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Morphine and Galectin-1 Modulate HIV-1 Infection of Human Monocyte-Derived Macrophages

Jessica L. Reynolds,* Wing Cheung Law,† Supriya D. Mahajan,* Ravikumar Aalinkeel,* Bindukumar Nair,* Donald E. Sykes,* Manoj J. Mammen,* Ken-Tye Yong,‡ Rui Hui,† Paras N. Prasad,† and Stanley A. Schwartz*

Morphine is a widely abused, addictive drug that modulates immune function. Macrophages are a primary reservoir of HIV-1; therefore, they play a role in the development of this disease, as well as impact the overall course of disease progression. Galectin-1 is a member of a family of β-galactoside–binding lectins that are soluble adhesion molecules and that mediate direct cell–pathogen interactions during HIV-1 viral adhesion. Because the drug abuse epidemic and the HIV-1 epidemic are closely interrelated, we propose that increased expression of galectin-1 induced by morphine may modulate HIV-1 infection of human monocyte-derived macrophages (MDMs). In this article, we show that galectin-1 gene and protein expression are potentiated by incubation with morphine. Confirming previous studies, morphine alone or galectin-1 alone enhance HIV-1 infection of MDMs. Concomitant incubation with exogenous galectin-1 and morphine potentiated HIV-1 infection of MDMs. We used a nanotechnology approach that uses gold nanorod–galectin-1 small interfering RNA complexes (nanoplexes) to inhibit gene expression for galectin-1. We found that nanoplexes silenced gene expression for galectin-1, and they reversed the effects of morphine on galectin-1 expression. Furthermore, the effects of morphine on HIV-1 infection were reduced in the presence of the nanoplex. The Journal of Immunology, 2012, 188: 3757–3765.

S ubstance abuse is a world-wide public health concern. Opiates are widely abused addictive drugs. The epidemics of addictive drug use and HIV-1 infection coincide with one another. Approximately 33.3 million people worldwide are living with HIV-1/AIDS, and 2.5 million people were estimated to contract HIV-1 in 2009 (1). Addictive drugs were shown to enhance HIV-1 infection of immune cells, including PBMCs, dendritic cells, and macrophages (2–5). HIV-1 infection is characterized by sustained activation of the immune system. Macrophages are one of the first lines of defense against pathogens, are permissive to HIV-1 infection, and contribute to viral persistence. Thus, these cells can serve as vehicles for dissemination and reservoirs of HIV-1 infection (6–9). Therefore, addictive drug use and its effects on macrophages impact the overall course of HIV-1 disease.

During the budding process, HIV-1 incorporates host-derived molecules, including ICAM-1, HLA-DR1, and MHC class II (10, 11), and may include galectins. Galectins are a family of β-galactoside–binding lectins that modulate cell-to-cell and cell-matrix interactions, cell adhesion, and cell signaling by cross-linking of cell surface glycol conjugates (12, 13). Galectins are expressed in a wide range of tissues, including muscle, heart, placenta, lymph nodes, bone marrow, and liver. They are expressed in epithelial cells, endothelial cells, dendritic cells, macrophages, and T and B cells (13). Galectins are located both extracellularly and intracellularly. Twelve galectins exist (galectin 1–12) (14). Galectin-1 is a 14-kDa monomer that consists of two identical carbohydrate-recognition domains. Receptors for galectin-1 include CD45, CD7, CD43, CD2, CD3, CD4, laminin, and integrins (15). Galectin-1 induces activation of mitogen-activated protein kinases, phospholipase C, an increase in intracellular calcium influx, and activation of transcription factor AP-1 (13, 16, 17). Galectin-1 elicits a broad spectrum of biological functions, including cell proliferation, fetomaternal tolerance, apoptosis, cell cycle arrest, cell matrix adhesion, and cell-to-cell adhesion (13, 15–17). With regard to HIV-1 viral adhesion and infection, galectin-1 is a soluble adhesion molecule that mediates direct cell–pathogen interactions enhancing HIV-1 infection of T cells and macrophage (18–20). Our laboratory showed that treatment of immature dendritic cells (IDCs) with methamphetamine increases the expression of galectin-1 (21). The present study, using a nanotechnology method (22–27), was conducted to investigate the potential role of galectin-1 in morphine-induced potentiation of HIV-1 infection of human monocyte-derived macrophages (MDMs).

