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Meteorin-like/Meteorin- β Is a Novel Immunoregulatory Cytokine Associated with Inflammation

Irina Ushach,^{*,†,1} Gerardo Arrevillaga-Boni,^{*,†,1} Gina N. Heller,^{*,†} Egest Pone,[‡] Marcela Hernandez-Ruiz,^{*,†} Jovani Catalan-Dibene,^{*,†} Peter Hevezi,^{*,†} and Albert Zlotnik^{*,†}

We have described a novel cytokine encoded by a gene called Meteorin-like (*Metrl*). *Metrl* is a small (~28 kDa) secreted protein expressed by activated macrophages and barrier tissues (mucosa and skin). *Metrl* production by bone marrow macrophages is induced by several cytokines including TNF- α , IL-17 α , IL-12, and IL-4 and inhibited by IFN- γ and TGF- β . *Metrl* expression in macrophages is also induced by LPS, and its levels in circulation are associated with inflammatory responses in vivo. Furthermore, *Metrl* regulates the production of several cytokines and chemokines in macrophages. We have produced a *Metrl*^{-/-} mouse, which is viable and shows normal development. However, it exhibits dysregulated cytokine production, alterations in IgG production, and is highly susceptible to LPS in a sepsis model. Furthermore, older *Metrl*^{-/-} mice develop inflammatory lesions, suggesting that *Metrl* participates in the control of inflammatory responses. Taken together, these observations indicate that *Metrl* encodes a novel immunoregulatory cytokine associated with inflammatory responses that we have designated Meteorin- β . *The Journal of Immunology*, 2018, 201: 3669–3676.

Cytokines are small secreted proteins that play key roles in many biological processes including hematopoiesis, embryonic development, and immune responses. Approximately 10% of the human genome is estimated to encode secreted proteins. Given the importance of cytokines in the immune system, we asked whether there were secreted proteins linked to the immune system that remained to be described. To address this question, we screened a comprehensive database of gene expression (body index of gene expression) (1), looking for genes encoding secreted proteins expressed by cells or organs of the immune system. This screen yielded several novel secreted proteins expressed by various cells of the immune system. One of these genes (*C17ORF99*) encodes a small secreted protein that we have recently identified as a novel B cell-associated cytokine, which we have called IL-40 (2). The same screen identified another gene with cytokine-like characteristics including another gene encoding a small secreted protein (~28 kDa) annotated as Meteorin-like (*Metrl*). This name reflects the fact that *Metrl* is

evolutionarily related to a gene that encodes a known neurotrophic factor called Meteorin (*Metrn*) that is expressed in the CNS (3). *Metrl* has recently been described to be a hormone (4) or adipokine (5) involved in metabolic responses. We have reported that *Metrl* is produced by activated macrophages, and that its expression is associated with several human autoimmune diseases, including psoriasis (3). Furthermore, *Metrl*, under homeostatic conditions, is mainly expressed in human barrier tissues, including skin and mucosal sites of the digestive and respiratory tract (3). We, therefore, hypothesized that *Metrl* encodes a novel cytokine whose functions likely include important roles in inflammation and in innate and acquired immunity (3). To confirm this hypothesis, we sought to continue the functional characterization of *Metrl* by producing and characterizing a *Metrl*^{-/-} mouse.

In the current study, we report that the production of *Metrl* in bone marrow-derived macrophages (BMM) is regulated by many cytokines (including TNF- α , IL17a, IL12, IL4, IL-1 β , IFN- γ , and TGF- β). Conversely, *Metrl* can also regulate the expression of several cytokines by macrophages. Importantly, the expression of *Metrl* is strongly induced in macrophages by TNF- α and LPS, and *Metrl* levels parallel the development and resolution of inflammatory responses in vivo. We have produced a *Metrl*^{-/-} mouse that lacks the capacity to express *Metrl* in all tissues. *Metrl*^{-/-} mice develop and reproduce normally, but exhibit multiple immune system abnormalities. They have lower levels of IgG in plasma and show dysregulation of cytokine and chemokine production. When tested in a model of sepsis induced by LPS, they show enhanced susceptibility. Furthermore, a significant proportion of older *Metrl*^{-/-} mice developed inflammatory lesions. Taken together, these observations support and expand our previous findings (3) and confirm that *Metrl* encodes a novel immunoregulatory cytokine associated with inflammation. *Metrl*^{-/-} mice do not show metabolic abnormalities when fed either a normal or high-fat diet, further suggesting that the main functions of *Metrl* are in the immune system. The last novel cytokines described include a new member of the IL-12 family that has been called IL-39 (6, 7) and our own recent description of IL-40/*C17ORF99* (2). The name *Metrl* only

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Abbreviations used in this article: BMM, bone marrow-derived macrophage; IL1RN, IL-1R antagonist; Metrn α , Meteorin- α ; Metrn β , Meteorin- β ; *Metrl*, Meteorin-like; WT, wild type.

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reflects its evolutionary relationship to *Meteorin*, but does not describe that it is a cytokine or that it plays an important role in immunity, inflammation, and likely other systems (4, 5, 8). We, therefore, propose that Meteorin should be renamed Meteorin- α (Metn α) and that Metnl should be renamed Meteorin- β (Metn β) (9), a designation that we will use in the present report.

Materials and Methods

Generation of *Metnl/Metrn β ^{-/-}* mice

To produce a *Metnl/Metrn β ^{-/-}* mouse, we obtained mouse embryonic stem cells from the Knockout Mouse Project (www.KOMP.org) containing a selection cassette that was inserted into the mouse *Metnl* locus by homologous recombination (Supplemental Fig. 1). These mouse embryonic stem clones were microinjected into C57BL/6 blastocysts, which were transferred into pseudo-pregnant female mice. Resulting chimeras were crossed with wild type (WT) C57BL/6 mice, and *Metn β Neo⁺* heterozygotes were subsequently bred with FLP α mice. The *Metn β Neo^{-/-}/loxP⁺* mice were then bred to CRE mice to delete the loxP-flanked target region. Finally, heterozygote *Metn β ^{+/+}* mice were intercrossed to generate homozygote *Metn β ^{-/-}* mice in the C57BL/6 background. (Supplemental Fig. 1).

