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Activation by Inflammatory Stimuli Increases Neutrophil Binding of Human Immunodeficiency Virus Type 1 and Subsequent Infection of Lymphocytes

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Resting neutrophils bind human immunodeficiency virus type 1 (HIV-1) and efficiently transfer infection to lymphocytes. The present study shows that a brief activation by inflammatory stimuli increases the neutrophil binding levels of both R5 and X4 isolates of HIV-1 at least twofold. The binding occurs independently of CD4, gp120, and incubation temperature and is observed with HIV-1 propagated either in lymphocytes or in HEK293 cells. Significantly, HIV-1 bound to the activated neutrophils accelerates the infection of activated lymphocytes compared to free HIV-1 or to HIV-1 bound to resting neutrophils. It is proposed that these events may contribute to the increased risk of HIV-1 transmission at sites of mucosal infection.

Mucosal inflammation is a risk factor for the transmission of human immunodeficiency virus type 1 (HIV-1) infection (5, 13, 17, 18). Neutrophils, through their role in innate immunity (22), are frequently prominent at sites of mucosal inflammation. Several neutrophil-derived mediators stimulate HIV-1 replication (9, 10, 12, 15, 19), and the possibility that neutrophils may directly promote HIV-1 infection is suggested by the finding that neutrophils bind both CCR5 and CXCR4 strains of HIV-1 and efficiently transfer infection to lymphocytes (16). Specifically, HIV-1 bound to neutrophils is approximately ninefold more infectious than is the same amount of free HIV-1, as measured by the p24 antigen levels in culture supernatants of phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC) after 7 days of culture (16). Because neutrophils are activated at sites of inflammation by microbial products and by cytokines such as tumor necrosis factor alpha (TNF- α) (22), the present study was performed to determine the effect of neutrophil activation on the capacity of neutrophils to bind HIV-1 and transfer infection to CD4-positive lymphocytes.

Neutrophils were isolated from the venous blood of healthy adults by density gradient centrifugation as described previously (21). The study protocol was approved by the Institutional Review Board of Rush University Medical Center. The neutrophils (3×10^6) were incubated in triplicate with 1 μ M formylated peptide (fMLP), 10 ng of TNF- α /ml, 100 ng of *Escherichia coli* lipopolysaccharide (LPS) serotype O55:B5/ml, or in HEPES-buffered Hanks' balanced salt solution, pH 7.4, containing 30 μ g of human serum albumin (Calbiochem, La Jolla, Calif.)/ml alone for 15 min at 37°C or, as a control for nonspecific effects, at 4°C. The cells were collected by centrifugation and were incubated in RPMI 1640 with HIV-1_{BaL} [CCR5-tropic] containing 2,000 pg of p24 for 2 h at 4°C. The HIV-1 was produced in PHA-stimulated PBMC as described

previously (16). The total incubation volume was 0.1 ml. Incubations were stopped by centrifugation, and the cells were washed extensively to remove unbound virus and were then lysed in 125 μ l of 0.5% Triton X-100 containing a cocktail of protease inhibitors to inhibit degradation of the p24 (16). Cell counting demonstrated that similar numbers of neutrophils (approximately 1.3×10^6) were recovered from resting and stimulated samples. HIV-1 was quantified by measurement of p24 antigen content in 100 μ l of the cell lysate by using an HIV-1 p24 antigen capture enzyme-linked immunosorbent assay (AIDS Vaccine Program, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Md.). The lower limit of the sensitivity of the assay as performed in our laboratory is 19 pg/ml. Results are presented as the net total amount of bound HIV-1 after the subtraction of nonspecific values for neutrophil lysate alone and for free virus alone, which were routinely <5 and 1 pg, respectively. The level of neutrophil binding of HIV-1_{BaL} was significantly increased by activation with 1 μ M fMLP or 10 ng of TNF- α /ml (two- or threefold, respectively) (Fig. 1A and B). In contrast, incubating the neutrophils with 100 ng of LPS/ml or with any of the stimuli at 4°C did not increase the level of HIV-1_{BaL} binding. Parallel experiments confirmed that the brief incubation with fMLP or TNF- α , but not LPS, activated the neutrophils, as measured by the increased expression of CD11b (Fig. 2). Additional results demonstrated that incubating neutrophils with 3 to 30 ng of TNF- α /ml stimulated a concentration-dependent increase in HIV-1 binding, with 30 ng of TNF- α /ml causing an approximately sevenfold increase in binding (Fig. 1C). In results not shown, all three concentrations of TNF- α , however, stimulated the same increase in CD11b expression as that shown for 10 ng of TNF- α /ml in Fig. 2. The latter finding indicates that increased expression of CD11b is not directly responsible for the increased binding of HIV-1, although an increase in CD11b affinity cannot be excluded.

