Innate Immunity

Matrix metalloproteinase 28 is regulated by TRIF- and type I IFN-dependent signaling in macrophages

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Abstract

Matrix metalloproteinases (MMPs) are transcriptionally regulated proteases that have multiple roles in modifying the extracellular matrix (ECM) and inflammatory response. Our previous work identified $Mmp28$ as a key regulator of inflammation and macrophage polarization during experimental models of pulmonary infection, fibrosis, and chronic smoke exposure. However, the signaling pathways responsible for regulation of macrophage $Mmp28$ expression remain undefined. This study utilized murine macrophages obtained from wild type, $Tlr2^{-/-}$, $Tlr4^{-/-}$, $MyD88^{-/-}$, $Ticam1^{Lps2}$ ($Trif_{mutant}$), and $Ifnar1^{-/-}$ mice to test the hypothesis that macrophage $Mmp28$ expression was dependent on TRIF and type I IFN. Our results support the hypothesis, demonstrating that increased macrophage $Mmp28$ expression was dependent on type I IFN after LPS and poly(I:C) stimulation. To gain further insight into the function of MMP28, we explored the inflammatory response of macrophages derived from wild type or $Mmp28^{-/-}$ mice to stimulation with poly(I:C). Our data support a role for MMP28 in regulating the macrophage inflammatory response to poly(I:C) because expression of $Ccl2$, $Ccl4$, $Cxcl10$, and $Il6$ were increased in $Mmp28^{-/-}$ macrophages. Together, these data support a model in which macrophages integrate TRIF- and type I IFN-dependent signaling to coordinate regulation of proteins with the capacity to modify the ECM.

Introduction

Macrophages are innate immune cells that play key regulatory roles in host inflammatory responses to infection and injury, controlling the inflammatory process in part through phagocytosis and killing of microbes and clearance of dying cells and debris.[1] In addition, macrophages detect changes in the extracellular environment by recognition of pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) to fine-tune the inflammatory response. In the lung, activation of epithelial cell pattern recognition receptors during infection can induce a robust type I IFN response, which is critical for development of a protective innate immune response to infection. Type I IFN also causes functional changes in macrophages, resulting in diverse phenotypes. In many circumstances, type I IFN increases the antimicrobial responses of

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macrophages.[2,3] Macrophages also produce and secrete type I IFN after detection of PAMP to stimulate autocrine and paracrine pro-inflammatory responses. For example, Gram-negative bacterial LPS activates the TLR4 signaling pathway to induce pro-inflammatory and type I IFN responses through a TRIF-dependent pathway,[4] and recognition of dsRNA by TLR3 also utilizes a TRIF-dependent pathway to induce a type I IFN response.[4,5]

Matrix metalloproteinases (MMPs) are proteases that are produced by multiple cell types, including macrophages, and function not only in regulating degradation of the extracellular matrix (ECM), but also in regulating immune responses, including antiviral responses.[6,7] Although transcriptional regulation of MMPs is one of the main mechanisms regulating their function,[6] the integration of PAMP and DAMP sensing by macrophages to coordinately regulate modifiers of the ECM remains an understudied area of investigation. Although recent studies have demonstrated that macrophage transcriptional regulation of versican is dependent on the type I IFN response,[8] the overall contribution of these stimuli to the regulation of macrophage MMPs has not been investigated.

Our previous studies have demonstrated that macrophage expression of Mmp28 is increased upon stimulation with LPS or poly(I:C) and MMP28 regulates inflammation and macrophage polarization during experimental models of pulmonary infection, fibrosis, and chronic smoke exposure.[9–11] Depending on the injury model, there are several consequences of MMP28 deletion. For example, during *Pseudomonas aeruginosa* pneumonia, $Mmp28^{-/-}$ mice have increased macrophage-specific pro-inflammatory responses with enhanced bacterial clearance.[9] In contrast, $Mmp28^{-/-}$ mice exposed to chronic cigarette smoke have blunted inflammatory responses and are protected from emphysema, which could be due in part to decreased macrophage M2 polarization.[10] Similarly, we have demonstrated that $Mmp28^{-/-}$ macrophages have impaired M2 polarization and are protected from bleomycin-induced fibrosis.[11] Because the airway epithelium, in addition to macrophages, expresses Mmp28, it is not surprising that the role of MMP28 in regulating pulmonary inflammation is likely complex and stimulus dependent.

In order to gain a better understanding of how macrophage MMP28 regulates the inflammatory response, we sought to address two unresolved questions. First, we wanted to define the molecular signaling pathway components required for macrophage Mmp28 expression in response to LPS and poly(I:C). Second, we were interested in determining whether the response of $Mmp28^{-/-}$ macrophages to poly(I:C) was altered in order to provide further insight into MMP28 function. We performed our studies in murine bone marrow-derived macrophages to utilize a genetic approach to test the hypothesis that type I IFN, a common feature of both LPS and poly(I:C) stimulation, was responsible for increased macrophage Mmp28 expression.

