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_J Immunol_ 1999; 162:400-406; ;
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Activation-Induced Resistance of Human Macrophages to HIV-1 Infection In Vitro

Gabriele Zybarth, Norbert Reiling, Helena Schmidtmaierova, Barbara Sherry, and Michael Bukrinsky

Cells of the monocyte/macrophage lineage are the first targets of HIV-1 in patients and also serve as reservoirs for the virus during the course of infection. We investigated the effects of cell activation on early events of HIV-1 infection of monocyte-derived macrophages. Addition of LPS, a potent stimulator of macrophages, at the time of infection stimulated entry of HIV-1 into monocyte-derived macrophages, as judged by accumulation of early products of RT, but inhibited the synthesis of late RT products and strongly repressed nuclear import of the viral DNA, resulting in protection from infection. This effect was mediated by the CD14 receptor and involved activation of the p38 mitogen-activated protein kinase pathway. Disruption of this signaling pathway using a specific inhibitor of the p38 mitogen-activated protein kinase (SB203580) restored HIV-1 infection in the presence of LPS. These results suggest a novel view of the role of macrophage activation in anti-HIV responses of the immune system. The Journal of Immunology, 1999, 162: 400–406.

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Received for publication May 14, 1998. Accepted for publication September 21, 1998.

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1 This work was supported in part by National Institutes of Health Grant R01AI38245 (to M.B.), funds from The Picower Institute, and Grant RE 1228/2-1 from the Deutscher Forschungsgemeinschaft (to N.R.).

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3 Abbreviations used in this paper: MAPK, mitogen-activated protein kinase; PIC, preintegration complex; MDM, monocyte-derived macrophage; LTR, long terminal repeat.

Materials and Methods

Reagents

HIV-1Ada was obtained from Dr. H. E. Gendelman, Department of Pathology and Microbiology, University of Nebraska Medical Center (Omaha, NE) (34). HIV-1 primary isolates 92US660, 92US657, and 92UG021 were obtained from the AIDS Research and Reference Reagent Program (Rockville, MD). LPS (Escherichia coli 011:B4), lipid A (Salmonella minnesota RE595), and polymyxin B sulfate were obtained from Sigma (St. Louis, MO). Compound 406 was a gift from Dr. A. J. Ulmer, Department of Immunology and Cell Biology, Research Center Borstel (Borstel, Germany). Anti-CD14 (MEM18) was purchased from Sanbio (AM Uden, The Netherlands). SB203580 and SB202474 were obtained from Alexis...
and Calbiochem (both in San Diego, CA). CNI-1493 was provided by Dr. K. J. Tracey, The Picower Institute for Medical Research (Manhasset, NY).

Isolation and differentiation of human MDMs

Human PBMC were isolated from buffy coats of healthy seronegative donors (Long Island Blood Services, Melville, NY) by Ficoll density gradient centrifugation (Ficoll-Paque PLUS, Pharmacia Biotech, Piscataway, NJ). PBMC were cultured in DMEM supplemented with 10% heat-inactivated human serum (BioWhittaker, Walkersville, MD), 2 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin (all from Life Technologies, Gaithersburg, MD) at 37°C in 5% CO₂. After 18 h, adherent cells were detached with ice-cold 10 mM EDTA/PBS and cultured in 24-well Primaria plates (Becton Dickinson, Franklin Lakes, NJ) for 7 days in the presence of recombinant human macrophage CSF (2 ng/ml; Sigma) at a density of 10⁶ cells/ml. Cells were >98% macrophages as judged by morphology and nonspecific esterase activity.

Infection with HIV-1

The macrophage-tropic strain HIV-1ADA was grown on PBMCs. Before infection, the virus was pretreated with RNase-free DNase (Boehringer Mannheim, Indianapolis, IN) for 1 h at room temperature, and then filtered through a 0.45-μm nitrocellulose membrane. Seven days after isolation, adherent macrophages were infected for 2 h at 37°C with an amount of virus corresponding to 1 × 10⁶ cpm reverse transcriptase activity/1 × 10⁶ cells. After infection, unabsorbed virus was washed away, and cells were cultured in the medium without macrophage CSF.