Materials and Methods

Human subjects

Blood donors were recruited at the University at Buffalo; consents were obtained consistent with the policies of the University at Buffalo Health Sciences Institutional Review Board and the National Institutes of Health. Peripheral blood samples from HIV-1–negative individuals were drawn into a syringe containing heparin (20 U/ml; Sigma-Aldrich, St. Louis, MO).

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The online version of this article contains supplemental material.

Abbreviations used in this article: CTAB, cetyltrimethylammonium bromide; GNR, gold nanorod; IDC, immature dendritic cell; MDM, monocyte-derived macrophage; Q-PCR, quantitative PCR; siRNA, small interfering RNA.

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Isolation of MDMs

Human PBMCs were separated by Ficoll-Paque (GE Healthcare, Piscataway, NJ) gradient centrifugation. CD14+ cells were isolated from PBMCs by direct positive isolation using Dynabeads CD14 (Invitrogen, Carlsbad, CA), according to the manufacturer’s instructions. CD14+ cells were cultured in RPMI medium (RPMI 1640, 10% FCS, 5% human AB serum, 10 mM HEPES, 1% penicillin-streptomycin, 10 ng/ml M-CSF [Millipore, Billerica, MA]) for 7 d for differentiation into MDMs.

Drug treatment

MDMs were treated with morphine sulfate (10−13–10−5 M) (Sigma-Aldrich) for 6, 12, 24, or 48 h. The concentrations of morphine used were based on previous dose-response in vitro studies that produced a maximum biological response without causing toxicity to the target cells (28, 29). For all experiments, cells treated with vehicle alone (media) were used as the untreated control.

RNA extraction and real-time quantitative PCR

MDMs (1 × 105 cells/ml) were cultured in six-well plates, and cytoplasmic RNA was extracted using TRizol (Invitrogen), according to the manufacturer’s specifications. The final RNA pellet was dried and resuspended in diethyl pyrocarbonate water, and the concentration of RNA was determined using a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Any DNA contamination in the RNA preparation was removed by treating the RNA with DNase (1 IU/μg RNA; Promega, Madison, WI) for 2 h at 37°C, followed by proteinase K digestion at 37°C for 15 min and subsequent extraction with phenol/chloroform and NH4OAc/ETOH precipitation. The isolated RNA was stored at −70°C until used. Relative abundance of each mRNA species was assessed using SYBR Green Master Mix (Strategene, La Jolla, CA) to perform quantitative PCR (Q-PCR). Differences in threshold cycle number were used to quantify the relative amount of PCR target contained within each tube. Relative mRNA species expression was quantitated and expressed as transcript accumulation index [2−ΔΔCT], which was calculated using the comparative CT method. All data were controlled for the constitutive expression of the housekeeping gene, β-actin (28, 29). Primers sequences used are shown in Table I.

Western blot

Briefly, 50 μg protein was separated by electrophoresis using 4–20% Tris-HEPES NuSep LongLife gels (Bioexpress, Kaysville, UT) and transferred to polyvinylidene fluoride membranes (Sigma-Aldrich). Membranes were blocked for 1 h with NAP-Blocker (G-Biosciences, Maryland Heights, MO) in TBST (150 mM NaCl, 20 mM Tris [pH 7.5], 0.1% Tween-20) and then incubated with primary Abs overnight at 4°C with gentle rocking. Primary Abs used were anti–galectin-1 rabbit polyclonal Ab (cat. #sc-28248; Santa Cruz Biotechnology, Santa Cruz, CA) and anti–β-actin rabbit polyclonal Ab (cat. #sc-130656; Santa Cruz Biotechnology). After incubation with primary Abs, membranes were washed and incubated with a biotin-conjugated secondary Ab (goat anti-rabbit IgG, cat. #BAF017; R&D Systems, Minneapolis, MN). Membranes were washed three times, for 10 min each, in TBST and then incubated for another 30 min with a streptavidin-alkaline phosphatase conjugate (Invitrogen), followed by colorimetric detection using NBT/BCIP reagent (Thermo Scientific, Rockford, IL). Densitometry analyses were done using a Syngene Image Analyzer with Gene Tools Analysis Software version 3.02.00 (Syngene, Frederick, MD). Data were normalized to protein expression levels of β-actin.