Flow cytometry and imaging flow cytometry

Single-cell suspensions were stained with the following Abs: CD11b (M1/70), Ly6G (IA8), CCR3 (J073E5), F4/80, (BM8), and class II MHC (M5/114.15.2) (BioLegend, San Diego, CA) and Siglec-F (eBioscience, Carlsbad, CA). Samples were processed in a NovoCyte (ACEA) and analyzed with FlowJo software (Tree Star). Imaging was performed using an imaging flow cytometer (ImageStream X Mark II, Amnis).

Mice

C57BL/6, FLP α , and Cre mice were obtained from Jackson Laboratories (Bar Harbor, ME). All animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of California, Irvine. WT mice used as controls in experiments using *Metn β ^{-/-}* mice were age- and sex-matched littermates of *Metn β ^{-/-}* mouse het/het crossings identified through genotyping.

Macrophages

Bone marrow was isolated from murine femurs and cultured in DMEM with 50 ng/ml M-CSF (BioLegend). After 3 d, nonadherent cells were removed, and fresh medium was added. BMM were used after 7 d in culture. For stimulation, BMM were cultured in the presence of TNF- α , IL-4, IFN- γ , TGF- β , IL-17A, IL-6, IL-10, or IL-12 (BioLegend). All cytokines were used at 50 ng/ml. Peritoneal macrophages were obtained from the mouse peritoneal cavity following lavage with 4 ml of balanced salt solution. For some experiments, macrophages were also incubated with recombinant Metnl/Metrn β (100 ng/ml; Bio-Techne, Minneapolis, MN).

Cytokine assays

For cytokine measurements, samples were analyzed using LEGENDplex mouse 13-plex kits (BioLegend). Plates were read using a NovoCyte (ACEA) instrument. The cytokines assessed by LEGENDplex included the following: CCL5, CCL20, CCL11, CCL17, CXCL1, CCL2, CXCL9, CXCL10, CL3, CCL4, CXCL13, CXCL5, CCL22, IL-23, IL-1 α , IFN- γ , TNF- α , IL-12p70, IL-1 β , IL-10, IL-6, IL-27, IL-17A, IFN- β , and GM-CSF.

ELISA

Mouse Metnl ELISA was purchased from R&D Systems (Minneapolis, MN). IL-6 and IL-10 ELISAs were obtained from BioLegend. All ELISAs were used according to each manufacturer's protocol.

Quantitative PCR

RNA was isolated from tissues using the QIAGEN RNeasy Kit, according to the manufacturer's instructions (QIAGEN, Valencia, CA). cDNA reactions were performed using QuantiTect Reverse Transcription (QIAGEN). Quantitative PCR was performed using the Roche LightCycler 480 Real-Time PCR System with probes designed to detect TNF- α (B cell marker) and GAPDH (housekeeping gene) (Roche, Pleasanton, CA).

Thioglycolate peritonitis model

Mice were injected i.p. with 3 ml 3% Brewer thioglycolate (Difco, Detroit, MI) in PBS. Serum and peritoneal exudate samples were collected at different time points postinjection on days 0, 2, 4, 6, and 9. Metn β levels in these samples were measured by ELISA (Bio-Techne) per the manufacturer's instructions.

Sepsis assay

This model has been characterized previously (10). Briefly, 8-wk-old female mice were injected i.p. with LPS derived from *Escherichia coli* Serotype 0111:B4 (Sigma-Aldrich, St. Louis, MO) dissolved in PBS (10 mg/kg body weight). Survival of the mice was monitored every 6 h during the day and until 9:00 PM for 72 h.

Metn β ^{-/-} mouse inflammatory lesions

Twenty female mice were followed and monitored for the development of inflammatory lesions. Two groups were euthanized at months 4 or 8. Tissues and, where applicable, cell infiltrates were characterized by flow cytometry. Expression of TNF- α was determined by quantitative PCR as previously described.

Database analyses

Comparative expression analyses of *Metn α* and *Metn β* in brain, cervix, colon, and esophagus tissues were performed using data generated or provided by the Genotype-Tissue Expression Project database, The Human Protein Atlas project, body index of gene expression database (1), and the Expression Atlas compilation.

<https://www.proteinatlas.org/ENSG00000176845-METRNL/tissue>.

<https://www.gtexportal.org/home/gene/METRNL>.

<https://www.ebi.ac.uk/gxa/query?geneQuery=Q641Q3>.