Neutrophils from approximately 12% of healthy individuals express CD4 (2), but flow cytometric analysis using fluorescein isothiocyanate-conjugated anti-human CD4 did not detect any CD4 expression by neutrophils isolated from five individuals,

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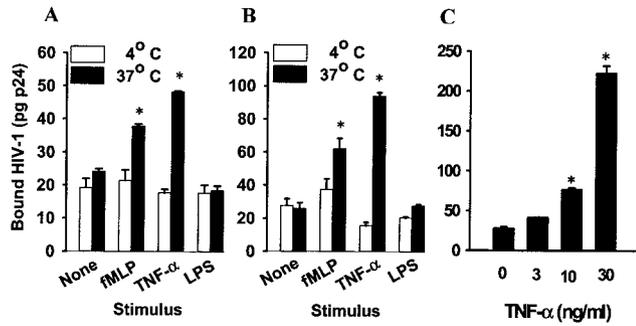


FIG. 1. Neutrophil activation increases HIV-1 binding. (A and B) Neutrophils were incubated with or without 1 μ M fMLP, 10 ng of TNF- α /ml, or 100 ng of LPS/ml for 15 min at 37°C (filled bars) or at 4°C (open bars) and then were incubated with 2,000 pg of HIV-1_{BaL} for 2 h at 4°C. Cells were lysed, and binding was quantified by measurement of viral p24 antigen content in the lysate. Results obtained in two separate experiments (A and B), each using neutrophils of a different individual, are shown as the means \pm standard errors (SE) for triplicate samples. Similar results were obtained for two additional experiments. (C) Neutrophils were incubated with or without the indicated concentrations of TNF- α for 15 min at 37°C, and binding of HIV-1_{BaL} was determined as described for panels A and B. Results are presented as the means \pm SE for triplicate samples in a single experiment. Similar results were obtained in two additional experiments. *, $P < 0.05$ compared with the value for resting neutrophils (None) by Student's paired t test.

including the three individuals who participated in the experiments for which results are shown in Fig. 1, before or after activation by 10 ng of TNF- α /ml (results not shown). Thus, HIV-1 binds to activated neutrophils independently of CD4. Other surface molecules, including C-type lectin receptors on dendritic cells (7, 23) as well as glycosaminoglycans (14) and galactosyl ceramides (1, 6), are also targets for gp120-mediated binding of HIV-1 to cells. The results presented in Fig. 3A indicate that C-type lectins do not mediate HIV-1 binding by the activated neutrophils, as chelation of calcium and other divalent cations by EDTA did not impair the binding. More directly, the results shown in Fig. 3B demonstrate that HIV-1 binds to the neutrophils independently of gp120. Activation of the neutrophils by 10 ng of TNF- α /ml increased the binding of gp120/41-negative HIV-1_{NL4-3} produced in HEK293 cells (American Type Culture Collection, Manassas, Va.) as described previously