Immunofluorescence

MDMs (1 × 105 cells/ml) cultured on Lab-Tek chambered coverglasses (Nalgene Nunc International, Rochester, NY) were fixed and permeabilized in cold 70% methanol for 30 min at 4°C. Cells were washed in PBS and incubated with Image-IT FX signal enhancer (Invitrogen) for 30 min at room temperature in a humid environment. Cells were then incubated with primary Abs (anti–galectin-1, cat. #sc-19726; isotype-matched control, cat. #sc-2028: 1:50; Santa Cruz Biotechnology) overnight at 4°C. Cells were washed with PBS and incubated for 1 h at room temperature with a secondary Ab conjugated to Alexa Fluor 647 (Alexa Fluor SFX Kits; Invitrogen). Cells were then counterstained with DAPI (Molecular Probes Invitrogen). MDMs were imaged by confocal microscopy using a Leica Confocal Laser Scanning Microscope (TCS SP2 AOBS; Leica Microsystems, Heidelberg, Germany) with a 63× oil-immersion objective lens. An HeNe 633-nm laser was applied to excite DAPI. Quantification of immunostaining was performed by densitometry using Image-J (1.3tc software version of Image J; National Institutes of Health, Bethesda, MD). Expression of galectin-1 was quantified by determining the positive area (index) as a percentage of total image area/microscopy field (32, 33).

ELISA

Total galectin-1 concentrations were measured by ELISA (NovoTeinBio, Cambridge, MA). The assay range was 0.01–1000 ng/ml for galectin-1. Absorbance values were read at 450 nm using a microtiter plate spectrophotometer, and the results are expressed in ng/ml.

Synthesis of gold nanorods

Gold seeds were synthesized by reducing gold salt in the presence of 99% hexadecyltrimethylammonium bromide (CTAB) as a capping agent (22–27, 34). Briefly, 10 ml 0.1 M CTAB solution was mixed with 200 μl 25 mM HAuCl4. Then, 1 ml ice-cold 0.01 M NaBH4 was quickly added, with vigorous stirring for 2 min. A light brown solution (seed solution) was formed, which was kept in a water bath at 33°C for further use. The average size of these Au seeds was 3–5 nm. Meanwhile, to synthesize gold nanorods (GNRs), 10 ml 0.1 M CTAB was mixed with 200 μl 25 mM HAuCl4, in a separate vial, in the presence of a small amount of Ag+ ion (8×10−5 M). Then, the moderate reductant, 100 μl 0.1 M ascorbic acid, was added at room temperature, resulting in the formation of colorless solution. This mixture is the so-called “growth solution.” Finally, the growth solution was heated to 33°C in the water bath, and 12 μl seed solution was gently added to it. Rod formation was permitted undisturbed at 33°C for at least 3 h. To remove excess CTAB from the prepared GNRs, the bilayer CTAB-coated GNRs were centrifuged at 9000 rpm for 15 min. GNRs were sequentially coated with two layers of polyelectrolytes: the negatively charged poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) and the positively charged poly(diallyldimethylammoniumchloride).

Formation of GNR–small interfering RNA nanoplexes and MDM transfection

Cationic GNRs were electrostatically attached to galectin-1 small interfering RNA (siRNA; Ambion), resulting in the formation of nanoplexes. Galectin-1 siRNA/RAM or scrambled control was reconstituted in DNaase/RNase-free water to a final concentration of 0.1 μM and mixed with GNR solution, incubated at room temperature for 15 min, and used for in vitro transfection at a final concentration of 200 pmol siRNA/30 μl (galectin-1 siRNA/GNR = nanoplexes). A total of 1 × 105 cells/ml was seeded onto six-well plates in Opti-MEM containing 4% FBS with no antibiotics. Twenty-four hours later, nanoplexes were added to cells, and transgene expression was monitored at 24–72 h posttransfection. Protein expression was analyzed at 24, 48, and 72 h posttransfection.

Dark-field microscopy imaging

The cellular uptake of the nanoparticle conjugated with galectin-1 small interfering RNA (siRNA) (nanoplex) was visualized using dark-field microscopy 24 h posttransfection. The light-scattering images were recorded using an upright Nikon Eclipse 800 microscope with a high numerical dark-field condenser (N.A. 1.20–1.43, oil immersion) and a 100/1.4 NA oil iris objective (Cfi Plan Fluor). In the dark-field configuration, the condenser delivers a narrow beam of white light from a tungsten lamp, and the high NA oil-immersion objective collects only the scattered light from the samples. The dark-field imaging was captured using a QImaging MicroPublisher 3.3 RTV color camera. QCapture software was used for image acquisition, and it has a feature for adjusting the white color balance for accurately capturing the color differences in samples.