Statistical analyses

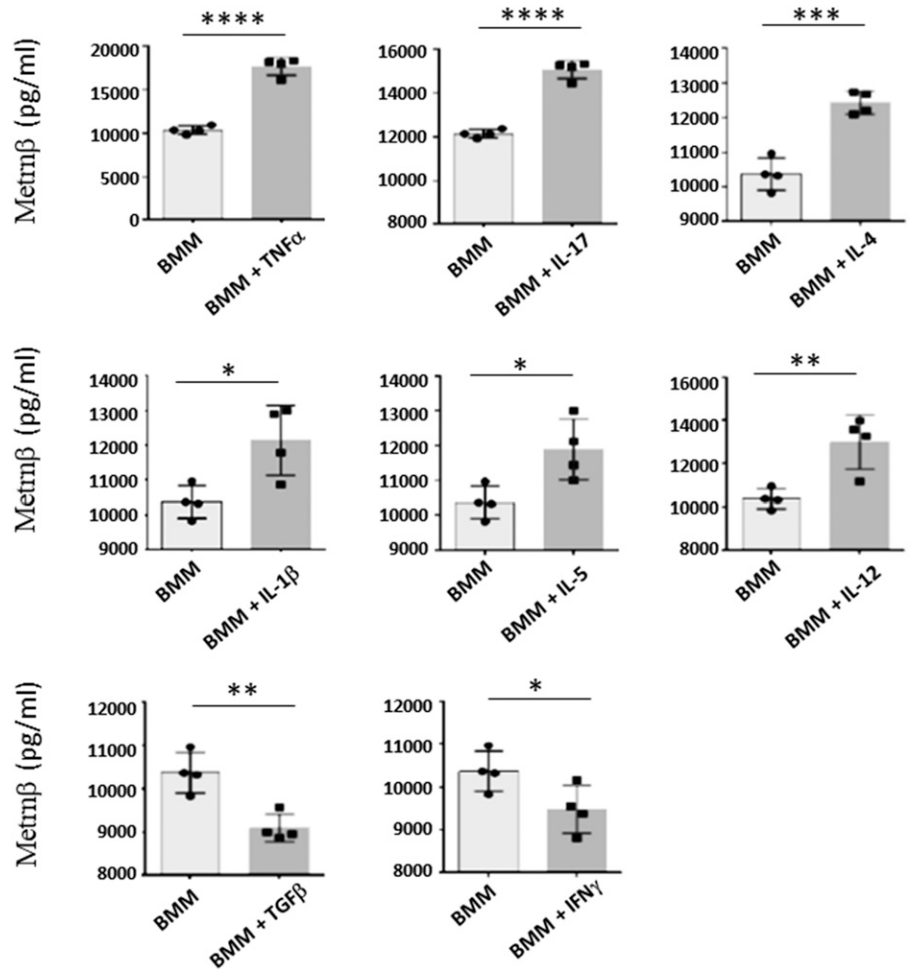
The significance of differences between two groups was determined by Student *t* test (two-tailed) or Gehan-Breslow-Wilcoxon test, which were performed using Prism software (GraphPad, La Jolla, CA). A *p* value <0.05 was considered significant.

Results

The production of Metn β by macrophages is regulated by multiple cytokines

We have reported the identification of a new cytokine, Metnl (or Metn β), which is highly expressed in barrier tissues (skin and mucosa) and activated macrophages (3). Depending on the stimuli present in their microenvironment, macrophages can assume distinct activation phenotypes (11) and have been classified into M1 (IFN- γ -activated macrophages) or M2 (IL-4- and/or IL-13-activated macrophages) states (12). However, these initially described states have been expanded into a more accurate spectrum model of macrophage activation in response to diverse stimuli (13). We initially reported that Metn β is produced by alternatively activated macrophages because its expression was upregulated in peritoneal macrophages cultured with IL-4 and inhibited by IFN- γ (3). However, we have now expanded these studies to test other cytokines (Fig. 1). We cultured mouse BMM with various cytokines and measured Metn β in the supernatant by ELISA. Interestingly, in BMM, TNF- α is the most potent inducer of Metn β production by macrophages, whereas, conversely, IFN- γ or TGF- β suppress its expression (Fig. 1). In addition, other cytokines, including IL-17A, IL12, IL4, and IL1 β , also induced Metn β expression in BMM (Fig. 1). These observations indicate that Metn β production by macrophages is under complex regulation, consistent with the hypothesis that it likely plays multiple functional roles in different immune responses. We should note that TNF- α is the most powerful inducer of Metn β expression in macrophages. This observation suggested that Metn β is produced during (and likely participates in) inflammatory responses. These results also suggested that Metn β is produced in response to

FIGURE 1. Metn β production by BMM is regulated by cytokines. Levels of Metn β production by BMM in response to various cytokines. TNF- α induced the highest expression of Metn β in BMM, but IL-17 α , IL-4, IL-1 β , IL-5, and IL-12 also induced significant increases in Metn β levels. IFN- γ and TGF- β reduced baseline production of Metn β by BMM. Bars represent mean \pm SEM. Results are representative of three experiments. * p < 0.05, ** p < 0.02, *** p < 0.01, **** p < 0.005.



inflammatory stimuli. We, therefore, investigated whether it is induced by LPS in macrophages. As shown in Fig. 2A, LPS induces strong Metn β expression in RAW 264.7 macrophages in a dose-dependent manner. We observed similar responses in peritoneal and BMM macrophages (data not shown). We then measured Metn β levels in circulation during an inflammatory response induced by

thioglycolate injection into the peritoneal cavity of mice. As shown in Fig. 2B and 2C, the levels of Metn β markedly increased in both serum and in the peritoneal exudate following thioglycolate challenge and subsided gradually, mirroring the resolution of inflammation. These observations indicate that Metn β is associated with inflammation in vivo.

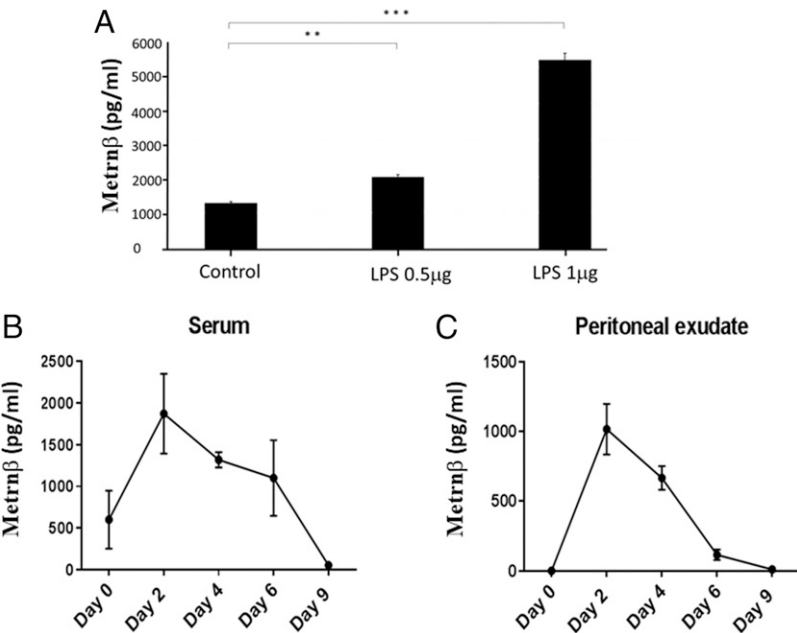


FIGURE 2. Metn β levels are associated with inflammation. (A) Metn β production is induced in macrophages by LPS. Levels of Metn β production by RAW 264.7 macrophages were measured by ELISA in response to 0.5 or 1 mg of LPS stimulus for 48 h. Metn β levels correlate with inflammation in vivo. Levels of Metn β in serum (B) and peritoneal exudate (C) were measured by ELISA over the course of sterile inflammation induced by i.p. thioglycolate injection. Bars represent mean \pm SEM. Results are representative of at least three experiments. ** p < 0.02, *** p < 0.01.