DNA PCR

Infected macrophages (10⁶ cells) were resuspended in 200 μl of 1× PCR buffer (50 mM KCl, 10 mM Tris-Cl (pH 8.3), 3.5 mM MgCl₂, and 0.1% Nonidet P-40, 0.45% Tween 20, and proteinase K (500 μg/ml; Boehringer Mannheim). Samples were digested overnight at 56°C, and proteinase K was inactivated at 95°C for 10 min. For each PCR replicate, 25 μl of sample was added to 25 μl of PCR mix containing 2.5 μl of 10× PCR buffer, 1 μl each of sense and antisense primers (from a 10-μM stock), 1 μl of 10 mM deoxynucleotide triphosphates (Life Technologies), 19.25 μl of H₂O, and 1.25 U of Taq polymerase (Perkin-Elmer, Norwalk, CT). The primers used in this study have been described recently (35).

Results

Inhibition of preintegration steps of HIV-1 replication by LPS

Several previous reports demonstrated that LPS inhibits replication of HIV-1 in long term cultures of MDMs (29–32). Consistent with these results, we observed that virus replication, as measured by the amount of viral reverse transcriptase activity released into the culture medium, was blocked when LPS was added to the cells together with the virus (Fig. 1A). To evaluate the effect of LPS on preintegration steps of HIV-1 replication, we used PCR analysis of virus-specific DNA. Monocytes from healthy, HIV-seronegative blood donors were differentiated into macrophages in vitro by adherence to plastic (34). After 7 days in culture, MDMs were infected with HIV-1ADA in the absence or the presence of increasing concentrations of LPS (0.01–10 ng/ml) for 2 h (Fig. 1B). MDMs were harvested 48 h postinfection, which is the time required to complete RT and nuclear import of the viral PICs in MDMs (36), and 2LTR circle forms of the viral DNA were amplified by PCR. These DNA forms are produced exclusively inside the nucleus after completion of RT and nuclear import (37) and are convenient markers in the analysis of preintegration steps of HIV-1 replication (38). As a control for the amount of DNA in each sample, PCR using tubulin-specific primers was performed in parallel on each sample (not shown). Results were scored in the linear phase of amplification, as demonstrated by PCR performed on serial dilutions of HIV-1-infected H9 cells (Fig. 1B, upper panel). After Southern blotting, the products of the PCR reaction were revealed by autoradiography (Fig. 1B, upper panel) and quantified on an Instant Imager (bar graph). As shown in Fig. 1B, LPS inhibited the appearance of the 2LTR circle forms of HIV-1 DNA in a dose-dependent manner. Maximal inhibition (80–90%) was typically observed at LPS concentrations from 1–10 ng/ml, in good correlation with the results obtained with long term cultures (Fig. 1A).

The results varied to some extent between different donors. Even at the low concentration (10 pg/ml), LPS consistently reduced the amounts of viral 2LTR circle DNA forms by >30%. This effect was not specific for the strain HIV-1ADA, as the same inhibition was observed with primary NSI isolates 92US660 and 92US657 (not shown). Because the sensitivity to the inhibitory effect of LPS was donor dependent, a concentration of 10 ng/ml was used in all additional experiments to ensure maximal inhibition. To control...
for possible cytotoxic effects due to simultaneous exposure of macrophages to both LPS and HIV-1, we used the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. No difference in the viability of HIV-1-infected MDMs with or without LPS was observed.


differential effects of LPS on the early and late products of HIV-1 RT

To define the effect of LPS in more detail, we followed the fate of the entering virus using PCR primers specific for the early, intermediate, and late products of viral RT and for the intranuclear forms of viral DNA, thus monitoring the stages from viral entry to nuclear import. LPS was added to cells either together with the virus or 2 h after infection. For the analysis of early RT products, cells were harvested 6 h postinfection, and viral DNA was analyzed with primers (U3/R) specific for the strong stop HIV-1 cDNA that is produced early in infection cycle (35, 39). Regardless of whether LPS was present only during infection or was added 2 h after infection, a dramatic, more than threefold, increase in the amount of early RT products was observed (Fig. 2A). LPS-enhanced HIV-1 entry was still dependent on virus interaction with receptors, as Ab to CD4 blocked entry into both unactivated and activated macrophages (not shown). In addition, treatments that down-regulate HIV receptors (e.g., pretreatment of macrophages with RANTES or LPS) also blocked HIV-1 entry (G. Franchin, B. Sherry, and M. B., manuscript in preparation). These results suggest that LPS promotes entry of the receptor-bound virus into human macrophages.

