cultured for another 24 h, at which time they were used in the infection assay. To pseudotype ASLV-A Env onto HIV-1 luciferase reporter viruses [HIV-1(ASLV-A)], 3 μ g of the pCB6/WTa vector expressing wild-type ASLV-A Env (a gift from Judy White, University of Virginia) was cotransfected with 1.5 μ g of the pCMV Δ P1 Δ envpA plasmid, 4.5 μ g of the pHIV-1Luc vector, and 1 μ g of the pc-Rev plasmid into 293T cells by using the Lipofectamine reagent, following a standard protocol (24). The pCMV Δ P1 Δ envpA plasmid encodes the Gag/Pol and Tat proteins of HIV-1. The pHIV-1Luc plasmid encodes an HIV-1 RNA genome that is defective in all HIV-1 genes except *tat* and contains a firefly luciferase reporter gene. The pc-Rev plasmid expresses the HIV-1 Rev protein to help export the viral RNA genome out of the nucleus. The viruses were harvested 2 days later and used as fresh stocks to measure infectivity. To conduct the single-round infection assay, 100 μ l of freshly prepared virus suspension in culture medium was added to each well after thoroughly removing the medium from the target cells. After 48 h, viral infectivity was quantified by measuring the luciferase activity using a luciferase detection kit (Pharmingen) and an automated luminometer (Centro 960; EG&G Berthod). To study the inhibition of virus entry, the culture medium of target 293T cells in 96-well luciferase assay plates was thoroughly removed from cells. Fresh growth medium containing twice the indicated concentration of 2D7 anti-CCR5 antibody or compound A (CompA) was added to each well of cells and incubated at 37°C for 1 h. The recombinant luciferase-expressing viruses with ASLV-A Env were suspended in growth medium supplemented with 2 μ M Polybrene and added to each well of the target cells. The infectivities of the virus as a function of the concentrations of 2D7 antibody or CompA are shown. To calculate residual infectivity, the luciferase activity from target cells treated with the 2D7 antibody or CompA was normalized to that from untreated cells. Results consistent with those shown were observed in repeated experiments; the means and ranges of variation of four parallel measurements from a typical experiment are shown.

the 2D7 antibody, a CCR5 ligand of high molecular mass, but not a smaller CCR5 ligand, neutralized virus entry mediated by the ASLV-A Env by binding to the CCR5 moiety within the Tva-R5 receptor.

We investigated whether binding of 2D7 to the Tva-R5 receptor would competitively block the binding of a soluble

FIG. 3. Effects of the 2D7 anti-CCR5 antibody on binding of the soluble ASLV-A Env protein to Tva-R5. The SUA-rIgG protein contains the first 338 amino acids of ASLV-A Env fused to the N terminus of amino acids 96 to 323 of the rIgG constant region (25). To express the recombinant SUA-rIgG protein, 1 mg of the plasmid expressing SUA-rIgG (a gift from John Young, Salk Institute) was transfected into 1 liter of 293F cells (Invitrogen), using the FreeStyle reagent (Invitrogen) according to the manufacturer's recommendations. Four days later, the culture medium containing the SUA-rIgG protein was harvested by centrifugation to remove the cells and cell debris. The recombinant SUA-rIgG protein in the culture medium was then bound to 20 ml of a protein A-Sepharose affinity gel (Pharmacia) and eluted in 0.1 M citric acid. After neutralization of the acid, the recombinant protein was concentrated with a Milliprep-30 filter (Millipore). At 2 days after transfection, 0.1 ml containing 1×10^5 293T cells expressing either Tva or Tva-R5, transfected as described in the legend to Fig. 1, was incubated first with 400 nM 2D7 anti-CCR5 antibody for 1 h at 37°C and then with increasing concentrations of SUA-rIgG for 1 h at 4°C. The binding of SUA-rIgG was quantitated by FACS after secondary staining with a 1:1,000 dilution of phycoerythrin-conjugated anti-rabbit antibody (Sigma-Aldrich). The geometric mean fluorescence intensity from all cells in one staining reaction was calculated. Consistent results were produced in two experiments, and the results from one set of experiments are shown.