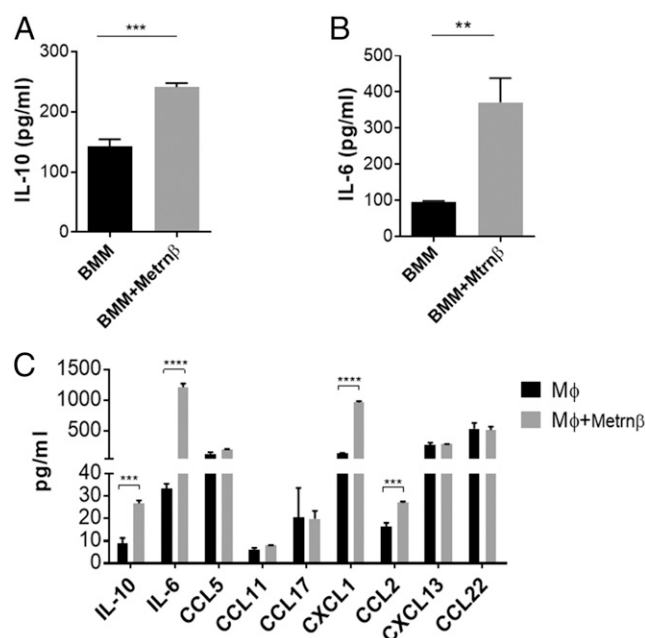


FIGURE 3. Metn β induces production of several cytokines and chemokines in macrophages. Induction of (A) IL-6 and (B) IL-10 in BMM by Metn β as measured by ELISA. BMM were incubated in control medium or 100 ng/ml Metn β for 24 h, and their supernatants were measured for IL-6 or IL-10 by ELISA. (C) Metn β induces expression of CXCL1 and CCL2 in peritoneal macrophages. Peritoneal cavity macrophages were incubated in the presence of Metn β (100 ng/ml) for 24 h prior to measuring levels of different cytokines and chemokines in supernatants using LEGENDplex (BioLegend). Bars represent the mean \pm SEM. Results are representative of at least three experiments. ** p < 0.02, *** p < 0.01, **** p < 0.005. M ϕ , macrophages.

We also explored whether Metn β can regulate cytokine production in macrophages. To this end, we incubated BMM with Metn β and measured several cytokines. As shown in Fig. 3A and

3B, Metn β induces the expression of IL-6 and IL-10 in BMM, and it also increases the expression of CXCL1 and CCL2 in peritoneal macrophages (Fig. 3C). We conclude that Metn β expression is associated with inflammation, that its production is regulated by several cytokines, and that it can also regulate the expression of other cytokines and chemokines. Taken together, these results indicate that Metn β is a new major player in inflammatory responses and is likely to play a role in the pathogenesis of human inflammatory diseases. In support of the latter hypothesis, we have already reported that Metn β is upregulated in several human autoimmune diseases, including psoriasis and rheumatoid arthritis (3).

Metn β -deficient mice exhibit immune system defects

The results shown in Figs. 1–3 strongly suggested that Metn β represents a novel cytokine associated with immune responses and inflammation. To learn more about its possible role in the immune system, we generated a full-body Metn β ^{−/−} mouse (Supplemental Fig. 1). The resulting Metn β ^{−/−} mouse exhibits normal development, gains weight normally (data not shown), breeds well, and is otherwise healthy. These results are similar to information reported by the Knockout Mouse Project for a knockout mouse of this gene (<http://www.mousephenotype.org/data/experiments?geneAccession=MGI:2384806>). Metn β has been reported to be involved in metabolic regulation of energy expenditure and control of glucose tolerance (4), but we have not observed abnormalities in weight gain in Metn β ^{−/−} mice (either under a normal diet or when fed a high-fat diet) (data not shown). These results confirm data from Li et al. (14, 15), who performed similar studies with an adipocyte-specific Metn β ^{−/−} mouse. Analyses of the lymphoid populations of immune organs (spleen, lymph nodes, thymus, and bone marrow) of Metn β ^{−/−} mice (compared with WT littermates) failed to reveal abnormalities in major lymphoid populations in these organs. It has been reported that Metn β overexpression results in eosinophil recruitment (4). The Metn β ^{−/−} mouse, however, shows normal numbers of eosinophils in blood, spleen, peritoneal cavity, and bone marrow, and the