Materials and methods

Animal ethics

The University of Washington Institutional Animal Care and Use Committee reviewed and approved all animal procedures prior to initiation of these studies. Wild type controls were gender matched for each knock-out mouse during independent experiments.

Cell culture medium and reagents

The macrophage medium used for culture of murine bone marrow-derived macrophages (BMDMs) was RPMI-1640 containing L-Glutamine and supplemented with 20% L929-conditioned medium, 10% heat-inactivated FBS, and 1% penicillin/streptomycin. High-molecular-mass poly(I:C) was from InvivoGen (San Diego, CA) and *Escherichia coli* LPS 0111:B4 was from Sigma-Aldrich (St. Louis, MO). Carrier-free recombinant mouse IFN-α1 and mouse IFN-β1 were from BioLegend (San Diego, CA). Mouse CCL2 (DY-479-05), CCL4 (DY451), CXCL10 (DY466-05), and IL-6 (DY460-05) DuoSet ELISA assays were from R&D Systems (Minneapolis, MN).

Isolation, culture, and stimulation of BMDMs

Bone marrow was isolated from the femurs and tibias of male and female wild type (C57BL/6) mice and $Mmp28^{-/-}$, $Tlr2^{-/-}$, $Tlr4^{-/-}$, $MyD88^{-/-}$, $Ticam1^{Lps2}$ ($Trif_{mutant}$), and $Ifnar1^{-/-}$ mice 8–12 wk of age as described previously.[12] Cells were plated overnight in macrophage medium. Non-adherent cells were collected and cultured for 6 d additional for growth and differentiation into murine BMDM, as described previously.[12] BMDM were re-seeded into 24-well tissue culture plates at 200,000 cells/well and allowed to adhere overnight before performing stimulations. Wild type, $Tlr2^{-/-}$ and $Tlr4^{-/-}$ macrophages were stimulated with 100 ng/ml LPS for 24 h. Wild type, $MyD88^{-/-}$, and $Trif_{mutant}$ macrophages were stimulated with 50 ng/ml LPS or 10 µg/ml poly(I:C) for 4 h. Wild type and $Ifnar1^{-/-}$ macrophages were stimulated with 10 ng/ml LPS or 10 µg/ml poly(I:C) for 4 h. A concentration of 100 U/ml of either murine recombinant IFN-α1 or IFN-β1 were used to stimulate wild type macrophages for 6 h to assess Mmp28 expression. Wild type and $Mmp28^{-/-}$ macrophages were stimulated with 10 µg/ml poly(I:C) or 100 U/ml IFN-α1 or IFN-β1 for 24 h to assess differential inflammatory responses. Non-stimulated cells were treated with equivalent volume of PBS for all experiments (control).

For cytokine measurements, cell supernatants were collected after 24 h and centrifuged for 10 min at 14,000 g to remove cellular debris.

Quantitative real-time PCR

RNA was isolated from cell cultures with the NucleoSpin RNA isolation kit (Clontech Laboratories, Mountain View, CA). A NanoDrop 1000 (Thermo Fisher Scientific, Waltham, MA) was used to determine RNA concentrations and equal amounts of RNA were used to synthesize cDNA with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). This cDNA was used as the template for quantitative real-time PCR (qPCR) reactions that used the SensiMix II Probe Hi-ROX Kit (Bioline, Taunton, MA) and validated TaqMan FAM primer probes for the murine genes *Hprt* Mm01545399, *Mmp2* Mm00439498, *Mmp3* Mm00440295, *Mmp8* Mm00439509, *Mmp9* Mm00600163, *Mmp10* Mm01168399, *Mmp11* Mm004 85048, *Mmp12* Mm00500554, *Mmp14* Mm00485054, *Mmp28* Mm00712992, *Tnf* Mm00443258, *Ccl2* Mm00441242, *Ccl4* Mm00443111, *Ccl5* Mm01302427, *Il1b* Mm00434228, *Il6* Mm00446190, and *Ifit1* Mm00515153 (Life Technologies, Carlsbad, CA). The ABI 7900HT Fast Real-Time PCR System was used to obtain the average cycle threshold (Ct) from duplicate replicates of each sample; ΔCt of each primer probe was calculated relative to *Hprt* as described previously.[12,13]

Statistics

Statistical analyses were performed using GraphPad Prism 7 software. One-way ANOVA with Dunnett's multiple-comparisons test or two-way ANOVA with Sidak's or Tukey's multiple-comparisons tests with a confidence of 95% were used as indicated; otherwise, an unpaired Student's *t* test was used to compare groups.

