The Journal of Immunology

Macrophage-Stimulating Protein, the Ligand for the Stem Cell-Derived Tyrosine Kinase/RON Receptor Tyrosine Kinase, Inhibits IL-12 Production by Primary Peritoneal Macrophages Stimulated with IFN- γ and Lipopolysaccharide

Amy C. Morrison, Caleph B. Wilson, Manujendra Ray and Pamela H. Correll

J Immunol 2004; 172:1825-1832; ;
doi: 10.4049/jimmunol.172.3.1825
http://www.jimmunol.org/content/172/3/1825

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Macrophage-Stimulating Protein, the Ligand for the Stem Cell-Derived Tyrosine Kinase/RON Receptor Tyrosine Kinase, Inhibits IL-12 Production by Primary Peritoneal Macrophages Stimulated with IFN-γ and Lipopolysaccharide[1]

Amy C. Morrison,* Caleph B. Wilson,† Manujendra Ray,‡ and Pamela H. Correll[2]*†‡

IL-12, produced by APCs during the initial stages of an immune response, plays a pivotal role in the induction of IFN-γ by NK and $\gamma\delta$T cells and in driving the differentiation of Th1 cells, thus providing a critical link between innate and acquired immunity. Due to the unique position occupied by IL-12 in the regulation of immunity, many mechanisms have evolved to modulate IL-12 production. We have shown previously that macrophage-stimulating protein (MSP), the ligand for the stem cell-derived tyrosine kinase/recepteur d'origine nantais (RON) receptor, inhibits NO production by macrophages in response to IFN-γ and enhances the expression of arginase. Mice lacking RON exhibit increased inflammation in a delayed-type hypersensitivity reaction and increased susceptibility to endotoxic shock. In this study we demonstrate that pretreatment of macrophages with MSP before IFN-γ and LPS results in the complete inhibition of IL-12 production due to suppression of p40 expression. This response is mediated by the RON receptor, and splenocytes from RON$^{-/-}$ animals produce increased levels of IFN-γ. MSP pretreatment of macrophages resulted in decreased tyrosine phosphorylation of Stat-1 and decreased expression of IFN consensus sequence binding protein in response to inflammatory cytokines. In addition to IL-12, the expression of IL-15 and IL-18, cytokines that are also dependent on IFN consensus sequence binding protein activation, is inhibited by pretreatment with MSP before IFN-γ and LPS. We also show that the ability of MSP to inhibit IL-12 production is independent of IL-10. Taken together, these results suggest that MSP may actively suppress cell-mediated immune responses through its ability to down-regulate IL-12 production and thus inhibit classical activation of macrophages. ***The Journal of Immunology*****, 2004, 172: 1825–1832.**

The production of proinflammatory mediators by macrophages must be tightly regulated to establish an effective immune response without causing damage to the host. The proinflammatory cytokine IL-12 plays a unique role in the immune response by bridging innate and adaptive immunity. IL-12 is produced primarily by APCs and stimulates IFN-γ production by NK and $\gamma\delta$T cells (1). This cytokine also plays a key role in promoting the development of Th1 cells as well as the inhibition of Th2 responses (reviewed in Ref. 2). Animals deficient in IL-12 or its receptor have decreased ability to produce IFN-γ and induce normal Th1 responses (3) and are more susceptible to infection with intracellular pathogens (4, 5). Although IL-12 helps drive cell-mediated immunity, overproduction of IL-12 has been implicated in granuloma formation from delayed-type hypersensitivity (DTH)[3] responses (6) as well as endotoxemia (7) and autoimmune disorders (8, 9).

The production of IL-12 by macrophages and other APCs is regulated by a variety of mechanisms. IL-12 can be produced in a T cell-independent manner in response to bacterial products such as LPS, where IFN-γ is thought to have a priming effect (10), as well as in a T cell-dependent manner via CD40/CD40 ligand interaction of APCs with naive CD4$^+$ T cells (reviewed in Refs. 11 and 12). The subsequent IFN-γ produced in response to IL-12 serves as a classical positive feedback regulator of IL-12 to maintain IL-12-induced Th-1 responses. To control IFN-γ-mediated augmentation of IL-12, there are also effective mechanisms to down-regulate IL-12 production. Cytokines such as IL-4, IL-10, and TGF-β; chemoattractants; activators of complement; and phagocytic receptors have all been shown to be inhibitors of IL-12 production (reviewed in Ref. 12). More recently, receptor tyrosine kinases from the Tyro 3 family, receptors for the growth factors Gas6 and protein S, have been shown to regulate IL-12. Macrophages from triple-mutant mice lacking Tyro3, Axl, and Mer receptors produce elevated levels of IL-12 (13).

Macrophage-stimulating protein (MSP) is an 80-kDa serum growth factor that was originally identified due to its ability to cause shape changes and induce responsiveness to chemoattractants in murine peritoneal macrophages (14, 15). MSP is most closely related to hepatocyte growth factor and belongs to a family of proteins characterized by a kringle domain and an inactive serine protease-like domain (16). MSP is primarily produced in the liver as a biologically inactive single-chain pro-MSP (17) and can be converted to its active form by several coagulation cascade enzymes (18), a serine protease found in wound fluids (19), and a macrophage plasma membrane-associated enzyme (20). In primary murine peritoneal macrophages, MSP can induce both C5a-mediated chemotaxis as well as act as a chemoattractant itself (21).

Department of Veterinary Science, *Graduate Program in Biochemistry, Microbiology, and Molecular Biology, and †Graduate Programs in Pathobiology and ‡Integrative Biosciences, Pennsylvania State University, University Park, PA 16802

Received for publication December 23, 2002. Accepted for publication November 18, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Institutes of Health Grant AI43367.

[2] Address correspondence and reprint requests to Dr. Pamela H. Correll, Department of Veterinary Science, 115 Henning, Pennsylvania State University, University Park, PA 16802. E-mail address: phc7@psu.edu

[3] Abbreviations used in this paper: DTH, delayed-type hypersensitivity; ICSBP, IFN consensus sequence binding protein; iNOS, inducible NO synthase; IRF, IFN regulatory factor; JAK, Janus kinase; MSP, macrophage-stimulating protein; SOCS, suppressor of cytokine signaling; SR-A, scavenger receptor A; STK, stem cell-derived tyrosine kinase; RON, recepteur d'origine nantais; RTK, receptor tyrosine kinase.

0022-1767/04/$02.00

In these cells MSP has also been shown to stimulate the phagocytosis of C3bi-coated SRBC (22).

The receptor for MSP is the recepteur d'origine nantais (RON) receptor tyrosine kinase, a member of the MET protooncogene family and shares structural similarities to the Tyro 3 receptor family (23). RON was originally isolated from a human keratinocyte cDNA library (23), and the murine homologue, also called stem cell-derived tyrosine kinase (STK), was cloned from hemopoietic stem cells (24). RON is a disulfide-linked heterodimer composed of an extracellular α-chain and a transmembrane β-chain with intrinsic kinase activity (25). Binding of MSP stimulates autophosphorylation of two C-terminal tyrosine residues in the multifunctional docking site (26). Signaling through this family of receptors has been shown to recruit a number of SH2-containing signaling molecules, including PLC-γ, phosphoinositol 3-kinase, Shc, and Grb2 (25, 27), as well as the adaptor protein Gab 1 (28). RON is expressed on tissue-resident macrophages, including resident peritoneal macrophages (29), human alveolar macrophages (30), osteoclasts (31), and dermal macrophages (19), but is not present on circulating monocytes or bone marrow macrophages (29). RON expression, which is regulated during inflammation, is inhibited by proinflammatory cytokines and NO (32), but is upregulated on day 3 peritoneal exudate macrophages (29) and burn wound exudate macrophages (19).

Recent data suggest that RON plays an important role in regulating the activities of macrophages during inflammation. Activation of RON by MSP results in the inhibition of inducible NO production by macrophages stimulated with LPS and inflammatory cytokines, such as IFN-γ and TNF-α (33), due to transcriptional repression of the inducible NO synthase (iNOS) gene (34) and is associated with the inhibition of NF-κB activation (33). Subsequently, mice with a targeted disruption in the RON gene exhibit increased susceptibility to LPS-induced septic shock associated with increased levels of serum NO (35, 36). We have recently shown that activation of the RON receptor by MSP also results in the up-regulation of arginase, an enzyme that shares a substrate with iNOS, L-arginine, but has opposing biological effects (37), suggesting another potential mechanism by which MSP/RON may regulate NO production. In addition to arginase, scavenger receptor A and IL-1R antagonist, other markers associated with alternative activation of macrophages, were induced by MSP (37).

