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Mycobacterium avium Complex Promotes Recruitment of Monocyte Hosts for HIV-1 and Bacteria

Hollie Hale-Donze,* Teresa Greenwell-Wild,* Diane Mizel,* T. Mark Doherty,† Delphi Chatterjee,§ Jan M. Orenstein,‡ and Sharon M. Wahl*§

In lymphoid tissues coinfectied with Mycobacterium avium complex (MAC) and HIV-1, increased viral replication has been observed. This study investigates the role of MAC in perpetuating both infections through the recruitment of monocytes as potential new hosts for bacteria and HIV-1. Increased numbers of macrophages were present in the lymph nodes of patients with dual infection as compared with lymph nodes from HIV+ patients with no known opportunistic pathogens. In a coculture system, monocyte-derived macrophages were treated with HIV-1 or M. avium and its constituents to further define the mechanism whereby MAC infection of macrophages initiates monocyte migration. Monocyte-derived macrophages treated with bacteria or bacterial products, but not HIV-1, induced a rapid 2- to 3-fold increase in recruitment of monocytes. Pretreatment of the monocytes with pertussis toxin inhibited the migration of these cells, indicating a G protein-linked pathway is necessary for induction of chemotaxis and thus suggesting the involvement of chemokines. Analysis of chemokine mRNA and protein levels from M. avium-treated cultures revealed MAC-induced increases in the expression of IL-8, macrophage-inflammatory protein (MIP)-1α, and MIP-1β with donor-dependent changes in monocyte chemotactic protein-1. Pyrrolidine dithiocarbamate, an antioxidant, inhibited the activation of NF-κB and significantly diminished the MAC-induced chemotaxis, concurrently lowering the levels of monocyte chemotactic protein-1 and MIP-1β. These data demonstrate that MAC induces macrophage production of multiple chemotactic factors via NF-κB to promote monocyte migration to sites of MAC infection. In vivo, opportunistic infection may act as a recruitment mechanism in which newly arrived monocytes serve as naive hosts for both MAC and HIV-1, thus perpetuating both infections. The Journal of Immunology, 2002, 169: 3854–3862.

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oinfection with opportunistic pathogens such as Mycobacterium avium complex (MAC)$ is a hallmark for HIV-mediated decline in immunocompetency responsible for AIDS (1–4). MAC represents a combination of several atypical mycobacteria in which M. avium dominates, and is considered an environmental pathogen based on early colonization patterns of the upper respiratory and gastrointestinal tracts before dissemination (5–7). Although rarely found in HIV-1-negative individuals, MAC infection occurs in many HIV-1-infected persons with a T cell count less than 100 CD4+ cells/mm³ and a high viral burden (5, 8). Early studies showed that reduced T cell numbers were indicative of the ability of MAC to disseminate, revealing that T cell function is needed to control the bacterial infection (8–12). Current data from clinical studies using highly active antiretroviral therapy (HAART) argue that viral burden is a better marker for potential opportunistic pathogen infections (OI) than T cell counts alone (13, 14). Recent advances in antiviral therapies that lowered the viral burden and increased the CD4 cell numbers dramatically reduced the occurrence of most OI (15–17); however, they do not eliminate lymph node (LN) MAC (18–20), nor is HAART universally available. These findings suggest that a dormant MAC infection may, upon diminished T cell function, become the impetus for launching a viral replication machine.

An increase in viral burden is often seen in sera from patients with M. avium coinfection, which correlates with increased levels of circulating TNF-α (21). The hosts’ natural defense of producing inflammatory cytokines such as TNF-α to rid the body of infection instead becomes the machinery for increased viral and bacterial infection. In LN from patients coinfected with MAC, macrophages emerge as hosts, in addition to CD4+ T cells for HIV-1 (22). Augmentation through the NF-κB pathway in both the production of inflammatory cytokines and the expression of CCR5 (the macrophage-tropic coreceptor for HIV-1) by MAC-infected or M. avium Ag (MAg)-induced macrophages provides a mechanism for increased HIV-1 replication observed in this population (23–25). Another potential device used for the amplification of viral burden during coinfection is the pronounced increase in numbers of macrophages present within these tissues that may magnify the population of virus-producing cells. Moreover, localized release of recruitment factors may alter the trafficking of cells bearing virus in addition to naive populations, further exacerbating viral replication. Understanding the underlying mechanisms whereby OI and/or inflammatory lesions (26) promote the recruitment of naive and/or infected host cells may provide strategies for disengaging this cyclic infectious process.
Materials and Methods