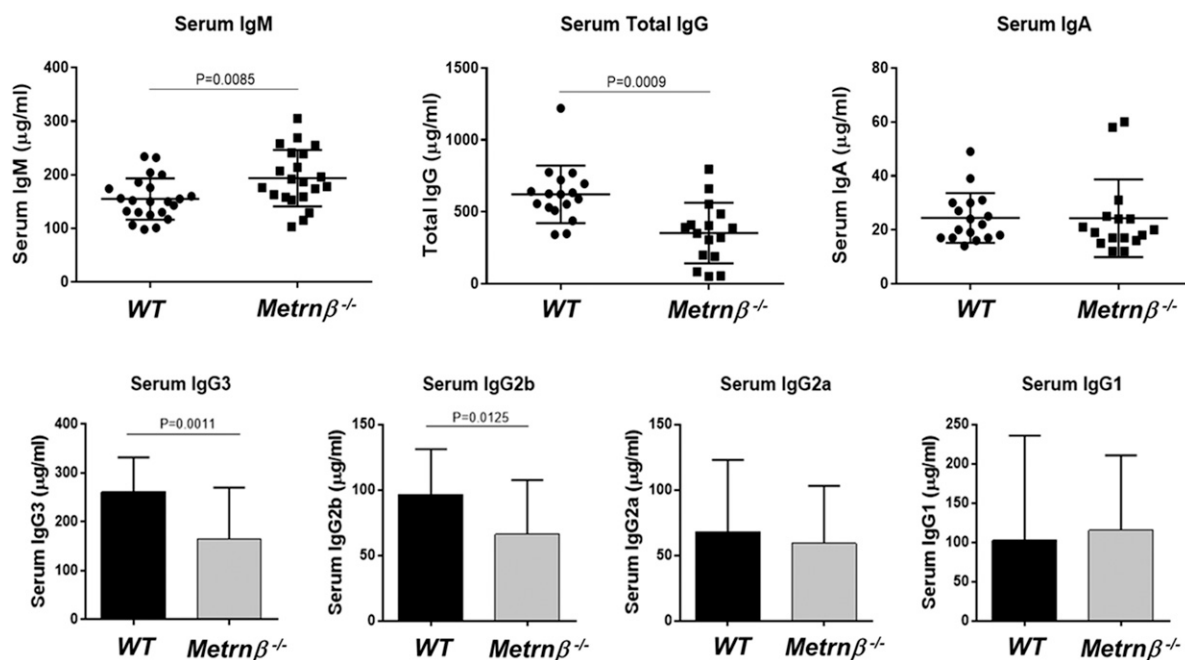


FIGURE 4. Metn β ^{−/−} mice have altered levels of serum Igs. The level of IgM is elevated in Metn β ^{−/−} mice when compared with WT littermate controls, whereas levels of total IgG decreased. Among four different IgG subtypes, levels of IgG3 and IgG2b are decreased in Metn β ^{−/−} mice, whereas levels of IgG2a and IgG1 are comparable between WT and Metn β ^{−/−} mice. Bars represent the mean \pm SEM.

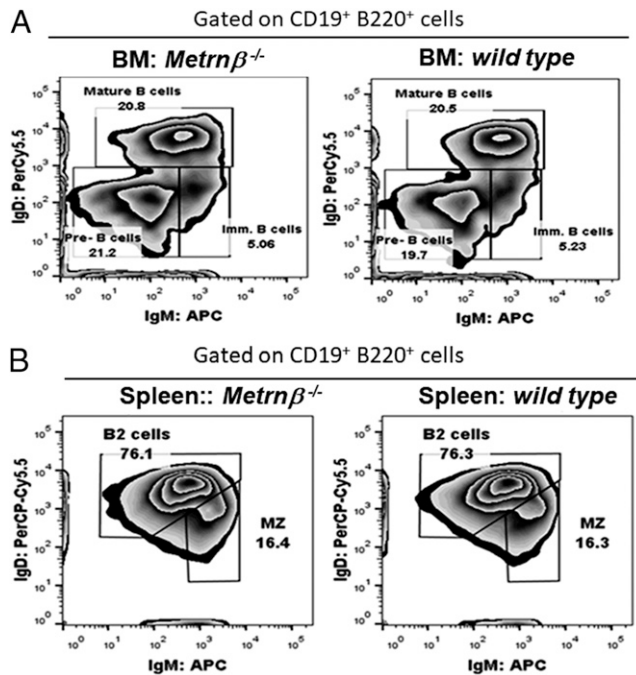


FIGURE 5. Normal B cell population frequencies in *Metrnβ*^{-/-} mice. (A) Staining shows frequencies of B cells in *Metrnβ*^{-/-} mice or WT littermate mice. The plot of B cell populations indicates the percentage of pre-, mature, or immature (imm.) B cells in bone marrow (BM). (B) Percentage of marginal zone (MZ) B cells and B2 cells in the spleen. Data are representative of three independent experiments.

numbers of WBCs in the blood of *Metrnβ*^{-/-} mice are also normal (data not shown). However, we did detect higher levels of IgM and lower levels of IgG in serum of *Metrnβ*^{-/-} mice. Further analyses indicate that the IgG isotypes affected were IgG3 and IgG2b (Fig. 4). We analyzed the spleen B cell populations in *Metrnβ*^{-/-} mice, but did not detect any abnormalities in these populations (Fig. 5). Because Ig class switching is largely determined by cytokines (16), we hypothesized that the defect in IgG production may be due to cytokine production abnormalities in *Metrnβ*^{-/-} mice.

Indeed, a LEGENDplex analysis of the cytokine and chemokine production by splenocytes from the *Metrnβ*^{-/-} mice revealed multiple abnormalities. Some of the most affected were CCL3 and CCL4. As shown in Fig. 6, we confirmed this result by measuring

the ability of spleen cells to produce CCL3 and CCL4 by ELISA. The results indicate that the ability of spleen cells (most likely T cells because the activating stimulus was anti-CD3 and anti-CD28) to produce these chemokines is strongly inhibited in *Metrnβ*^{-/-} mice (Fig. 6).

*IFN-γ induces higher levels of class II MHC in peritoneal macrophages in *Metrnβ*^{-/-} mice*

Interestingly, IFN-γ inhibits the expression of *Metrnβ* by macrophages (Fig. 1) (3). However, IFN-γ is known to induce expression of class II MHC in macrophages (17). We, therefore, investigated whether *Metrnβ* had an effect on the expression of class II MHC in macrophages. To this end, we analyzed the levels of class II MHC in peritoneal macrophages from WT or *Metrnβ*^{-/-} mice in the presence or absence of IFN-γ. As shown in Fig. 7, IFN-γ induced a significantly higher level of class II MHC expression in macrophages from *Metrnβ*^{-/-} mice, suggesting that *Metrnβ* normally has a dampening effect on the expression of class II MHC by macrophages during immune responses. We confirmed this hypothesis by testing the capacity of recombinant *Metrnβ* to inhibit IFN-γ-induced expression of class II MHC in peritoneal macrophages. As shown in Supplemental Fig. 2, the presence of *Metrnβ* during IFN-γ induction of class II MHC in peritoneal macrophages from *Metrnβ*^{-/-} mice inhibited the expression of class II MHC.

