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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

Amy C. Morrison* and Pamela H. Correll2*†

Regulation of macrophage activities in response to inflammatory stimuli must be finely tuned to promote an effective immune response while, at the same time, preventing damage to the host. Our lab and others have previously shown that macrophage-stimulating protein (MSP), through activation of its receptor RON, negatively regulates NO production in response to IFN-γ and LPS by inhibiting the expression of inducible NO synthase (iNOS). Furthermore, activated macrophages from mice harboring targeted mutations in RON produce increased levels of NO both in vitro and in vivo, rendering them more susceptible to LPS-induced endotoxic shock. In this study, we demonstrate that stimulation of murine peritoneal macrophages with MSP results in the RON-dependent up-regulation of arginase, an enzyme associated with alternative activation that competes with iNOS for the substrate L-arginine, the products of which are involved in cell proliferation and matrix synthesis. Expression of other genes associated with alternative activation, including scavenger receptor A and IL-1R antagonist, is also up-regulated in MSP-stimulated murine macrophages. Stimulation of cells with IFN-γ and LPS blocks the ability of MSP to induce arginase activity. However, pretreatment of cells with MSP results in the up-regulation of arginase and inhibits their ability to produce NO in response to IFN-γ and LPS, even in the presence of excess substrate, suggesting that the inhibition of NO by MSP occurs primarily through its ability to regulate iNOS expression.


Balanced macrophage activation is essential to the health of an organism. Much like the dual activation paradigm found in Th cells, macrophages also possess a similar dichotomy. A shift toward classically activated macrophages is likely to result in tissue damage and autoimmunity disorders, while an imbalance toward alternatively activated macrophages would decrease an organism’s ability to fight infection (reviewed in Ref. 1). Th1 cytokines and LPS are the major mediators of the classical macrophage activation pathway, while Th2 cytokines and glucocorticoids induce alternative macrophage activation (2). The cytokines involved in activation not only inhibit the expression of proinflammatory molecules associated with classical activation, but up-regulate molecules that enable these cells to actively participate in healing processes (reviewed in Ref. 3).

While classically activated macrophages produce many inflammatory mediators including NO, which exert microbialidal activities, macrophages activated through the alternative pathway produce enhanced levels of arginase. Arginase and inducible NO synthase (iNOS),3 the enzyme that produces NO, share a common substrate, L-arginine. Metabolism of arginine through iNOS yields the products citrulline and NO, while metabolism through arginase yields urea and ornithine. While these enzymes catalyze the same substrate, their products have opposing biological effects. NO is cytotoxic to microbes, parasites, and tumors, and is generally antiproliferative. In contrast, ornithine is a precursor for proline and polyamine synthesis, molecules that are involved in cell growth and proliferation (4). Th2 cytokines, specifically IL-4 and IL-10, markedly down-regulate NO production in macrophages, and it has been proposed that they do so by the dual mechanism of suppressing the induction of iNOS gene expression in response to proinflammatory mediators like IFN-γ and by enhancing arginase activity, which reduces intracellular arginine accessible to iNOS (5).

Recent data suggest that macrophage-stimulating protein (MSP) plays an important role in regulating the activities of macrophages during inflammation. MSP is an 80-kDa serum protein that was originally identified due to its ability to cause shape changes and induce responsiveness to chemotactants in murine peritoneal macrophages (6, 7). 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 (8). MSP is primarily produced in the liver as a biologically inactive single-chain pro-MSP (9) and can be converted to its active form by several coagulation cascade enzymes (10), a serine protease found in wound fluids (11), and a macrophage plasma membrane-associated enzyme (12). In primary murine peritoneal macrophages, MSP can both induce C5a-mediated chemotaxis and act as a chemotactant itself (13). In these cells, MSP has also been shown to stimulate the phagocytosis of C3bi-coated SRBCs (14) activated protein kinase; MSP, macrophage-stimulating protein; SR-A, scavenger receptor A; DI, 1,1’-dioctadecyl-1,3,3’,3’-tetramethylindocyanine.
arginine II PCR are as follows, as described previously (30): 95°C for 5 min, followed by 35 cycles of 20 s at 95°C, 5 s at 54°C for 45 s, and 72°C for 60 s, with a final extension at 72°C for 5 min. The sequences of primers used are: sense, 5'-CTGAGGCGCTGTCAT-3', and antisense, 5'-CTGAGGCGCTGTCAT-3'. The conditions for IL-1Ra are as follows, as described previously (31); 99°C for 5 min followed by 30 cycles of 95°C for 15 s, 54°C for 45 s, and 72°C for 60 s, with a final extension at 72°C for 5 min. The sequences of primers used are: sense, 5'-CTTTTTGGAAGAACCTTTGGAAGAAG-3', and antisense, 5'-GAACCTTGGACATAAGGGACTCT-3'. The conditions for L-6 and IL-10 are as follows, as described previously (32); 95°C for 5 min followed by 30 cycles of 95°C for 15 s, 58°C for 20 s, and 72°C for 30 s, with a final extension at 72°C for 6 min. The sequences of primers used are: IL-6 sense, 5'-CAAGAAGCAATGCGGAGAGAAGCAGA-3', and IL-6 antisense, 5'-CTGACTGTGCTGAAGCTACTGAGATTGTGCG-3'. The PCR products were run on a 1% agarose gel.