Results

LPS and poly(I:C) induce macrophage Mmp28 expression by a TRIF-dependent pathway

We previously demonstrated that several inflammatory stimuli, including *E. coli* LPS, *P. aeruginosa*, and *Chlamydophila pneumoniae*, increase murine BMDM expression of *Mmp28*.[9,11] In addition, treatment of murine BMDM with poly(I:C) also increases *Mmp28* expression; however, the signaling pathways required for *Mmp28* expression in macrophages have not been fully characterized. LPS-induced TLR4 signaling occurs through utilization of both MyD88-TIRAP and TRIF-TRAM adapter proteins,[4] whereas utilization of a TRIF-dependent signaling pathway is a shared feature of TLR3 activation by poly(I:C).[5]

Based on the ability of both LPS and poly(I:C) to increase *Mmp28* expression, we hypothesized that stimulation of *Mmp28* expression by LPS was primarily due to a TRIF-dependent pathway. To test this hypothesis, we used murine macrophages derived from $Tlr2^{-/-}$, $Tlr4^{-/-}$, $MyD88^{-/-}$, $Trif_{mutant}$, and wild type mice. Because standard LPS could be contaminated with bacterial lipopeptides that interact with TLR2, we first tested *Mmp28* gene expression in LPS-stimulated cells deficient in TLR2 or TLR4. We found that, in response to LPS stimulation, TLR4, but not TLR2, was required for *Mmp28* expression (Figure 1a). Next, we utilized murine macrophages deficient in MyD88 or expressing non-functional TRIF. Whereas $MyD88^{-/-}$ macrophages retained *Mmp28* expression in response to LPS (Figure 1b), $Trif_{mutant}$ macrophages had significantly reduced *Mmp28* expression compared with wild type controls (Figure 1c), thus demonstrating a selective LPS pathway required for *Mmp28* expression. Further, we demonstrate that
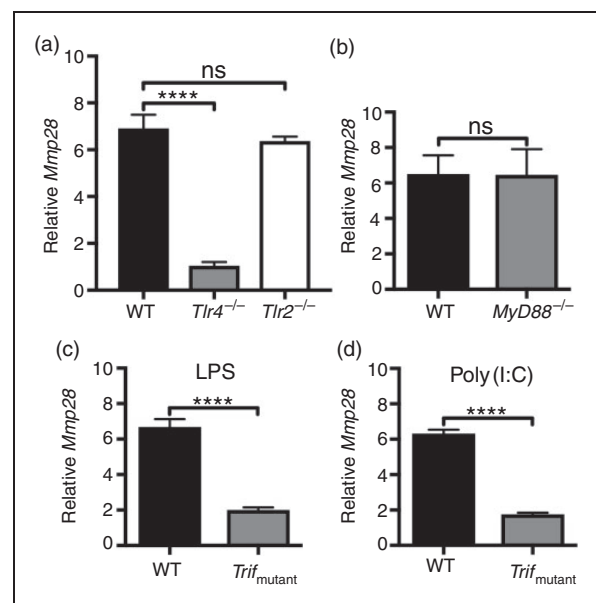
Figure 1. LPS and poly(I:C) increase *Mmp28* gene expression by a TRIF-dependent pathway. (a–d) BMDM cells from wild type (WT), $Tlr4^{-/-}$, $Tlr2^{-/-}$, and $Trif_{mutant}$ mice were stimulated with LPS for 24 h (a) or LPS (b,c) and poly(I:C) (d) for 4 h and processed to determine mRNA levels of *Mmp28* relative to *Hprt*. Results are shown as the fold change in *Mmp28* expression relative to non-stimulated genotype-matched control cells. (a) LPS-induced *Mmp28* expression was dependent on TLR4 but not TLR2. (b,c) LPS-induced *Mmp28* expression was independent of MyD88 but required TRIF. (d) Poly(I:C)-induced *Mmp28* expression was also TRIF dependent. Data represent mean ± SEM from a representative experiment (a) or independent experiments (b–d) with three to four biological replicates per genotype. Statistical analysis was performed using a one-way ANOVA with Dunnett's multiple-comparisons test (a) or Student's unpaired *t* test (b–d). ****$P < 0.0001$; ns, not significant ($P > 0.05$).

poly(I:C)-stimulated *Mmp28* expression was also dependent on TRIF (Figure 1d). Taken together, these data support our initial hypothesis that macrophage *Mmp28* expression is TLR4 and TRIF dependent and TLR2 and MyD88 independent.

Macrophage Mmp28 expression is type I IFN dependent

Because TRIF-dependent pathways activate type I IFN expression and secretion, we investigated whether *Mmp28* expression is a type I IFN-dependent gene using two independent approaches. First, we directly tested the ability of type I IFNs, IFN-α or IFN-β, to

