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MIP-1α Induces Differential MAP Kinase Activation and IκB Gene Expression in Human B Lymphocytes

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ABSTRACT

The chemokine macrophage inflammatory protein-1α (MIP-1α) stimulates migration of B cells and affects B cell immunoglobulin production. However, the molecular mechanisms by which MIP-1α modulates these biologic effects have not been completely defined. Previously, we demonstrated that treatment of B cells with MIP-1α induced the transcription factor, nuclear factor (NF)-κB, to bind to DNA, concomitant with the degradation of IκBα, a cytoplasmic inhibitor of NF-κB activation. Here, we report that MIP-1α treatment of tonsil B cells induced IκB gene expression that was dependent on MIP-1α-mediated activation of a pathway(s) involving NF-κB and phosphatidylinositol-3 kinase (PI3K). The NF-κB pathway is understood to be controlled in an autoregulatory fashion, so expression of IκB is thought to provide a means by which B cells modulate this pathway after stimulation with MIP-1α. Although the idea of NF-κB autoregulation is not novel, this is the first report to suggest the regulation of B cell gene expression by MIP-1α. In addition, we observed the activation of Jun N-terminal kinase (JNK) and p38 mitogenic-activated protein kinase (MAPK), but not extracellular signal-related kinase (ERK) in response to MIP-1α. Although p38 and NF-κB activity were both necessary for B cell migration, IκB gene expression was not affected by p38 inhibition, suggesting that p38 is involved in a separate MIP-1α-mediated signal transduction pathway.

INTRODUCTION

MACROPHAGE INFLAMMATORY PROTEIN-1α (MIP-1α) belongs to a family of structurally related proteins called chemokines (chemoattractant cytokines) that play multiple roles in attracting, activating, and suppressing various cells of the immune system.[1,2] MIP-1α belongs to the C-C chemokine family that shares a similar structure containing two conserved cysteine residues at nearly identical locations in the primary amino acid sequence.[3] In early studies, MIP-1α was shown to inhibit the proliferation of stem cells as well as epidermal keratinocytes.[4] More recently, it has been reported that MIP-1α can regulate HIV infection,[5] activate leukocytes,[6] control fetal development,[7,8] and induce mobilization and proliferation of hematopoietic progenitor cells.[9,10] MIP-1α is a known chemoattractant for T cells[11] and is involved in T cell signal transduction by inducing the activation of signal transducers and activators of transcription (Stat) proteins.[12] In human tonsil B cells, we have demonstrated that MIP-1α induces activation of the transcription factor nuclear factor (NF)-κB[13] as well as other signaling molecules, such as proline-rich tyrosine kinase (PYK-2) and phosphatidylinositol-3 kinase (PI3K).[14] MIP-1α also has been shown to induce B cell chemotaxis[11,14,15] and selectively enhance interleukin-4 (IL-4)-mediated IgE and IgG4 production in a dose-dependent manner.[16] However, the complete signaling pathway(s) by which B cell biologic events occur in response to MIP-1α are not fully understood.

B cell activation following antigen recognition,[17,18] CD40 ligation,[19] and cytokine stimulation[20,21] is known to rely on signaling cascades involving the activation of mitogen-activated protein kinases (MAPK). The activation of MAPK is also thought to link G-protein coupled receptors to the activation of specific transcription factors, resulting in the regulation of gene expression.[22] Signal transduction pathways activated by a number of chemokines have been shown to access members of the MAPK family, which includes extracellular signal-related kinase (ERK), p38, and Jun N-terminal kinase (JNK). Some ex-

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amples include IL-8 in neutrophils,[23] stromal cell-derived factor-1α (SDF-1α) in T cells, and megakaryoblasts[24,25] and MIP-3α in eosinophils.[26] In addition, chemokine-mediated signals that regulate chemotaxis of monocytes and eosinophils[27,28] are dependent on MAPK activation. Because engagement of MAPK pathways has been linked to PI3K and PYK-2 activity,[29,30] which we have demonstrated to be involved in MIP-1α signaling in B cells,[14] we investigated the role of MAPKs in B cell signaling following MIP-1α stimulation.

