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Systemic Morphine Administration Suppresses Genes Involved in Antigen Presentation

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ABSTRACT

Administration of opioids in both humans and animal models results in significant alterations in immune system responsiveness. Although the majority of studies have focused on phenotypic changes in immune cells after short- and long-term morphine administration, few studies have determined whether alterations in gene expression profiles accompany these effects. To address this question, rats were treated with either morphine (20 mg/kg) or saline, and changes in gene expression and function in blood leukocytes were examined. Within 2 h, morphine administration resulted in a decrease in blood leukocyte expression of the major histocompatibility complex class II (MHC II RT1.B β) (−3.27-fold) and related molecules, including the MHC II invariant chain (−2.73-fold). Furthermore, these changes in gene expression were accompanied by a significant decrease in surface MHC II RT1.B β protein expression, specifically on B lymphocytes. Morphine administration was also found to inhibit IL-4 induced up-regulation of MHC II RT1.B β cell surface expression on B lymphocytes. This is the first demonstration that receptors involved in antigen presentation are modified after systemic morphine administration. We propose that the inability of B lymphocytes to up-regulate key immune proteins, such as the MHC II molecule, after exposure to antigen-induced cytokine production may account for the increase in the susceptibility to bacterial and viral infections such as HIV in both drug abusers and patients receiving morphine.

Opioid drugs such as morphine and heroin have been shown, in both human and animal models, to suppress many different types of immune cell activities, resulting in the modulation of both innate and acquired immune responses (Bayer et al., 1990; Eisenstein and Hilburger, 1998; Fecho and Lysle, 2002; Guo et al., 2002; Houghtling and Bayer, 2002). A fundamental component common to these particular immune responses is the formation and surface display of antigen-derived peptide-major histocompatibility complex (MHC) II complexes (Thomas et al., 1979; Gosselin et al., 1993; Luder et al., 1998). Constitutive expression of MHC II is restricted to antigen presenting cells such as dendritic cells, B cells, and macrophages. In the innate immune response, MHC II molecules present antigen to T lymphocytes leading to T lymphocyte activation and differentiation (Benacerraf and Germain, 1981). In acquired immune responses, MHC class II molecules on B lymphocytes present processed antigen to T lymphocytes, resulting in a cytokine feedback loop, thereby causing B lymphocytes to proliferate and differentiate into antibody-producing plasma cells (Abbas et al., 2002). Therefore, modulation of either the expression or function of the MHC II molecule by morphine may result in significant alterations in immune responses, which could increase susceptibility to pathogens.

An excellent method for measuring innate immunity, where antigen is presented by the MHC II molecule on the macrophage to the T lymphocyte, resulting in cytokine production, is delayed type hypersensitivity (Eisenstein and Hilburger, 1998). Interestingly, morphine treatment suppresses T lymphocyte responses as assessed by inhibition of delayed-type hypersensitivity reactions (Bryant and Roudebush, 1990). These findings suggest that the MHC II molecule may be down-regulated on the macrophage, resulting in inefficient antigen presentation to the T lymphocyte. Similarly, B lymphocyte activities involved in acquired immunity are also suppressed in that heroin administration results in a decrease in lipopolysaccharide-stimulated proliferation (Fecho et al., 2000) as well as a decrease in antibody production (Eisenstein et al., 1998). Although functional changes in antigen processing and presentation have been measured after in vivo morphine administration, effects on gene ex-

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ABBREVIATIONS: MHC, major histocompatability complex; rr, rat recombinant; IL, interleukin; FITC, fluorescein isothiocyanate; FACS, fluorescence-activated cell sorting; PBS, phosphate-buffered saline; RT, reverse transcription; PCR, polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Ii, major histocompatability complex II invariant chain; U50,488H, [(*trans*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide.

pression and intracellular pathways that may contribute to these changes in leukocytes have not been elucidated.