Tissues, in situ hybridization, and immunohistochemistry

Lymphoid tissue biopsies (LN) were obtained with consent from patients with AIDS-defining OI, HIV-1-seropositive subjects without evidence of OI, and HIV-1-seronegative donors. Tissues were fixed in 10% neutral buffered Formalin, paraffin embedded, and sectioned. In situ hybridization using 35S-labeled sense and antisense probes for HIV-1 RNA was used to assess the HIV-1+ cells in the LN, as described (22, 25). For identification of macrophages, deparaffinized sections were blocked with 1.5% normal serum and subsequently incubated with Abs against CD685 (DAKO; Carpinteria, CA). Sections were washed, incubated with secondary Abs, followed by peroxidase staining (ABC elite; Vector Laboratories, Burlingame, CA). Enzymatic visualization was achieved using 3-amin-9-ethylcarbazole (Vector Laboratories) and counterstained with methyl green. The expression of inflammatory protein (MIP)-1 fl, monocyte chemotactic protein (MCP)-1, RANTES, and MIP-1 fl (mAb; R&D Systems, Minneapolis, MN) in these tissues was detected with enhanced immunohistochemical staining using tyramide signal amplification (NEN Life Science Products, Boston, MA), followed by colorimetric detection of streptavidin peroxidase with Vector Red (Vector Laboratories), and counterstained with methyl green. Similarly, detection of HIV-1 was assessed using an Ab against p24 (DAKO) and Ag retrieval methods with tyramide signal amplification enhancement, and then visualized with diamobenzidine and hematoxylin. Quantification of positive cells in LN sections was performed using MetaMorph Imaging Software (Universal Imaging, West Chester, PA). For each LN, two independent images captured at ×20 were used to determine the average number of positive cells. Cells within two 900 × 350-μm regions per image were counted for a total of four fields for each section. The final cell numbers were defined per 7.59 × 105 μm2 (one rectangle).

M. avium complex

MAC, a virulent smooth transparent morphotype strain 2-151, was grown, as previously described (25, 27), and viable organisms were added to adherent monocytes in the chemotaxis assays or plated into 24-well plates (Costar, Cambridge, MA). Enzymatic visualization was achieved using 3-amino-9-ethylcarbazole (Vector Laboratories) and counterstained with methyl green. The expression of MAC inclusions was disrupted by three 10-s pulses with a sonicator probe. In addition (Heat Systems, Farmingdale, NY), the protein concentration was determined by bicinchoninic acid assay (28). M. avium liporabinominannam (LAM) was generated as previously described (29). In this study, LAM isolated from M. avium, strain 2-151, smooth transparent morphotype was used.

Monocytes and MDM cultures

Human peripheral blood cells, obtained by leukapheresis of healthy volunteers (Department of Transfusion Medicine, National Institutes of Health), were density sedimented, and the monocytes were purified from the mononuclear cell population by elutriation (30, 31). Freshly elutriated monocytes were resuspended in DMEM containing 2 mM L-glutamine and 50 μg/ml gentamicin (BioWhittaker, Walkersville, MD) and used as nonadherent monocytes in the chemotaxis assays or plated into 24-well plates (Costar, Cambridge, MA) at a concentration of 1 × 106 cells/well or into 100-μm petri dishes (Falcon; BD Biosciences, Franklin Lakes, NJ) at 50 × 104/dish. The plated monocytes were allowed to adhere for 3 h at 37°C with 5% CO2 before FCS (Life Technologies, Grand Island, NY) was added for a final concentration of 10%. The monocytes were differentiated into MDM by culturing for 7 days at 37°C with 5% CO2.

