HIV-1 gp120 and chemokines activate ion channels in primary macrophages through CCR5 and CXCR4 stimulation

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HIV type 1 (HIV-1) uses the chemokine receptors CCR5 and CXCR4 as coreceptors for entry into target cells. Here we show that the HIV-1 envelope gp120 (Env) activates multiple ionic signaling responses in primary human macrophages, which are important targets for HIV-1 *in vivo*. Env from both CCR5-dependent JRFL (R5) and CXCR4-dependent IIIB (X4) HIV-1 opened calcium-activated potassium (K_{Ca}), chloride, and calcium-permeant nonselective cation channels in macrophages. These signals were mediated by CCR5 and CXCR4 because macrophages lacking CCR5 failed to respond to JRFL and an inhibitor of CXCR4 blocked ion current activation by IIIB. MIP-1β and SDF-1α, chemokine ligands for CCR5 and CXCR4, respectively, also activated K_{Ca} and Cl^- currents in macrophages, but nonselective cation channel activation was unique to gp120. Intracellular Ca^{2+} levels were also elevated by gp120. The patterns of activation mediated by CCR5 and CXCR4 were qualitatively similar but quantitatively distinct, as R5 Env activated the K_{Ca} current more frequently, elicited Cl^- currents that were $\approx$2-fold greater in amplitude, and elevated intracellular Ca^{+2} to higher peak and steady-state levels. Env from R5 and X4 primary isolates evoked similar current responses as the corresponding prototype strains. Thus, the interaction of HIV-1 gp120 with CCR5 or CXCR4 evokes complex and distinct signaling responses in primary macrophages, and gp120-evoked signals differ from those activated by the coreceptors' chemokine ligands. Intracellular signaling responses of macrophages to HIV-1 may modulate postentry steps of infection and cell functions apart from infection.

HIV type 1 (HIV-1) entry into target cells is initiated by viral envelope (Env) gp120 binding to CD4, which leads to conformational changes in gp120 that enable it to interact with one of several G-protein coupled chemokine receptors (reviewed in refs. 1 and 2). Additional conformational changes then enable the transmembrane subunit gp41 to trigger fusion between the viral and target cell membranes. CCR5 and CXCR4 are the principal coreceptors used by macrophage (M)-tropic (R5) and T cell line (T)-tropic (X4) isolates of HIV-1, respectively. Although HIV-1 has subverted these chemokine receptors for its own use, their natural chemokine ligands normally activate intracellular signaling processes that induce leukocyte migration and activation (reviewed in refs. 3 and 4).

Macrophages are important targets for HIV-1 *in vivo*. Infected macrophages serve as reservoirs for viral persistence, and aberrant functions of both infected and uninfected macrophages have been implicated in pathogenesis (5–8). Macrophages express CD4, CCR5, and CXCR4 (9, 10), but the ability of macrophage chemokine receptors to support HIV-1 infection is both cell and strain-specific. Macrophages are permissive for M-tropic R5 and dual-tropic R5X4 but not prototype T-tropic X4 strains, although some primary X4 isolates can use macrophage CXCR4 (11, 12). Conversely, all primary simian immunodeficiency virus strains use CCR5 for entry (13–15), but determinants in Env regulate their ability to proceed through postentry steps of infection in macrophages (16). The basis for cell- and strain-specific differences in macrophage coreceptor function is not known.

In addition to facilitating viral entry, Env-coreceptor binding may also initiate signaling events that affect postentry stages of infection or modulate cellular functions even in the absence of infection. Previous studies have demonstrated gp120-induced chemokine receptor-mediated intracellular calcium elevations and protein phosphorylation in T cells (17, 18), but, in many cells, G protein-coupled receptors are linked to ionic signaling pathways (19, 20). Because macrophages are important targets for both direct and indirect effects of HIV-1, we used single cell patch-clamp recording and calcium-sensitive fluorescence methods to define the ionic signaling pathways linked to the chemokine receptors in macrophages. We found that gp120 activated several distinct ion channels and elevated intracellular calcium in primary macrophages through CCR5 and CXCR4. The signaling pattern depended on the chemokine receptor engaged, and, unexpectedly, the pathways activated by gp120 differed from those elicited by chemokine activation of CCR5 and CXCR4.

Methods

Isolation and Maintenance of Primary Human Macrophages. Monocytes were isolated from peripheral blood mononuclear cells of healthy donors by selective adherence and were maintained as described (21). Cells were plated on glass coverslips coated with poly-D-lysine (50 μg/ml) at a density of 10^6 cells per 22-mm coverslip and were cultured for 5–7 days before study to allow differentiation into monocyte-derived macrophages (MDM). Donors were screened for the CCR5 $\triangle$32 mutation by PCR (22), and only donors homozygous for the wild-type allele were used for these studies, except for specified experiments in which CCR5-deficient macrophages were obtained from donors homozygous for the mutant allele.