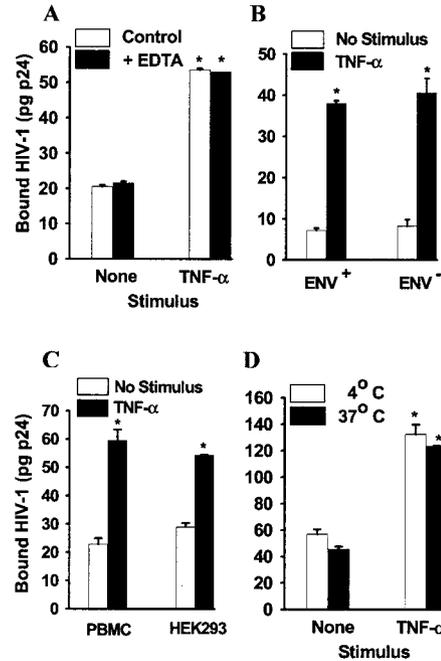


FIG. 3. HIV-1 binding to activated neutrophils occurs independently of calcium, gp120/41, and incubation temperature. Neutrophils were incubated without (None) or with 10 ng of TNF- α /ml at 37°C for 15 min. The neutrophils were then incubated for 2 h with 2,000 pg of HIV-1_{BaL} in the presence or absence of 10 mM EDTA at 4°C (A), 2,000 pg of wild-type HIV-1_{NL4-3} (ENV⁺) or gp120/41-negative HIV-1_{NL4-3} particles (ENV⁻) at 4°C (B), 2,000 pg of HIV-1_{NL4-3} produced in PBMC or HEK293 cells at 4°C (C), or 2,000 pg of HIV-1_{BaL} for 2 h at 4°C or at 37°C (D). The results shown were obtained in separate experiments and are presented as the means \pm SE for triplicate samples in a single experiment. Similar results were obtained in two additional experiments for each protocol. *, $P < 0.05$ compared with the value to resting neutrophils (None) by Student's paired t test.

(20). The results presented in Fig. 3C indicate that a host-derived molecule(s) common at least to lymphocytes and HEK293 cells mediates the binding of HIV-1 to neutrophils, as TNF- α stimulation produced similar increases in the binding of HIV-1_{NL4-3} propagated either in lymphocytes or in HEK293 cells (20). Lastly, incubating resting and TNF- α -stimulated neutrophils with HIV-1

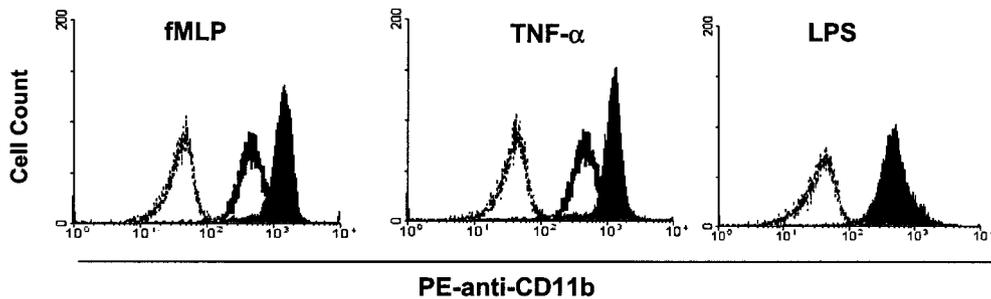


FIG. 2. TNF- α and fMLP increase neutrophil expression of CD11b. Neutrophils were incubated without (thick line) or with (filled peak) 1 μ M fMLP, 10 ng of TNF- α /ml, or 100 ng of LPS/ml for 15 min at 37°C. The cells were washed and incubated with phycoerythrin (PE)-conjugated mouse anti-CD11b (BD Biosciences, San Diego, Calif.) (thick line; filled peak) or the phycoerythrin-conjugated isotype (immunoglobulin G2a) control (thin line) as described previously (4). Results of a single experiment are shown. Similar results were obtained in three additional experiments, each using neutrophils from a different donor.

