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Constitutive Expression of p50 Homodimer in Freshly Isolated Human Monocytes Decreases with In Vitro and In Vivo Differentiation: a Possible Mechanism Influencing Human Immunodeficiency Virus Replication in Monocytes and Mature Macrophages

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Human immunodeficiency virus type 1 (HIV-1) replicates more efficiently in vitro in differentiated macrophages than in freshly isolated monocytes. We investigated whether this may be partly explained by changes in expression of NF-κB with monocyte differentiation. We demonstrated that constitutive expression of NF-κB in primary human monocytes changed significantly with differentiation in vitro to monocyte-derived macrophages (MDMs) and differentiation in vivo to alveolar macrophages (AMs). Freshly isolated monocytes constitutively expressed high levels of transcriptionally inactive p50 homodimer which decreased with time in culture in favor of the transcriptionally active p50/p65 and p50/RelB heterodimers. As in MDMs, AMs constitutively expressed p50/p65 and p50/RelB although at lower levels. HIV infection of fresh monocytes failed to induce p50/p65 as seen in MDMs. The replacement of p50 homodimers with transcriptionally active heterodimers following time in culture may partially explain the progressive increase in susceptibility of monocytes to HIV infection during in vitro culture. The change in NF-κB components with monocyte differentiation in vivo may also explain the different transcriptional activities of these cell populations in HIV-infected individuals.

NF-κB is a transcription factor (2) which strongly activates transcription of human immunodeficiency virus (HIV) provirus in T cells (22) and monocytic cells (1, 10, 33). The NF-κB/Rel family of proteins is a group of structurally and functionally related transcription factors and includes p50 (NF-KB1), p52 (NF-KB2), p65 (RelA), RelB, and c-Rel, which can form a variety of homo- and heterodimers. There is a homologous region within each member of this family known as the Rel homology domain which is responsible for DNA binding, dimerization, and nuclear localization (33, 34). The carboxyl-terminal regions of p65, c-Rel, and RelB contain transcription activation domains (3, 26, 34), while p50 and p52 lack these domains. p50 has been shown to inhibit transactivation of several genes including those controlled by the HIV long terminal repeat (LTR) (7, 8, 34). A family of serine proteases expressed exclusively in myelomonocytic cells has been described, which cleaves the C terminus of the NF-κB subunit p65 in vitro (6); these proteases may explain previous reports of novel fast-migrating NF-κB-specific complexes associated with restricted HIV expression in certain myelomonocytic cells (23, 27–29).

Monocytes and macrophages differ in their ability to support HIV replication both in vitro (24, 30, 39) and in vivo (16, 37). In general, HIV replication in monocytes appears to require at least partial differentiation (30, 36, 38, 39) and is enhanced by factors that promote differentiation (14, 17, 35). Recently, a block prior to the initiation of reverse transcription has been demonstrated to occur in fresh monocytes. However, reverse transcription was initiated if the cells were maintained in culture for as little as 24 h prior to infection, and increasing the time in culture prior to exposure to HIV resulted in a greater percentage of cells infected (38). Monocyte-derived macrophages (MDMs) and alveolar macrophages (AMs) express fewer surface CD4 receptors than do freshly isolated monocytes (19, 24, 39) but are more susceptible to infection (30). However, the differences following HIV infection of fresh monocytes, MDMs, and AMs in the numbers of initiated and complete viral transcripts do not fully explain the relative differences in permissiveness to infection (30). Therefore, monocyte differentiation, although associated with changes in events prior to reverse transcription, may also involve intracellular changes which enhance HIV replication.

We report here that differentiation of fresh monocytes, either in vitro or in vivo, was associated with significant changes in the constitutive expression of NF-κB. Fresh monocytes predominantly express a p50 homodimer; with differentiation, p50 homodimer expression decreases and is replaced by p50/p65 and p50/RelB heterodimers. The replacement of p50 homodimers with transcriptionally active heterodimers following time in culture may partially explain the progressive increase in susceptibility of monocytes to HIV infection during in vitro culture.

