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Negative Regulation of Bacterial fMLP-induced Pro-inflammatory Cytokine Gene Expression via MKP-1-dependent Inhibition of NF-κB

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Abstract

The stimulated release of pro inflammatory cytokines (i.e. TNFα and IL-1β) by bacterial fMet-Leu-Phe- (fMLP) in human peripheral blood monocytes and macrophages is an important component of the inflammatory process and plays a critical role in sepsis and septic shock. The signaling mechanisms utilized by fMLP to stimulate the release of cytokines are still incompletely understood. We previously demonstrated that the key positive signaling pathways involved in the activation of nuclear factor κB (NF-κB) and the resultant inflammatory response by bacterial fMLP. We now present evidence that MKP- 1, a MAP kinase phosphatase, negatively regulates fMLP-induced NF-κB-dependent inflammatory response both *in vitro* and *in vivo*. Interestingly, our data indicate that anti- inflammatory agent, dexamethasone, a synthetic member of the glucocorticoid class of hormones, inhibits fMLP-induced TNFα in monocytes through increasing MKP-1 expression, which occurs at the level of IKK *via* the glucocorticoid receptor (GR). These results suggest a potential role of MKP-1 in the regulation of pro inflammatory cytokine expression, and provide the first evidence that such regulation may occur through the NF-κB pathway in fMLP-stimulated human peripheral blood monocytes.

Keywords: Blood monocytes; Signaling mechanisms; Signaling pathways; Inflammatory response; Bacterial components; Gene expression; Pro-inflammatory

Introduction

The inflammatory response to bacteria or bacterial components produced by infecting bacteria involves leukocyte transcription activation and specific gene expression. Pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNFα) and interleukin (IL)-1β are considered major pro-inflammatory cytokines during bacterial infections. Although beneficial to host defense at the time of infection, cytokine production, when inappropriate or exaggerated, can contribute to numerous pathological conditions [1-4]. To avoid detrimental activation of leukocytes in bacterial infections, intracellular signaling must be tightly regulated. Negative feedback regulation plays a critical role in regulation of leukocytes by directly inhibiting the key signaling pathway(s), which in turn leads to subsequent inhibition of the production of inflammatory cytokines [5]. MKP-1, a mitogen activated protein (MAP) kinase phosphatase [6-8], which is expressed as a 39 kDa protein in a wide variety of cell types, recently has been shown to act as a negative regulator for inflammatory cytokine expression [9-14]. MKP-1 has been shown to dephosphorylate p38 and C-Jun N-terminal kinase (JNK), with less effect on extracellular signal-regulated kinases (ERK) [15,16]. The molecular mechanisms of MKP-1 in the regulation of NF-κB- dependent inflammatory cytokine gene expression still remain unclear.

Glucocorticoids are highly effective in the control of many inflammatory and immune diseases. In addition to their broad use in the treatment of immune and inflammatory diseases, adjunctive glucocorticoid therapy has also been used in a variety of bacterial infections, including sepsis and septic shock [17-19]. Because of the importance of glucocorticoids in the treatment of immune and inflammatory diseases as well as bacterial infections, much effort has been made toward demonstrating the effect of glucocorticoids on inflammatory cytokine gene expression [20-23]. While glucocorticoids may be administered during severe septic shock to augment blood pressure (through the support of adrenal function), the molecular basis for any inhibitory effect of glucocorticoids directly on bacterial infections is still not fully understood.