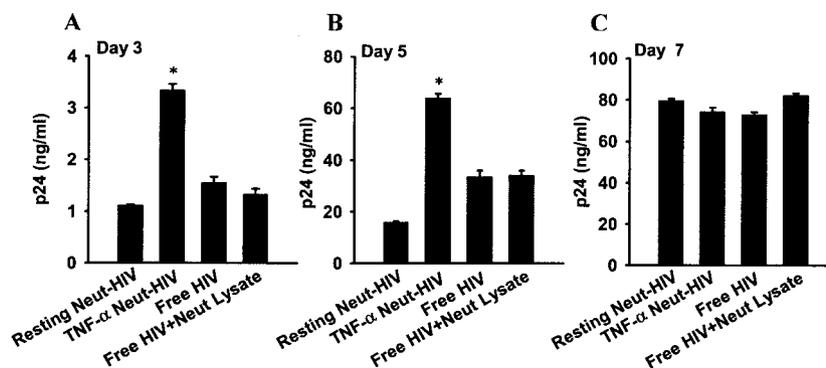


FIG. 4. Neutrophil activation accelerates infection of activated lymphocytes by neutrophil-bound HIV-1. Neutrophils (Neut) were incubated in the absence or presence of 10 ng TNF- α /ml at 37°C and were then incubated with 2,000 pg of HIV-1_{BaL} as described in the legend for Fig. 1. The cells were then cultured with PHA-activated PBMC for 7 days. The PHA-activated PBMC were also cultured with 300 pg of free HIV-1_{BaL} alone and in the presence of a freeze-thaw lysate of neutrophils (2×10^6 cells). Virus replication was measured as p24 antigen content in the culture supernatants at days 3 (A), 5 (B), and 7 (C). Results are presented as the means \pm SE for triplicate samples in a single experiment. Similar results were obtained in three additional experiments. *, $P < 0.05$ compared with the value for the resting neutrophil-HIV-1 conjugates by Student's paired t test.

at 37°C yielded the same levels of HIV-1 binding as observed after incubating neutrophils with the virus at 4°C (Fig. 3D). This finding suggests that the binding of HIV-1 by the neutrophils is relatively specific, as incubation at 37°C would facilitate nonspecific binding. This finding also argues against the internalization of HIV-1, at least during the 2-h incubation period.

The results depicted in Fig. 4 show that HIV-1 bound to activated neutrophils produced a threefold increase in the p24 content in the culture supernatant after 3 days of culture compared to infection caused by HIV-1 bound to resting neutrophils or to 300 pg of free HIV-1_{BaL}. For these experiments, neutrophils were incubated with or without 30 ng of TNF- α /ml for 15 min at 37°C and were then incubated with 2,000 pg of HIV-1_{BaL} for 2 h at 4°C in the standard manner. After washing, the neutrophil-HIV-1_{BaL} conjugates were cultured with heterologous PBMC that had been pre-activated by PHA as described previously (16). As a control, the PHA-stimulated PBMC were also cultured with 300 pg of free HIV-1_{BaL}, a concentration previously established to cause maximum infection after 7 days of culture (16), alone and in combination with a freeze-thaw lysate of 2×10^6 neutrophils. HIV-1 bound to the activated neutrophils continued to cause a significantly greater level of virus production after 5 days of culture, but no further increase was noted after 7 days of culture, when virus production reached a plateau. As a consequence, HIV-1 bound to either resting or activated neutrophils produced similar levels of p24 after 7 days of culture. HIV-1_{BaL} bound to TNF- α -stimulated neutrophils also accelerated the infection of autologous PHA-stimulated PBMC ($n = 2$; results not shown). Although several neutrophil products (9, 10, 12), including some preformed mediators (15, 19), stimulate HIV-1 replication in infected cells, the addition of a freeze-thaw lysate of 2×10^6 neutrophils to the PBMC cultures did not increase the level of replication caused by the free virus. Instead, it is likely that the increased infection levels observed with HIV-1 bound to activated neutrophils result from the enhanced transfer of the HIV-1 by the neutrophils to the lymphocytes. The possibility that the enhanced transfer simply reflects the increased amount of HIV-1 bound to the activated neutrophils (approx-

imately 117 pg for the experiment in Fig. 4) cannot be excluded. Similarly, because HIV-1 production in activated PBMC may not be increased linearly, the possibility that HIV-1 bound to activated neutrophils has an even greater effect on infection of PBMC per se cannot be excluded.