Recombinant gp120 Proteins and Chemokines. Recombinant gp120 was produced as described (23) in 293 T cells infected with gp120-expressing recombinant vaccinia viruses vSC60 (IIIB, BH8 clone) or vCB28 (JR-FL). For the X4 primary isolate UG021, standard methods were used to insert a stop codon after

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Abbreviations: MDM, monocyte-derived macrophage; NSC, nonselective cation.

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gp120 into the T7-driven gp160 plasmid pCRII-92UG021.16 (24) provided by B. Hahn through the National Institutes of Health AIDS Reagent Program. 293 T cells were then infected with the T7 polymerase-expressing recombinant vaccinia virus vTF1.1 followed by transfection with the gp120 plasmid as described (23). Supernatants were clarified by centrifugation and were filtered (0.45 μm pore size). Virus was inactivated (0.1% Triton X-100), and gp120 was purified by using *Galanthus nivalis* lectin-coupled agarose beads (Vector Laboratories) followed by protein concentration and buffer exchange. Env integrity was confirmed by Western blot analysis with a rabbit polyclonal antibody. Recombinant gp120 generated in baculovirus was kindly provided by J. Leary [SmithKline Beecham (25); strains JRFL and IIIB]; and through the National Institutes of Health AIDS Reagent Program by ImmunoDiagnostics (Bedford, MA) (strain IIIB) and MicroGeneSys (West Haven, CT) [R5 Chiang Mai strain (26)]. To neutralize gp120, preparations were incubated with affinity-purified anti-Env polyclonal Ig derived from pooled AIDS patient sera or polyclonal rabbit and goat antisera kindly provided by K. Steimer (Chiron). Chemokine signaling was investigated with MIP-1β and SDF-1α (1 μg/ml) (Peprotech, Rocky Hill, NJ).

Patch-Clamp Experiments. MDM on coverslips were placed into a temperature-controlled recording chamber (Brook Industries, Lake Villa, IL) on the stage of an inverted fluorescence microscope (Nikon). The bath solution contained 140 mM NaCl, 4.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, and 10 mM Hepes (pH 7.4). The standard pipette solution contained 145 mM KCl, 0.5 mM EGTA, 0.5 mM $MgCl_2$, and 5 mM Hepes (pH 7.4). Voltage clamp experiments were performed by using standard whole-cell recording techniques as described (27). Agonists were applied directly onto cells by using a pressure-controlled micropipette (Eppendorf) at the final concentrations indicated. In experiments using multiple reagents, the initial reagent was added to the bath solution, and the second reagent was applied by using the microapplication system.

To measure the instantaneous current reversal potentials, 300-ms ramp depolarizations were applied from −100 mV to 75 mV. Control currents were obtained immediately before agonist application and were subtracted from steady-state agonist-induced currents. Alternatively, step voltage pulses (1 s) were applied from a holding potential of −50 mV to potentials of −100 mV to 75 mV in 25-mV increments, with 500-ms steps back to the holding voltage. For clarity, ramps were removed from all macroscopic current plots shown. Channel selectivity was determined by using ion substituted solutions, including low chloride bath (110 mM Na-gluconate and 35 mM NaCl) and pipette (120 mM Cs-aspartate and 25 mM CsCl instead of KCl) solutions, or low sodium bath solution (110 mM *N*-methyl-D-glucamine and 30 mM NaCl). A high Ca^{2+} bath solution was used to determine the Ca^{2+} permeability of nonselective cation (NSC) channels (100 mM $CaCl_2$ and no NaCl). The pharmacological inhibitors NPPB, IAA, niflumic acid, and DIDS were obtained from Calbiochem, and charybdotoxin was obtained from Alomone Laboratories (Jerusalem).

Intracellular Calcium Measurements. MDM were loaded with the cell permeant calcium indicator fura-2 AM (6.0 μM) (Molecular Probes) for 10 min at 37°C in the microscope recording chamber. Discrete bandwidth excitation light (340 ± 10 nm, 380 ± 10 nm) from a xenon source coupled to a computer-controlled monochromator (TILL, Applied Scientific Imaging, Eugene, OR) was directed to the epifluorescence attachment of the microscope through a quartz fiberoptic guide. Excitation light was directed through a fluorescence objective (40× or 100×) (Nikon) via a dichroic mirror. The emitted fluorescence from fura-2 loaded cells passed through a 470-nm pass filter, and images were obtained with an intensified charge-coupled video camera [Hammamatsu (Bridgewater, NJ) Model C2400–68] connected to the side port of the microscope (28).