Treatment of MDM

After seven days, MDM culture supernatants were removed and one of the following added: fresh medium, MAC at a ratio of 5:1 or 2.5:1, 1–25 μg/ml MAg, 0.1–10 μg/ml LAM, or 1 × 105 tissue culture infectious dose 50 (BD) (HIV-1; Advanced Biotechnologies, Columbia, MD). MDM cultures were incubated for 1 h at 37°C. After the incubation, wells were washed three times with PBS, and 1 ml fresh DMEM containing gentamicin, L-glutamine, and 10% FCS was added (complete medium). In some experiments, MDM were incubated for 2 h with cycloheximide (10 μg/ml; Sigma-Aldrich; St. Louis, MO) and washed in PBS before MAg exposure. Pyrrolidine dithiocarbamate (PDT; Sigma-Aldrich), an antioxidant that has been shown to inhibit NF-κB pathways, was added from 0.6 to 60 μM to the cultures 30 min before MAg introduction (32, 33). Mitogen-activated protein kinase (MAPK) p38 inhibitor SB203580 (Calbiochem, San Diego, CA) was added at 5 μM to MDM cultures 30 min before MAg. In additional experiments, MDM were first treated with MAg for 1 h and washed, and fresh medium was added so that only newly released chemotractants would be present at the time of coculture. Neutralizing Abs against the following were then added to the MDM cultures in the lower chambers for 30 min before performing the chemotaxis assay, thus allowing time for Abs to bind to any released chemotractant before the addition of naive monocytes to the upper chambers: RANTES (1 μg/ml; BioSource International, Camarillo, CA), MCP-1, MIP-1α, MIP-1β (all 1 μg/ml; R&D Systems), IL-8 (10 μg/ml; R&D Systems), and mouse IgG1 isotype control (1 or 10 μg/ml; Vector Laboratories). Abs against MCP-1, MIP-1α, and MIP-1β were added in combination in some experiments. All experimental conditions were done in duplicate. Viability of cells after treatment was assessed using trypan blue dye.

Chemotaxis assay

Sterile 3-μm polycarbonate insert transwells (Corning, Costar) were placed into the 24-well plates that contained treated 7-day adherent MDM. Monocytes (1 × 106) that had been fluorescently labeled using Red Fluorescent Cell Linker kit (Sigma-Aldrich) were then added into the top of the transwells and cocultured for 1 h at 37°C. The transwells were removed, and migrated cells were viewed and quantified using an inverted fluorescence microscope (Olympus, Melville, NY). Following photomicrography, the supernatants were collected for further analysis. For some experiments, the monocytes were pretreated for 1 h with 10 ng/ml pertussis toxin (Biomol Research Laboratories, Plymouth Meeting, PA) or MAC, or 30 min with PDTC (60 μM) or SB203580 (5 μM) before fluorescent labeling. All experimental conditions were performed in duplicate.

To determine chemotaxis due to the stimuli themselves, 0.4 ml medium containing MAC (5:1), and MAC (25 μg/ml) was added to the bottom of the transwell system. MCP-1 (NCI Biological Resources Branch, Frederick, MD) or FMLP (Sigma-Aldrich; 2 nM) was used as the positive control, and medium alone as a negative control. Monocytes were resuspended at 3 × 105 in chemotaxis buffer (1% HBSS with calcium and magnesium and 0.5% BSA) and placed in the upper transwell before the 90-min incubation at 37°C. The transwells were removed, and 40 μl 10× chemotaxis fixative (PBS containing 100 mM EDTA plus 10% formalde-hyde) was added to the lower chamber; the cells were collected and counted using a Corixa (Seattle, WA) counter.

Emsa

MDM cultured for 7 days in 10 × 20-mm petri dishes (50 × 105/dish) were treated with PTDc, MAg, and PTDc+MAC, SB203580, SB with MAC, or medium, as described earlier. Nuclear extracts were prepared and the EMSA run using a radiolabeled NF-κB consensus oligonucleotide probe (Promega, Madison, WI), as described previously (25). The binding reactions were run on non-denaturing 6% polyacrylamide gels (Novex, San Diego, CA) in 0.25× Tris borate EDTA buffer, dried, and analyzed with a PhosphorImager using IMAGEQUANT software (Molecular Dynamics, Sunnyvale, CA).

Rnase protection assay (RPA)

Total cellular RNA was extracted using the RNeasy minikit (Qiagen, Chatsworth, CA) from 6 × 105 cells/well of control or treated MDM. Three micrograms of total RNA were used with the hCK-5 template of the BD PharMingen Riboquant MultiProbe RPA system (San Diego, CA) and developed using phosphor imaging. Band densities were normalized to GAPDH housekeeping gene using IMAGEQUANT software (Molecular Dynamics).