stimulate macrophage *Mmp28* expression. Both IFN-α and IFN-β increased *Mmp28* expression, whereas neither recombinant IFN elicited *Tnf* expression (Figure 2a). To further confirm that type I IFN was responsible for LPS- and poly(I:C)-induced *Mmp28* expression, we stimulated macrophages from mice lacking the type I IFN receptor ($Ifnar1^{-/-}$) compared with wild type cells (Figure 2b). Although *Mmp28* expression was induced by both LPS and poly(I:C) in wild type macrophages, it was not increased by these stimuli in $Ifnar1^{-/-}$ macrophages. We also confirmed upregulation of the type I IFN-responsive transcript *Ifit1* in LPS- and poly(I:C)-treated wild type cells that was absent in similarly treated $Ifnar1^{-/-}$ cells. In
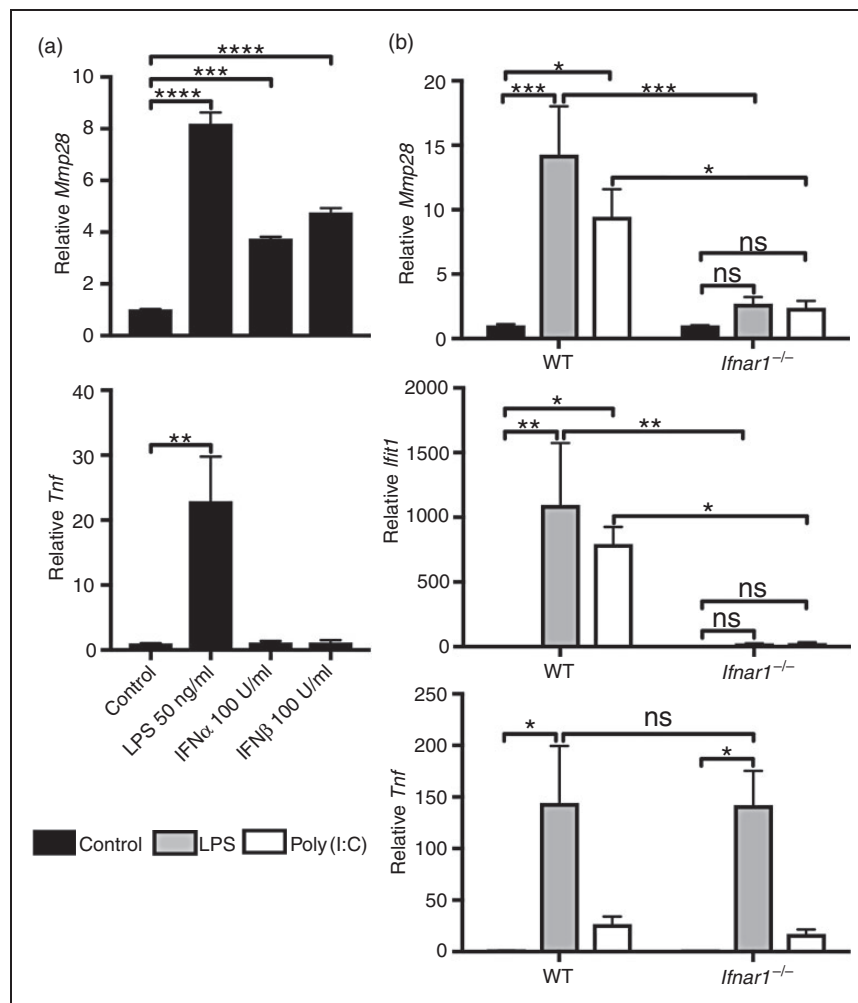

Figure 2. Macrophage *Mmp28* is regulated by type I IFN. (a) Wild type (WT) BMDMs were stimulated with PBS (control), LPS (50 ng/ml), IFN-α (100 U/ml), or IFN-β (100 U/ml) for 6 h. The mRNA levels of *Mmp28* and *Tnf* were measured relative to *Hprt* and normalized to control. All three stimuli, LPS, IFN-α, and IFN-β, increased *Mmp28* gene expression. Data are expressed as mean ± SEM ($n = 5$ independent experiments). Statistical analysis used one-way ANOVA with Dunnett's multiple-comparisons test. (b) WT and $Ifnar1^{-/-}$ BMDMs were stimulated with PBS (control), LPS (10 ng/ml), or poly(I:C) (10 μg/ml) and assessed for *Mmp28*, *Ifit1*, and *Tnf* gene expression at 4 h. Both *Mmp28* and *Ifit1*, but not *Tnf*, were dependent on IFNAR1 (mean ± SEM; $n = 3$–5 mice/genotype). Statistical analyses used two-way ANOVA with Sidak's multiple-comparisons test between WT and $Ifnar1^{-/-}$ samples and Tukey's multiple-comparisons test to compare treatment groups within each genotype *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$; ns, not significant ($P > 0.05$).

contrast, LPS-induced increase in *Tnf* was unaltered in *Ifnar1*$^{-/-}$ macrophages compared with wild type (Figure 2b). Together, these data support the hypothesis that type I IFN secretion following either LPS or poly(I:C) activation of macrophages results in signaling through IFNAR1 to increase expression of *Mmp28*.