In the present study, we hypothesize that genes expressed in blood leukocytes that are involved in immune function would be modulated after short-term in vivo morphine treatment. To examine whether gene expression changes occur concurrently with these functional changes, microarray analysis was performed on whole blood from animals undergoing short-term morphine treatment. Surprisingly, the majority of genes that were shown to be down-regulated are involved in antigen presentation and processing, including multiple chains of the MHC II molecule. Using flow cytometry, these changes in MHC II protein were further shown to be cell-type specific. Finally, morphine administration attenuated the up-regulation of the expression of the MHC II gene after stimulation with a cytokine. These data suggest that regulation of the expression of MHC II by morphine may be related to the increased susceptibility to both bacterial (Bhaskaran et al., 2001) and viral (Guo et al., 2002) infectious diseases, such as HIV, upon drug exposure.

Materials and Methods

Animals. Pathogen-free male Sprague-Dawley rats (200–225 g upon receipt) were obtained from Taconic Laboratories (Germantown, NY). Animals were housed 3 per cage with microisolator tops and provided food (Purina rat chow) and water ad libitum. The light cycle was regulated automatically (lights on at 6 AM, off at 6 PM), and temperature was maintained at 23 ± 1°C. All animals were allowed to acclimate to this environment for 1 week before any experimental manipulations. To avoid complications of circadian changes in corticosteroid levels, experiments were performed between 8 AM and 10 AM, when corticosterone levels are at a nadir.

Reagents. Morphine sulfate was generously provided by the National Institute on Drug Abuse (Research Triangle Park, NC). Recombinant rat (rr) IL-4 was purchased from Peprotech Inc. (Rocky Hill, NJ). Mouse anti-rat CD45RA-PE clone OX33 and mouse anti-rat monocyte/granulocyte clone R2-1Aba-FITC were purchased from Caltag (Burlingame, CA). MHC II RT1.Bβ mouse anti-rat antibodies were purchased from BD Pharmingen (San Diego, CA).

Leukocyte Isolation and Cell Counts. Animals were sacrificed via decapitation, and whole trunk blood was diluted 1:4 with RPMI-1640 medium (Invitrogen, Carlsbad, CA) and 1% fetal calf serum (Biofluids, Rockville, MD) and placed on a Ficoll-Hypaque (Amersham Biosciences, Piscataway, NJ) gradient (3 ml of Ficoll for every 10 ml of diluted blood). The gradient was then centrifuged for 30 min at 1500 rpm, and circulating leukocytes were obtained from the buffy coat in the interface between the Ficoll and the media. After a series of washes, the cell pellet was resuspended in 1 ml of RPMI-1640 medium and 1% fetal calf serum, and cells were counted using a Z1 counter (Beckman Coulter, Fullerton, CA) (20 μl of diluted leukocytes added to 10 ml of Isoton II counting solution with 100 μl of LAS lysing reagent (Fisher Scientific Co., Chicago, IL). Although previous studies in our laboratory have shown significant differences in lymphocyte number after morphine treatment (Flores et al., 1995), no significant differences in cell numbers were observed between morphine- and saline- treated animals in these studies.

Determination of MHC II RT1.B β Expression on Leukocyte Populations (FACS). Phosphate-buffered saline (PBS; 80 μl) (Beckman Coulter) was added to polystyrene tubes. Anti-rat MHC II RT1.B β-FITC (OX-6; 10 μl) diluted with PBS (1:10) was added to tubes containing PBS. Anti-rat CD45RA-PE (OX33; 10 μl) was added to a set of tubes containing PBS. Rats were sacrificed by rapid decapitation, and trunk blood was collected into heparinized tubes. Whole blood or isolated blood leukocytes (100 μl) were added to tubes containing PBS and antibody solutions. Samples were incubated (60 min at 4°C), washed twice with PBS, centrifuged at 1200 rpm, and supernatants were aspirated. Samples were vortexed; immunolyse (1 ml; Beckman Coulter) was added to each tube and the samples were vortexed again. Samples were fixed with 250 μl of 9.25% formaldehyde and vortexed (60 s). Samples were resuspended with PBS, centrifuged (1200 rpm), and supernatants were aspirated. A final volume of 100 μl of PBS was added to each tube and vortexed. As positive controls, cell suspensions were incubated separately with each monoclonal labeled antibody. Samples were protected from light and stored at 4°C until analysis using a FACStar flow cytometer (BD Biosciences, San Jose, CA) with a single excitation source (200-mW argon laser). An analysis gate was set to include cells with the forward-and-side scatter characteristics of lymphocytes and monocytes.

