JOURNAL OF VIROLOGY, Aug. 2002, p. 8455–8459 Vol. 76, No. 16
0022-538X/02/$04.00+0 DOI: 10.1128/JVI.76.16.8455–8459.2002

NOTES

Nef Enhances Human Immunodeficiency Virus Type 1 Infectivity and Replication Independently of Viral Coreceptor Tropism

Armin Papkalla,[1] Jan Münch,[2] Claas Otto,[2] and Frank Kirchhoff[2]*

Institute for Clinical and Molecular Virology, University of Erlangen-Nürnberg, 91054 Erlangen,[1] and Department of Virology, University of Ulm, 89081 Ulm,[2] Germany

Received 21 February 2002/Accepted 17 May 2002

We investigated the infectivities and replicative capacities of a large panel of variants of the molecular human immunodeficiency virus type 1 (HIV-1) NL4-3 clone that differ exclusively in the V3 region of the viral envelope glycoprotein and the *nef* gene. Our results demonstrate that Nef enhances virion infectivity and HIV-1 replication independently of the viral coreceptor tropism.

Nef is a myristoylated membrane-associated protein that is important for efficient replication and pathogenicity of human and simian immodeficiency viruses (HIV and SIV, respectively) (15, 25, 27). Nef increases viral spread both indirectly by mechanisms of immune evasion and directly by enhancing viral infectivity and replication (reviewed in references 14, 17, and 37). Enhancement of virion infectivity and stimulation of viral replication are well-conserved properties of primary *nef* alleles and most likely contribute to HIV type 1 (HIV-1) virulence (7). The exact molecular mechanisms that mediate both of these Nef functions remain elusive. The ability of Nef to enhance viral infectivity involves enhanced proviral DNA synthesis (2, 12). It has been proposed that targeting to lipid rafts (43, 45), increased Env incorporation (30), altered viral Env function (1, 9, 46), enhanced efficiency of reverse transcription (26, 41), or particle disassembly (32, 40) might account for the more effective initiation of proviral DNA. Nef is found in the core of the virions and is cleaved by the viral protease (5, 28, 36, 44). However, cleavage seems not to be required for the enhancement of infectivity (10), and the relevance of virion association is unclear. It has been proposed that Nef-mediated CD4 down-regulation is important for enhanced virion infectivity and effective release of viral particles (30, 39). Furthermore, down-modulation of CD4 correlates with the efficiency of viral replication in peripheral blood mononuclear cells (PBMC) and in human lymphoid tissue ex vivo (7, 21, 31). However, Nef also enhances the infectivity of HIV-1 particles produced from CD4-negative cells (6, 21). Several recent studies suggest that the effect of Nef on infectivity might not account for the enhanced growth kinetics of HIV-1 containing a functional open *nef* gene in primary lymphocyte culture (7, 21, 31). The enhanced replication kinetics of *nef*-open viruses are observed predominantly in PBMC that have been infected immediately after isolation and stimulated several days later and not in prestimulated lymphocyte cultures or in most immortalized T-cell lines (11, 34, 42). It is well established that Nef alters intracellular signaling pathways (reviewed in reference 22) and that increased replication of *nef*-open virus is most likely due to lymphoid cell activation (3, 19).

Infection of cells by HIV-1 requires binding of the viral envelope glycoprotein to CD4 and subsequent interactions with G protein-coupled coreceptors, usually CCR5 (R5) or CXCR4 (X4) (reviewed in reference 4). Some aspects of Nef and Env functions are overlapping. For example, both proteins reduce cell surface expression of CD4 (8, 20), alter CD3 signaling (23, 24), and affect apoptosis (reviewed in reference 38). It has been suggested that Nef might support virus-cell fusion or change the envelope structure, resulting in enhanced release of the core into the cytoplasm (32, 40, 46). The interaction of the envelope glycoprotein with CD4 and coreceptors transduces intracellular signals (13) and might cause cellular activation independently of Nef function. For example, it has been described that both the gp120 signaling cascade and Nef can activate the mitogen-activated protein kinase pathway (16, 33).

