HIV-1 Proteins Preferentially Activate Anti-Inflammatory M2-Type Macrophages

Takashi Chihara, Michihiro Hashimoto, Abu Osman, Yuka Hiyoshi-Yoshidomi, Ikuko Suzu, Nopporn Chutiwitoonchai, Masateru Hiyoshi, Seiji Okada and Shinya Suzu

J Immunol 2012; 188:3620-3627; Prepublished online 9 March 2012; doi: 10.4049/jimmunol.1101593
http://www.jimmunol.org/content/188/8/3620

Supplementary Material
http://www.jimmunol.org/content/suppl/2012/03/13/jimmunol.1101593.DC1

References
This article cites 65 articles, 32 of which you can access for free at:
http://www.jimmunol.org/content/188/8/3620.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
HIV-1 Proteins Preferentially Activate Anti-Inflammatory M2-Type Macrophages

Takashi Chihara,1 Michihiro Hashimoto,1 Abu Osman, Yuka Hiyoshi-Yoshidomi, Ikuko Suzuki, Nopporn Chutiwitoonchai, Masateru Hiyoshi, Seiji Okada, and Shinya Suzu

HIV-1 proteins, including Tat, gp120, and Nef, activate macrophages (MΦ), which is consistent with the fact that HIV-1 infection is characterized by sustained immune activation. Meanwhile, MΦ are functionally classified into two types: proinflammatory M1-MΦ and anti-inflammatory M2-MΦ. We show that HIV-1 proteins, particularly Nef, preferentially activate M2-MΦ. Extracellular Tat, gp120, and Nef activated MAPK and NF-κB pathways in human peripheral blood monocyte-derived MΦ. However, the activation was marked in M-CSF–derived M2-MΦ but not GM-CSF–derived M1-MΦ. Nef was the most potent activator, and its signaling activation was comparable to that by TNF-α. Indeed, Nef was internalized more rapidly by M2-MΦ than by M1-MΦ. The myristoylation and proline-rich motif of Nef were responsible for the observed signaling activation. Consistent with the activation of MAPK/NF-κB pathways, Nef stimulated the production of a number of proinflammatory cytokines/chemokines by M2-MΦ. However, Nef reduced the expression of CD163 and phagocytosis, the characteristic markers of M2-MΦ, indicating that Nef drives an M2-like to M1-like phenotypic shift. Because the differentiation of most tissue MΦ depends on M-CSF and its receptor, which is the essential axis for the anti-inflammatory M2-MΦ phenotype, the current study reveals an efficient mechanism by which HIV-1 proteins, such as Nef, induce the proinflammatory MΦ.

The online version of this article contains supplemental material.
Preparation of primary human monocyte-derived MΦ

Heparinized venous blood was collected from healthy donors after informed consent had been obtained in accordance with the Declaration of Helsinki. The approval for this study was obtained from the Kumamoto University Medical Ethical Committee. Monocytes were essentially as described previously (10, 35). Briefly, mononuclear cells obtained using Pansorbin reagent (PAN Biotech, Aidienbach, Germany) were suspended in RPMI 1640 medium-1% FCS at 1 × 10⁶ cells/ml and seeded in 24-well plates. Monocytes were enriched by adherence to plates for 1 h at 37°C, and nonadherent cells were removed by extensive washing with PBS. Then, the adherent monocytes were differentiated into MΦ by culturing them with RPMI 1640-10% FCS containing 100 ng/ml of GM-CSF or 10 ng/ml of GM-CSF (36). After 3 d, the cultures were replaced with fresh complete media after extensive washing with PBS to remove nonadherent cells and incubated for another 2 d. The purity of the day-5 MΦ prepared by this method was routinely >95%, according to the expression of CD14 (data not shown). The differentiated MΦ were stimulated and subjected to subsequent analyses.

In a selected experiment (see Fig. 4E), the MΦ were incubated for 1 h at 37°C with pharmacological inhibitors prior to their treatment with Nef, P22 (Src kinase inhibitor; Wako), SU6565 (Src kinase inhibitor; Calbiochem), Src Kinase Inhibitor I (Calbiochem), LY294002 (PI3K inhibitor; Calbiochem), and IPA-3 (p21-activated kinase inhibitor; Calbiochem) were used in this study. All of the inhibitors were dissolved in DMSO (Wako, Osaka, Japan) equipped with Plan-Fluor ELWD 20×0.45 objective lenses (Nikon). Image processing was performed with a BZ-Analyzer (Keyence) and Adobe Photoshop software (Adobe Systems).

