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HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages

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Abstract

Infection of macrophage lineage cells is a feature of primate lentivirus replication, and several properties of primate lentiviruses seem to have evolved to promote the infection of macrophages. Here we demonstrate that the accessory gene product Nef induces the production of two CC-chemokines, macrophage inflammatory proteins 1α and 1β, by HIV-1-infected macrophages. Adenovirus-mediated expression of Nef in primary macrophages was sufficient for chemokine induction. Supernatants from Nef-expressing macrophages induced both the chemotaxis and activation of resting T lymphocytes, permitting productive HIV-1 infection. These results indicate a role for Nef in lymphocyte recruitment and activation at sites of virus replication.

CD4$^+$ T cells and tissue macrophages are the principal target cells for primate lentivirus replication in the infected host[1–5]. Although virus replication within CD4$^+$ T lymphocytes is generally accepted to account for many aspects of viral pathogenicity, the contribution of macrophage lineage cells to virus replication and pathogenicity are less well-understood. Several lines of evidence support the idea that macrophage lineage cells may be important for certain aspects of primate lentivirus replication. CCR5 is the principal co-receptor through which infection of macrophages by HIV-1 is mediated[6–10]. People with homozygous deletions in the CCR5 gene are resistant to infection[11,12]. The selective sexual transmission of monocytotropic viruses[13–15] indicates that macrophage tropism may facilitate establishment of the virus in the newly infected host. One of the more compelling pieces of evidence indicating macrophages are involved in viral replication is that primate lentiviruses have acquired specific characteristics to allow their replication within these

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cells. Although retroviruses only infect dividing cells[16,17], the actions of several primate lentiviral proteins permit efficient infection of nondividing macrophages[18–22]. HIV-2/SIV_{SM} variants that inefficiently infect macrophages *in vitro* were considerably impaired in replication and pathogenicity *in vivo*[23].

To gain further insight into how macrophages may facilitate virus replication, we determined whether macrophage function was influenced by HIV-1 infection. We demonstrate that the accessory gene product Nef induces CC- chemokine production by macrophages. Supernatants of macrophages expressing HIV-1 Nef showed chemotactic activity for T cells. Resting T cells, which are normally refractory to productive infection, became activated and permitted productive HIV-1 infection when exposed to supernatants of Nef-expressing macrophages. These results indicate a mechanism that allows primate lentiviruses to recruit substrate T lymphocytes to sites of virus replication. These results may also explain how Nef, by mediating lymphocyte chemotaxis and activation, can promote primate lentivirus replication in the infected host.

Nef mediates chemokine induction by infected macrophages

We infected monocyte-derived macrophages obtained from three different donors with monocytotropic wild-type HIV-1 ($HIV\text{-}1_{ADA}$) or with a variant containing an inactivating mutation in the *nef* gene($HIV\text{-}1_{ADA}$ ΔNef). We monitored culture supernatants for virus production (reverse transcriptase activity) and chemokine and cytokine production. The replication of wild-type and ΔNef viruses was indistinguishable, indicating that at these multiplicities of infection, Nef was dispensable for efficient replication within these cells (Fig. 1a). Virus replication resulted in increased production of the chemokines macrophage inflammatory protein (MIP)-1α and MIP-1β (Fig. 1a). Although the cells had similar levels of virus replication, chemokine production was impaired in cultures infected with HIV-1 ΔNef (Fig. 1a). Stimulation of chemokine production correlated with an increase in the level of CC-chemokine RNA in macrophages infected with wild-type, but not those infected with ΔNef (Fig. 1b). Production of monocyte colony-stimulating factor, which is induced after HIV-1 infection of macrophages[24], was identical in macrophages infected with wild-type or ΔNef virus (Fig. 1a). The increase in chemokine production was not a generalized consequence of macrophage activation, as the inflammatory cytokines tumor necrosis factor α and interleukin (IL)-1β were not induced by HIV-1 infection (not shown). In addition, there was no detectable induction of RANTES, monocyte chemotactic peptide-1, IL-2, IL-4, IL-7, IL-8, IL-12 or IL-15 by HIV-1 infection. Although induction of chemokine release was impaired in ΔNef-infected macrophages, there was a higher level of chemokine production in these than in mock-infected cultures (Fig. 1a). This indicated the presence of additional factors in HIV-1-infected macrophages that influenced CC-chemokine production.

Nef is sufficient for chemokine induction

To determine whether Nef was sufficient for CC-chemokine induction, we expressed the Nef gene product in the absence of other viral proteins in primary macrophages. Nonviral gene transfer methods transduce primary macrophages extremely inefficiently. HIV-1 based lentiviral vectors can transduce nondividing cells[25], albeit inefficiently; however, the

