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Upregulation of IP-10(CXCL10) mRNA Expression by Interleukin-18

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─Abstract─

Background : Interleukin-18 (IL-18) is one of the principal inducers of interferon-γ (IFN-γ) in lymphocytes.

Materials and Methods : The effect of IL-18 on the expression of chemokine IP-10(CXCL10) mRNA in C57BL/6 mouse peritoneal macrophages was studied by using Northern blot analysis, enzyme linked immunosobent assay and electrophoretic mobility shift assay.

Results : IL-18 was determined to exert no direct effect on the expression of IP-10(CXCL10) mRNA. However, IL-18 pretreatment was determined to play a cooperative role in the synergistic induction of LPS-induced IP-10(CXCL10) mRNA expression. The effect associated with IL-18 pretreatment with regard to the synergistic induction of LPS-induced IP-10 (CXCL10) mRNA expression was detected after 16 hr of IL-18 pretreatment, administered prior to LPS stimulation. The pattern of NF-kB binding activity during IL-18 pretreatment with LPS stimulation was found to coincide with the expression of IP-10(CXCL10) mRNA.

Conclusion : Although IL-18 alone exerts no direct effect on the expression of chemokine IP-10(CXCL10), a definite period of IL-18 pretreatment induces the synergistic expression of LPS-induced IP-10(CXCL10) mRNA. NF-kB activation is a component of this synergistic effect of IL-18 pretreatment. These results provide useful information, which may facilitate the elucidation of the action mechanisms underlying IL-18 effect on the expression of IP-10(CXCL10) mRNA.

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Key Words: IP-10(CXCL10), Interleukin-18, Lipopolysaccharide

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Introduction

The inflammatory response to lipopoly-saccharide (LPS) stimulation is known to be mediated, at least in part, by the secretion of chemokines (chemoattractant cytokines) at incipient inflammation sites. Virtually every type of immune cell carries the potential to generate abundant amounts of different chemokine types. Many studies have provided data which indicates that individual chemokine genes can be regulated differentially in response to LPS stimulation.[1-4]

Interleukin-18 (IL-18) has been classified as part of the IL-1 family, primarily by virtue of its structural similarity to IL-1, as well as the fact that it represents one of the principal inducers of Interferon-γ (IFN-γ) in natural killer cells (NK cell) and in T lymphocytes.[5] Until the discovery of IL-18, the dominant IFN-γ-inducing factor in the macrophages was thought to be the heterodimeric cytokine, IL-12.[6] IL-18 acts synergistically with IL-12, inducing the generation of IFN-γ in a variety of immune cells.[7-9] Interferon-γ-inducible protein 10 kilodaltons (IP-10(CXCL10)) is a representative chemokine which is induced by IFN-γ.[10] This chemokine performs a relevant function in the inflammatory reactions, acting as a chemoattractant for lymphocytes, and also exhibits anti-tumor activity with an angiostatic function.[11, 12]

Although IL-18 has been demonstrated to induce IFN-γ production in a variety of ways, IL-18's effects on the expression of IFN-γ-induced chemokine genes has yet to become the focus of a concerted study. Therefore, the objective of this study was to characterize IL-18's effect on chemokine IP-10(CXCL10) expression in mouse peritoneal macrophages.

Materials and Methods

Reagents. Brewer's thioglycollate broth was obtained from Difco Laboratories (Detroit, MI, U.S.A.). RPMI 1640 medium, Hank's balanced salt solution (HBSS) and Dulbecco's phosphate-buffered saline (PBS) were all acquired from Gibco BRL (Life Technologies, Gaithersburg, MD, U.S.A.). Fetal bovine serum (FBS) was purchased from Hyclone (Logeln, UT, U.S.A.). The magna nylon transfer membrane was obtained from Micron Separation, Inc. (Westboro, KS, U.S.A.). The high prime kit was acquired from Boehringer Mannheim (Indianapolis, IN, U.S.A.). Dupont-New England Nuclear (Boston, MA, U.S.A.) was the source for the $[\alpha-^{32}P]dCTP$. The recombinant mouse IFN-γ, IL-18, and IL-12 were all acquired from Bio-source (Camarillo, CA, U.S.A.). *Escherichia coli* LPS (O111:B4), trihydroxymethyl aminomethane (Tris) and sodium dodecyl sulfate (SDS) were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). RNA-bee for total RNA isolation was obtained from TEL-TEST (Friendswood, TX, U.S.A.). The plasmid which encodes for

the IP-10(CXCL10), Mig(CXCL9), and GAPDH genes was kindly provided by Dr. Hamilton at the department of immunology, Lehner Research Institute, Cleveland Clinic Foundation, U.S.A..