Cell-viability assay

MDMs (10,000 cells/ml/well) were incubated with morphine (10−7 M), or galectin-1 (2 μM; R&D Systems), and nanoplex for 30 min or 2, 12, 24, 48, and 72 h. MDMs were subsequently incubated with MTT (Sigma-Aldrich) for ∼3 h, followed by addition of a detergent solution to lyse the cells and solubilize the colored crystals. The samples were read using an ELISA plate reader at a wavelength of 570 nm.

Infection of MDM with HIV-1

In one series of experiments, MDMs (1 × 105 cells/ml) were treated with morphine (10−7 M) for 24 h and then recombinant galectin-1 (2 μM) for 30 min prior to infection with HIV-1. In another series of experiments, MDMs (1 × 105 cells/ml) were treated with nanoplex for 24 h and then morphine (10−7 M) for 24 h prior to infection with HIV-1.
Cells were washed and subsequently infected with HIV-1 BaL virus (Advanced Biotechnologies, Columbia, MD) at a multiplicity of infection of 0.05 for 2 h and were washed three times with HBSS (Invitrogen, Grand Island, NY) before being returned to culture for 5, 10, or 15 d. The culture supernatants were assayed for p24 Ag using a p24ELISA kit (ZeptoMetrix, Buffalo, NY) on days 5, 10, and 15. Cells were harvested for RNA analyses at day 10 for quantification of p24 and HIV–LTR-R/U5 gene expression (34–36).

Statistics

Multiple comparisons were calculated using ANOVA, followed by the Bonferroni post hoc test (SPSS). Data represent the mean ± SD.

Results

Galectin-1 expression is increased by morphine

It is well established that addictive drugs modulate the expression of various genes and proteins (37, 38). We showed that treatment of IDCs and astrocytes with methamphetamine and heroin, respectively, increases the expression of galectin-1 (21, 39). Therefore, we sought to determine whether morphine could modulate galectin-1 gene expression in human MDMs. Using the primers shown in Table I, Q-PCR was done to analyze gene expression for galectin-1. As shown in Fig. 1A, morphine significantly increased galectin-1 gene expression at 10^{-7} and 10^{-5} M at 12 h, with no change in expression at 6 h. At 24 h, gene expression for galectin-1 was significantly increased at 10^{-9}, 10^{-7}, and 10^{-5} M, as well as at 10^{-9} and 10^{-7} M at 48 h. These data demonstrate that morphine modulates galectin-1 gene expression at various times and doses in MDMs. We next performed Western blotting to validate the changes in gene expression of galectin-1 in MDMs. Morphine significantly increased galectin-1 protein expression at 10^{-7} and 10^{-5} M at 12, 24, and 48 h, with no change at 6 h (Fig. 1B). β-actin, used as an internal control, showed an equivalent protein content among all samples (data not shown). These data are consistent with gene-expression data. Because the maximal effects of morphine on gene and protein expression were observed at 10^{-7} M, further validation in the changes in expression of galectin-1 were done using immunofluorescence and subsequent confocal microscopy using 10^{-7} M morphine. Data demonstrate immunostaining for galectin-1 in MDMs, with cell nuclei labeled.

![Figure 1](http://www.jimmunol.org/)

**Figure 1.** Morphine regulates galectin-1 gene and protein expression in MDMs. MDMs were incubated with morphine (10^{-13}–10^{-5} M) for 6, 12, 24, or 48 h. (A) Gene expression for galectin-1 was determined using Q-PCR (n = 4–6). (B) Galectin-1 protein expression was determined using Western blotting. Immunoreactive protein bands were semiquantified by densitometric analysis and normalized to β-actin levels. Data are expressed as relative protein levels; control values were arbitrarily set to 100 relative protein units. Inset, Representative Western blot (n = 4). (C) Representative confocal images of galectin-1 protein expression following morphine (10^{-7} M) incubation. Isotype-matched control Ab (a), control (b), 6 h morphine (c), 12 h morphine (d), 24 h morphine (e) and 48 h morphine (f). Percentage of galectin-1+ cells is shown in each panel (n = 5). Red: galectin-1 protein; blue: cell nuclei labeled with DAPI. (D) Supernatants were assayed for galectin-1 protein using ELISA (n = 4). Data represent the mean ± SD. *p < 0.001, #p < 0.05, ANOVA, followed by Bonferroni post hoc test. Gal, Galectin-1; MO, morphine.