In the past few years, we focused on investigating the key positive signaling pathways involved in activation of nuclear factor κB (NF-κB) and the resultant inflammatory response by bacterial component N-formylated peptides (fMLP) [21-26]. Recently many laboratories found that MKP-1 negatively regulates the inflammatory cytokines production [11]. We therefore investigated the role of MKP-1 in the fMLP- stimulated signaling events that lead to pro-inflammatory cytokine gene expression in human peripheral blood monocytes. In this report we show that bacterial fMLP induces NF-κB-dependent inflammatory cytokines (TNFα and IL-1β) expression. At the same time, fMLP also stimulates the expression of MKP-1, which in turn terminates inflammatory response through inactivating NF-κB

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to prevent overactive inflammatory responses during bacterial infection. Interestingly, dexamethasone, a glucocorticoid used clinically as an anti-inflammatory agent, inhibited fMLP-induced TNFα in monocytes through increasing MKP-1 expression, which occurs at the level of IKK via the glucocorticoid receptor. These studies provide new insight into the molecular mechanism of inflammation and may lead to novel therapeutic strategies for restraining the production of pro-inflammatory cytokines in patients with bacterial infections.

Materials and Methods

Reagents

FMLP, Dexamethasone and RU486 were obtained from Sigma (St. Louis, MO). Triptolide was obtained from Calbiochem (San Diogo, CA). A rabbit polyclonal antibody against MKP-1 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). An antibody against phospho-IκBα and an antibody against IKK were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Preparation of monocytes from human peripheral blood and monocytic cell lines

Heparinized human peripheral blood from health donors was fractionated on Percoll (Pharmacia) density gradients. Monocytes were prepared from the mononuclear cell population as described [27]. The monocytic cell line THP-1 cells were differentiated by incubation with 1, 25(OH) 2D3 for 3 days [28]. THP1 cells were cultured in RPMI 1640 (Irvine Scienfic, Santa Ana, CA) with 10% (V/V) heat-inactivated fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM; Irvine Scienfic, Santa Ana, CA) and 2-mercaptoethanol (complete media).

Real-Time Quantitative PCR (RTQ-PCR) analysis

Cells were stimulated with fMLP for 2 hours. Total RNA was isolated by using TRIzol reagent (Invitrogen) by following the manufacturer's instructions. For the reverse transcription reaction, TaqMan reverse transcription reagents (Applied Biosystems) were used. The reverse transcription reaction was performed for 60 min at 37°C, followed by 60 min at 42°C by using oligo (dT) and random hexamers. PCR amplification was performed by using TaqMan Universal Master Mix. Pre developed TaqMan assay reagents (probe and primer mixture of TNFα, IL-1β or MKP-1) were used to detect expression of the gene. In brief, reactions were performed in duplicate containing 2 x Universal Master Mix, 2 μl of template cDNA, 200 nM primers, and 100 nM probe in a final volume of 25 μl, and they were analyzed in a 96-well optical-reaction plate (Applied Bio systems). Probes include a fluorescent reporter dye, 6-carboxyfluorescein (FAM), on the 5' end and labeled with a fluorescent quencher dye, 6-carboxytetramethyl-rhodamine (TAMRA), on the 3' end to allow direct detection of the PCR product. Reactions were amplified and quantified by using an ABI 7500 sequence detector and the manufacturer's corresponding software (Applied Biosystems). Relative quantity of TNFα, IL-1β or MKP-1 mRNA was obtained by using the comparative Ct Method (for details, see User Bulletin 2 for the ABI PRISM 7500 sequence-detection system) and was normalized by using pre developed TaqMan assay reagent human cyclophilin as an endogenous control (Applied Biosystems).

Immunoprecipitation and immunoblotting

Cell lysates were incubated with an appropriate amount of antibody for three hours and then precipitated following absorption onto protein A-Sepharose (Amersham, Arlington Heights, IL). Precipitates were washed three times, separated by SDS-PAGE and transferred to Hybond-ECL nitrocellulose (Amersham, Arlington Heights, IL). Filter strips were incubated with primary antibody for 30 min at room temperature, followed by addition of peroxidase-conjugated IgG (1:10,000 dilution, 30 min) and then analyzed for peroxidase using enhanced chemiluminescence reagents (DuPont/NEN Research Products, Boston, MA).

