would help make this determination are observations over a greater range of magnetic latitudes, to look for changes suggesting a connection with magnetospheric processes; at both east and west elongations, to see if the patches are fixed on Io's surface; during eclipse, to verify that solar H I Lyman-α (whether by diffuse reflection or resonant scattering) is responsible for the emission; and at high spectral resolution, to determine whether the emission from Io has the distinctive solar lineshape and to separate the Ionian emission from terrestrial airglow with a suitable velocity difference.

References and Notes

1. F. Scherb and W. H. Smyth, *J. Geophys. Res.* **98**, 18729 (1993).
2. J. T. Trauger, K. R. Stapelfeldt, G. E. Ballester, J. T. Clarke, WFPC2 Science Team, *Bull. Am. Astron. Soc.* **29**, 1002 (1997).
3. G. E. Ballester *et al.*, *Astrophys. J.* **319**, L33 (1987).
4. G. E. Ballester *et al.*, *Bull. Am. Astron. Soc.* **29**, 980 (1997).
5. J. T. Clarke, J. Ajello, J. Luhmann, N. M. Schneider, I. Kanik, *J. Geophys. Res.* **99**, 8387 (1994).
6. S. T. Durrance *et al.*, *Astrophys. J.* **447**, 408 (1995).
7. G. K. Fox *et al.*, *Astronom. J.* **113**, 1158 (1997); B. R. Sandel and A. L. Broadfoot, *J. Geophys. Res.* **87**, 212 (1982); J. R. Spencer and N. M. Schneider, *Annu. Rev. Earth Planet. Sci.* **24**, 125 (1996).
8. B. E. Woodgate *et al.*, *Proc. Astron. Soc. Pacific* **110**, 1183 (October 1998).
9. R. A. Kimble *et al.*, *Astrophys. J.* **492**, L83 (1998).
10. In time-tag mode, each photon's arrival time and position in the STIS multi-anode microchannel array (MAMA) are recorded individually.
11. M. Voit, Ed., *HST Data Handbook* (Space Telescope Science Institute, Baltimore, ed. 3.0, 1997).
12. The slit was effectively reduced to 30 arc sec by 2 arc sec (September) and 25 arc sec by 2 arc sec (October) by the finite size of the MAMA detectors.
13. The STIS is equipped with two MAMAs, which have different photocathodes sensitive to different wavelength ranges (*8*). Unfortunately, the NUV MAMA (covering 1650 to 3100 Å) was found after launch to have a dark rate exceeding design specification by about an order of magnitude, as a result of excessive phosphoresence in its MgF_2 window (*9*). Consequently, our G230M data (orbits 1 and 2) are noisy, and only the brightest features can be distinguished; these data are largely excluded from this paper.
14. It is unclear whether the near-Io emission at ~1250 Å is predominately S I λ1251 or S II λ1256.
15. A. S. McEwen *et al.*, *Geophys. Res. Lett.* **24**, 2443 (1997).
16. T. E. Smith, M. A. McGrath, P. Sartoretti, *Bull. Am. Astron. Soc.* **27**, 1157 (1995).
17. G. E. Ballester *et al.*, in *Magnetospheres of the Outer Planets* (Boulder, CO, March 1997), unpublished proceedings.
18. One Rayleigh is 10^6 photons 4π sr^{-1} cm^{-2} s^{-1}.
19. Io's electrodynamic interaction with the plasma torus is typical of plasma flowing past an obstacle. In this case it is sub-Alfvénic ($v_{plasma} \sim 57$ km/s, $v_A \sim 300$ km/s), with no bowshock forming and standing magnetohydrodynamic Alfvén waves in Io's rest frame. The corotational electric field of 0.114 V/m produces a ~400-kV voltage drop across Io, driving a current of a few million amperes through Io's ionosphere; Alfvén waves carry the current along the essentially equipotential magnetic field lines into Io's inner hemisphere and away from its outer hemisphere. (Electrons, the likely current carriers, would of course flow in the opposite direction.) The current loop can be completed in Jupiter's ionosphere only if the round trip travel time for an Alfvén wave is short compared with the time it takes for torus plasma to sweep past Io; otherwise, the current loop is closed in the plasma torus (*45*).
20. J. Saur, F. M. Neubauer, D. F. Strobel, M. E. Summers, in preparation.
21. M. G. Kivelson *et al.*, *Science*, **273**, 337 (1996); M. G. Kivelson *et al.*, *ibid.* **274**, 396 (1996).
22. P. E. Geissler *et al.*, *Bull. Am. Astron. Soc.* **30**, 1116 (1998).
23. A. S. McEwen *et al.*, *Icarus* **135**, 181 (1998).
24. Jupiter's magnetic equator is tilted 9.6° with respect to its spin equator and Io's orbital plane. Jupiter's centrifugal equator—the locus of points that, of all points on a magnetic field line, are the farthest from Jupiter's spin axis—lies between the magnetic and spin equators. The Io plasma torus is densest at, and approximately symmetric about, the centrifugal equator.
25. R. C. Woodward Jr. and W. H. Smyth, *Bull. Am. Astron. Soc.* **26**, 1139 (1994).
26. L. A. Frank *et al.*, *Science* **274**, 394 (1996).
27. F. Bagenal *et al.*, *Geophys. Res. Lett.* **24**, 2119 (1997).
28. R. J. Oliversen *et al.*, *Bull. Am. Astron. Soc.* **29**, 1315 (1997).
29. F. Scherb, K. D. Retherford, R. C. Woodward Jr., W. H. Smyth, *ibid.* **28**, 1155 (1996).
30. D. L. Judge and R. W. Carlson, *Science* **183**, 317 (1974).
31. F. M. Wu, P. Gangopadhyay, D. L. Judge, *J. Geophys. Res.* **100**, 3481 (1995).
32. The terrestrial glow still contributed significant photon noise to our data, especially because we made these observations 2 months after opposition, and a fraction of the Earth's exosphere along our line of sight was therefore sunlit. This effect was most pronounced at the beginning of each orbit.
33. E. Lellouch, *Icarus* **124**, 1 (1996).
34. No correction has been made for the fact that Io blocks part of the H I Lyman-α emission from the interplanetary medium; all Io H I Lyman-α intensities given in this research article could therefore be 200 to 900 Rayleighs too low. Fluxes given in row 2 of Table 3 are not subject to this correction.
35. D. B. Nash and R. R. Howell, *Science* **244**, 454 (1989).
36. A. S. McEwen, T. V. Johnson, D. L. Matson, L. A. Soderblom, *Icarus* **75**, 450 (1988).
37. E. Lellouch *et al.*, *ibid.* **98**, 271 (1992).
38. F. Salama *et al.*, *ibid.* **107**, 413 (1994); R. W. Carlson *et al.*, *Geophys. Res. Lett.* **24**, 2479 (1997).
39. D. J. Williams *et al.*, *Science* **274**, 401 (1996).
40. J. R. Spencer and N. M. Schneider, *Annu. Rev. Earth Planet. Sci.* **24**, 125 (1996).
41. S. A. Manatt and A. L. Lane, *J. Quant. Spectrosc. Radiat. Transfer* **50**, 267 (1993).
42. M. C. Wong and R. E. Johnson, *J. Geophys. Res.* **101**, 23243 (1996).
43. T. Chust, A. Roux, S. Perraut, D. A. Gurnett, *Ann. Geophys.* **15**, C824 (1997).
44. L. A. Frank, W. R. Paterson, K. L. Ackerson, S. J. Bolton, *Eos* **79**, S201 (1998).
45. D. A. Wolf-Gladrow, F. M. Neubaer, M. Lussem, *J. Geophys. Res.* **92**, 9949 (1987).