Metrnβ^{-/-} mice are highly susceptible to endotoxin shock

The data presented so far indicate that *Metrnβ* is associated with inflammation, is produced by macrophages exposed to LPS, and is also an immunoregulatory cytokine. We, therefore, hypothesized that *Metrnβ* is produced and plays a role during sepsis. To test this hypothesis, we tested *Metrnβ* or WT mice in a model of endotoxemia by injecting them with LPS and monitoring their responses. As shown in Fig. 8, *Metrnβ*^{-/-} mice are more susceptible to endotoxic shock than WT mice and started to die more than 24 h before WT mice. This result strongly suggests that *Metrnβ* normally plays an anti-inflammatory role during sepsis.

Metrnβ^{-/-} mice develop inflammatory lesions

As we developed our colony of *Metrnβ*^{-/-} mice, we noticed that several *Metrnβ*^{-/-} mice developed inflammatory lesions. The most common one was unilateral inflammatory lesions of one horn of the uterus, but we also observed inflammatory lesions in kidneys and liver. As shown in Fig. 9, these lesions typically contained large numbers of neutrophils and high levels of TNF-α consistent with inflammatory responses. To quantify this effect,

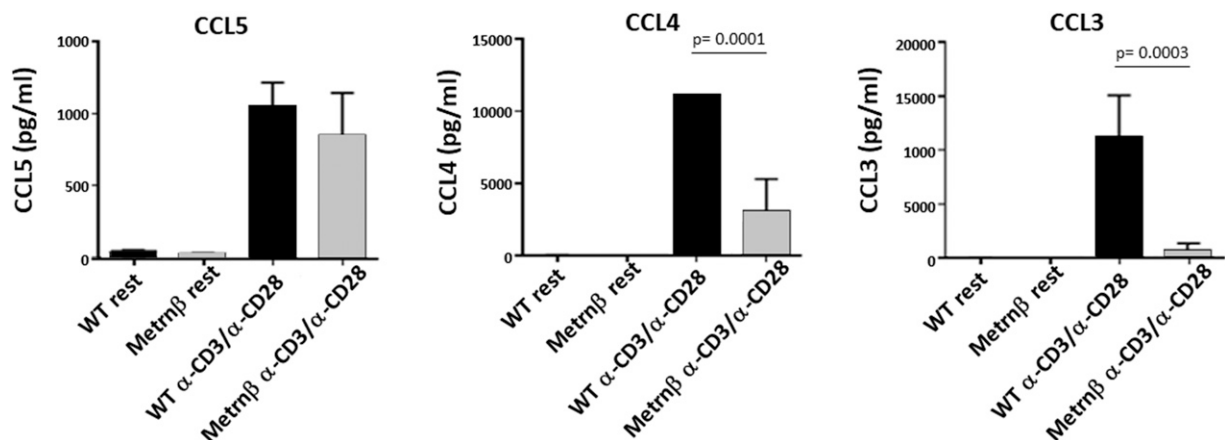


FIGURE 6. Splenocytes from *Metrnβ*^{-/-} mice produce altered levels of CCL3 and CCL4. Splenocytes isolated from *Metrnβ*^{-/-} or WT littermate mice were activated with α-CD3/α-CD28 for 24 h prior to measuring the production of different chemokines (CCL5, CCL4, and CCL3) by ELISA. Bars represent the mean ± SEM. Results are representative of at least three experiments.

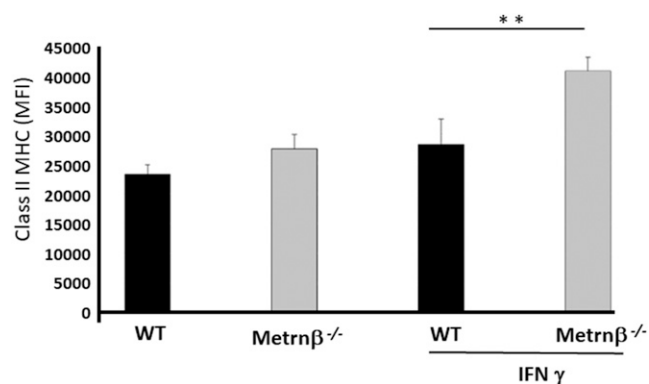


FIGURE 7. IFN- γ induces higher levels of class II MHC in *Metn β ^{-/-}* peritoneal macrophages. Levels of class II MHC in peritoneal macrophages from *Metn β ^{-/-}* or WT littermate mice in the presence or absence of IFN- γ . Macrophages were stained for class II MHC upon harvest (left) or following stimulation with IFN- γ (250 ng/ml) for 24 h and analyzed by flow cytometry. Bars represent the mean \pm SEM. Results are representative of at least three experiments. ** $p < 0.02$. MFI, mean fluorescence index.

we selected a cohort of 20 *Metn β ^{-/-}* mice that were followed for several months and monitored for the development of inflammatory lesions. At 4 mo, 12 mice were sacrificed, and three of them (25%) showed large inflammatory lesions in the uterus; the rest of the mice (8) were sacrificed at 8 mo, and again, two of them (25%) showed inflammatory lesions. In contrast, none of their WT littermates developed lesions. These observations indicate that the lack of *Metn β* favors the development of these inflammatory lesions. Given these observations, we hypothesized whether *Metn β* could affect the production of TNF- α by activated macrophages. However, as shown in Supplemental Fig. 3, the addition of *Metn β* to macrophages following activation with LPS failed to modify TNF- α production.