LPS-induced expression of macrophage Mmp10 is IFNAR1 dependent

Similar to our previous studies with *Mmp28*, recent data also indicate that *Mmp10* expression is increased in macrophages following stimulation with LPS or *P. aeruginosa*.[14] To determine whether macrophage regulation of *Mmp10* expression or other *Mmps*, including *Mmp2, Mmp3, Mmp8, Mmp9, Mmp11, Mmp12*, and *Mmp14*, are similar to *Mmp28*, we measured respective mRNA levels by qPCR in wild type and *Ifnar1*$^{-/-}$ macrophages stimulated with LPS or poly(I:C). We did not find detectable levels of *Mmp2, Mmp3, Mmp11*, or *Mmp14* in either resting or LPS- or poly(I:C)-activated macrophages (data not shown). Although *Mmp8, Mmp9*, and *Mmp12* were detected, no increase in expression due to LPS or poly(I:C) or dependence on IFNAR1 at this time point was observed (Figure 3). However, consistent with previous findings, LPS increased macrophage *Mmp10* expression in wild type cells but was significantly reduced in *Ifnar1*$^{-/-}$ cells, suggesting that LPS-induced expression of both *Mmp10* and *Mmp28* is similar and dependent on type I IFN signaling (Figure 3). Although, in contrast to *Mmp28, Mmp10* expression was not increased following poly(I:C) stimulation, suggesting that its transcription has additional, as yet undefined, stimulus-specific regulation. Overall, these findings indicate that type I IFN is integral to macrophage regulation of MMPs.

MMP28 restrains macrophage pro-inflammatory responses to poly(I:C)

In previous studies, we observed increased pro-inflammatory responses in LPS-stimulated *Mmp28*$^{-/-}$ macrophages.[9,11] However, the role of MMP28 in regulating the inflammatory response to type I IFN or poly(I:C) has not been investigated. To determine whether MMP28 influenced the macrophage response to poly(I:C) stimulation, we measured expression of several inflammatory genes 24 h after PBS or poly(I:C) stimulation of wild type compared with *Mmp28*$^{-/-}$ murine BMDMs. Expression of *Ccl2, Ccl4, Cxcl10*, and *Il6* was increased in *Mmp28*$^{-/-}$ macrophages compared with wild type macrophages after stimulation with poly(I:C) (Figure 4a). In contrast, expression of *Ccl5, Il1b*, and *Ifit1* were similar in wild type and *Mmp28*$^{-/-}$ macrophages (data not shown). We further measured protein levels in cell supernatants to
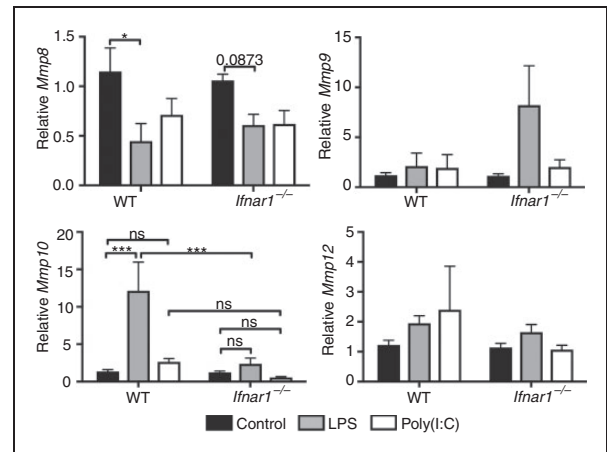

Figure 3. LPS-stimulated increase in macrophage *Mmp10* expression is dependent on type I IFN. Wild type (WT) and *Ifnar1*$^{-/-}$ BMDMs were stimulated with PBS (control), LPS (10 ng/ml), or poly(I:C) (10 μg/ml) and mRNA levels of *Mmp8, Mmp9, Mmp10*, and *Mmp12* relative to *Hprt* were measured at 4 h. LPS-induced *Mmp10* expression was dependent on IFNAR1; however, poly(I:C) did not increase *Mmp10* expression. LPS or poly(I:C) did not increase *Mmp8, Mmp9*, or *Mmp12*. Data represent mean ± SEM from five to six mice/genotype. Statistical analyses used two-way ANOVA with Sidak's multiple-comparisons test between WT and *Ifnar1*$^{-/-}$ samples and Tukey's multiple-comparisons test to compare treatment groups within each genotype. ***$P < 0.001$; ns, or otherwise not indicated, are not significant ($P > 0.05$).

