Stromelysin-2 (MMP10) Moderates Inflammation by Controlling Macrophage Activation

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Abstract

Several members of the matrix metalloproteinase (MMP) family control a range of immune processes, such as leukocyte influx and chemokine activity. Stromelysin-2 (MMP10) is expressed by macrophages in numerous tissues after injury; however, little is known of its function. Here, we report that MMP10 is expressed by macrophages in human lungs from patients with cystic fibrosis and induced in mouse macrophages in response to *Pseudomonas aeruginosa* infection both *in vivo* and by isolated resident alveolar and bone marrow-derived macrophages (BMDM). Our data indicates that macrophage MMP10 serves a beneficial function in response to acute infection. Whereas wildtype mice survived infection with minimal morbidity, 50% of *Mmp10*^{−/−} mice died and all showed sustained weight loss (morbidity). Although bacterial clearance and neutrophil influx did not differ between genotypes, macrophage numbers were about 3-fold greater in infected *Mmp10*^{−/−} lungs than in wildtypes. Adoptive transfer of wildtype BMDM normalized infection-induced morbidity in *Mmp10*^{−/−} recipients to wildtype levels demonstrating that the protective effect of MMP10 was due to its production by macrophages. Both *in vivo* and in cultured alveolar macrophages and BMDM, expression of several M1 macrophage markers was elevated, while M2 markers were reduced in *Mmp10*^{−/−} tissue and cells. Global gene expression analysis revealed that infection-mediated transcriptional changes persisted in *Mmp10*^{−/−} BMDM long after they were down-regulated in wildtype cells. These results indicate that MMP10 serves a beneficial role in response to acute infection by moderating the pro-inflammatory response of resident and infiltrating macrophages.

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Introduction

Macrophages are critical effector cells of the immune system and play essential, yet distinct roles in both promoting and resolving inflammation and in facilitating tissue repair and contributing to its destruction (1). That a single cell type can serve opposing functions may seem counterintuitive, but dramatic phenotypic changes occur when macrophages respond to local stimuli (1–4). Based on patterns of gene and protein expression and function, macrophages are commonly classified as classically activated (M1) or alternatively activated (M2) cells, as well as a variety of M2 subtypes (1, 4, 5). M1 macrophages are induced by infection and pro-inflammatory T_H1 cytokines, are effective at killing bacteria, and release pro-inflammatory cytokines, such as IL-1β, IL-12, and TNFα. M2 macrophages are induced by the T_H2 cytokines IL-4 and IL-13 and other factors, release anti-inflammatory factors, such as IL-10, are weakly microbicidal, and promote repair. We recognize, however, that dividing macrophages into M1 and M2 classes oversimplifies the complex continuum of functional and reversible states in which these cells exist (6–9).

Several proteins influence macrophage behavior, including some members of the matrix metalloproteinase (MMP) gene family. For example, MMP12 and MMP28, both macrophage products, either promote or restrict macrophage influx into lung (10, 11), and MMP8 promotes M2 polarization (12). In addition, we reported that MMP28 and TIMP3, an MMP inhibitor, moderate M1 activation of macrophages in models of lung infection and fibrosis (13, 14). As their name implies, MMPs are thought to degrade extracellular matrix proteins, a function that is indeed performed by some members (15–17). However, matrix degradation is neither the shared nor predominant function of these enzymes. Rather, individual MMPs have been shown to regulate specific immune processes, such as leukocyte influx and activation, antimicrobial activity, and restoration of barrier function, typically by processing of a range of non-matrix protein substrates (18–25).

MMP10 (stromelysin-2) is not expressed in developing or normal adult tissues, including lung (26, 27). However, in both human conditions and mouse models, MMP10 is induced in response to injury, infection, or transformation in essentially all tissues (28–33). In a meta-analysis of gene array experiments involving numerous different host-pathogen interactions, MMP10 was identified as a common host response gene (34). The widespread expression of MMP10 among tissues suggests that this proteinase serves critical roles in the host response to environmental insults. As we show here, MMP10 serves a protective role in acute infection by moderating the pro-inflammatory activity of macrophages.

Recently, we reported that macrophage MMP10 promotes the ability of M2 macrophages to clear scar tissues in normal skin wounds by controlling the expression of collagenolytic MMPs, particularly MMP13 (35). However, because sterile excision wounds are not associated with a profound inflammatory response, the stimulus (i.e., a clean wound) may not have been sufficiently robust to reveal other MMP10-dependent roles in macrophage inflammation. Thus, for the studies reported here, we challenged *Mmp10*$^{-/-}$ mouse lungs with *Pseudomonas aeruginosa*, an opportunistic pathogen and common cause of hospital-acquired infections and pneumonia (36) and the major pathogen in lungs of patients with cystic fibrosis (CF) (37). Compared to wildtype mice, we found that *Mmp10*$^{-/-}$ mice were

much more susceptible to airway infection and that MMP10 influenced activation of resident and infiltrated macrophages by curbing M1 polarization and *P. aeruginosa*-induced transcriptional changes. Our findings indicate that MMP10 is a critical cell autonomous mediator controlling macrophage activation.

Materials and Methods

Animals

Mmp10⁻/⁻ mice and wildtype littermates (C57BL/6J, male and female, 8–16 wk old) were used for these studies. All procedures were approved by the Office of Animal Welfare at the University of Washington and the IACUC at Cedars-Sinai. *Mmp10*⁻/⁻ mice were generated by targeting exons 3–5 that code the catalytic domain as described (38). *Mmp10*⁻/⁻ mice breed and appear normal, with similar litter sizes. We also generated *Mmp10*⁻/⁻ mice (*Mmp10*^tm1Lex/Mmucd) with embryonic stem cells from the Mutant Mouse Resource & Research Center (MMRRC; https://www.mmrrc.org/catalog/sds.php?mmrrc_id=11737). These cells were originally generated by Lexicon Pharmaceuticals (The Woodlands, TX), who used a targeting strategy distinct from ours.

Infection Model

Mice were challenged with live *P. aeruginosa* strain K either by aerosolization in a whole animal nebulizer chamber for 30 min to produce a deposition of bacteria between 10^5 to 10^6 cfu/left lung or by oropharyngeal aspiration as described (11, 39). Briefly, bacteria were grown in LB overnight, washed twice, and suspended in sterile PBS. For oropharyngeal aspiration, bacteria were quantified by OD_{600} and suspended at 2×10^8 cells/ml, and 50 μl was instilled per mouse. After exposure by either method, the lungs of 3–5 mice were immediately harvested and homogenized to determine 0-h bacterial counts (CFU). CFU were determined by plating serial dilutions of left lungs or spleens homogenized in 1 ml PBS on LB agar plates. Duplicate plates of each dilution were incubated 24–48 h at 37°C and counted using a Quebec Colony Counter (Reichert Technologies, Depew NY).

Sample Collection and Analyses

The left lung hilum was ligated, and the left lung and spleen were removed for determination of bacterial load. Bronchoalveolar lavage (BAL) was collected by flushing the right lung via the trachea 4 times with 0.5 ml saline. The recovered lavage (typically 1.7 ml) was centrifuged at 400×g, 4°C for 10 min. Supernatants were collected and frozen at −70°C for later analysis. Total protein in BAL was quantified using the Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford IL) and IgM by ELISA (Bethyl Laboratories, Montgomery TX). Pelleted cells were resuspended in DMEM with 10% FBS, counted in a hemocytometer, and a 100-μl aliquot was cytospun onto slides and differentially stained with LeukoStat (Fisher Scientific, Pittsburgh PA). At least 300 cells were counted per lavage. Lungs from separate mice were used for RNA isolation or flow cytometry (below).