MATERIALS AND METHODS

Nuclear extracts. Nuclear extracts were prepared by a high-salt extraction method (4a) using some modifications described by Bellas et al. (4). This method allowed the use of small numbers of cells and minimized proteolysis, a common problem in the manipulation of myelomonocytic cells. Briefly, 5×10^6 to 10×10^6 cells were collected, washed in ice-cold phosphate-buffered saline (Cytosystems, Sidney, Australia), and resuspended in 2 ml of buffer A (10 mM Tris-HCl [pH 7.5], 2 mM $MgCl_2$, 5 mM KCl, 10% glycerol, 1 mM EDTA), which was then

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adjusted to a final concentration of 0.5% Nonidet P-40 by the addition of an equal volume of buffer A plus 1.0% Nonidet P-40. Following incubation on ice for 5 min, nuclei were pelleted at 500 × *g* for 5 min at 4°C. Nuclei were extracted in high-salt buffer (10 mM Tris-HCl [pH 7.5], 0.5 M NaCl, 1 mM EDTA, 1 mM dithiothreitol [DTT]) for 30 min on ice. All buffers were supplemented with the following protease inhibitors: phenylmethylsulfonyl fluoride (PMSF), 2 mM; aprotinin, 0.2 μg/ml; leupeptin, 0.5 μg/ml; pepstatin, 0.7 μg/ml; antipain, 50 μg/ml; trypsin inhibitor, 50 μg/ml; and chymostatin, 20 μg/ml (all from Boehringer Mannheim, Mannheim, Germany). Cell debris was removed by centrifugation at 13,000 × *g*, and the supernatant was dialyzed for 2 h against buffer B (10 mM Tris-HCl [pH 7.5], 90 mM KCl, 10% glycerol, 1 mM EDTA, 50 mM NaCl, 2 mM PMSF, 1 mM DTT). Samples were frozen in liquid nitrogen and stored at −70°C. The concentrations of protein in nuclear extract samples were determined by using bovine serum albumin (BSA) as the standard.

Electrophoretic mobility shift assays (EMSA). Two to 4 μg of protein extract was incubated with 10^4 cpm (0.1 to 0.5 ng) of radiolabelled double-stranded oligonucleotide. The double-stranded probes were end filled with [α-^{32}P]dCTP by using Klenow fragment (Promega). A final volume of 20 μl contained binding buffer (10 mM Tris-HCl [pH 8.0], 5 mM $MgCl_2$, 1 mM EDTA, 1 mg of BSA per ml, 1 mM DTT) and 0.1 μg of poly(dI-dC). The final concentration of NaCl was 60 mM. Reaction mixtures were incubated for 20 min at room temperature and electrophoresed on 6% (acrylamide/bisacrylamide ratio, 29:1) prerun (1 h, 250 to 350 V) polyacrylamide gels in 0.25× TBE (Tris-borate-EDTA) and visualized by autoradiography. DNA binding competition was assessed by preincubating the reaction mixture prior to the addition of the probe, with a 100-fold excess of either unlabelled oligonucleotide (specific competitor) or unlabelled oligonucleotide in which the NF-κB binding sites had been mutated (nonspecific competitor). The oligonucleotide used in the binding reaction corresponded to the NF-κB binding sequence present within the enhancer of the HIV LTR (5′-TACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAGGCGTG-3′). The sequence of the nonspecific competitor oligonucleotide was 5′-TACAACTCACTTTCCGCTGCTCACTTTCCAGGGAGGCGTG-3′. The oligonucleotide containing an Sp1 binding site, 5′-GATCGATCGGGGCGGGGCGATC-3′, (a kind gift from Anna Peeters, Macfarlane Burnet Centre for Medical Research, Fairfield, Australia) was end labelled with [γ-^{32}P]dATP. All oligonucleotides were synthesized on an Applied Biosystems model 381A DNA Synthesizer by using phosphoramidite chemistry.

Antibodies. The antibodies used in the supershift assays included mouse polyclonal anti-p50 (a kind gift from K. P. LeClair) and anti-p65, anti-p52, anti-c-Rel, and anti-RelB (Santa Cruz Biotechnology, Santa Cruz, Calif.). Control antibodies included preimmune mouse and rabbit immunoglobulin G (Sigma Immunochemicals, St. Louis, Mo.).