Western blot analysis
A total of 1 × 10^7 cells was incubated in the presence or absence of 300 ng/ml MSP for 24 h. After incubation, cells were harvested with a cell scraper, washed with PBS, pelleted, 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 mM of 10 mM leupeptin, 1 µg/ml pepstatin A, and 2 µg/ml aprotinin. The protein contents were determined using the dendritic cell protein assay kit (Bio-Rad, Richmond, CA). Absorbance was measured at 750 nm with a Beckman DU530 spectrophotometer (Beckman Coulter, Palo Alto, CA). Proteins were mixed with 5× SDS sample buffer, SDS-PAGE, using 12.5% bis-acrylamide gel for separation, which was performed with a MiniProtein II Cell (Bio-Rad) at 65 V for 15 min, then at 150–160 V for 1 h. Inhobillon P polynylidenedifluorode membranes (Millipore, Bedford, MA) were washed briefly in methanol and then ddH2O, then equilibrated in trans-blotting buffer (48 mM Tris, 39 mM glycine, and 20% methanol) for 5 min. The gels were washed in ddH2O, then in trans-blotting buffer for 15 min. Trans-blotting 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. The blots were then incubated overnight at 4°C with mouse anti-rat arginase I Ab (Transduction Laboratories, Lexington, KY), according to manufacturer’s instructions, followed by anti-mouse peroxidase-conjugated secondary Ab. Protein was then detected by incubating the blots in chemiluminescence substrates (Amersham, Piscataway, NJ) and exposing to x-ray film.

Flow cytometry
Cells were harvested, incubated in the presence or absence of 100 ng/ml MSP for 24 h, and washed with PBS. A total of 5 × 10^6 cells/100 µl was resuspended in PBS plus 2% newborn calf serum on ice. FCr were blocked using 1 µl of anti-mouse CD32/16 (FcγIII/IIIR; BD Pharmingen, San Diego, CA) for 5 min on ice. Scavenger receptor A (SR-A) expression was then detected using a FITC-conjugated rat anti-mouse macrophage scavenger receptor Ab (Serotec, Oxford, UK) and analyzed by flow cytometry (XL; Coulter, Hialeah, FL).

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 (27), 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 overnight and then washed with PBS to eliminate nonadherent cells. All cell cultures were maintained in RPMI 1640 plus 10% FBS at 37°C in a humidified incubator containing 5% CO2.

**Reagents**

MSP, IL-10, and IL-1Ra were obtained from R&D Systems (Minneapolis, MN). IFN-γ was purchased from PeproTech (Rocky Hill, NJ). LPS and L-arginine were obtained from Sigma-Aldrich (St. Louis, MO).

**Arginase and nitrite assays**

Arginase activity was measured in cell lysates, as described by Corraliza et al. (29), with modifications as described previously (30). Briefly, cells were lysed with 100 µl of 0.1% Triton X-100. After 30 min on a shaker, 100 µl of 25 mM Tris-HCl was added. To 100 ml of this lysate, 10 µl of 10 mM MnCl2 was added, and the enzyme was activated by heating for 10 min at 55°C. Arginine hydrolysis was conducted by incubating the lysates with 100 µl of 0.5 M L-arginine (pH 9.7) at 37°C for 60 min. The reaction was stopped with 800 µl of H2SO4 (96%/H3PO4 (85%)/H2O (1/3/7, v/v/v). The H2SO4 concentration was measured at 550 nm after addition of 40 µl of α-isonitrosopropiophenone (Sigma-Aldrich) (dissolved in 100% ethanol), followed by heating at 100°C for 30 min. One unit of enzyme activity is defined as the amount of enzyme that catalyzes the formation of 1 µmol urea/min.