These previous studies suggest that the interaction of the HIV-1 Env protein with different coreceptors might have an impact on the effect of Nef on both virion infectivity and viral replication. To evaluate this possibility, we generated a series of *nef*-open and *nef*-defective mutants of the molecular HIV-1 NL4-3 clone containing the V3 regions derived from 28 primary isolates or molecular HIV-1 clones with differential coreceptor tropism (Table 1). Splice overlap extension PCR was used to replace the V3 loop sequence of NL4-3, and virus stocks were generated by transient transfection of 293T cells as described previously (35). Coreceptor utilization of the HIV-1 NL4-3 V3 variants was determined by infection of X4.15 and R5.3 cells as described previously (29, 35). Infectivity assays performed in triplicate with three independent virus stocks demonstrated that 13 of these NL4-3 V3 recombinants were R5-tropic, 3 were X4-tropic, 2 were dualtropic, and 10 were noninfectious (Table 1). As expected, the ability to utilize X4 correlated with a high positive charge of the V3 loop amino acid sequence (Table 1).

* Corresponding author. Mailing address: Department of Virology, University of Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany. Phone: 49-731-50023344. Fax: 49-731-50023389. E-mail: frank.kirchhoff@medizin.uni-ulm.de.

TABLE 1. Properties of HIV-1 NL4-3 V3 loop recombinants

Clone	V3 loop sequence[a]	Charge	Tropism	Replication[b]	
				PM-1	PBMC
	CTRPNNNTRKSIHIQRGPGRAFYTTGEI..IGDIRQAHC				
NL4–3wt	----------r---------v-i-k-...-nm-----	+8	X4	++	++
005pf130	----------g-n-..----------..----------	+5	R5	(+)	(+)
005pf135	----------g---..----------..----------	+5	R5	+	+
011jr101	----------p-..----------..--g------	+5	R5	(+)	(+)
046jm109	----------p-..----------..----------	+5	R5	+	(+)
165dh103	----s----------..-q--------dv..----------	+5	R5	(+)	+
92th014.12	----------1..-----w----q-..----------	+5	R5	++	+
92ug037.8	----------vr-..---qt--a--d-..----------	+5	R5	+	++
91us005.11	----g-------p-..-------a--d-..----------	+5	R5	++	++
93br025.9	----------r-..---q---a----..----------	+5	R5	+	+
92rw020.5	----------gvr-..---q---a--g-..----------	+5	R5	+	+
92br020.4	----------..-------a--d-..----------	+5	R5	+	+
YU2	----------n-..-----l------..----------	+5	R5	++	+
93br029.2	----------q-..----------..-----k---	+6	R5	+	++
93br020.17	----------r-sl..----v---a---..-----k---	+7	R5X4	+	++
92ht593.1	--------s-r-s-..------ra-.k-..--n------	+7	R5X4	++	++
P59–S/27	----gspq-r--r-..-----wlwyargng----------	+7	X4	++	++
P51–S[c]	----g-k--r-ms-..------ia-rq-..-----k---	+8	X4	+	++
P34–S	------his-r-s-..------ra-er-..-----k---	+8	X4	++	++
032an108	----------..a--------ed-..t--------	+5	−	−	−
161kc105	----------pm..---k---a--d-..----------	+5	−	−	−
166pw101	----s----------..a------a----..----------	+5	−	−	−
167rw103	---------rg--f..---ktl---.dv..----------	+5	−	−	−
92ug024–2	----y--i-qrtp-..-l-q-l---.rr..-e---r---	+7	−	−	−
P59–S/25	----gsip-r--r-..-----wlwyargggmen------	+7	−	−	−
P61–A	------y---g-s-..----s-vaark-..----------	+7	−	−	−
P58–A	-l--g-----r--f..--rs-l---eq-..v--tkr---	+7	−	−	−
SG3–1	-----kk---r-tt..----vy------..v--------	+8	−	−	−
89–6	---------rrls-..-------arrn-..----------	+8	−	−	−

[a] All sequences are shown in comparison to the V3 consensus sequence (top row). Dashes indicate amino acid identity, and dots indicate gaps introduced to optimize the alignment. All recombinants differ only in the V3 sequence from the parental X4-tropic NL4-3 isolate.