Immunostaining and *In Situ* Hybridization

De-identified human lung specimens were obtained with approval of the University of Washington Institutional Review Board. Sections (5 μm) were stained with antibodies

specific for human MMP10 (Abcam, Cambridge MA) as described (40). Mouse lungs were perfused-fixed with PBS-buffered formalin via the trachea under 25-cm pressure for 5 min, incubated in fixative at room temperature for 48 h, dehydrated through graded ethanol, and embedded in paraffin. Deparaffinized sections (5 μm) underwent heat-induced (95°C, 30 min) antigen retrieval in pH 7.0 citrate. Endogenous peroxidase activity was quenched with 3% H_2O_2 for 10 min. Sections were incubated with 4% normal rabbit serum for 1 h then overnight at 4°C with anti-Mac2 antibody (1:500 dilution; clone M3/38.1.2.8 HL.2, ATCC, Manassas VA). Bound antibody was detected using a Vectastain Elite ABC (Rat IgG) kit (Vector Laboratories, Burlingame CA) and 3,3′-diaminobenzidine peroxidase substrate and counterstained with hematoxylin. For combined *in situ* hybridization/immunostaining, deparaffinized rehydrated sections were treated with 10 μg/ml proteinase K in RNase-free PBS for 20 min at ambient temperature, incubated with 0.1M triethanolamine in RNase-free water, washed in RNase-free PBS, then probed overnight at 55°C with either a 570-bp sense or antisense digoxigenin-labeled RNA probe targeting bases 1050–1620 of murine *Mmp10*. Probes were labeled using DIG RNA Labeling Mix and SP6/T7 DIG Labeling Kit (Roche Diagnostics, Indianapolis IN). After hybridization, slides were washed 3 times in 2× SSC/50% formamide, for 15 min at 50°C, once in 2× SSC at ambient temperature for 20 min, and once in 0.5× SSC for 30 min then developed with anti-digoxigenin-alkaline phosphatase using NBT/BCIP as substrate. Slides were washed with PBS and images were captured using an Olympus BX51 with DP70 digital camera (Olympus America, Center Valley, PA). Sections were then processed for immunostaining with anti-Mac-2 as described above minus antigen retrieval and counterstaining.

Flow Cytometry

Whole lungs were perfused with 10 ml ice-cold PBS through the left ventricle and then harvested, minced into tiny pieces using a razor blade, and digested in RPMI containing 2.5 mg/ml Collagenase Type IV (Gibco) for 45 min at 37°C with shaking. The digestion was stopped with addition of FBS to 10%. The lung cell suspension was applied to a 70-μm cell strainer and washed with cold RPMI. The sample was subjected to RBC lysis (ACK Lysing Buffer; Thermo-Fisher), followed by washing in RPMI and cell counting using a Bio-Rad Cell Counter. Lung cells were stained with fluorophore-conjugated antibodies and analyzed using a BD LSRFortessa or sorted using a BD FACSARIA III (BD Biosciences, San Jose CA). Cell populations in the lungs were identified using the following combinations of cell surface markers: alveolar macrophages, F4/80$^+$ CD11c$^+$ SiglecF$^+$; interstitial macrophages, F4/80$^+$ CD11b$^+$ CD11c$^+$ MHCII$^+$ SiglecF$^-$; Ly6C$^+$ monocytes/macrophages, F4/80$^+$ Ly6C$^+$ CD11b$^+$ CD11c$^+$ MHCII$^-$ SiglecF$^-$, and neutrophils, F4/80$^-$ Ly6G$^+$ CD11b$^+$. Sorted cells were kept at 4°C during sorting, centrifuged, and immediately processed for RNA isolation as described below. SiglecF antibody was from BD Biosciences; all others were from eBioscience (San Diego CA).

Macrophage Culture, Transfer, and Treatments

Bone marrow-derived macrophages (BMDM) and alveolar macrophages were isolated and cultured as described (11, 39). To generate BMDM, marrow cells were differentiated in CSF-1-containing medium for 7 d (referred to as M0 macrophages). To generate M1-biased macrophages, M0 BMDM (1.5×10^6 per well in 6-well plates) were stimulated with 100

ng/ml *E. coli* 0111:B4 lipopolysaccharide (LPS) for 24 h or with live *P. aeruginosa* strain K at a multiplicity of infection of 5 bacteria per macrophage for 1 h before washing away bacteria and then culturing in medium with 75 μg/ml gentamicin to kill live extracellular bacteria. For M2 polarized macrophages, BMDM were stimulated with 10 ng/ml each of IL-4 and IL-13 for 48 h. For adoptive transfer, recipient mice were given 2×10^6 wildtype or *Mmp10*$^{-/-}$ M0 BMDM in 100 μl PBS via retro-orbital 24 h post infection. For the microarray experiments, M0 BMDM cells were exposed to *P. aeruginosa* strain K as described above. The cells were then cultured in medium containing gentamicin, 100 IU/ml penicillin, and 100 μg/ml streptomycin, and RNA was collected 6 h or 24 h later. For alveolar macrophages, cells in BAL (98% macrophages) from 4 groups of 5 naïve wildtype mice/group were pooled, and 2×10^5 cells were cultured in 24-well plates overnight in RPMI containing 10% FBS and penicillin/streptomycin before stimulating with LPS or bacteria. Phagocytosis assay is described in the supplement.

mRNA Assays

Total RNA was isolated from lungs or macrophages and RT-PCR was performed and quantified as described (41). Unlabeled PCR primers and TaqMan probes (FAM dye-labeled) were used to detect mRNAs for *Nos2, Ifng, Ccl2, Ccl3, Ccl5, Il6, Tnf, Ccr2, Arg1, Il10, Mrc1, Clec10a, Retnla, Mmp3, 7, 8, 10, 12,* and *28*, and *Hprt* (Applied Biosystems, Foster City CA). The data are expressed as relative quantification, which is the fold change and calculated as $2^{-\Delta Ct}$.

Cytokine/Chemokine Assays

Lung samples were homogenized in 2 ml lysis buffer: 150 mM NaCl, 15 mM Tris (pH 7.2), 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1% Triton X-100, and Complete Mini Protease Inhibitor Cocktail (Roche Molecular Biochemicals, Mannheim, Germany). Homogenates were centrifuged at $10,000 \times g$ for 15 min at 4°C, and the supernatants were collected. Multiplex reagents were from R&D Systems (Minneapolis MN) including the mouse Fluorokine MAP kit and analytes for IFNγ, IL-1β, IL-6, IL-10, IL-12, IL-17, TNFα, CXCL1, CXCL2, and CCL2.

Microarray Analysis

Total RNA from BMDM was purified using RNeasy Mini-kits (Qiagen, Hilden, Germany). All RNA samples were of high quality as assessed by an Agilent 2100 Bioanalyzer, with RIN 9.8. Each RNA sample was converted to cDNA, labeled, and hybridized to GeneChip Mouse Gene 2.0 ST arrays (Affymetrix, Santa Clara CA) following the manufacturer's protocols. The whole genome GeneChip Mouse Gene 2.0 ST is comprised of 35,240 transcripts including 26,515 unique genes. Hybridized arrays were scanned with an Affymetrix Gene Chip 3000 scanner, and image generation and feature extraction were performed using Affymetrix GeneChip Operating Software. All arrays passed the manufacturer's quality specifications with respect to background and percent present call rates. Gene expression levels were estimated from probe intensities using the robust multi-array analysis method with quantile normalization and background adjustment (42). Differential gene expression between wildtype and *Mmp10*-null samples at each time point was determined using a Bayesian implementation of the parametric *t*-test (43) and multiple

hypothesis testing was controlled using false discovery rate analysis (q-value) (44). A q-value < 0.01 was used to identify significant differential gene expression. Functional enrichment analysis of differentially expressed genes was performed with WebGestalt program (45) based on multiple publicly available resources including Gene Ontology and Kyoto Encyclopedia of Genes and Genomes. Enrichment p-values were determined using Fisher's exact test and corrected for multiple testing using Benjamini-Hochberg's method (46) with a significance adjusted p-value < 0.01. Minimal Information About Microarray Experiments (MIAME)-compliant data have been submitted to the NIH-GEO (accession no. GSE78175; http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=epkvmssmvhmrlmf&acc=GSE78175).

Statistics

Statistical analysis was performed using 2-way ANOVA with Bonferroni post-test where appropriate. Data are presented as mean ± SEM, with p 0.05 considered statistically significant. Mortality data was analyzed by Kaplan-Meier log rank test. Prism 5 software (GraphPad Software, La Jolla CA) was used for all statistical analyses.

Results

MMP10 is Expressed in Infected Lungs

We stained de-identified samples of CF lungs – all colonized with *P. aeruginosa* – from transplant recipients ($n = 5$) and normal human lungs ($n = 3$) for MMP10 protein using a human-specific antibody. In all CF samples, signal for MMP10 was seen in most mononuclear cells (Fig. 1B,C). The large, pale nuclei of these cells and their accumulation within interstitial areas are characteristic features of macrophages. No signal for MMP10 was detected in normal human lung, including resident alveolar macrophages (Fig. 1A).

We used *in situ* hybridization coupled with immunostaining to identify the cell sources of *Mmp10* mRNA expression in mouse lung. Similar to human lung, we detected no signal for *Mmp10* mRNA in naïve mouse lungs (Fig. 1E). However, at 72 h post-infection with *P. aeruginosa*, we detected prominent signal for *Mmp10* mRNA (Fig. 1F) in cells that stained positive for Mac-2, a pan-macrophage marker (11), demonstrating that all cells strongly positive for *Mmp10* mRNA were macrophages (Fig. 1G, G'). No specific signal was seen in sections processed with sense RNA probe (Fig. 1H). These findings demonstrate that MMP10 is not expressed in healthy lung but is induced by macrophages in human and murine lung in response to bacterial infection.