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<th>Table I. Primer sequences for real-time Q-PCR</th>
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<td><strong>β-actin</strong></td>
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<td><strong>HIV-1 LTR RU/5</strong></td>
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The Journal of Immunology 3759

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in blue (DAPI) (Fig. 1C). Morphine increased galectin-1 protein expression at $10^{-7}$ M at 12 h (Fig. 1Cd), 24 h (Fig. 1Ce), and 48 h (Fig. 1 Cf) compared with control (Fig. 1Cb). These data confirm both Q-PCR and Western blotting data. Because galectin-1 is secreted by macrophages, we next investigated the effects of morphine on unbound galectin-1 in the supernatant. MDMs were treated with various concentrations of morphine ($10^{-13}$–$10^{-5}$ M) for 6, 12, 24, and 48 h and then assessed for galectin-1 protein release by ELISA. As shown in Fig. 1D, there was a concentration-dependent increase in galectin-1 protein release after morphine treatment at all time points, with significance occurring at $10^{-7}$ M morphine at 6, 12, 24, and 48 h. Collectively, Q-PCR, Western blotting, immunostaining, and ELISA demonstrate that morphine enhances the expression and release of galectin-1 from human MDMs.

In vitro uptake of nanoplexes in MDMs and RNA interference

Previously, we showed that delivery and gene-silencing efficiency of siRNA using GNRs are superior to a commercially available transfection reagent (22). Therefore, we used GNRs as a carrier to deliver galectin-1–specific siRNA to MDMs. Cationic GNRs were electrostatically attached to anionic galectin-1–specific siRNA-FAM, resulting in the formation of nanoplexes. The strong orange-red plasmonic scattering associated with GNRs was used to monitor cellular delivery of the nanoplex using dark-field microscopy (26, 27), and fluorescent microscopy was used to evaluate the delivery of siRNA using FAM, a fluorescent label attached to siRNA. Fig. 2A shows 100× dark-field images (Fig. 2Aa–d) of MDMs 24 h posttransfection with nanoplexes, as well as the corresponding fluorescent images (Fig. 2Ae–h). Cellular uptake of the nanoplexes was observed from the strong orange-red scattering of nanoplex-treated MDMs (Fig. 2Ad) compared with untreated MDMs (Fig. 2Aa). Fluorescent microscopy (e-h) of FAM-labeled siRNA was also used to confirm the cellular entry of the nanoplexes. Uptake of nanoplex was observed at 24 h posttransfection (Fig. 2Ah) compared with control (Fig. 2Ae) and free galectin-siRNA-FAM (Fig. 2Ag). These data demonstrated delivery of the nanoplex to MDMs. We next investigated the viability of MDMs following incubation with nanoplexes, morphine ($10^{-7}$ M), and galectin-1 (2 μM) for various times (Fig. 2B). Data demonstrate that cell viability was not affected in nanoplex-, morphine-, or galectin-1–treated MDMs compared with untreated control MDMs. These data confirm that the paradigms that we are investigating are not toxic to MDMs. We next investigated RNA interference for galectin-1 using the nanoplexes in MDMs. Endogenous galectin-1 gene expression, in the absence of morphine or HIV-1, was investigated using Q-PCR at 24, 48, and 72 h posttransfection of nanoplexes in MDMs. As shown in Fig. 2C, the nanoplexes significantly decreased gene expression for galectin-1 at 24, 48, and 72 h compared with control. GNRs alone and scrambled siRNA/GNRs had no effect on galectin-1 gene expression. The decrease in gene expression was further validated using Western blotting. Representative Western blots and densitometry quantification are shown in Fig. 2D. The levels of en-

FIGURE 2. Effect of nanoplex on MDMs. MDMs were transfected with GNRs, free siRNA, scrambled siRNAs, and nanoplexes for various times. (A) Representative dark-field images with DAPI nuclear staining (a–d) and corresponding fluorescent images (e–h) of untransfected MDMs (a, e), GNRs alone (b, f), free galectin-siRNA-FAM (c, g), and nanoplexes-FAM (d, h) 24 h posttransfection (original magnification ×100). (B) Cell viability, as determined by MTT cell-proliferation assay (n = 4). (C) Gene expression for galectin-1 in MDMs following transfection of galectin-1 siRNA/GNR nanoplexes (n = 6) in the absence of morphine or HIV-1 infection. (D) Galectin-1 protein expression following transfection with galectin-1 siRNA/GNR nanoplexes in the absence of morphine or HIV-1 infection. Immunoreactive protein bands were semiquantified by densitometric analysis and normalized to β-actin levels. Data were expressed as relative protein levels; control values were arbitrarily set to 100 relative protein units. Representative Western blot is shown (n = 4). *p < 0.001, ANOVA followed by Bonferroni post hoc test. Data represent the mean ± SD. C, Control; GAL, galectin-1; MO, morphine ($10^{-7}$ M); NP, nanoplex; SCR, scrambled.
dogenous galectin protein expression were significantly decreased 24, 48, and 72 h posttransfection of the nanoplex in the absence of morphine or HIV-1. GNRS alone or scrambled siRNA had no effect on protein expression. These data demonstrate that nanoplexes are able to silence gene expression for galectin-1, as well as prevent translation for galectin-1 protein.