[b] ++, highly efficient; +, efficient; (+), low; −, not significant.

[c] The P51-S *env* construct was kindly provided by A. Werner (Langer, Germany).

Nef-defective forms of the infectious NL4-3 V3 recombinants containing either a deletion of the *nef* unique region (Δnef) (6) or premature stop codons (*nef**) at positions 73 and 74 of the *nef* open reading frame were generated by standard cloning techniques. P4-CCR5 cells expressing CD4 and both R5 and X4 were infected with aliquots of virus stocks containing 20 ng of p24 antigen as described previously (18). Consistent with the results obtained with the X4.15 and R5.3 cells, the infectivities of the various molecular clones varied considerably, independently of the viral coreceptor tropism (Fig. 1). Importantly, however, all viral particles produced in the presence of an intact *nef* gene were more infectious than the corresponding *nef*-defective forms (Fig. 1). Compared to the respective Δ*nef* mutants, an intact *nef* open reading frame enhanced the infectivity of R5-tropic forms 8.1 ± 6.3-fold (mean ± standard deviation) (n = 108, where n is the number of infections performed), the infectivity of X4-tropic variants 17.3 ± 5.9-fold (n = 36), and the infectivity of X4R5-tropic recombinants 8.5 ± 2.5-fold (n = 18). Similar results were obtained when the infectivity of the *nef*-open NL4-3 V3 recombinants was compared with that of the corresponding *nef*-deleted forms: for R5-tropic forms, 11.0 ± 2.5-fold (n = 78); for X4-tropic forms, 14.3 ± 7.8-fold (n = 24); and for X4R5-tropic forms, 7.9 ± 1.2-fold (n = 12). Concordant with previous results obtained with HIV-1 particles pseudotyped with one X4-tropic and one R5-tropic HIV-1 Env protein (9), our data demonstrate that Nef increases the infectivity of HIV-1 particles independently of the viral coreceptor tropism.

We then investigated whether the stimulation of viral replication in PBMC cultures is affected by the gp120 interaction with different entry cofactors. $CD4^+$ T cells were isolated using magnetic beads as described in the protocol of the manufacturer (Miltenyi, Bergisch-Gladbach, Germany). Cells were either prestimulated with phytohemagglutinin (PHA; 3 μg/ml) for 3 days prior to infection or infected with virus stocks containing 20 ng of p24 antigen immediately after isolation and then stimulated with PHA 3 days later. All NL4-3 V3 recombinants replicated in prestimulated PBMC (data not shown). Under these experimental conditions, an intact *nef* gene increased the efficiency of viral replication only moderately. As expected from previous studies (12, 34), HIV-1 NL4-3 V3 recombinants expressing functional *nef* genes replicated with considerably higher efficiency than the corresponding *nef*-defective forms in PBMC infected immediately after isolation and stimulated 3 days later (Fig. 2). The effect of Nef on viral replication was to some extent dependent on the PBMC donor (data not shown). Altogether, however, infection of PBMC derived from six different donors with different virus stocks clearly demonstrated that an intact *nef* gene enhances HIV-1 replication in primary blood monocytes independently of the viral coreceptor tropism (see Fig. 2 for examples). Substantial