determine whether differences in gene expression between wild type and *Mmp28*$^{-/-}$ macrophages resulted in changes in chemokine/cytokine secretion. Consistent with the changes in gene expression, there was a significant increase in CCL4, CXCL10, and IL-6 in the supernatants of *Mmp28*$^{-/-}$ macrophages compared with wild type (Figure 4b). To further evaluate whether loss of MMP28 altered regulation of other MMPs, the mRNA levels of *Mmp9, Mmp10*, and *Mmp12* were measured 24 h after poly(I:C) stimulation. Compared with wild type, *Mmp28*$^{-/-}$ macrophages had increased *Mmp9* and decreased *Mmp10* after poly(I:C) (Figure 4c). To determine whether any of the increased inflammatory responses in *Mmp28*$^{-/-}$ macrophages were the result of differences in the response to type I IFN, we stimulated macrophages with either recombinant IFN-α1 or IFN-β1 and measured inflammatory gene expression. Similar to poly(I:C) treatment, stimulation of *Mmp28*$^{-/-}$ macrophages with either recombinant IFN-α1 or IFN-β1 resulted in increased *Il6* compared with wild type macrophages; however, there was no difference in *Ccl2, Ccl4*, or *Cxcl10* (Figure 4d). These results suggest that, whereas there are some intrinsic differences in the response of *Mmp28*$^{-/-}$ macrophages to type I IFN, these differences likely do not completely explain the increased pro-inflammatory response to poly(I:C). Potentially, these effects could be due to the altered activation of
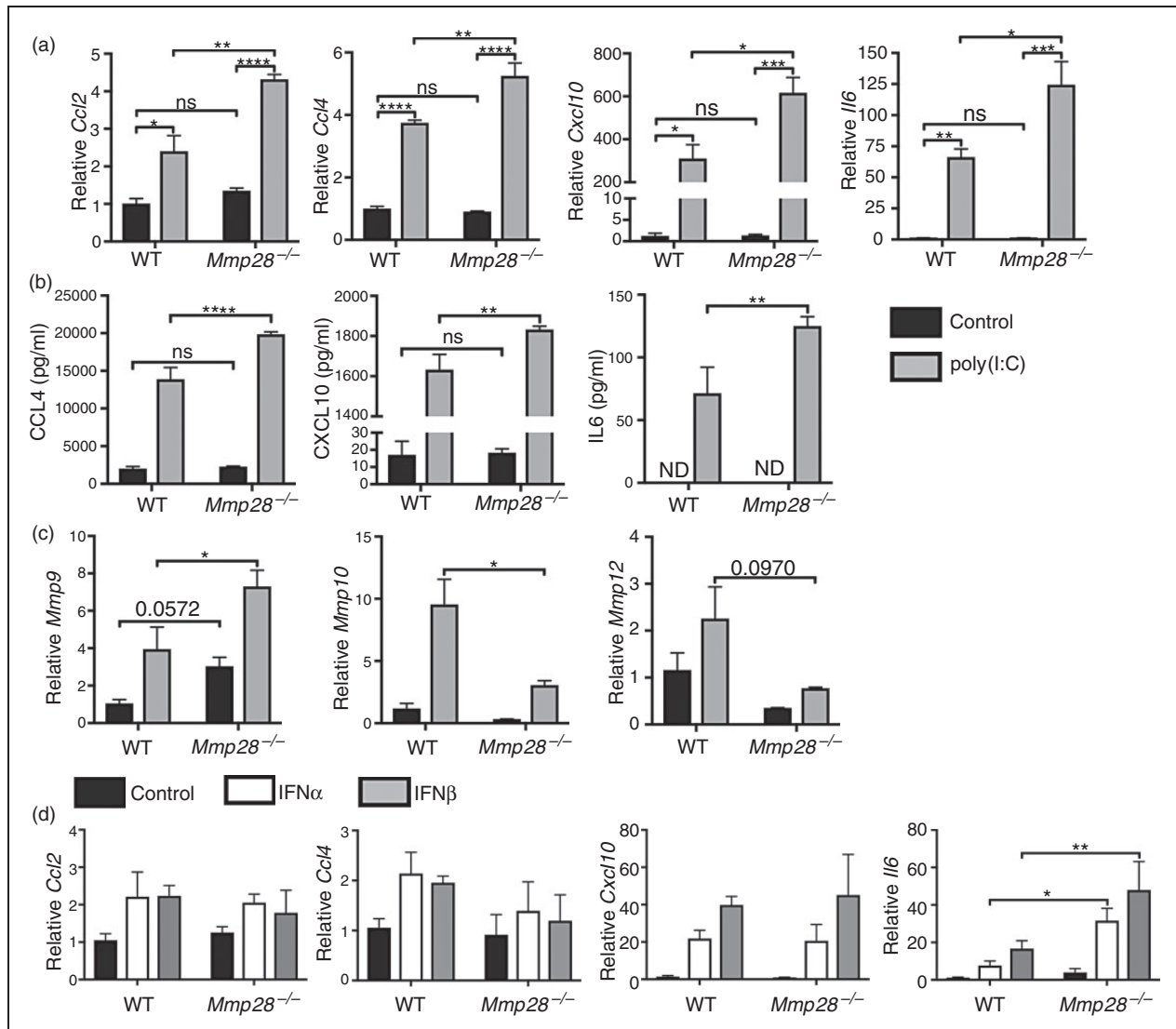
Figure 4. Macrophage MMP28 restrains the pro-inflammatory response to poly(I:C). BMDMs from wild type or *Mmp28*$^{-/-}$ mice were stimulated with media containing (a,b) poly(I:C) (10 μg/ml) or (c) IFN-α (100 U/ml), IFN-β (100 U/ml), or control media containing PBS for 24 h. The levels of *Ccl2, Ccl4, Cxcl10,* and *Il6* were measured to *Hprt* and expression was normalized to control treated wild type samples. (a) There was a significant increase in *Ccl2, Ccl4, Cxcl10,* and *Il6* gene expression and (b) in CCL4, CXCL10, and IL-6 protein levels in the cell supernatants in poly(I:C)-treated *Mmp28*$^{-/-}$ cells compared with similarly treated wild type cells. (c) The levels of *Mmp9* and *Mmp10* but not *Mmp12* were significantly altered relative to *Hprt* at 24 h in wild type and *Mmp28*$^{-/-}$ macrophages. (d) In contrast to poly(I:C), only *Il6* expression was differentially regulated by MMP28 in response to recombinant type I IFN. Data represent mean ± SEM; $n = 3-4$ mice per genotype. ND indicates below limit of detection. Statistical analyses used two-way ANOVA with Sidak's multiple-comparisons test. $P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