presence of other HIV-1 gene products could potentially influence chemokine induction. We constructed adenovirus vectors (D.F. *et al.*, in preparation) expressing green fluorescent protein (Adeno GFP) or Nef (Adeno Nef). Adenovirus-mediated transduction of primary macrophages was very efficient: 60–70% of the cells expressed green fluorescent protein within 18 hours of infection with Adeno GFP (Fig. 2a). Macrophages infected with the Adeno Nef but not Adeno GFP virus expressed Nef protein (Fig. 2b) and had increased levels of MIP-1α and MIP-1β RNA (Fig. 2b). By limiting dilution PCR analysis, MIP-1α and MIP-1β RNA levels were increased by 16-fold and 64-fold, respectively (Fig. 2b). Although we cannot exclude the possibility of effects due to protein or mRNA stability, the increase in chemokine RNA levels is more consistent with stimulation of chemokine gene expression in the presence of Nef. *In situ* hybridization with an fluorescein isothiocyanate (FITC)-conjugated, Nef-specific probe and a rhodamine-conjugated, MIP-1α-specific probe demonstrated that Nef and MIP-1α were co-expressed (Fig. 2c), and MIP-1α RNA expression seemed to be limited to those cells which also expressed Nef. In addition, the production of MIP-1α and MIP-1β proteins was considerably higher in macrophages infected with Adeno Nef than in those infected with Adeno GFP (Fig. 2d). This pattern of chemokine induction was very consistent in an additional six different donor macrophage cultures (Table 1). Chemokine induction was Nef-specific: Adeno GFP-infected macrophages or mock-infected macrophages released low or undetectable levels of chemokines (Fig. 2d and Table 1). In addition, an adenovirus vector lacking ‘foreign’ inserts did not induce the release of chemokines from macrophages (Fig. 2d). The induction of chemokines by Nef was transient; levels peaked 18 hours after Adeno Nef infection and returned to baseline levels around 36–72 hours after infection (Fig. 2d). MIP-1α and MIP-1β are upregulated through CD40 ligand-mediated CD40 stimulation[26]. Treatment of macrophages with CD40 ligand[27] also induced a transient chemokine response: levels peaked within 4 hours of CD40 ligand stimulation and returned to baseline levels within 48 hours of stimulation (Fig. 2e). Thus, the transient duration of Nef-mediated chemokine induction parallels that when chemokines are induced by a physiological stimulus. The pattern of cytokine induction in macrophages infected by Adeno Nef reflected that produced by macrophages infected by wild-type HIV-1_{ADA} in that there was no induction of IL-1α/β, IL-2, IL-4, IL-7, IL-12, IL-15 or of tumor necrosis factor α or RANTES (not shown). In addition, expression of Nef after infection with Adeno Nef did not influence monocyte colony-stimulating factor release (not shown). These results indicate that Nef is sufficient for the specific induction of CC-chemokines from macrophages.

Analysis of lymphocyte chemotaxis induced by Nef

The CC-chemokines mediate lymphocyte transport to sites of inflammation. Thus, we evaluated whether supernatants of HIV-1-infected macrophages and of macrophages expressing Nef protein promoted lymphocyte chemotaxis. Supernatants of macrophages infected by wild-type HIV-1_{ADA} (collected at the peak of CC-chemokine production) induced lymphocyte chemotaxis (Fig. 3a). In contrast, there was no substantial chemotactic activity in supernatants from macrophages infected by HIV-1_{ADA} ΔNef (Fig. 3a). Expression of HIV-1 Nef was sufficient to reconstitute chemotactic activity of infected macrophage culture supernatants: supernatants from macrophages infected by Adeno Nef, but not those

infected by Adeno GFP or mock-infected, promoted lymphocyte chemotaxis (Fig. 3a). An identical pattern of chemotaxis was obtained whether unstimulated lymphocytes (Fig. 3) or CD3-activated lymphocytes (not shown) were used in chemotaxis assays. The chemotactic activity of culture supernatants from macrophages expressing HIV-1 Nef was mediated by MIP-1α and MIP-1β, as antibodies against these proteins blocked the chemotactic activity of macrophage culture supernatants expressing Nef (Fig. 3b).

Effect of Nef on lymphocyte activation

Supernatants of macrophages expressing the Nef protein were equally capable of promoting chemotaxis of resting as well as activated peripheral blood lymphocytes. As T cells are refractory to productive HIV-1 infection (review, ref. 28), we evaluated whether supernatants of macrophages expressing Nef protein influenced the activation state of T cells and their susceptibility to productive virus infection. Resting T lymphocytes incubated with supernatants of macrophages expressing HIV-1 Nef were stimulated to enter cell cycle, as shown by ^{3}H-thymidine uptake (Fig. 4a). In addition, some of these cells had activation markers (approximately 7% CD4/CD69; 2% CD4/HLA-DR double-positive). In contrast, supernatants of macrophages infected with Adeno GFP or mock-infected did not influence lymphocyte cycling status (Fig. 4a). Immunodepletion of MIP-1α and MIP-1β from supernatants of Nef-expressing macrophages did not diminish their ability to mediate lymphocyte stimulation (Fig. 4a). These supernatants did not contain known cytokines or interleukins that influence lymphocyte activation independent of co-stimulation. In addition, lymphocyte activation was not due to the presence of Nef protein in culture supernatants, as immunodepletion of Nef from supernatants of macrophages expressing Nef also did not diminish their ability to induce lymphocyte stimulation (not shown). Resting T cells exposed to supernatants of macrophages expressing Nef (Adeno Nef infection) permitted productive HIV-1 infection (Fig. 4b). In contrast, resting T cells exposed to supernatants from macrophages expressing green fluorescent protein (Adeno GFP) or from mock-infected macrophages remained incapable of supporting productive HIV-1 infection (Fig. 4b).

Patterns of chemokine production *in vivo*

SIV and HIV-1 encephalitis are both characterized by multifocal accumulations of mononuclear cells that are mainly of macrophage/microglial lineage. The increased expression of CC-chemokines in these lesions may be involved in leukocyte recruitment to the brain in SIV-infected macaques[29]. We determined whether the chemokine expression was localized to macrophages and whether it paralleled the pattern in HIV-infected macrophages *in vitro* (that is, expression of MIP-1α/β but not RANTES). These multifocal accumulations in the brain of an SIV-infected macaque with SIV encephalitis comprised CD14^{+} macrophages, which contained abundant viral antigen and viral nucleic acids (Fig. 5a–c). These same aggregates of SIV-infected macrophages also expressed MIP-1α (Fig. 5d) and MIP-1β (Fig. 5e). In contrast, RANTES expression was limited in these aggregates and was confined mainly to vascular endothelium (Fig. 5f). Most of the cells in these aggregates were infected and most of the macrophages in these aggregates expressed MIP-1α and MIP-1β, indicating that the infected macrophage is the source of the CC-chemokine

production. Chemokine expression was minimal or absent in the brain tissue of uninfected age- and sex-matched monkeys (Fig. 5g–I).