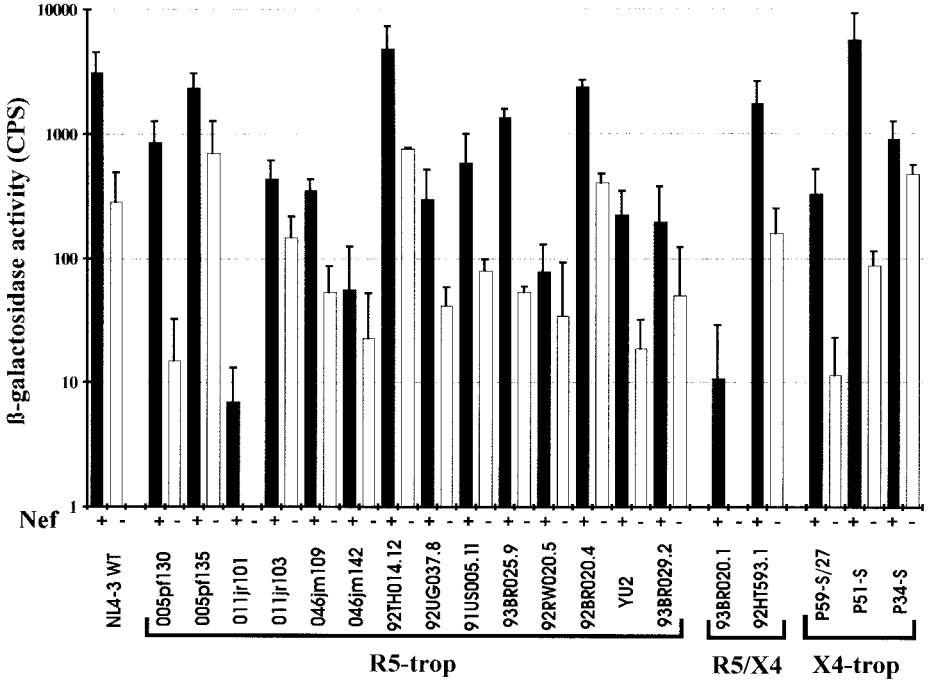

FIG. 1. Nef enhances HIV-1 infectivity independently of the viral coreceptor tropism. P4-CCR5 cells were infected with NL4-3 variants containing an intact *nef* gene or a deletion in the *nef* unique region. Infections were performed in triplicate with three different virus stocks containing 20 ng of p24 antigen. Shown are average values of nine measurements ± standard deviations. Comparable results were obtained with the variants containing a premature stop codon in the *nef* gene. CPS, counts per second; WT, wild type.

variations were observed between the different HIV-1 NL4-3 V3 recombinants analyzed. Altogether, however, the infectivity ($P < 0.0001$) and replicative capacity ($P = 0.0008$) of the *nef*-defective viruses correlated significantly with the replicative capacity of the respective *nef*-open constructs. On average, the *nef*-open HIV-1 variants showed about 1-log increases in infectivity and levels of viral replication relative to those of the *nef*-defective viruses.