To determine the levels of NO production, macrophages were plated in a 96-well plate at a concentration of 1 × 10^5 cells/well. Following stimulation, the production of NO was determined by assaysing culture supernatants for NO2−, a stable reaction product of NO with molecular oxygen. Briefly, 100 ml of culture supernatant was reacted with an equal volume of reagent (1% sulfanilamide/0.1% naphthylenediamine dihydrochloride/2.5% H3PO4) at room temperature for 10 min, after which the absorbance at 550 nm was determined.

**RNA extraction and RT-PCR**

Arginase, IL-10, IL-6, and IL-1Ra expression in resting and MSP-activated resident peritoneal macrophages was analyzed by RT-PCR. Briefly, 1 × 10^6 cells were activated with 100 ng/ml MSP for 24 h, after which they were harvested for RNA isolation using the guanidinium thiocyanate method. Reverse transcription was conducted for 15 min at 42°C using random hexamers from 0.3 µg of total RNA. Conditions for arginase I and II PCR are as follows, as described previously (30); 95°C for 5 min, followed by 35 cycles of 20 s at 95°C, 20 s at 56°C, and 30 s at 72°C, with a final extension at 72°C for 10 min. The sequences of primers used are: arginase I sense, 5'-CAGAAGAATGGAAGGATCAG-3', and antisense, 5'-CAGATATGCGAGGGATCACC-3'; and arginase II sense, 5'-TGATTTGCGAAAGGGCGAAGG-3', and antisense, 5'-CTAGGAGTAGAAGGTTGTC-3'. The conditions for IL-1Ra are as follows, as described previously (31); 99°C for 5 min followed by 30 cycles of 95°C for 15 s, 54°C for 45 s, and 72°C for 60 s, with a final extension at 72°C for 5 min. The sequences of primers used are: sense, 5'-CTCTTCTGGGAAAAGACCCCTGGAAGAAG-3', and antisense, 5'-GAACCTTGGACATAAGGGACTCT-3'. The conditions for IL-6 and IL-10 are as follows, as described previously (32); 95°C for 5 min followed by 30 cycles of 95°C for 15 s, 58°C for 20 s, and 72°C for 30 s, with a final extension at 72°C for 6 min. The sequences of primers used are: IL-6 sense, 5'-CAAGAAGCGAGTCTCTGAGAG-3', and IL-6 antisense, 5'-CTAGGTGTCGCACTATGACTC-3'; IL-10 sense, 5'-CTAGGAGCCCGTCCTCATCGATT-3', and IL-10 antisense, 5'-AGTGGCTGGACTCCAGACA-3'. PCR products were run on a 1% agarose gel.

**Flow cytometry**

Cells were harvested, incubated in the presence or absence of 100 ng/ml MSP for 24 h, and washed with PBS. A total of 5 × 10^6 cells/100 µl was resuspended in PBS plus 2% newborn calf serum on ice. FCr were blocked using 1 µl of anti-mouse CD32/16 (FcγIII/IIIR; BD Pharmingen, San Diego, CA) for 5 min on ice. Scavenger receptor A (SR-A) expression was then detected using a FITC-conjugated rat anti-mouse macrophage scavenger receptor Ab (Serotec, Oxford, UK) and analyzed by flow cytometry (XL; Coulter, Hialeah, FL).
AcLDL endocytosis assay

Cells were harvested, incubated in the presence or absence of 100 ng/ml MSP and 5 ng/ml IL-10 for 24 h, and washed with PBS. A total of 5 × 10⁶ cells was then incubated with 2 μg/ml 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocyanine (DiI)-acylated low density lipoprotein (AcLDL; Intrace, Rockville, MD) for 4 h in serum-free RPMI plus 2 mg/ml BSA. Cells were then washed with PBS, harvested in PBS plus 2% newborn calf serum, and analyzed by flow cytometry.