In addition to the activation of the PI3K and MAPK pathways, MIP-1α also induces the activation of NF-κB.[13] The NF-κB family of transcription factors includes p65/RelA, RelB, p105/p50, p100/p52, and c-Rel, each of which exists in an inactive state, bound to the inhibitor IκB in the cytoplasm of quiescent cells.[31,32] When the cell is stimulated, IκB is phosphorylated and rapidly degraded, allowing the nuclear translocation of NF-κB proteins. These proteins form homodimers or heterodimers, bind to regulatory regions of DNA, and participate in modulation of gene expression. The multifaceted interactions among members of the NF-κB family enable them to regulate a wide variety of genes. Such genes include those involved in cell growth (such as c-*myc*), cellular adhesion (such as *ICAM-1*), and the immune response (*IL-2* and antibody production).[32] We demonstrated previously that MIP-1α activates NF-κB transcription factors in tonsil B cells, and this appeared to correspond with IκB degradation in peripheral blood lymphocyte (PBL) B cells.[13] Although transcription factor activation has been associated with MIP-1α treatment in T and B lymphocytes,[12,13] the effect MIP-1α has on regulation of B cell gene expression has not been investigated.

In the present study, we demonstrate differential activation of three MAPK family members in response to MIP-1α. We report here that MIP-1α induced the activation of p38 and JNK but not the activation of ERK MAPK. Furthermore, MIP-1α activation of JNK led to the phosphorylation of c-Jun, which is known to dimerize and to create a form of the transcription factor, activator protein-1 (AP-1).[33] We also show that MIP-1α modulates the expression of the IκB gene in human B lymphocytes. MIP-1α-induced IκB gene expression was linked to the activation of NF-κB and PI3K but was not affected by p38 or ERK activity, suggesting that the MAPK pathway is separate from the pathway involving NF-κB and PI3K activation in response to MIP-1α. Additionally, we show that MIP-1α-induced B cell migration is dependent on the activation NF-κB and p38. This report extends our knowledge of the molecular components required for MIP-1α signaling in B cells and is the first to present evidence suggesting a direct role for MIP-1α in the modulation of B lymphocyte gene expression.

MATERIALS AND METHODS

Cell isolation and culture

Tonsil B cells were obtained from patients undergoing routine tonsillectomy. Patients were informed, and written consent was obtained by the surgical staff at Lawrence Memorial Hospital under the auspices of the University of Kansas Human Subjects Committee. Tonsil B cells were prepared as we have described previously.[34] Briefly, cells were minced and passed through a cell strainer. Cells were diluted in tissue culture phosphate-buffered saline (TC-PBS), pH 7.2 (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 10 mM dextrose, 0.68 mM $CaCl_2$, 0.5 mM $MgCl_2$), supplemented with 2% fetal bovine serum (FBS) (Atlanta Biologicals, Norcross, GA), 2 mM glutamine (GIBCO Life Technologies, Grand Island, NY), 50 U/ml penicillin (GIBCO), and 50 μg/ml streptomycin (GIBCO). Mononuclear cells from tonsil or peripheral blood were isolated by Ficoll-Paque™ Plus (Amersham Pharmacia Biotech, Uppsala, Sweden) density gradient centrifugation. Mononuclear cells were then separated into B cell and T cell populations by E-rosette depletion, and monocytes were removed from the B cell fraction by plastic adherence for 1 h in RPMI 1640 (Cellgro™, Mediatech, Inc., Herndon, VA) supplemented with 10% FBS, 2 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin at 37°C, 5% CO_2. B cells were then rested overnight in RPMI at 37°C, 5% CO_2. The purified B cell population was routinely >95% $CD22^+$ as measured by flow cytometry. The Ramos B cell line was obtained from American Type Cell Culture Collection (ATCC, Rockville, MD) and cultured in RPMI 1640 supplemented with 10% FBS, 2 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin.

Cell stimulation

Tonsil B cells were left nontreated or stimulated with MIP-1α (Peprotech, Norwood, MA) at the times and concentrations indicated in a volume of 500 μl of spent medium at 37°C on a rotator, washed once in TC-PBS, and lysed for 15 min on ice in 200 μl lysis buffer (1% Triton X-100, 10 mM Tris, pH 8.0, 140 mM NaCl, 0.2 mM Na_3VO_4, 100 mM NaF, 1.0 μg/ml leupeptin, 1.0 μg/ml pepstatin, and 1.0 mM PMSF). For inhibitor studies, cells were pretreated with parthenolide (Biomol, Plymouth Meeting, PA), SB202190, or PD98059 (Calbiochem, La Jolla, CA) at the indicated concentrations for 30 min at 37°C prior to MIP-1α treatment. In some experiments, *Staphylococcus aureus* Cowan's strain (SAC) (Calbiochem) was used as a B cell mitogen for positive control of activation. Typically, tonsil B cells were stimulated at concentrations of 5–20 $\times$ 10^6 cells/ml in a rotator at 37°C.