The expression of surface molecules on M-CSF–derived MΦ cultured in the presence or absence of Nef for 2 d was also determined by flow cytometry. The following labeled Abs were used: FITC-labeled anti-HLA-DR (HLDR01; Caltag), FITC-labeled anti-CD204, FITC-labeled anti-CD14 (38), and PE-labeled anti-M-CSFR (3A-4A4; Santa Cruz Biotechnology). The phagocytic activity of MΦ was determined by measuring the uptake of fluorescent microspheres (Fluoresbrite Carbocylate Microspheres, 0.7 μm in diameter; Polysciences, Warrington, PA). MΦ cultured on 24-well tissue culture plates were incubated with the fluorescent microspheres (1/2000 dilution) for 1 h at 37°C, washed extensively with PBS, detached from the wells using enzyme-free Cell Dissociation Buffer, and immediately subjected to flow cytometric analysis (38).

Cytokine/chemokine production

The relative levels of multiple cytokines and chemokines in the supernatants of MΦ were analyzed using a human cytokine array (R&D Systems), according to the manufacturer’s instructions. Briefly, M-CSF–derived day-5 MΦ were cultured in the presence or absence of Nef for 2 d, and the culture supernatants collected after centrifugation (100 µl) were added to duplicate wells to which the Abs had been added in duplicate. After incubation with the secondary Ab mixture, the signals were detected using Immunostar LD Western blotting detection reagent and an ImageQuant LAS 4000 image analyzer. The intensity of the spots was quantified using ImageQuant TL software (GE Healthcare).

Results

Soluble HIV-1 proteins activate MAPK and NF-κB pathways in M-CSF–derived M2-MΦ more strongly than in GM-CSF–derived M1-MΦ

M-CSF–derived and GM-CSF–derived MΦ prepared in this study showed spindle-like and egg-liked-like morphologies, respectively (Supplemental Fig. 1A). Phagocytic activity was higher in M-CSF MΦ, whereas the expression of transferrin receptor (CD71) was higher in GM-CSF MΦ (data not shown), which were consistent with previous reports (36, 39). Using the widely used method to prepare M2-MΦ (M-CSF derived) and M1-MΦ (GM-CSF derived) (13–16), we examined whether soluble Tat, gp120, or Nef differentially activated these MΦ by comparing the activation of MΦ, such as p38, JNK, and ERK. As reported (18–27), all soluble HIV-1 proteins clearly activated p38 (Supplemental Fig. 1B). However, we found that p38 activation was more marked in M2-MΦ for all HIV-1 proteins (Supplemental Fig. 1B). Neither Tat- nor Nef-induced p38 activation was inhibited by polymyxin B, the concentration of which (10 μg/ml) completely inhibited 10 ng/ml LPS-induced p38 activation (data not shown), indicating that the observed effect was not due to endotoxin contamination. The recombinant Nef preparation did not induce p38 activation after immunodepletion with anti-Nef Abs (Supplemental Fig. 1C), confirming that the observed effect was specific to Nef. Because gp120 required a higher concentration (2.5 μg/ml) to activate p38 compared with Tat (50 ng/ml) or Nef (100 ng/ml), and Nef was more potent in signaling activation than Tat in our system, we focused on Nef in the subsequent experiments.

To confirm the marked signaling activation in M2-MΦ by Nef, we performed more detailed time-course analyses. M1-MΦ showed higher basal ERK activation, which was not enhanced by Nef, whereas M2-MΦ showed lower ERK activation, which was enhanced by Nef (Fig. 1, p-ERK blot). The activation of p38 was marked and rapid in M2-MΦ (Fig. 1, p-p38 blot). Moreover, the activation of JNK was detectable only in M2-MΦ (Fig. 1, p-JNK blot). We also analyzed the activation of the NF-κB pathway. In NF-κB pathway activation, IKKα/β are activated through serine phosphorylation, which phosphorylates IkB, leading to its degradation and the nuclear translocation of NF-κB (26). Thus, we
We next analyzed the signaling activation in M2-ΜΦ by Nef by varying its concentration. The activation of p38, JNK, and ERK was detectable at 3 ng/ml (Fig. 2A). In contrast, higher concentrations were required to detect IKKα/β phosphorylation (100 ng/ml) or IκB degradation (30 ng/ml) (Fig. 2B). Importantly, the activation of these signaling pathways by Nef was comparable to that by TNF-α, the well-defined proinflammatory cytokine (40). Compared at the peak (30 and 10 min for Nef and TNF-α, respectively), the degrees of p38 activation, ERK activation, and IκB degradation induced by Nef were similar to those induced by TNF-α (Fig. 2C).