RNA interference prevents morphine-induced potentiation of galectin-1 expression

Because we showed that morphine potentiates galectin-1 gene and protein expression (Fig. 1), we next investigated the effect of the nanoplexes on this. Twenty-four hours posttransfection with nanoplexes, MDMs were treated with morphine (10^{-7} M); RNA and protein were isolated 24 h later. As shown in Fig. 3A, the effects of morphine on galectin-1 gene expression were reversed in the presence of the nanoplex compared with morphine alone. We validated this finding using Western blotting and immunofluorescent staining. Protein expression levels of galectin-1 were decreased in the presence of the nanoplex alone (Fig. 3B). Furthermore, the effects of morphine on galectin-1 protein expression were reversed in the presence of the nanoplex. Immunofluorescence confirmed these findings. Data demonstrate immunostaining

![Graph showing gene expression](image)

![Western blot showing protein levels](image)

![Confocal images of galectin-1 expression](image)

**FIGURE 3.** The effect of the nanoplex on morphine modulation of galectin-1 expression from MDMs. MDMs were transfected with galectin-1 siRNA/GNR nanoplexes (24 h) and then incubated in the presence of 10^{-7} M morphine (24 h). (A) Gene expression for galectin-1 (n = 6). (B) Galectin-1 protein expression. Immunoreactive protein bands were semiquantified by densitometric analysis and normalized to β-actin levels. Data are expressed as relative protein levels; control values were arbitrarily set to 100 relative protein units. Representative Western blot is shown (n = 4). (C) Representative confocal image of galectin-1 protein (original magnification ×100). Control (a), 24-h morphine (b), 48-h nanoplex (c), and 48-h nanoplex + 24-h morphine (d). Percentage of galectin-1^+^ cells is shown in each panel (n = 6). Red: galectin-1 protein; blue: cell nuclei labeled with DAPI. Data represent the mean ± SD. Statistical significance was calculated using ANOVA, followed by Bonferroni post hoc test. *Compared with control (p < 0.05), †compared with morphine alone (p < 0.05). C, Control; MO, morphine; NP, nanoplex.
for galectin-1 in MDMs (red), with cell nuclei labeled in blue (DAPI). Immunofluorescent immunostaining demonstrated that 48 h posttransfection (Fig. 3Cc), nanoplexes decreased galectin-1 protein expression compared with control (Fig. 3Ca). A 24-h incubation with morphine potentiated galectin-1 protein expression (Fig. 3Cb), which was reversed in the presence of the nanoplex (Fig. 3Cd).

HIV-1 analyses

Previous studies demonstrated that, individually, both galectin-1 and addictive drugs enhance HIV-1 infection of MDMs (2, 3, 5, 18–20). Confirming these previous studies, either exogenous recombinant galectin-1 (2 μM, 30-min incubation) or morphine (10⁻⁷ M, 24 h) alone prior to HIV-1 infection enhanced p24 Ag production at 5, 10, and 15 d postinfection of MDMs (Fig. 4A). Because both galectin-1 and morphine alone increased HIV-1 infection, we next investigated the effects of their concomitant administration on levels of HIV-1 p24 Ag. We found that concomitant incubation of morphine (10⁻⁷ M, 24 h) and recombinant galectin-1 (2 μM, 30 min) prior to infection had a synergistic effect on levels of p24 Ag produced by MDMs at 5, 10, and 15 d postinfection. Because maximal levels of p24 Ag were produced at 10 d postinfection by galectin-1 and galectin-1 + morphine, we investigated p24 Ag gene expression at this time point using the previous paradigm. We found that either morphine or galectin alone increased p24 Ag gene expression compared with control (Fig. 4B). Furthermore, concomitant incubation with galectin-1 and morphine significantly enhanced gene expression for p24 Ag compared with control, morphine alone, and galectin alone. We next investigated changes in amplification of the HIV-LTR-R/U5 region using Q-PCR on day 10 postinfection. This method is designed to detect early stages of reverse transcription of HIV-1 (36). We found that either morphine or galectin alone increased gene expression for HIV-LTR-R/U5 compared with control (Fig. 4C). Furthermore, concomitant incubation with galectin-1 and morphine significantly enhanced gene expression for HIV-LTR-R/U5 compared with control, morphine alone, and galectin alone. These data—p24 Ag assay, p24 gene expression, and HIV-LTR-R/U5 gene expression—indicate that concomitant morphine and galectin-1 enhance HIV-1 infection of MDMs.