ASLV-A Env protein to the Tva moiety of Tva-R5. The SUA-rIgG protein contains the first 338 amino acids of ASLV-A Env fused to the N terminus of amino acids 96 to 323 of the rabbit immunoglobulin G (rIgG) constant region (25). The recombinant SUA-rIgG protein was transiently expressed in 293F cells (Invitrogen) and purified using a protein A-Sepharose affinity gel (Pharmacia), as described previously (25). At 2 days after transfection, 0.1 ml containing 1×10^5 293T cells transiently expressing either Tva or Tva-R5 was incubated first with 400 nM 2D7 anti-CCR5 antibody for 1 h at 37°C and then with increasing concentrations of SUA-rIgG for 1 h at 4°C. The binding of SUA-rIgG was quantitated by FACS after secondary staining with phycoerythrin-conjugated anti-rabbit antibody. Preliminary experiments indicated that SUA-rIgG binding to the Tva or Tva-R5 receptors in this experimental setting is specific for the Tva moieties, because SUA-rIgG did not stain mock-transfected 293T cells (data not shown). Within a wide range of SUA-rIgG concentrations (0.4 to 400 nM), the 2D7 antibody prebound to the CCR5 protein of Tva-R5 had little effect on the binding of the ASLV-A SU glycoprotein to the Tva moiety of Tva-R5 (Fig. 3).

We also investigated the effect of 2D7 binding to Tva-R5 on HIV-1(ASLV-A) virion attachment to the target cells. One

FIG. 4. Effect of the 2D7 antibody on HIV-1(ASLV-A) virion attachment to target cells. One million 293T cells expressing Tva-R5, transfected as described in the Fig. 1 legend, were incubated in 100 μ l PBS with 6 μ g of 2D7, SUA-rIgG, or BSA for 1 h at 4°C. HIV-1(ASLV-A) stocks produced by transfecting two 10-cm culture plates of 293T cells were concentrated up to 20-fold using a Milliprep-30 filter. Then, 100 μ l of the concentrated HIV-1(ASLV-A) viruses were added to 100 μ l of the target cells, prepared as described above, and incubated for 1 h at 4°C. After three washes with 1 $\times$ PBS, the cell/virus complex was lysed with 300 μ l of NP-40 buffer (0.5% NP-40, 0.15 M NaCl, 20 mM Tris-HCl, pH 7.4). After clarification of the cell debris by centrifugation, the cell-bound viruses in duplicate samples were quantitated by measuring the HIV-1 p24 level using a custom-made enzyme-linked immunosorbent assay. In the enzyme-linked immunosorbent assay, purified Ig from polyclonal anti-HIV-1 p24 serum (produced by injecting a rabbit with 200 μ g of purified HIV-1 p24 in Freund's adjuvant three times) was used to capture the p24 protein from the cell lysates, and the 71-31 anti-HIV-1-p24 monoclonal antibody (a gift from the AIDS Reagent Repository) (6) was used for detection. This enzyme-linked immunosorbent assay has a detection sensitivity of 3 ng/ml of recombinant HIV-1 p24 (data not shown). As a positive control for enzyme-linked immunosorbent assay detection, 10% of the input virus was lysed and studied in parallel. Target cells without virus incubation were run as a negative control. HIV-1(HXBc2) viruses were produced and tested for binding to the Tva-R5-expressing target cells as an additional control. The optical densities at 450 nm (OD₄₅₀) from the enzyme-linked immunosorbent assay detection are reported for a single experiment. The experiment was repeated, and consistent results were observed.

million 293T cells expressing Tva-R5 were incubated in 100 μ l phosphate-buffered saline (PBS) with 6 μ g of 2D7 (400 nM), SUA-rIgG, or bovine serum albumin (BSA) for 1 h at 4°C. Then, 100 μ l of 20-fold-concentrated HIV-1(ASLV-A) was added and incubated for 1 h at 4°C. After three washes with 1 $\times$ PBS, the cell/virus complexes were lysed with 300 μ l of NP-40 lysis buffer. The cell-bound viruses were quantitated by measuring the HIV-1 p24 level using a custom-made sandwich capture assay, which has a detection sensitivity of 3 ng/ml (data not shown). Fewer than 10% of virions bound to the target cells in this format (Fig. 4). In multiple experiments, prebinding of SUA-rIgG or 2D7 to the Tva-R5 receptor did not sig-

nificantly reduce HIV-1(ASLV-A) binding to the target cells. This result is not surprising, because the HIV-1 virions carrying HIV-1(HXBc2) gp160 were able to bind to the Tva-R5-expressing target cells with an efficiency comparable to that of HIV-1(ASLV-A) (Fig. 4). Thus, the efficiency with which HIV-1 virions can bind to target cells is not dependent on the recognition of cognate receptors on the cells, as reported previously (13, 18, 20). Moreover, the binding of 2D7 to Tva-R5 did not prevent virion attachment to the target cells.