In Vitro IL-4 Assays. After leukocyte isolation, 1×10^6 cells were plated out into each well in a 12-well plate. Cells were then treated with varying concentrations of rrIL-4. After 24 h, cells were harvested using a cell scraper and 1% trypsin-EDTA solution. Cells were then centrifuged for 5 min at 1100 rpm, and the resulting cell pellet was resuspended in 200 μl of media. MHC II RT1.B β and CD45RA levels were then measured using FACS analysis.

RNA Extraction. Blood leukocytes were separated from whole blood by the Ficoll-Hypaque (Amersham Biosciences) method. Total cellular RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. This procedure uses the phenol and guanidine isothiocyanate method described by Chomczynski and Sacchi (1987). Briefly, 1 ml of the TRIzol reagent was used per 10×10^6 cells. Cells were homogenized in TRIzol and were incubated at room temperature for 5 min. A chloroform extraction (0.2 ml) was performed followed by centrifugation at 12,000*g* for 15 min at 4°C. The aqueous phase (containing RNA) was removed and the RNA was precipitated by addition of 0.5 ml of isopropyl alcohol per 1 ml of TRIzol. The RNA was precipitated for 10 min at room temperature and centrifuged at 12,000*g* for 10 min at 4 °C. The supernatant was removed and the pellet was washed once with ethanol (75%) and centrifuged at 7500*g* for 5 min at 4 °C. The supernatant was removed and the pellet was allowed to air dry for 10 min. The pellet was resuspended in 20 μl of diethyl pyrocarbonate-treated water, and the sample was incubated at 60°C for 10 min. Each RNA sample was quantified using an UV spectrophotometer (Beckman Coulter).

To determine the integrity of the RNA sample, RNAs (2 μg) were electrophoresed in a 1% agarose gel containing ethidium bromide. Samples that produced clear 18S and 28S bands were considered intact and were used for the oligonucleotide microarray and reverse-transcription-polymerase chain reaction (RT-PCR).

Affymetrix Microarray. The differential gene expression patterns of in vivo morphine- and saline-treated circulating blood leukocytes were studied using a microarray expression assay. RNA samples from rats whose whole blood lymphocyte proliferation assay showed at least 50% suppression of proliferation compared with control were pooled and analyzed using a rat Affymetrix oligonucleotide microarray chip (U34). Differences in gene expression levels of at least 2-fold were considered significant (2-fold up- or down-regulated).

Poly(A^+) mRNA was extracted and purified from total RNA (5 to 100 μg) using a Micro poly(A^+) kit (Ambion, Austin, TX). cDNA was synthesized (from 0.5 to 5 μg of mRNA) using the Superscript Choice kit (Invitrogen), incorporating a T7- (dT) 24 primer (Genset, Paris, France). Purified cDNA (up to 2 μg) was in vitro-transcribed using the MEGAscript T7 Kit (Ambion), incorporating Biotin-11-cytidine triphosphate and Biotin-16-uridine triphosphate (final concentration, 1.875 mM; Sigma/Enzo, New York, NY). After purification, in vitro cRNA was fragmented in buffer containing magnesium at 94°C. Labeled cRNA was hybridized to the microarray while rotating at 60 rpm for approximately 16 h at 45°C. After hybridization, the microarray was washed using the Affymetrix Fluidics Station in buffer containing biotinylated anti-streptavidin antibody (10 min at 25°C; Vector Laboratories, Burlingame, CA) and stained with streptavidin

phycoerythrin (final concentration, 10 μg/ml; Molecular Probes, Eugene, OR) for 10 min at 25°C. Subsequently, the microarray was washed, restained with streptavidin phycoerythrin (10 min at 25°C), and washed again before measuring the fluorescence bound to the microarray at 570 nm in an Affymetrix scanner. Data were analyzed using an Affymetrix MicroDB Software Version 3.0 program and the Affymetrix Data Mining Tool program.