We investigated whether a mechanism of enhanced HIV-1 infection by morphine may be by modulating endogenous galectin-1 expression. MDMs were transfected with nanoplexes, incubated for 24 h, and treated with morphine (10⁻⁷ M) for 24 h. After morphine treatment, MDMs were infected with HIV-1 for 2 h, washed, and returned to culture. The levels of p24 Ag were measured at days 5, 10, and 15 postinfection. Morphine enhanced p24 Ag production compared with control MDMs (Fig. 5A). Incubation with nanoplexes before morphine treatment partially reversed the potentiation in p24 Ag production compared with morphine alone. We next investigated p24 Ag and HIV-LTR-R/U5 gene expression at 10 d postinfection, using the above previous paradigm. We found that morphine increased p24 Ag and HIV-LTR-R/U5 gene expression compared with control (Fig. 5B, 5C), and these were

**FIGURE 4.** Effect of galectin and morphine on HIV-1 infection. MDMs were incubated with morphine alone (10⁻⁷ M, 24 h), recombinant galectin-1 alone (2 μM, 30 min), or morphine + recombinant galectin-1 prior to infection with HIV-1. (A) Supernatants were collected on days 5, 10, and 15 postinfection and assayed for p24 Ag. Data are presented as p24 Ag levels (pg/ml). Inset represents expansion of box in graph at 5 d postinfection (n = 6). (B) RNA was isolated on day 10 postinfection; p24 Ag gene expression was quantitated using Q-PCR (n = 6). (C) RNA was isolated on day 10 postinfection; HIV-LTR-R/U5 Ag gene expression was quantitated using Q-PCR (n = 6). Data represent the mean ± SD. Statistical significance was calculated using ANOVA, followed by Bonferroni post hoc test (p < 0.05). *Compared with control, †compared with morphine alone, ††compared with recombinant galectin-1 alone. C, Control; GAL, galectin-1; MO, morphine.
partially reversed in the presence of the nanoplex. These data indicate that the mechanism by which morphine enhances HIV-1 infection of MDMs is not regulated by galectin-1 alone. The nanoplex significantly reduced HIV-1 infection compared with morphine alone, but it did not return levels to those of untreated MDMs. Future studies are necessary to elucidate with which proteins galectin-1 may act concomitantly to regulate morphine-induced HIV-1 infections of MDMs. However, these studies implicate the important role that galectin-1 plays in addictive drug-induced infections.

Discussion

Addictive drugs modulate the immune response. Martin et al. (40) demonstrated in a murine model of substance abuse that morphine treatment impedes neutrophil and macrophage recruitment to wound sites. Chronic morphine administration to mice reduces both the volume of spleen and inguinal lymph nodes and the number of WBCs (41). In vivo morphine suppresses rat NK cells (42). Systemic morphine administration to mice reduces both the volume of spleen and inguinal lymph nodes and the number of WBCs (41). In vivo morphine suppresses rat NK cells (42). With regard to HIV-1 infection, morphine has differential effects on cells of the immune system. Peterson et al. (44) showed that morphine stimulates the replication of HIV-1 within PBMCs. In CD4+ T cell cultures, treatment with morphine for 30 min inhibited HIV-1 expression, whereas pretreatment for 24 h stimulated viral expression (45). Morphine amplifies HIV-1 expression in the chronically infected promonocytic clone U1 when cocultured with LPS-stimulated human fetal brain cells (46). Morphine stimulated HIV-1 replication in primary cultures of human Kupffer cells (47). Morphine significantly enhanced HIV-1 R5 strain infection of MDMs but had little effect on X4 strain infection (5). Morphine significantly enhanced HIV-1 infection of neonatal MDMs, which was suppressed by naltrexone, the opioid receptor antagonist (48). The opioid antagonist methylnaltrexone abrogated morphine-induced HIV-1 Bal strain infection of MDMs (49). These studies demonstrate the role of morphine as an immunomodulator. The current study demonstrates that morphine potentiates HIV-1 replication of MDMs, confirming previous studies.