Plasmids, transfection and luciferase assay

The (plasmids IKKβ, and NF-κB luciferase were described previously [26]. All transient transfections were carried out in triplicate using amaxa electroporation technique (Amaxa Biosystems, Gaithersburg, MD) following the manufacturer's protocol. The transfected cells were treated with fMLP for 5 h before being harvested for luciferase assay. The luciferase activity was measured by utilizing the Dual-Luciferase Reporter Assay System (Promega, Madison, WI) according to the protocol provided and the Monolight 3010 luminometer (BD Biosciences/ Pharmingen San Jose, CA).

ELISA

The media stimulated with fMLP (100 nM) were collected and secreted TNFα was measured by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Genzyme Corp.) according to the manufacturer's recommended protocol. The quantities of secreted TNFα in the test samples were determined by using a standard curve generated with purified TNFα.

Small interfering RNA (siRNA) approach

Custom designed small interfering RNA duplexes for MKP (siRNA MKP-1), and siRNA controls (non-targeting and RISC-free) were purchased from Dharmacon (Lafayette, CO). A final concentration of 100 nM of siRNA MKP-1 or non-target or RISC free siRNA was transfected in to the human monocytes using amaxa electroporation technique (human monocyte nucleofector kit, Amaxa Bio systems, Gaithersburg, MD). 30 hours post transfection they were stimulated with fMLP, mRNA fold induction was analyzed by QRT- PCR.

Mouse and animal experiments

C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME), and all animal experiments were approved by the Institutional Animal Care and Use Committee at the Scripps Research Institute and Southern Medical University. Mice were treated intranasally with fMLP (0.5 mg/kg) in 50 μl of sterile PBS (control), administered under light anesthesia. Mice were sacrificed after fMLP inoculation, and total RNA was isolated from the lung tissues using TRIzol® Reagent following manufacturers' instruction. Reverse transcription and Q-PCR analysis of mouse TNFα and MKP-1 were conducted as described above.

Statistical analysis

Statistical analysis was performed with Student t-test. P values of less than 0.05 were considered statistically significant.

Results

MKP-1 is a negative regulator for bacterial fMLP-induced inflammatory cytokine gene expression in human blood monocytes and in mice in vivo: To determine whether MKP-1 acts as a negative regulator for inflammatory cytokine gene expression by bacterial fMLP, we examined the effects of MKP-1 on TNFα and IL-1β expression induced by fMLP in human blood monocytes by using a siRNA approach. We first confirmed the efficiency of MKP-1-specific siRNA (siRNA-MKP-1) in reducing MKP-1 expression in human blood monocytes co-transfected with siRNA-MKP-1 or empty vector by using the Amaxa System. As expected, the endogenous MKP-1 protein was markedly reduced by siRNA-MKP-1 (Figure 1A).

We then assessed the effect of siRNA-MKP-1 on TNFα or IL-1β expression by fMLP. As shown in Figure 1, MKP-1 knockdown by siRNA-MKP-1 greatly enhanced induction of TNFα or IL-1β by fMLP in human blood monocytes (Figure 1B) and THP1 cells (Figure 1C), respectively. Similarly, induction of TNFα and IL-1β stimulated by other known inflammatory cytokine inducers LPS and peptidoglycan was also enhanced by siRNA- MKP-1 (data not shown). To further confirm whether MKP-1 is also negatively regulation of cytokine in vivo, we next determined the effects of MKP-1 in fMLP-induced TNFα expression in the lungs of the mice. As shown in Figure 1D, 0.5 n mol of siRNA-MKP-1 greatly enhanced induction of TNFα by fMLP in the lungs of the mice. Taken together, these in vitro and in vivo data indicate that MKP-1 is indeed a negative regulator for inflammatory cytokine expression induced by bacterial fMLP.