cDNA Synthesis and Semiquantitative PCR. Two genes exhibiting significant increases in expression after short-term morphine treatment and 2 genes exhibiting significant down-regulation in expression after short-term morphine treatment (2 h) were subjected to secondary analysis by semiquantitative RT-PCR. RNA was pooled from three rats per treatment group from four independent experiments. RT was performed by converting 1 μg of total RNA to cDNA using an OmniScript kit (QIAGEN, Valencia, CA) as directed. cDNA was amplified in a 50-μl reaction containing 10 mM concentrations of each primer and 1× master mix *Taq* polymerase (QIAGEN). Cycle conditions were 94°C for 4 min; 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s, and 72°C for 5 min. Ten-microliter aliquots were taken after cycles 25, 28, 35, and 40. Primers used were MHC II RT1.B β, 5′-attaggaacggggactggac-3′, 5′-aggagaccttgctggaggag-3′; MHC II invariant chain, 5′-tggctactgctggtgtgtgt-3′, 5′-aatggggtgtgatgttaggg-3′, and GAPDH, 5′-gaacatcatccctgcatcca-3, 5′-ccagtgagcttcccgttca-3′. These were visualized by ethidium bromide gel electrophoresis and quantified using a densitometer (Stratagene, La Jolla, CA). All results were normalized to GAPDH.

Results

Comparison of Blood Leukocyte Gene Expression in Morphine- and Saline-Treated Animals. To determine whether morphine administration resulted in altered gene expression in blood leukocytes, male Sprague-Dawley rats received either saline or morphine (20 mg/kg) subcutaneously and were sacrificed 2 h later. Pooled RNA samples from peripheral blood leukocytes (seven rats per treatment group) was hybridized to Affymetrix (U34) oligonucleotide chips to measure changes in gene expression. Suppression of lymphocyte proliferation was seen in all morphine-treated animals relative to control animals. Approximately 60% of all animals treated with morphine showed a 50% or greater suppression of lymphocyte proliferation, and samples from these animals were pooled for microarray analysis ([^{3}H]thymidine incorporation was 19,520 ± 920.0 cpm for saline-treated animals and 5839 ± 699.1 cpm for morphine-treated animals).

After scanning the probe arrays, the resulting digitized images produced by the GeneChip analysis software were used to calculate an average intensity and assigned a given *x/y* coordinate. Up and down-regulated genes were detected by regression analysis, and after applying a second algorithm, the expression level of a given gene was calculated. Changes were considered significant for genes whose expression significantly deviated from the regression line by at least 2-fold (Fig. 1). Only genes that were significantly altered in both arrays were further evaluated. Two independent microarray experiments were carried out, and the average -fold difference of up or down-regulated genes for both microarrays was calculated. It was found that 23 known genes were up-regulated, 40 expressed sequence tags were up-regulated, 26 known genes were down-regulated, and 54 expressed sequence tags were down-regulated. Interestingly, of 26 down-regulated genes, nearly half play a role in antigen processing and presentation. Furthermore, it was of particular interest that six of nine antigen processing genes encoded for various chains of the MHC II molecule (Table 1). The MHC II molecule plays a crucial role in presenting antigen peptides to T lymphocytes, resulting in activation of the T cell and subsequent proliferation and cytokine production. A down-regulation of the MHC II gene (α and β chains) could result in a significant decrease in the ability of antigen-presenting cells to activate T lymphocytes, resulting in immunosuppression.

Reverse-Transcription-Polymerase Chain Reaction for Verification of Microarray Analysis. To verify by an independent measure the effects of morphine on gene expression, experiments were repeated to generate new RNA samples, different from that used in the microarray studies. RNA samples from three to four rats were pooled for each sample, and four samples were run per cycle. Samples were removed at different cycle points as indicated (25, 28, 35, and 40 cycles). In all comparisons, differences in gene expression were determined at cycle 35. To verify by an independent measure the effects of morphine on gene expression, experiments were repeated to generate new RNA samples, different from that used in the microarray studies. RNA samples from