Monocytes, MDMs, AMs, and monocytic cell lines. Monocytes were isolated on the day of blood collection from HIV-seronegative buffy packs (Red Cross Blood Bank, Melbourne, Australia) by density gradient purification and plastic adherence as detailed previously (5, 39). Monocyte cultures were 90% ± 3% monocytes as determined by flow cytometric analysis using anti-CD11c monoclonal antibody (Becton Dickinson, Mountain View, Calif.). The nonadherent cells were collected and are referred to here as peripheral blood lymphocytes (PBLs). In some experiments monocytes were obtained from peripheral blood mononuclear cells (PBMCs) by using a magnetic activated cell sorter (MACS; Miltenyi, Bergisch Gladbach, Germany). PBMCs were incubated with anti-CD3 and CD19 antibodies (hybridomas OKT3 and FMC63) and goat anti-mouse antibody bound to magnetic beads (Miltenyi). These monocytes contained only 2 to 3% CD3- or CD19-positive cells as assessed by flow cytometry. MDMs were cultured in polytetrafluoroethylene Teflon pots (Savillex, Minnetonka, Minn.) and harvested after 5 to 7 days in suspension culture.

AMs were obtained from bronchoalveolar lavage from healthy donors who gave informed consent (as approved by our institution's Human Ethics Committee). AMs were obtained as previously described (19). They were then purified by either adherence or immunomagnetic bead selection as described above. Nuclear extraction was performed on the day of isolation.

RC2A monocytic cells (kind gift from W. Boyle, University of Melbourne, Melbourne, Australia) were grown in RPMI 1640 with 10% heat-inactivated fetal calf serum (Flow Laboratories).

All solutions and media used in purification and culture were endotoxin free as assessed by a chromogenic *Limulus* amoebocyte lysate assay (Whittaker Bioproducts, Walkersville, Md.). In experiments assessing the response to lipopolysaccharide (LPS), 0.1 μg of LPS per ml was added to fresh monocytes and MDMs and nuclear extracts were harvested 4 h later.

Protein electrophoresis. Protein (10 μg) was denatured in 2-mercaptoethanol at 100°C for 2 min and run on sodium dodecyl sulfate–10% polyacrylamide gel at 150 V for 1 h. The gel was fixed with methanol-glacial acetic acid and stained with Coomassie brilliant blue (Bio-Rad) overnight.

HIV infection. Fresh monocytes and MDMs were infected with HIV type 1 (HIV-1) isolate HIV-$1_{Ba\text{-}L}$ (9) or a primary macrophage-tropic patient isolate HIV-1_{676} (kindly donated by Dale McPhee, Macfarlane Burnet Centre for Medical Research) at a multiplicity of infection of 0.01 (as assessed by end point titration in MDMs). Virus stock suspensions were prepared by a single passage of HIV-$1_{Ba\text{-}L}$ through MDMs followed by a single amplification in PBMCs. The primary isolate HIV-1_{676} had been passaged through PBMCs no more than three times. HIV-1 virus stocks were treated with 10 U of RNase-free DNase (Boehringer-Mannheim) per ml and 10 mM $MgCl_2$ for 20 min at room temperature and then passed through a 0.20-μm-pore-size filter prior to use in experiments. Control cultures were mock infected with supernatant that had been prepared in parallel with virus amplification by using the same donor PBMCs. The cells were pulsed with virus or mock supernatant for 4 h at 37°C and then washed twice in PBS. The cells were collected 24 h later for nuclear extraction. At the same time, 10^6 cells were lysed, heated, and subjected to PCR amplification using primers to *gag* (SK38 and SK39) and HLA-DQ (GH26 and GH27) as previously published (18). Cells (10^6 per well) were allowed to adhere to a 24-well plate (Costar), and supernatant was collected at days 7 and 14. p24 antigen was assessed by using the Abbot p24 antigen quantitation kit following the manufacturer's instructions.