Our study demonstrates that the binding of an antibody to a receptor region not directly involved in the virus entry process can result in significant inhibition of infection. In the chimeric Tva-R5 receptor, the artificially introduced CCR5 moiety serves to anchor the protein and provides an epitope for the anti-CCR5 antibody, 2D7. The binding of a small CCR5 ligand, compound A, exerted no effect on Tva-R5-mediated entry of the recombinant HIV-1 pseudotyped with the ASLV-A envelope glycoproteins. By contrast, the binding of the large 2D7 antibody significantly inhibited infection by this virus. Thus, the nature of the bound ligand apparently influences the degree of inhibition achieved.

With respect to the potential mechanism of neutralization in our system, we observed no significant effects of the 2D7 anti-CCR5 antibody on internalization of the Tva-R5 fusion proteins (data not shown), the binding of the ASLV-A envelope glycoprotein to the receptor, or the attachment of virions to the cells. The binding of the antibody in proximity to the virus entry machinery likely establishes a steric block to one or more steps in the virus entry process. More generally, any antibody bound within sufficient proximity to the virus entry machinery would be expected to exert some inhibitory activity due to the steric effects of the antibody molecule. For some antibodies, this basal level of neutralization resulting from steric hindrance may be supplemented by the effects of bound antibody on specific entry-related events.

Some anti-CD4 antibodies can block HIV-1 entry without disrupting gp120 binding (8, 17, 19). Of note, not all antibodies that recognize domains 3 and 4 of CD4, which are dispensable for efficient gp120 binding, inhibited cell-cell fusion or virus entry mediated by the HIV-1 envelope glycoproteins (10, 17, 19). Why anti-CD4 antibodies that allow HIV-1 envelope glycoprotein binding differ in virus neutralization is not understood, but the binding affinity, the distance from the gp160/CD4 D1 domain, or the orientation of the bound antibody could influence whether the entry process is sterically blocked. Further studies will better define these aspects of the mechanisms of antibody-mediated neutralization of retroviruses.

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REFERENCES

1. Bates, P., L. Rong, H. E. Varmus, J. A. Young, and L. B. Crittenden. 1998. Genetic mapping of the cloned subgroup A avian sarcoma and leukosis virus receptor gene to the TVA locus. *J. Virol.* 72:2505-2508.