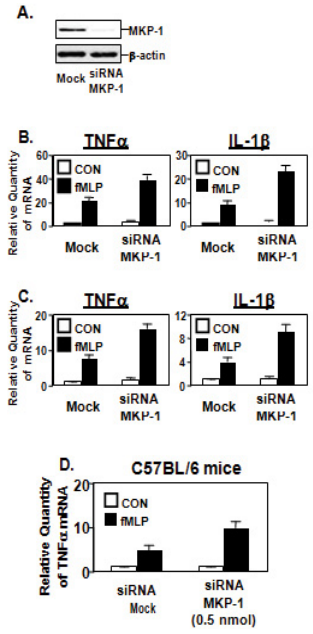

Figure 1: MKP-1 is a negative regulator for bacterial fMLP-induced inflammatory cytokine gene expression in human blood monocytes and in the lungs of C57BL/6 mice *in vivo*. Human blood monocytes (A) and (B) or THP1 cells (C) were co-transfected with siRNA-MKP-1 or control siRNA by using the Amaxa System. 30 hours post transfection, cells were stimulated with fMLP (100 nM) for 2 h and the endogenous MKP-1 protein levels were markedly reduced by siRNA-MKP-1 (A). (B) and (C) siRNA-MKP-1 enhanced the induction of TNFα (left panel) and IL-1β (right panel) induced by fMLP at the mRNA level as assessed by Q-PCR in human blood monocytes (B) and THP1 cells (C). (D) siRNA-MKP-1 enhanced the induction of TNFα *in vivo*. Mice were i.v. injected with siRNA-MKP-1 (0.5 nmol) for 24 h, then were treated intranasally with fMLP (0.5 mg/kg) in 50 μl of sterile saline. TNFα expression was induced by fMLP in the lungs of C57BL/6 mice *in vivo*. Values are means + S.D. (n=5).

MKP-1 in induced by fMLP in vitro and in vivo: Because a variety of genes involved in the inflammatory response undergo changes in expression pattern after initiation of inflammation, and the endogenous expression of MKP-1 is relatively low in monocytes/macrophages, we hypothesized that MKP-1 is induced by a variety of inflammation stimuli such as bacteria and bacterial components/products. We thus tested our hypothesis by assessing the effects on MKP-1 expression of fMLP, a highly potent inducer of inflammatory cytokines. As shown in Figure 2, fMLP strongly induced MKP-1 expression at the protein level in human monocytes (A), THP1 cells (B, upper panel) and RAW274.7 cells (B, lower panel) by Western blotting analysis. Interestingly, only the MKP- 1 isoform (not MKP-3 or 5) is induced by fMLP in human monocytes (Figure 2A, right panel).

To test whether MKP-1 is also induced in vivo, we next determined the effects of fMLP on MKP-1 expression in the lungs of the mice. As shown in Figure 2C, fMLP induced MKP-1 expression in a time-dependent manner. The expression of MKP-1 was greatly up- regulated at 1 h, and returned to baseline level by 4 h after inoculation of fMLP. Collectively, these data demonstrate that MKP-1 is induced by bacterial fMLP in vitro and in vivo.

Induction of MKP-1 correlates with the inactivation of NF-κB in fMLP-stimulated monocytes: The kinetics of NF-κB activation in human blood monocytes stimulated with fMLP (100 nM) were examined by Western blotting using Abs specifically recognizing

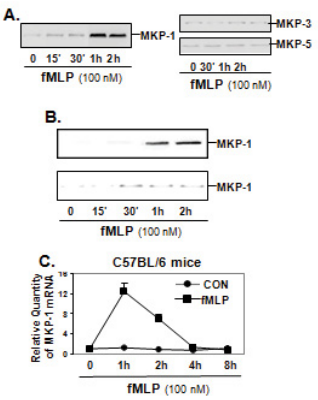

Figure 2: MKP-1 is induced by fMLP *in vitro* and *in vivo*. A, only MKP-1 isoform, not MKP-3 or MKP-5, was induced by fMLP in human blood monocytes (A), THP1 cells (B) upper panel) and RAW274.7 cells (B, lower panel) by Western blotting analysis. (C) MKP-1 expression was induced by fMLP in the lungs of C57BL/6 mice *in vivo* in a time-dependent manner. Values are the means ± S.D. (n=5).