IL-12 produced by APCs is the principal mediator of cell-mediated immunity and DTH responses. In addition to its central role in protection against mycobacterial infection, IL-12 augments tissue damage resulting from a DTH response (reviewed in Ref. 6). RON$^{-/-}$ animals also exhibit increased inflammation in a DTH response (35, 38). The increased inflammation in the RON$^{-/-}$ animals suggests that MSP and RON may play a role in regulating IL-12 production. In this study we demonstrate that pretreatment of peritoneal macrophages with MSP before stimulation with IFN-γ and LPS results in the complete inhibition of IL-12 production. MSP inhibits IL-12 in a manner similar to IL-10; however, MSP acts independently of IL-10. We also observe increased IFN-γ levels in the spleens of RON$^{-/-}$ animals, suggesting that the inhibition of IL-12 by MSP is an important mechanism of regulating inflammation in vivo.

Materials and Methods

Cells and animals

The mouse strains used for these experiments were CD-1, CD-1 mice with a targeted mutation in the RON gene (35), C57/B6, and C57/B6 mice with a deletion in the IL-10 gene (The Jackson Laboratory, Bar Harbor, ME). Murine resident peritoneal macrophages were obtained by peritoneal lavage with 10 ml of RPMI 1640 containing 10% FBS (Life Technologies, Gaithersburg, MD). Cells were incubated 2–3 h and then washed with PBS to eliminate nonadherent cells. Splenic cells were mechanically harvested from control and RON$^{-/-}$ mice. RBC were lysed using a buffer containing 17 mM Tris-HCl and 140 mM NH_4Cl, pH7.2. All cell cultures were maintained in RPMI 1640 and 10% FBS at 37°C in a humidified incubator containing 5% CO_2.

Reagents and Abs

MSP was obtained from R&D Systems (Minneapolis, MN). IFN-γ was purchased from PeproTech (Rocky Hill, NJ). LPS was obtained from Sigma-Aldrich (St. Louis, MO). Anti-phospho-STAT1 S727 was purchased from Upstate Biotechnology (Lake Placid, NY). Anti-phospho-STAT1 Y701 and anti-STAT1 were purchased from Cell Signaling Technology (Beverly, MA).

RNA extraction and RT-PCR

IL-12 p35, p40, TNF-α, IL-15, IL-18, IFN regulatory factor-1 (IRF-1), and IFN consensus sequence binding protein (ICSBP) expression in resting and IFN-γ- and LPS-activated resident peritoneal macrophages with or without pretreatment with MSP was analyzed by RT-PCR. Briefly, 1×10^6 cells were activated with 100 ng/ml MSP for 8–10 h, after which they were stimulated with IFN-γ and LPS, then harvested for RNA isolation using RNeasy (Qiagen, Valencia, CA). RT was conducted for 15 min at 42°C using random hexamers from 0.1 μg of total RNA. Conditions for PCR are as follows: IL-12 p40 (39) at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 54°C for 1 min, and 72°C for 2 min, with a final extension at 72°C for 10 min (IL-12 p40 sense, 5′-ATCGTTTTGCTGGTGTCTCC-3′; IL-12 p40 antisense, 5′-AGTCCCTTTGGTCCAGTGTG-3′). Conditions for IL-12 p35, IL-15, IL-18, and TNF-α (40) as well as IRF-1 (34) and ICSBP (41) were previously described.

Western blot analysis

Cells (5×10^6) were incubated with the indicated cytokines. After incubation cells were washed with PBS and lysed for 15 min on ice in 500 μl of lysis buffer containing 150 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.5% Triton X-100, and protease inhibitors (1 μg/ml leupeptin, 1 μg/ml pepstatin A, and 2 μg/ml aprotinin). The protein contents were determined using the DC protein assay kit (Bio-Rad, Richmond, CA). Absorbance was measured at 750 nm with a DU530 spectrophotometer (Beckman Coulter, Palo Alto, CA). Proteins were mixed with 5× SDS sample buffer. SDS-PAGE was performed using 12.5% bis-acrylamide gel for separation with a Mini-Protean II Cell (Bio-Rad) at 65 V for 15 min, then at 150–160 V for 1 h. Immobilon P polyvinlydifluoride membranes (Millipore, Bedford, MA) were washed briefly in methanol, then in ddH_2O, and was equilibrated in transblotting buffer (48 mM Tris, 39 mM glycine, and 20% methanol) for 5 min. The gels were washed in ddH_2O, then in transblotting buffer for 15 min. Transblotting was performed using the Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad) for 1 h at 100 V. The blots were then placed in blocking buffer containing 5% nonfat dry milk for 1 h at room temperature and incubated overnight with primary Ab according to manufacturer's instructions. Anti-rabbit or anti-goat Abs conjugated with HRP were added at 1/8000 for 1 h. Protein was then detected by incubating the blots in chemiluminescence substrates (Amersham Pharmacia Biotech, Piscataway, NJ) and exposing them to x-ray film.

Cytokine measurement

Cells (1×10^5) were incubated with the cytokines indicated in a 96-well plate, and the cytokines in the supernatant were detected by standard sandwich ELISA. The OptELIA IL-10 mouse set was obtained from BD PharMingen (San Diego, CA), and the IL-12 p70, TNF-α, IL-1β, and IL-6 and IFN-γ Quantikine murine ELISAs were obtained from R&D Systems and were performed according to the manufacturer's instructions. Colorimetric changes in enzyme substrates were detected at 430 nm using a HTS 7000 Bio Assay Reader (PerkinElmer, Shelton, CT).

Results

Pretreatment of primary murine peritoneal macrophages with MSP inhibits IL-12 production in response to IFN-γ and LPS

IL-12 produced by macrophages plays an important role in cell-mediated immunity and early IFN-γ production. To determine whether MSP regulates IL-12 production, we harvested peritoneal macrophages and incubated them for 10 h with or without 100 ng/ml MSP before stimulation with IFN-γ and LPS, and measured IL-12 p70 production by ELISA (Fig. 1*A*). Although MSP given at