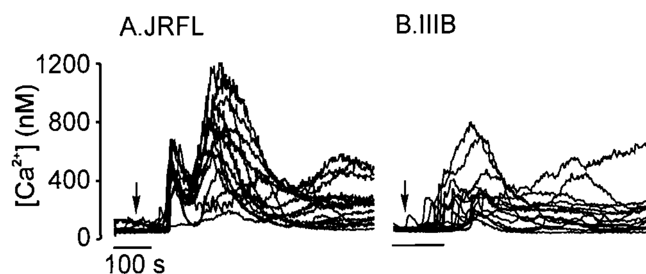

Fig. 1. Elevation of cytoplasmic calcium in MDM induced by HIV-1 gp120. Macrophages were loaded with fura-2 AM and were stimulated with gp120 (200 nM) from HIV-1 JRFL (*A*) or IIIB (*B*). Intracellular calcium in individual cells was calculated from F340/F380 ratios.

Results

HIV-1 gp120 Elicits Ca^{2+} Signals in Macrophages. Previous studies showed that R5 but not X4 gp120 elevated $[Ca^{2+}]_i$ in human lymphocytes (17). We used recombinant gp120 from the R5 isolate JRFL and X4 isolate IIIB to determine whether Env evoked a rise in $[Ca^{2+}]_i$ in primary human macrophages (Fig. 1). Both JRFL and IIIB gp120 elicited an increase in $[Ca^{2+}]_i$. However, the mean peak $[Ca^{2+}]_i$ induced by JRFL gp120 (590 ± 66 nM, $n = 44$ cells) was nearly twice as high as the response to IIIB (323 ± 56 nM, $n = 32$ cells). JRFL-induced Ca^{2+} elevations also typically decayed to a higher elevated steady-state level than IIIB-induced Ca^{2+} elevations.

JRFL and IIIB gp120 Activate Ionic Currents in Macrophages. We next examined receptor-mediated ionic currents in voltage-clamped MDM (Fig. 2). JRFL gp120 activated a brief outward current followed by a slowly developing, longer duration inward current. gp120 from IIIB also evoked an inward current but was less likely to activate the initial outward current. Among all cells exhibiting currents, JRFL activated the initial outward current in 53% of cells whereas IIIB produced an outward current in only 19% (Table 1). There was also a difference in peak amplitude of the inward current, as that evoked by JRFL was approximately twice that elicited by IIIB (−474 ± 118 pA vs. −243 ± 41 pA) (Table 1).

Several approaches confirmed that these ionic signaling responses were specifically elicited by gp120. The same currents were observed with different preparations of vaccinia-generated JRFL and IIIB gp120, JRFL gp120 from another source generated in baculovirus, and baculovirus IIIB gp120 from two different sources (data not shown). On the other hand, no currents were elicited by a mock prep prepared in an identical manner from 293 T cells infected with vaccinia virus lacking gp120 (data not shown). Finally, ion current activation was neutralized by preincubating Env preparations with a polyclonal anti-gp120 antiserum (Fig. 2 *C* and *D*) or with polyclonal rabbit and goat anti-gp120 sera but not by non-immune sera (data not shown).

Chemokines MIP-1β and SDF-1α Activate Ionic Currents in Macrophages. To compare the Env-induced ionic currents with those activated by CCR5 and CXCR4 chemokine ligands, we used MIP-1β, the most specific of several chemokines that signal through CCR5, and SDF-1α, the only known CXCR4 ligand. Both chemokines elicited a transient outward current followed by a more sustained inward current (Fig. 2 *E* and *F*). Unlike