Western blotting

Cells were stimulated as described and lysed in NEB1X Lysis Buffer (New England Biolabs, Beverly, MA). SDS-PAGE and protein blotting were performed as we have described previously.[35] Briefly, 100 μg B cell lysate was resolved on 8% SDS-PAGE and transferred electrophoretically to a nitrocellulose membrane (Life Science Products, Denver, CO). Membranes were blocked for 1 h in 3% nonfat milk and 0.1% Tween-20 in Tris-buffered saline (50 mM Tris, 150 mM NaCl, pH 7) and probed with the indicated antibodies for 1 h in blocking solution. In some cases, blots were stripped by agitation in 0.1 M glycine for 15 min and then reprobed. Proteins were visualized by enhanced chemiluminescence (ECL) (Amersham, Arlington Heights, IL). Antiphosphospecific ERK, antiphosphospecific p38, antiphosphospecific Jun, and anti-p38 were purchased from New England Biolabs. Anti-ERK1,2 and anti-Jun were purchased from Transduction Laboratories (Lexington, KY). A JNK Kinase Assay Kit (New England Biolabs) was used to ob-

serve JNK activity by detecting phosphorylated c-Jun fusion protein as a substrate according to the manufacturer's protocol. Proteins were visualized by ECL.

RNA extraction

RNA was extracted by the vanadyl ribonucleoside complex (VRC) method as we have described previously.[36] Briefly, cells were pelleted and resuspended in 200 μl TSM (10 mM Tris, pH 7.6, 0.15 M NaCl, 2 mM $MgCl_2$) lysis buffer (TSM, 0.5% NP-40, 10 mM VRC) and lysed on ice for 5 min. Nuclei were removed by centrifugation, and the cytoplasmic fraction was separated and combined with 200 μl TSE (10 mM Tris, pH 7.6, 0.15 M NaCl, 5 mM EDTA, and 1% SDS). RNA was extracted with two phenol:chloroform:isoamylalcohol (25:24:1) extractions and one chloroform:isoamylalcohol (24:1) extraction. RNA was precipitated overnight at −20°C in 1:16 volume 3 M sodium acetate and 2.2 volumes 100% ethanol. This precipitation was repeated once, and a third precipitation was performed overnight at −20°C in 1:25 volume 5 M NaCl and 2.2 volumes 100% ethanol. After final precipitation, RNA was resuspended in RNase-free dH_20, and absorbance readings were taken at 260 and 280 nm. RNA (10 μg) was electrophoresed on a 1% denaturing agarose gel containing 5% formaldehyde and 1× MOPS (20 mM morpholinopropane sulfonic acid, 10 mM sodium acetate, 1 mM EDTA, pH to 7.0) to assess ribosomal RNA integrity.

Gene expression analysis

Tonsil B cells were treated with or without MIP-1α for 3 h in a rotator at 37°C. RNA was extracted as described, and cDNA was synthesized using SuperScript II reverse transcriptase (RT) (GIBCO). The cDNA probe was biotin labeled and hybridized to premanufactured arrays using the manufacturer's protocol for the GEArray™ Q series KIT (SuperArray Simplicity, Bethesda MD). Hybridized biotin-labeled probe was visualized by ECL, and differential gene expression was determined by pixel summation densitometry using Bit Image (BioImaging Technologies, Brookfield, WI).

RT-PCR

Tonsil B cells were treated with or without MIP-1α for 30 min in a rotator at 37°C. RNA was extracted as described, and cDNA was synthesized by combining 2 μg cytoplasmic RNA, 5 U RNAsin (Promega, Madison, WI), and 0.5 μg oligo(dT) at 35°C and incubating for 60 min. SuperScript II RT (200 U) was then added along with 0.2 mM dNTP (Finnzyme, Watertown MA) and incubated at 35°C for 90 min. The RT reaction was stopped by heating the reaction to 95°C for 5 min. PCR was performed using the DyNAzyme EXT™ PCR kit (Finnzyme) according to the manufacturer's protocol. Amplification was performed in a Perkin-Elmer (Alameda, CA) thermocycler under the following conditions: 1 hold at 94°C for 2 min, 30 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 2.5 min, and a final hold at 72°C for 7 min. The sequences of primers (GIBCO) used for PCR were: IκB: forward, 5′-CAGATCAGCTCCTAAGGGGG-3′, reverse 5′-CTCCAGCCTGCTTCTGTCTC-3′; GAPDH: forward, 5′-GAATCTACTGGCGTCTTCACC-3′, reverse 5′-GTCATGAGCCCTTCC- ACGATGC-3′.

Migration assay

B cells were pretreated in the presence or absence of 0.1–10 μM parthenolide, 0.2–20 μM PD98059, or 0.3–30 μM SB202190 for 30 min. Cells (50,000) were placed into the top wells of a migration chamber constructed using one described by others[37] (Neuro Probe, Bethesda, MD) as a model. The membrane used was Nucleopore Polycarbonate (Costar, Cambridge, MA) with a 5-μM pore size. The upper chamber volume allows for a 50-μl sample, and the lower chamber volume was 20 μl. MIP-1α was placed into the bottom wells at a concentration of 100 ng/ml. Cells were allowed to migrate for 3 h at 37°C, and the number of migrated cells was enumerated microscopically.