Galectin-1 was shown to play a role in immunomodulation. Studies found an increase in the expression of galectin-1 in activated macrophages (50). Galectin-1 modulates constitutive and inducible FcγRI expression on monocytes/macrophages and FcγRI-dependent phagocytosis through an ERK1/2-dependent pathway (51). Galectin-1 initiates the activation of JNK, MAPK kinase 4, and MAPK kinase 7 as upstream JNK activators in Jurkat T cells (52). Galectin-1 suppresses T cell proliferation and alloreactivity in human multipotent mesenchymal stromal cells (53). Galectin-1 inhibits the secretion of cytokines typical of Th1 and Th17 cells while promoting Th2-type cytokine secretion (54–56). Studies suggest that, during the course of HIV-1 infection, a bias toward Th2-like responses and, hence, Th1 inhibition, may contribute to the loss of control of the immune system over HIV-1 infection (54–57). Therefore, galectin-1 may regulate the immune system’s response to HIV-1 infection.

Studies showed that galectin-1 enhances HIV-1 infectivity and replication by allowing efficient binding of virus particles to target cells (18–20). Galectin-1 stabilizes virus–cell interaction and
promotes virus replication in PBMCs and CD4+ T cells (19). The investigators also reported that galectin-1 facilitates HIV-1 infection by increasing the kinetics of HIV-1 binding to its target cell (19). They also demonstrated that galectin-1 acts as a soluble adhesion molecule by facilitating attachment of HIV-1 to the cell surface. Therefore, they proposed that galectin-1 can cross-link HIV-1 and target cells and promote a firmer adhesion of the virus to the cell surface, thereby augmenting the efficiency of the infection process (18–20). Studies by our laboratory showed that addictive drugs enhance galectin-1 expression in ICDS and normal human astrocytes, resulting in enhanced HIV-1 infection (21, 39). The current study demonstrates that morphine enhances galectin-1 expression and enhances HIV-1 infection of MDMs. Furthermore, we show that exogenous recombinant galectin-1, in combination with morphine, synergistically enhances HIV-1 viral infection of MDMs compared with exogenous galectin-1 alone. Because morphine is known to activate macrophages (5, 7, 48, 49), and activated macrophages secrete galectin-1 (50), these conditions are likely to exist in the drug-using population’s blood milieu (i.e., morphine + galectin-1). These studies demonstrate the profound potential effects that concomitant morphine and galectin-1 have on HIV-1 infection in the drug-using population. We further investigated whether morphine regulation of galectin-1 may play a role in enhanced infection of MDM. We demonstrate that gene silencing of galectin-1 partially reversed the potentiation of HIV-1 infection induced by morphine. Our current study supports the idea that galectin-1 stabilized HIV-1 interactions with MDMs. Because viral attachment is a rate-limiting step for virus entry into a cell, we propose that morphine facilitates HIV-1 attachment to MDMs by regulating galectin-1 expression and release. However, our study also found that HIV-1 replication in the presence of the nanoplex and morphine was still enhanced compared with control. These data suggest a redundancy in the regulation of HIV-1 infectivity, such that galectin-1 may act concomitantly with another protein or molecule to regulate HIV-1 infection. Several studies showed that morphine modulates the HIV-1 coreceptor CCR5, and enhanced expression of CCR5 plays a role in increased HIV-1 infection in macrophage (5, 48, 49). We found that morphine enhances CCR5 expression, and siRNA against galectin-1 did not affect CCR5 expression in the absence of morphine. Further, the nanoplex had no effect on morphine-induced potentiation of CCR5 expression (Supplemental Fig. 1). Future studies are necessary to elucidate the interaction between galectin-1 and CCR5. We propose that concomitant silencing of galectin-1 and CCR5 may be ideal candidates for therapeutic prevention of HIV-1 infection in addictive drug users.

Disclosures

The authors have no financial conflicts of interest.

References


MDM were incubated in the presence of $10^{-7}$M morphine (24 hr, MO) or were transfected with galectin-1 siRNA/GNR nanoplexes (24 hr, NP) or were transfected with NP for 24 hr then incubated with $10^{-7}$M morphine for 24 hr (NP + MO). Gene expression for CCR5 was investigated using Q-PCR (n = 4). Results: Morphine enhanced CCR5 gene expression in MDM. The nanoplex had no effect on CCR5 gene expression. Further in the presence of the nanoplex, morphine significantly potentiated CCR5 gene expression. These data demonstrated the specificity of the nanoplexes for galectin-1 and demonstrate that the nanoplex has no effect on already known morphine targets (CCR5).