Analysis of later RT products was performed 18 h after infection with primers specific for the HIV-1 pol gene. Surprisingly, there was a marked reduction (~70%) of pol-specific PCR products in all LPS-treated samples (Fig. 2B). An even stronger inhibition (up to 90%) was observed at the level of nuclear HIV-1 DNA, detected 48 h after infection using primers specific for the 2LTR circle DNA (Fig. 2C). Taken together, these results suggest that addition of LPS during or immediately after infection stimulates virus entry, but strongly inhibits RT and nuclear import of the viral PIC.

Disruption of the activation pathway restores HIV-1 infection of LPS-treated macrophages

The inhibitory effect of LPS on HIV-1 infection was probably mediated by LPS-induced signaling events. LPS initiates intracellular signaling through the CD14 molecule, a glycosylphosphatidylinositol-anchored membrane protein of myeloid cells (40). Although other surface proteins have been suggested to function as LPS receptors, CD14 is the only protein of fully defined structure that binds LPS and mediates LPS-induced cell activation. To investigate the role of CD14-mediated LPS signaling in the inhibition of HIV-1 infection of MDMs, we used inhibitors of LPS-CD14 interaction. Compound 406, also called precursor Ia or lipid IVa, is a precursor in the synthesis of lipid A and has been shown to bind to CD14. In contrast to lipid A, compound 406 has no biologic activity, but effectively inhibits the effects of LPS, such as induction of cytokine production in human monocytes (41). Preincubation of MDMs with compound 406 for 30 min before the simultaneous addition of LPS (10 ng/ml) and HIV-1ADA completely eliminated the inhibitory effect of LPS on HIV-1 infection, as measured by 2LTR circle-specific PCR (Fig. 3A). As a control, compound 406 alone had no effect on infection. A similar result was obtained with a mAb MEM-18 directed against CD14 (Fig. 3B). This Ab has been shown to specifically block LPS binding and signaling (42). Together, these results suggest that CD14-mediated signal
transduction events regulate the LPS-mediated effect on HIV-1 infection of human macrophages.

One of the major signal transduction pathways activated by LPS is p38 MAPK-dependent signaling (14, 43). To determine its role in protection of macrophages from HIV-1 infection, we used compound SB203580, a highly specific inhibitor of p38 MAP kinase (16). Pretreatment of MDMs with this compound led to a reversal of LPS-mediated inhibition of viral 2LTR circle DNA formation (Fig. 4A). Interestingly, the inhibitor alone had a weak stimulatory effect on HIV-1 infection (see below). Cell viability in the presence of the inhibitor (used at 1 μM) was measured by trypan blue exclusion and by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay and was 95% of the viability of control (untreated) cultures. In addition, the fact that cells treated with the compound supported higher levels of virus replication than untreated cells attests to their high metabolic activity and viability.

A small multivalent guanylhydrazone compound, CNI-1493, has been shown recently to suppress activation of macrophages (44) by a mechanism involving inhibition of the MAPK pathway (45). Incubation with CNI-1493 (1 μg/ml) resulted in a complete reversal of LPS-mediated inhibition of HIV-1 infection (Fig. 4B). Cell viability was 93% that of control cells. Taken together, these results indicate that activation of macrophages, and of the p38 MAPK pathway in particular, is critical in mediating the inhibitory effect of LPS on the preintegration steps of HIV-1 infection of human macrophages.

Inhibition of p38 MAP kinase stimulates replication of HIV-1 in human macrophages

Since it has been reported that compound SB203580 blocks transcriptional activation of the HIV-1 LTR by cytokines and stress (46), while we observed a stimulatory effect on the preintegration steps of viral infection (Fig. 2A), we investigated the cumulative effect of this compound on HIV-1 replication in MDMs. Macrophages were infected with HIV-1ADA or HIV-1 92UG021 (a primary SI isolate) in the presence of SB203580 or its inactive control, SB202474. The amount of the virus released into the medium was determined by reverse transcriptase activity in culture supernatants. Figure 5 shows that SB203580 stimulated replication of both virus isolates (HIV-1ADA by more than twofold and HIV-1 92UG021 by about fivefold). A similar stimulation of virus replication was observed with the compound CNI-1493 or when LPS and inhibitor were added together (data not shown). These results suggest that, in contrast to infection of primary T cells (45), inhibition of p38 MAPK (and of macrophage activation) enhances virus replication, most likely through stimulation of virus entry.