RESULTS

MIP-1α has a differential effect on activation of ERK and p38 MAPKs

MAPKs are thought to be important signaling molecules downstream of G-protein coupled receptors[22] and have been implicated in chemokine-induced signal trasduction pathways.[24,26] This led us to investigate the possible role of MAPK activation in B cell signaling in response to MIP-1α. Here, we show that MIP-1α treatment of tonsil B cells for up to 30 min did not cause phosphorylation of ERK (Fig. 1A). However, ERK was activated by 15 min with antigen stimulation (SAC), which served as a positive control for ERK activity (Fig. 1A). In contrast, MIP-1α treatment of tonsil B cells resulted in a modest increase in p38 phosphorylation by 2.5–5 min compared with nontreated cells (Fig. 1B,C). These data suggest a differential involvement of p38 and ERK MAPKs in the B cell signaling cascade induced by MIP-1α.

MIP-1α-mediated B cell migration is dependent on p38 activity but does not rely on ERK activation

To further investigate the roles of ERK and p38 in MIP-1α-induced signaling, we examined the effect of ERK and p38 inhibition on MIP-1α-mediated B cell migration. Figure 1D shows that MIP-1α-induced B cell migration was approximately 3-fold greater than spontaneous migration (open bars). Tonsil B cells pretreated with SB202190 (black bars), which inhibits p38 kinase activity, decreased MIP-1α-mediated migration in a dose-dependent manner, with maximal inhibition occurring at a concentration of 30 μM. In contrast, PD98059 (stippled bars), which inhibits the activation of ERK, had no effect on MIP-1α-mediated B cell migration at any of the concentrations tested. To determine if the effect of these inhibitors was due to cytotoxicity, cell viability was assessed by trypan blue exclusion. None of the concentrations of SB202190 or PD98059 tested had a negative effect on cell viability during these times (data not shown). These data corroborate the data from Figure 1A,B and further suggest that MIP-1α-mediated B cell migration is dependent on a signal transduction pathway that requires p38 activity but not the activation of ERK.

MIP-1α induces an increase in JNK activity in tonsil B cells

Because of our observations showing that MIP-1α induces the phosphorylation of p38 but not ERK (Fig. 1), we investi-