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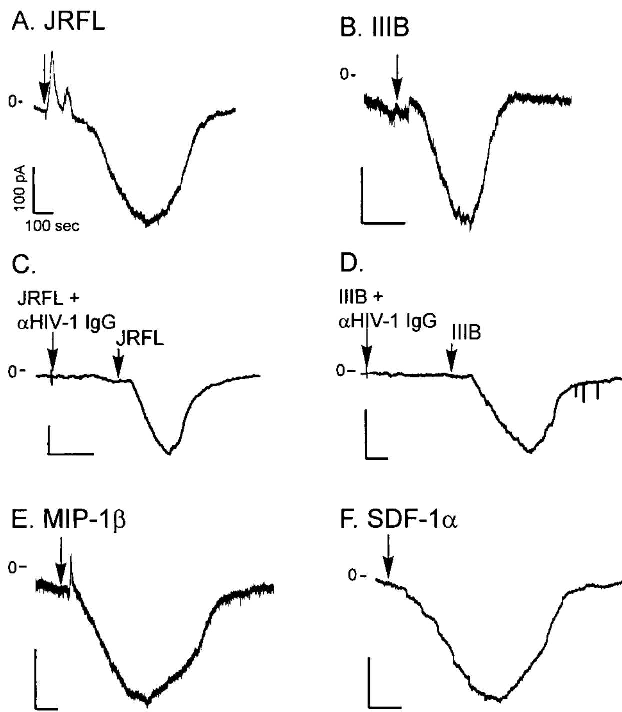

Fig. 2. Activation of ionic currents in MDM by gp120 and by chemokines. Patch clamp recordings were carried out on MDM stimulated with gp120 (200 nM) from HIV-1 JRFL (*A*) and IIIB (*B*). Incubation of gp120 with anti-Env antiserum blocked current activation but did not prevent subsequent activation by non-neutralized gp120 (*C* and *D*). MDM were also stimulated with the chemokines (1 μg/ml) MIP-1β (*E*) and SDF-1α (*F*).

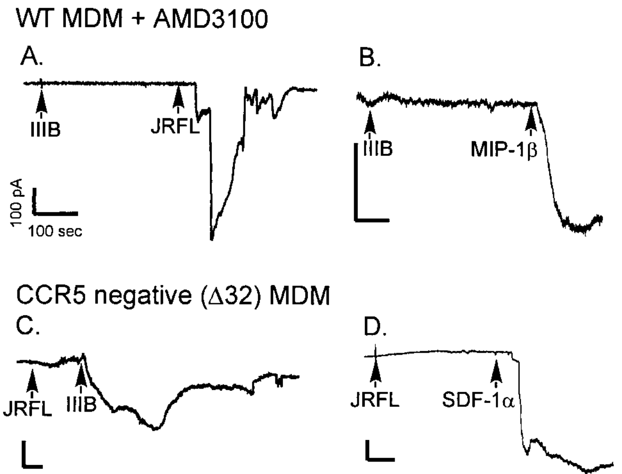

Fig. 3. CCR5 and CXCR4 dependence of MDM ion current activation by gp120. Incubation of MDM from CCR5 wild-type donors (WT) with the CXCR4 inhibitor AMD3100 (5 μg/ml) blocked currents elicited by IIIB gp120 but did not inhibit responses to JRFL or MIP-1β (*A* and *B*). In MDM from donors homozygous for the CCR5 Δ32 allele, no current was evoked by JRFL gp120 whereas both IIIB and SDF-1α elicited ion currents (*C* and *D*).

gp120, the outward current was evoked by MIP-1β in all tested cells and by SDF-1α in ≈50% of cells (Table 1). The inward currents activated by the chemokines were slowly developing and sustained, like those elicited by HIV-1 Env. Thus, SDF-1α and MIP-1β, which activate CXCR4 and CCR5 independent of CD4, elicited both inward and outward currents.

JRFL and IIIB gp120-Evoked Currents Are Mediated by CCR5 and CXCR4. The chemokine receptor specificity of gp120 ion channel activation was then determined by using AMD3100, which blocks the HIV-1 coreceptor capability of CXCR4 and SDF-1α signaling (29), and macrophages from donors homozygous for the CCR5 Δ32 allele, which abrogates CCR5 surface expression, chemokine signaling, and coreceptor function (22, 30). AMD3100 completely blocked IIIB gp120 ion current activation in macrophages but had no effect on JRFL and MIP-1β-elicited currents (Fig. 3 *A* and *B*). In CCR5-negative macrophages, no current was evoked by JRFL gp120, but the cells responded normally to IIIB gp120 and SDF-1α (Fig. 3 *C* and *D*). Furthermore, no current was activated by SDF-1α in AMD3100-treated macrophages or by MIP-1β in CCR5-negative macrophages (data not shown). Thus, CCR5 and CXCR4 mediate the responses to gp120 as well as chemokines. Because neither CXCR4 antagonism nor lack of functional CCR5 would affect gp120 binding to CD4, these results also show that CD4 binding in the absence of chemokine receptor stimulation is not sufficient to activate ionic signaling.