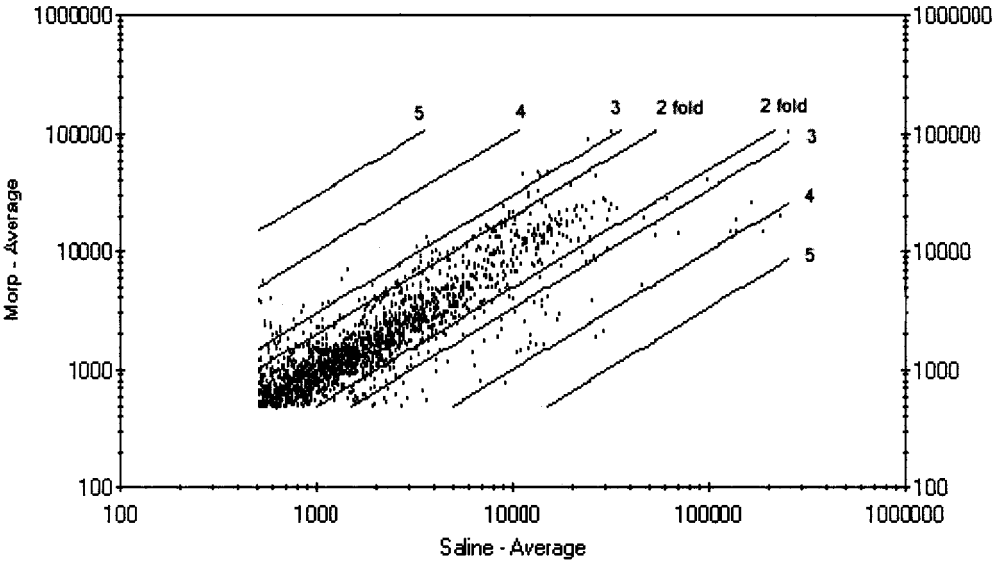

Fig. 1. In vivo morphine treatment causes differential regulation of genes in blood leukocytes. Scatter correlation diagram representing gene expression after morphine treatment (20 mg/kg; 2 h) versus saline-treated control mice (average of two individual microarray experiments using pooled animals, n = 14/group).

three to four rats were pooled for each sample and 4 samples were run per cycle. Samples were removed at different cycle times as indicated (25, 28, 35, and 40 cycles). In all comparisons, differences in gene expression were determined at cycle 35. Semiquantitative PCR analysis showed the MHC II RT1.B β chain to be down-regulated, comparing it with GAPDH (Fig. 2.), We estimated a 3.4-fold down-regulation ($p < 0.05$) (Fig. 2.), which was in good agreement with the -3.27-fold changes observed for this gene in the microarray experiments (Table 1). In addition, the MHC II invariant chain was down-regulated somewhat less, and we estimate a -2.1-fold down-regulation ($p < 0.05$), as shown by RT-PCR, which also is similar to the decrease observed in the microarray analysis (Fig. 2). There were, however, no significant changes in GAPDH expression in any of these assays. In both cases, RT-PCR confirmed the microarray results indicating a down-regulation in antigen presenting and processing genes.

Cell-Specific Down-Regulation of MHC class II RT1.B β Molecules on Blood Leukocytes after Short-Term Morphine Treatment. To determine whether the decrease in gene expression was accompanied by a decrease in protein expression and, if so, to identify the type of cells involved, FACS analysis of blood samples after morphine treatment was performed. Specifically, cells were labeled with antibodies against MHC II RT.1B β and those identifying either monocytes or B lymphocytes. Interestingly, 2 h after morphine treatment, a decrease in the MHC II RT.1B β chain expression was seen only on B lymphocytes compared with saline-treated animals ($p < 0.05$; Student's t test) (Fig. 3a). No significant change was observed in MHC II RT.1B β levels on blood monocytes after a 2-h morphine treatment (Fig. 3b). Because the MHC II RT1.B β gene is translationally down-regulated, as indicated by a decrease in protein expression, the net effect could indicate a decreased ability of B lymphocytes to effectively present antigen to $CD4^+$ T lymphocytes.