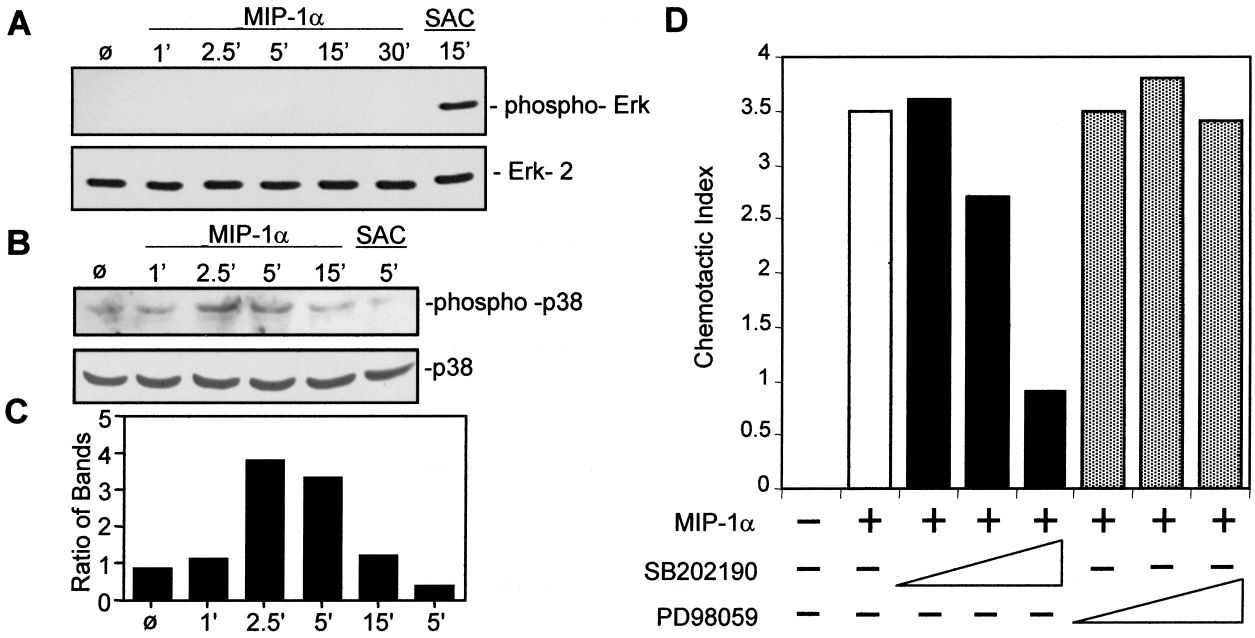

FIG. 1. MIP-1α induces the activation of p38 but not ERK MAPK, which have differing roles in MIP-1α-mediated B cell migration. Tonsil B cells were left nontreated or stimulated with MIP-1α for the indicated times. Blots were probed with either antiphospho-ERK (**A**) or antiphospho-p38 (**B**) and visualized by ECL. The same blots were stripped, tested to certify removal of residual signals, then reprobed with anti-ERK2 (**A**) or anti-p38 (**B**). A ratio of phospho-p38 to total p38 is presented in graphic form for comparison (**C**). (**D**) PBL B cells were introduced into the top wells of the migration chamber, having been first nontreated (open bars) or pretreated for 30 min with 0.3, 3.0, or 30.0 μM SB202190 (black bars) or 0.2, 2.0 or 20.0 μM PD98059 (stippled bars). The lower chamber contained medium alone or 100 ng/ml MIP-1α. Cells were allowed to migrate for 3 h at 37°C and enumerated microscopically. Results are expressed as chemotactic index (number of cells migrated in response to the sample divided by the number of cells migrated to the control). The typical number of cells migrated to MIP-1α was 10,000 cells/ml. Data are representative of more than three separate experiments.

gated whether MIP-1α had an effect on the activity of the MAPK family member JNK. Based on *in vitro* kinase assays, MIP-1α induced an increase in the phosphorylation of the JNK substrate c-Jun by 1 min (Fig. 2). These results reinforce the idea that MIP-1α has a differential effect on the activation of MAPK family members. Furthermore, as c-Jun is part of the transcription factor AP-1,[33] these data suggest that MIP-1α may have an effect on B cell gene expression.

MIP-1α induces an increase in IκB gene expression in tonsil B cells

Based on our previous data showing that MIP-1α regulates the activation of the transcription factors NF-κB[13] and c-Jun (Fig. 2), we investigated the possible role MIP-1α may have in regulating B cell gene expression. To increase the number of genes that could be analyzed simultaneously, we employed a nitrocellulose gene array membrane. For Figure 3A,B, two identical gene array membranes were probed with labeled cDNA from mRNA extracted from tonsil B cells that were left either nontreated (tetrads 1–4) or treated with 10 ng/ml MIP-1α (tetrads 5–8) for 3 h. In addition to the regulation of other genes (not shown), we observed an approximate 2.4-fold increase in the signal intensity of the IκB gene tetrad after treatment with MIP-1α compared with nontreated cells (Fig. 3A, compare 1 and 5). Differences were determined by densitometry and normalized to the manufacturer's control gene tetrads (Fig. 3B, tetrads 1–8), which were not regulated by MIP-1α.

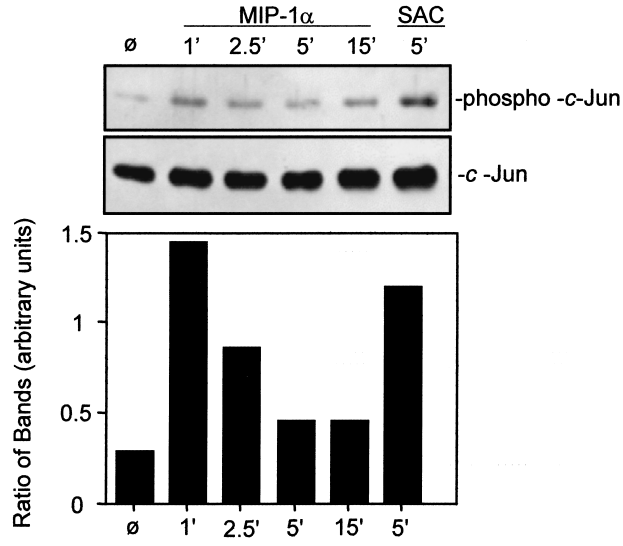

FIG. 2. MIP-1α induces JNK activation. Tonsil B cells were left nontreated or stimulated with MIP-1α for the indicated times. B cell lysates (500 ng) were immunoprecipitated with anti-c-Jun then subjected to an *in vitro* JNK assay, as described in Materials and Methods, resolved on 8% SDS-PAGE, then transferred to a nitrocellulose membrane. The membranes were probed with antiphospho-c-Jun and visualized by ECL. Blots were then stripped and reprobed with anti-c-Jun and visualized by ECL. A ratio of phospho-c-Jun/total c-Jun is presented in graphic form for comparison. Data are representative of more than three separate experiments.