Cytokine/chemokine ELISA

The supernatants from the 7-day MDM cultures after 1-h MAC treatment were analyzed for IL-8, MIP-1α, MIP-1β, RANTES, TGF-β, MCP-3 (BioSource International), and MIP-1β (R&D Systems) production by ELISA. Additionally, supernatants were analyzed for MIP-1α, MCP-1, and MIP-1β after 15, 30, 60, or 120 min of MAC, LAM, or MAg exposure.

Statistical analysis

Nonparametric statistical analysis was determined by the two-tailed, Mann-Whitney t test at 95% confidence level.

Results

MAC-induced cell migration

By immunohistochemical analysis, dramatically increased numbers of CD685 macrophages were evident within LN from patients coinfected with MAC and HIV-1 as compared with those HIV-1 infected with no OI or with neither HIV-1 nor OI (Fig. 1, A–D).

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Consistent with this finding was the increased presence of HIV-1-infected macrophages in the MAC tissues and around the high endothelial venules within these coinfected LN as compared with the LN from the HIV-1/H11001 donor with no OI (Fig. 1, E–H) (22, 25), suggesting an active process of adhesion and recruitment. Because at the time of OI infection there is diminished T cell function, we focused on the role of macrophages in enlisting new hosts to carry out viral replication. To ascertain whether M. avium induces migration of circulating monocytes into the LN, chemotaxis studies were performed (Fig. 2A). In the coculture system, macrophages that were treated with MAC for 1 h before the chemotaxis assay released a soluble factor within the subsequent 60 min of the assay that induced nonadherent monocytes to migrate an average of 4-fold over control MDM (Fig. 2B). The presence of migrating monocytes in the control wells in this dynamic system is consistent with the fact that macrophages may constitutively express low levels of some chemoattractants (Fig. 2A) (34, 35). The MAC-induced response did not significantly increase in the subsequent 4 h from that observed after MDM were stimulated for 2 h; however, there was a significant difference with MAC infection after 8 h (data not shown), presumably due to continued MAC infection and replication (T. Greenwell-Wild, unpublished observations), and/or release of de novo synthesized recruitment factors. Because of the rapid response to MAC, we focused on this initial generation of chemotactic activity.

To determine whether M. avium viability and/or infection are required to induce chemotactant production in MDM, bacterial lysates were used to treat the MDM. A 2- to 3-fold increase in...
chemotaxis occurred within 1 h in the MAg-treated cultures (Fig. 2B), suggesting that bacterial components could initiate the rapid MDM response. Based on the dose-response curves, 1 μg/ml lysate was sufficient to induce the secretion of chemotactic factors (Fig. 2C), but higher doses (25 μg/ml) provided consistent stimulation in all donors. Purified LAM, a cell wall lipoglycan of MAC with known immunomodulatory effects (reviewed in Ref. 29), was assessed for its ability to induce the MDM production of chemotactants. Monocytic migration into the LAM-treated MDM wells was on average 2-fold greater than control MDM (Fig. 2B), with induction of activity at 0.1 μg/ml and optimal at 10 μg/ml (Fig. 2C). Exposure of macrophages to a macrophage-tropic strain of HIV-1, Bal, did not induce chemotaxis above control levels within this 60-min time interval (Fig. 2B). The question of whether MAC or MAg was directly chemotactic or induced a chemotactic factor(s) was addressed by making dilutions of intact bacteria or bacterial Ags in chemotaxis buffer and adding these dilutions in the absence of MDM into the bottom transwell, and monocyte migration was measured. Neither induced chemotaxis independently (data not shown), indicating that MAC initiates signaling in MDM to release a chemotactant factor(s). Because MAg was as effective as viable M. avium in inducing the MDM to produce chemotactic activity, subsequent experiments used MAg for defining the recruitment factors.