phosphorylated IκBα (Figure 3A). NF-κB was rapidly activated by fMLP, reaching its maximal activities within 15 min. The activity then decreased and plunged to nearly basal levels by 60 min. MKP-1 protein in human monocytes was examined by Western blotting (Figure 3A, lower panel). In unstipulated cells, the MKP-1 protein level was very low, essentially below the limit of detection. In response to fMLP stimulation, MKP-1 protein increased dramatically, reaching its maximal level about 1 h, and then decreased slightly. The reciprocal relationship between MKP-1 and the NF-κB, supports the notion that MKP-1 may play a role in the inactivation of NF-κB. To further examine the effect of MKP-1 on the inactivation of NF-κB and regulation of cytokine gene expression, a mammalian vector expressing MKP-1 was transfected into human monocytes. The cells expressing MKP-1 exhibited a profoundly attenuated NF-κB luciferase activity (Figure 3B), and a reduced TNFα mRNA level (Figure 3C) in response to fMLP stimulation. To further understand the MKP-1 induction and NF-κB inactivation, we used that triptolide, a diterpenoid triepoxide, that potently blocked MKP-1 induction by fMLP (Figure 3D). This blockade of MKP-1 protein accumulation was associated with a reciprocal increase in the level of phosphorylated IκBα (Figure 3D). Our results suggest that triptolide completely blocked MKP-1 induction and prevented the inactivation of NF-κB. These observations strengthen the link between MKP-1 induction and the inactivation of NF-κB. Taken together, these results indicate that expression of MKP-1 inhibits the production of TNFα via inactivation of NF-κB.

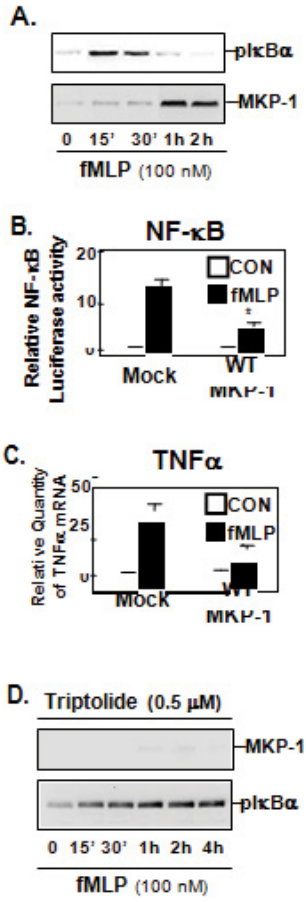

Figure 3: Induction of MKP-1 correlates with the inactivation of NF-κB in fMLP- stimulated monocytes. (A) Human peripheral blood monocytes were stimulated with fMLP (100 nM) for different times. The NF-κB activation and MKP-1 expression were examined by Western blotting using Abs specifically recognizing phosphorylated IκBα or MKP-1 respectively. (B) and (C) Exogenous expression of MKP-1 in THP1 cells inhibits fMLP- activated NF-κB (B) and TNFα expression (C). (D) Human peripheral blood monocytes were pretreated with triptilide (0.5 μM), then stimulated with fMLP for different times. Western blots were performed using Abs for MKP-1 (A, upper panel) or phosphorylated IκBα (A, lower panel). *p<0.05 compared with Vector.

NF-κB acts as a signaling component downstream of MKP-1: The relationship between MKP-1 induction and subsequent inactivation of NF-κB was further investigated using a co-transfection with expression vectors encoding a constitutively active form of IKKβ, and either a wt MKP-1 or siRNA MKP-1, and an NF-κB-dependent luciferase reporter plasmid (Figure 4). The constitutively active IKKβ induced a strong luciferase activity (Figure 4, lane 2), but this activity was inhibited by co-expression of the wt MKP-1 (Figure 4, lane 3) while co-expression of the siRNA MKP-1 enhanced NF-κB binding activity (Figure 4, lane 4). These data also indicate the relationship between MKP-1 induction and inactivation of NF-κB, and that NF-κB acts as a target of MKP-1.