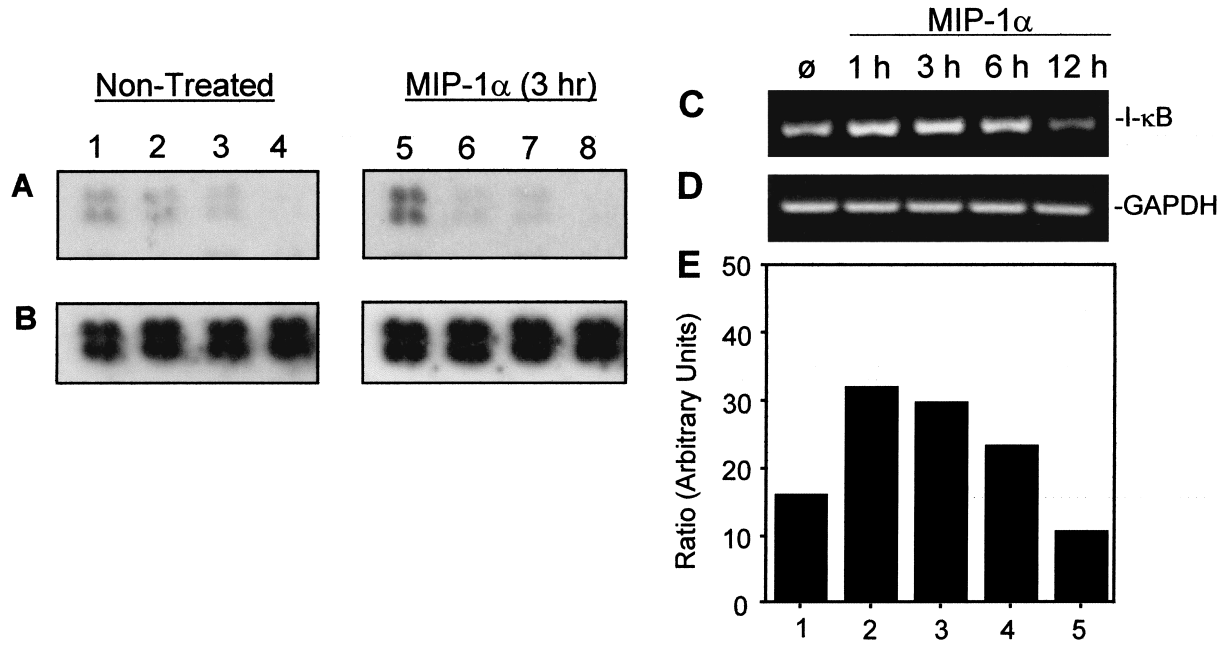

FIG. 3. MIP-1α induces an increase in IκB gene expression. Tonsil B cells were left nontreated (**A, B**, lanes 1–4) or treated with 10 ng/ml MIP-1α (**A, B,** lanes 5–8) for 3 h at 37°C. RNA was extracted and used to prepare a biotin-labeled cDNA probe. Known genes are arranged in tetrad formation. The gene tetrads in **A**, lane 1, and **A**, lane 5 represent IκB. Differential expression of experimental genes was determined by densitometric comparison to a control gene, cyclophilin A (**B**, lanes 1–8). The other tetrads shown represent nonmetastatic cell 4 expressed protein (**A**, lanes 2 and 6), platelet-derived growth factor-α (**A**, lanes 3 and 7), and platelet-derived growth factor-BB (**A**, lanes 4 and 8). The same RNA was also analyzed for study of kinetic IκB gene expression by RT-PCR using IκB primers (**C**) and GAPDH primers (**D**). (**E**) A ratio of IκB expression/GAPDH expression is presented in graphic form for comparison. Data are representative of three separate experiments.

To support the observation that MIP-1α caused increased expression of IκB, we used specific IκB DNA primers to examine gene expression by RT-PCR. Here, IκB expression was increased following 1 h of MIP-1α treatment (Fig. 3C, first and second lanes) and was reduced slightly after 6 h (Fig. 3C, fourth lane). The level of MIP-1α-induced IκB expression returned to that of nontreated cells between 6 and 12 h (Fig. 3C, compare first lane 1 with fourth and fifth lanes). A graphic representation of the ratio of IκB bands/GAPDH bands (Fig. 3D) is presented in Figure 3E.