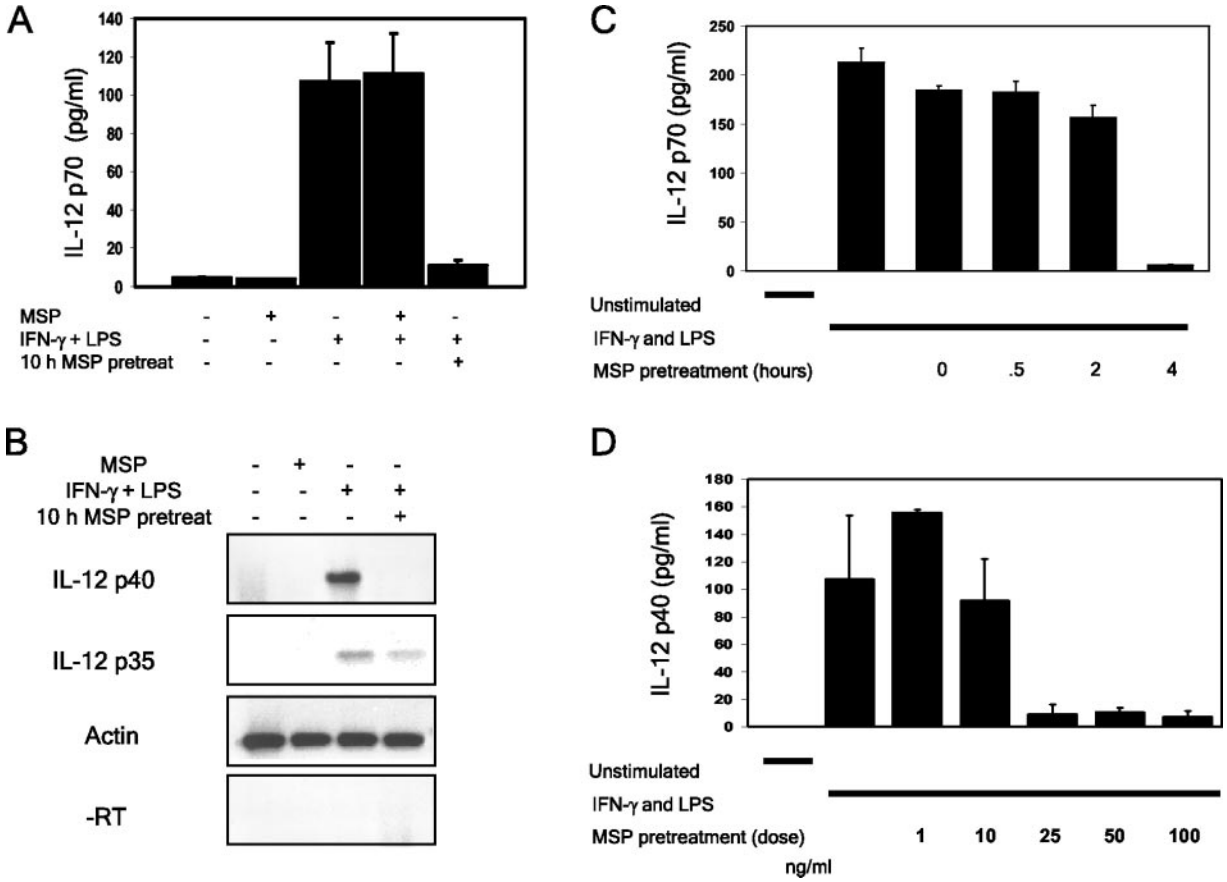

FIGURE 1. MSP inhibits IL-12 production by primary peritoneal macrophages. *A*, Peritoneal macrophages were treated with or without 100 ng/ml MSP for 10 h before stimulation with 10 U/ml IFN-γ, 0.1 μM LPS, and/or 100 ng/ml MSP for 24 h. Supernatants were collected, and IL-12 p70 production was measured by ELISA. *B*, Peritoneal macrophages were treated with or without 100 ng/ml MSP for 10 h. RNA was collected from macrophages 6 h after stimulation with either 10 U/ml IFN-γ and 0.1 μM LPS or MSP, and IL-12 p40 and p35 subunit expression was determined by RT-PCR. *C*, Supernatants from macrophages were pretreated with 100 ng/ml MSP for the times indicated before stimulation with IFN-γ and LPS for 24 h, and IL-12 p70 production was measured ELISA as described in *A*. *D*, Supernatants from macrophages were pretreated with the indicated dose of MSP for 10 h before stimulation with IFN-γ and LPS for 24 h, and IL-12 p40 production was measured by ELISA.

the same time as IFN-γ and LPS was unable to suppress IL-12 production, pretreatment for 10 h with MSP completely suppressed IL-12 p70 secretion. IL-12 is a heterodimeric cytokine made up of a p40 and a p35 subunit. The IL-12 p35 chain is constitutively produced at a low level by a variety of cell types, whereas the p40 chain is only produced by cells making biologically active IL-12 (42). The transcription of both chains as well as the IL-12 p70 heterodimer are inducible by many pathogens and their components, including LPS (1, 42). Although it does not induce IL-12 by itself, IFN-γ exerts a potent priming effect on IL-12 p40 production in cultured macrophages stimulated with LPS (10, 43). In this study we show that pretreatment of macrophages with MSP before IFN-γ and LPS completely inhibits the up-regulation of IL-12 p40 RNA, with an ~2-fold decrease in IL-12 p35 RNA as determined by densitometry (Fig. 1*B*).

The inhibition of IL-12 by MSP requires pretreatment. Therefore, we performed a time course of MSP pretreatment to determine the amount of time needed for MSP to exert its effects. As seen in Fig. 1*C*, ~2–4 h of pretreatment with MSP is required before IFN-γ and LPS stimulation to inhibit the production of IL-12. We also performed a dose-response curve to determine the minimal concentration of MSP required to inhibit IL-12. As shown in Fig. 1*D*, pretreatment with 25 ng/ml MSP is sufficient to inhibit IL-12 in response to IFN-γ and LPS. In comparison, pretreatment of peritoneal macrophages with MSP only partially inhibits TNF-α RNA and protein expression (Fig. 2, *A* and *B*). IL-1β is unaffected by MSP (Fig. 2*C*), whereas pretreatment with MSP before IFN-γ and LPS enhances IL-6 production (Fig. 2*D*).

MSP mediates its effects on macrophages through the STK/RON receptor tyrosine kinase. Thus, we examined the effects of MSP pretreatment on IL-12 production in RON$^{-/-}$ macrophages. In this study we show that the effect of MSP on IL-12 production is dependent on signaling through the RON receptor, as macrophages from the knockout animals produce IL-12 in the presence of MSP (Fig. 3*A*). During the early stages of an innate immune response, IL-12 stimulates IFN-γ production by NK and γδ T cells. It has also been suggested that dendritic cells and macrophages themselves can produce IFN-γ in response to IL-12 (44, 45). To determine whether the inhibitory effect of MSP on IL-12 production plays a role in the regulation of IFN-γ production in vivo, we isolated splenic white cells from RON$^{-/-}$ mice and their wild-type littermates and examined IFN-γ production by unstimulated splenocytes cultured in vitro for 24 h by ELISA (Fig. 3*B*). Splenocytes from RON$^{-/-}$ mice produce significantly elevated levels of IFN-γ compared with splenic cells from wild-type animals.

MSP pretreatment inhibits the tyrosine phosphorylation of STAT1 and expression of ICSBP/IRF-8

Both IRF-1$^{-/-}$ and ICSBP$^{-/-}$ mice are deficient in IL-12 production, suggesting that these are both critical transcription factors in

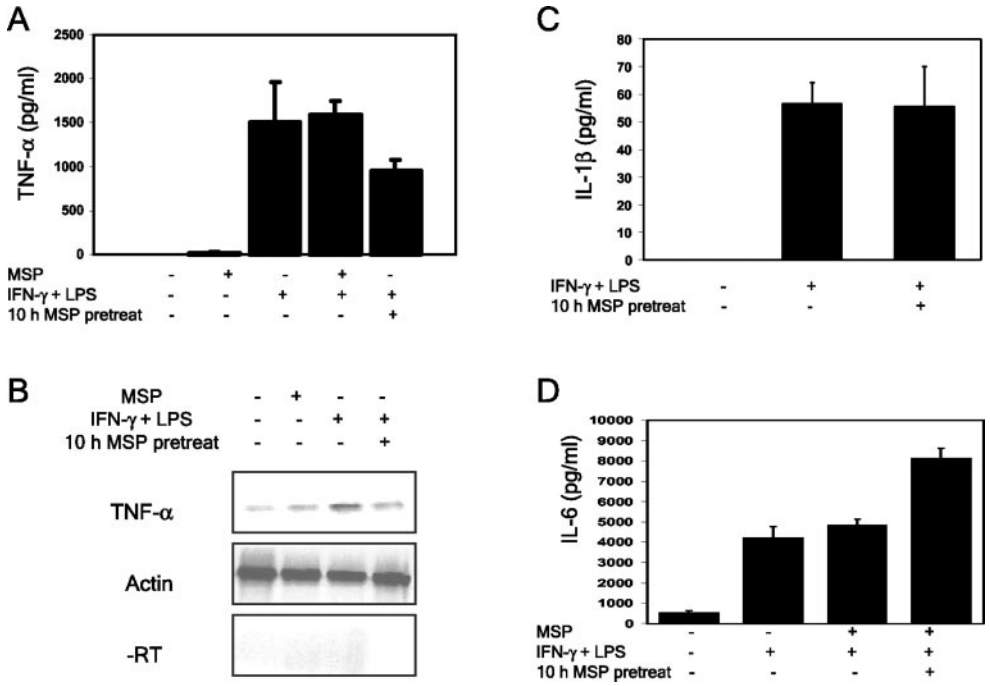

FIGURE 2. MSP alters the profile of inflammatory cytokines. Supernatants were collected from macrophages 24 h after stimulation with inflammatory cytokines and/or MSP as described in Fig. 1. TNF-α (*A*), IL-1β (*C*), and IL-6 (*D*) production was measured by ELISA. *B*, RNA was collected from macrophages 6 h after stimulation as described in Fig. 1, and TNF-α expression was determined by RT-PCR.

the regulation of IL-12 p40 (46–48). These transcription factors are regulated, in turn, by IFN-γ-induced STAT1 activation. STAT1 is phosphorylated in response to IFN-γ on both Y701 and S727, and phosphorylation at both of these residues is required for maximal transcriptional activity of STAT1 (49). Although tyrosine phosphorylation of STAT1 in response to IFN-γ is not affected by simultaneous treatment of macrophages with MSP (34), Fig. 4*A* demonstrates that 10 h of MSP pretreatment inhibits the tyrosine phosphorylation of STAT1 after stimulation with IFN-γ and LPS. Serine phosphorylation appears to be unaffected at both 30 min and 6 h after stimulation with both IFN-γ and LPS (Fig. 4*A*). The inhibition of STAT1 tyrosine phosphorylation by pretreatment with MSP is mediated by the RON receptor as MSP pretreatment of macrophages from RON$^{-/-}$ animals has no effect on STAT1 tyrosine phosphorylation (Fig. 4*B*).