Mice. Specific pathogen-free (SPF) female inbred C57BL/6 mice, all from 8-10 weeks of age, were purchased from Hyeunchang Science (Daegu, Korea) and maintained in microisolator cages in the animal center at the Yeungnam Medical College. All experimental animals received autoclaved food and bedding in order to minimize any exposure to viral or microbial pathogens, and to ensure that the degree to which tissue macrophages were spontaneously activated would also be minimal.

Preparation of Mouse Peritoneal Macrophages and Cell Culture. Thioglycollate (TG)-elicited macrophages were then obtained via the previously-described method.1) Peritoneal lavage from the C57BL/6 mice was performed as described, using 10 ml of ice-cold HBSS which contained 5 U/ml of heparin. The macrophages were plated in 100 mm dishes, and incubated for 2 hr at 37℃ in an atmosphere containing 5% CO_2, then washed three times with HBSS in order to remove any nonadherent cells. The macrophages were then cultured overnight in RPMI 1640 containing 10% FBS, at a temperature of 37℃ with an atmosphere containing 5% CO_2.

After being allowed to stand overnight, the medium was replaced with serum-free RPMI 1640 medium. The cells were then cultured in either the presence or absence of stimuli at the indicated doses for the indicated times.

Preparation of Total RNA and Northern Hybridization Analysis. Total cellular RNA was extracted with RNA-bee solution, in accordance with the manufacturer's instructions.

For Northern blot analysis, equal amounts of RNA (7 μg/sample) were used in each lane of the gel. The RNA was then denatured, separated via electrophoresis in 1% agarose/ 2.2 M formaldehyde gel, and then transferred to a nylon membrane. The blots were then prehybridized for 8 hr at 42℃ in prehybridization solution (50% formamide, 1% SDS, 1×Denhardt's, 0.25 mg/ml denatured salmon sperm DNA, and 50 mM sodium phosphate). Hybridization was conducted for 16-18 hr at 42℃ with 2×10^7 cpm of denatured plasmid DNA, which contained the appropriate specific cDNA inserts. The blots were then rinsed for 30 min at 42℃ and for 15 min at 65℃ with a 0.5×SSC-0.1% SDS solution. The filters were then dried and exposed with X-ray film at -70℃.

Electrophoretic Mobility Shift Assay (EMSA). The nuclear extracts were prepared via modified version of the methods developed by Harris et al.[13] The cells were then washed

three times with cold PBS (Ca^{++}, Mg^{++}), and harvested via centrifugation. The cells were resuspended and incubated on ice for 15 min in 400 µl of a hypotonic buffer A (10 mM HEPES, 10 mM KCl, 1.5 mM MgCl$_2$, 0.5 mM DTT, 0.1 mM PMSF, 10 µg/µl pepstatin, 10 µg/µl leupeptin, 10 µg/µl autipain, 10 µg/µl aprotinin). Nonidet P-40 was then added to a final concentration of 2.5%, and the cells were vortexed for 10 sec. The nuclei were separated from the cytosol via 15 sec of centrifugation at 12,000 x g. The pellets were then resuspended in 40 µl of a hypotonic buffer C (20 mM HEPES, 25% glycerol, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 0.5 mM DTT, 0.1 mM PMSF, 10 µg/µl pepstatin, 10 µg/µl leupeptin, 10 µg/µl autipain, 10 µg/µl aprotinin). The samples were sonicated at level 3-4 for 2-3 sec, and then centrifuged at 4℃ for 10 min. The nuclear protein concentrations were measured via Bradford assay (Bio-Rad, Richmond. CA, U.S.A.).