Results

MSP induces arginase activity in primary murine peritoneal macrophages

Previously, we and others have shown that the MSP/RON signaling pathway inhibits NO production in response to IFN-γ and LPS (25, 26). We have also seen that MSP can inhibit the up-regulation of iNOS gene in response to IFN-γ and LPS in resident peritoneal macrophages (data not shown), as seen previously by Wang et al. (15). Several Th2 cytokines, including IL-4 and IL-10, that inhibit NO production by activated macrophages also up-regulate expression of arginase, which competes with iNOS for the substrate, l-arginine. To determine whether MSP can induce arginase activity, resident peritoneal macrophages were incubated with MSP for 24 h, and arginase activity was measured. MSP stimulation consistently increased arginase activity approximately 2- to 3-fold (Fig. 1A). To determine whether the up-regulation of arginase by MSP is mediated by the RON receptor, macrophages from RON-deficient mice were utilized (Fig. 1B). MSP was unable to induce arginase activity in RON-deficient macrophages, suggesting that the observed increase in arginase activity in response to MSP is mediated by the RON receptor. Although RON-deficient macrophages did not show increased arginase activity in response to MSP, these cells were capable of up-regulating arginase activity in response to the Th2 cytokine IL-4 (Fig. 1C).

Two distinct isoforms of arginase are expressed in macrophages. Arginase I is identical to the liver-type arginase and is present in the cytosol, while type II is identical to the kidney-type arginase and is contained in the mitochondria (4). To determine whether the observed increase in arginase activity was due to increased expression of one or both forms of arginase, we examined the levels of arginase I and II RNA by RT-PCR following stimulation with MSP for 24 h (Fig. 2A). The induction of arginase activity in response to MSP appears to be primarily due to an increase in arginase I expression. These results were confirmed by quantitative real time RT-PCR. In addition, arginase I protein was induced by MSP stimulation, as shown by Western blot analysis (Fig. 2B).

MSP induces the expression of SR-A and IL-1Ra

Up-regulation of arginase activity in macrophages is an event associated with alternative activation. Therefore, we set out to determine whether the expression of other genes associated with alternative activation of macrophages was also up-regulated in response to MSP. While classically activated macrophages primarily express IgG receptors, alternatively activated macrophages express a variety of immune pattern recognition receptors such as the mannose receptor (2), the β-glucan receptor (33), and the scavenger receptor type I (34), which give these cells enhanced phagocytic ability by recognizing a wide variety of foreign Ags. SR-A plays a role in binding and endocytosis of AcLDL and oxidized LDL, phagocytosis of apoptotic cells, cell adhesion, and host defense function (reviewed in Ref. 35). SR-A expression on macrophages is up-regulated following differentiation from monocytes to macrophages; however, stimulation with MSP for 24 h resulted in increased expression of this receptor, as seen by flow cytometry (Fig. 3A). This observed increase in expression was mediated by the RON receptor, as macrophages from RON-deficient mice did not show an increase in SR-A expression in response to MSP (data not shown). Expression of CD-11b, a marker of macrophage differentiation, remained unchanged in these cells when stimulated with MSP (data not shown). In addition to increased expression of SR-A, we also observe an increase in the number of macrophages able to endocytose DiI-labeled AcLDL when stimulated with MSP, as measured by flow cytometry (Fig. 3C).
In addition to the expression of receptors involved in phagocytosis, cytokine production is also coordinately regulated in macrophages. While classically activated macrophages primarily secrete cytokines that function as inflammatory mediators, including IL-1 and TNF-α, alternatively activated macrophages preferentially express cytokines with anti-inflammatory effects, such as IL-1Ra (36) and IL-10. IL-1Ra inhibits the action of IL-1α and IL-1β by competitively binding to IL-1Rs but has no agonist activity (reviewed in Ref. 37). To determine whether MSP induced IL-1Ra expression in primary peritoneal macrophages, we analyzed IL-1Ra RNA expression by RT-PCR. Our results demonstrate that IL-1Ra RNA expression was consistently induced by MSP stimulation (Fig. 3B).