Characterization of Ionic Currents Evoked by gp120 and Chemokines in MDM. To define the ionic nature of Env-activated currents, instantaneous current-voltage relationships were obtained by using a voltage ramp protocol. Fig. 4*A* shows a typical plot of the current/voltage relationship of the initial outward current evoked by JRFL gp120. The subtracted instantaneous current was linear over the voltage range and reversed at -77 ± 2 mV ($n = 3$), which is close to the predicted equilibrium potential for K^+ ions (predicted $E_k = -87$ mV) and indicates that it is carried

Table 1. Amplitude and frequency of currents activated in macrophages

	JRFL gp120	CM gp120	IIIB gp120	UG021 gp120	MIP-1β	SDF-1α
I_{inward}*	−474 ± 118§	−481 ± 18¶	−243 ± 41§	−180 ± 46¶	−392 ± 158	−287 ± 70
Percent†	77	78	100	88	40	71
$I_{outward}$*	260 ± 88	134 ± 46	384 ± 160	215 ± 57	273 ± 63	298 ± 59
Percent†	53	56	19	33	100	58
n‡	17	9	16	22	20	24
$I_{[cat]}$						
Percent†	100	100	100	100	0	0

*Peak current amplitude (pA) (mean ± SEM).

†Percent of responding cells exhibiting each current.

‡Number of responding cells examined, using cells from ≥6 donors for each ligand except UG021, which was tested in 3 donors.

§Statistically significant ($P \leq 0.05$) by Student's *t* test.

¶Statistically significant ($P \leq 0.05$) by Student's *t* test.

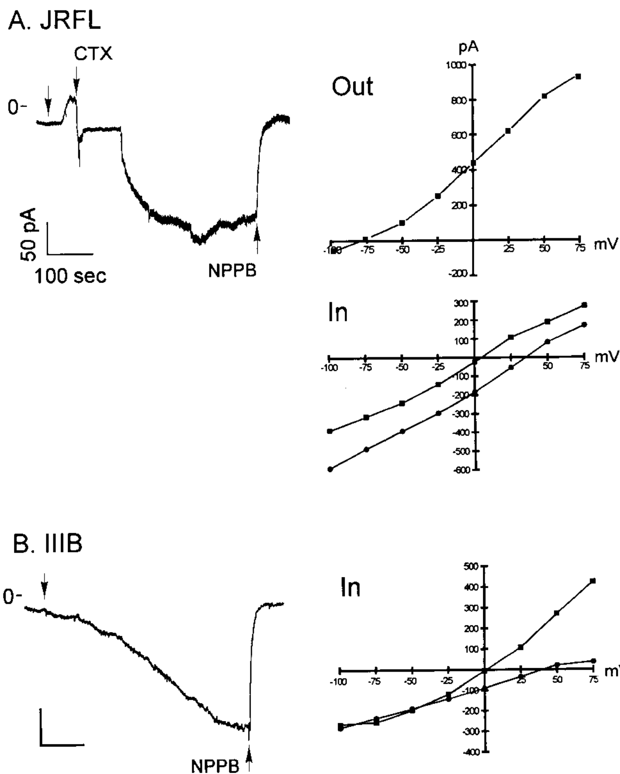

Fig. 4. Potassium and chloride currents evoked by gp120 in MDM. The ionic nature of currents elicited in MDM by gp120 from JRFL (*A*) and IIIB (*B*) was determined from the reversal potential (right) and with channel inhibitors (left). At peak activation, the outward JRFL current reversed direction at ≈−78 mV (*A*, top right) whereas the peak inward currents elicited by JRFL (*A*, bottom right) and IIIB (*B*, right) reversed at ≈5 mV (squares). In low Cl^- bath solution, the current voltage relationship of the inward current elicited by both Envs shifted to ≈+40 mV (circles). Charybdotoxin (100 nM) blocked the JRFL-evoked outward current, and NPPB (10 μM) blocked the inward currents activated by both Envs (*A* and *B*, left).

by K^+. The outward K^+ current was shown to be a Ca^{2+}-activated because it was inhibited by the addition of EGTA to the pipette solution (data not shown). The current was blocked by charybdotoxin, a peptidyl blocker of K channels (Fig. 4*A*), but not by iberiatoxin (data not shown), which suggests activation of an intermediate conductance K_{Ca} channel such as that expressed in human lymphocytes (31).