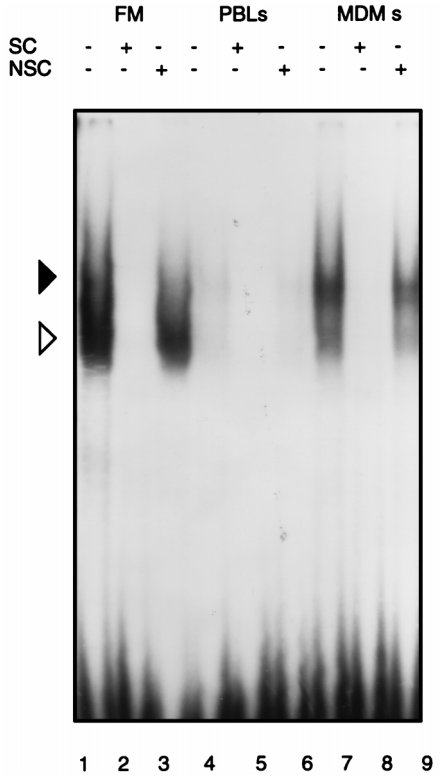

FIG. 1. Nuclear extracts from fresh monocytes (FM), PBLs, and MDMs contain distinctive NF-κB complexes (2 to 4 μg/lane). Nuclear extracts from fresh monocytes (lanes 1 to 3), PBLs (lanes 4 to 6), and MDMs (lanes 7 to 9) were analyzed by EMSA with the radiolabelled probe alone (lanes 1, 4, and 7) or in the presence (+) of a specific competitor (SC) (lanes 2, 5, and 8) or a nonspecific competitor (NSC) (lanes 3, 6, and 9). A fast-migrating complex (▷) is observed in abundance in nuclear extracts from fresh monocytes, while a slower-migrating doublet (▶) is observed in those from MDMs.

RESULTS

Freshly isolated monocytes constitutively express high levels of a fast-migrating NF-κB complex. EMSA of nuclear extracts from freshly isolated monocytes, unstimulated PBLs, and MDMs showed that specific NF-κB complexes differed in these cell populations. An abundant fast-migrating complex was observed in nuclear extracts from freshly isolated monocytes, while the predominant complex in MDM extracts migrated more slowly (Fig. 1). The appearance of the fast-migrating complex did not alter with different methods of monocyte purification, either adherence or magnetic bead negative selection (data not shown). The fast-migrating complex persisted even when nuclear extracts were prepared in the presence or absence of chymostatin (data not shown). Low levels of constitutively expressed, slow-migrating NF-κB were observed in unstimulated PBLs as previously described (12).

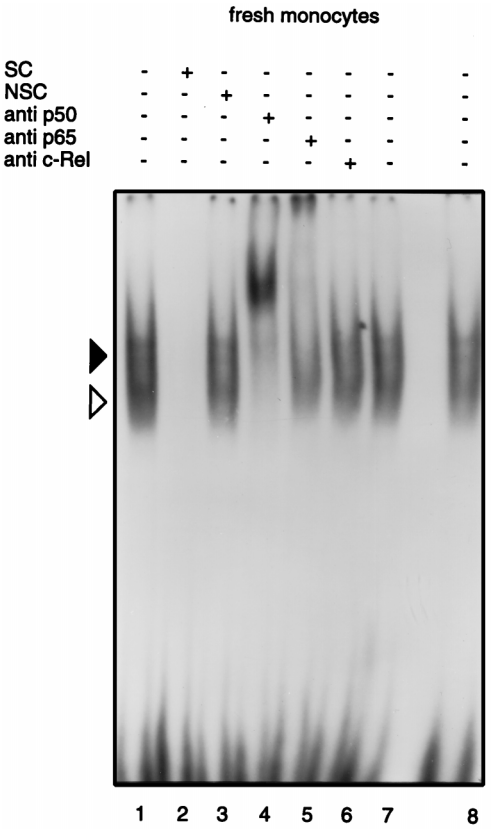

FIG. 2. EMSA and supershift analysis on nuclear extracts from fresh monocytes (2 to 4 μg/lane). Nuclear extracts were incubated with probe alone (lane 1) or in the presence (+) of a specific competitor (SC) (lane 2) or a nonspecific competitor (NSC) (lane 3). Antibodies used included antibodies to p50 (lane 4), p65 (lane 5), and c-Rel (lane 6), and control antibodies were mouse (lane 7) and rabbit (lane 8) preimmune sera. Symbols: ▶, p50/p65 heterodimer; ▷, p50 homodimer.

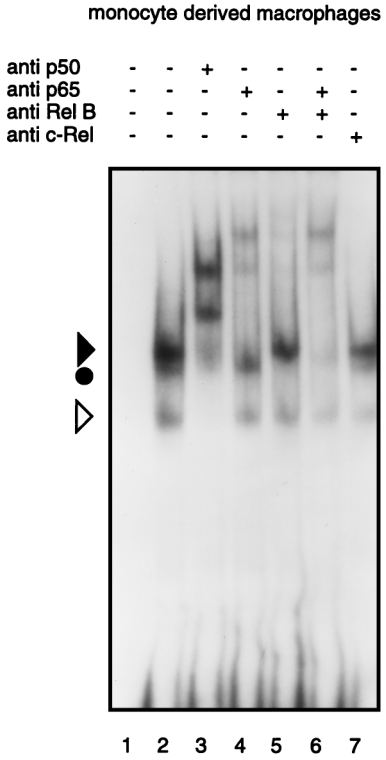

FIG. 3. Supershift analysis of nuclear extracts from MDMs (2 to 4 μg/lane). Antibodies used included antibodies to p50 (lane 3), p65 (lane 4), RelB (lane 5), p65 together with RelB (lane 6), and c-Rel (lane 7). Symbols: ▶, p50/p65 heterodimer; ●, p50/RelB heterodimer; ▷, p50 homodimer.