2. **Burton, D. R., R. A. Williamson, and P. W. Parren.** 2000. Antibody and virus: binding and neutralization. *Virology* **270**:1–3.
3. **Earp, L. J., S. E. Delos, R. C. Netter, P. Bates, and J. M. White.** 2003. The avian retrovirus avian sarcoma/leukosis virus subtype A reaches the lipid mixing stage of fusion at neutral pH. *J. Virol.* **77**:3058–3066.
4. **Finke, P. E., B. Oates, S. G. Mills, M. MacCoss, L. Malkowitz, M. S. Springer, S. L. Gould, J. A. DeMartino, A. Carella, G. Carver, K. Holmes, R. Danzeisen, D. Hazuda, J. Kessler, J. Lineberger, M. Miller, W. A. Schleif, and E. A. Emini.** 2001. Antagonists of the human CCR5 receptor as anti-HIV-1 agents. 4. Synthesis and structure-activity relationships for 1-[N-(methyl)-N-(phenylsulfonyl)amino]-2-(phenyl)-4-(4-(N-(alkyl)-N-(benzyloxycarbonyl)amino)piperidin-1-yl)butanes. *Bioorg Med. Chem. Lett.* **11**:2475–2479.
5. **Gilbert, J. M., L. D. Hernandez, J. W. Balliet, P. Bates, and J. M. White.** 1995. Receptor-induced conformational changes in the subgroup A avian leukosis and sarcoma virus envelope glycoprotein. *J. Virol.* **69**:7410–7415.
6. **Gorny, M. K., V. Gianakakos, S. Sharpe, and S. Zolla-Pazner.** 1989. Generation of human monoclonal antibodies to human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* **86**:1624–1628.
7. **Hale, J. J., R. J. Budhu, E. B. Holson, P. E. Finke, B. Oates, S. G. Mills, M. MacCoss, S. L. Gould, J. A. DeMartino, M. S. Springer, S. Siciliano, L. Malkowitz, W. A. Schleif, D. Hazuda, M. Miller, J. Kessler, R. Danzeisen, K. Holmes, J. Lineberger, A. Carella, G. Carver, and E. Emini.** 2001. 1,3,4-Trisubstituted pyrrolidine CCR5 receptor antagonists. 2. Lead optimization affording selective, orally bioavailable compounds with potent anti-HIV activity. *Bioorg Med. Chem. Lett.* **11**:2741–2745.
8. **Healey, D., L. Dianda, J. P. Moore, J. S. McDougal, M. J. Moore, P. Estess, D. Buck, P. D. Kwong, P. C. Beverley, and Q. J. Sattentau.** 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.
9. Reference deleted.
10. **Hoxie, J. A., J. L. Rackowski, B. S. Haggarty, and G. N. Gaulton.** 1988. T4 endocytosis and phosphorylation induced by phorbol esters but not by mitogen or HIV infection. *J. Immunol.* **140**:786–795.
11. **Klasse, P. J., and Q. J. Sattentau.** 2002. Occupancy and mechanism in antibody-mediated neutralization of animal viruses. *J. Gen. Virol.* **83**:2091–2108.
12. **Parren, P. W., and D. R. Burton.** 2001. The antiviral activity of antibodies in vitro and in vivo. *Adv. Immunol.* **77**:195–262.
13. **Pudney, J., and M. J. Song.** 1994. Electron microscopic analysis of HIV-host cell interactions. *Tissue Cell* **26**:539–550.
14. **Ren, X., J. Sodroski, and X. Yang.** 2005. An unrelated monoclonal antibody neutralizes human immunodeficiency virus type 1 by binding to an artificial epitope engineered in a functionally neutral region of the viral envelope glycoproteins. *J. Virol.* **79**:5616–5624.
15. **Rong, L., and P. Bates.** 1995. Analysis of the subgroup A avian sarcoma and leukosis virus receptor: the 40-residue, cysteine-rich, low-density lipoprotein receptor repeat motif of Tva is sufficient to mediate viral entry. *J. Virol.* **69**:4847–4853.
16. **Samson, M., G. LaRosa, F. Libert, P. Paindavoine, M. Dethaux, G. Vassart, and M. Parmentier.** 1997. The second extracellular loop of CCR5 is the major determinant of ligand specificity. *J. Biol. Chem.* **272**:24934–24941.
17. **Sattentau, Q. J., A. G. Dalgleish, R. A. Weiss, and P. C. Beverley.** 1986. Epitopes of the CD4 antigen and HIV infection. *Science* **234**:1120–1123.
- 17a. **Snitkovsky, S., and J. Young.** 1998. Cell-specific viral targeting mediated by a soluble retroviral receptor-ligand fusion protein. *Proc. Natl. Acad. Sci. USA* **95**:7063–7068.
18. **Tardif, M. R., and M. J. Tremblay.** 2003. Presence of host ICAM-1 in human immunodeficiency virus type 1 virions increases productive infection of CD4⁺ T lymphocytes by favoring cytosolic delivery of viral material. *J. Virol.* **77**:12299–12309.
19. **Truneh, A., D. Buck, D. R. Cassatt, R. Juszczak, S. Kassis, S. E. Ryu, D. Healey, R. Sweet, and Q. Sattentau.** 1991. A region in domain 1 of CD4 distinct from the primary gp120 binding site is involved in HIV infection and virus-mediated fusion. *J. Biol. Chem.* **266**:5942–5948.
20. **Ugolini, S., I. Mondor, P. W. Parren, D. R. Burton, S. A. Tilley, P. J. Klasse, and Q. J. Sattentau.** 1997. Inhibition of virus attachment to CD4⁺ target cells is a major mechanism of T cell line-adapted HIV-1 neutralization. *J. Exp. Med.* **186**:1287–1298.
21. **Wu, L., N. P. Gerard, R. Wyatt, H. Choe, C. Parolin, N. Ruffing, A. Borsetti, A. A. Cardoso, E. Desjardin, W. Newman, C. Gerard, and J. Sodroski.** 1996. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. *Nature* **384**:179–183.
22. **Wu, L., W. A. Paxton, N. Kassam, N. Ruffing, J. B. Rottman, N. Sullivan, H. Choe, J. Sodroski, W. Newman, R. A. Koup, and C. R. Mackay.** 1997. CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, in vitro. *J. Exp. Med.* **185**:1681–1691.
23. **Yang, X., S. Kurteva, S. Lee, and J. Sodroski.** 2005. Stoichiometry of antibody neutralization of human immunodeficiency virus type 1. *J. Virol.* **79**:3500–3508.
24. **Yang, X., S. Kurteva, X. Ren, S. Lee, and J. Sodroski.** 2005. Stoichiometry of envelope glycoprotein trimers in the entry of human immunodeficiency virus type 1. *J. Virol.* **79**:12132–12147.
25. **Zingler, K., and J. A. Young.** 1996. Residue Trp-48 of Tva is critical for viral entry but not for high-affinity binding to the SU glycoprotein of subgroup A avian leukosis and sarcoma viruses. *J. Virol.* **70**:7510–7516.