46. We thank D. Hall for valuable assistance in planning this experiment, and J. Corliss and M. Freed for long hours spent in data reduction. We also gratefully acknowledge the cooperation and assistance of our co-workers on the STIS Instrument Development Team, and the many people at the Space Telescope Science Institute whose work made these observations possible. We thank anonymous reviewers for valuable comments on the manuscript. A portion of D.F.S.'s research was accomplished at the Observatoire de Paris-Meudon; he thanks Département de Recherche Spatiale for its hospitality. This work was supported by NASA grants NAGW-3319, NAG-4168, and NAGW-6546, and NASA contracts NAS5-30131 and NAS5-30403.

24 August 1998; accepted 10 December 1998

Fusion-Competent Vaccines: Broad Neutralization of Primary Isolates of HIV

Rachel A. LaCasse, Kathryn E. Follis, Meg Trahey, John D. Scarborough,* Dan R. Littman, Jack H. Nunberg†

Current recombinant human immunodeficiency virus (HIV) gp120 protein vaccine candidates are unable to elicit antibodies capable of neutralizing infectivity of primary isolates from patients. Here, "fusion-competent" HIV vaccine immunogens were generated that capture the transient envelope-CD4-coreceptor structures that arise during HIV binding and fusion. In a transgenic mouse immunization model, these formaldehyde-fixed whole-cell vaccines elicited antibodies capable of neutralizing infectivity of 23 of 24 primary HIV isolates from diverse geographic locations and genetic clades A to E. Development of these fusion-dependent immunogens may lead to a broadly effective HIV vaccine.

The expanding epidemic of HIV infection threatens to engulf more than 40 million persons worldwide by the year 2000 (*1*). The need for an effective HIV vaccine is urgent, but progress toward this goal has been slowed in part by the inability of any vaccine candidate to elicit antibodies capable of neutralizing infectivity of primary HIV isolates (PIs) from infected individuals (*2, 3*).

Because the HIV envelope protein mediates the early binding and entry steps in infection, many vaccine strategies have focused on this target. In 1993, two recombinant forms of the surface gp120 subunit of the HIV envelope protein (rgp120) were advanced as candidate vaccines for a large-scale efficacy study sponsored by the National Institutes of Health

R. A. LaCasse, K. E. Follis, M. Trahey, J. H. Nunberg, The Montana Biotechnology Center and Division of Biological Sciences, University of Montana, Missoula, MT 59812, USA. J. D. Scarborough and D. R. Littman, Howard Hughes Medical Institute, Skirball Institute of Biomolecular Medicine, New York University Medical Center, 540 First Avenue, New York, NY 10016, USA.

*Present address: Oregon Health Sciences University, Portland, OR 97201, USA.

†To whom correspondence should be addressed. E-mail: nunberg@selway.umt.edu

(NIH). In previous clinical studies, these rgp120 vaccines had been shown to be safe and to elicit antibodies capable of potently neutralizing related laboratory-adapted isolates of HIV (*4*). The inability of rgp120 vaccine sera to neutralize PI viruses, however, interrupted this momentum (*5*) and highlighted many central questions that have continued to impede development of an HIV vaccine candidate.

In contrast to antibodies elicited by rgp120 vaccines, antibodies from persons actively infected with HIV are able to neutralize infectivity of PI viruses, albeit incompletely (*6*). Surveys of patient sera typically report low-level neutralization of 30 to 50% of PIs, although breadth and titers vary. We speculated that this ability to neutralize PI viruses might be related to the presentation of functioning envelope protein in active infection, as compared with the static, nonfunctioning presentation of the envelope protein in rgp120 vaccines.

HIV Fusion and Fusion-Competent Vaccine Immunogens

The HIV envelope protein orchestrates a complex series of protein-protein interactions and structural changes that ultimately result in fusion of the virus and cell membranes and infection of the cell. Upon binding to CD4, the envelope protein undergoes conformational change that facilitates subsequent interaction with one of several coreceptor molecules, predominantly the CC chemokine receptor 5 (CCR5) or the CXC chemokine receptor 4 (CXCR4) (*7*). Interaction with either coreceptor induces further conformational change in the envelope protein and exposure of the hydrophobic fusion domain of the transmembrane gp41 subunit, which then mediates fusion of the apposed cell and virus membranes. On the basis of this dynamic model of HIV binding and entry, we undertook to develop HIV vaccine immunogens that explicitly incorporate these functional intermediate structures.

One measure of envelope protein function is the ability to mediate cell-cell fusion. When cells expressing envelope protein are cocultured with cells expressing CD4 and coreceptor, multinucleated syncytia form over the course of 6 to 24 hours. For our vaccine studies, we asked whether this process of binding and fusion might be captured in progress by formaldehyde cross-linking before extensive syncytium formation. In these studies, the functioning envelope protein was derived from a T lymphocytotropic PI virus obtained from the Amsterdam Cohort (ACH168.10; 168P) (*8*). The molecularly cloned envelope protein, as well as the parental syncytium-inducing (SI) virus, uses both CCR5 and CXCR4 coreceptors. COS-7 cells were transfected to express the envelope protein (COS-env) and subsequently cocultured with human U87 glioma cells that express CD4 and CCR5 coreceptor (U87-CD4-CCR5) (*9*). To capture transitional intermediates during the process of binding and fusion, we fixed cocultures in 0.2% form-