In contrast, the inward current activated by JRFL (Fig. 4*A*) and IIIB (Fig. 4*B*) Env reversed near 0 mV (JRFL E_R = 3.8 ± 2.4 mV, n = 5; IIIB E_R = 0.6 ± 2.1 mV, n = 7), which is consistent with activation of either a Cl^- or nonselective cation (NSC) channel. To define the inward current Cl^- selectivity, we replaced extracellular Cl^- on an equimolar basis with the impermeant anion gluconate. This substitution shifted the reversal potential of inward currents evoked by both JRFL gp120 (Fig. 4*A*; 31 ± 3 mV, n = 3) and IIIB gp120 (Fig. 4*B*; 44 ± 9 mV, n = 4) close to that predicted for Cl^- (E_{Cl} = 45 mV), indicating a Cl^- current. Consistent with this finding, the Cl^- channel blocker NPPB (10–100 μM) rapidly blocked the HIV-1 Env currents (Fig. 4 *A* and *B*), as did additional Cl^- channel blockers including DIDS (500 μM), niflumic acid (50 μM), and IAA-96 (250 μM) (data not shown). Of note, EGTA in the patch pipette did not block channel activation (data not shown), indicating this Cl^- channel was not Ca^{2+}-gated.

The MIP-1β and SDF-1α-induced initial outward current also reversed at ≈−75 mV (data not shown), similar to the JRFL-induced outward K_{Ca} current. Also like Env, the chemokine-induced inward current was a Cl^- current, based on its reversal potential and E_{Cl} shift with Cl^- replacement (Fig. 5), and inhibition by NPPB (data not shown). Thus, both HIV-1 Env and chemokines activate K_{Ca} and Cl^- channels in human macrophages.

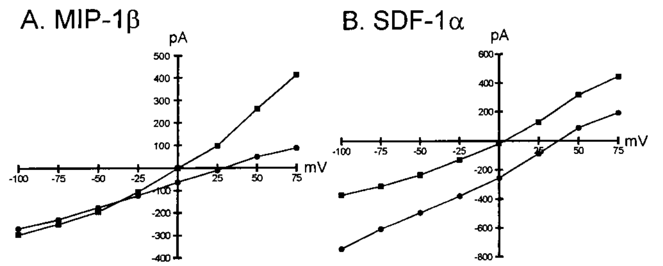

Fig. 5. Potassium and chloride currents evoked by chemokines in MDM. The ionic nature of currents elicited by MIP-1β (*A*) and SDF-1α (*B*) was determined from the reversal potential in normal (squares) and low Cl^- (circles) bath solution and the effect of channel inhibitors as described in Fig. 4.

HIV-1 Env, but not Chemokines, Activate a Nonselective Cation Current in MDM. Because both Cl^- and NSC currents may reverse at or near 0 mV, we asked whether the inward current elicited by Env might also include a NSC current component. To isolate non-chloride conductances, we voltage clamped cells at −45 mV and adjusted the ionic conditions to set E_{Cl} to that potential, added NPPB to the bath to block any residual Cl^- current, and blocked K^+ currents by replacing K^+ in the pipette solution with Cs^+ (Fig. 6 *A* and *B*). Under these conditions, JRFL and IIIB Env evoked an inward current in every cell tested. The reversal potential of the activated current was 0.3 ± 0.3 mV (n = 3) for JRFL and −1 ± 2 mV (n = 4) for IIIB. The current was identified as a NSC current based on the shift in E_R when Na^+ was partially replaced by *N*-methyl-D-glucamine in the bath solution (Fig. 6 *A Lower* and *B Lower*). In low Na^+ external solution, the reversal potential of the Env current was shifted close to the monovalent cation equilibrium potential (E_{cation} predicted = −40 mV; JRFL = −45 ± 5 mV, n = 3; IIIB = −39 ± 6 mV, n = 4). In contrast, under identical conditions, neither MIP-1β nor SDF-1α elicited currents (Fig. 6 *C* and *D*), indicating that the NSC current was unique to Env stimulation.

Because NSC channels differ in Ca^{2+} permeability, and intracellular Ca^{2+} elevations mediate many physiological responses to receptor stimulation, we determined the Ca^{2+} permeability of the gp120-activated NSC channel. Macrophage currents were recorded under conditions in which the only extracellular cations were Ca^{2+} ions, the NSC current was isolated by holding the membrane potential at E_{Cl} (−50 mV), and NPPB was present to block Cl^- channels. As shown in Fig. 7, both JRFL and IIIB gp120 evoked a transient inward Ca^{2+} current. Neither MIP-1β nor SDF-1α elicited a Ca^{2+} current in macrophages under these conditions, as expected given their failure to evoke a NSC current in sodium containing solution. Together, these data indicate that JRFL and IIIB Env, but not MIP-1β or SDF-1α, activate a Ca^{2+}-permeant NSC channel in primary macrophages.