**Rapid uptake of Nef by M2-ΜΦ**

We next sought to clarify why Nef preferentially activated M2-ΜΦ. It was shown that soluble Nef enters ΜΦ or dendritic cells and accumulates in perinuclear regions (41, 42) through endocytosis, pinocytosis, or as-yet-unknown mechanisms (26). Of importance, we found that FITC-labeled Nef entered M2-ΜΦ more rapidly than it entered M1-ΜΦ (Fig. 3A, left and middle panels, respec-

**Activation of MAPK and NF-κB pathways in M2-ΜΦ by soluble Nef is comparable to that by TNF-α**

The differential activation of MAPK and NF-κB pathways by Nef were obtained by culturing peripheral blood monocytes from a single donor for 5 d with 100 ng/ml M-CSF (M2-ΜΦ) or 10 ng/ml GM-CSF (M1-ΜΦ) stimulating with 100 ng/ml soluble Nef for the indicated times; they were then analyzed by Western blotting using the indicated Abs. The actin blot is a loading control. In (A), the well-defined proinflammatory cytokine (40). Compared at the peak (30 and 10 min for Nef and TNF-α, respectively), the degrees of p38 activation, ERK activation, and IκB degradation induced by Nef were similar to those induced by TNF-α (Fig. 2C).

**FIGURE 3.** The differential uptake kinetics of labeled Nef between M2-ΜΦ and M1-ΜΦ. (A) ΜΦ were obtained by culturing peripheral blood monocytes from a single donor for 5 d with 100 ng/ml M-CSF (M2-ΜΦ) or 10 ng/ml GM-CSF (M1-ΜΦ) and incubating them with 100 ng/ml FITC-labeled Nef for 15, 30, or 60 min; they were then subjected to flow cytometric analysis to monitor their Nef uptake. ΜΦ were incubated at 37˚C (left and middle panels) or 4˚C (right panels) and detached from the wells using trypsin or enzyme-free Cell Dissociation Buffer (Enzyme-free). The mean fluorescence intensity (MFI) is shown. The experiments were repeated with ΜΦ obtained from different donors, and the data shown are representative of two independent experiments with similar results. (B) ΜΦ were obtained by culturing peripheral blood monocytes for 5 d with 100 ng/ml M-CSF (M2-ΜΦ) or 10 ng/ml GM-CSF (M1-ΜΦ) and incubating with 100 ng/ml FITC-labeled Nef for 2 h; they were then analyzed by fluorescent microscopy. The signals for Nef (green) were detected in the perinuclear regions and were observed in most ΜΦ. Original magnification ×60.

---

**FIGURE 1.** The differential activation of MAPK and NF-κB pathways by Nef between M2-ΜΦ and M1-ΜΦ. ΜΦ were obtained by culturing peripheral blood monocytes from a single donor for 5 d with 100 ng/ml M-CSF (M2-ΜΦ) or 10 ng/ml GM-CSF (M1-ΜΦ) and stimulating with 100 ng/ml soluble Nef for the indicated times; they were then analyzed by Western blotting using the indicated Abs. The actin blot is a loading control. In (A), the well-defined proinflammatory cytokine (40). Compared at the peak (30 and 10 min for Nef and TNF-α, respectively), the degrees of p38 activation, ERK activation, and IκB degradation induced by Nef were similar to those induced by TNF-α (Fig. 2C).

**FIGURE 2.** A comparison of the degree of activation of MAPK and NF-κB pathways induced by Nef and TNF-α. (A–C) ΜΦ were obtained by culturing peripheral blood monocytes for 5 d with 100 ng/ml M-CSF (M2-ΜΦ). In (A) and (B), ΜΦ were stimulated with soluble Nef for 30 min at the concentrations indicated and analyzed by Western blotting using the indicated Abs. The actin blot is a loading control. In (C), the M-CSF–derived day-5 ΜΦ were stimulated with 100 ng/ml soluble Nef for 30 min or with 10 ng/ml TNF-α for 3, 10, or 20 min and analyzed as in (A) and (B). All experiments were repeated with ΜΦ obtained from different donors, and the data shown are representative of two independent experiments with similar results.
The actin blot is a loading control. 