signaling pathways that are activated by poly(I:C), but not by recombinant type I IFN per se (Figure 5), or that multiple changes due to the loss of MMP28-function combine to change the inflammatory response.[2,15,16]

Discussion

Although MMP regulation of ECM has long been recognized, recently described diverse roles of MMPs in regulating other cellular responses have increased the implications for MMPs involved in a number of inflammatory diseases.[6] Although prior reports from us[9,11] and others[17] demonstrated that macrophage *Mmp28* expression increases following LPS or poly(I:C) stimulation, the pathways required for this response have not been described previously. In this report, we identified *Mmp28* as a type I IFN-responsive gene in murine macrophages (Figure 4). Using a genetic approach, we demonstrated that TRIF and IFNAR1 are required for macrophage expression of *Mmp28* during LPS or poly(I:C) stimulation. In addition, stimulation of macrophages with either recombinant IFN-α or IFN-β was sufficient to increase *Mmp28*
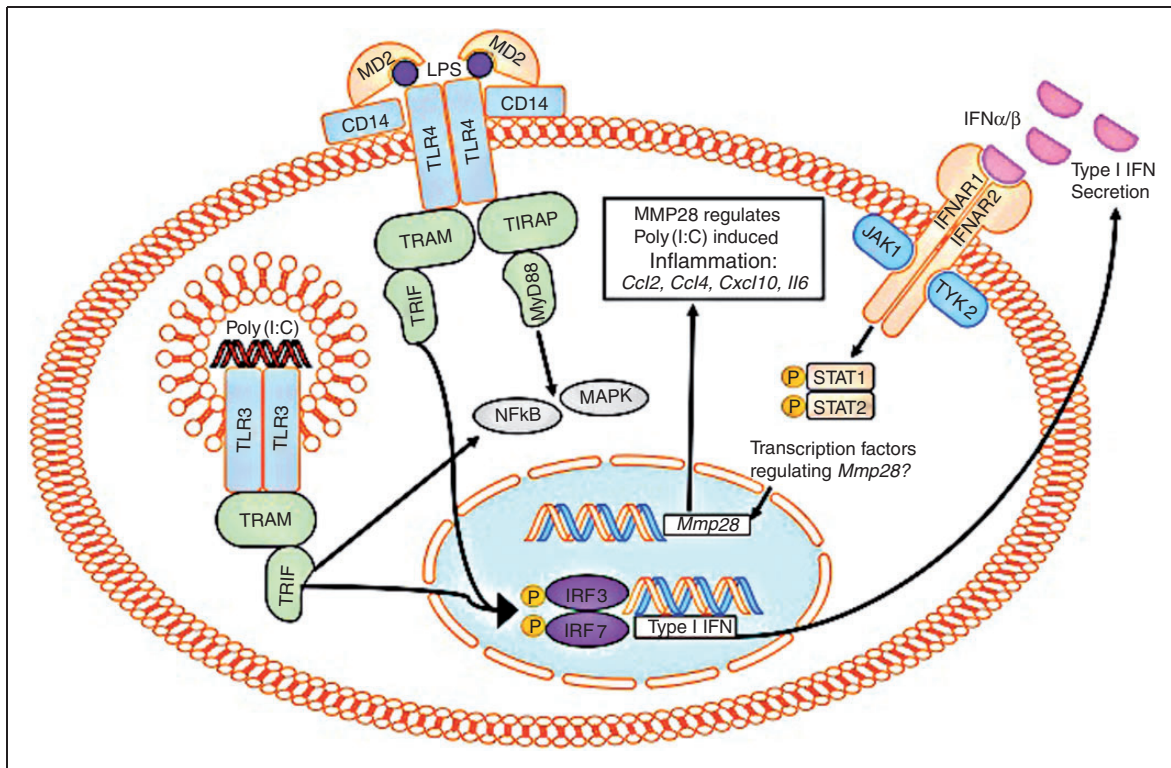
Figure 5. Type I IFN plays an integral role in macrophage regulation of *Mmp28* following stimulation with PAMP. Macrophage *Mmp28* expression occurs following stimulation with LPS following a TLR4-TRIF-IFNAR1 dependent pathway that occurs independently of TLR2. In addition, poly(I:C) stimulation of macrophage *Mmp28* occurs by a TRIF-IFNAR1-dependent pathway. Macrophages derived from *Mmp28*$^{-/-}$ mice have increased *Ccl2, Ccl4, Cxcl10*, and *Il6* compared with wild type macrophages following poly(I:C) stimulation.