Freshly isolated monocytes constitutively express high levels of p50 homodimer, while MDMs constitutively express p50/p65 and p50/RelB heterodimers. The fast-migrating NF-κB complex in fresh monocytes was supershifted by antibodies to p50 but not by antibodies to p65 or c-Rel, or by mouse or rabbit preimmune sera (Fig. 2) or by antibodies to p52 or RelB (data not shown), showing that the fast-migrating complex was a p50 homodimer. Because the p65 antibody used was directed against the N terminus and failed to shift the fast-migrating complex (Fig. 2, lane 5), we conclude that this complex did not contain p65 truncated at the carboxyl-terminal region as previously reported for monocytic cell lines (6). The slower-migrating complex in fresh monocytes was a doublet. The upper band was supershifted by antibodies to p50 and p65, indicating that it consists of p50/p65 heterodimers (Fig. 2, lanes 4 and 5), while the lower band was supershifted by antibodies to p50 and RelB (data not shown). The slower-migrating complex in MDMs was a doublet, with the upper band supershifted by antibodies to p50 and p65 and the lower band supershifted by antibodies to p50 and RelB (Fig. 3). The doublet disappeared when nuclear extracts were preincubated with antibodies to both p65 and RelB (Fig. 3, lane 6).

Constitutive expression of p50 homodimer decreases with differentiation in vitro to MDMs. Nuclear extracts from monocyte cultures from the same donor were taken immediately postisolation and then following 1, 3, and 6 days in suspension culture. A progressive reduction in the relative amount of the fast-migrating p50 homodimer complex was observed with time in culture (Fig. 4). The identity of the complex was demonstrated in previous experiments by specific and nonspecific competition ($n = 6$) (Fig. 1).

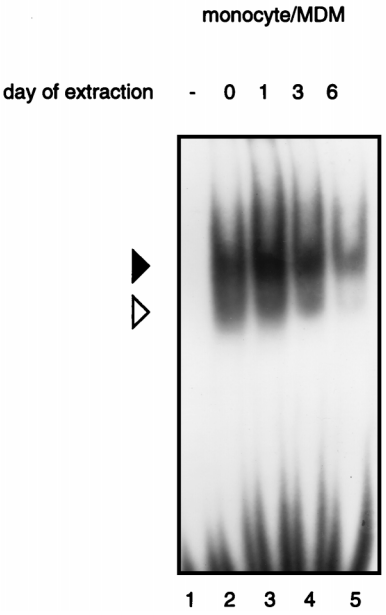

FIG. 4. Nuclear extracts from monocytes taken on the day of isolation (-) (lane 2) and following 1 (lane 3), 3 (lane 4), and 6 (lane 5) days in culture (2 to 4 μg/lane). Symbols: ▶, p50/p65 heterodimer; ▷, p50 homodimer.