Neutrophils are present constitutively in the gingival mucosa in the oral cavity (3) and are also enriched at sites of inflammation in the oral and genital tract mucosae (3, 25). Although it is possible that the neutrophils simply serve to clear the virus, as proposed previously for CD4-negative cells (14), the results presented here and those reported previously (16) indicate that HIV-1 binding to neutrophils may contribute to the transmission of HIV-1 infection. Indeed, neutrophils may act in a manner analogous to dendritic cells (8, 11, 18) and epithelial cells (24), although whether neutrophils also sequester the virus intracellularly remains to be determined. In summary, the results presented here demonstrate that the activation of neutrophils by inflammatory stimuli increases the binding of HIV-1, that the binding occurs independently of CD4 or gp120, and that HIV-1 bound to activated neutrophils rapidly infects lymphocytes. It is proposed that these events contribute to the increased risk of HIV-1 transmission at sites of mucosal infection.

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REFERENCES

- Bhat, S., S. L. Spitalnik, F. Gonzalez-Scarano, and D. H. Silberberg. 1991. Galactosyl ceramide or a derivative is an essential component of the neural receptor for human immunodeficiency virus type 1 envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. USA* **88**:7131-7134.
- Biswas, P., B. Mantelli, A. Sica, M. Malnati, C. Panzeri, A. Sacconi, H. Hasson, A. Vecchi, A. Saniabadi, P. Lusso, A. Lazzarin, and A. Beretta. 2003. Expression of CD4 on human peripheral blood neutrophils. *Blood* **101**:4452-4456.
- Delima, A. J., and T. E. Van Dyke. 2003. Origin and function of the cellular components in gingival crevice fluid. *Periodontol.* **2000** **31**:55-76.
- Deriy, L. V., J. Chor, and L. L. Thomas. 2000. Surface expression of lactoferrin by resting neutrophils. *Biochem. Biophys. Res. Commun.* **275**:241-246.
- Embree, J. E., S. Njenga, P. Datta, N. J. Nagelkerke, J. O. Ndinya-Achola, Z.