**FIGURE 4.** The ability of Nef mutants to activate MAPKs and NF-κB pathways, the activation of Hck by WT Nef, and the effects of kinase inhibitors on WT Nef-induced activation of MAPK and NF-κB pathways.

(A) The His-tagged Nef mutants used (G2A, AxxA, and LL/AA) are shown schematically. To verify that the concentrations of these Nef preparations were equivalent, the same volume of these preparations was analyzed by Western blotting using anti-His tag Abs. (B) MΦ were obtained by culturing peripheral blood monocytes for 5 d with 100 ng/ml M-CSF (M2-ΜΦ) and stimulating for 30 min with the WT or the Nef mutants at the concentrations indicated; they were then analyzed by Western blotting using the indicated Abs. The actin blot is a loading control. (C) The uptake kinetics of labeled Nef mutants were also analyzed. MΦ were obtained by culturing peripheral blood monocytes for 5 d with 100 ng/ml M-CSF (M2-ΜΦ) and stimulated for 30 min with WT or Nef mutants at the concentrations indicated; they were then analyzed by Western blotting using the indicated Abs. The actin blot is a loading control. (D) MΦ were obtained by culturing peripheral blood monocytes from a single donor for 5 d with 100 ng/ml M-CSF (M2-ΜΦ) or 10 ng/ml GM-CSF (M1-ΜΦ) and stimulating with 100 ng/ml soluble Nef, and analyzed by Western blotting using the indicated Abs. The actin blot is a loading control. (E) The inhibition of Akt activation state by the PI3K inhibitor LY294002 was analyzed. M-CSF-derived MΦ were treated for 1 h with LY294002 at the concentrations indicated. The lysates were analyzed by Western blotting using anti-p-specific Akt (p-Akt) or anti-actin Abs. The experiments in (B)–(F) were repeated with MΦ obtained from different donors, and the data shown are representative of two independent experiments with similar results. total pTyr, Total phosphotyrosine.
which is highly expressed in Mφ. Hck activation is mediated through the PxxP motif of Nef (47–49). Indeed, we found that soluble Nef also induced the activation of Hck (Fig. 4D, p-Hck blot). Of importance, such Hck activation was obvious in M2-Mφ but not in M1-Mφ (Fig. 4D). Nevertheless, its kinetics was slow, and it peaked later than the activation of p38 and JNK (Fig. 4D), implying that Hck activation was not the cause of Nef-induced MAPK and NF-κB pathways. Furthermore, Src family kinase inhibitors (PP2, SU6656, and Src Inhibitor I) failed to block Nef-induced p38 activation, IKKα/β phosphorylation, and IκB degradation (Fig. 4E), although at least SU6656 markedly reduced the basal protein tyrosine-phosphorylation level (total pTyr blot). The P13K inhibitor (LY294002) and the p21-activated kinase inhibitor (IPA-3) also failed to block the signaling activation induced by Nef (Fig. 4E). The concentration of LY294002 used in this study (10 μM) was sufficient to inhibit the activation of Akt, a downstream molecule of P13K (Fig. 4F), and the concentration of IPA-3 (10 μM) was shown to inhibit the activation of p21-activated kinase (50). These results indicated that the strong activation of MAPK and NF-κB pathways in M2-Mφ by Nef was dependent on its proline-rich PxxP motif but independent of the activation of Hck, P13K, or p21-activated kinase.

Effect of soluble Nef and its mutants on phenotypes of M2-Mφ

Finally, we examined how Nef modulated the phenotypes of M2-Mφ. M2-Mφ cultured with soluble Nef for 2 d showed obvious morphological changes (i.e., the appearance of a number of Mφ with protrusions) (Fig. 5A, arrows). Based on the finding that soluble Nef strongly activates MAPK and NF-κB pathways in M2-Mφ (Figs. 1, 2), we next analyzed whether the treatment of M2-Mφ with Nef led to the production of proinflammatory cytokines or chemokines using the semiquantitative array. Nef-treated M2-Mφ produced higher amounts of proinflammatory cytokines, such as TNF-α and IL-6, and chemokines, such as MIP-1α and MIP-1β, in two donors tested (Fig. 5B). It was shown that Nef activates Stat family proteins in Mφ through the release of proinflammatory cytokines and chemokines (24, 27). Indeed, we found the activation of Stat1 and Stat3 in Nef-treated M2-Mφ (Fig. 5C), the kinetics of which were delayed compared with those related to the activation of MAPK and NF-κB pathways (Fig. 1).