To further define the mechanism whereby M. avium triggers the release of chemoattractants, cycloheximide, an inhibitor of protein translation, was added before MAg in the MDM cultures. This inhibitor decreased the chemotactic activity, implying that the M. avium-stimulated chemotactant(s) released from MDM is at least in part synthesized de novo (Fig. 3A). Because MAg activates NF-κB pathways (25), an antioxidant that has been shown to inhibit this pathway, PDTC (32), was incubated with the MDM before MAg activation. Although PDTC did not completely eliminate the response, it caused a significant dose-dependent reduction (Fig. 3A, 60 μM shown) in the MAg-mediated production of chemotactic molecules, which corresponded with the reduced MAg activation of NF-κB (Fig. 3B, 60 μM shown). The possibility that activation of p38 MAPK signal transduction induced chemotactic factors that may be independent of NF-κB binding was also assessed (36, 37). Addition of SB203580, an inhibitor of p38 MAPK, to the cultures did not significantly decrease MAg-induced chemotaxis nor influence NF-κB (Fig. 3, A and B). These data provide evidence that in macrophages, activation of the NF-κB pathway and subsequent cellular events are involved in the regulation of rapid MAg-induced MDM chemotactic production.

Analysis of MAC-induced chemotactic factor(s)

To define the molecules involved in recruitment, the migrating monocytes were pretreated with pertussis toxin, an inhibitor of G protein-mediated pathways. The pertussis toxin-treated monocytes failed to migrate in response to the MAg-induced chemotaxant, suggesting that the receptor mediating this response is pertussis toxin sensitive (Fig. 3C), and thus, pointing to a chemokine receptor (35). Surprisingly, treatment of monocytes with PDTC blocked both the MAg-induced, as well as the background chemotaxis, indicating that the NF-κB pathway may be involved in monocyte movement in this system. The p38 MAPK inhibitor also reduced monocytic chemotaxis, albeit to a lesser extent, thus implicating several pathways in the monocytic response to the soluble chemotactant(s) generated by MAg activation of macrophages.

The production of several members of the chemokine family, including MCP-1, RANTES, and MIP-1α, is known to occur rapidly, and their production is inhibited by cycloheximide (reviewed in Refs. 34, 35, and 38). Additionally, the receptors for these chemokines are sensitive to pertussis toxin (34, 35, 38), which inhibited the monocytic response to the MDM-derived factor. Because the data suggested a role for G protein-mediated signaling, MAg-stimulated MDM mRNA was analyzed by RPA using probes for known chemokines. RNA analysis indicated that MAg up-regulated mRNA for MIP-1α, MIP-1β, and IL-8 (Fig. 4A) from 5- to 76-fold compared with the control during the first 3 h. Similar results were also obtained with LAM treatment of MDM (Fig. 4A, lower panel). Increases in RANTES and MCP-1 mRNA were donor dependent (Fig. 4), being highly up-regulated in some donors, but not induced in others.

![FIGURE 3](http://www.jimmunol.org/)

Effect of signaling inhibitors on MAg-induced chemotaxis. A, MDM were pretreated with cycloheximide (10 μg/ml) for 2 h, washed extensively with PBS, and treated with MAg (1 h after washing); fresh medium was added, and the chemotaxis assay was performed. Data are expressed as the mean of three independent studies ± SEM. MDM were also pretreated with pertussis toxin (PT, 10 ng/ml), or SB302508 (SB, 5 μM) before the addition of MAg. Data are expressed as the mean of five independent studies ± SEM, *p ≤ 0.05; **, p ≤ 0.001; ***, p ≤ 0.0001. B, To assess NF-κB activation by MAg (25 μg/ml), EMSA was performed on nuclear extracts of MDM-treated cells. Activation of NF-κB was evidenced by increases in the p65/50 band. MAg-induced NF-κB activation was inhibited by PDTC treatment (60 μM), but not SB302508 (5 μM). C, In addition to treating the macrophages in the bottom wells as described in A with cellular inhibitors, nonadherent monocytes were pretreated for either 60 min with pertussis toxin (PT, 10 ng/ml), or 30 min with SB302508 (SB, 5 μM) or PDTC (60 μM) before being fluorescently labeled and washed thoroughly. The treated monocytes were added to the upper chamber of wells of MDM treated with MAg (25 μg/ml). Data are expressed as the mean of two independent studies SEM, and represent the ratio of the number of labeled monocytes that migrated in the treated wells to that in the control wells.
Supernatants from the chemotaxis assays were analyzed for chemokine protein levels by ELISA. Consistent with the mRNA analysis, elevated MIP-1α, MIP-1β, and IL-8 proteins were measured in the MDM treated with MAg supernatants (Fig. 4B). The increase for MIP-1α averaged 18.3 ± 2.3-fold in eight independent experiments, MIP-1β from 11.9 ± 4.5-fold (n = 10), and IL-8 from 7.1 ± 3.77-fold (n = 8) over controls. Similar to the RPA data, MCP-1 levels varied with donor, dependent in part on the constitutive levels of expression. Neither RANTES protein nor mRNA levels were typically elevated and did not significantly increase above the control in this 2-h assay. MCP-3 and TGF-β were not detected in control supernatants or after MAg stimulation. To determine the kinetics of the rapid expression of MCP-1, MIP-1α, or MIP-1β after MAC, LAM, or MAg treatment, supernatants were analyzed after 15-, 30-, 60-, and 120-min intervals. MCP-1 levels were present in the control supernatants at 15 min, and these levels did not change significantly after treatment at these early time points (data not shown). Detectable levels of MIP-1α and MIP-1β (Fig. 4C) were observed only after 60 min of exposure with MAC, LAM, or MAg. These results suggest that MCP-1 may be preformed and ready to ignite early migratory events. However, MIP-1α and MIP-1β appear to be made rapidly in response to MAC or its components. Taken together, results from the neutralizing Ab and the kinetic studies suggest that these multiple chemokines may work interdependently to recruit monocytes.