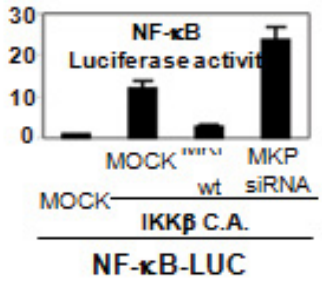

Figure 4: NF-κB is a signaling component acting as a target of MKP-1. THP1 cells were co transfected with NF-κB-LUC and additional constructs: empty vector, vector expressing a constitutive active form of IKKβ and vector expressing a wt MKP-1 or a MKP- 1 siRNA. Luciferase gene activity was measured using the 3010 illuminometer.

Glucocorticoids inhibit fMLP-induced TNFα expression via up regulation of MKP-1: Although many laboratories have shown that glucocorticoids inhibit inflammatory cytokine expression [11], a key issue that has yet to be addressed is how glucocorticoids negatively regulate inflammatory cytokine gene expression. To investigate whether in human monocytes enhanced MKP-1 expression is involved in the anti-inflammatory activity of glucocorticoids, we first assessed the effect of triptolide on the dexamethasone- mediated inhibition of TNFα expression. As shown in Figure 5A, dexamethasone-mediated inhibition of fMLP-induced TNFα expression was counteracted by triptolide in both mRNA level (Figure 5A) and protein level (Figure 5B) in human blood monocytes.

To determine whether glucocorticoids can induce MKP-1 expression, monocytes were treated with 100 nM of dexamethasone. MKP-1 protein was analyzed by Western blotting. MKP-1 protein levels were elevated after a 2 h dexamethasone treatment and lasted for the entire time period examined (Figure 5C). Our results suggest that glucocorticoids inhibited TNFα in monocytes through increasing MKP-1 expression. These data

also provide a mechanistic rationale for the therapeutic use of glucocorticoids in the treatment of inflammatory diseases.

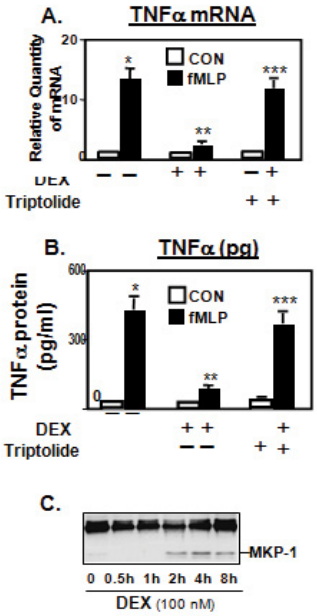

Figure 5: Glucocorticoids inhibit fMLP-induced TNFα expression via up regulation of MKP-1. Human peripheral blood monocytes were pretreated with or without DEX (100 nM) for 2 h in the presence or absence of 0.5 mM triptilide were then stimulated with fMLP (100 nM) for 2 h (A) or 4 h (B). TNFα mRNA was assessed by Q-PCR (A) and the secreted TNFα was measured by ELISA (B). (C) MKP-1 is induced by dexamethasone. Human blood monocytes were stimulated with 100 nM of dexamethasone for indicated times. MKP-1 protein was detected by Western blot analysis. Values in A and B are means ± SD (n=3). *p < 0.001 compared with CON, **p < 0.001 compared with fMLP, ***p < 0.001 compared with DEX.