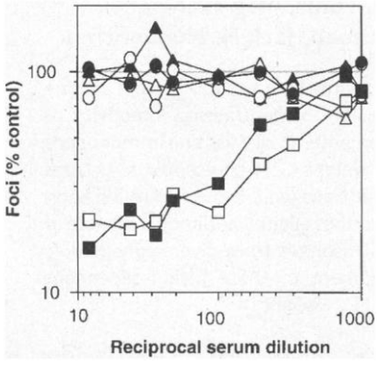

Fig. 1. Neutralization of the homologous 168P PI virus by FC vaccine sera. Transgenic mice (hu $CD4^+$, hu $CCR5^+$, mouse $CD4^+$) in studies 1 to 3 were immunized with FC immunogen (COS-env with U87-CD4-CCR5) (squares; n = 3 mice) or with cell controls (U87-CD4-CCR5 cells alone or cocultured with mock-transfected COS cells) (circles; n = 3 mice). Unimmunized mice were also used (triangles; n = 2 mice). Sera were tested for neutralization of 168P with U87-CD4 cells expressing either CXCR4 (black symbols) or CCR5 (white symbols). Data represent averages of three to six neutralization assays with serum obtained 2 weeks after the second or third immunization.

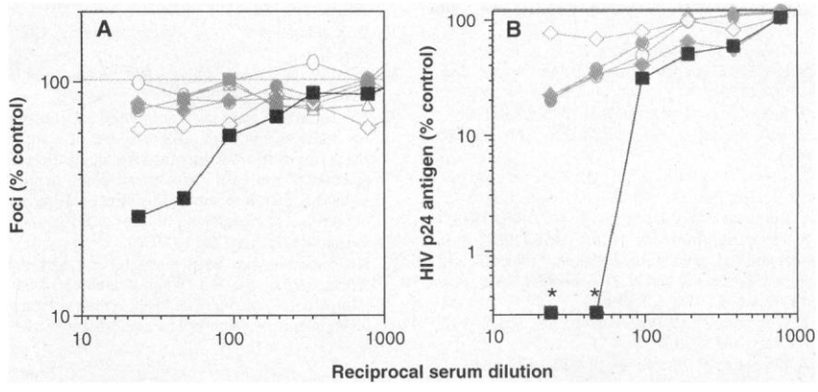

Fig. 2. Neutralization of 168P by FC, but not FI, vaccine sera. (**A**) Transgenic mice in study 4 were immunized with FC immunogen (black squares, n = 4), FI immunogens (COS-env with U87 cells, gray circles, n = 4; COS-env with U87-CD4 cells, gray diamonds, n = 3; COS-env with sCD4, white diamonds n = 2; COS-env with U87-CD4-CCR5 cells, each fixed separately before mixing for immunization, gray squares, n = 2), or mock-transfected COS cell immunogen (cocultured with U87-CD4-CCR5 cells) (white circles; n = 2). Unimmunized control mice (white triangles; n = 2) were also used. Neutralization was independent of specific coreceptor use (Fig. 1), and data here represent averages of three to six neutralization assays in U87-CD4-CXCR4 or -CCR5 cells. In some cases, sera that had been analyzed individually were pooled in equal proportions in order to conserve limited amounts of sera. (**B**) Neutralization by FC and FI vaccine sera was also determined in human PBL culture. PBLs were isolated, stimulated with phytohemagglutinin, and grown in the presence of interleukin-2; neutralization was determined as described (*13*). HIV p24 antigen was determined after 5 days of culture by ELISA, and values were normalized to the virus control (36 ng/ml). Asterisks indicate p24 antigen concentrations below the limit of detection at the dilution used in the ELISA. Vaccine groups are as defined above, and sera were pooled for this assay.

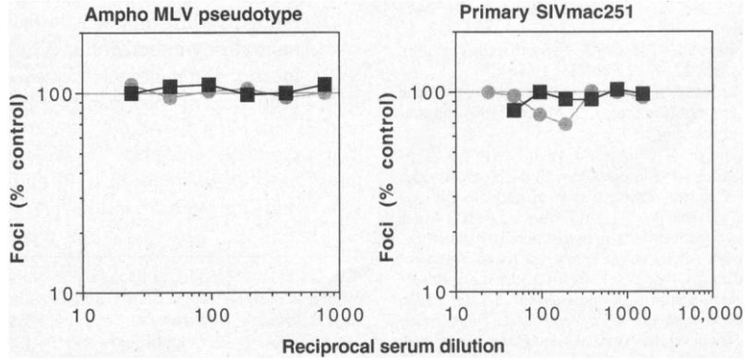

Fig. 3. FC vaccine serum does not neutralize pseudotyped HIV virions bearing amphotropic MLV envelope protein (*19*) or primary SIVmac251 (*20*). For HIV bearing an amphotropic MLV envelope protein (ampho MLV pseudotype), neutralization sensitivity with pooled FC and FI antisera was determined in U87-CD4-CXCR4 cells. For primary isolate SIVmac251, neutralization was determined in U87-CD4-CCR5 cells. Symbols are as defined in Fig. 2: FC immunogen (black squares) and FI immunogen (COS-env + U87 cells, gray circles).

aldehyde after 5 hours (*10*) when few if any multinucleate cells were evident. This inactivated whole-cell preparation was used as the fusion-competent (FC) immunogen.

To test the ability of these complex immunogens to elicit neutralizing antibodies, it was necessary to restrict the immune response to viral and virus-induced epitopes. Otherwise, antibodies to CD4 and CCR5 would be generated that would themselves block infectivity. Therefore, it was essential to use an animal model that was immunologically tolerant to the human (hu) CD4 and CCR5 components of the vaccine, as would also be the case in human immunization. Thus, immunogenicity studies were performed with transgenic mice that express hu CD4 and hu CCR5 coreceptor (*11*).