Discussion

The *Nef* gene product facilitates HIV-1 and SIV replication and pathogenicity. HIV-1 and SIV Nef mutants are substantially attenuated *in vivo* both in virus replication and virulence[30–32]. In addition to modulating expression of CD4 and MHC class I (refs. 33,34), Nef may be involved in altering the activation status of the infected cell (review, ref. 35). A highly conserved Src homology region 3 domain in Nef, which may be involved in the activation of Src family protein tyrosine kinases (review, ref. 35), may be dispensable for chemokine induction by Nef (J.-M.J. *et al.*, unpublished results). Mutagenesis studies to identify an 'effector domain' in Nef that regulates chemokine induction are in progress. Our studies support the possibility of a role for Nef in lymphocyte activation that is mediated indirectly through macrophage infection. The ability of Nef to induce the release of a lymphocyte stimulating factor by macrophages indicates that Nef promotes an environment in which lymphocytes, without additional stimuli, permits productive HIV-1 infection. This indicates that Nef promotes viral replication in the host by increasing the 'pool' of substrate lymphocytes permissive to infection. Monocytotropism promotes amplification and dissemination of SIV_{SM} in macaques[23]. The results here point to a possible mechanism through which macrophages facilitate virus dissemination and indicate Nef is involved in this mechanism. The CC-chemokines, and MIP-1β in particular, induce preferential chemotaxis of $CD4^+$ T lymphocytes[36–38]. As these cells are also the main targets of HIV-1 infection, the release of chemokines from HIV-1-infected macrophages would promote the chemotaxis of substrate T lymphocytes to sites of virus production. Induction of cytokine and chemokine production by HIV-1 and SIV-infected macrophages through inflammatory action may contribute to viral pathogenesis[29,39–43]. In addition, MIP-1α and MIP-1β are CCR5 ligands and competitive inhibitors of HIV-1 binding to CCR5 (refs. 6–10,44,45). However, the CC-chemokine concentrations required for inhibition of CCR5-dependent virus infection of macrophages are 1.5 logs higher than peak CC-chemokine levels induced by HIV-1 infection or by adenovirus-mediated Nef expression. Perhaps Nef, through induction of chemokine release, does not directly affect the efficiency of viral replication in the target cell itself but instead promotes an environment that facilitates virus dissemination from infected macrophages to substrate T lymphocytes. As resting lymphocytes were activated after incubation with Nef-expressing macrophage supernatants, this Nef-induced environment may also influence the 'permissiveness' of those neighboring T lymphocytes to HIV-1 infection.

Methods

Cells, viruses and reagents.

Mononuclear leukocyte-rich cell preparations were obtained by leukapheresis from normal donors seronegative for HIV-1 and hepatitis B (ref. 46). Monocytes were isolated by countercurrent centrifugal elutriation, which separates cells on the basis of cell size and density[46,47]. Monocytes were cultured in 24-well plates or in T-25 flasks as adherent

monolayers for 2 d in medium containing 1,000 units of monocyte colony-stimulating factor (R & D Systems, Minneapolis, Minnesota) and for an additional 5 d in medium lacking monocyte colony-stimulating factor. Monocyte-derived macrophages were infected 7 d after being plated. CEM cells (human $CD4^+$ T-cell line; provided by National Institutes of Health AIDS Research and Reference Reagent Program, National Institute of Allergy and Infectious Diseases) and 293 cells (provided by B. Panning, Whitehead Institute, Cambridge, Massachusetts) were maintained in RPMI and DMEM, respectively, supplemented with 10% FCS. As the interaction of CD40 ligand with CD40 on macrophages induces chemokine production[48], viruses were obtained from transfected CV-1 cells, which were determined to be lacking the CD40 ligand (recombinant CD40 ligand provided by M. Kehry, Boehringer). For generation of viral stocks, CV-1 cells were transfected with 30 μg proviral DNA by calcium phosphate/DNA co-precipitation and were incubated at 37 °C. Virions in culture supernatants were used directly for macrophage and T-cell infections. HIV-1 gag p24 protein was quantified by ELISA (Coulter, Hialeah, Florida). COS 7 and CV-1 cells were obtained from American Type Culture Collection (Rockville, Maryland). Recombinant MIP-1α/β proteins, ELISA assays, and antibodies specific for MIP-1α/β and MIP-1α/β were obtained from R & D Systems (Minneapolis, Minnesota).

RT–PCR analysis.

Cytoplasmic RNA (treated with DNAse I) was isolated just after peak reverse transcriptase activity in infected macrophage cultures or at the peak of chemokine production in macrophage cultures infected with Adeno Nef. RNA was reverse-transcribed using random hexamers (GeneAmp RNA PCR kit; Perkin-Elmer, Norwalk, Connecticut) and amplified by PCR using primers specific for MIP-1α, MIP-1β and GAPDH. After initial denaturation (95 °C, 9 min), amplification proceeded for 42 cycles (94 °C for 45 s; 56 °C for 45 s; 72 °C for 90 s), followed by a final 7-minute extension at 72°C. Primers span at least one intron–exon border, to exclude amplification of genomic sequences. PCR products were separated by 1.6% agarose gel electrophoresis, stained with ethidium bromide and quantified from the negative of the photographic image. Relative levels of chemokine RNA production were compared after limiting dilution of substrate RNA. Gel bands were scanned and quantitated by volume integration using Molecular Dynamics ImageQuant software (Molecular Dynamics, Sunnyvale, California). Primer sequences have been described for MIP-1α/β (ref. 49) and GAPDH (ref. 50).

Lymphocyte chemotaxis assay.