Inhibition of fMLP-induced TNFα expression by glucocorticoids occurs at the level of IKK: Having identified that the glucocorticoid dexamethasone inhibits fMLP-induced TNFαexpression via up regulation of MKP-1, we next sought to determine the molecular mechanism by which glucocorticoids inhibit TNFα up-regulation by using a co- immune precipitation assay (Figure 6A) and co-transfection with expression vectors encoding a dominant-negative form of IKKβ in human monocytes (Figure 6B). As shown in Figure 6, endogenous IKK is functionally associated with the MKP-1 protein in fMLP-stimulated monocytes (Figure 6A). Both dexamethasone and dominant-negative of IKKβ reduced fMLP-induced phosphorylated IκBα (Figure 6B). In view of the molecular basis of glucocorticoids effects, the GR, a member of the steroid hormone receptor superfamily located in the cytoplasm, plays an important role in glucocorticoids-mediated cellular responses [29,30]. We thus sought to determine the involvement of the glucocorticoid receptor in glucocorticoid-mediated TNFα down-regulation upon fMLP treatment. As shown in Figure 6C, pretreatment with 1 μM of RU486, a glucocorticoid receptor antagonist [31,32], prior to fMLP treatment counteracted the inhibitory effect of dexamethasone on fMLP-induced TNFα transcription at an equimolar concentration, which effectively blocked dexamethasone-induced MKP-1 up regulation (data not shown). Taken together, these data demonstrate that inhibition

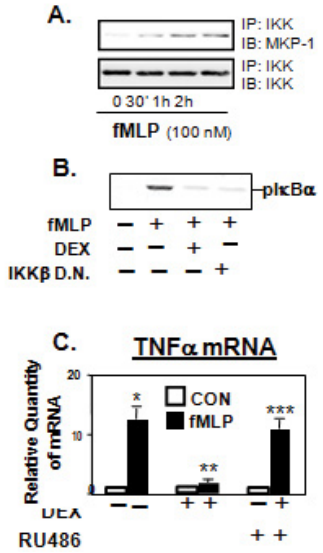

Figure 6: Inhibition of fMLP-induced TNFα expression by glucocorticoids occurs at the level of IKK. (A) Endogenous IKK is functional associated with the MKP-1 protein in fMLP-stimulated monocytes. Human monocytes were stimulated with fMLP for indicated times, the cell lysates were incubated with anti-IKK antibody. The immune precipitations (IP) were probed with either anti-MKP-1 (upper panel) or anti-IKK antibodies (lower panel). (B) THP1 cells were transiently transfected with a dominant negative form of IKKβ and were treated with fMLP (100 nM) for 15 min with or without DEX (100 nM) pretreatment. The NFκB activation was examined by Western blotting using Abs specifically recognizing phosphorylated IκBα. (C) Human monocytes were pretreated with or without DEX (100 nM) for 2 h in the presence or absence of RU486 (1 μM) and were treated with fMLP for 2 h. Values in C are means ± SD (n=3). *p < 0.001 compared with CON, **p < 0.001 compared with fMLP, ***p < 0.001 compared with DEX.

of TNFα expression by dexamethasone occurs at the level of IKK via the glucocorticoid receptor [33].

Discussion and Conclusion

In this report we have shown that the bacterial chemo attractant fMLP stimulates diverse signaling intermediates involved in the regulation of inflammatory cytokines through fMLP activated MKP-1, which acts as a critical negative regulator for inflammatory cytokine gene expression in vitro and in vivo. Our results not only demonstrate that bacteria can induce inflammatory responses, but also can concurrently stimulate the expression of negative regulators that play a critical role in preventing overactive inflammatory responses during bacterial infection. The control of inflammation is likely best understood by the molecular mechanisms underlying the tight regulation of host response to bacteria. We feel this represents an important molecular mechanism occurring during bacterial infection. During evolution, the host has developed a variety of strategies to prevent detrimental inflammatory response during bacterial infections. Negative feedback regulation is thought to play a critical role in preventing overactive host response by tightly regulating the activity of the key signaling molecules that are specifically activated by bacteria. Therefore, increasing the expression of negative regulators could be an interesting and effective therapeutic strategy for overactive inflammatory response. Indeed, glucocorticoids are highly effective in the

control of many inflammatory and immune diseases. In addition to their broad use in the treatment of immune and inflammatory diseases, adjunctive glucocorticoid therapy has also been used in a variety of bacterial infections [17-19]. However, the molecular basis for the inhibitory effect of glucocorticoids on inflammatory cytokine expression still incompletely understood. Our results provided the direct evidence that the synthetic glucocorticoid hormone, dexamethasone, inhibits fMLP-induced TNFα in human blood monocytes. Inhibition of fMLP-induced TNFα expression by dexamethasone occurs at the level of IKK via up regulation of MKP-1. These studies also provide a mechanistic rationale for the therapeutic use of glucocorticoids in the treatment of inflammatory diseases and may lead to the development of a new therapeutic agent to minimize host injury following bacterial infection.