Consensus sequences for the NF-*k*B DNA binding site (5'-agttgaggggactttcccaggg-3'), AP-1 DNA binding site (5'-cgcttgatgactcag ccggaa-3') and STAT-1 DNA binding site (5'-catgttatgcatattcctgtaagtg-3') were then labeled with [α-^{32}P]dCTP, using random primed DNA labeling (Roche, Mannheim, Germany). The labeled DNA was purified over a S-200HR column (Pharmacia, Piscutaway, NJ, U.S.A.) in order to remove the unbound nucleotides. Nuclear protein extracts were incubated, at a concentration of 10 µg, at room temperature for 20 min, using ~50000 cpm of the labeled oligonucleotides in binding buffer (200 mM HEPES, 500 mM KCl, 10 mM EDTA, 50% glycerol, 10 mM DTT, 1 mg/ml BSA, 1 µg/µl poly(dI-dC)). The samples were resolved on 4% polyacrylamide gel at 150 V, then exposed to film at -70℃

Results

Effects of IL-18 on the Chemokine IP-10 (CXCL10) mRNA Expression in Mouse Peritoneal Macrophages. IFN-ɣ and LPS were employed for their potent induction of IP-10(CXCL10) mRNA expression, and the expression of Mig(CXCL9) mRNA, another IFN-ɣ-induced chemokine, was also observed, in order to compare it with the expression of IP-10(CXCL10) mRNA. After the thioglycollate (TG)-elicited C57BL/6 peritoneal macrophages were stimulated with IL-18 (50 ng/ml), LPS (100 ng/ml), and IFN-ɣ (100 U/ml) alone, or with IL-18 plus IFN-ɣ (IL-18/IFN-ɣ), IL-18 plus LPS (IL-18/LPS) simultaneously for 4 hr, we conducted Northern analysis on all samples. As is shown in Fig. 1, IL-18 alone exerted no effects on IP-10(CXCL10) and Mig(CXCL9) mRNA expression. Also, the levels at which IL-18/LPS or IL-18/IFN-ɣ-induced IP-10 (CXCL10) mRNA were expressed were almost identical to the levels of IP-10(CXCL10) mRNA induced by LPS or IFN-ɣ alone.
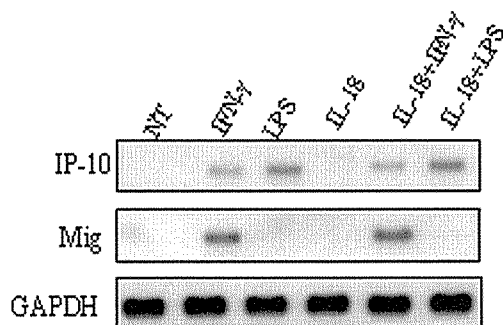
Fig. 1. Expression of chemokine IP-10(CXCL10) and Mig(CXCL9)mRNA in mouse peritoneal macrophages. Thioglycollated (TG)-elicited C57BL/6 mouse peritoneal macrophages (PeM Φ) were untreated (NT) or treated with IFN-ɣ (100 U/ml), LPS (100 ng/ml), IL-18 (50 ng/ml), or with IL-18 plus IFN-ɣ, or IL-18 plus LPS simultaneously for 4 hr. Total RNA was isolated and the levels of the IP-10(CXCL10) and Mig(CXCL9) mRNA were analyzed by Northern hybridization. These data are a representative of three similar experiments.
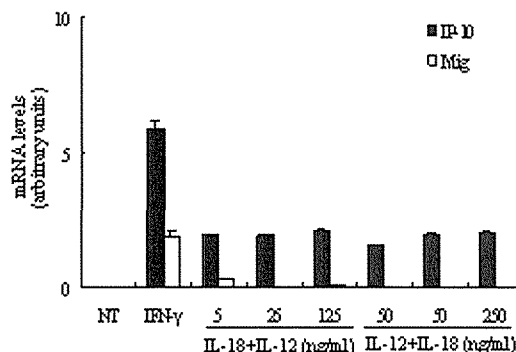

Fig. 2. Dose effect of IL-18 and IL-12 on the expression of IP-10(CXCL10) and Mig(CXCL9) mRNAs in the mouse peritoneal macrophages. TG-elicited peritoneal macrophages were untreated (NT) or treated with IFN-ɣ (100 U/ml) alone, IL-18 (50 ng/ml) with various concentrations of IL-12 (5, 25, 125 ng/ml), or IL-12 (25 ng/ml) with various concentrations of IL-18 (5, 50, 250 ng/ml) simultaneously for 4 hr. Total RNA was prepared, and Northern blot analysis was performed. The blots were quantified by Statview software (SAS Institute, Cary, NC) analysis, IP-10 (CXCL10) mRNA levels normalized for GAPDH content of each sample and expressed as fold induction compared with untreated cells. Similar results were obtained in two separate experiments. Bars represent mean ± SEM from two separate experiment.