gp120 from Primary Isolates also Activate Macrophage Ion Channels. Finally, we tested the macrophage current responses to HIV-1 primary isolates by using gp120 from the R5 Chiang Mai (CM) isolate (26) and the X4 isolate UG021 (24). Both primary isolates also activated K_{Ca}, Cl^-, and NSC currents (Table 1). Responses elicited by CM gp120 were comparable to JRFL, with relatively

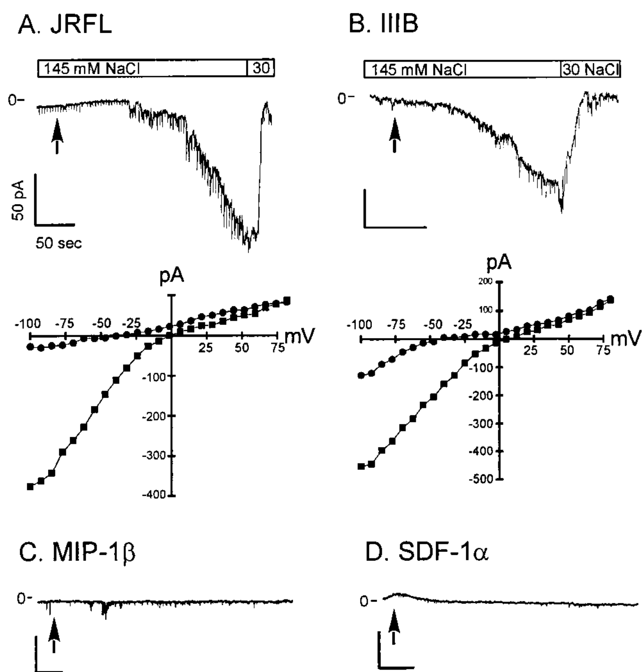

Fig. 6. Activation of a nonselective cation channel in MDM by gp120 but not by MIP-1β or SDF-1α. Currents were recorded by using a pipette solution in which Cs^+ replaced K^+, aspartate was substituted for Cl^-, and the cell was voltage-clamped at the calculated E_{Cl} of −45 mV. NPPB (10 μM) was added to block any residual Cl^- current. Cells were stimulated with JRFL gp120 (*A*), IIIB gp120 (*B*), MIP-1β (*C*), and SDF-1α (*D*). At the peak of gp120-activated currents, the bath solution was replaced with a low sodium solution (30 mM) as indicated by the bars (*A* and *B*). The reversal potentials for currents evoked by gp120 were determined in normal (squares) and low sodium (circles) bath solutions (lower panels of *A* and *B*).

frequent activation of the K_{Ca} currents (56% of cells) and large amplitude Cl^- currents (−481 ± 18 pA). Similarly, the current responses elicited by UG021 gp120 resembled those elicited by IIIB, with less frequent K_{Ca} activation (33%) and smaller Cl^- currents (−180 ± 46 pA) than R5 Env. Both CM and UG021 gp120 also activated the NSC current that was unique to Env stimulation.

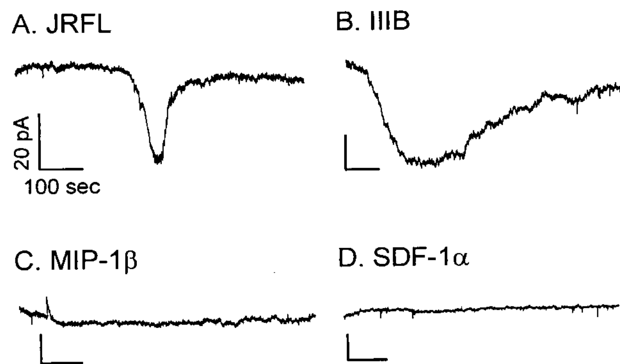

Fig. 7. Calcium-permeant nature of the nonselective cation channel activated by gp120. Currents were recorded by using a pipette solution containing 120 mM Cs-aspartate and 25 mM CsCl, and extracellular bath containing 100 mM $CaCl_2$ and no other cations. The membrane was voltage-clamped at the calculated E_{Cl} (−50 mV), and NPPB (10 μM) was added to the bath. An inward calcium current was elicited by gp120 from JRFL (*A*) and IIIB (*B*) but not by MIP-1β (*C*) or SDF-1α (*D*).