In pilot studies, mice were immunized with either FC immunogen or with cell controls (U87-CD4-CCR5 cells, alone or cocultured with mock-transfected COS cells) (*12*). Sensitivity of the homologous 168P virus to neutralization by vaccine sera was determined with U87-CD4 cells expressing either CCR5 or CXCR4 coreceptor (*13*). No inhibition of infectivity was observed in sera from mice immunized with cell controls, suggesting that the transgenic mice were in fact tolerant to hu CD4 and CCR5 and that other adventitious cellular reactivities did not interfere with the virus infectivity assay (Fig. 1). Sera from mice immunized with FC immunogens were able to neutralize the homologous 168P PI virus. This neutralization activity was antibody-mediated and could be adsorbed to, and subsequently eluted from, a solid support containing protein A and protein G (*14*). Furthermore, neutralization of the 168P virus by FC serum was observed regardless of the coreceptor used in the U87-CD4 cell infection assay (Fig. 1). Several reports have demonstrated that, in general, neutralization sensitivity is independent of specific

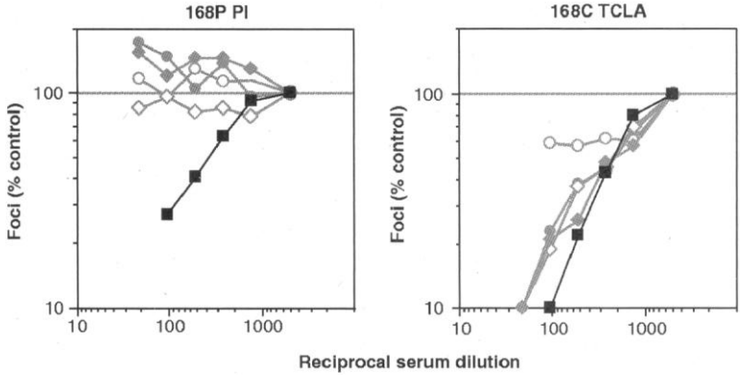

Fig. 4. Neutralization of TCLA 168C virus by FI vaccine sera. Neutralization sensitivity of the 168P PI virus and its TCLA derivative 168C were tested in U87-CD4-CXCR4 cells with pooled sera: FC immunogen (black squares), FI immunogens (COS-env + U87 cells, gray circles; COS-env + U87-CD4 cells, gray diamonds; COS-env + sCD4, white diamonds), and mock-transfected cell controls (white circles).

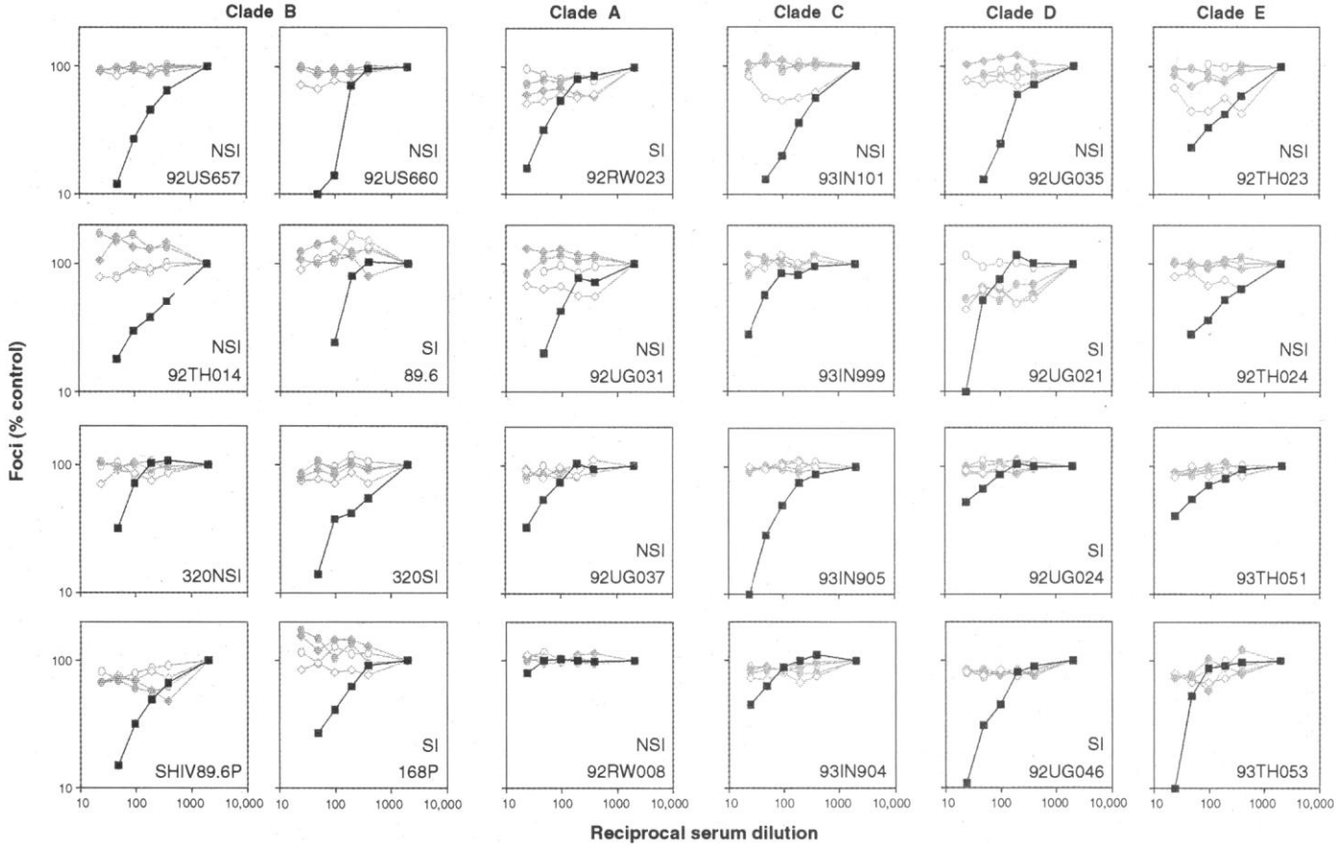

Fig. 5. Neutralization of diverse PI viruses from clades A to E. Primary isolates were expanded in human PBLs, and neutralization was determined in permissive U87-CD4-CCR5 (or -CXCR4) cells with pooled sera: FC immunogen (black squares), FI immunogens (COS-env + U87 cells, gray circles; COS-env + U87-CD4 cells, gray diamonds; COS-env + sCD4, white diamonds, and mock-transfected cell controls (white circles). Viral biotype is indicated if known.

coreceptor use (*15–17*). The fact that neutralization is observed in this study with CXCR4, a coreceptor to which the animal had not been exposed, argues that neutralization does not directly target the CCR5 component of the vaccine.

To explore the role of fusion-dependent determinants in the induction of PI virus neutralization, we expanded our studies to include fusion-incompetent (FI) immunogens—cocultures that do not undergo cell-cell fusion. These include COS-env cocultured with U87 cells (no CD4 or CCR5 coreceptor), COS-env cocultured with U87-CD4 cells, and COS-env cells to which soluble CD4 (sCD4) was complexed (*18*). An additional FI immunogen comprised COS-env and U87-CD4-CCR5 cells that were separately fixed with formaldehyde before mixing during the formulation of the vaccine.

In marked contrast to FC immunogens, all FI immunogens were unable to elicit significant neutralization of the homologous PI virus (Fig. 2A). These results are consistent with the well-documented failure of rgp120 vaccines to elicit PI virus neutralization. The difference in neutralization by FC and FI vaccine sera was also observed in assays with human primary blood lymphocytes (PBLs) (Fig. 2B).