Of importance, we found that Nef significantly reduced the surface expression of CD163 scavenger receptor in M2-Mφ (Fig. 6A), the high expression of which is one of the characteristics of M2-Mφ (51, 52). Although the surface expression levels of another scavenger receptor CD204, as well as HLA-DR, were also reduced, the degree was moderate compared with that of CD163 (Fig. 6A). The downregulation of CD163 was also observed with the Nef LL/AA mutant (Fig. 6B). However, the G2A and AxxA mutants failed to induce CD163 downregulation (Fig. 6B). Moreover, we found that Nef also reduced phagocytic activity (Fig. 6B), which is another characteristic of M2-Mφ (13, 36). As was the case for CD163 downregulation, the reduced phagocytic activity was observed with the LL/AA mutant but not with the G2A or AxxA mutants (Fig. 6B). In this study, we found that CD163 downregulation and reduced phagocytic activity by soluble Nef were not blocked by the pretreatment of M2-Mφ with p38 inhibitor (SB 239063), JNK inhibitor (JNK inhibitor II), or NF-κB inhibitor (DHMEQ) (data not shown). Instead, soluble Nef strongly downregulated the surface expression of M-CSF receptor (Fig. 6C), as observed with Nef-expressing Mφ (10). Therefore, it was possible that CD163 downregulation and reduced phagocytic activity by soluble Nef were due to the downregulation of M-CSFR, followed by an impaired response of Mφ to M-CSF, which is important for the M2-phenotype of Mφ (7, 8, 51, 52).

In summary, the current study strongly suggests that HIV-1 proteins, particularly Nef, efficiently enter M2-Mφ, activate

**FIGURE 5.** The morphology of Nef-treated Mφ and their cytokine and chemokine production levels. (A and B) Mφ were obtained by culturing peripheral blood monocytes for 5 d with 100 ng/ml M-CSF (M2-Mφ) and for 2 d in the presence or absence of 100 ng/ml soluble Nef. (A) The arrows indicate the Mφ with protrusions. (B) The relative levels of multiple cytokines and chemokines in the Mφ supernatants were analyzed using a human cytokine array. The culture supernatants collected after centrifugation (100 μl) were added to blots onto which the capture Abs had been spotted in duplicate. After incubation with the secondary Ab mixture, the signals were detected using Western blotting detection reagent. Data shown in the bar graph are the results of densitometric analysis with Mφ obtained from two donors. The cytokines and chemokines whose levels were below the detection limit of the test are listed. (C) The activation of Stat family proteins in Nef-treated M2-Mφ was confirmed. Mφ obtained by culturing peripheral blood monocytes for 5 d with 100 ng/ml M-CSF were stimulated with 100 ng/ml Nef for the indicated times. The lysates were analyzed by Western blotting using the indicated Abs. The experiments were repeated with Mφ obtained from different donors, and the data shown are representative of two independent experiments with similar results.
their MAPK and NF-κB pathways through the PxxP motif, and drive them toward MΦ with an M1-like phenotype.

**Discussion**

Studies with different mice (M-CSF-deficient op/op mice, M-CSFR knockout mice, and GM-CSF knockout mice) clearly demonstrated that the development or survival of most tissue MΦ is dependent on the M-CSFR system (30, 31, 53) and its ligands, including M-CSF and possibly the newly identified alternative ligand IL-34 (54). Moreover, it was suggested that, under normal conditions, peripheral blood monocytes are predisposed toward an M2 phenotype and are mostly devoted to tissue repair as a result of their stimulation by the relatively high levels of M-CSF present in sera (6, 55). Indeed, a transcriptome analysis showed that M2 polarization involved a minimal alteration in MΦ steady-state mRNA expression compared with M1 polarization (14). It was also shown that, unlike T cells, MΦ polarization is transient and highly reversible (56). Therefore, more marked activation of MAPK and NF-κB pathways in M2-MΦ by soluble HIV-1 proteins, such as gp120 (Supplemental Fig. 1B), Tat (Supplemental Fig. 1B), and Nef (Fig. 1), appears to be an efficient mechanism by which HIV-1 induces the production of proinflammatory MΦ.

Such a response of M2-MΦ to soluble HIV-1 proteins might be a rapid process, because it occurs independently of viral replication within the MΦ.