STAT1 activation leads to stimulation of ICSBP and IRF-1 promoter activity through the IFN-γ activation site element (50, 51). ICSBP can, in turn, potentiate STAT1-dependent activation of IFN-γ-responsive promoters through the IFN-γ activation site element (52). To determine the effect of MSP on IRF-1 and ICSBP expression, we examined the expression of these transcription factors by RT-PCR. Macrophages pretreated with MSP before IFN-γ and LPS stimulation exhibit decreased expression of ICSBP, but no relative change in IRF-1 expression (Fig. 5*A*). The time period of pretreatment with MSP required for the inhibition of ICSBP expression was also determined (Fig. 5*B*). Similar to the inhibition of IL-12 production, pretreatment of macrophages with MSP for at least 2–4 h before stimulation with IFN-γ and LPS was required for decreased ICSBP expression, with complete inhibition observed at 6 h. In addition to IL-12, IL-15 and IL-18 are cytokines that required ICSBP for their maximal expression (53, 54). Pretreatment of macrophages with MSP before IFN-γ and LPS inhibits the up-regulation of both IL-15 and IL-18 mRNA (Fig. 5*C*).

MSP inhibits IL-12 production independently of IL-10

IL-10 is a potent inhibitor of IL-12 production (55). We have previously shown that stimulation of peritoneal macrophages with 100 ng/ml MSP does not result in the up-regulation of IL-10 (37). However, LPS alone or combined with other signals can induce IL-10 production. In Fig. 6*A* we tested the effects of MSP combined with LPS on the production of IL-10. Treatment of macrophages with MSP simultaneously or before LPS stimulation did not result in any significant changes in IL-10 production. We also examined the effect of MSP on IL-12 production in IL-10$^{-/-}$ mice (Fig. 6*B*). Pretreatment of macrophages with MSP before stimulation with IFN-γ and LPS resulted in complete inhibition of IL-12 production in these mice, suggesting that MSP acts independently of IL-10 in its ability to inhibit IL-12 production.

Discussion

The regulation of IL-12 p40 is incompletely understood; however, some transcriptional regulatory elements have been described. The human p40 gene contains both NFκB (-122 to -132) and Ets (-212 to -207) sites that are important for promoter activation by IFN-γ and LPS (56, 57). PU.1, IRF-1, and ICSBP have all been shown to cooperate and bind to the Ets site on the p40 promoter (58, 59). It has also been shown that C/EBP (-96 to -88 murine) and AP-1 (-79 to -74 murine) sites are important for the regulation of both murine and human IL-12 p40 in response to LPS (60, 61). In addition, AP-1 family members have been shown to synergize with C/EBPβ to induce p40 downstream of LPS (61). More recently, IRF-1 and ICSBP were found to cooperatively activate transcription of the murine p40 promoter at an ISRE-like site downstream from the NF-κB and C/EBP sites (62). ICSBP has been shown to be important at yet another site on this promoter, cooperating with NF-AT (-68 to -54 murine). This site is functionally important for p40 promoter activation by LPS plus INF-γ

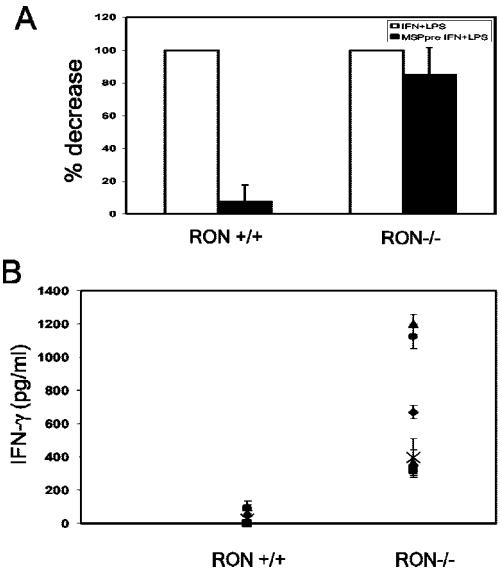

FIGURE 3. MSP inhibition of IL-12 production is dependent on the RON receptor. *A*, Supernatant was collected from macrophages from wild-type or $STK^{-/-}$ mice treated with or without MSP for 10 h before stimulation with 10 U/ml IFN-γ and 0.1 μM LPS for 24 h. IL-12 p70 production was determined by ELISA, and the percent change was calculated. *B*, Unstimulated splenic cells were isolated from wild-type and $STK^{-/-}$ mice, and IFN-γ production was determined by ELISA 24 h after isolation.

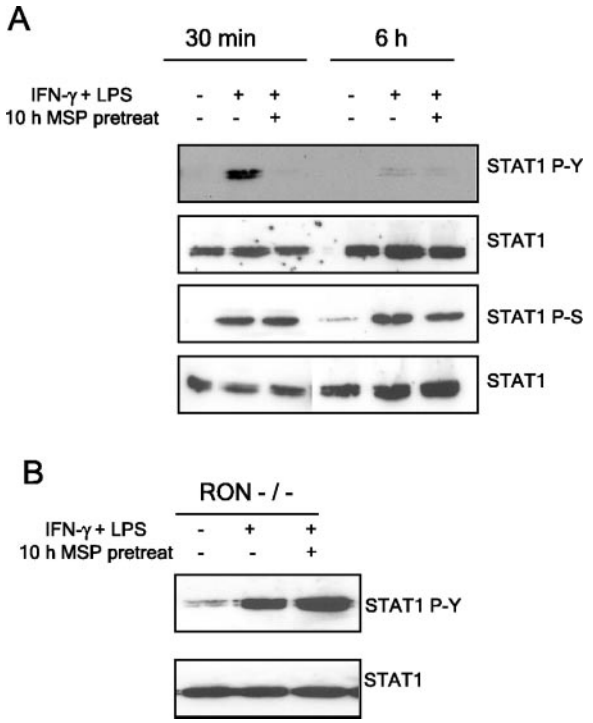

FIGURE 4. MSP inhibits STAT1 tyrosine phosphorylation. *A*, Peritoneal macrophages were treated with or without 100 ng/ml MSP for 10 h before stimulation with 10 U/ml IFN-γ and 0.1 μM LPS for the indicated time. Whole-cell extracts were analyzed by Western blot for STAT1 phosphorylation and then striped and reprobed for STAT1 protein to ensure equal protein loading. *B*, Peritoneal macrophages from $RON^{-/-}$ mice were treated as described in *A* for 30 min, and STAT1 tyrosine phosphorylation was determined by Western blot.

(63). In this study we demonstrate that MSP inhibits IL-12 production primarily by repressing the expression of the p40 subunit.

Although both ICSBP and IRF-1 have been shown to be activated by STAT1, it has also been demonstrated that IRF-1 can be activated by STAT1-independent mechanisms. Retinoic acid is able to induce IRF-1 via a STAT1-independent pathway that involves NF-κB (64). STAT1 responses have also been inhibited in the absence of an effect on IRF-1. In studying the effect of IL-10 on IFN-γ-inducible genes, Ito et al. (65) observed that STAT1 tyrosine phosphorylation was inhibited; however, IRF-1 expression was unaffected by IL-10. In our studies we show that MSP inhibits ICSBP induction, probably through the inhibition of STAT1 tyrosine phosphorylation, with little or no effect on IRF-1 expression. The cytokines affected by MSP pretreatment all require ICSBP for their expression, whereas the cytokines that are either unaffected or slightly affected do not. Macrophages from $ICSBP^{-/-}$ animals show a selective impairment in the mRNA expression of IL-12 p40, but not in that of IL-1-α, IL-1-β, IL-6, IL-10, or TNF-α, in response to LPS (5). In addition, dendritic cells from $ICSBP^{-/-}$ mice did not express either IL-12 p40 or IL-15 (53). IL-18 up-regulation is also dependent on ICSBP (54, 66). In our studies we have shown that pretreatment with MSP before stimulation with IFN-γ and LPS inhibits the expression of IL-12 p40, IL-15, and IL-18, but not IL-1-β, IL-6, or IL-10 and only slightly inhibits TNF-α. This corresponds to the cytokines that require ICSBP for their induction.