NF-κB-dependent chemokine production

Because several chemokines and cytokines, including MCP-1, are regulated through NF-κB (34, 35), and inhibition of the NF-κB pathway decreased MAg-stimulated MDM recruitment of monocytes, we evaluated the supernatants from the PDTC-treated MDM for chemokine protein expression (Fig. 5). The decrease seen in the levels of MIP-1β and MCP-1, but not MIP-1α after PDTC treatment corresponded to the levels of functional chemotactic activity (Fig. 3). Moreover, when neutralizing Abs against MIP-1α, MIP-1β, IL-8, MCP-1, RANTES, and an irrelevant isotype control were preincubated with the MAg-treated MDM before the chemotaxis assay was performed, anti-MIP-1β and anti-MCP-1, but not anti-RANTES, anti-IL-8, anti-MIP-1α, or the isotype control, significantly inhibited the MAg-induced chemotaxis (Fig. 5 and data not shown).
mRNA, rapid transcription, translation, and secretion of multiple cytokines (21, 25, 29, 39–44), investigations into chemokines and recruitment are limited (45).

Knowledge concerning cell migration and chemokine production has been primarily derived from work with the more virulent Mycobacterium tuberculosis (46–50). However, due to the innate differences in the infection processes and virulence of Mycobacterium spp., as well as differences in receptor use (51), cytokine and chemokine induction may not be comparable (52, 53). Previous reports indicate that M. tuberculosis can induce neutrophil, eosinophil, monocyte, and lymphocyte migration (46, 48, 49, 52), as well as chemokine up-regulation in infected monocytes/macrophages (47, 49, 50). Mycobacterial infections have been shown to activate NF-κB via Toll-like receptors, resulting in the enhanced expression of TNF-α, CCR5, and IL-8 (25, 54–57). In this investigation, we report that MAC or its cellular constituents quickly activate NF-κB and the induction of several chemokines. We observed significant increases in the production of multiple chemokines, including MIP-1α, MIP-1β, IL-8, and MCP-1. At least two chemokines investigated, MIP-1β and MCP-1, appear to coordinate promote the recruitment of blood monocytes both in vitro and in vivo, consistent with the striking accumulation of mononuclear phagocytes in the MAC-infected tissues.

These data are compatible with signaling via Toll-like receptor 2, a recently identified mycobacterial receptor (51, 58), which can lead to NF-κB signaling (56). We demonstrate that the activation of NF-κB pathway by MAC is required for the induction of recruitment factors, including MCP-1 and MIP-1β, and plays a role in the responding monocytes. The p38 MAPK, however, appears not essential for induction of chemotactic factors, but may be involved in the signaling pathways induced in the responding monocytes. It is conceivable that the activation or cross talk of multiple signaling pathways, including those involving p38 MAPK, occurs due to the complexity of chemokines and other mediators in initiating this cellular migration.