Next, we attempted to characterize the synergistic effect of IL-18/IL-12 at several concentrations on the expression of IP-10 (CXCL10) or Mig(CXCL9) mRNA (Fig. 2). After the TG-elicited macrophages had been stimulated with IFN-ɣ (100 U/ml) alone, IL-18 (50 ng/ml) with various concentrations of IL-12 (5, 25, 125 ng/ml), or IL-12 (25 ng/ml) with various concentrations of IL-18 (5, 50, 250 ng/ml) simultaneously for 4 hr, the total RNAs were analyzed via Northern blot analysis. Although IP-10(CXCL10) mRNA expression was detected at trace levels in all cases in which IL-18/IL-12 stimulation was applied, enhanced IP-10(CXCL10) mRNA expression was not detected, as compared to the expression of IP-10(CXCL10) in cells that had been stimulated with IFN-ɣ. Mig(CXCL9) mRNA expression also proved to be undetectable in all cases in which IL-18/IL-12 stimulation was applied.

The time courses of IL-18/LPS and IL-18/IFN-ɣ-induced IP-10(CXCL10) mRNA expression were observed at a variety of time points. The TG-elicited macrophages

71

were stimulated using IFN-γ, LPS, IL-18/ IFN-γ, or IL-18/LPS simultaneously, for 2, 4, 8, and 16 hr. The overall time course patterns and levels of IP-10(CXCL10) mRNA expression as the result of stimulation with
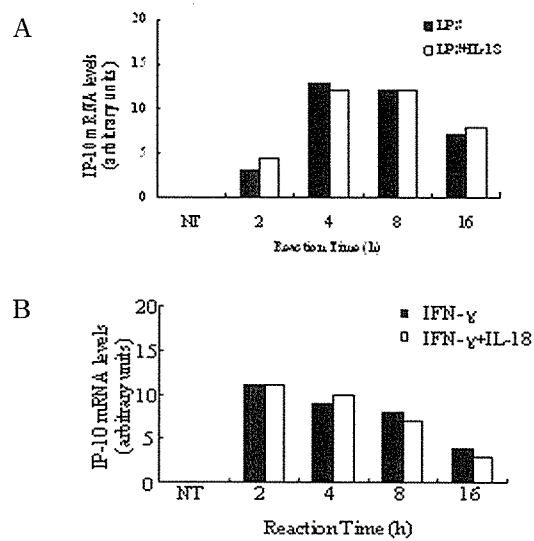

Fig. 3. Time course of LPS/IL-18 or IFN-γ /IL-18-induced IP-10(CXCL10) mRNA expression in mouse peritoneal macrophages. (A) TG-elicited peritoneal macrophages were untreated (NT) or treated with LPS (100 ng/ml) alone or IL-18 (50 ng/ml) plus LPS simultaneously for the indicated times. (B) TG-elicited peritoneal macrophages were untreated or treated with IFN-γ (100 U/ml) alone or IL-18 (50 ng/ml) plus IFN-γ simultaneously for the indicated times. Total RNA was prepared and the level of IP-10(CXCL10) mRNA was analyzed by Northern hybridization. The blots were quantified by Statview software (SAS Institute, Cary, NC) analysis, IP-10(CXCL10) mRNA levels normalized for GAPDH content of each sample and expressed as fold induction compared with untreated cells (bar graph). These data are a representative of three similar experiments.

IL-18/LPS or IL-18/IFN-γ were almost identical to those of IP-10(CXCL10) mRNA expression induced by LPS or IFN-γ alone. The IP-10(CXCL10) mRNA expression induced by LPS alone- or by the combination of IL-18/LPS reached a peak 4 hr after treatment, and this level persisted for 8 h (Fig. 3A). The IP-10(CXCL10) mRNA expression induced by IFN-γ alone or by the IL-18/IFN-γ combination reached a maximum level as early as 2 hr after treatment, and then declined gradually until the 16 hr mark (Fig. 3B).