Discussion

The results reported in this paper demonstrate that activation of macrophages, in particular via the p38 MAPK-dependent pathway, induces resistance to HIV-1 infection. This appears to be in contrast to the situation in T lymphocytes, where inactivation of p38

FIGURE 3. Compound 406 or anti-CD14 mAb restores HIV-1 infection in the presence of LPS. MDMs were preincubated for 30 min with 1 μg/ml of compound 406 (A) or 5 μg/ml of anti-CD14 mAb MEM 18 (B), and then infected with HIV-1ADA in the presence of LPS (10 ng/ml) and compound 406 or Ab, respectively. The experiment was quantified as described in Fig. 1. Shown are the results of one representative experiment of three performed with cells from different donors.

FIGURE 4. Disruption of the p38 MAPK signal transduction pathway reverses the inhibitory effect of LPS. MDMs were preincubated for 30 min with 1 μM SB203580 (A) or 1 μg/ml of CNI-1493 (B), and then infected with HIV-1ADA in the presence of LPS (10 ng/ml) and compound. The experiment was quantified as described in Fig. 1.

FIGURE 5. Inhibition of p38 MAP kinase stimulates the replication of HIV-1 in human MDMs. MDMs from the same donor were infected with virus isolates of HIV-1ADA (left graph) or HIV-1 92U6921 (right graph) either with no drug added (solid bars labeled a) or in the presence of 1 μM SB203580 (bars with light hatching labeled c) or its inactive control, SB202474 (bars with dark hatching labeled b). Every 3 days, half of the culture medium was replaced with fresh medium containing inhibitor or control. Virus replication was followed by RT activity in the supernatants and was expressed as counts per minute. Each bar represents the mean activity from two replicates. Shown are the results of one representative experiment of three performed with cells from different donors.
MAPK was shown to suppress HIV-1 infection (45, 46), and underscores differences between these two major targets of HIV-1. While cell activation is required for productive viral infection of T lymphocytes (39, 47, 48), our data and those of others demonstrate that the opposite is true for primary macrophages (49, 50).

A recently published report (51) demonstrated that inhibition of p38 reduces IL-1β-induced HIV-1 production by promonocytic cell line U1. This seeming conflict with our results is probably explained by the fact that the U1 cell line represents undifferentiated monocytes that contain two copies of integrated defective HIV-1 genome. Therefore, early (preintegration) steps of viral infection cannot be studied on these cells. As the inhibitory effect of LPS on HIV-1 replication (mediated, at least in part, by p38) occurs at the step of virus entry, it conceals any stimulatory effect of p38 activation at the later step. In addition, transcription of the wild-type HIV-1 genome in primary macrophages might be regulated differently from that of a Tat-defective virus (52) in the U1 cell line and might depend less on p38 activation. These differences are underscored by diametrically opposite effects of LPS on HIV-1 replication in primary macrophages (29–32) and in U1 cells (33).

It has been shown recently (31) that preexposure of macrophages to LPS suppresses HIV-1 infection through the release of C-C chemokines, which can compete with the virus for the CCR5 receptor. This mechanism, however, is unlikely to account for the effects described in this study for several reasons. First, we added LPS to cells during or after addition of the virus, thus allowing virus-receptor interaction to occur. Second, in contrast to chemokine-mediated inhibition (53, 54), we observed an enhancement, rather than an inhibition, of virus entry in LPS-treated macrophages (Fig. 2A). Third, the low LPS concentrations used in our experiments (100-fold lower than those used by Verani et al. (31)) did not induce the high levels of C-C chemokines required for inhibition of HIV-1 entry into macrophages (35) (results not shown).