Another interesting finding in our study is the evidence that the primary target of MKP-1 in terminating pro-inflammatory cytokine expression in fMLP-stimulated human monocytes appears to be the NF-κB. Previous studies identify MKP-1 plays a critical role in the de-phosphorylation of MAP kinases in some cell systems. Dr. Liu and his group found that MKP-1 act as a critical negative regulator of the inflammatory cytokine expression induced by LPS, peptidoglycan or lipoteichoic acid [9-16], and that MKP-1 preferentially de-phosphorylates p38 and JNK in LPS-stimulated RAW264.7 cells [15,16]. In the present study, we showed that MKP-1 is a negative regulator for inflammatory cytokine expression induced by bacterial fMLP, and MKP-1 inhibits the production of TNFα via inactivation of NF-κB. Furthermore, our data indicated that glucocorticoids inhibited fMLP-induced TNFα in monocytes through increasing MKP-1 expression, which occurs at the level of IKK via glucocorticoid receptor. Our results, for the first time, provide evidence that the NF-κB acts as a target of MKP-1 in fMLP-stimulated human peripheral blood monocytes. Our studies have concentrated on NF-κB; however, NF-κB may not be the only target of MKP-1. For example, the literature suggests multiple possible relationships between MKP-1 and MAP kinases, including: 1) MKP-1 may regulate the activation of MAP kinases [34-36,2] MKP-1 may act as a downstream MAP kinase [37,38]. The information regarding the role of MAP kinases is still controversial. It appears that differences exist between cell lines or primary cells and different ligand stimulation. Dr. Newton and his group indicated that MKP-1 inhibits NF-κB through p38 MAPK [38]. Our preliminary results indicated that fMLP could induce ERK, p38 and JNK activity in human monocytes (data not shown). These results suggest that both NF-κB and MAP kinase(s) pathways may involve in inflammatory cytokine gene regulation mediated by MKP-1.

In summary, we have shown that bacterial fMLP induces NF-κB activation and inflammatory cytokine gene expression. At the same time, fMLP also stimulates the expression of MKP-1, which in turn terminates inflammatory response through inactivating NF-κB to prevent overactive inflammatory responses during bacterial infection. Interestingly, the synthetic glucocorticoid dexamethasone inhibited fMLP-induced TNFα in monocytes through increasing MKP-1 expression, which occurs at the level of IKK via the glucocorticoid receptor. Schematic representation of the signaling pathways involved in glucocorticoid-mediated inhibition of fMLP-induced TNFα expression via MKP-1-dependent inhibition of NF-κB in human peripheral blood monocytes is shown in (Figure 7). These findings provide evidence that fMLP-induced inflammatory cytokine gene expression via activation of the positive NF-κB pathway and regulation of the negative MKP-1 pathway. Our results also suggest that fMLP induced signaling pathway is distinct from the signaling pathway utilized by LPS. The relationship between NF-κB, MAP kinase and MKP-1, however, remains to be determined.

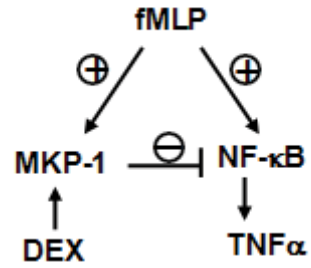

Figure 7: Schematic representation of the signaling pathways involved in glucocorticoid- mediated inhibition of fMLP-induced TNFα expression via MKP-1-dependent inhibition of NF-κB in human peripheral blood monocytes.

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