These data indicate that *Metn β* is an important novel immunoregulatory cytokine expressed in mucosal tissues and skin (Fig. 10) that is likely involved in various types of immune responses through its ability to regulate the production of cytokines and chemokines. Other cytokines exhibit anti- and proinflammatory effects. For example, IL-10 can inhibit T cell activation by inhibiting the ability of macrophages to present Ag (18), but it also enhances development of cytotoxic T cells (19). Our data indicate that multiple cytokines are able to regulate the production of *Metn β* in macrophages and, conversely, that *Metn β* is able to regulate the production of other cytokines and chemokines by macrophages. Taken together, we conclude that *Metn β* is an important novel immunoregulatory cytokine involved in inflammation.

Discussion

Metnl is a poorly characterized small (~27 kDa) secreted protein produced by activated macrophages and homeostatically expressed by barrier tissues. In the latter, the main cellular sources are epithelial cells in the mucosa (20) and fibroblasts in the skin (3). This expression pattern is consistent with a homeostatic function in innate immunity. Moreover, we show in this study that *Metn β* expression is also associated with inflammatory responses. The latter results strongly suggest that it must have functions during both homeostasis and inflammatory responses. *Metn β* has also been reported to be a hormone that regulates thermogenesis and adipocyte metabolism (4). However, we have not observed significant differences in the development of *Metnl^{-/-}* mice. Overall, the main phenotype of *Metn β ^{-/-}* mice is associated with the immune system, supporting the view that the

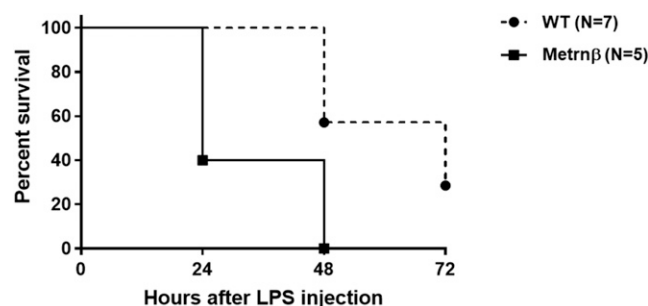


FIGURE 8. *Metn β ^{-/-}* mice are highly susceptible to endotoxemia. Survival curve of WT littermate (dashed line) or *Metn β ^{-/-}* (solid line) mice following an LPS challenge (10 mg/kg body weight) ($n = 7$ for WT mice and $n = 5$ knockout [KO] mice). Differences in survival curves (between WT and *Metn β ^{-/-}* mice) were statistically significant, $p \leq 0.01$. One out of two experiments with similar results is shown.

gene currently annotated as *Metnl* encodes a novel cytokine that we recommend should be renamed *Metn β* to distinguish it from the original neurotrophic factor Meteorin, which we suggest should be renamed *Metn α* . *Metn α* is expressed in the CNS, whereas *Metn β* is expressed mainly in skin and mucosal tissues, as we have previously reported (3). We confirmed their expression patterns by analyzing their expression in several tissue databases (Fig. 10). Based on these results, we conclude that *Metn α* and *Metn β* represent two cytokines that likely represent the evolutionary offspring of an ancestral gene that duplicated to give rise to these cytokines, a phenomenon that has previously been described in other cytokine families (21). These observations suggest that *Metn α* and *Metn β* may have similar functions, except that *Metn α* is primarily acting in the CNS, whereas *Metn β* may be part of innate immunity. In support of this conclusion, Wen et al. (22) have described the disulfide structure of *Metn α* and observed high conservation of the 10 Cys residues between *Metn α* and *Metn β* , suggesting that the disulfide linkages in these proteins are highly conserved. A corollary to these observations is that structures and their functions may be similar, although *Metn α* will likely exert these effects in the CNS where it is predominantly expressed, whereas *Metn β* may mediate similar functions in skin, mucosa, and inflammatory responses. These observations also predict that their receptor(s) should be evolutionary linked as well.

Taken together, our results strongly suggest that the main cell associated with *Metn β* inflammatory functions is the macrophage. In support of this, Gong et al. (23) have reported that *Metnl* affects bone development likely through effects on osteoblasts, which are related to macrophages by lineage. Our data indicate that many cytokines regulate *Metn β* expression in macrophages, and conversely, *Metn β* can also regulate the production of several cytokines and chemokines in macrophages (Fig. 3). Importantly, the fact that TNF- α is the most potent inducer of *Metn β* production in macrophages (Fig. 1) strongly supports an important role for *Metn β* in inflammation. Our data suggest that *Metn β* plays an anti-inflammatory role during normal homeostasis, for example, by modulating cytokine and chemokine production and expression of class II MHC. This potential role would explain its expression in the normal mucosa and skin, important sites for innate immunity that receive a large number of antigenic stimuli. Interestingly, other anti-inflammatory molecules, such as IL-1R antagonist (IL1RN), show an expression pattern in the human body similar to *Metn β* . Its highest expression is in the oral mucosa and esophagus and is also strongly expressed by activated macrophages (Supplemental Fig. 4). These are tissues where *Metn β* is also highly expressed. These data

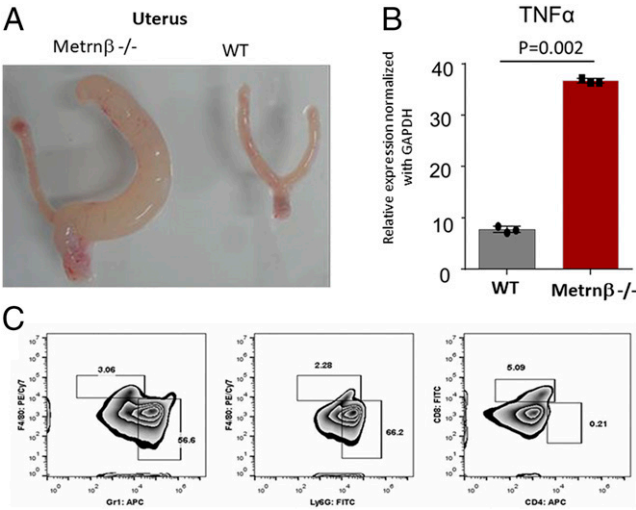


FIGURE 9. Recurring lesions observed in uterus of *Metnrβ*^{-/-} mice show inflammation. **(A)** Pathologic condition observed in one of the horns of a uterus from an *Metnrβ*^{-/-} mouse compared with WT littermate uterus. **(B)** TNF-α upregulation in *Metnrβ*^{-/-} mouse uterus was measured by quantitative PCR (qPCR). **(C)** Flow cytometry analysis revealed leukocytic infiltrate, including neutrophils (Gr1; Ly6G) and macrophages (F4/80). Bars represent mean ± SEM. Results are representative of at least three experiments.