Despite the apparent requirement for fusion competency in the immunogen, we wanted to exclude the possibility that FC vaccine sera inhibited viral infectivity in a nonspecific manner. Thus, FC vaccine sera were shown not to inhibit infectivity of pseudotyped HIV virions bearing an amphotropic murine leukemia virus (MLV) envelope protein (*19*), nor to neutralize a PI of the simian immunodeficiency virus SIVmac251 (*20*) (Fig. 3).

As with conventional rgp120 vaccines, FI vaccines were able to elicit neutralization of a related laboratory-adapted isolate of HIV, the T cell line–adapted derivative of 168P, 168C (*2, 15*) (Fig. 4). Neutralization titers of the TCLA 168C virus were comparable between FC and FI vaccine sera, as were titers of antibodies to gp120 (anti-gp120), suggesting a similar degree of inherent immunogenicity among the vaccines.

The failure of FI vaccines to elicit PI virus neutralization in the transgenic mouse model highlights the specificity of the neutralization elicited by FC vaccines (*21*). Furthermore, the consistent failure of FI vaccine sera to inhibit PI virus infectivity strongly indicates that the immune response is not directed to adventitious human cellular targets, such as those that confounded early studies of inactivated SIV vaccines (*22*). Rather, we suggest that FC immunogens present unique fusion-dependent determinants that mediate neutralization of PI viruses.

Broad Neutralization of Primary Isolates

A critical issue in HIV vaccine development centers on the ability of vaccine antisera to neutralize a broad range of diverse PI viruses. To determine the breadth of PI virus neutralization elicited by FC immunogens, we examined the sensitivity of a panel of representative PI viruses from five prevalent and geographically diverse phylogenetic clades (*23*). As depicted in Fig. 5, FC sera elicited by a functioning clade B envelope protein were able to neutralize 23 of 24 PI viruses tested—monocytotropic (non-SI; NSI) and T lymphocytotropic (SI viruses) from North America and Europe (clade B), Africa (clades A and D), Thailand (clades B and E), and India (clade C). Despite the sequence diversity among these isolates, most were similarly sensitive to neutralization by FC vaccine sera. One isolate (92RW008) failed to attain >50% neutralization and two others (93IN904 and 92UG024) showed limited neutralization beyond 50%; these exceptions to the otherwise broad pattern of neutralization further indicate that FC immunogens target primarily viral, rather than cellular, determinants. FI sera were uniformly unable to neutralize these heterologous PI viruses, in keeping with the failure of rgp120 immunogens. The broad and uniform neutralization of diverse PI viruses suggests that the critical determinants presented by FC immunogens are highly conserved and may be intimately tied to the basic functioning of the envelope protein in binding and fusion.

Molecular Target for PI Virus Neutralization

We next sought to define the molecular target for neutralization by FC vaccines. We had shown that the elicitation of PI virus–neutralizing antibodies required as immunogen a functional envelope-CD4-CCR5 interaction. Nonetheless, these neutralizing antibodies might recognize native, nonfunctioning envelope protein as antigen. A similar instance has been reported for monoclonal antibody (mAb) 17b, which targets a CD4-induced epitope on the gp120-CD4 complex but which also binds isolated gp120 (*24*). In contrast, another gp120-CD4–specific mAb CG10 (*25*) does not recognize isolated gp120 or CD4. Thus, we tested whether neutralizing antibodies could be removed from FC vaccine sera on incubation with envelope protein expressed on the surface of transfected COS cells. Formaldehyde-fixed COS cells expressing 168P envelope protein were incubated with FC serum, and the recovered serum was then tested for PI virus neutralization (*26*). Neutralization activity in FC vaccine serum was removed by incubation with envelope-expressing cells, but only minimally reduced by incubation with COS cell controls (Fig. 6). Although the static form of the envelope protein does not function as an effective immunogen, we found that some aspects of the critical fusion-dependent epitopes are sufficiently represented on the static protein to allow binding. This initial binding to nonfunctioning envelope protein may facilitate subsequent access of antibody to transient fusion-dependent conformations that arise during binding and fusion. Importantly, these data strongly suggest that PI virus neutralizing antibodies target, at least in part, the HIV envelope protein.

These data also provide independent support that neutralizing antibodies do not target adventitious cellular proteins, which would be equally recognized (or not recognized) on control COS cells. To further exclude this possibility, we examined whether neutralization activity could be removed by adsorption to U87-CD4-CCR5 cells. FC serum was incubated with intact U87-CD4-CCR5 cells, and the recovered serum was then tested for PI virus neutralization (*27*). As demonstrated in Fig. 7, no reduction in FC serum neutralization was

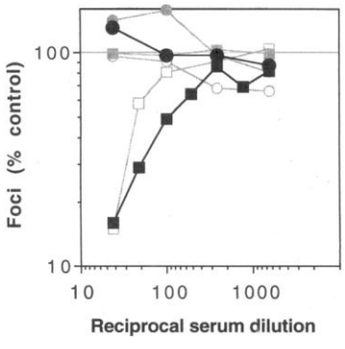

Fig. 6. Adsorption of PI virus neutralization activity by formaldehyde-fixed COS-env cells. FC vaccine serum was repeatedly incubated with formaldehyde-fixed COS-env (gray squares) or control COS (white squares) cells. Serum obtained before FC immunization was similarly adsorbed (gray and white circles, respectively). The starting FC and preimmunization sera are indicated as black squares or black circles, respectively. Sera were tested for neutralization of 168P with U87-CD4-CXCR4 cells.

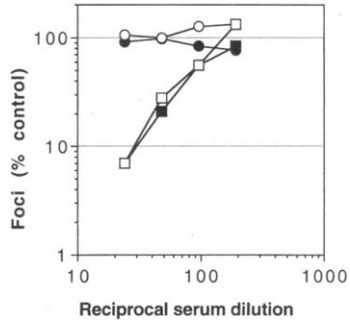

Fig. 7. PI virus neutralization activity is not adsorbed by intact U87-CD4-CCR5 cells. Pooled FC vaccine serum was incubated with U87-CD4-CCR5 cells (white squares) or in an empty microculture well (mock; black squares). Pooled FI serum (COS-env + U87-CD4) was similarly treated (white and black circles, respectively). Sera were tested for remaining neutralization of 168P with U87-CD4-CCR5 cells.

observed on adsorption to U87-CD4-CCR5 cells.