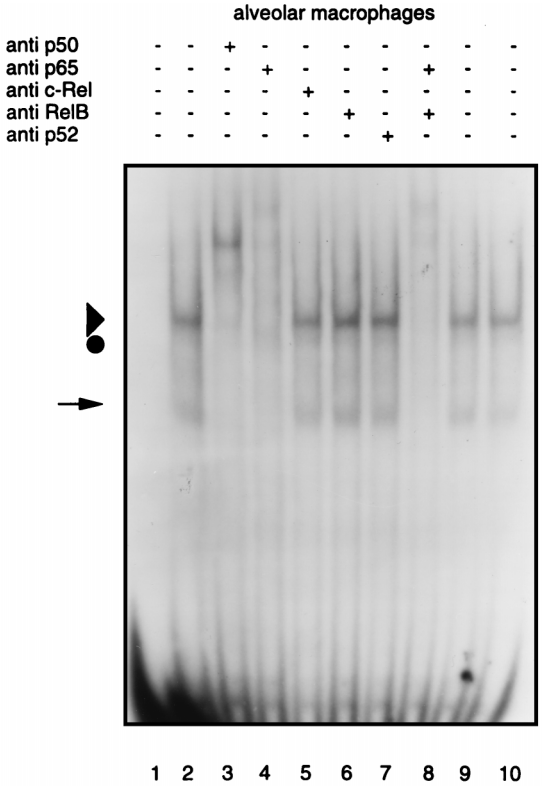

FIG. 5. Supershift analysis of nuclear extracts from AMs demonstrate the constitutive expression of p50/p65 and p50/RelB (5 to 10 μg/lane). EMSA is shown with the radiolabelled probe alone (lane 2) or in the presence (+) of antibodies to p50, p65, c-Rel, RelB, p52, or both p52 and RelB. Control antibodies included mouse (lane 9) and rabbit (lane 10) preimmune sera. Symbols: ▶, p50/p65 heterodimer; ●, p50/RelB heterodimer; ⟶, p50/p65 breakdown product.

Human AMs constitutively express low levels of p50/p65 and p50/RelB. Nuclear extracts from AMs analyzed by EMSA revealed a doublet which was specific for NF-κB as demonstrated by specific and nonspecific competition experiments using the oligonucleotides noted in the legend to Fig. 1 (data not shown). The upper band supershifted by antibodies to both p50 and p65, and the lower band supershifted by antibodies to p50 and RelB (Fig. 5). This supershift is best demonstrated when both p65 and RelB are incubated with the nuclear extract together (Fig. 5, lane 8). Low levels of expression were observed despite high levels of extracted nuclear protein (5 to 10 μg of nuclear protein per lane). A small amount of degradation of p50/p65 was observed as a fast-migrating complex (Fig. 5) that shifted with both p50 and p65. This was observed in only one of three AM nuclear extracts. Coomassie brilliant blue staining of sodium dodecyl sulfate-polyacrylamide gel electrophoresis-resolved nuclear extracts from AMs, fresh monocytes, and a differentiated monocytic cell line, RC2A, revealed a similar banding pattern without nonspecific smearing (data not shown), showing that the reduced levels of NF-κB in AM were not a consequence of a generalized protein degradation by cellular proteases occurring during nuclear extraction.

EMSA with a radioactively labelled oligonucleotide containing the Sp1 binding site showed that AMs had a binding pattern similar to that of the RC2A cell line but markedly reduced in intensity (data not shown).

In vitro infection by HIV-1-induced p50 homodimer and p50/p65 in MDMs but not in fresh monocytes. In vitro infection of MDM with the macrophage-tropic laboratory isolate HIV-1_{Ba-L} induced p50 homodimer and p50/p65 expression (Fig. 6A, right panel), as previously reported (20, 40). In contrast, in vitro infection of fresh monocytes with HIV-1_{Ba-L} and a low-passage, macrophage-tropic primary isolate HIV-1_{676} failed to induce p50/p65 (Fig. 6A, left panel). These nuclear extracts were taken following 24 h in culture, which may explain the lower relative amount of p50 homodimer seen for the fresh monocytes than that seen in previous experiments (Fig. 1 and 2). Fresh monocytes are less permissive to in vitro HIV-1