MMP10 Protects against Lethality Induced by Lung Infection

To determine the role of MMP10 in the host response to infection, we exposed wildtype and *Mmp10*$^{-/-}$ mice to live *P. aeruginosa* by aerosolization or oropharyngeal aspiration. Unchallenged *Mmp10*$^{-/-}$ mice develop normally and reveal no overt phenotype in any tissue (33, 35, 38). Consistent with our *in situ* hybridization data (Fig. 1E), *Mmp10* mRNA levels were very low (average Ct = 38) in naïve wildtype lung, indicating that this transcript was not expressed by any resident cell, including alveolar and interstitial macrophages in unchallenged lung. In response to *P. aeruginosa* infection, expression of *Mmp10* mRNA was

induced in wildtype lungs, peaked 3 d after infection, and remained elevated at 7 d post infection (Fig. 2A). The pattern and extent of MMP10 expression were equivalent between the two infection protocols and mirrored the 0–24 h induction response we reported in mice infected by nasal inoculation with *P. aeruginosa* strain PA51673, CF clinical isolate (38). In contrast, expression of other MMPs increased modestly (MMP3, MMP12), if at all (MMP28), or transiently (MMP7, MMP8) (Fig. S1A). MMP8, which is produced by many cell types and is particularly abundant in neutrophils, quickly increased then fell (Fig. S1B), likely reflecting the rapid influx and efflux of neutrophils in this model (11). Thus, among MMPs, MMP10 revealed a potent and sustained response to infection.

Whereas infection with *P. aeruginosa* led to no mortality in wildtype mice, half of *Mmp10*$^{−/−}$ mice had died by 48 h post *P. aeruginosa* exposure (Fig. 2B). Among the survivors, *Mmp10*$^{−/−}$ mice were more susceptible to infection than wildtype mice as manifested by a reproducible and significant delay in their ability to regain body weight (Fig. 2C). Lethality and delayed weight recovery in *Mmp10*$^{−/−}$ mice coincided with the marked upswing in *Mmp10* expression in wildtype mice (Fig. 2A). In addition, we observed greater mortality (Fig. S1C) and delayed weight gain (data not shown) in *Mmp10*^{tm1Lex}/Mmucd generated with embryonic stem cells in which the *Mmp10* locus was targeted by a gene-trap approach. Thus, the phenotypes we observed in our *Mmp10*$^{−/−}$ mice were not due to an off-target artifact. These data indicate that MMP10 serves a critical and protective role in the host response to infection.

Lethality and morbidity in *Mmp10*$^{−/−}$ mice were not due to impaired bacterial clearance. By 48 h, both wildtype and *Mmp10*$^{−/−}$ mice had effectively cleared the infection (Fig. 2D). In addition, bacteremia, as measured by the recovery of live bacteria from spleen (Fig. 2E) and spleen weights (data not shown) before and during infection did not differ between genotypes. Furthermore, infection-induced increase in lung permeability, a marker of acute lung injury, also did not differ between genotypes (Fig. S1D). We did not see any indication (edema, hemorrhage, inflammation) that other tissues (liver, intestines) were involved (data not shown).

Whereas mortality of *Mmp10*$^{−/−}$ mice was more pronounced when *P. aeruginosa* was administered by aerosolization (50%; Fig. 2B, S1C) compared to oropharyngeal aspiration (14%; *n* > 90 mice/genotype from several experiments; data not shown), morbidity as gauged by degree of weight loss and delay in weight recovery was similar by either method of *P. aeruginosa* infection. Thus, to minimize a potential survival bias, our subsequent *in vivo* data were obtained with mice infected by oropharyngeal aspiration.

Increased Macrophage Influx in Lungs of *Mmp10*$^{−/−}$ Mice

Because lethality in *Mmp10*$^{−/−}$ mice was not associated with impaired bacterial clearance, we predicted that *Mmp10*$^{−/−}$ mice died due to altered or excessive inflammation. We examined BAL and lung sections at various times post infection. Consistent with the similar rate of bacterial clearance (Fig. 2D), neutrophils – which are critical for clearance of *P. aeruginosa* – were the predominant cell in BAL post infection (>95% of total cells), and their numbers did not differ between genotypes (Fig. 3B). Lymphocyte numbers, which increased in response to infection, also did not differ significantly between genotypes (data

not shown). In contrast, we detected 3-fold more macrophages in BAL (Fig. 3C) at 48 h post-infection, coincident with the upswing in MMP10 expression (Fig. 2A), and even more in tissue sections from *Mmp10*$^{-/-}$ mice (Fig. 3E). At 4 and 24 h post-infection, before the significant increase in MMP10 expression, the number of macrophages in BAL did not differ between genotypes (Fig. 3C). Flow cytometry of CD45$^+$ cells from non-lavaged lung demonstrated that numbers of alveolar (F4/80$^+$ CD11c$^+$ SiglecF$^+$) and interstitial (F4/80$^+$ CD11b$^+$ CD11c$^+$ MHCII$^+$ SiglecF$^-$) macrophages did not differ between naïve wildtype and *Mmp10*$^{-/-}$ mice and that the influx of macrophages into lungs of both genotypes was predictably due to infiltrated cells (F4/80$^+$ Ly6C$^+$ CD11b$^+$ CD11c$^+$ MHCII$^-$ SiglecF$^-$; data not shown). Similarly, we observed a 2-fold increase in BAL macrophages in *Mmp10*$^{tm1Lex/}$Mmucd mice post-infection (data not shown).

The number and differential of circulating leukocytes did not differ between wildtype and *Mmp10*$^{-/-}$ mice (data not shown). In addition, we reported no difference in the ability of wildtype and *Mmp10*$^{-/-}$ macrophages to migrate toward serum or other chemotactic stimuli (35) indicating that MMP10 does not affect the migratory machinery of macrophages. We also assessed the expression of various chemokines and cytokines that could affect macrophage influx or that have pro-inflammatory activity. We found no differences in the levels for TNFα, CXCL1/2, IL-6, or IL-17 in BAL or lung homogenates (data not shown). In contrast, we found elevated expression of CCL2/MCP1, a potent macrophage chemokine (47, 48), in *Mmp10*$^{-/-}$ lungs (see Fig. S3A), a finding that was mirrored in isolated macrophages (below).

MMP10 Expression by Macrophages

Macrophages that influx into the lung – or any tissue – in response to infection or injury are distinct from the resident population (49, 50). We found that both resident and recruited macrophages expressed MMP10 at fairly similar levels post-infection (data not shown). We then assessed if macrophages in culture respond as these cells did *in vivo*. Consistent with our *in vivo* data, *Mmp10* mRNA was not expressed by alveolar macrophages (Fig. 4B) or bone marrow (Fig. 4C) freshly isolated from naïve wildtype mice. Upon CSF-1-mediated differentiation into macrophages (M0), MMP10 was induced in bone marrow-derived macrophages (BMDM) (Fig. S2A, Fig. 4C). MMP10 was not induced by marrow cells cultured with GM-CSF (Fig. S2A). As GM-CSF promotes differentiation into dendritic cells (5), these negative data indicate further that macrophages are the source of MMP10. Expression of MMP10 by both alveolar macrophages and BMDM was stimulated with LPS and even more so by exposure to live *P. aeruginosa* (Fig. 4B,C) reaching levels that were quite similar between cell types and comparable to the levels detected for *Mmp10* mRNA in lung in response to airway infection (see Fig. 2A). Together, these findings indicate that both resident alveolar and infiltrated macrophages express similar levels of MMP10 in response to infection.

Rescue of *Mmp10*$^{-/-}$ Phenotype

We performed adoptive transfer experiments to determine if wildtype macrophages could ameliorate morbidity in infected *Mmp10*$^{-/-}$ mice. Indeed, adoptive transfer of wildtype BMDM 24 h after infection with *P. aeruginosa* resulted in significantly more rapid recovery

of weight loss – used as a surrogate for morbidity – comparable to that seen in wildtype mice (Fig. 5). *Mmp10*$^{-/-}$ BMDM did not affect weight loss in either wildtype or *Mmp10*$^{-/-}$ recipients (Fig. 5). These findings demonstrate that macrophages are the key cells mediating the MMP10-dependent phenotypes in response to *P. aeruginosa* lung infection.