MSP-induced arginase activity and SR-A expression are independent of IL-10 production

Arginase has previously been shown to be induced by stimulation of macrophages with IL-4 and IL-10 (38). While IL-4 is primarily produced by T-cells, IL-10 can be produced by macrophages. LPS has also been shown to induce arginase activity, but recent results suggest that this induction is mediated by the ability of LPS to induce IL-10 production by macrophages (30). In addition, IL-10 is also considered a marker of alternative activation (3). To elucidate the mechanism by which MSP induces arginase activity, we wanted to determine whether MSP induces production of cytokines known to stimulate this activity. To determine whether MSP-induced arginase activity is dependent on IL-10 production, macrophages were isolated from mice with a targeted mutation in the IL-10 gene. When these macrophages were stimulated with MSP, the levels of arginase activity were similar to those seen in wild-type macrophages (Fig. 4A). Similarly, SR-A expression on IL-10 knockout macrophages is also induced by MSP at levels comparable with those seen in wild-type macrophages (Fig. 4B). We also analyzed RNA from macrophages stimulated with MSP for 24 h and saw no observable increase in IL-10 expression (Fig. 4D).

There was also no increase in IL-10 production from MSP-stimulated cells, as determined by ELISA (data not shown). Therefore, the ability of MSP to induce arginase activity and SR-A expression is independent of IL-10.

In addition to IL-10, we sought to determine whether MSP induction of arginase activity could be mediated by the up-regulation of IL-6. Although IL-6 had not been previously shown to induce arginase activity, we hypothesized that it would be able to induce activity due to the importance of CCAAT/enhancer-binding protein/β sites in the arginase I promoter (39). IL-6 stimulation (10 ng/ml) of murine resident peritoneal macrophages for 24 h showed...
a similar 2- to 3-fold increase in arginase activity as seen with MSP (Fig. 4C); however, RNA analyzed by RT-PCR from MSP-stimulated macrophages did not reveal any IL-6 expression in these cells (Fig. 4D).

**Antagonistic production of NO and arginase in response to proinflammatory cytokines and MSP**

We and others have shown previously that expression of the RON receptor in RAW 264.7 cells results in a decrease in the transcriptional activation of iNOS in response to cytokine stimulation (25, 26). To determine whether the ability of MSP to inhibit NO production is also due to its ability to up-regulate arginase activity and compete with iNOS for the substrate, L-arginine, we stimulated murine peritoneal macrophages with IFN-γ and LPS in the presence and absence of MSP for 24 h and measured arginase activity and NO production (Fig. 5). MSP was able to inhibit NO production in response to IFN-γ and LPS, as previously reported (15); however, in these cells, MSP failed to enhance arginase activity. However, pretreatment of macrophages for 9 h with MSP before stimulation with IFN-γ and LPS enhanced arginase activity and resulted in an even larger decrease in NO production (Fig. 5, A and B). Macrophages prestimulated with MSP were refractory to IFN-γ and LPS treatment even in the absence of additional MSP. The addition of excess arginine (Fig. 5, C and D) did not affect the ability of MSP to inhibit NO production. Similar results were seen with up to 12 mM exogenous arginine (data not shown). These data suggest that the ability of MSP to inhibit NO production in response to IFN-γ and LPS under these conditions is not due to competition for substrate by arginase. In a larger context, these studies demonstrate that Th1 cytokines and MSP act in an antagonistic manner in their ability to stimulate macrophages to produce NO and increase arginase activity, respectively.

**Discussion**

The data presented in this work demonstrate that, in addition to its role as a suppressor of NO production in IFN-γ-activated macrophages, MSP can also induce arginase activity. This increase in arginase activity is a result of an increase in arginase RNA and protein expression. Arginase activity is a marker of alternative activation in macrophages. Other markers, such as SR-A and IL-1Ra, also appear up-regulated by MSP stimulation. Taken together, these results suggest that MSP may play a role in the alternative activation process. While it remains speculative, the ability of MSP to induce genes associated with alternative activation in macrophages may play a role in its ability to regulate septic shock.