Peripheral blood lymphocytes (5×10^5; obtained by leukapheresis) were placed over filters with pores 5 μm in diameter in the top chambers of 96-well microchemotaxis plates (Neuropore, Gaithersburg, Maryland). Infected culture supernatants, media controls and recombinant chemokines were added to either the top or bottom chambers or to both chambers and incubated at 37 °C for 2 h. Cells remaining above the filter were removed by washing with PBS–EDTA, and cells that migrated to bottom chambers were collected by centrifugation (3 min at 500*g*) and counted in a hemocytometer.

Adenovirus recombinants.

The parental adenovirus vector is a derivative of pJM17 (ref. 51) in which the entire E1 and E3 coding sequences have been deleted (D.F. *et al.*, in preparation). The adenovirus shuttle vector (452/JL1) (D.F. *et al.*, in preparation) was constructed from pXC/JL1 (ref. 51). HIV-1 Nef (from HIV-1_{SF2}) and green fluorescent protein coding regions were inserted in the shuttle vector under control of the CMV immediate early promoter. The green fluorescent protein reporter gene was subcloned from the plasmid, pEGFP (Clontech, Palo Alto, California). The recombinant Adeno GFP and Adeno Nef vectors were generated by homologous recombination of the parent adenovirus vector with the corresponding shuttle vector after calcium phosphate transfection of early passage 293 cells. Recombinants were propagated on 293 cells and purified through two rounds of plaque purification and concentrated on cesium chloride gradients. Titers of recombinant viruses were determined by plaque formation on 293 cells[52].

In situ hybridization and immunohistochemistry.

Monocyte-derived macrophages 7 days old, plated on chamber slides, were infected with Adeno Nef or Adeno GFP or were mock infected. At 15 h after infection, cells were rinsed with 10 mM $MgCl_2$ in PBS, fixed in 4% paraformaldehyde for 60 min at room temperature in the dark, then washed 5 min with 70% ethanol and permeabilized with a solution of 0.5% Triton-X100 and 10 mM $McCl_2$ in PBS for 10 min at room temperature. Cells were then incubated with prehybridization solution (2X SSC, 40% formamide) for 30 min at room temperature and hybridized overnight at 37 °C with 20 pmol fluorescent labeled oligonucleotides (Nef probe, complimentary to nucleotides 8,805–8,854 of HIV-1_{SF2}, 5′–FITC–TCCCTTATAGCAGACCATCCACCCATACTACGTTTTGACCACTTGCC–3′; MIP-1α probe, complimentary to nucleotides 2,129-2,153 in exon I and 2,842-2,866 in exon II, 5′–rhodamine–CAGGCGGTCGGCGTGTCAGCAGCAAGTGATGCAGAGAACTGGTTGCAGAG-3′) in 2X SSC, 40% formamide, 400 μg/ml BSA, 1 mg/ml denatured tRNA, 1 mg/ml denatured salmon sperm DNA, 10% dextran sulfate and 100 mM vanadyl complex. After hybridization, slides were treated with 1X SSC and 40% formamide for 30 min at 37 °C and washed three times for 10 min with 1X SSC, once with 10 mM $MgCl_2$ in PBS, then stained with 4,6-diamidino-2-phenylindole (DAPI) solution. Finally, cells were washed once with 10 mM $MgCl_2$ in PBS for 10 min and visualized on a Zeiss UV microscope, and images were captured by digital photography.

Brain tissue was obtained from a macaque that was persistently infected with SIV_{MAC} 251 and had SIV encephalitis. Brain tissue was collected at the time the monkey was killed and was used for histopathology, *in situ* hybridization and immunohistochemistry as described[5,29]. Representative samples of tissue were examined for viral nucleic acid by *in situ* hybridization and viral envelope expression by immunohistochemistry as described[5]. Macrophages were identified by immunohistochemistry using monoclonal antibody against CD14 (Becton Dickinson, Franklin Lakes, New Jersey). Chemokine expression on adjacent tissue sections was assessed using monoclonal antibodies against MIP-1α and MIP-1β (ref. 29).

Lymphocyte activation assay.

Elutriated peripheral blood lymphocytes (4×10^6/ml) were washed twice with Hanks′ balanced salt solution (HBSS) and cultured for 3 d with supernatant from macrophages infected with Adeno Nef or Adeno GFP or mock-infected. As a positive control, cells were incubated with phytohemagglutinin (1 μg/ml). Cells were washed twice in HBSS, then divided into aliquots and incubated with ^{3}H-thymidine (1 μCi per 1×10^6 cells) for 16 h. Cycling status was determined by the amount of label incorporated into TCA-insoluble material by measurement scintillation. In parallel, aliquots of cells were infected with HIV-1_{NL4-3} (about 1 μg per 1×10^6 cells, based on gag p24) for 16 h, washed and maintained in medium containing IL-2 (20 U/ml). Virus replication was monitored by reverse transcription assay.

Chemokine immunodepletion.

Supernatants of mock-infected macrophage cultures or macrophages infected with Adeno Nef or Adeno GFP viruses (collected 24 h after infection) were incubated with 5 μg/ml mouse antibody against MIP-1α, 7.5 μg/ml goat antibody against MIP-1β and mouse antibody against MIP-1α (5 μg/ml) and goat antibody against MIP-1β (7.5 μg/ml). Immune complexes were absorbed by incubation with detergent-free protein A/G for 4 h at 4 °C. Immune complexes were pelleted (2,500*g* for 3 min). Supernatants were filtered (0.22 μm, pore diameter) and incubated with resting elutriated peripheral blood lymphocyte cultures as described above. Immunodepletion with irrelevant isotype and species-matched antibodies served as controls. All chemokine antibodies were obtained from R&D Systems (Minneapolis, Minnesota). Irrelevant isotype and species-matched antibodies were obtained from PharMingen (San Diego, California) and Jackson ImmunoResearch (West Grove, Pennsylvania). The efficiency of chemokine immunodepletion was determined by ELISA measurement of chemokine concentrations in culture supernatants before and after immunodepletion.