MMP10 Regulates Macrophage Activation

We assessed if MMP10 influences the state of macrophage activation by measuring the *in vivo* expression of M1 and M2 markers (5). Compared to levels in wildtype mice, we found elevated expression of mRNAs for the M1 markers IFNγ, iNOS, and CCL2 (Fig. S3A) and reduced expression levels of M2 markers arginase-1 and IL-10 in infected *Mmp10*$^{-/-}$ lungs (Fig. S3C). In contrast, IL-12a, an M1 marker, was reduced in *Mmp10*$^{-/-}$ lungs (Fig. S3A), and protein levels of TNFα and IL-1β did not differ between genotypes (Fig. S3B). In addition, as we found in a skin wound model (35), expression of the M2 marker resistin-like α (*Retnla*, FIZZ1) was not affected by MMP10 (Fig. S3D). Overall, the *in vivo* phenotypes we saw in *Mmp10*$^{-/-}$ mice, as summarized in Table I, support the idea that MMP10 mitigates the pro-inflammatory activity of macrophages.

We next assessed if MMP10 plays a cell autonomous role in regulating macrophage polarization. We treated wildtype and *Mmp10*$^{-/-}$ M0 BMDM with live *P. aeruginosa* to stimulate M1 differentiation or with IL-4 and IL-13 for M2 differentiation and assessed expression of various markers. Expression of MMP10 by M0 macrophages was stimulated by M1 agonists (Fig. 4C, S2B) but was not further elevated by M2 agonists (Fig. S2B), consistent with findings in human monocytes (51). In M0 and M2-polarized BMDM, we saw no difference in the expression of M1 and M2 markers between wildtype and *Mmp10*$^{-/-}$ BMDM (Table II, Fig. S4), with one notable exception. IFNγ expression was elevated about 10-fold in *Mmp10*$^{-/-}$ M0 BMDM compared to wildtype M0 cells (Fig. S4A), suggesting that in the absence of MMP10 macrophages are primed toward a pro-inflammatory phenotype.

We did observe significant MMP10-dependent effects in M1-polarized macrophages (Table II). In particular, the induction of CCL2 in response to *P. aeruginosa* was markedly greater in *Mmp10*$^{-/-}$ alveolar macrophages and BMDM compared to wildtype cells (Fig. 6). In addition, expression of mRNAs for the M1 markers iNOS, IFNγ, and CCL5 and the ability to phagocytize *E. coli* particles – an M1 property – were significantly elevated in M1-polarized *Mmp10*$^{-/-}$ macrophages (Fig. S4A). Expression of IL-6 did not differ between genotypes (Table I, Fig. S4B). Furthermore, expression of the M2 markers arginase-1 and IL-10 was stimulated in *P. aeruginosa*-treated BMDM, a response that was significantly diminished in *Mmp10*$^{-/-}$ BMDM (Fig. S4C). CD206 (mannose receptor) was down-regulated in M1-polarized wildtype BMDM and significantly more so in *Mmp10*$^{-/-}$ BMDM (Table I, Fig. S4C). The levels of other M2 markers, including CCR2 (Fig. S4D) and resistin-like α (35), did not differ between M1-polarized wildtype and *Mmp10*$^{-/-}$ BMDM. Collectively, these data indicate that MMP10 functions to moderate several characteristic responses of M1 macrophage activation.

MMP10 Moderates Bacteria-induced Transcriptional Changes in Macrophages

To gain insight into potential mechanisms by which MMP10 influences the macrophage response to bacterial infection, we performed global gene expression analysis. Wildtype and *Mmp10*⁻/⁻ BMDM were exposed to *P. aeruginosa* for 1 h, and cells were harvested at 6 h and 24 h later to assess acute and persistent gene expression changes. We identified many genes differentially expressed (q < 0.01) compared to untreated controls at 6 h after infection in both wildtype and *Mmp10*⁻/⁻ BMDM. As expected, *P. aeruginosa* infection caused a robust transcriptional response in both wildtype (12,672 genes) and *Mmp10*⁻/⁻ (15,164 genes) BMDM. The vast majority of differentially expressed genes (n = 10,286) were common between wildtype and *Mmp10*⁻/⁻ BMDM (Fig. 7A). Moreover, the direction and pattern of differential expression of the common genes were similar in wildtype and *Mmp10*⁻/⁻ cells, indicating that *P. aeruginosa* infection was the dominant cause of early transcriptional changes (Fig. 7B). Functional enrichment analysis of the smaller subsets of genes that were differentially expressed only in wildtype or *Mmp10*⁻/⁻ at 6 h did not reveal coherent phenotypes (data not shown).

Because gene expression is a temporally dynamic process, we postulated that the role of *Mmp10* in regulating transcriptional programs induced by bacterial challenge may be revealed by examining a later time point. Thus, we focused on the large set of common differentially expressed genes at 6 h and assessed if their levels remained significantly altered at 24 h post-infection. Of the 10,286 common genes, 5,609 remained differentially expressed 24 h after infection (Fig. 7C). Notably, we observed that the majority (3,981) of genes that remained differentially expressed at the 24-h time point were unique to *Mmp10*⁻/⁻ BMDM, implying that MMP10 plays a distinct role in moderating the post-infection transcriptional response. Of the 3,981 genes that remained differentially expressed only in *Mmp10*⁻/⁻ BMDM, 1,386 were up-regulated and 2,505 were down-regulated at 24 h. We applied functional analysis using WebGestalt to identify biological processes enriched within the subset of persistently up-regulated genes (Fig. 7D). We found that the absence of *Mmp10* in *P. aeruginosa*-exposed BMDM was associated with persistent activation of a wide repertoire of pathways mapping to immunity, inflammation, apoptosis, and cell signaling. In contrast, functional analysis of the persistently down-regulated genes showed enrichment of processes involved in metabolism, transcription, and translation.

Discussion

Here, we report on the expression and function of macrophage MMP10 in the host response to infection. Our findings indicate that MMP10 is not expressed in healthy, unchallenged human and mouse lung but is markedly induced in both resident and infiltrated macrophages following infection with *P. aeruginosa*. With a combination of *in vivo* and primary macrophage models, we demonstrate that MMP10 protects mice from morbidity and mortality induced by acute *P. aeruginosa* lung infection. By assessing the expression of various markers and overall gene expression, our findings indicate that MMP10 functions to drive the activation of macrophages from proinflammatory (M1-like) cells into immunosuppressive (M2-like) cells. Our findings complement observations we reported with others showing greater mortality and delayed weight gain in *Mmp10*⁻/⁻ mice following

acute liver injury (52) and more macrophages biased towards M1 activation and more tissue injury in *Mmp10^−/−* mice subjected to acute colon injury (33). Furthermore, *Mmp10^−/−* mice are much more susceptible to the lethal effects of acute lung injury caused by bleomycin toxicity than are wildtype animals (unpublished observations). Thus, across organ systems, MMP10 appears to play a beneficial role in mitigating the deleterious responses to injury and infection by promoting the conversion of pro-inflammatory macrophages toward immunosuppressive cells. Our findings support the general concept that in response to acute injury or infection macrophage activation evolves from potentially detrimental M1-based cells to suppressive M2-biased macrophages (6, 39, 50).

Although more macrophages emigrated into *Mmp10^−/−* lungs in response to infection, we found no difference in the ability of wildtype and *Mmp10^−/−* macrophages to migrate toward serum or wound-tissue homogenate (35) indicating that MMP10 does not affect the migratory machinery of macrophages. With others (53), we reported that *Mmp10^−/−* macrophages have an impaired ability to migrate over fibronectin and to invade into Matrigel. Although these findings are seemingly opposed to our *in vivo* observations, there are two important caveats with these *in vitro* studies. First, it is not clear if macrophages migrate over fibronectin on their way into and through the interstitial space. Although fibronectin would be present in the area, it appears that other matrix components, particularly versican and hyaluronan (54), serve as the interstitial substrata that macrophages follow as they migrate through tissue (55, 56). Second, Matrigel is a highly dense material that does not mirror the more porous architecture of interstitial matrix (57, 58). In fact, Matrigel does not contain interstitial matrix components; it is comprised of misassembled basement membrane components (57). Thus, unlike how they move through the interstitium *in vivo*, macrophages may require proteases to burrow through a thick plug of Matrigel in a culture dish. As we reported that MMP10 activates matrix-remodeling programs in macrophages (35, 59), *Mmp10^−/−* cells may not possess the enzymes needed to invade through the artificial setting of Matrigel. We conclude that MMP10 does not affect macrophage migration *per se* in physiologic settings.