In conclusion, our study demonstrates that Nef increases

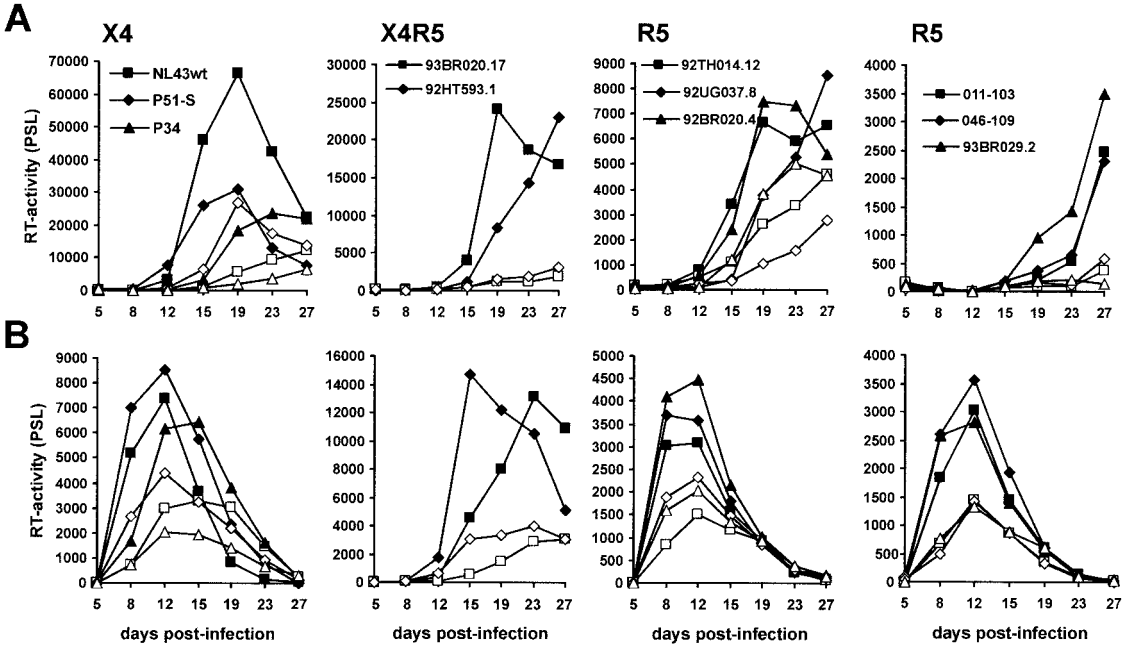

FIG. 2. Nef increases replication of R5-tropic, X4-tropic, and dualtropic HIV-1 NL4-3 V3 variants in human PBMC. Unstimulated PBMC were infected with HIV-1 variants containing intact *nef* genes (filled symbols) and either a deletion (A) or a premature stop codon (B) in *nef* (empty symbols) immediately after isolation and stimulated 3 days later. Virus production was monitored by a reverse transcriptase (RT) assay. Results were derived from a single experiment using PBMC from two different donors. Similar results were obtained in five independent experiments with different virus stocks and PBMC from different donors. PSL, photon-stimulated light emission.

virion infectivity and viral replication independently of the viral coreceptor tropism. The finding that Nef enhances the infectivity of R5-tropic, X4-tropic, and dualtropic forms is consistent with recent findings that this function is generally preserved among primary *nef* alleles obtained at different clinical stages of HIV-1 infection (7). Enhancement of virion infectivity by Nef is obviously also advantageous for the spread of HIV-1 variants that show an expanded coreceptor tropism. Our findings do not exclude the possibility that the interaction of the viral Env protein with R5 and/or X4 induces intracellular signals which already cause some activation of the cell during the entry process and lead to accelerated viral replication. Our data clearly demonstrate, however, that Nef is generally required for the full replicative potential of HIV-1 in primary human cells.

We thank Mandy Krumbiegel for excellent technical assistance, Bernhard Fleckenstein and Thomas Mertens for constant support and encouragement, and Ingrid Bennett and Stefan Pöhlmann for critical reading of the manuscript. Molecular HIV-1 clones and *env* genes from primary isolates were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH.

This work was supported by the Wilhelm-Sander Foundation and the Deutsche Forschungsgemeinschaft.