Taken together, our data provide evidence that FC serum neutralization targets highly conserved envelope protein structures that arise transiently during binding and fusion. Nonetheless, absolute exclusion of fusion-induced cellular targets, and the ultimate definition of specific envelope protein determinants, must await the analysis of PI virus–neutralizing mAbs to FC immunogens.

On the basis of the requirement for interaction with coreceptor, we speculate that neutralization may target a late event in virus binding and entry. Upon binding to coreceptor, the envelope protein must mediate fusion of the viral and cell membranes. Cryptic but highly conserved determinants may be exposed during this process. One possible target for neutralization might involve the trimeric coiled-coil structure that mediates membrane fusion and is highly conserved among Orthomyxoviridae (Influenza), Filoviridae (Ebola), and Retroviridae (HIV) (*28*). The fusion-active structure of the HIV envelope protein is believed to form subsequent to CD4 and coreceptor binding by the collapse of two helical coils within each gp41 monomer. This trimeric coiled-coil structure is thought to drive membrane insertion of the hydrophobic fusion domain of gp41 and to initiate membrane fusion. Synthetic peptides that comprise either of the gp41 helical coils are able to bind the cognate helical region and broadly inhibit viral infectivity (*29*). Broadly neutralizing antibodies to FC immunogens may likewise target structures involved in the activation of fusion.

The potency and breadth of neutralization by FC immunogens appears to surpass that observed in sera from infected individuals or in the more broadly neutralizing human mAbs (*30*). Perhaps formaldehyde fixation traps critical fusion-dependent structures that are only transiently presented during active infection. It remains to be determined whether FC immunogens target the same neutralizing determinants as active infection.

Implications for HIV Vaccine Development

The ability of antibodies induced by FC immunogens to neutralize a broad range of PI viruses necessitates a shift in our thinking on HIV vaccines. Whereas previous discussions regarding the possible number of HIV serotypes were moot in the absence of any vaccine-induced neutralization, it had been widely accepted that multiple envelope protein immunogens might be needed to span the range of HIV sequence diversity. We now show that an appropriately presented clade B envelope protein can elicit potent neutralization against most PI viruses from multiple HIV clades. We show that not only is PI virus neutralization achievable, we suggest that broad vaccine protection may not require an unlimited number of HIV serotypes.

Although the immunological basis for potential HIV vaccine efficacy is presently unknown and controversial, there is ample reason to believe that preexisting neutralizing antibody may offer protection against infection or disease. Passively administered neutralizing antibodies have been shown to exert potent antiviral effects in several experimental models of HIV infection, as manifested by complete or partial protection (*31*). Antibody-mediated protection is, of course, well accepted in other viral infections and vaccines (*32*). With the ability to elicit potent HIV neutralization will come the opportunity to determine the role of preexisting neutralizing antibody in HIV prophylaxis. Concerns regarding the diversity of HIV populations are lessened by the breadth of neutralization elicited by FC immunogens. Still, one may raise the concern that extant antibodies in HIV-infected persons do not protect against disease progression. Whether the ultimate disease course is modified by the ongoing antibody response is difficult to assess. In any case, an established chronic infection presents special challenges in terms of host competence and virus load and diversity, challenges that are distinct from those envisioned in prophylactic immunization. The effect of preexisting antibodies in exposure to a minimal infectious dose of virus in an immunocompetent host remains to be determined in preclinical and clinical studies.

We recognize that in its current form an inactivated whole-cell FC vaccine is not practical for clinical development. Nonetheless, FC formulations that incorporate critical fusion-dependent determinants of PI virus neutralization can be envisioned. For example, recombinant viral vectors that respectively express envelope and CD4 with coreceptor could be coadministered to drive critical fusion events in vivo. Alternatively, purified fusion-active complexes could be developed as an inactivated subunit vaccine. This concept of FC immunogens may also be applicable to other enveloped viruses where protection has been difficult to generate other than by live attenuated virus immunization.