An open question is if increased morbidity and mortality in *Mmp10^−/−* animals was due to more macrophage numbers, to a bias toward M1 activation, or a combination of these two processes. We propose that altered activation of both resident and infiltrated macrophages towards an M1, pro-inflammatory state led to worse outcomes for infected *Mmp10^−/−* mice and this deleterious effect was augmented by the presence of more M1-biased cells. Consistent with this conclusion, Johnston *et al.* reported that the macrophage phenotypes begins to evolve toward an M2 state about 48 h after lung infection with *P. aeruginosa* (39), and D'Alessio *et al.* recently demonstrated that therapeutically promoting macrophage differentiation toward M2 cells (via instillation of IL-4) markedly reduces lethality and morbidity in response to acute *P. aeruginosa* infection (60). In *Mmp10^−/−* mice and macrophages, we found increased expression levels of M1 markers and decreased levels of M2 markers (Table I, II), such as the increased and prolonged expression of CCL2 (MCP-1), which was also seen in injured *Mmp10^−/−* colons (33). CCL2, a potent macrophage chemokine, is expressed predominantly by resident lung macrophages (47, 48), and over-expression of this factor may account for the excess macrophage inflammation seen in *Mmp10^−/−* mice. The diminished expression of IL-10 and Arg-1, both M2 markers, in

infected *Mmp10⁻ᐟ⁻* lungs and BMDM provides further evidence that MMP10 broadly affects macrophage polarity to moderate inflammation.

Neutrophil influx, which accounted for about 95% of the cells recovered from BAL, and the levels of CXCL1 and CXCL2, critical neutrophil chemokines, did not differ between wildtype and *Mmp10⁻ᐟ⁻* mice in response to infection. Similarly, Koller *et al.* found no difference in neutrophil numbers between wildtype and *Mmp10⁻ᐟ⁻* mice over the first 14 days post colon injury (33). However, they did find that elevated neutrophil counts persisted in *Mmp10⁻ᐟ⁻* mice at 21 days post-injury, at which time their numbers had dropped in wildtype colons. Although sustained neutrophil influx may have been secondary to worse injury of *Mmp10⁻ᐟ⁻* colons, it is also possible that a bias toward M1 macrophages maintained neutrophil influx that, in turn, led to more tissue damage. In our lung infection model, the lack of difference in neutrophil numbers and indices of acute lung injury between genotypes indicates that neutrophils did not contribute to worse outcomes in *Mmp10⁻ᐟ⁻* mice. A potential caveat to this conclusion is if MMP10 influences the state of neutrophil activation. For example, in other studies, we found that MMP7 shedding of syndecan-1/ CXCL1 complexes from injured lung epithelium permits both neutrophil migration through the mucosal barrier and their subsequent activation (20, 61). Without this processing mechanism, *Mmp7⁻ᐟ⁻* mice are markedly protected from the lethal effects of neutrophil-mediated lethality in response to acute lung injury (20). However, we propose that the similar rate of bacterial clearance in wildtype and *Mmp10⁻ᐟ⁻* mice does not support an MMP10-dependent role in neutrophil activation.

While MMP10 can also be expressed by injured/infected epithelium, including in lung (38 and unpublished observations), we do not think that the enzyme produced by these cells contributed to the key phenotypes and responses we report here. Indeed, adoptive transfer of wildtype macrophages into *Mmp10⁻ᐟ⁻* recipients reduced post-infection morbidity to levels seen in wildtypes. Similarly, using bone marrow transplantation, Koller *et al.* concluded that the lack of MMP10 in macrophages led to worse tissue damage in a model of acute colon injury (33). Furthermore, the macrophage polarization changes we observed *in vivo* (Table I) were largely duplicated with isolated macrophages in culture (Table II). These findings support the conclusion that MMP10 is a cell autonomous regulator of macrophage activation. In contrast, the function of epithelial MMP10 remains unanswered. In skin wound models, we found no re-epithelialization phenotype in *Mmp10⁻ᐟ⁻* mice (35) or in mice over-expressing active MMP-10 in basal keratinocytes (32).

We recently contributed to a multi-center genome-wide association study that identified *MMP10* as a candidate gene for chronic obstructive pulmonary disease (59). Using a model of chronic (6 mo) exposure to cigarette smoke, we found that *Mmp10⁻ᐟ⁻* mice are fully resistant to the development of emphysema. In addition, MMP10 is produced by macrophages from human smokers with emphysema (62) and is one of two genes whose expression correlates with reduced lung function (i.e., FEV_1) in smokers (63). These findings indicate that macrophage MMP10 contributes to disease progression in emphysema, which is seemingly opposed to the protective role for this MMP in acute infection we reported here. However, there are important differences between these models, especially with respect to macrophage biology. Macrophages that function early in

inflammation are functionally distinct from those that function late in inflammation or in a persistent inflammatory response (4, 6, 50, 64, 65), and this concept has been demonstrated in models of liver (66), vascular (67), cardiac (68), and kidney disease (69). In addition, whereas acute infection and injury bias toward an M1 phenotype (4), cigarette smoke promotes expansion of M2 macrophages (70). Macrophages are considered to be the destructive cell in emphysema (71, 72), and our studies in skin wounds indicate that MMP10 promotes expression of matrix-degrading proteinases in M2 macrophages (35). Thus, in an acute setting, MMP10 is beneficial by moderating the pro-inflammatory activity of M1-biased macrophages and by stimulating the ability of M2-biased macrophages to remodel scar tissue. But in a chronic setting, MMP10-driven matrix remodeling could be excessive and detrimental, as suggested in our smoke-exposure studies. Still, an important and shared conclusion among these models is that MMP10 functions to control macrophage behavior.

Significant differences in expression of M1 and M2 markers were only observed between infected (M1-biased) wildtype and *Mmp10*$^{-/-}$ macrophages but not between M0 or M2 cells (Table II). Consistent with these data, transcriptomic analysis did not reveal significant enrichment of any inflammation-related pathways in genes differentially expressed between M0 wildtype and *Mmp10*$^{-/-}$ BMDM (data not shown). Thus, the effects of MMP10, at least in terms of polarization, are more significant in M1-activated macrophages. However, MMP10 does shape some functional properties of M2-biased macrophages, such as their ability to clear collagen (35). The *P. aeruginosa*-induced transcriptional changes between wildtype and *Mmp10*$^{-/-}$ BMDM were quite similar at 6 h post infection but diverged significantly at 24 h. Nearly 4,000 of the genes that were differentially expressed in both genotypes at 6 h remained significantly changed only in *Mmp10*$^{-/-}$ BMDM at 24 h. These data indicate that MMP10 functions to broadly moderate or quench the temporal transcriptional response induced by bacteria in infiltrating macrophages. The genes that remained persistently up-regulated only in *Mmp10*$^{-/-}$ macrophages at 24 h were significantly enriched for inflammation and immune-related pathways, consistent with our observations in the infected *Mmp10*$^{-/-}$ lung.

In summary, our data demonstrate a novel role for MMP10 in moderating lung inflammation by altering the balance of M1/M2 transcripts and broadly suppressing immune-related pathways in macrophages. Importantly, the patterns of MMP10 expression are conserved between human and mouse lung and macrophages. The critical functions of MMP10 in lung inflammation are largely attributable to infiltrated macrophages and may therefore be relevant in other organs and inflammatory conditions. Given the importance of macrophage polarization in the initiation and resolution of inflammation, further study of MMP10 function is warranted in other disease models, especially those involving bacterial infection. Though beyond the scope of this study, identification of the physiologic substrates cleaved by MMP10 will be essential to determine the mechanisms by which MMP10 regulates macrophage polarization. Schlage *et al.* have identified multiple MMP10 substrates in primary mouse fibroblasts (73), but we predict that macrophage-derived MMP10 may cleave a limited and distinct set of substrates, some of which may only be expressed in an inflamed microenvironment. Determining the relevant MMP10 cleavage events in the lung is a significant challenge but a priority for our future work.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations used in this paper