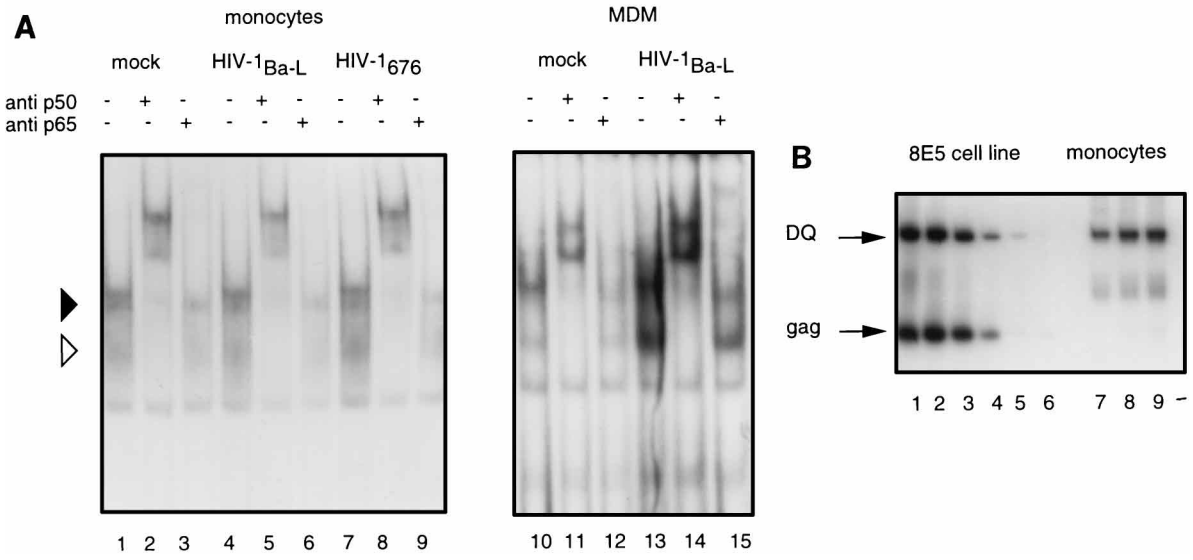

FIG. 6. (A) p50/p65 heterodimers are induced with HIV-1 infection of MDMs, but induction does not occur with HIV-1 infection of fresh monocytes. EMSA were done with fresh monocytes and MDMs incubated with mock supernatant (lanes 1 to 3 and 10 to 12) or infected with HIV-1_{Ba-L} (lanes 4 to 6 and 13 to 15) or HIV-1_{676} (lanes 7 to 9). Supershift analyses were done with antibodies to p50 (lanes 2, 5, 8, 11, and 14) and p65 (lane 3, 6, 9, 12, and 15). Symbols: ▶, p50/p65 heterodimer; ▷, p50 homodimer. (B) Autoradiograph of radiolabelled products from PCR amplification of extracts from fresh monocytes that were mock infected (lane 7) or treated with HIV-1_{Ba-L} (lane 8) or HIV-1_{676} (lane 9) and 8E5 cell extracts with the equivalent of 10^5, 10^4, 10^3, 10^2, 10^1, and 10^0 cells (lanes 1 to 6, respectively). –, control (no DNA).

infection than MDMs (39), but although no *gag* proviral DNA was seen at the time of nuclear extraction, 24 h postinfection (Fig. 6B), low-level infection of fresh monocytes was confirmed by the presence of p24 antigen in culture supernatant taken 7 days postinfection. The mean p24 level 7 days following infection with HIV-$1_{Ba\text{-}L}$ of fresh monocytes was 719 pg/ml (range, 97 to 2,094 pg/ml; $n = 5$), and following infection of MDMs, it was 4,862 pg/ml (range, 642 to 8,845 pg/ml; $n = 4$). In contrast, we found that LPS induced p50/p65 expression in fresh monocytes but not in MDMs (data not shown).

DISCUSSION

This study demonstrates that fresh monocytes, macrophages derived from monocytes by in vitro culture (MDMs) and AMs each have a distinctive pattern of constitutive expression of NF-κB. This is the first analysis of NF-κB expression in primary differentiated human macrophages, namely, in AMs. In fresh monocytes, the predominant NF-κB species expressed is p50 homodimer, while the predominant species expressed in macrophages which have differentiated in vitro (MDM) or in vivo (AM) are p50/p65 and p50/RelB. Furthermore, as fresh monocytes differentiate in vitro, there is a progressive loss of p50 homodimer, altering the balance in favor of p50/p65 and p50/RelB.

Our findings of a predominant p50 homodimer in fresh monocytes and the replacement of this homodimer by p50/p65 and p50/RelB may explain the progressive increase in susceptibility to HIV infection these cells undergo during differentiation (30, 39). The p50 homodimer has DNA binding activity but is thought, under most circumstances, to be transcriptionally inactive and possibly inhibitory to transactivation of the HIV LTR (8, 34). Although it has been demonstrated that the block in HIV replication in fresh monocytes is prior to reverse transcription (38), this block is in fact overcome when monocytes are cultured for only 24 h prior to HIV infection, and a progressive increase in permissiveness to infection is observed with subsequent time in culture (36, 38, 39). Therefore, a block to proviral transcription could further and independently modulate replication of the HIV particles that manage to enter and integrate into the host genome in monocytes cultured for 24 h or more.