Effects of IL-18 Pretreatment on the IP-10(CXCL10) mRNA Expression. We attempted to determine the effect of IL-18 pretreatment on LPS induced-IP-10(CXCL10) mRNA expression. The TG-elicited peritoneal macrophages were treated with IL-18 (or LPS) at 16, 8, 4, and 0 (simultaneously) hr prior to the administration of LPS (or IL-18). The most effective synergy of IL-18 pretreatment with LPS stimulation on the expression of IP-10(CXCL10) mRNA was recorded when IL-18 pretreatment was administered 16 hr prior to LPS stimulation, and the most effective synergy between LPS pretreatment and IL-18 stimulation was detected when LPS was applied 4 hr prior to the addition of IL-18 (Fig. 4A).

We also administered an experimental combination of IL-18 and IFN-γ pretreatment. In this case, we determined there to be no

effect of IL-18 pretreatment on the IFN-ɣ -induced expression of IP-10(CXCL10) mRNA. The overall levels of IP-10(CXCL10) mRNA expression in cases in which IL-18 pretreatment was applied were higher than the levels seen in the cases in which IFN-ɣ pretreatment was applied. The most pronounced IP-10(CXCL10) mRNA expression was detected when IL-18 and IFN-ɣ stimulation were simultaneously applied (Fig. 4B).
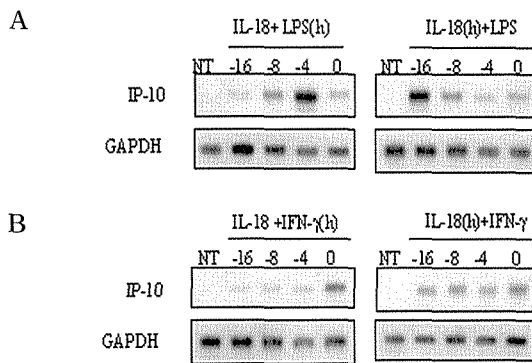
Synergistic Mechanisms of IL-18 Pretreat-

Fig. 4. Effect of IL-18, LPS, or IFN-ɣ pretreatment on the expression of IP-10(CXCL10) mRNA in mouse peritoneal macrophages. (A) TG-elicited peritoneal macrophages were treated with LPS (100 ng/ml) or IL-18 (50 ng/ml) at 16 (-16), 8 (-8), 4 (-4), or 0 hr prior to the stimulation with IL-18 or LPS for 2 hr. (B) TG-elicited peritoneal macrophages were treated with IFN-ɣ(100 U/ml) or IL-18 (50 ng/ml) at 16 (-16), 8 (-8), 4 (-4), or 0 hr prior to the stimulation with IL-18 or IFN-ɣ for 2 hr. Total RNA was prepared and the level of IP-10(CXCL10) mRNA was analyzed by Northern hybridization. These data are a representative of three similar experiments.

ment on LPS-Induced IP-10(CXCL10) mRNA Expression. In order to elucidate the mechanisms underlying the synergistic effect of IL-18 pretreatment on the LPS-induced expression of IP-10(CXCL10) mRNA, we assessed the activities of transcription factors, NF-*k*B and AP-1. After the TG-elicited peritoneal macrophages had been pretreated with IL-18 or LPS as was previously described in Fig. 4 method, we conducted an electrophoretic mobility shift assay using the NF-*k*B or AP-1 consensus sequences. As was shown in Fig. 5A, the most profound binding activity of NF-*k*B in the cases in which IL-18 pretreatment was coupled with LPS stimulation was detected when the IL-18 was applied 16 hr prior to LPS stimulation. Similarly, the most pronounced NF-*k*B binding activity in the cases in which LPS pretreatment was coupled with IL-18 stimulation was detected at when the LPS pretreatment was applied 4 hr prior to the IL-18 stimulation. These results correlated with the results of the expressions of the IP-10(CXCL10) mRNA, as was shown in Fig. 4A. However, AP-1 activity could not be confidently correlated with the results of the expressions of IP-10(CXCL10) mRNA, as is shown in Fig. 4A.