MMP matrix metalloproteinase

BMDM bone marrow-derived macrophage

BAL bronchoalveolar lavage

iNOS nitric oxide synthase 2

CF cystic fibrosis

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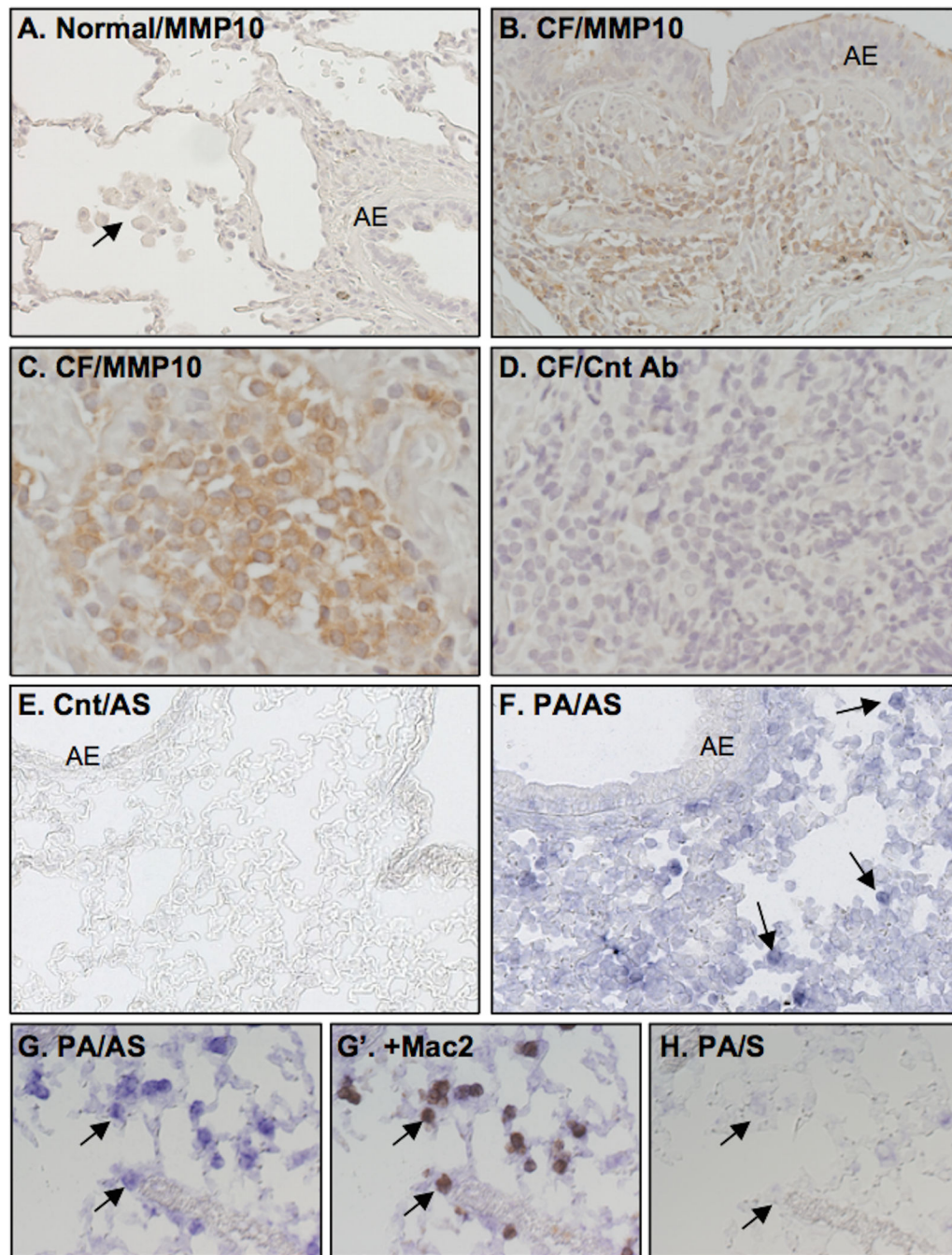
Figure 1. MMP10 is expressed in the lungs of patients with cystic fibrosis (CF) and frzom mice infected with *P. aeruginosa*

(A–C) Sections of normal or CF human lungs were stained for MMP10 protein. (A) MMP10 was not detected in alveolar macrophages (arrow), airway epithelium (AE), or elsewhere in normal human lung. (B) MMP10 protein is expressed strongly in mononuclear cells; weak signal was seen in some AE. (C) Higher magnification shows a nest of mononuclear cells with strong MMP10 expression. (D) No specific signal was seen in CF sections processed with pre-immune serum (Cnt Ab). (E–H) Wildtype mice were infected with *P. aeruginosa* (PA) by oropharyngeal aspiration and controls (Cnt) received an equal volume of saline.

Lungs were harvested 72 h later, and sections were probed for *Mmp10* mRNA by hybridization with digoxigenin anti-sense (AS) or sense (S) RNA probes. Images were collected, and sections were stained with Mac-2 antibody. (E) No signal for *Mmp10* mRNA was seen in lung sections from control mice. (F) Signal for *Mmp10* mRNA was detected in mononuclear cells (arrows) in infected lungs, whereas weak signal was observed in AE cells. Higher magnification images show *Mmp10* RNA expression before (G) and after co-staining with Mac-2 antibody (G'). (H) The section serial to that in G was hybridized with sense RNA and showed no signal.
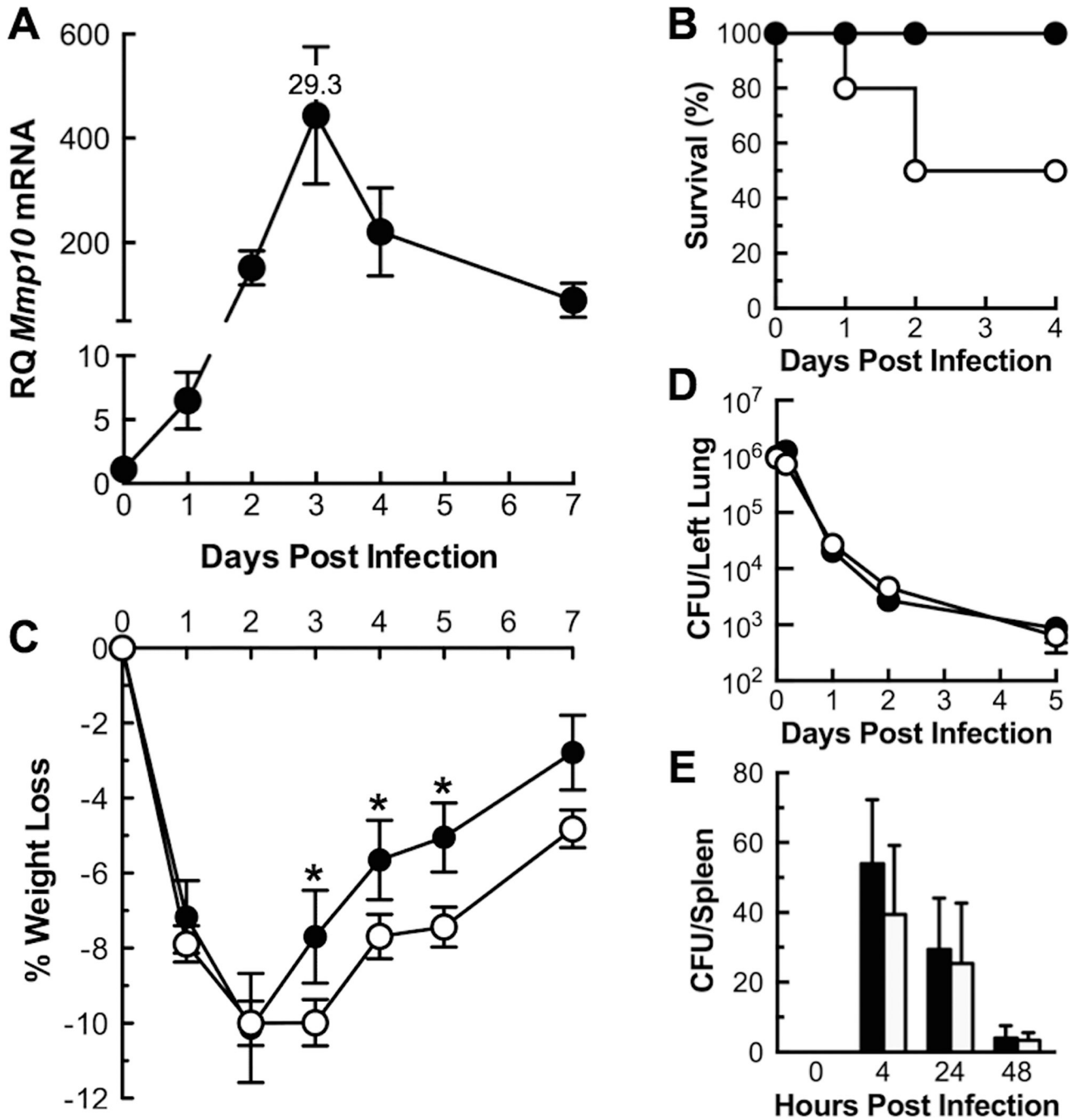
Figure 2. MMP10 is induced in *P. aeruginosa*-infected mouse lung and protects against the lethal effects of lung infection