A variety of cytokines and anti-inflammatory molecules have previously been shown to inhibit IL-12 production. These molecules affect the expression of IL-12 at the level of both transcription and message stability. The type 1 IFNs (IFN-$\alpha\beta$) primarily target the transcription factor PU.1 (67, 68), whereas the cytokine TGF-β appears to reduce the stability of IL-12 p40 mRNA (69). Molecules such as PGE_2 and histamine act by inducing cAMP pathways to inhibit IL-12 production (70, 71). The Th2 cytokines IL-10 and IL-4 act by signaling through STAT pathways. IL-4 inhibits IL-12 by both STAT6-dependent and -independent mechanisms (72).

IL-10, a key regulator of IL-12, is produced by LPS and inhibits IL-12 as part of a negative feedback mechanism (73). It is also produced in response to anti-inflammatory molecules such as glucocorticoids and catecholamines, which inhibit IL-12 (74). IL-10, which mediates all of its effects through STAT3 (75), induces suppressors of cytokine signaling (SOCS3), which is able to inhibit both IFN-γ and LPS responses (65, 76). In a microarray experiment IL-10 was also shown to down-regulate the expression of ICSBP in bone marrow macrophages treated with LPS (75). The inhibition of STAT1 tyrosine phosphorylation and ICSBP expression by IL-10 is similar to our results with MSP. However, we have shown that MSP can inhibit IL-12 by an IL-10-independent mechanism, suggesting that MSP is not inducing IL-10, but may be sharing a similar signaling pathway.

The phagocytosis of a number of microbial pathogens has been associated with IL-12 production from macrophages. LPS, lipoteichoic acid, and bacterial DNA are all known inducers of IL-12 (10, 77, 78). However, ligation of phagocytic receptors, such as CR3, FcγR, and scavenger receptor-A (SR-A), inhibits the production of IL-12 (79). Calcium fluxes and the production of PGE_2 are implicated in FcR-mediated inhibition of IL-12 production (80). IL-10 is also partially responsible for this inhibition; however, FcR-mediated inhibition of IL-12 can occur in $IL\text{-}10^{-/-}$ mice (81). Ligation of FcγRs also disrupts binding of PU.1 to the Ets site on the IL-12 p40 promoter (59). We and others have shown that MSP regulates the expression and/or activity of several phagocytic receptors involved in the down-regulation of IL-12, including CR3, C5aR, and SR-A (22, 37). In addition, CR3 stimulation by C3bi

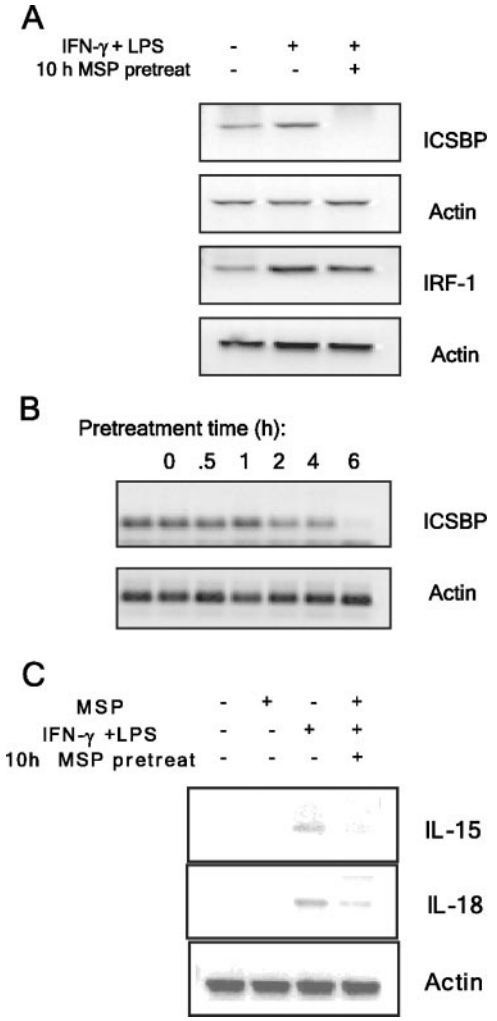

FIGURE 5. MSP inhibits the up-regulation of ICSBP expression. *A*, Peritoneal macrophages were treated with or without 100 ng/ml MSP for 10 h before stimulation with 10 U/ml IFN-γ and 0.1 μM LPS for 1.5 h. RNA was extracted and analyzed by RT-PCR. *B*, Peritoneal macrophages were stimulated with MSP for the time indicated before treatment with IFN-γ and LPS for 1.5 h, and ICSBP expression was determined by RT-PCR. *C*, Macrophages were treated with or without MSP for 10 h before stimulation with IFN-γ and LPS for 4 h, and IL-15 and IL-18 expression was determined by RT-PCR.

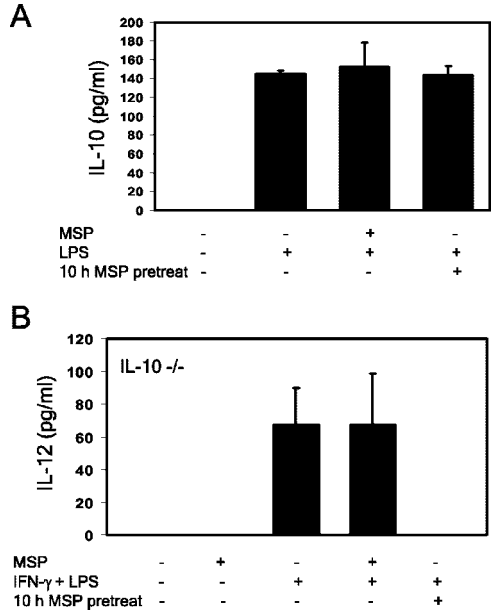

FIGURE 6. MSP inhibition of IL-12 production is independent of IL-10. *A*, Peritoneal macrophages were treated with or without 100 ng/ml MSP for 10 h before stimulation with 0.1 μM LPS and/or 100 ng/ml MSP for 24 h. Supernatants were collected, and IL-10 production was measured by ELISA. *B*, Peritoneal macrophages from IL-10$^{-/-}$ mice were stimulated as described in Fig. 1. IL-12 p70 production was measured by ELISA.

results in the suppression of IFN-γ-stimulated tyrosine phosphorylation (82). Therefore, MSP/RON may be able to regulate the production of IL-12 in part by inducing the expression of receptors such as SR-A or through the activation of complement receptors such as CR3.

Recent evidence has suggested that receptor tyrosine kinase (RTKs) play an important role in immune regulation. Mice deficient in either the Mer receptor alone or all three receptors of the Tyro-3 family, Tyro-3, Axl, and Mer, exhibit decreased phagocytosis and clearance of apoptotic cells (83, 84) as well as increased susceptibility to LPS-induced endotoxic shock (85). Macrophages from Tyro-3-, Axl-, and Mer-deficient animals also express increased levels of MHC class II and produce elevated amounts of IL-12 (13). In this study we show that the RON receptor tyrosine kinase is another RTK that regulates IL-12 production and thus the production of IFN-γ in the spleen. Both the RON receptor and the Mer receptor have been shown to inhibit the nuclear translocation of NF-κB after LPS stimulation (34, 86, 85), which is important for the regulation of iNOS, cyclooxygenase-2, and TNF-α production by macrophages. Previous studies have implicated the phosphoinositol 3-kinase pathway in the regulation of iNOS by MSP (87). We will continue to look at the signaling pathways downstream of RTKs that regulate immune responses.