Activation of arginase has also been shown to play an important role in helping to alleviate endotoxic shock. NO production by macrophages is dependent on an extracellular level of L-arginine (40), and arginase can decrease NO production by reducing L-arginine availability to iNOS (41). Intravenously added arginase has also been shown to decrease NO formation in the liver, lung, spleen, and kidney in LPS-treated rats as well as plasma nitrite levels (42). However, in the studies described in this work, the addition of excess arginine did not overcome the ability of MSP to inhibit NO production. While MSP is able to activate arginase...
expression, it also directly inhibits the transcription of iNOS in response to IFN-γ and LPS. Therefore, it appears that the iNOS and arginase signaling pathways may be independent targets of MSP. Preliminary data from our lab have suggested that different mitogen-activated protein kinases (MAPKs) may be required for the regulation of arginase and iNOS. We have seen that chemical inhibitors of p38 MAPK can inhibit arginase induction, but have no effect on NO production. Recent work has also shown that p38 MAPK and cAMP/protein kinase A are required for IL-13-induced arginase activity (43). On the other hand, chemical inhibitors of mitogen-activated protein/extracellular signal-related kinase kinase can inhibit the ability of MSP to inhibit NO production, but have no effect on the ability of MSP to induce arginase.

A role for SR-A in protection against endotoxic shock has also been demonstrated. Mice with a targeted mutation in the gene encoding SR-A are more sensitive to endotoxic shock and are more susceptible to infection with Listeria monocytogenes (44), both phenotypes that were also observed in the RON-deficient mice (27, 51). It has been suggested that the SR-A knockout mice may be more susceptible to endotoxic shock due to the ability of the SR-A to act as an alternate binding site for LPS and quench excess endotoxin away from CD-14 (45). Studies in transgenic and knockout mice also indicate that IL-1Ra is important for protection from endotoxin-induced sepsis (46). In vivo studies have shown that treatment with rIL-1Ra reduces the severity of experimental sepsis, chemically induced colitis, arthritis, and diabetes (37). The ability of MSP to up-regulate both SR-A and IL-1Ra may also prove important in protection from endotoxic shock.

Experimental evidence has suggested that RON/MSP may also play a role in wound healing. Previous studies have shown increased levels of active MSP in human burn wound fluid, as well as the up-regulation of RON on keratinocytes and dermal macrophages at wound sites (11). In experimental rat wounds, decreased levels of arginine in the wound fluid were accompanied by increased levels of ornithine and high arginase activity (47). It has been suggested that the cellular localization of the arginase isoforms dictates their functions. Ornithine decarboxylase, like arginase type I, is located in the cytosol and utilizes ornithine in the production of polyamines (48). In contrast, ornithine aminotransferase, the enzyme that initiates the conversion of ornithine to proline, which is essential for collagen synthesis, is located within the mitochondria (4). The production of both polyamines and collagen

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**FIGURE 5.** Antagonistic production of NO and arginase in response to MSP. Murine peritoneal macrophages were preincubated for 9 h in the presence or absence of 100 ng/ml MSP and then stimulated either with 10 U/ml IFN-γ and 0.1 μM LPS or with MSP, IFN-γ, and LPS. NO production was measured from the supernatant using Griess reagent (A and C) and arginase activity was measured from the cellular lysates (B and D). The effect of excess arginine was also determined (C and D).
is important in the healing wound. The ability of MSP to induce expression of arginase I provides further evidence to suggest that this pathway may play an important role in wound healing.

While cytokines such as IL-10 and IL-6 can induce arginase activity, results demonstrated in this work show that MSP-induced arginase activity is not dependent on IL-10 or IL-6 production. However, IL-10, IL-6, and MSP appear to induce arginase activity to similar levels. One downstream signaling pathway shared by these diverse receptors is the activation of STAT3. STAT3 has been shown to be downstream of c-Met receptor signaling (49). Conditional macrophage knockouts of STAT3 have defects in their inflammatory response, similar to defects seen in both the RON- and IL-10-deficient mice. STAT3 macrophage knockouts are highly susceptible to endotoxic shock and show increased production of inflammatory cytokines such as TNF-α, IFN-γ, and IL-1 (50). It will be of interest to determine whether these macrophages are defective in their ability to up-regulate arginase activity in response to IL-10, IL-6, or MSP.

Much like the dichotomy found in Th cells, macrophages are activated down two distinct pathways. The regulation of RON may play an important role in this balanced macrophage activation. The inability of MSP to induce arginase activity when cells were stimulated simultaneously with IFN-γ and LPS may result from the suppressive effects of these inflammatory cytokines on RON expression. Based on the data presented in this work, we suggest a model in which down-regulation of RON by inflammatory cytokines during the initial stages of an immune response may enhance classical activation, while its up-regulation during later stages of an immune response and in wounds may facilitate alternative activation and promote healing.

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