Wildtype and *Mmp10*^{−/−} mice were infected with *P. aeruginosa* by oropharyngeal aspiration (A,C), or by aerosolization (B, D, E). (A) Wildtype mice were sacrificed at 0 (uninfected), 1, 2, 3, 4, and 7 d post-infection ($n = 5$ mice/time point). RNA was isolated and processed for RT-PCR for *Mmp10* mRNA. Data are presented as relative quantification (RQ). The number above the 3-d time point is the average Ct value for those samples. For the 0-d samples, the Ct > 35. (B) Survival was significantly reduced in *Mmp10*^{−/−} post infection ($n = 11$ wildtype, 10 *Mmp10*^{−/−} mice; Kaplan-Meier log rank test, $X^2 = 5.775$, $p = 0.0163$). (C)

Surviving *Mmp10*$^{-/-}$ (n = 15) mice had delayed recovery of body weight post-infection compared to wildtype mice (n = 13). *p 0.0288 by 2-way paired ANOVA between genotypes. (D) Clearance of bacteria from lungs did not differ between genotypes. (E) Septicemia as measured by live bacteria counts in spleen also did not differ between genotypes.
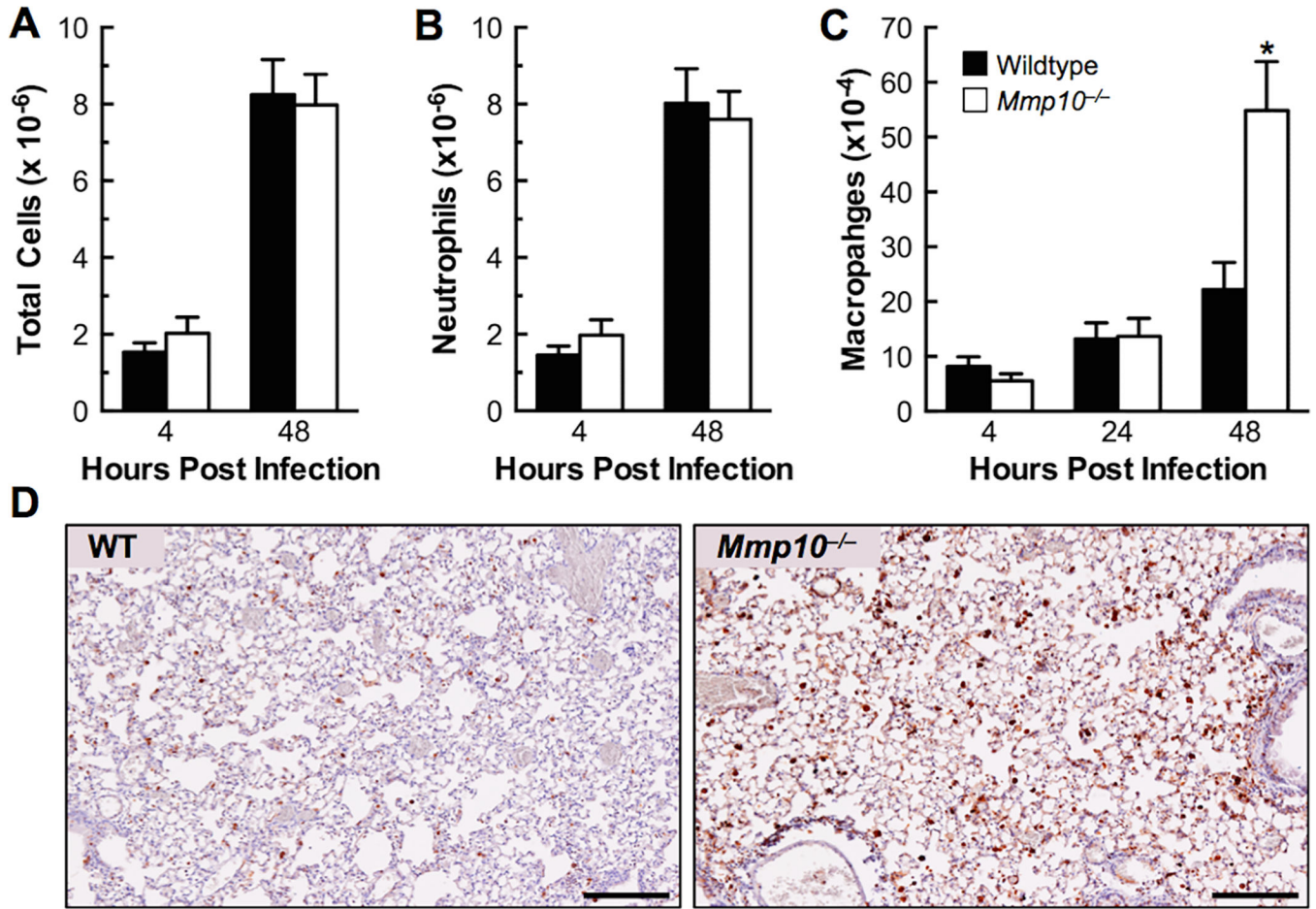
Figure 3. Increased macrophage influx in *Mmp10*^{−/−} lungs