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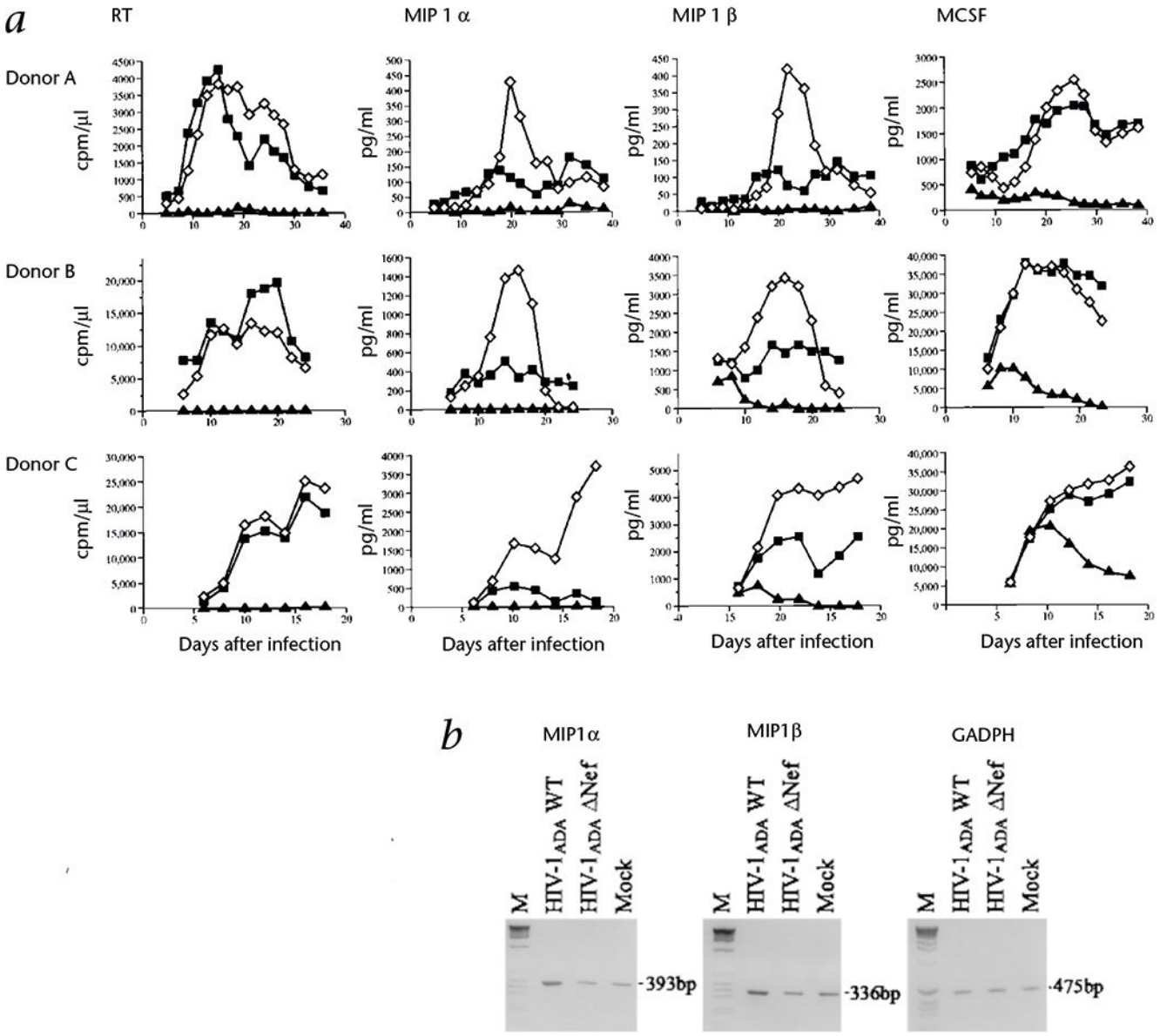

Fig. 1.

HIV-1 Nef influences CC-chemokine production by HIV-1-infected macrophages. ***a***, Chemokine and cytokine profiles in mock-infected macrophages (▲) and in macrophages infected with wild-type (◇) or ΔNef (■) HIV-1_{ADA} (1 μg virus, based on gag p24, was used to infection of 1×10^6 cells). RT, reverse transcriptase; MCSF, monocyte colony-stimulating factor. ***b***, Chemokine RNA levels in macrophages infected with wild-type (WT) HIV-1_{ADA} or HIV-1_{ADA} ΔNef or mock-infected at the peak of supernatant reverse transcriptase activity. M, molecular size markers.