STAT-1 binding activity was also assessed, in order to determine whether the results of IL-18 pretreatment with IFN-ɣ stimulation Mig(CXCL9) might be related to STAT-1 activity. As is shown in Fig. 5B, the

STAT-1 binding activity in the cases in which IL-18 pretreatment was coupled with IFN-γ stimulation were found to be similar at all IL-18 pretreatment time points. However, the STAT binding activity in the IFN-γ pretreatments coupled with IL-18 stimulation were not detected except in the cells which were stimulated simultaneously with IL-18/IFN-γ. The overall patterns of STAT-1 binding activity were similar to those of IP-10(CXCL10) mRNA expression, as shown in Fig. 4B. As a consequence, IL-18 has no effect on the IFN-γ-induced

Fig. 5. The binding activities of NF-kB, AP-1 and STAT-1 according to the pretreatment of IL-18, LPS or IFN-γ. (A) TG-elicited peritoneal macrophages were treated with LPS (100 ng/ml) or IL-18 (50 ng/ml) at 16 (-16), 8 (-8), 4 (-4), or 0 hr prior to the stimulation with IL-18 or LPS for 2 hr. (B) TG-elicited peritoneal macrophages were treated with IFN-γ (100 U/ml) or IL-18 (50 ng/ml) at 16 (-16), 8 (-8), 4 (-4), or 0 hr prior to the stimulation with IL-18 or IFN-γ for 2 hr. Nuclear extracts were prepared, and specific binding activity of NF-kB, AP-1 or STAT-1 was assessed by electrophoretic mobility shift assay (EMSA). m; mutant probe, Ab; anti NF-kB Ig, anti AP-1 Ig, or anti STAT-1 Ig. These data are a representative of three similar experiments.

expression of IP-10(CXCL10) mRNA, and IFN-γ-induced IP-10(CXCL10) mRNA expression in mouse peritoneal macrophages is dependent on STAT-1 activation.

Discussion

Interleukin-18 is a well known IFN-γ inducer, and IFN-γ is a primary IP-10 (CXCL10) inducer.[5, 10] Accordingly, the first objective of this study was to evaluate the notion that IL-18 is able to directly induce chemokine IP-10(CXCL10) in mouse peritoneal macrophages. However, IL-18 was determined to have no direct effect on the expression of IP-10(CXCL10) mRNA in mouse peritoneal macrophages, and was also shown to exert no synergistic effect with IL-12 in this regard. Also, in a set of time course for IL-18/LPS or IL-18/IFN-γ-induced IP-10 (CXCL10) mRNA expression, we noted almost identical patterns of IP-10(CXCL10) expression when stimulated by LPS or IFN-γ alone. IL-18 was not determined to exert a synergistic effect on LPS or IFN-γ -induced IP-10(CXCL10) mRNA expression. Therefore, IL-18 itself appeared to have no direct effects on the IP-10(CXCL10) mRNA expression in the mouse peritoneal macrophages. This negative reaction of IP-10(CXCL10) mRNA expression might be a result of the differential response of macrophages to differing reaction times concentrations of stimuli, and/or specific cell-type patterns of stimulus
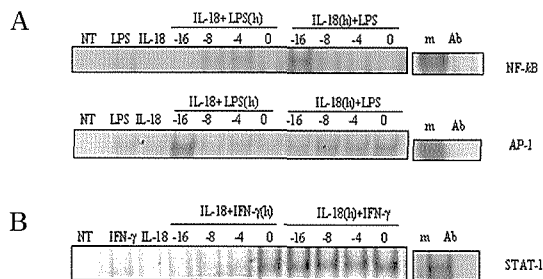
sensitivity. However, surprisingly, the synergistic induction of IP-10(CXCL10) mRNA was detected in cells which had been pretreated with IL-18 for 16 hr prior to the administration of LPS stimulation. In cases in which LPS was administered prior to IL-18 stimulation, the pattern of IP-10 (CXCL10) mRNA expression at each of the pretreatment time points was similar to those recorded in Fig. 3A. This result can be attributed to the fact that the expression of IP-10(CXCL10) mRNA in cases of LPS pretreatment is not due to the synergistic reaction of LPS and IL-18, but rather to the effect of LPS stimulation itself. In the case of a combination of IL-18 and IFN-γ pretreatment, the effects of IL-18 on the induction of IP-10(CXCL10) mRNA were not detected. All of the levels of IP-10(CXCL10) mRNA expression in cases of IL-18 pretreatment were almost identical to those detected in cases in which IL-18 and IFN-γ stimulation were simultaneously applied, and the levels of IP-10(CXCL10) mRNA expression in cases in which IFN-γ pretreatment was applied were quite weak, as compared to the levels seen in case in which IL-18 and IFN-γ stimulation were simultaneously administered. Therefore, LPS, and not IFN-γ, was determined to perform a cooperative function in the synergistic induction of IP-10(CXCL10) mRNA in cells which had received IL-18 pretreatment.