expression. We further discovered that LPS-induced expression of macrophage *Mmp10* was dependent on IFNAR1; however, in contrast to *Mmp28, Mmp10* expression was not increased in poly(I:C)-treated wild type macrophages, suggesting that the regulation of *Mmp10* may be more complex than *Mmp28*. Although previous examination of the promoter region of *Mmp28* from both humans and mice concluded that the DNA sequence does not contain a TATA box or AP-1-binding site close to the transcription start site (classified as a group III MMP promoter region), there are Sp1 and Sp3 sites present that are likely important for transcription.[18–22] In addition, there are predicted STAT-binding sites in the promoter region of some MMPs, including MMP-28; however, the role of STATs in regulating the transcription of MMPs has not been well characterized.[19,22] A previous study examined transcriptional expression of all MMPs during a murine model of RSV infection and found that expression of several MMPs, including *Mmp28*, were increased during RSV infection due to a TRIF- and MAVS-dependent response.[23] However, this analysis was only performed on whole lung homogenates, whereas our study provides a novel demonstration of macrophage MMP transcriptional activation that is dependent on the type I IFN response. In summary, our data

demonstrate that type I IFN is integral to macrophage expression of *Mmp10* and *Mmp28*.

The contribution of type I IFN to regulation of MMPs remains relatively unexplored, with the exception of previous studies that identified that IFNβ-1b reduced *MMP9* expression and protein activity in human peripheral blood mononuclear cells[24] and human monocyte-derived dendritic cells.[25] In contrast, whole-lung homogenate *Mmp9* expression was increased during RSV infection by a MAVS-dependent but TRIF-independent mechanism and SAE cells treated with recombinant IFN-β had increased *Mmp9* expression.[23] Although the underlying mechanism that accounts for differences in these findings are not clear, they highlight the notion that MMPs likely have cell-type-specific functions that will need to be investigated in order to fully understand how MMPs exert their biological functions.[6] Our data demonstrating differential regulation of macrophage *Mmp10* during stimulation with LPS or poly(I:C) underscore this idea and these differences should be considered when designing murine *in vivo* models of disease.

In addition to advancing the understanding of macrophage *Mmp28* regulation by type I IFN, our work here further investigated whether loss of MMP28 altered macrophage responses to poly(I:C) or recombinant type I IFN. Our results demonstrate

that $Mmp28^{-/-}$ macrophages have increased pro-inflammatory responses to poly(I:C) stimulation and at least part of this response, such as $Il6$, is increased in $Mmp28^{-/-}$ macrophages when stimulated with either recombinant IFN-α or IFN-β. Activation of the NF-κB p65 and ERK1/2 pathways was examined at 5 and 24 h after poly(I:C) stimulation. No statistical differences between wild type and $Mmp28^{-/-}$ macrophages were observed (data not shown). Therefore, the precise underlying mechanism by which MMP28 regulates this inflammatory response remains to be determined. In our previous study, we observed increased $Mmp9$ mRNA in macrophages obtained from the lungs of cigarette-smoke exposed $Mmp28^{-/-}$ mice compared with wild type.[10] Here, we further demonstrate that, after poly(I:C) stimulation, $Mmp28^{-/-}$ macrophages have increased $Mmp9$ mRNA and decreased $Mmp10$ mRNA, demonstrating that alterations in MMP28 function likely influence a network of changes that may result in differential regulation of the inflammatory response.

A limitation of our current study is the use of murine cells as a model system compared with human cells. We have found that CD14$^+$ human monocyte-derived macrophages differentiated with M-CSF have very low to undetectable expression of $MMP28$ (data not shown); therefore, the ability to evaluate whether the regulation of human macrophage $MMP28$ is similar to the findings we have described here for murine macrophages remains a challenge for the field. However, we have detected MMP28 in alveolar macrophages in human lung samples[10] and also in human alveolar macrophage samples from critically ill patients by qPCR (unpublished observations). Multiple studies have described large differences in expression profiles between $in\ vitro$ cultured human monocyte-derived macrophages, peripheral blood monocytes, and alveolar macrophages;[26,27] therefore, future studies examining human alveolar macrophage $MMP28$ responses to type I IFN pathway activation would be of interest. In summary, our findings suggest a model by which the expression and function of macrophage MMP28 is integrated into to the type I IFN response and future investigation into the function of MMP28 will focus on responses to stimulation of these pathways. In addition, further studies designed to investigate the cell-specific responses of MMPs to determine how they integrate into the overall IFN response will likely yield new insights into the pathogenesis of diseases that have previously been shown to involve the functions of MMPs or type I IFN.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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