suggest that the tissues from the oral cavity to the esophagus are strongly anti-inflammatory environments. This makes sense because of the large number of substances that mammals place in their mouths. Likely their function is to regulate the extent of potential inflammatory responses in barrier tissues. This hypothesis is further supported by the effectiveness that IL1RN shows in certain autoimmune/inflammatory diseases, such as Schnitzler syndrome (24, 25). However, *Metnrβ* is also strongly expressed in the skin, whereas IL1RN is not, suggesting that it may exhibit other functions in the skin. The ability of *Metnrβ* to regulate

cytokine and chemokine expression by macrophages further supports this hypothesis. Several results from our study, including the high sensitivity of *Metnrβ*^{-/-} mice to LPS (Fig. 8) and the development of inflammatory lesions in older *Metnrβ*^{-/-} mice (Fig. 9) strongly support an anti-inflammatory function for *Metnrβ*. In this sense, it is noteworthy that we have also observed that it is capable of enhancing IL-10 production by macrophages (Fig. 3B), which also points to an anti-inflammatory cytokine.

Our results also suggest that *Metnrβ* is likely to play important roles in acquired immunity. For example, the ability of IL-4 and IL-17A to induce *Metnrβ* production in macrophages, along with the inhibitory effect of IFN-γ on *Metnrβ* production strongly suggests that *Metnrβ* may play specific roles in the development of Th1, Th2, and Th17 responses. We also detected abnormalities in cytokine production in the *Metnrβ*^{-/-} mouse, suggesting abnormalities in acquired immunity. For example, as shown in Fig. 6, the production of CCL3 and CCL4 by *Metnrβ*^{-/-} mouse splenocytes activated with anti-CD3 and anti-CD28 is strongly inhibited. This suggests abnormalities in the T cell compartment as well. Interestingly, the ImmGen database (<https://www.immgen.org/>) indicates that *Metnrβ* is produced by thymic medullary epithelial cells. These observations suggest that *Metnrβ* may also have effects on T cell development.

Overall, the main phenotype detected in *Metnrβ*^{-/-} mice includes immune system abnormalities as well as abnormal inflammatory responses. We should note that *Metnrβ* does not inhibit TNF-α production by macrophages (Supplemental Fig. 3), suggesting that the inflammatory lesions observed in older *Metnrβ*^{-/-} mice are not due to dysregulation of TNF-α production. Instead, we hypothesize that many immune and inflammatory control mechanisms are likely altered in this mouse, resulting in the failure to control inflammatory lesions, a phenotype that may become more accentuated with age. Taken together, we hypothesize that *Metnrβ* has both homeostatic and inflammatory functions. Its homeostatic functions, however, are likely to include the prevention of development of both immune responses and inflammation.

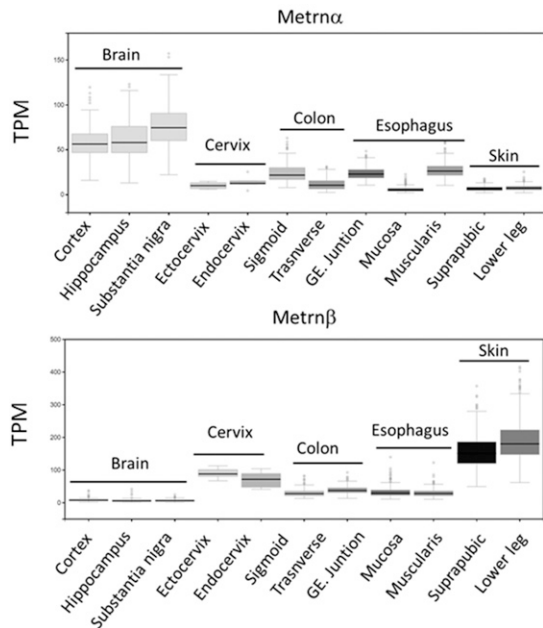


FIGURE 10. Comparative expression of *Metnrα* and *Metnrβ* in brain, cervix, colon, and esophagus using the Genotype-Tissue Expression (GTEx) Project database (left) and its correlation with data generated by The Human Protein Atlas (HPA) project, body index of gene expression (BIGE) database, and Expression Atlas (GXA) compilation. GE (gastroesophageal) TPM (transcripts per kilobase million) (right). There is general agreement between these databases on the expression of *Metnrα* and *Metnrβ* in these tissues, with *Metnrα* expressed mainly in the brain, whereas *Metnrβ* is expressed mainly in mucosal tissues and skin. *Very low but detectable expression.

Dataset	Brain	Cervix	Colon	Esophagus	Skin
Metnrα					
GTEx	++++	-	Low*	-	-
HPA	++++	-	-	-	-
GXA	++++	-	-	Low*	NA
BIGE	++++	-	-	-	-

Dataset	Brain	Cervix	Colon	Esophagus	Skin
Metnrβ					
GTEx	-	+++	++	++	++++
HPA	Low*	++	++	++	++++
GXA	-	+++	+++	++++	NA
BIGE	-	+++	+++	+++	++++

We, therefore, conclude that *Metrn β* encodes a novel cytokine, which we suggest should be called Metrn β , that is involved in immunity and inflammation. We hope that our results will encourage other studies that should expand and clarify the potential functional roles of Metrn β in both innate and acquired immune responses.

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Disclosures

I.U. and A.Z. are named inventors in a patent application of Meteorin-like filed by the Regents of the University of California. The other authors have no financial conflicts of interest.

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