Previous reports suggest that HIV-1 induces both T cells and monocytes to migrate in response to viral infection or Ags, including Nef, gp120, and Tat (59–64). In our system, HIV-1 binding and early entry events do not trigger MDM to produce detectable chemotactic factors within 60 min. However, from in vitro kinetic assays of the HIV-1-induced chemotactic response, we found that
as HIV-1 begins to replicate (as evidenced by p24 levels), chemotaxis to HIV-1-infected MDM occurs (64, 65). Kinetic analyses also revealed that MAC replication and reinfection of macrophages sustained the production of elevated chemotactic factors, including an increase in MCP-1 production (H. Hale-Donze, unpublished data), consistent with in vivo observations (Fig. 6). These findings would indicate that dual infection, in vitro and in vivo, maintains the production of a chemoattractant gradient over the course of infection.

That neutralizing individual chemokines significantly affected the MAC-induced chemotaxis, even in the presence of other chemokines that can themselves elicit monocyte migration, is consistent with studies in the MCP-1-deficient mouse. Even in the presence of other inducible chemokines, monocyte recruitment to sites of infection was impaired in these mice (66). Despite the modest detection of MAC-induced MCP-1 protein expression above the constitutive expression, Abs against this chemokine markedly reduced the MAC-elicited chemotaxis. Potentially, complex formation or structural alterations of MCP-1 may render it undetectable by ELISA. Posttranslational modifications in MCP-1, which occur naturally, can influence its chemotactic potency (67), because glycosylated MCP-1 was shown to be less chemotactic than nonglycosylated forms, whereas NH2-terminally truncated forms lost bioactivity (68). Less severe modifications to the NH2-terminal domains cannot only change the chemopotency, but also alter the effector cell population (69). Taken together, posttranslational regulation of MCP-1 may be as critical as increased production for inflammatory responses.

Our findings implicate an essential role for multiple factors in a systematic pathway to set up chemotactic gradients for the recruitment of monocytes into sites of inflammation. Some of these factors may act directly as chemokines; others may act indirectly to cause the release of additional chemokines, recruitment of other cell types, or, in the case of mycobacterial infections, the induction of granulomas. For instance, induction of IL-8 and MIP-1β in Mycobacterium infections has been postulated to control the recruitment of T cells and neutrophils involved in granuloma formation (49, 70–72). MIP-1α and IL-8 levels were also reportedly
elevated in adherent blood monocyte cultures after infection with *M. tuberculosis*; however, neutralizing Abs against these chemokines did not significantly reduce the migration of monocytes (49). In addition, elevated MIP-1α levels were not detected in bronchoalveolar lavage fluid from patients with active pulmonary tuberculosis (49), nor did we find increased expression in our MAC LN. These findings indicate that MIP-1α may be less critical in recruitment of monocytes and/or function in other processes of *Mycobacterium* infection. In this regard, IL-8 along with MCP-1 were recently found to induce firm adhesion of monocytes to vascular epithelium, indicating that IL-8 may indeed be important for monocytosis extravasation from the circulation into sites of inflammation (73).

The coculture chemotaxis system has allowed the initial dissection of the complex mechanism involved in recruitment of monocytes by infected macrophages. Multiple factors, including MIP-1α, MIP-1β, MCP-1, RANTES, IL-8, and TNF-α, as well as other yet undefined components, are likely to interact in blood monocyte recruitment. These results have clearly demonstrated the need to further define the interactions of multiple factors that initiate monocyte chemotaxis to understand potential interventions that would prevent the spread of viral or bacterial infections.

*M. avium* infection has been an OI that plagued patients with HIV-1 before the advent of HAART (74). Clinical data suggest that T cell function together with phagocytic cells are needed to control MAC and other OI infections (8–12, 15). Unfortunately, HAART, which often partially restores T cell function in infected individuals, is not taken or accessible to all HIV-1 patients throughout the world; therefore, the threat from this pathogen still persists for these and other immunocompromised individuals (75). In the later stages of HIV-1 infection, when T cell function is diminished, the composition of immunoregulatory cells is dramatically changed and OIs including MAC become a serious health risk (74). Macrophages are the primary target for infection and replication of *M. avium* and become an additional reservoir for HIV-1 (22, 23, 25). Understanding the impact of the bacteria on this cell population is fundamental to developing strategies of intervention.

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**References**