The need for 2–4 h of pretreatment with MSP before IFN-γ and LPS to inhibit responses suggests that there may be new protein being made. It will be of interest to determine whether MSP is inducing the expression of SOCS proteins. SOCS proteins have been shown to regulate cytokine signals transduced by the Janus kinase (JAK)/STAT pathway through a mechanism that involves their up-regulation by STAT proteins. Both SOCS1 and SOCS3 inhibit JAK tyrosine kinase activity, which is important for IFN-γ signaling. SOCS-1 binds directly to the activation loop of JAKs through the SH2 domain, whereas SOCS3 binds to the cytokine receptors (88, 89). Thus, the SOCS proteins are able to effectively down-regulate IFN-γ signaling.

Based on previous data from our laboratory and others, we proposed a model in which the expression of the RON receptor on tissue-resident macrophages may provide protection from excess inflammation through the inhibition of iNOS expression and NO production in response to IFN-γ. Much like Th cells, the polarization of macrophages has recently been described. IFN-γ is the primary mediator of classical macrophage activation, resulting in the up-regulation of inflammatory cytokines such as IL-12, IL-1, and TNF-α, as well as bactericidal molecules, such as NO. In contrast, IL-4 is the primary mediator of alternative macrophage activation associated with the expression of genes involved in the inhibition of inflammation and the promotion of healing (90). We have shown previously that MSP induces arginase activity, potentially providing a second mechanism to regulate NO production. In addition, MSP can induce the expression of SR-A and IL-1R antagonist, which are markers of alternatively activated macrophages (37). In this study we demonstrate for the first time that the MSP/RON signaling pathway regulates the expression of IL-12 by APCs, thus, limiting IFN-γ production. Therefore, in addition to

limiting the effector functions of classically activated macrophages and promoting the expression of genes associated with alternative activation, MSP may also regulate the induction of classical activation through down-regulation of the IL-12/IFN-γ axis.

Acknowledgments

We thank Dr. Margherita Cantorna for providing the IL-10$^{-/-}$ animals.

References

1. Kobayashi, M., L. Fitz, M. Ryan, R. Hewick, S. Clark, S. Chan, R. Loudon, F. Sherman, B. Perussia, and G. Trinchieri. 1989. Identification and purification of natural killer cell stimulatory factor (NKSF), a cytokine with multiple biologic effects on human lymphocytes. *J. Exp. Med. 170:827*.
2. Trinchieri, G. 1995. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu. Rev. Immunol. 13:251*.
3. Magram, J., S. Connaughton, R. Warrier, D. Carvajal, C.-Y. Wu, J. Ferrante, C. Stewart, U. Sarmiento, D. Faherty, and M. Gately. 1996. IL-12 deficient mice are defective in IFN gamma production and type 1 cytokine responses. *Immunity 4:471*.
4. Mattner, F., J. Magram, J. Ferrante, P. Launois, K. Dipadova, R. Behin, M. Gately, J. Louis, and G. Alber. 1996. Genetically resistant mice lacking interleukin-12 are susceptible to infection with *Leishmania major* and mount a polarized Th2 cell response. *Eur. J. Immunol. 26:1553*.
5. Sharton-Kersten, T., T. Wynn, E. Denkers, S. Bala, E. Grunvald, S. Hieny, R. Gazzinelli, and A. Sher. 1996. In the absence of endogenous IFN-γ, mice develop unimpaired IL-12 responses to *Toxoplama gondii* while failing to control acute infection. *J. Immunol. 157:4045*.
6. Kobayashi, K., K. Kaneda, and T. Kasama. 2001. Immunopathogenesis of Delayed-Type Hypersensitivity. *Microsc. Res. Technol. 53:241*.
7. Wysoka, M., M. Kubin, L. Vieira, L. Ozmen, G. Garotta, P. Scott, and G. Trinchieri. 1995. Interleukin-12 is required for interferon-γ production and lethality in lipopolysaccharide-induced shock in mice. *Eur. J. Immunol. 25:672*.
8. Germann, T., J. Szeliga, H. Hess, S. Storkel, F. Podlaski, M. Gately, E. Schmitt, and E. Rude. 1995. Administration of interleukin 12 in combination with type II collagen induces severe arthritis in DBA/1 mice. *Proc. Natl. Acad. Sci. USA 92:4823*.
9. Trembleau, S., G. Penna, E. Bosi, A. Mortara, M. Gately, and L. Adorini. 1995. Interleukin 12 administration induces T helper type 1 cells and accelerates autoimmune diabetes in NOD mice. *J. Exp. Med. 181:817*.
10. Ma, X., J. Chow, G. Gri, G. Carra, F. Gerosa, S. Wolf, R. Dzialo, and G. Trinchieri. 1996. The interleukin 12 p40 gene promoter is primed by interferon gamma in monocytic cells. *J. Exp. Med. 183:147*.
11. Braun, M., and B. Kelsall. 2001. Regulation of interleukin-12 production by G-protein-coupled receptors. *Microbes Infect. 3:99*.
12. Ma, X. 2001. TNF-α and IL-12: a balancing act in macrophage functioning. *Microbes Infect. 3:121*.
13. Lu, Q., and G. Lemke. 2001. Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family. *Science 293:306*.
14. Leonard, E., and A. Skeel. 1976. A serum protein that stimulates macrophage movement, chemotaxis, and spreading. *Exp. Cell Res. 102:434*.
15. Leonard, E., and A. Skeel. 1978. Isolation of macrophage stimulating protein (MSP) from human serum. *Exp Cell Res. 114:117*.
16. Yoshimua, T., N. Yuhki, M. Wang, A. Skeel, and E. Leonard. 1993. Cloning, sequencing, and expression of human macrophage stimulating protein (MSP) confirms MSP as a member of the family of kringle proteins and locates the MSP gene on chromosome 3. *J. Biol. Chem. 268:15462*.
17. Bezerra, J., D. Witte, B. Aronow, and S. Degan. 1993. Hepatocyte specific expression of the mouse hepatocyte growth factor like protein. *Hepatology 18:394*.
18. Wang, M., T. Yoshimura, A. Skeel, and E. Leonard. 1994. Proteolytic conversion of single chain precursor macrophage stimulating protein to a biologically active heterodimer by contact enzymes of the coagulation cascade. *J. Biol. Chem. 269:3436*.
19. Nanney, L., A. Skeel, J. Luan, S. Polis, A. Richmond, M. Wang, and E. Leonard. 1998. Proteolytic cleavage and activation of pro-macrophage stimulating protein and upregulation of its receptor in tissue injury. *J. Invest. Dermatol. 111:573*.
20. Wang, M., A. Skeel, and E. Leonard. 1996. Proteolytic cleavage and activation of pro macrophage stimulating protein by resident peritoneal macrophage membrane proteases. *J. Clin. Invest. 97:720*.
21. Skeel, A., and E. Leonard. 1994. Action and target cell specificity of human macrophage stimulating protein. *J. Immunol. 152:4618*.