Short-Term Morphine Treatment Decreases the Ability of B Lymphocytes to Up-Regulate MHC II RT1.B β Expression after in Vitro IL-4 Stimulation. In the previously mentioned experiments, suppression of MHC II RT1.B β by morphine was observed in unstimulated blood B lymphocytes. To investigate whether stimulation of MHC II processing and antigen presentation pathways would also be modulated after morphine treatment, we used IL-4 as an inducer of MHC II transcription. IL-4 is a potent cytokine that has been shown to up-regulate MHC II receptor expression predominately on B lymphocytes (Roos et al., 1998). Blood leukocytes were taken from morphine- and saline-treated animals and separated using a Ficoll gradient. Cells were treated for 24 h with varying concentrations of rr IL-4 (0, 1, 10, 100, and 1000 ng/ml) and then incubated with CD45RA-PE (OX33) and MHC II RT1.B β-FITC (OX-6) antibodies for flow cytometry analysis. We discovered that blood B lymphocytes taken from saline-treated animals showed a 2- and 3-fold increase in MHC II RT1.Bβ with a treatment of 100 or 1000 ng/ml rr IL-4, respectively (Fig. 4.) However, blood B lymphocytes taken from morphine treated animals were not able to up-regulate MHC II RT1.B β expression as efficiently as saline-treated animals after a 24-h in vitro stimulation with rr IL-4 (100 and 1000 ng/ml) ($p < 0.05$). During HIV and other viral and bacterial infections, IL-4 levels are increased to combat infection (Sukwit et al., 2001). Therefore, a decrease in the ability of B lymphocytes to respond to IL-4 stimulation by up-regulating MHC II expression may contribute to the increased susceptibility to infections in drug abusers and patients receiving morphine for pain after surgery.

TABLE 1

Morphine-induced down-regulation (>2-fold) of gene expression in blood leukocytes

Oligonucleotide array analysis (Affymetrix U34) of gene expression patterns in rat blood leukocytes 2 h after treatment with morphine (20 mg/kg). Samples were pooled from each treatment group. Values represent differences in expression in morphine animals compared with saline-injected control animals and are the average of two separate experiments.

GenBank Accession no.	Antigen Presentation	Fold change
U65217	MHC II antigen RT1.B β chain	-3.27
M22366	MHC II RT1.B α chain	-2.05
M36151	MHC II A-β RT1.B-b-β gene	-2.75
X56596	MHC II antigen RT1.B-1 β chain	-2.33
X53054	MHC II RT1.D β chain	-2.02
X14254	MHC II-associated invariant chain	-2.73

Discussion

We used an expression profiling approach and incorporated oligonucleotide microarray technology to search for candidate genes that are specifically altered in blood leukocytes in response to in vivo treatment with morphine. As

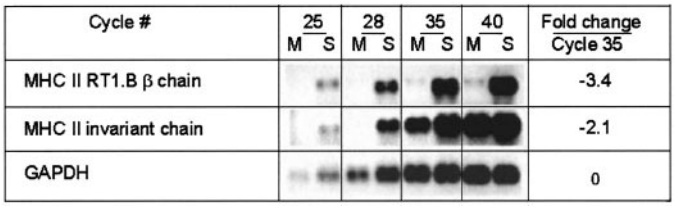

Fig. 2. RT-PCR confirmation of reduced MHC II RT1.B β and MHC II invariant chain expression on blood leukocytes after 2-h in vivo morphine treatment. Representative experiment using semiquantitative PCR of selected down-regulated genes identified by microarray analysis [pooled blood leukocytes from three rats per saline treatment group (S) and morphine (20 mg/kg; 2 h) treatment group (M)]. The degree of down-regulation (fold change) was determined by densitometric analysis of the bands of the morphine-treated group relative to the saline-treated group. ($n = 5$ samples per cycle; three to four rats pooled per sample).