MIP-1α-mediated B cell migration is dependent on NF-κB activity

Thus far, we have demonstrated that MIP-1α-mediated B cell migration was dependent on the activation of both p38 (Fig. 1C) and PI3K[14] and that MIP-1α activates a pathway leading to NF-κB.[13] We also determined that formation of protein/DNA complexes containing NF-κB was almost completely inhibited in cells pretreated with 100 μM parthenolide,[13] which prevents activation of NF-κB by inhibiting IκB kinase.[38] In an effort to link these observations, we investigated the role that NF-κB activity might play in MIP-1α-mediated B cell migration. B cell migration was inhibited in a dose-dependent manner when B cells were pretreated with as little as 0.1 μM parthenolide (Fig. 4) and was nearly eliminated with parthenolide concentrations >0.5 μM. This suggests that treatment of B cells with higher concentrations of parthenolide may inhibit

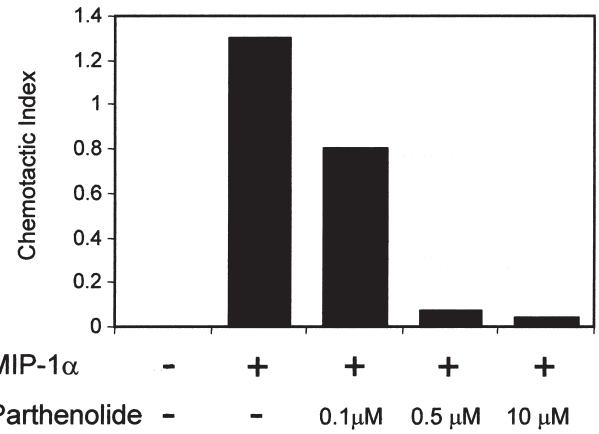

FIG. 4. MIP-1α-induced B cell migration is dependent on NF-κB activity. Tonsil B cells were introduced into the top wells of the migration chamber, having been left nontreated or pretreated for 30 min with 0.1, 0.5, or 10.0 μM parthenolide. The lower chamber contained medium alone or 100 ng/ml MIP-1α. Cells were allowed to migrate for 3 h at 37°C and were enumerated microscopically. Results are expressed as chemotactic index (number of cells migrated in response to the sample divided by the number of cells migrated to the control). The typical number of cells migrated to MIP-1α was 3×10^5 cells/ml. MIP-1α-induced migration was significant compared with medium alone, as determined by the Student's *t*-test. Data are representative of three separate experiments.

other important pathways in addition to or downstream of NF-κB. Not suprisingly, treatment of cells with 100 μM parthenolide for 12–24 h led to a reduction in cell viability, as assessed by trypan blue exclusion. However, parthenolide did not affect cell viability after 3 h at concentrations <10 μM (data not shown). These data suggest that NF-κB activity is involved in a migratory signal transduction pathway induced by MIP-1α in B cells.

IκB gene expression is dependent on PI3K and NF-κB but independent of p38 and ERK MAPKs

To determine the involvement of specific signaling molecules in MIP-1α-mediated IκB gene modulation, we pretreated tonsil B cells with specific inhibitors of NF-κB, PI3K, p38, and ERK MAPKs. The effect this inhibition had on the expression of IκB was determined by RT-PCR using specific IκB primers. Figure 5 shows, once again, that IκB gene expression is upregulated in B cells treated with MIP-1α. However, when the NF-κB and PI3K dependent pathways were inhibited with parthenolide or wortmannin, respectively, the expression of IκB was reduced to levels below that for nontreated cells. In contrast, inhibitors of p38 or ERK had no effect on MIP-1α-mediated IκB gene expression. This suggests that MIP-1α-mediated IκB gene expression is dependent on a pathway that involves both NF-κB and PI3K activation but is independent of p38 and ERK activity.