References and Notes

1. UNAIDS Report (available at www.unaids.org/highband/document/epidemio/wadr98e.pdf).
2. T. Wrin, T. P. Loh, J. Charron-Vennari, H. Schuitemaker, J. H. Nunberg, *J. Virol.* **69**, 39 (1995).
3. J. P. Moore and D. D. Ho, *AIDS* **9** (suppl. A), S117 (1995); J. R. Mascola *et al.*, *J. Infect. Dis.* **173**, 340 (1996); R. I. Connor *et al.*, *J. Virol.* **72**, 1552 (1998); B. S. Graham *et al.*, *J. Infect. Dis.* **177**, 310 (1998).
4. R. B. Belshe *et al.*, *J. Am. Med. Assoc.* **272**, 475 (1994); J. O. Kahn *et al.*, *J. Infect. Dis.* **170**, 1288 (1994).
5. J. Cohen, *Science* **262**, 980 (1993); *ibid.* **264**, 1839 (1994).
6. J. R. Mascola *et al.*, *AIDS Res. Hum. Retrovir.* **12**, 13198 (1996); J. P. Moore *et al.*, *J. Virol.* **70**, 427 (1996); L. G. Kostrikis, Y. Cao, H. Ngai, J. P. Moore, D. D. Ho, *ibid.*, p. 445; J. Weber, E. M. Fenyo, S. Beddows, P. Kaleebu, A. Bjorndal, *ibid.*, p. 7827.
7. Reviewed in E. A. Berger, *AIDS* **11** (suppl. A), S3 (1997); J. P. Moore, A. Trkola, T. Dragic, *Curr. Opin. Immunol.* **9**, 551 (1997); B. J. Doranz, J. F. Berson, J. Rucker, R. W. Doms, *Immunol. Res.* **16**, 15 (1997).
8. The molecularly cloned envelope gene of ACH168.10 was isolated by polymerase chain reaction with the pCR3.1-Uni plasmid (Invitrogen) [(*2*, *15*); M. Tersmette *et al.*, *J. Virol.* **63**, 2118 (1989)].
9. The plasmid encoding expression of the functional 168P envelope protein (168P23) was transfected into COS-7 cells (American Type Culture Collection, Manassas, VA) by calcium phosphate precipitation (20 μg of DNA per 10^6 cells per 10-cm culture dish) [M. Jordan, A. Schallhorn, F. M. Wurm, *Nucleic Acids Res.* **24**, 596 (1996)]. Transiently expressing COS-7 cells were harvested 2 days later with 0.5 mM EDTA in phosphate-buffered saline (PBS), and U87-CD4-CCR5 fusion partners [C. M. Hill *et al.*, *J. Virol.* **71**, 6296 (1997)] were prepared with 0.1 mM EDTA in PBS. Cocultures were initiated by mixing the two cell types (1.5×10^6 cells each) in 10-cm culture dishes. The time course of cell-cell fusion was monitored microscopically and by immunochemical staining (HIVIG) in parallel cocultures (*15*). Cocultures were harvested by formaldehyde fixation at 4 to 5 hours, when little or no overt syncytium formation was evident.
10. Cultures were fixed in situ with 0.2% formaldehyde in PBS at 4°C overnight [J. K. Yamamoto *et al.*, *AIDS Res. Hum. Retrovir.* **7**, 911 (1991); E. J. Verschoor *et al.*, *Vet. Immunol. Immunopathol.* **46**, 139 (1995)]. Cells were subsequently scraped, washed twice with PBS, resuspended at a nominal density of 3×10^6 cells/0.1 ml in PBS containing 10% dimethyl sulfoxide, and frozen at −80°C.
11. J. D. Scarborough, W. Ellmeier, D. R. Littman, unpublished results. Construction of a CD4 targeted deletion and hu CD4 transgenic mouse has been described [N. Killeen, S. Sawada, D. R. Littman, *EMBO J.* **12**, 1547 (1993)]. Briefly, a hu CCR5 transgenic mouse was constructed by molecularly cloning a 1.15-kb hu CCR5 cDNA into an engineered Sal I site in exon 2 of a murine CD4 expression cassette [construct c in S. Sawada, J. D. Scarborough, N. Killeen, D. R. Littman, *Cell* **77**, 917 (1994)]. This minigene contains the murine CD4 enhancer, the CD4 promoter, the first (noncoding) exon, and intron 1 with an internal deletion that eliminates the CD4 silencer. Transgenic founders were identified by flow cytometry with a monoclonal antibody (mAb) to CCR5. These animals were bred to hu CD4 transgenic mice to yield progeny expressing hu CD4, hu CCR5, and mouse CD4. Pups were screened for expression of hu CD4, hu CCR5, and mouse CD4 by flow cytometry with a Coulter EPICS ELITE flow cytometer. The following antibody reagents were used: mouse antibody to human CD4 conjugated to CyChrome (Pharmingen), mouse antibody to human CCR5 mAb 180 (R&D Systems) with goat antibody to mouse immunoglobulin conjugated to fluorescein isothiocyanate (Caltag), and rat antibody to mouse CD4 L3T4 conjugated to phycoerythrin.
12. Vaccines comprised formaldehyde-fixed whole cells (3×10^6 cells/0.1 ml) formulated with an equal volume of Ribi adjuvant (R-700; reconstituted in half the recommended volume of PBS); in some experiments, the initial immunization was with adjuvant containing cell wall material (R-730). Mice received 0.05 ml of vaccine in four subcutaneous sites. Booster immunizations were at 3-week intervals, and mice were bled from the tail at 10 to 28 days after immunizations. Ultimately, mice were boosted and exsanguinated by cardiac puncture in order to obtain larger quantities of serum. Animal care was in accordance with institutional guidelines. Serum antibodies directed to gp120 were quantitated by gp120 enzyme-linked immunosorbent assay (ELISA) [J. Moore, L. Wallace, E. Follett, J. McKeating, *AIDS* **3**, 155 (1989)].
13. This rapid PI virus neutralization assay has been validated relative to our standard neutralization assay in peripheral blood lymphocyte (PBL) culture (*15*, *17*) and performs well in the presence of mouse serum. All sera were heat inactivated before use in neutralization assays.