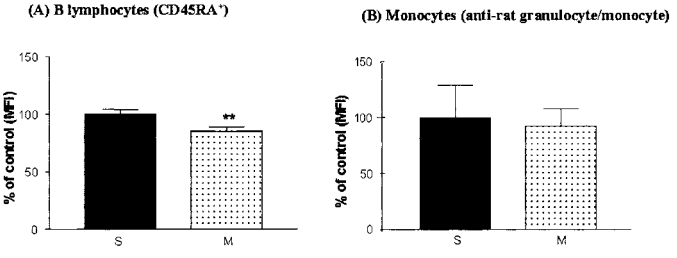

Fig. 3. Effect of morphine on MHC II RT1.B β receptor expression on B lymphocytes and monocytes. Rats were treated with morphine (20 mg/kg) and sacrificed 2 h later. Blood leukocytes were isolated as described under *Materials and Methods* and were stained with antibodies against MHC II RT1.B β and costained with CD45RA and/or monocyte antibodies (B lymphocyte or monocyte cell populations, respectively). A, effect of short-term treatment with morphine (M) or saline (S) on MHC II RT1.B β receptor expression on blood B lymphocytes ($n = 12$ rats per treatment group). B, effect of short-term morphine treatment on MHC II RT1.B β receptor expression on blood monocytes ($n = 12$ rats per treatment group) Data were expressed as a percentage of saline (S)-treated control mice (99.5 ± 4.45 for $n = 12$ rats per group). (**, $p < 0.05$ as determined by Student's t test)

shown by two independent oligonucleotide microarrays, short-term morphine treatment resulted in a significant down-regulation of potential candidate genes involved in antigen-presenting cells. Furthermore, both the MHC II RT1.B β and MHC II invariant chain genes were subjected to secondary analysis using semiquantitative PCR. Differential expression of both genes between morphine-treated animals and saline-treated control animals was in good agreement with the -fold changes observed in the average of the microarrays. Because there may be differences between RNA and protein levels because of translational control and altered protein stability, we measured MHC II RT1.B β surface protein levels using flow cytometry. Our results indicate that the MHC II RT1.B β protein is also decreased on the cell surface. Interestingly, down-regulation of MHC II RT1.B β protein was found only on B lymphocytes and not monocytes 2 h after morphine administration. Although B lymphocytes are not the major antigen-presenting cell in humans, they are the most abundant and major antigen presenting cell in rat blood. Therefore, we further investigated this 2-h response to morphine on B lymphocytes to choose the most direct effect of morphine on immune cells and to discover upstream gene products that drive this decrease in immune responsiveness. It remains to be determined whether these initial gene changes after short-term exposure to morphine contribute to the overall immunosuppression that accompanies long-term exposure.

Additional studies were carried out to begin to identify potential signal transduction pathways involved in mediating the down-regulation of MHC II RT1.B β expression on stimulated B lymphocytes. Our results demonstrated that IL-4–regulated transcription and further translation of MHC II RT1.B β were suppressed, suggesting that morphine administration is a modulator of signal transduction of the IL-4 pathway and/or transcriptional regulators of MHC II expression.

In addition to the specific effects on MHC II, other genes involved both directly and indirectly in the synthesis of the MHC II molecule and antigen presentation were also down-regulated, including the MHC II-associated invariant (Ii) chain. Newly synthesized MHC class II α and β chains associate with Ii for the assembly of MHC II complexes. Ii also assists in the export of the MHC II molecule from the endoplasmic reticulum and directs MHC II molecules to endosomes, where antigens are loaded onto the MHC II molecule (Stumptner-Cuvelette and Benaroch, 2002). A down-regulation of the Ii gene and protein could potentially result in improper folding and trafficking of the MHC II molecule and consequently a decrease in the expression of MHC II on the cell surface.