REFERENCES

1. **Aiken, C.** 1997. Pseudotyping human immunodeficiency virus type 1 (HIV-1) by the glycoprotein of vesicular stomatitis virus targets HIV-1 entry to an endocytic pathway and suppresses both the requirement for Nef and the sensitivity to cyclosporin A. J. Virol. **71:**5871–5877.
2. **Aiken, C., and D. Trono.** 1995. Nef stimulates human immunodeficiency virus type 1 proviral DNA synthesis. J. Virol. **69:**5048–5056.
3. **Alexander, L., Z. Du, M. Rosenzweig, J. J. Jung, and R. C. Desrosiers.** 1997. A role for natural simian immunodeficiency virus and human immunodeficiency virus type 1 *nef* alleles in lymphocyte activation. J. Virol. **71:**6094–6099.
4. **Berger, E. A., P. M. Murphy, and J. M. Farber.** 1999. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu. Rev. Immunol. **17:**657–700.
5. **Bukovsky, A. A., T. Dorfman, A. Weimann, and H. G. Gottlinger.** 1997. Nef association with human immunodeficiency virus type 1 virions and cleavage by the viral protease. J. Virol. **71:**1013–1018.
6. **Carl, S., R. Daniels, A. J. Iafrate, P. Easterbrook, T. C. Greenough, J. Skowronski, and F. Kirchhoff.** 2000. Partial "repair" of defective NEF genes in a long-term nonprogressor with human immunodeficiency virus type 1 infection. J. Infect. Dis. **181:**132–140.
7. **Carl, S., T. C. Greenough, M. Krumbiegel, M. Greenberg, J. Skowronski, J. L. Sullivan, and F. Kirchhoff.** 2001. Modulation of different human immunodeficiency virus type 1 Nef functions during progression to AIDS. J. Virol. **75:**3657–3665.
8. **Cefai, D., M. Ferrer, N. Serpente, T. Idziorek, A. Dautry-Varsat, P. Debre, and G. Bismuth.** 1992. Internalization of HIV glycoprotein gp120 is associated with down-modulation of membrane CD4 and p56lck together with impairment of T cell activation. J. Immunol. **149:**285–294.
9. **Chazal, N., G. Singer, C. Aiken, M. L. Hammarskjold, and D. Rekosh.** 2001. Human immunodeficiency virus type 1 particles pseudotyped with envelope proteins that fuse at low pH no longer require Nef for optimal infectivity. J. Virol. **75:**4014–4018.
10. **Chen, Y. L., D. Trono, and D. Camaur.** 1998. The proteolytic cleavage of human immunodeficiency virus type 1 Nef does not correlate with its ability to stimulate virion infectivity. J. Virol. **72:**3178–3184.
11. **Chowers, M. Y., C. A. Spina, T. J. Kwoh, N. J. Fitch, D. D. Richman, and J. C. Guatelli.** 1994. Optimal infectivity in vitro of human immunodeficiency virus type 1 requires an intact *nef* gene. J. Virol. **68:**2906–2914.
12. **Chowers, M. Y., M. W. Pandori, C. A. Spina, D. D. Richman, and J. C. Guatelli.** 1995. The growth advantage conferred by HIV-1 nef is determined at the level of viral DNA formation and is independent of CD4 downregulation. Virology **212:**451–457.
13. **Cicala, C., J. Arthos, A. Rubbert, S. Selig, K. Wildt, O. J. Cohen, and A. S. Fauci.** 2000. HIV-1 envelope induces activation of caspase-3 and cleavage of focal adhesion kinase in primary human CD4(+) T cells. Proc. Natl. Acad. Sci. USA **97:**1178–1183.
14. **Collins, K. L., and D. Baltimore.** 1999. HIV's evasion of the cellular immune response. Immunol. Rev. **168:**65–74.
15. **Deacon, N. J., A. Tsykin, A. Solomon, K. Smith, M. Ludford-Menting, D. J. Hooker, D. A. McPhee, A. L. Greenway, A. Ellett, and C. Chatfield.** 1995. Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. Science **270:**988–991.
16. **Del Corno, M., Q. H. Liu, D. Schols, E. de Clercq, S. Gessani, B. D. Freedman, and R. G. Collman.** 2001. HIV-1 gp120 and chemokine activation of Pyk2 and mitogen-activated protein kinases in primary macrophages mediated by calcium-dependent, pertussis toxin-insensitive chemokine receptor signaling. Blood **98:**2909–2916.
17. **Desrosiers, R. C.** 1999. Strategies used by HIV that allow persistent viral replication. Nat. Med. **5:**723–725.
18. **Detheux, M., L. Standker, V. Vakili, J. Münch, U. Forssmann, K. Adermann, S. Pöhlmann, G. Vassart, F. Kirchhoff, M. Parmentier, and W. G. Forssmann.** 2000. Natural proteolytic processing of hemofiltrate CC chemokine 1 generates a potent CC chemokine receptor (CCR)1 and CCR5 agonist with anti-HIV properties. J. Exp. Med. **192:**1501–1508.
19. **Du, Z., S. M. Lang, V. G. Sasseville, A. A. Lackner, P. O. Ilyinskii, M. D. Daniel, J. U. Jung, and R. C. Desrosiers.** 1995. Identification of a *nef* allele that causes lymphocyte activation and acute disease in macaque monkeys. Cell **82:**665–674.