Among those tested in our system (gp120, Tat, and Nef), Nef was the most potent activator of MAPK and NF-κB pathways of M2-MΦ, the degree of which was comparable to that of TNF-α (Fig. 2C). Although the concentration of Nef required for the optimal activity (100–300 ng/ml) was higher than that detected in patients’ sera (1–10 ng/ml) (29), the activation of p38 was detectable at a minimal concentration of 3 ng/ml (Fig. 2A). The mechanism and significance of the sustained activation of ERK in M2-MΦ by Nef remain unclear (Fig. 1A), but such sustained ERK activation has been found in several stimuli such as TPA-induced megakaryocytic differentiation of K562 cells (57–59). Soluble Nef was shown to activate MAPK and NF-κB pathways in MΦ (25–27). However, the in vitro preparations of differentiated MΦ used in these studies varied; for instance, monocytes were cultured with GM-CSF and then FCS alone (26) or were cultured with a high concentration of FCS alone (27). To our knowledge, the current study is the first report in which the response to exogenous Nef was compared between two major MΦ populations. In this study, we showed that Nef markedly stimulated the production of...
proinflammatory cytokines/chemokines, such as TNF-α, IL-6, MIP-1α, and MIP-1β, in M2-MΦ (Fig. 5B). Of interest, Nef did not stimulate the production of IL-12 or IL-23 in M2-MΦ (Fig. 5B), the higher expression level of which is one of the characteristics of M1-MΦ (13). More functional and transcriptional analyses, including the expression of transcription factors, such as IRF4 (60) and IRF5 (61), are needed to understand the phenotypic changes induced in M2-MΦ by Nef. Despite the unresolved issue, our novel finding that Nef reduced the expression of CD163 and phagocytic activity of M2-MΦ (Fig. 6), both of which are characteristics of M2-MΦ (13, 36, 51, 52), strongly suggests that Nef drives anti-inflammatory M2-MΦ toward MΦ with an M1-like phenotype.

The reason why soluble Nef activates MAPK and NF-κB pathways in M2-MΦ more strongly than in M1-MΦ might be because Nef rapidly and efficiently enters M- MΦ (Fig. 3). Indeed, it was shown that the N-terminal myristoylation of Nef at the glycine residue is required for Nef to target the cellular membrane (62), and we found that the nonmyristoylated Nef G2A mutant neither activated the MAPK/NF-κB pathways (Fig. 4B) nor reduced the surface expression of CD163 or phagocytic activity (Fig. 6B). Of interest, the proline-rich PxXp motif-disrupted AxxA mutant also lost these abilities (Figs. 4B, 6B). The proline-rich motif of Nef was shown to bind the Src homology 3 (SH3) domains of a subset of cellular Src family tyrosine kinases, such as Hck, Lyn, and possibly c-Src (47–49, 63). Among them, the interaction with Hck is important because it causes the activation of Hck kinase activity (47–49). However, the activation kinetics (Fig. 4D) and the results of our pharmacological inhibition analyses (Fig. 4E) did not support the idea that the activation of Hck or other Src kinases was involved in the soluble Nef-mediated activation of MAPK and NF-κB pathways. Because the proline-rich sequence acts as a canonical SH3 domain-binding motif, these results suggest that an unidentified SH3-containing cellular protein mediates the signaling activation of MΦ by soluble Nef. When expressed endogenously, Nef activates PI3K (64) and p21-activated kinase (65). However, our pharmacological inhibition analysis did not support the idea that these kinases were involved in the soluble Nef-mediated activation of MAPK and NF-κB pathways (Fig. 4E). Mangino et al. (66) recently showed that the pathway involving TNFR-associated factors was required for soluble Nef-induced activation of Stat family proteins, which may provide another explanation for why Nef preferentially activates M2-MΦ and clarify why the PxXp motif-disrupted Nef mutant fails to activate M2-MΦ.

The activation of MΦ by soluble HIV-1 proteins is thought to contribute to the sustained activation of the immune system observed in HIV-1 infection (6, 17). Our finding that HIV-1 proteins, particularly Nef, preferentially target M2-MΦ, which predominate under normal conditions, and drive them toward MΦ with an M1-like phenotype provides a novel mechanism by which HIV-1 efficiently and rapidly induces the sustained immune activation. More detailed phenotypic characterization of MΦ treated with gp120, Tat, and Nef will clarify the pathological significance of the activation of MΦ by HIV-1 proteins and the molecular mechanisms by which MΦ differentiation is physiologically regulated.

Acknowledgments

We thank F. Koutaki and H. Motoyama for secretarial assistance.

Disclosures

The authors have no financial conflicts of interest.