Restriction of infection in activated macrophages appears to occur at the postentry step, but before integration. To monitor these steps of HIV-1 replication, we used PCR analysis of HIV-1 DNA. The 2LTR circle forms of viral DNA were used as a marker for successful completion of RT and translocation of the PICs into the nucleus. Because these HIV-1 DNA forms are produced exclusively inside the nucleus (55), they are an excellent indicator for the completion of preintegration steps. Although previously this approach has been used to monitor specifically nuclear importation of the viral PIC under one-cycle conditions (38, 56), there is a good correlation between the efficiency of production of 2LTR circle DNA forms and the level of HIV-1 replication in a long term culture (57–61). With PCR-based analyses there is always a risk of contaminating material. In our case, macrophage populations contained 2% of contaminating cells, some of which might be HIV-1-susceptible CD4+ T lymphocytes. However, as we have demonstrated previously (35), the contribution of T cells to HIV-1 replication in our culture conditions is minimal.

Our results indicate that whereas HIV-1 entry into activated macrophages was enhanced, subsequent steps of replication (RT and nuclear import of the PIC) were greatly inhibited (Fig. 2). The most likely explanation of this result is that LPS treatment switched internalization of receptor-bound virus from a normally used fusion between the viral and cellular membranes to endocytosis. This hypothesis is consistent with the previously reported LPS-mediated increase in phagocytic and endocytic activity of MDMs (62). In addition, LPS has previously been reported to rapidly reduce the number of binding sites for MCP-1, the ligand for CCR2 (63). LPS also down-regulates CCR5 (J. Franchin, B. Sherry, and M. Bukrinsky, unpublished result), most likely via internalization of surface molecules.

While internalized virus was able to initiate RT (Fig. 2A), subsequent steps of replication in activated macrophages were suppressed (Fig. 2, B and C), suggesting that virus particles did not gain access to the right subcellular compartment that normally supports viral RT and nuclear import. As a result, LPS-treated macrophages became resistant to productive infection.

The effect of LPS on HIV-1 infection appears to be mediated by signaling from the CD14 molecule (Fig. 3), the principal receptor for LPS (40, 42). One of the best documented pathways of LPS-induced intracellular signal transduction is the rasraf/38 MAPK pathway, by which p38 MAPK is phosphorylated rapidly during activation with LPS and is crucial in mediating LPS-specific effects (14, 43). It was thus conceivable that the LPS effect on HIV-1 infection was also mediated by p38. Consistent with this hypothesis, preincubation of MDMs with a specific inhibitor of p38 MAPK activation, compound SB203580, restored HIV-1 infection in the presence of LPS (Fig. 4A), indicating the central role of p38 MAPK in LPS-induced resistance to HIV-1 infection. Additional experiments will be necessary to determine the link between activation of the p38 MAPK by LPS and inhibition of virus replication, but two major mechanisms may be envisioned. First, LPS, via activation of p38, might induce rearrangement of actin filaments (22–24), thus stimulating virus internalization via endocytosis. Virus internalized via this mechanism would be targeted to lysosomes, where it would be rapidly degraded. This mechanism would explain both the enhanced uptake and the decreased RT/ nuclear import observed after LPS treatment. Second, p38 activation might directly affect viral RT and/or nuclear import through phosphorylation of downstream targets of p38. For instance, another member of the MAPK family, ERK, has been shown recently to phosphorylate HIV-1 matrix protein (64), thus regulating PIC formation and RT.

The idea of LPS-induced resistance is consistent with a recent report (65) demonstrating that macrophages isolated from mucosal surfaces of the gastrointestinal tract, where exposure to bacterial LPS is very likely, showed reduced susceptibility to HIV-1 infection compared with blood-derived monocytes. Of course, our results should not be interpreted as an indication that LPS or bacterial infections can help fight HIV-1 disease, since such treatments lead to the secretion of proinflammatory cytokines and give rise to activated T cells that are highly permissive for HIV-1. However, alternative, less radical, methods of macrophage stimulation, especially those that target the p38 MAPK pathway, might prove useful in future therapeutic and vaccine approaches.

Acknowledgments

We thank Arthur J. Ulmer for the generous gift of compound 406, and Kevin Tracey for CNI-1493. We thank Tang Hao and Larisa Dubrowsky for expert technical assistance, and the members of Bukrinsky laboratory for discussions and valuable suggestions on the manuscript. Primary HIV-1 isolates were obtained through the AIDS Research and Reference Reagent Program; Division of AIDS, National Institute of Arthritis and Infectious Diseases, National Institute of Health.

References


4. Connor, R. I., and D. D. Ho. 1994. Human immunodeficiency virus type 1 variants with increased replicative capacity develop during the asymptomatic stage before disease progression.