20. **Garcia, J. V., and A. D. Miller.** 1991. Serine phosphorylation-independent downregulation of cell-surface CD4 by nef. Nature **350:**508–511.
21. **Glushakova, S., J. Münch, S. Carl, T. C. Greenough, J. L. Sullivan, L. Margolis, and F. Kirchhoff.** 2001. CD4 down-modulation by human immunodeficiency virus type 1 Nef correlates with the efficiency of viral replication and with $CD4^+$ T-cell depletion in human lymphoid tissue ex vivo. J. Virol. **75:**10113–10117.
22. **Herna, R. G., and K. Saksela.** 2000. Interactions of HIV-1 Nef with cellular signal transducing proteins. Front. Biosci. **5:**268–283.
23. **Hubert, P., G. Bismuth, M. Korner, and P. Debre.** 1995. HIV-1 glycoprotein gp120 disrupts CD4-p56lck/CD3-T cell receptor interactions and inhibits CD3 signaling. Eur. J. Immunol. **25:**1417–1425.
24. **Iafrate, A. J., S. Bronson, and J. Skowronski.** 1997. Separable functions of Nef disrupt two aspects of T-cell receptor machinery: CD4 expression and CD3 signaling. EMBO J. **16:**673–684.
25. **Kestler, H. W., D. J. Ringler, K. Mori, D. L. Panicali, P. K. Sehgal, M. D. Daniel, and R. C. Desrosiers.** 1991. Importance of the *nef* gene for maintenance of high virus loads and for development of AIDS. Cell **65:**651–662.
26. **Khan, M., M. Garcia-Barrio, and M. D. Powell.** 2001. Restoration of wild-type infectivity to human immunodeficiency virus type 1 strains lacking Nef by intravirion reverse transcription. J. Virol. **75:**12081–12087.
27. **Kirchhoff, F., T. C. Greenough, D. B. Brettler, J. L. Sullivan, and R. C. Desrosiers.** 1995. Absence of intact nef sequences in a long-term, nonprogressing survivor of HIV-1 infection. N. Engl. J. Med. **332:**228–232.
28. **Kotov, A., J. Zhou, P. Flicker, and C. Aiken.** 1999. Association of Nef with the human immunodeficiency virus type 1 core. J. Virol.**73:**8824–8830.
29. **Labrosse, B., A. Brelot, N. Heveker, N. Sol, D. Schols, E. De Clercq, and M. Alizon.** 1998. Determinants for sensitivity of human immunodeficiency virus coreceptor CXCR4 to the bicyclam AMD3100. J. Virol **72:**6381–6388.
30. **Lama, J., A. Mangasarian, and D. Trono.** 1999. Cell-surface expression of CD4 reduces HIV-1 infectivity by blocking Env incorporation in a Nef- and Vpu-inhibitable manner. Curr. Biol. **9:**622–631.
31. **Lundquist, C. A., M. Tobiume, J. Zhou, D. Unutmaz, and C. Aiken.** 2002. Nef-mediated downregulation of CD4 enhances human immunodeficiency virus type 1 replication in primary T lymphocytes. J. Virol. **76:**4625–4633.
32. **Luo, T., J. L. Douglas, R. L. Livingston, and J. V. Garcia.** 1998. Infectivity enhancement by HIV-1 Nef is dependent on the pathway of virus entry: implications for HIV-based gene transfer systems. Virology **241:**224–233.
33. **Manninen, A., G. H. Renkema, and K. Saksela.** 2000. Synergistic activation of NFAT by HIV-1 nef and the Ras/MAPK pathway. J. Biol. Chem. **275:**16513–16517.
34. **Miller, M. D., M. T. Warmerdam, I. Gaston, W. C. Greene, and M. B. Feinberg.** 1994. The human immunodeficiency virus-1 *nef* gene product: a positive factor for viral infection and replication in primary lymphocytes and macrophages. J. Exp. Med. **179:**101–114.
35. **Münch, J., L. Ständker, S. Pöhlmann, F. Baribaud, A. Papkalla, O. Rosorius, R. Stauber, G. Sass, N. Heveker, K. Adermann, S. Escher, E. Klüver, R. W. Doms, H. G. Forssmann, and F. Kirchhoff.** 2002. Hemofiltrate CC chemokine 1[9–74] causes effective internalization of CCR5 and is a potent inhibitor of R5-tropic human immunodeficiency virus type 1 strains in primary T-cells and macrophages. Antimicrob. Agents Chemother. **46:**982–990.
36. **Pandori, M. W., N. J. S. Fitch, H. M. Craig, D. D. Richman, C. A. Spina, and J. C. Guatelli.** 1996. Producer-cell modification of human immunodeficiency virus type 1: Nef is a virion protein. J. Virol. **70:**4283–4290.
37. **Peter, F.** 1998. HIV nef: the mother of all evil? Immunity **9:**433–437.
38. **Roshal, M., Y. Zhu, and V. Planelles.** 2001. Apoptosis in AIDS. Apoptosis **2001:**103–116.
39. **Ross, T. M., A. E. Oran, and B. R. Cullen.** 1999. Inhibition of HIV-1 progeny virion release by cell-surface CD4 is relieved by expression of the viral Nef protein. Curr. Biol. **9:**613–621.
40. **Schaeffer, E., R. Geleziunas, and W. C. Greene.** 2001. Human immunodeficiency virus type 1 Nef functions at the level of virus entry by enhancing cytoplasmic delivery of virions. J. Virol. **75:**2993–3000.