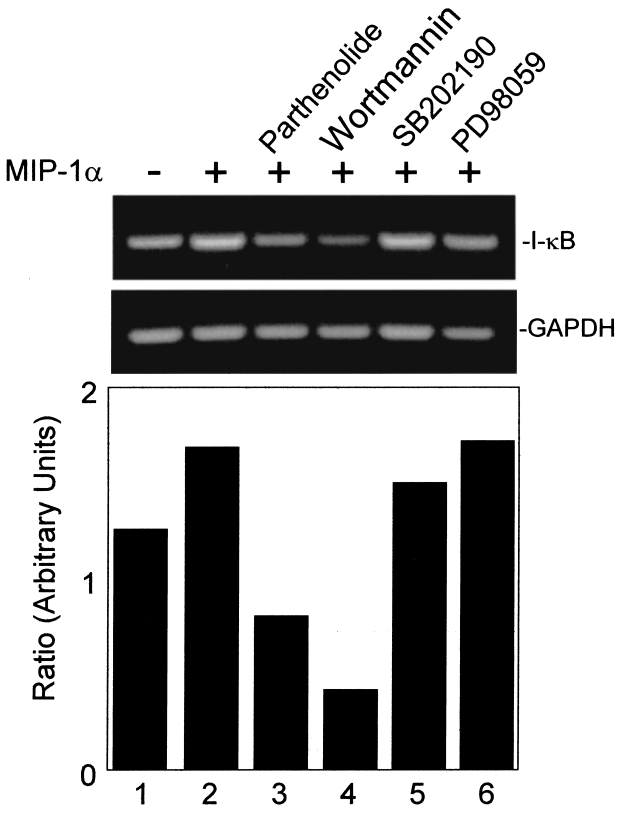

FIG. 5. The effect of signaling inhibition on IκB gene expression. Tonsil B cells were left nontreated or pretreated with 10 μM parthenolide, 50 nM wortmannin, 3 μM SB202190, or 20 μM PD98059 for 30 min at 37°C. After pretreatment, cells were left nontreated or treated with 10 ng/ml MIP-1α at 37°C for 3 h. RNA was extracted and used to prepare cDNA. PCR was performed using IκB primers and GAPDH primers as indicated. A ratio of IκB expression/GAPDH expression is presented in graphic format. Data are representative of two separate experiments.

DISCUSSION

MAPKs are involved in chemokine-induced signal transduction pathways[26] as well as chemotaxis in a number of cell types.[27,28] However, these cell types differ in both function and chemokine-mediated response from B cells. Therefore, we investigated whether MAPK activation was important in MIP-1α-mediated signaling and chemotaxis in human tonsil B cells. Here, we observed that p38 is activated within 2.5 min after MIP-1α treatment, and inhibition of p38 kinase activity led to inhibition of MIP-1α-induced B cell migration. This suggested that p38 is an important component in MIP-1α-mediated signaling. We also observed the activation of JNK and the resulting phosphorylation of the transcription factor c-Jun. In contrast, we were not able to detect the phosphorylation of ERK in response to MIP-1α. Similarly, inhibition of ERK activation had no effect on MIP-1α-mediated B cell migration. Thus, ERK does not appear to be involved in MIP-1α signaling in B cells.

MIP-1α-induced activation of the transcription factor c-Jun suggests that B cell gene expression may be regulated as a result of MIP-1α signaling. We have observed previously MIP-1α-induced activation of the transcription factor NF-κB in human B cells and binding of NF-κB-specific nuclear factors to κB DNA sequences.[13] Because MIP-1α induced NF-κB activation and other transcription factors downsteam of JNK and possibly p38, we examined the effects of MIP-1α on B cell gene expression. By gene array analysis and RT-PCR, we observed an MIP-1α-mediated increase in IκB gene expression. Kinetically, this increase in IκB gene expression correlated with our previous observation of MIP-1α-mediated IκB protein degradation,[13] suggesting that MIP-1α is simultaneously directing both an increase in IκB gene expression and a decrease in IκB protein levels. This apparent paradox is explained by previous work indicating that degradation of IκB and the resulting activation of NF-κB occur in an autoregulatory manner.[39,40] Therefore, MIP-1α-mediated NF-κB activity is inhibited soon after activation, presumably in an effort to minimize the duration of its effects.

Further supporting the idea of an autoregulatory relationship, MIP-1α-mediated IκB gene expression was dependent on a signal transduction pathway involving the activation of NF-κB. Unexpectedly, MIP-1α regulation of IκB gene expression was also dependent on activation of PI3K, as inhibition of PI3K activity caused a reduction of IκB expression. Conversely, MIP-1α-induced activation of the p38 MAPK pathway had no effect on IκB gene expression but, instead, seemed to play an important role in an MIP-1α-initiated B cell migratory signal. Our data also suggest that NF-κB activity may play a role in the MIP-1α-induced migration signal. Thus, MIP-1α appears to activate several signaling cascades in human B cells, producing multiple biologic effects.

Our observation that MIP-1α activates transcription factors and has the ability to regulate gene expression in B lymphocytes is novel, although not unexpected, and represents a previously undescribed role for MIP-1α in B cell biology. By the work described here, we also have begun to elucidate some of the signaling events that lead to MIP-1α-induced B cell migration. Therefore, this work expands our understanding of the mechanisms required for MIP-1α-induced B cell biologic activity and is the first to present evidence for the function MIP-1α has in regulation of B cell gene expression. Besides a lack of general insight into the specific relationship between MIP-1α and B cells, the role chemokines have in gene expression has only begun to be examined. Therefore, this work has implications for all chemokines and their relationship to the immune system and cell biology as a whole.

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