22. Skeel, A., S. Yoshimura, S. Showatler, E. Tanaka, E. Appella, and E. Leonard. 1991. Macrophage stimulating protein: purification, partial amino acid sequence, and cellular activity. *J. Exp. Med. 173:1227*.
23. Ronsin, C., F. Muscatelli, M. Mattei, and R. Breathnach. 1993. A novel putative receptor protein tyrosine kinase of the met family. *Oncogene 8:1195*.
24. Iwama, A., K. Okano, T. Sudo, U. Matsuda, and T. Suda. 1994. Molecular cloning of a novel receptor tyosine kinase gene, STK, derived from enriched hematopoietic stem cells. *Blood 83:3160*.
25. Ponzetta, C., A. Bardelli, Z. Zhen, F. Maina, P. dalla Zonca, S. Fiordano, A. Graziani, G. Panayotou, and P. Comoglio. 1994. A multifunctional docking site mediates signaling and transformation by the hepatocyte growth factor/scatter factor receptor family. *Cell 77:261*.
26. Wang, M., C. Ronsin, M. Gesnel, L. Coupey, A. Skeel, E. Leonard, and R. Breathnach. 1994. Identification of the ron gene product as the receptor for the human macrophage stimulating protein. *Science 266:117*.
27. Iwama, A., N. Yamaguchi, and T. Suda. 1996. STK/RON receptor tyrosine kinase mediates both apoptotic and growth signals via the multifunctional docking site conserved among the HGF receptor family. *EMBO J. 15:5866*.
28. Weidner, K., S. DiCesare, M. Sachs, V. Brinkmann, J. Behrens, and W. Birchmeier. 1996. Interaction between Gab 1 and c-Met receptor tyrosine kinase is responsible for epithelial morphogenesis. *Nature 384:173*.
29. Iwama, A., M. Wang, N. Yamaguchi, N. Ohno, K. Okano, T. Sudo, M. Takeya, F. Gervais, C. Morissette, E. Leonard, et al. 1995. Terminal differentiation of murine resident peritoneal macrophages is characterized by expression of the STK protein tyrosine kinase, a receptor for macrophage stimulating protein. *Blood 86:3394*.
30. Brunelleschi, S., L. Penengo, L. Lavagno, C. Santoro, D. Colangelo, I. Viano, and G. Gaudino. 2001. Macrophage stimulating protein (MSP) evokes superoxide anion production by human macrophages of different origin. *Br. J. Pharmacol. 134:1285*.
31. Kurihara, N., A. Iwama, J. Tatsumi, K. Ikeda, and T. Suda. 1996. Macrophage stimulating protein activates STK receptor tyrosine kinase on osteoclasts and facilitates bone resorption by osteoclast-like cells. *Blood 87:3704*.
32. Wang, M., H. Fung, and Y. Chen. 2000. Regulation of the RON receptor tyrosine kinase expression in macrophages: blocking the RON gene transcription by endotoxin-induced nitric oxide. *J. Immunol. 164:3815*.
33. Wang, M., G. Cox, T. Yoshimura, L. Sheffler, A. Skeel, and E. Leonard. 1994. Macrophage stimulating protein inhibits induction of nitric oxide production by endotoxin or cytokine stimulated mouse macrophages. *J. Biol. Chem. 269:14027*.
34. Liu, Q., K. Fruit, J. Ward, and P. Correll. 1999. Negative regulation of macrophage activation in response to IFN-γ and LPS by the STK/RON receptor tyrosine kinase. *J. Immunol. 163:6606*.
35. Correll, P., A. Iwama, S. Tondat, G. Mayrhofer, T. Suda, and A. Bernstein. 1997. Deregulated inflammatory response in mice lacking the STK/RON receptor tyrosine kinase. *Genes Funct. 1:69*.
36. Muraoka, R., W. Sun, M. Colbert, S. Waltz, D. Witte, J. Degan, and S. Degan. 1999. The RON/STK receptor tyrosine kinase is essential for peri-implantation development in the mouse. *J. Clin. Invest. 103:1277*.
37. Morrison, A., and P. Correll. 2002. Activation of the stem cell-derived tyrosine kinase/RON receptor tyrosine kinase by macrophage-stimulating protein results in the induction of arginase activity in murine peritoneal macrophages. *J. Immunol. 168:853*.
38. Waltz, S., L. Eaton, K. Toney-Earley, K. Hess, B. E., J. Ihlendorf, M.-H. Wang, K. Kaestner, and S. Degan. 2001. Ron-mediated cytoplasmic signaling is dispensable for viability but is required to limit inflammatory responses. *J. Clin. Invest. 108:567*.
39. Perera, P.-Y., T. Mayadas, O. Takeuchi, S. Akira, M. Zaks-Zilberman, S. Goyert, and S. Vogel. 2001. CD11b/CD18 acts in concert with CD14 and Toll-like receptor (TLR) 4 to elicit full lipopolysaccharide and toxol-inducible gene expression. *J. Immunol. 166:574*.
40. Todt, J., J. Sonstein, T. Polak, G. Seitzman, B. Hu, and J. Curtis. 2000. Repeated intratracheal challenge with particlulate antigen modulates murine lung cytokines. *J. Immunol. 164:4037*.
41. Barber, S., M. Fultz, C. Salkowski, and S. Vogel. 1995. Differential expression of interferon regulatory factor 1 (IRF-1), IRF-2, and interferon consensus sequence binding protein genes in lipopolysaccharide (LPS)-responsive and LPS hyporesponsive macrophages. *Infect. Immun. 63:601*.
42. Gubler, U., A. Chua, D. Schoenhaut, C. Dwyer, W. McComas, R. Motyka, N. Nabavi, A. Wolitzky, P. Quinn, P. Familletti, et al. 1991. Co-expression of two distinct genes is required to generate secreted bioactive cytotoxic lymphocyte maturation factor. *Proc. Natl. Acad. Sci. USA 88:4143*.
43. Hayes, M., J. Wang, and M. Norcross. 1995. Regulation of interleukin-12 expression in human monocytes: selective priming by interferon-γ of lipopolysaccharide-inducible p35 and p40 genes. *Blood 86:646*.
44. Ohteki, T., T. Fukao, K. Suzue, C. Maki, M. Ito, M. Nakamura, and S. Koyasu. 1999. Interleukin 12-dependent interferon γ production by CD8α$^+$ lymphoid dendritic cells. *J. Exp. Med. 189:1981*.
45. Munder, M., M. Mallo, K. Eichmann, and M. Modolell. 1998. Murine macrophages secrete interferon γ upon combined stimulation with interleukin (IL)-12 and IL-18: a novel pathway of autocrine macrophage activation. *J. Exp. Med. 187:2103*.
46. Lohoff, M., D. Ferrick, H. Mittrucker, G. Duncan, S. Bischof, M. Rollinghoff, and T. Mak. 1997. Interferon regulatory factor-1 is required for a T helper 1 immune response in vivo. *Immunity 6:681*.
47. Taki, S., T. Sato, K. Ogasawara, T. Fukuda, M. Sato, S. Hida, G. Suzuki, M. Mitsuyama, E.-H. Shin, S. Kojima, et al. 1997. Multistage regulation of Th1-type immune responses by the transcription factor IRF-1. *Immunity 6:673*.
48. Holtschke, T., J. Lohler, Y. Kanno, T. Fehr, N. Giese, F. Rosenbauer, J. Lou, K.-P. Knobeloch, L. Gabriele, J. Waring, et al. 1996. Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. *Cell 87:307*.
49. Wen, Z., Z. Zhong, and J. J. Darnell. 1995. Maximal activation of transcription by Stat1 and Stat 3 requires both tyrosine and serine phosphorylation. *Cell 82:241*.
50. Politis, A., K. Ozato, J. Coligan, and S. Vogel. 1994. Regulation of IFN-γ induces nuclear expression of IFN consensus sequence binding protein in murine peritoneal macrophages. *J. Immunol. 152:2270*.