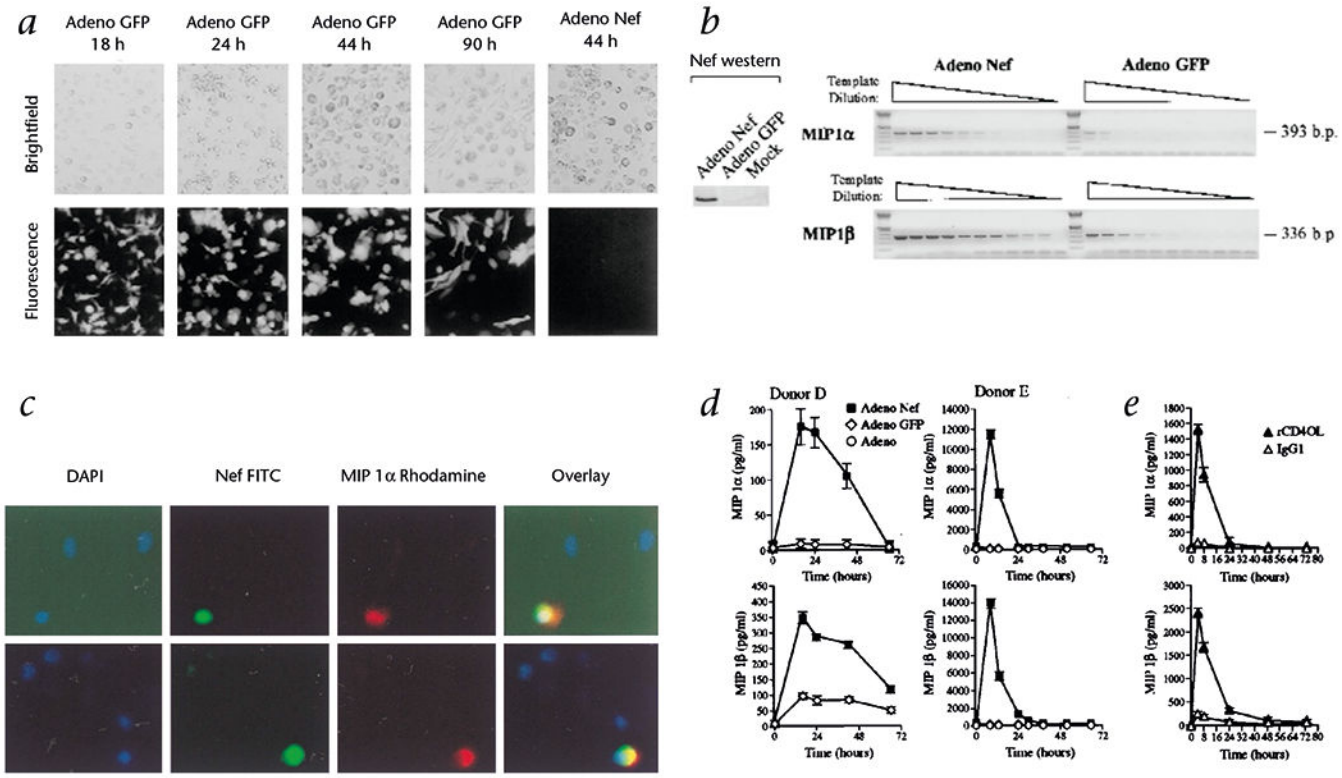

Fig. 2.

Nef expression is sufficient for induction of CC-chemokine expression. ***a***, Monocyte-derived macrophages were infected with an adenovirus vector expressing green fluorescent protein (Adeno GFP, 1,000 plaque-forming units/cell). Cells were photographed under fluorescent (bottom row; absorbance 495 nm; emission, 520 nm) and brightfield (top row) illumination. Above photos, times after infection. Original magnification, ×400. Adeno Nef, macrophages infected with an adenovirus vector expressing HIV-1 Nef (control). ***b***, HIV-1 Nef expression (right, western blot analysis) and chemokine RNA levels (left, RT–PCR) 16 h after infection in mock-infected macrophages and in macrophages infected with Adeno Nef or Adeno GFP virus. By limiting dilution analysis (wedges; doubling dilutions of substrate RNA), induction of MIP-1α and MIP-1β RNA in macrophages infected with Adeno Nef relative to those infected with Adeno GFP was 16-fold and 64-fold, respectively. ***c***, *In situ* analysis of Nef and MIP-1α co-expression in primary macrophages 15 h after infection with an adenovirus vector expressing HIV-1 Nef. Rows represent two independent cultures. ***d***, Chemokine production by macrophages infected with Adeno Nef (■) or Adeno GFP (◇) viruses or with an Adeno vector lacking a ‘foreign’ insert (○). ***e***, Chemokine production by macrophages after stimulation with recombinant CD40 ligand (▲; 25 μg/ml) or with a control protein (Δ; IgG1; 25 μg/ml).