41. **Schwartz, O., V. Marechal, O. Danos, and J. M. Heard.** 1995. Human immunodeficiency virus type 1 Nef increases the efficiency of reverse transcription in the infected cell. J. Virol. **69:**4053–4059.
42. **Spina, C. A., T. J. Kwoh, M. Y. Chowers, J. C. Guatelli, and D. D. Richman.** 1994. The importance of nef in the induction of human immunodeficiency virus type 1 replication from primary quiescent CD4 lymphocytes. J. Exp. Med. **179:**115–123.
43. **Wang, J. K., E. Kiyokawa, E. Verdin, and D. Trono.** 2000. The Nef protein of HIV-1 associates with rafts and primes T cells for activation. Proc. Natl. Acad. Sci. USA **97:**394–399.
44. **Welker, R., H. Kottler, H. R. Kalbitzer, and H.-G. Krausslich.** 1996. Human immunodeficiency virus type 1 Nef protein is incorporated into virus particles and specifically cleaved by the viral proteinase. Virology **219:**228–236.
45. **Zheng, Y. H., A. Plemenitas, T. Linnemann, O. T. Fackler, and B. M. Peterlin.** 2001. Nef increases infectivity of HIV via lipid rafts. Curr. Biol. **11:**875–879.
46. **Zhou, J., and C. Aiken.** 2001. Nef enhances human immunodeficiency virus type 1 infectivity resulting from intervirion fusion: evidence supporting a role for Nef at the virion envelope. J. Virol. **75:**5851–5859.