- Mohammed, S. Ramdahin, J. J. Bwayo, and F. A. Plummer. 2000. Risk factors for postnatal mother-child transmission of HIV-1. *AIDS* **14**:2535–2541.
6. Fantini, J., D. G. Cook, N. Nathanson, S. L. Spitalnik, and F. Gonzalez-Scarano. 1993. Infection of colonic epithelial cell lines by type 1 human immunodeficiency virus is associated with cell surface expression of galactosylceramide, a potential alternative gp120 receptor. *Proc. Natl. Acad. Sci. USA* **90**:2700–2704.
 7. Geijtenbeek, T. B. H., D. S. Kwon, R. Torensma, S. J. van Vliet, G. C. F. van Duinhoven, J. Middel, I. L. M. H. A. Cornelissen, H. S. L. M. Nottet, V. N. KewalRamani, D. R. Littman, C. G. Figdor, and Y. van Kooyk. 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances *trans*-infection of T cells. *Cell* **100**:587–597.
 8. Gummuluru, S., M. Rogel, L. Stamatatos, and M. Emerman. 2003. Binding of human immunodeficiency virus type 1 to immature dendritic cells can occur independently of DC-SIGN and mannose binding C-type lectin receptors via a cholesterol-dependent pathway. *J. Virol.* **77**:12865–12874.
 9. Ho, J. L., S. He, A. Hu, J. Geng, F. G. Basile, M. G. Almeida, A. Y. Saito, J. Laurence, and W. D. Johnson, Jr. 1995. Neutrophils from human immunodeficiency virus (HIV)-seronegative donors induce HIV replication from HIV-infected patients' mononuclear cells and cell lines: an in vitro model of HIV transmission facilitated by *Chlamydia trachomatis*. *J. Exp. Med.* **181**:1493–1505.
 10. Klebanoff, S. J., and C. M. Headley. 1999. Activation of the human immunodeficiency virus-1 long terminal repeat by respiratory burst oxidants of neutrophils. *Blood* **93**:350–356.
 11. Kwon, D. S., G. Gregorio, N. Bitton, W. A. Hendrickson, and D. R. Littman. 2002. DC-SIGN-mediated internalization of HIV is required for *trans*-enhancement of T cell infection. *Immunity* **16**:135–144.
 12. Lane, B. R., K. Lore, P. J. Bock, J. Andersson, M. J. Coffey, R. M. Strieter, and D. M. Markovitz. 2001. Interleukin-8 stimulates human immunodeficiency virus type 1 replication and is a potential new target for antiretroviral therapy. *J. Virol.* **75**:8195–8202.
 13. Martin, H. L., B. A. Richardson, P. M. Nyange, L. Lavreys, S. L. Hillier, B. Chohan, K. Mandaliya, J. O. Ndinya-Achola, J. Bwayo, and J. Kreiss. 1999. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *J. Infect. Dis.* **180**:1863–1868.
 14. Mondor, I., S. Ugolini, and Q. J. Sattentau. 1998. Human immunodeficiency virus type 1 attachment to HeLa CD4 cells is CD4 independent and gp120 dependent and requires cell surface heparans. *J. Virol.* **72**:3623–3634.
 15. Moriuchi, H., M. Moriuchi, and A. S. Fauci. 2000. Cathepsin G, a neutrophil-derived serine protease, increases susceptibility of macrophages to acute human immunodeficiency virus type 1 infection. *J. Virol.* **74**:6849–6855.
 16. Olinger, G. G., M. Saifuddin, and G. T. Spear. 2000. CD4-Negative cells bind human immunodeficiency virus type 1 and efficiently transfer virus to T cells. *J. Virol.* **74**:8550–8557.
 17. Plummer, F. A., J. N. Simonsen, D. W. Cameron, J. O. Ndinya-Achola, J. K. Kreiss, M. N. Gakinya, P. Waiyaki, M. Cheang, P. Piot, A. R. Ronald, et al. 1991. Cofactors in male-female sexual transmission of human immunodeficiency virus type 1. *J. Infect. Dis.* **163**:233–239.
 18. Pope, M., and A. T. Haase. 2003. Transmission, acute HIV-1 infection and the quest for strategies to prevent infection. *Nat. Med.* **9**:847–852.
 19. Ryckman, C., G. A. Robichaud, J. Roy, R. Cantin, M. J. Tremblay, and P. A. Tessier. 2002. HIV-1 transcription and virus production are both accentuated by the proinflammatory myeloid-related proteins in human CD4+ T lymphocytes. *J. Immunol.* **169**:3307–3313.
 20. Saifuddin, M., M. L. Hart, H. Gewurz, Y. Zhang, and G. T. Spear. 2000. Interaction of mannose-binding lectin with primary isolates of human immunodeficiency virus type 1. *J. Gen. Virol.* **81**:949–955.
 21. Shenoy, N. G., G. J. Gleich, and L. L. Thomas. 2003. Eosinophil major basic protein stimulates neutrophil superoxide production by a class IA phosphoinositide 3-kinase and protein kinase C- ζ -dependent pathway. *J. Immunol.* **171**:3734–3741.
 22. Smith, J. A. 1994. Neutrophils, host defense, and inflammation: a double-edged sword. *J. Leukoc. Biol.* **56**:672–686.
 23. Turville, S. G., P. U. Cameron, A. Handley, G. Lin, S. Pohlmann, R. W. Doms, and A. L. Cunningham. 2002. Diversity of receptors binding HIV on dendritic cell subsets. *Nat. Immunol.* **3**:975–983.
 24. Wu, Z., Z. Chen, and D. M. Phillips. 2003. Human genital epithelial cells capture cell-free human immunodeficiency virus type 1 and transmit the virus to CD4+ cells: implications for mechanisms of sexual transmission. *J. Infect. Dis.* **188**:1473–1482.
 25. Yudin, M. H., S. L. Hillier, H. C. Wiesenfeld, M. A. Krohn, A. A. Amortegui, and R. L. Sweet. 2003. Vaginal polymorphonuclear leukocytes and bacterial vaginosis as markers for histologic endometritis among women without symptoms of pelvic inflammatory disease. *Am. J. Obstet. Gynecol.* **188**:318–323.