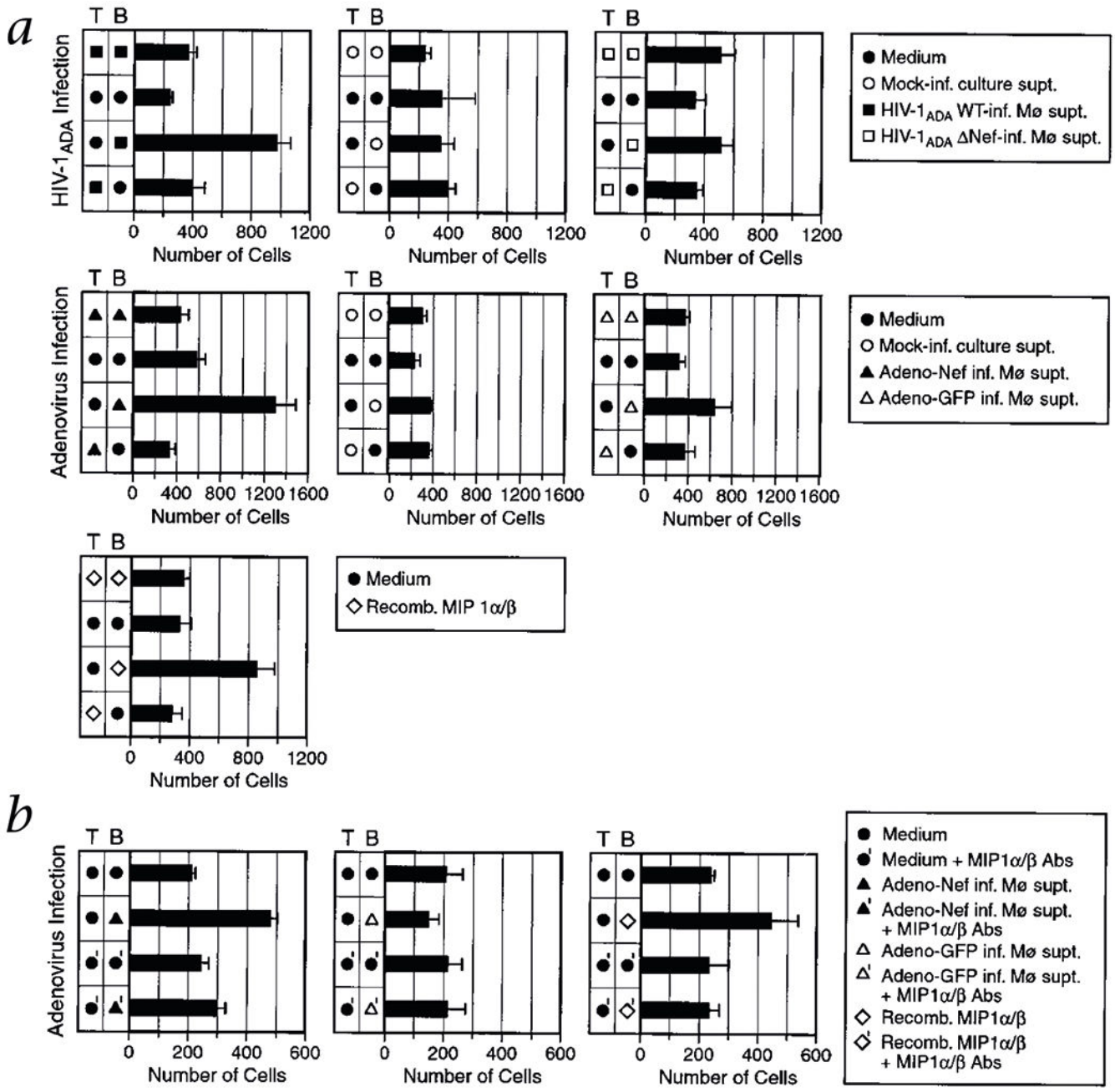

Fig. 3.

Induction of lymphocyte chemotaxis by supernatants from HIV-1-infected and Nef-expressing macrophage cultures. ***a***, Culture supernatants of macrophages infected with wild-type HIV-1_{ADA} or HIV-1_{ADA} ΔNef or mock-infected (top row) were collected at the peak of viral replication (10 d after infection). Supernatants of macrophages infected (inf. Mϕ supt.) with Adeno Nef or Adeno GFP (middle row) were collected at 18 h after infection and added to top (T) or bottom (B) or both chambers of microchemotaxis plates. The ability of these culture supernatants to promote chemotaxis of unstimulated peripheral blood lymphocytes was compared with the chemotactic activity of recombinant MIP-1α

and MIP-1β proteins (bottom row). ***b***, Antibodies against MIP-1α and MIP-1β block lymphocyte chemotactic activity of HIV-1 Nef-expressing macrophage culture supernatants (Adeno-Nef inf. Mϕ supt.). Chemotaxis was assessed in the presence (′) and absence of antibodies against MIP-1α (2.5 μg/ml) and MIP-1β (10 μg/ml). T, top chamber; B, bottom chamber.

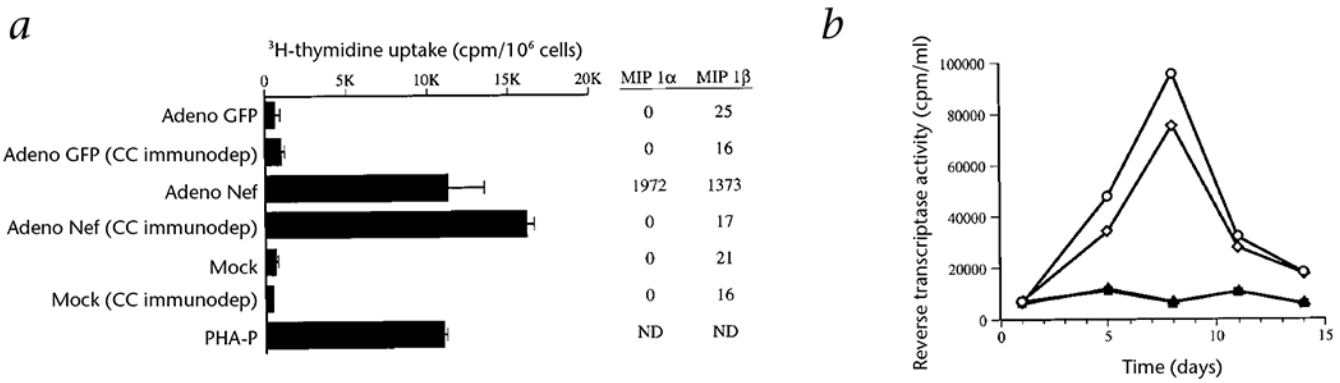

Fig. 4.

Induction of lymphocyte activation and 'permissiveness' to HIV-1 infection by Nef-expressing macrophage supernatants. ***a***, Culture supernatants from macrophages infected with Adeno Nef or Adeno GFP or mock-infected were collected 24 h after infection. Chemokines were immunodepleted from a portion of these culture supernatants using MIP-1α and MIP-1β antibodies conjugated to protein A/G sepharose. Right, MIP-1α and MIP-1β levels (in pg/ml) in macrophage supernatants before and after immunodepletion. Resting peripheral blood lymphocytes were cultured in immunodepleted and non-immunodepleted macrophage supernatants for 3 d, then cell division was evaluated by ^{3}H-thymidine incorporation. Lymphocytes activated with the mitogen phytohemagglutinin (PHA-P) served as a positive control. ND, not determined. ***b***, Resting lymphocyte cultures were incubated for 3d with supernatants derived from Adeno Nef (◇), Adeno GFP (■) or mock-infected (▲) macrophages cultures. Cells were then infected with HIV-1_{NL4-3} and virus replication was monitored by measurement of reverse transcriptase activity in culture. ○, phytohemagglutinin.