Puren et al.[14, 15] reported that the combination of low LPS concentration plus IL-18 could induce IFN-γ production at a level 3 to 5 times as high as could be induced by either of the stimulants alone. Therefore, we conducted ELISA for IFN-γ production, in order to determine whether the effect of IL-18 pretreatment on the synergistic induction of LPS-induced IP-10(CXCL10) mRNA expression was related to the production of IFN-γ during the reaction time inherent to IL-18 pretreatment. However, the overall levels of IFN-γ production during IL-18 pretreatment were quite low (data not shown). Consequently, the effects of IL-18 pretreatment on the synergistic expression of LPS-induced IP-10(CXCL10) mRNA expression may be mediated by mechanisms other than the direct activation of IFN-γ.

The expression of chemokine genes appears to be initiated by the activation of a variety of transcription factors. Nuclear factor-kB (NF-kB) and activator protein-1 (AP-1) are both transcription factors which are primarily involved in the trans-activation of pro-inflammatory genes.[16] IL-18 also activates NF-kB and AP-1 in target gene activation,[7, 17, 18] and induces the production of IFN-γ via these two transcription factors. The functional importance of AP-1 with regard to IL-18-dependent IFN-γ promoter activity has already been well-elucidated, as has that of signal transducer and activator of transcription (STAT)-1.19) STAT-1 is a well-known signal transcription factor, which

is activated by IFN-γ.20) Therefore, the activation and/or cooperation of NF-kB, AP-1, and/or STAT-1 may help to explain the mechanism underlying the effects of IL-18 pretreatment on the synergistic induction of LPS or IFN-γ-induced IP-10 (CXCL10) mRNA expression. Interestingly, the pattern of NF-kB binding ability in the cases in which IL-18 pretreatment was coupled with LPS stimulation coincided with the pattern of IP-10(CXCL10) mRNA expressions in the cases in which IL-18 pretreatment was coupled with LPS stimulation. AP-1 activation was also observed, but the pattern of AP-1 binding activity did not coincide with the expressions of IP-10(CXCL10) mRNA. These results indicate that the effects of IL-18 pretreatment on the synergistic induction of LPS-induced IP-10(CXCL10) mRNA in mouse peritoneal macrophages are not related to AP-1 activation, but rather to that of NF-kB. Meanwhile, the patterns of STAT-1 binding activity in the cases in which IL-18 and IFN-γ were combined with the expressions of IP-10(CXCL10) mRNA under of IP-10(CXCL10) mRNA under the same conditions. This result confirms that the IFN-γ-induced IP-10(CXCL10) mRNA expression in the mouse peritoneal macrophages is dependent on STAT-1 activation.

This report is, to the best of our knowledge, the first to focus on chemokine IP-10(CXCL10) expression being induced by IL-18 in C57BL/6 mouse peritoneal macrophages.

Although IL-18 alone has no direct effects on the expression of IP-10(CXCL10) mRNA, a definite period of IL-18 pretreatment clearly synergistically enhances LPS-induced IP-10(CXCL10) mRNA expression, and NF-kB activation appears to be important for IL-18's synergistic effect on the expression of IP-10(CXCL10) mRNA.

요   약

Interferon-γ (IFN-γ)의 주된 생산세포는 림프구이며 주로 Interleukin-18(IL-18)에 의해 생산이 된다. IP-10은 IFN-γ에 의해 유도, 생산되는 대표적인 케모카인이다.

따라서 본 연구는 마우스 복강내 대식세포에서의 IL-18에 의한 IP-10의 생산 여부를 관찰하고자 하였다.

IL-18은 마우스 복강내 대식세포에서 IP-10의 발현을 직접적으로 유도 하지는 않았다. 그러나 대식세포에 지다당질을 처리하기 전 IL-18을 전 처리 시킨 결과 지다당질에 의해 유도된 IP-10의 발현이 항진되어 나타남을 확인하였다. 이러한 항진 효과는 IL-18 전처리 16시간에 나타났으며, 이때 NF-kB의 활성이 IP-10의 발현 항진과 일치함을 확인하였다.

비록 IL-18이 IP-10을 직접적으로 발현시키지는 못하나 NF-kB의 활성을 통하여 IL-18의 적정시간에 따른 전 처리시 IP-10 발현의 항진은 케모카인 발현에있어 IL-18의 작용기전을 이해하는데 유용한 자료가 될 것이다.

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