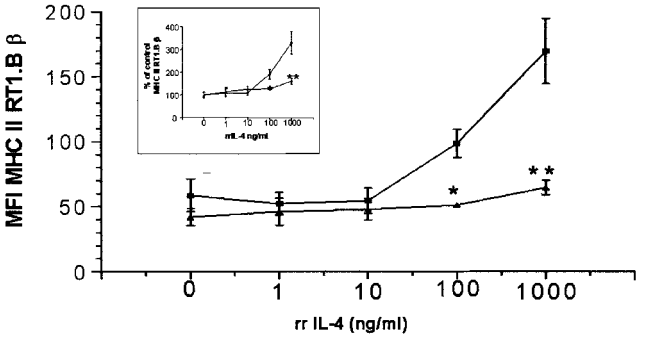

Fig. 4. Short-term morphine treatment inhibits ability of blood B lymphocytes to up-regulate MHC II RT1.B β after a 24-h in vitro treatment with rr IL-4. Blood leukocytes were isolated from the whole blood of morphine-treated rats (20 mg/kg; 2 h) and incubated in vitro with increasing doses of rr IL-4 (nanograms per milliliter). After 24 h, cells were stained with CD45RA-PE and MHC II RT1.B β-FITC for FACS analysis. Data are expressed as the mean fluorescent intensity (MFI) for saline-treated control group (■) and morphine treatment group (▲). Results are representative of one of three experiments. (*, $p < 0.05$ compared with saline control mice at 100 ng/ml rr IL-4; **, $p < 0.05$ compared with saline-treated control mice at 1000 ng/ml rr IL-4 by one-way analysis of variance; $n = 5–6$ samples per concentration and pooled leukocytes of three rats per sample)

A number of functional studies have indicated a potential role of the MHC II molecule in immunosuppression resulting from a single dose of morphine or other opioid receptor agonists. For example, Guan et al. (1997) determined that murine thymocytes stimulated with staphylococcal enterotoxin B exhibited a significant reduction in the production of IL-2 after administration of U50,488H (κ-opioid agonist) in vitro. IL-2 is a cytokine produced by antigen-activated T lymphocytes that acts in an autocrine manner to stimulate T lymphocyte proliferation (Lord et al., 2000). This study suggested that IL-2 production is suppressed as a result of the MHC II molecule's being down-regulated and therefore unable to mediate binding of staphylococcal enterotoxin B to the T lymphocyte receptor and MHC II molecule on the antigen-presenting cell. Furthermore, a single dose of morphine in vivo has also been shown to decrease antibody production against keyhole limpet hemocyanin from B lymphocytes, thereby decreasing humoral immune responses (Lockwood et al., 1996). MHC II molecules on B lymphocytes are responsible for presenting specific antigens to T lymphocytes, and activation of T lymphocytes results in secretion of cytokines that act in concert with CD40L to stimulate B lymphocyte proliferation and production of antibodies of different isotypes (Dadgostar et al., 2002). Collectively, these results suggest a potential

The mechanism whereby morphine decreases MHC II expression remains unknown. Morphine administration has been shown to be accompanied by an increase in several plasma factors that may contribute to the observed decrease in MHC II expression in blood leukocytes. For example, morphine has been shown to stimulate the transient release of nitric oxide (NO) in vitro (Stefano et al., 2001). Nitric oxide has been shown to cause a decrease in interferon-γ induced MHC II expression in monocytes in vitro (Grimm et al., 2002). Morphine has also been shown to increase prostaglandin E2 synthesis in monocytes/macrophages (Lu et al., 1996). Prostaglandins cause a decrease in interferon-γ induced MHC II expression by increasing PKA activity via an increase in cAMP and consequently causing an increase in the phosphorylation of CIITA (Class II transactivator) in monocyte cell lines (Li et al., 2001). Phosphorylation of CIITA by PKA results in a decrease in the transcription rate of MHC II. Studies have shown that corticosterone levels increase after a 2-h administration of morphine. Because glucocorticoids such as dexamethasone have been shown to decrease MHC II expression in dendritic cells after a 3-day incubation (Pan et al., 2001), the hypothalamus-pituitary-adrenal axis may play a role in the morphine-induced down-regulation of MHC II expression (Mellon and Bayer, 1999). In addition, in vitro studies have shown that interferon-γ induction of the MHC II molecule in peritoneal macrophages exposed to corticosterone was inhibited (Kimura et al., 1995).

mechanism by which both the cellular and humoral immune response may be severely suppressed by morphine. Furthermore, the inability of B lymphocytes to up-regulate key immune proteins, such as the MHC II molecule, after exposure to antigen-induced cytokine production may cause an increase in the susceptibility to bacterial and viral infections, such as HIV, in both drug abusers and patients receiving therapeutic morphine.

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