Discussion

In this report, we demonstrate that HIV-1 gp120 acts as a functional ligand of CCR5 and CXCR4 in primary macrophages. Env activates at least three distinct ion channels, eliciting a calcium-activated K^+ current, a Cl^- current, and a Ca^{2+}-permeant nonselective cation current, and also elevates $[Ca^{2+}]_i$. Ion channel activation by gp120 stimulation has not previously been examined in primary cells, nor has gp120-mediated Ca^{2+} signaling been reported in macrophages. In lymphocytes, Weissman *et al.* observed $[Ca^{2+}]_i$ elevations by gp120 from M-tropic R5 HIV-1 and simian immunodeficiency virus strains, but not by T-tropic X4 HIV-1 or R5 simian immunodeficiency virus strains that do not replicate in macrophages (17). Others did not detect gp120-induced $[Ca^{2+}]_i$ elevations in bulk cell populations, although both R5 and X4 gp120 induced phosphorylation of the tyrosine kinase Pyk2 in T lymphocytes (18). In contrast, Env blocked chemokine activation of a G protein-coupled K^+ channel, which was co-expressed with CD4 and chemokine receptors in *Xenopus* oocytes (32). Thus, our results in macrophages, together with these data, suggest that distinct intracellular signals may be elicited by Env in different cells.

Unexpectedly, we found that Env and chemokines that use the same receptors evoked distinct ionic signaling responses. Most striking was our observation that neither SDF-1α nor MIP-1β activated nonselective cation channels that were opened by R5 and X4 gp120. In addition, Env activated a Cl^- current more frequently and K_{Ca} current less frequently than the corresponding chemokines. Env-specific channel activation is not caused by signaling through CD4 alone because no current was evoked by Env binding to CD4 in CCR5-negative MDM stimulated with JRFL or in AMD3100-treated macrophages stimulated with IIIB gp120. It is possible that co-engagement of CD4 along with the chemokine receptor may cooperate to initiate unique signals not elicited by either receptor alone, or that CD4-initiated signals may modify signals initiated by chemokine receptor stimulation, as occurs with CD4 and the TCR in lymphocytes (33). Although the CD4-associated T cell tyrosine kinase $p56^{lck}$ is not expressed in macrophages (34), gp120 binding of macrophage CD4 may activate other pathways (35). However, antibody engagement of macrophage CD4, followed by SDF-1α or MIP-1β stimulation, did not elicit a NSC current (data not shown). Thus, if activation of NSC channels results from CD4 and chemokine receptor co-engagement, it may require specific restrictive geometry, stoichiometry, or timing of engagement.

Several quantitative differences were identified between R5 and X4 gp120-elicited ion currents. JRFL Env elicited higher peak and steady-state $[Ca^{2+}]_i$ elevations than IIIB, more frequent K_{Ca} activation, and larger inward Cl^- currents. The more frequent K_{Ca} activation is likely caused by the greater mean $[Ca^{2+}]_i$ elevations (590 nM for JRFL vs. 323 nM for IIIB) because the EC_{50} for activation of macrophage K_{Ca} channels is 400–500 nM (36). Quantitative differences may relate to the fact that the K_D of CD4-activated JRFL gp120 for CCR5 is ≈4 nM (37), compared with a K_D of ≈300 nM for IIIB gp120 and CXCR4 (R.W.D., unpublished work). Furthermore, the similarity in activation patterns elicited by primary and prototype strains suggests that HIV-1 Env-mediated stimulation of each chemokine receptor may elicit distinct response patterns that are determined primarily by the coreceptor rather than by the specific Env.

Coreceptor signaling is dispensable for infection in cell lines (13, 38–40), but indirect evidence suggests that it might be involved in certain primary cells. Macrophage tropism of simian immunodeficiency virus is determined by Env but at a level subsequent to CCR5-mediated entry (13, 16), and cross desensitization of CCR5 signaling was recently shown to be associated

with inhibition of lymphocyte infection by R5 isolates (41). Thus, signaling pathways, including those identified here in primary macrophages, may be involved in cell- and virus-specific mechanisms that modulate infection subsequent to fusion.

Finally, macrophages are important components of the innate immune system, and defects of macrophage function are observed in HIV-1 infection *in vivo* and *in vitro* that can be induced by viral particles or soluble gp120 even in the absence of infection (5–7). In addition, X4 gp120 can up-regulate macrophage membrane-bound TNF-α, which induces CD8 T cell apoptosis and may contribute to CD8 cell depletion *in vivo* (42). In the central nervous system, HIV-1 infection is associated with activation of brain macrophages and microglia and induction of macrophage secretory products and neuronal toxicity (43, 44). K_{Ca}, Cl^-, and NSC channels similar to those activated by gp120 have been identified previously in human macrophages (45) and are involved in triggering phagocytic, secretory, and respiratory burst activity (46–50). Thus, virion particles or shed gp120 may activate signaling pathways linked to physiological and immunological responses in uninfected macrophages that contribute to pathogenesis in AIDS.

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