14. R. A. LaCasse *et al.*, data not shown. Serum was adsorbed sequentially to protein A Sepharose (Sigma) and protein G agarose (Sigma) at 4°C. Adsorption of antibody was confirmed by gp120 ELISA. The solid supports were combined and antibodies were eluted with 100 mM glycine, pH 2.5. The eluate was neutralized and dialyzed by centrifugal ultrafiltration (Microcon-100; Amicon).
15. R. A. LaCasse *et al.*, *J. Virol.* **72**, 2491 (1998).
16. A. Trkola *et al.*, *ibid.*, p. 1876; D. C. Montefiori *et al.*, *ibid.*, p. 3427.
17. K. E. Follis, M. Trahey, R. A. LaCasse, J. H. Nunberg, *ibid.*, p. 7603.
18. Envelope-expressing cultures were incubated with sCD4 [E. A. Berger, T. R. Fuerst, B. Moss, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2357 (1988)] (5 μg/ml; 1 hour at 37°C) and subsequently washed to remove unbound sCD4. All FI immunogens were fixed with formaldehyde as described in (*10*).
19. Envelope-defective HIV NL4-3-Luc-R$^-$E$^-$ provirus was pseudotyped with amphotropic MLV envelope protein [H. Deng *et al.*, *Nature* **381**, 661 (1996)].
20. A primary isolate of SIVmac251 [A. L. Langlois *et al.*, *J. Virol.* **72**, 6950 (1998)] was produced in rhesus PBLs.
21. A statistical comparison was performed on data comprising all experimental animals and all virus neutralization assays. A simple model for virus-antibody binding was used to calculate a "binding constant" K for each assay, and a mean K value was determined for each mouse. Two-sample unpooled t tests were performed on log-transformed K values to compare groups pairwise. Bonferroni-adjusted comparison demonstrated a significant difference in mean neutralization between FC and FI immunogens ($P = 0.001$). This analysis included 26 independent neutralization assays of FC sera, from all mice receiving FC immunogen, and similarly, 35 assays of FI sera. In all experiments, responses within experimental groups were consistent and uniform.
22. M. P. Cranage *et al.*, *AIDS Res. Hum. Retrovir.* **9**, 13 (1993); P. Putkonen *et al.*, *J. Med. Primatol.* **22**, 100 (1993); L. O. Arthur *et al.*, *Science* **258**, 1935 (1992).
23. Infectious proviruses ACH320.2A.1.2 (320SI) and ACH320.2A.2.1 (320NSI) [M. Groenink *et al.*, *J. Virol.* **65**, 1968 (1991); C. Guillon *et al.*, *AIDS Res. Hum. Retrovir.* **11**, 1537 (1995)] were obtained through the National Institute for Biological Standards and Controls (NIBSC, United Kingdom) AIDS Reagent Program from Hanneke Schuitemaker (Netherlands Red Cross). HIV89.6 [R. Collman *et al.*, *J. Virol.* **66**, 7517 (1992)], SHIV89.6, and SHIV89.6P [K. A. Reimann *et al.*, *ibid.* **70**, 3198 (1996)] were provided with permission by D. Montefiori (Duke University Medical Center). All other primary isolates were obtained through the NIH AIDS Research and Reference Reagent Program and the UNAIDS Network for HIV-1 Isolation and Characterization. PI viruses were subjected to limited expansion in phytohemagglutinin-activated PBLs (*2*).
24. M. Thali *et al.*, *J. Virol.* **67**, 3978 (1993); N. Sullivan *et al.*, *ibid.* **72**, 4694 (1998).
25. J. M. Gershoni *et al.*, *FASEB J.* **7**, 1185 (1993); S. Lee *et al.*, *J. Virol.* **71**, 6037 (1997).
26. FC vaccine serum (1:10 dilution in cell culture medium) was sequentially adsorbed four times with ~10^6 formaldehyde-fixed COS cells expressing 168P envelope protein. Incubations were for 1 hour at 4°C with rocking. Controls included prebleed serum and formaldehyde-fixed, mock-transfected COS cells. Adsorption of bulk anti-gp120 was monitored by gp120 ELISA. Final sera were tested for neutralization of HIV 168P with U87-CD4-CXCR4 cells. Parallel studies with intact but nonfixed cells yielded concordant results.
27. Pooled FC and FI sera (1:8 dilution) were incubated with attached U87-CD4-CCR5 cells (2×10^4 cells per 96-well microculture) for 1 hour at 37°C with occasional mixing. Supernatants were tested for neutralization of HIV 168P with U87-CD4-CCR5 cells.
28. W. Weissenhorn *et al.*, *Nature* **387**, 426 (1997); D. C. Chan, D. Fass, J. M. Berger, P. S. Kim, *Cell* **89**, 263 (1997); W. Weissenhorn, L. J. Calder, S. A. Wharton, J. J. Skehel, D. C. Wiley, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6032 (1998).
29. C. Wild, T. Oas, C. McDanal, D. Bolognesi, T. Matthews, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10537 (1992); R. A. Furuta, C. T. Wild, Y. Weng, C. D. Weiss, *Nature Struct. Biol.* **5**, 276 (1998).
30. M. P. D'Souza *et al.*, *J. Infect. Dis.* **175**, 1056 (1997).
31. A. M. Prince *et al.*, *AIDS Res. Hum. Retrovir.* **7**, 971 (1991); E. A. Emini *et al.*, *Nature* **355**, 728 (1992); A. J. Conley *et al.*, *J. Virol.* **70**, 6751 (1996); M. C. Gauduin *et al.*, *Nature Med.* **3**, 1389 (1997).
32. R. M. Krause, N. J. Dimmock, D. M. Morens, *J. Infect. Dis.* **176**, 549 (1997).
33. J.H.N. was supported by targeted research grant 02560-23-RGV from the American Foundation for AIDS Research (AmFAR), funded in part by Concerned Parents for AIDS Research (CPFA). Additional funds were provided by The University of Montana, NIH AREA grant AI41165, and the M. J. Murdock Charitable Trust. D.R.L. was supported by NIH grant AI33856 and by a grant from AmFAR and is an investigator of the Howard Hughes Medical Institute. We thank E. Walker (Ribi ImmunoChem Research, Incorporated, Hamilton, MT) and L. Griggs for flow cytometry, C. Mackay (Leukosite) and M. Tsang for CCR5 mAbs, W. Ellmeier for the transgenic mouse CCR5 construct, D. Montefiori (Duke University Medical Center) for providing SIVmac251, HIV 89.6, and related SHIV viruses, and C. Weiss (U.S. Food and Drug Administration) for providing amphotropic MLV envelope protein pseudotyped HIV virions. Primary HIV isolates and other reagents were obtained from the NIH AIDS Research and Reference Reagent Program, the NIBSC (UK) AIDS Reagent Program, and the UNAIDS Network for HIV-1 Isolation and Characterization. We are grateful to D. A. Patterson (University of Montana, Department of Mathematics) for statistical analysis of neutralization data, and to J. Moore and D. Montefiori for constructive review of the manuscript. Discussions with C. Barbas III and E. Berger were important in the development of these studies.

13 October 1998; accepted 10 December 1998

Enhancement of Cation Diffusion Rates Across the 410-Kilometer Discontinuity in Earth's Mantle

S. Chakraborty,*† R. Knoche, H. Schulze, D. C. Rubie, D. Dobson, N. L. Ross, R. J. Angel

Rates of cation diffusion (magnesium, iron, and nickel) have been determined in olivine and its high-pressure polymorph, wadsleyite, at 9 to 15 gigapascals and 1100° to 1400°C for compositions that are relevant to Earth's mantle. Diffusion in olivine becomes strongly dependent on composition at high pressure. In wadsleyite, diffusion is one to two orders of magnitude faster than in olivine, depending on temperature. Homogenization of mantle heterogeneities (chemical mixing) and mineral transformations involving a magnesium-iron exchange will therefore occur considerably faster in the transition zone than at depths of less than 410 kilometers.

The diffusion of atoms in crystalline solids at high temperature is a process that is controlled by the concentration and mobility of point defects. In Earth's interior, solid state creep (through which mantle convection occurs) and electrical conduction, which is used as a probe of the structure and composition of Earth, are physical processes that depend on diffusion (*1*). The rates of chemical processes (such as the mixing of mantle heterogeneities, mineral transformations, and element partitioning during partial melting) are also determined by diffusion rates. Even the sharpness of mantle discontinuities may be controlled by cation diffusion during mantle convection, because the kinetics of phase transformations are likely to be diffusion controlled in such regions (*2*, *3*).

However, diffusion coefficients for high-pressure mantle phases (for example, wadsleyite, ringwoodite, and silicate perovskites) are poorly known because of the difficulties of performing appropriate experiments at the pressures (P) and temperatures (T) at which these phases are stable (*4*, *5*). Here, we report the diffusion behavior of major elements (Fe

S. Chakraborty, Institut für Mineralogie und Geochemie, Universität zu Köln, Zülpicher Strasse 49B, 50674 Köln, Germany. R. Knoche, Institut für Mineralogie und Petrographie, Universität Innsbruck, Innrain 52, A-6020, Innsbruck, Austria. H. Schulze, D. C. Rubie, R. J. Angel, Bayerisches Geoinstitut, Universität Bayreuth, 95440 Bayreuth, Germany. D. Dobson and N. L. Ross, Mantle Petrology Group, Department of Geological Sciences, University College London, Gower Street, London WC1E 6BT, UK.

*To whom correspondence should be addressed. E-mail: Sumit.Chakraborty@rz.ruhr-uni-bochum.de
†Present address: Institut für Mineralogie, Ruhr-Universität Bochum, D-44780 Bochum, Germany.