51. Li, X., S. Leung, S. Qureshi, J. Darnell, and G. Stark. 1996. Formation of STAT1-STAT2 heterodimers and their role in the activation of IRF-1 gene transcription by interferon-α. *J. Biol. Chem. 271:5790*.
52. Contursi, C., I. Wang, L. Gabriele, M. Gadina, J. O'Shea, H. I. Morse, and K. Ozato. 2000. IFN consensus sequence binding protein potentiates STAT1-dependent activation of IFNγ-responsive promoters in macrophages. *Proc. Natl. Acad. Sci. USA 97:91*.
53. Schiavoni, G., F. Mattei, P. Sestili, P. Borghi, M. Venditti, H. C. Morse III, F. Belardelli, and L. Gabriele. 2002. ICSBP is essential for the development of mouse type I interferon-producing cells and for the generation and activation of $CD8\alpha^+$ dendritic cells. *J. Exp. Med. 196:1415*.
54. Kim, Y. M., H. S. Kang, S. G. Paik, K. H. Pyun, K. L. Anderson, B. E. Torbett, and I. Choi. 1999. Roles of IFN consensus sequence binding protein and PU.1 in regulating IL-18 gene expression. *J. Immunol. 163:2000*.
55. D'Andrea, A., M. Aste-Amezaga, N. Valiante, X. Ma, M. Kubin, and G. Trinchieri. 1993. Interleukin 10 (IL-10) inhibits human lymphocyte interferon γ-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J. Exp. Med. 178:1041*.
56. Murphy, T., M. Cleveland, P. Kuleza, J. Magram, and K. Murphy. 1995. Regulation of interleukin 12 p40 expression through an NFκB half-site. *Mol. Cell Biol. 15:5258*.
57. Ma, X., M. Neurath, G. Gri, and G. Trinchieri. 1997. Identification and characterization of a novel Ets-2-related nuclear complex implicated in the activation of the human interleukin-12 p40 gene promoter. *J. Biol. Chem. 272:10389*.
58. Wang, I.-M., C. Contursi, A. Masumi, X. Ma, G. Trinchieri, and K. Ozato. 2000. An IFN-γ-inducible transcription factor, IFN consensus sequence binding protein (ICSBP), stimulates IL-12 p40 expression in macrophages. *J. Immunol. 165:271*.
59. Cappiello, M., F. Sutterwala, G. Trinchieri, D. Mosser, and X. Ma. 2001. Suppression of IL-12 transcription in macrophages following Fcγ receptor ligation. *J. Immunol. 2001:4498*.
60. Plevy, S., J. Gemberling, S. Hsu, A. Dorner, and S. Smale. 1997. Multiple control elements mediate activation of the murine and human interleukin 12 p40 promoters: evidence of functional synergy between C/EBP and Rel proteins. *Mol. Cell. Biol. 17:4572*.
61. Zhu, C., K. Gagnidze, J. Gemberling, and S. Plevy. 2001. Characterization of an activation protein-1-binding site in the murine inteleukin-12 p40 promoter: demonstration of novel functional elements by a reductionist approach. *J. Biol. Chem. 276:18519*.
62. Masumi, A., S. Tamaoki, I.-M. Wang, K. Ozato, and K. Komuro. 2002. IRF-8/ICSBP and IRF-1 cooperatively stimulate mouse IL-12 promoter activity in macrophages. *FEBS Lett. 531:348*.
63. Zhu, C., K. Rao, H. Xiong, K. Gagnidze, F. Li, C. Horvath, and S. Plevy. 2003. Activation of the murine interleukin-12 p40 promoter by functional interactions between NFAT and ICSBP. *J. Biol. Chem. 278:39372*.
64. Percario, Z., V. Giandomenico, G. Fiorucci, M. Chiantore, S. Vannucchi, J. Hiscott, E. Affabris, and G. Romeo. 1999. Retinoic acid is able to induce interferon regulatory facrtor 1 in squamous carcinoma cells via a STAT-1 independent signalling pathway. *Cell Growth Differ. 10:263*.
65. Ito, S., P. Ansari, M. Sakatsume, H. Dickensheets, N. Vazquez, R. Donnelly, A. Larner, and D. Finbloom. 1999. Interleukin-10 inhibits expression of both interferon α- and interferon γ-induced genes by suppressing tyrosine phosphorylation of STAT1. *Blood 93:1456*.
66. Kim, Y. M., J. Y. Im, S. H. Han, H. S. Kang, and I. Choi. 2000. IFN-γ upregulates IL-18 gene expression via IFN consensus sequence-binding protein and activator protein-1 elements in macrophages. *J. Immunol. 165:3198*.
67. Cousens, L., J. Orange, H. Su, and C. Biron. 1997. Interferon-α/β inhibition of interleukin 12 and interferon-γ production in vitro and endogenously during viral infection. *Proc. Natl. Acad. Sci. USA 94:634*.
68. McRae, B., R. Semnani, M. Hayes, and G. van Seventer. 1998. Type I IFNs inhibit human dendritic cell IL-12 production and Th1 cell development. *J. Immunol. 160:4298*.
69. Du, C., and S. Sriram. 1998. Mechanism of inhibition of LPS-induced IL-12 p40 production by IL-10 and TGF-β in ANA-1 cells. *J. Leukocyte Biol. 64:92*.
70. van der Pouw Kraan, T., L. Boeije, R. Smeenk, J. Wijdenes, and L. Aarden. 1995. Prostaglandin-E_2 is a potent inhibitor of human interleukin 12 production. *J. Exp. Med. 181:775*.
71. Elenkov, I., E. Webster, D. Papanicolaou, T. Fleisher, G. Chrousos, and R. Wilder. 1998. Histamine potently suppresses human IL-12 and stimulates IL-10 production via H2 receptors. *J. Immunol. 161:2586*.
72. Levings, M., and J. Schrader. 1999. IL-4 inhibits the production of TNF-α and IL-12 by STAT6-dependent and -independent mechanisms. *J. Immunol. 162:5224*.
73. Aste-Amezaga, M., X. Ma, A. Sartori, and G. Trinchieri. 1998. Molecular mechanisms of the induction of IL-12 and its inhibition by IL-10. *J. Immunol. 160:5936*.
74. Elenkov, I., D. Papanicolaou, R. Wilder, and G. Chrousos. 1996. Modulatory effects of glucocorticoids and catecholamines on human interleukin-12 and interleukin-10 production: clinical implications. *J. Clin. Invest. 100:1513*.
75. Lang, R., D. Patel, J. Morris, R. Rutschman, and P. Murray. 2002. Shaping gene expression in activated and resting primary macrophages by IL-10. *J. Immunol. 169:2253*.
76. Berlato, C., M. Cassatella, I. Kinjyo, L. Gatto, A. Yoshimura, and F. Bazzoni. 2002. Involvement of suppressor of cytokine signaling-3 as a mediator of the inhibitory effects of IL-10 on lipopolysaccharide-induced macrophage activation. *J. Immunol. 168:6404*.
77. Cleveland, M., J. Gorham, T. Murphy, E. Tuomanen, and K. Murphy. 1996. Lipoteichoic acid preparations of Gram-positive bacteria induce interleukin-12 through a CD14-dependent pathway. *Infect. Immun. 64:1906*.
78. Klinman, D., A. Yi, S. Beaucage, J. Conover, and A. Kreig. 1996. CpG motifs present in bacteria DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon γ. *Proc. Natl. Acad. Sci. USA 93:2879*.
79. Sutterwala, F., G. Noel, R. Clynes, and D. Mosser. 1997. Selective suppression of interleukin-12 induction after macrophage receptor ligation. *J. Exp. Med. 185:1977*.
80. Berger, S., R. Chandra, H. Ballo, R. Hildenbrand, and H. Stutte. 1997. Immune complexes are potent inhibitors of interleukin-12 secretion by human monocytes. *Eur. J. Immunol. 27:1977*.
81. Gerber, J., and D. Mosser. 2001. Reversing lipopolysaccharide toxicity by ligation the macrophage Fcγ receptors. *J. Immunol. 166:6861*.
82. Marth, T., and B. Kelsall. 1997. Regulation of interleukin-12 by complement receptor 3 signaling. *J. Exp. Med. 185:1987*.
83. Scott, R., E. McMahon, S. Pop, E. Reap, R. Caricchio, P. Cohen, H. Earp, and G. Matsushima. 2001. Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature 411:207*.
84. Lu, Q., M. Gore, Q. Zhang, T. Camenisch, S. Boast, F. Casagranda, C. Lai, M. Skinner, R. Klein, G. Matsushima, et al. 1999. Tyro-3 family receptors are essential regulators of mammalian spermatogenesis. *Nature 398:723*.
85. Camenisch, T., B. Koller, H. Earp, and G. Matsushima. 1999. A novel receptor tyrosine kinase, Mer, inhibits TNF-α production and lipopolysaccharide-induced endotoxic shock. *J. Immunol. 162:3498*.
86. Zhou, Y., Y. Chen, J. Fisher, and M. Wang. 2002. Activation of the RON receptor tyrosine kinase by macrophages-stimulating protein inhibits inducible cyclooxygenase-2 expression in murine macrophages. *J. Biol. Chem. 277:38104*.
87. Chen, Y., J. Fisher, and M. Wang. 1998. Activation of the RON receptor tyrosine kinase inhibits inducible nitric oxide synthase (iNOS) expression by murine peritoneal exudate macrophages: phosphatidylinositol-3 kinase is required for RON-mediated inhibition of iNOS expression. *J. Immunol. 161:4950*.
88. Yasukawa, H., H. Misawa, and H. Sakamoto. 1999. The JAK-binding protein JAB inhibits *Janus* tyrosine kinase activity through binding in the activation loop. *EMBO J. 18:1309*.
89. Sasaki, A., H. Yasukawa, A. Suzuki, S. Kamizono, T. Syoda, I. Kinjyo, M. Sasaki, J. Johnston, and A. Yoshimura. 1999. Cytokine-inducible SH2 protein-3 (CIS3/SOCS3) inhibits *Janus* tyrosine kinase by binding through the N-terminal kinase inhibitory region as well as SH2 domain. *Genes Cells 4:1309*.
90. Goerdt, S., and C. Orfanos. 1999. Other functions, other genes: alternative activation of antigen-presenting cells. *Immunity 10:137*.