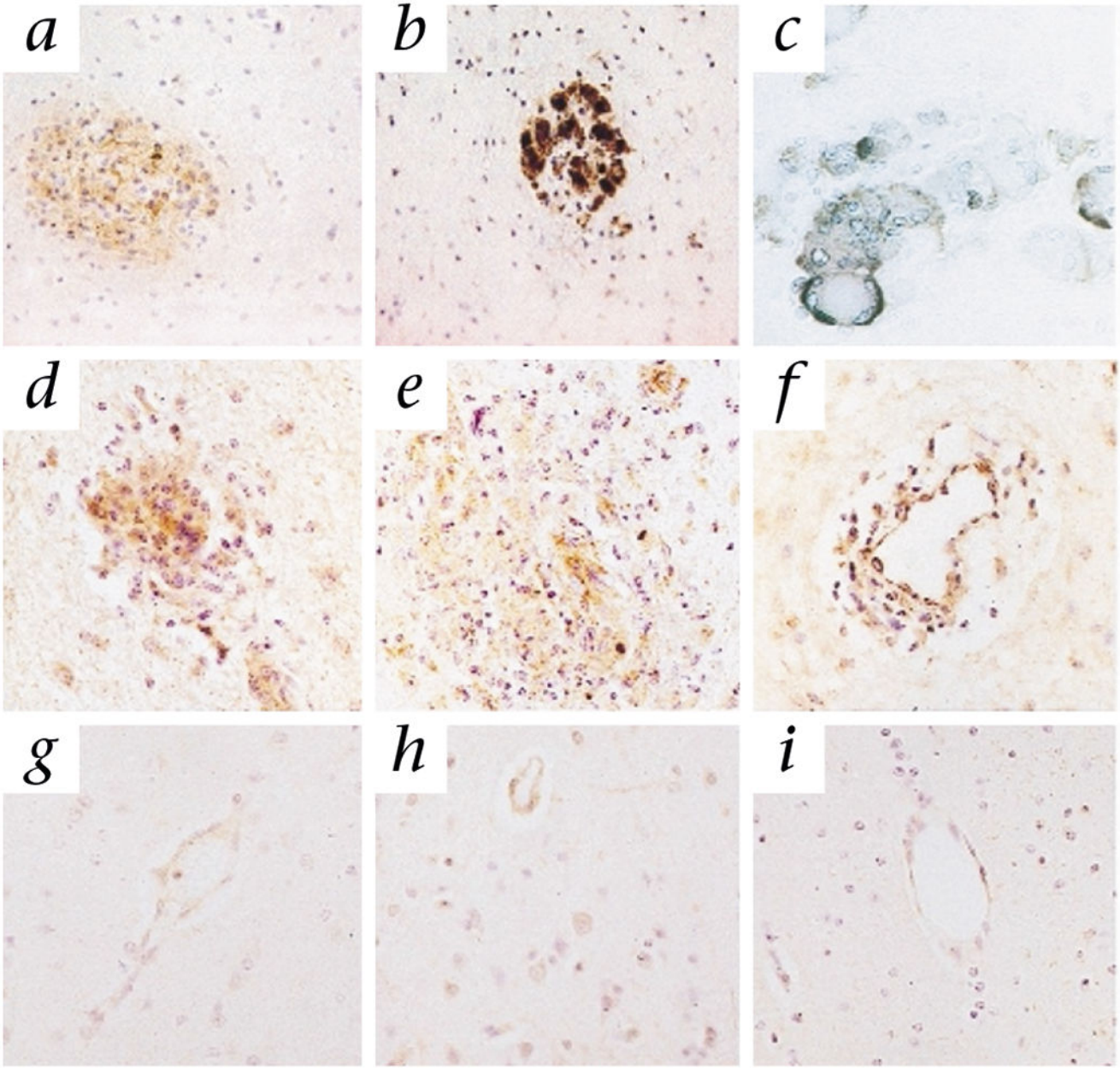

Fig. 5.

β chemokine expression associated with SIV-infected macrophages. ***a***, An aggregate of macrophages in the brain of an animal with SIV encephalitis immunostained for CD14. ***b*** and ***c***, The macrophages in the lesions in *a* contained abundant viral antigen (*b*) and viral nucleic acid (*c*). ***d*** and ***e***, perivascular aggregates of cells in adjacent tissue sections expressed high levels of MIP-1α (*d*) and MIP-1β (*e*), with expression of RANTES limited primarily to vascular endothelium (*f*). ***g–I***, Expression of MIP-1α (*g*) and MIP-1β (*h*) and RANTES (*i* was minimal or absent in normal brain from age and sex-matched control animals.

Table 1

Chemokine production by mock-infected macrophages and by macrophages infected with Adeno Nef or Adeno GFP viruses

	Adeno Nef		Adeno GFP		Mock	
	MIP1α (pg/ml)	**MIP1β (pg/ml)**	**MIP1α (pg/ml)**	**MIP1β (pg/ml)**	**MIP1α (pg/ml)**	**MIP1β (pg/ml)**
Donor F	15926	17606	496	1496	374	1192
Donor G	39470	33070	0	114	0	100
Donor H	20668	17801	0	19	0	29
Donor I	14378	9809	0	79	0	0
Donor J	17580	17520	3	185	3	240
Donor K	8600	6300	0	120	0	25

Chemokine levels in culture supernatants were determined 18 h post-infection. Results for macrophage cultures from six different donors are shown.