Our experiments show that nuclear extracts from fresh monocytes contained chiefly p50 homodimer with small amounts of p50/p65 and p50/RelB heterodimers. It has been reported that some monocytic cell extracts contain a form of p50/p65 in which p65 has been truncated at the C terminus, giving rise to an altered heterodimer which comigrates with the p50 homodimer (6). This cleavage event evidently took place during cell extraction, as this complex did not appear if particular protease inhibitors (chymostatin, 3,4-dichloroisocoumarin, diisopropyl fluorophosphate, or alpha 1-antitrypsin) were included in the extraction buffers. The p50 homodimer we observed was clearly not the aberrant p50/p65 heterodimer described by Franzoso et al. (6). In our work, having chymostatin present during cell extraction did not eliminate the p50 homodimer. In addition, the anti-p65 we used was a rabbit polyclonal antibody raised against a peptide corresponding to amino acids 3 to 19, within the amino terminus of p65; this antibody failed to shift the fast-migrating complex. We did, however, see evidence of a truncated complex in one nuclear extract from AMs as shown in Fig. 5.

Griffin et al. (10) analyzed NF-κB in monocytes, adherent macrophages, and mouse peritoneal macrophages and found NF-κB was constitutively expressed in monocytes and macrophages. However, the age and mode of purification of the cells were not specified, and supershift assays to determine the constituents of the NF-κB complex were not performed. Suzan et al. (40) describe clear differences in the NF-κB subunits expressed in uninfected and HIV-infected monocytes and macrophages, but the nuclear extracts were prepared from cells cultured for at least 3 days, at a time when our data show a predominance of p50/p65 heterodimers. Differences in reported constitutive expression of NF-κB in primary monocytes have been attributed to mode of purification and culture conditions (33, 40). In light of the finding that expression of IκBα (MAD-3) is induced within 30 min of monocyte adherence to plastic (11), we prepared monocytes by adherence and also by immunomagnetic bead negative selection (to ensure minimal activation) and found similar levels of expression of p50 homodimer in fresh monocytes prepared either way (data not shown).

Our finding of constitutive expression of p50/p65 and p50/RelB in AMs is the first direct demonstration of NF-κB expression in primary human macrophages and is consistent with data from mouse peritoneal macrophages (10). The functional significance of NF-κB-activated transcription in the HIV life cycle is controversial. The single NF-κB site of simian immunodeficiency virus SIV_{macEm} is essential for efficient replication of SIV_{macEm} in macaque AMs but not in macaque PBLs (4). Although the NF-κB sites of HIV-1 are a critical activator of transcription in monocytic cell lines when assessed by an HIV LTR-CAT expression vector (10, 21), their importance for replication of HIV in differentiated macrophages is unknown. However, constitutive expression of p50/p65 and p50/RelB would clearly favor productive HIV replication in these cells. In fact, in vivo HIV viral load studies have shown that AMs produce infectious virus more frequently than circulating monocytes (37).

HIV infection induces sustained NF-κB binding activity in certain HIV-infected monocytic cell lines (1, 25, 29, 31, 32, 40) and in MDMs (20, 40). In contrast to monocytic cell lines and MDMs, no change in NF-κB was demonstrated following exposure of fresh monocytes to HIV. The failure of HIV to induce p50/p65 in fresh monocytes may be due simply to a block prior to reverse transcription as recently demonstrated (38). However, binding alone without entry and reverse transcription may be sufficient to induce NF-κB, since induction of NF-κB can be inhibited in $CD4^+$ T cells by CD4 ligands such as CD4 antibodies or gp120 (13). Although a previous study has shown a block to HIV infection of fresh monocytes (38), virus binding almost certainly occurs (as there is abundant surface CD4), and other groups have demonstrated entry and reverse transcription (15, 30, 41). Productive HIV infection of fresh monocytes may appear to occur if the virus inoculum is allowed to persist beyond 24 h, at which time entry and reverse transcription can occur. We have shown this by infecting fresh monocytes with HIV and then culturing them for 14 days in the presence of recombinant soluble CD4. The late rise in supernatant p24 antigen which occasionally occurs in untreated monocytes was completely inhibited by recombinant soluble CD4 (data not shown).

In conclusion, we have identified heretofore unrecognized differences in constitutive and inducible expression of NF-κB in primary monocytes and macrophages. These differences may be partially responsible for the relative permissiveness of monocytes and macrophages to HIV infection in vitro and the different transcriptional activities of these cell populations in vivo.

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