Mmp10$^{-/-}$ and wildtype mice ($n = 6$–8/genotype/time point) were infected with *P. aeruginosa*, and BAL and lung tissue were collected 4 and 48 h later. In BAL samples, total cell (A) and neutrophil (B) counts did not differ between genotypes. (C) Macrophage numbers in BAL did not differ at 4 h post-infection, but at 48 h, about 3-fold more macrophages were detected in *Mmp10*$^{-/-}$ BAL compared to wildtype. *$p = 0.0045$ by unpaired t-test. (D) Sections of wildtype (WT) and *Mmp10*$^{-/-}$ lungs collected at 48 h post-infection were stained for macrophages with Mac-2 antibody.
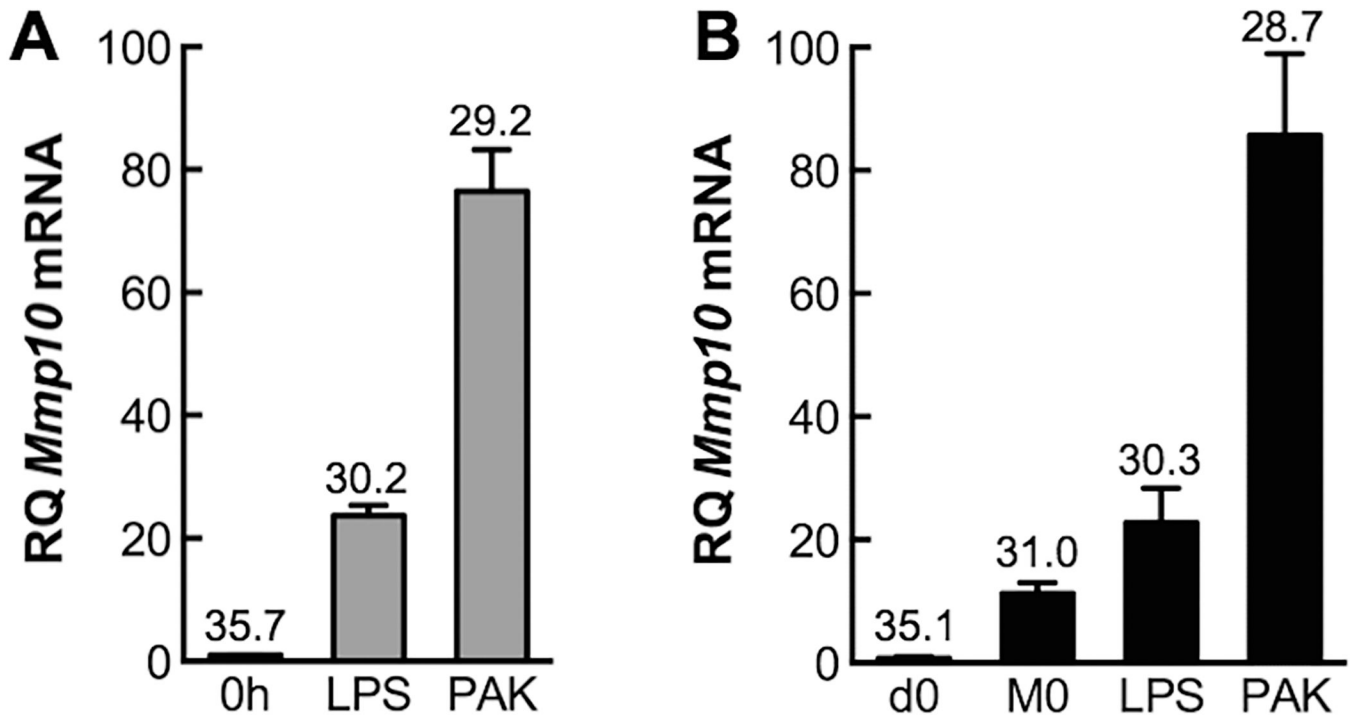
Figure 4. MMP10 is expressed by both resident and infiltrated macrophages
(A) Alveolar macrophages were isolated from BAL of naïve wildtype mice, cultured for 24 h, and exposed to 100 ng/ml LPS for 24 h or to live *P. aeruginosa* (Pa) at an MOI 5:1 for 1 h then washed and refed as described under Methods. RNA was collected 24 h later, and *Mmp10* expression was determined by RT-PCR. The numbers above the bars are the average Ct values. (*n* = cells from 4 mice/condition) (B) RNA was isolated from fresh wildtype bone marrow (d0), from cells following differentiation to macrophages in culture for 7 d (M0), and from M0 macrophages exposure to LPS or *P. aeruginosa*. (*n* = marrow from 3 mice/condition)
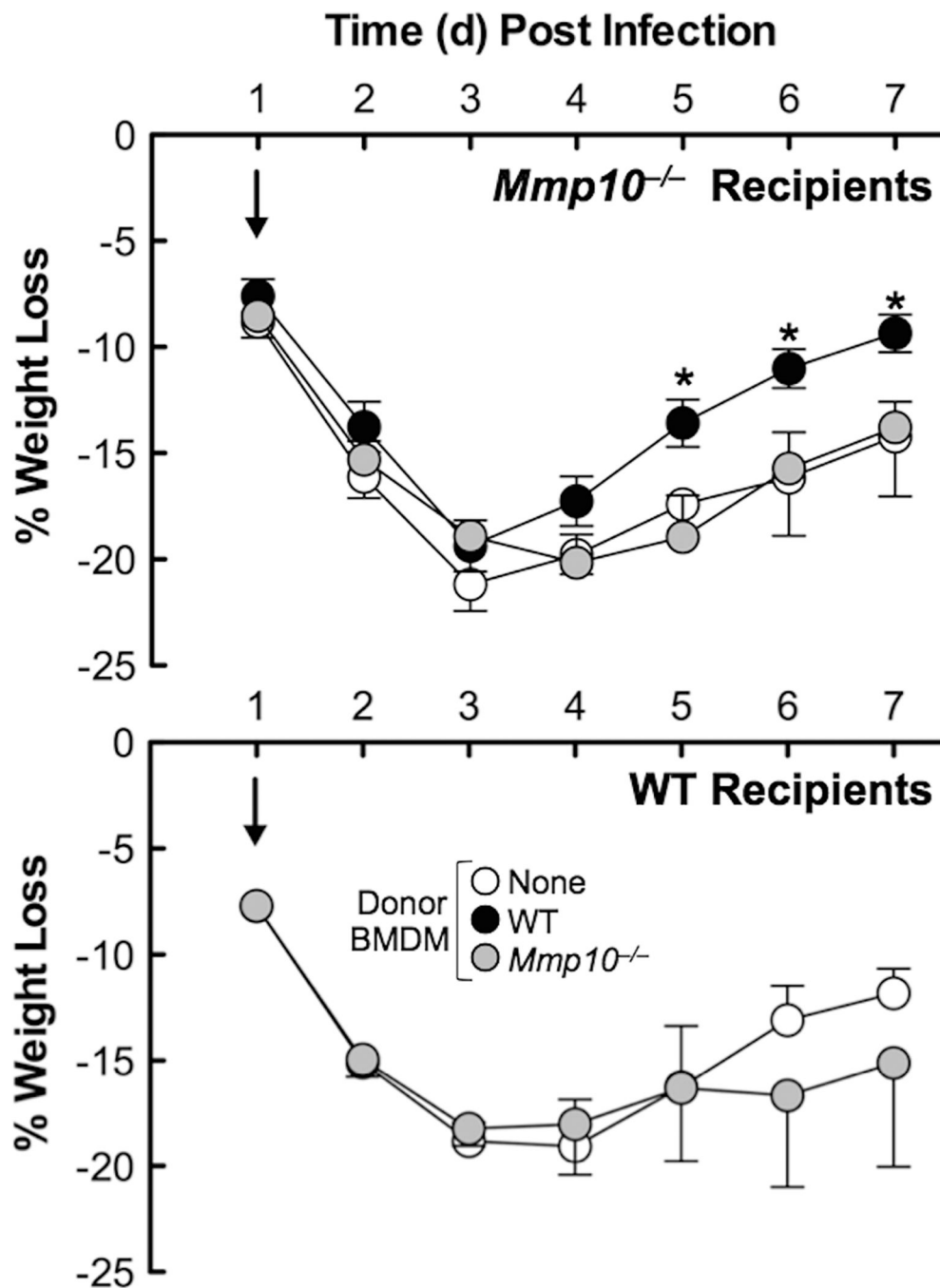
Figure 5. Adoptive transfer of wildtype macrophages improves body weight recovery
Wildtype and *Mmp10*^−/−^ mice ($n = 6$–7 mice/group) were exposed to *P. aeruginosa* by oropharyngeal aspiration and 24 h later were injected retro-orbitally with vehicle (100 μl saline) or with 2×10^6 wildtype or *Mmp10*^−/−^ BMDM. Mice were weighed and monitored daily for 7 d post infection. *p ≤ 0.03 between genotypes by unpaired t-test.
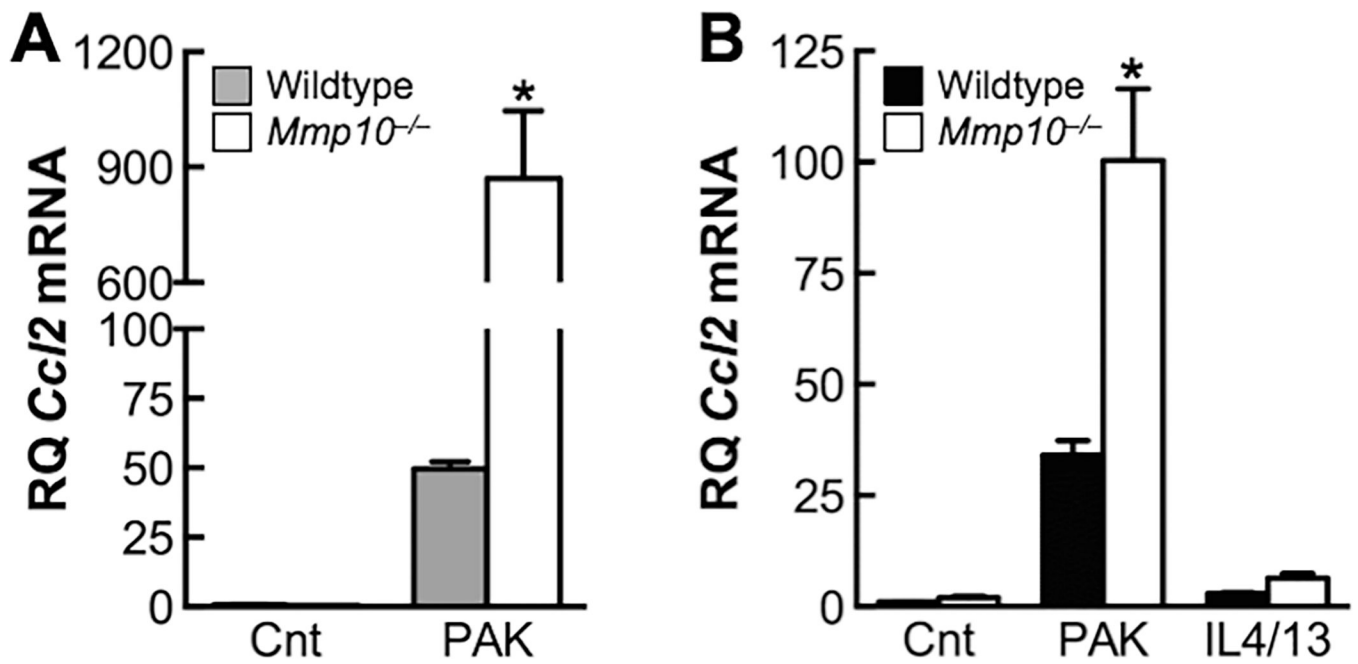
Figure 6. Elevated expression of CCL2 by *Mmp10$^{-/-}$* macrophages

(A) Alveolar macrophages (n = cells from 4 mice/genotype) and (B) BMDM (n = marrow from 8 mice/genotype) from naïve wildtype and *Mmp10$^{-/-}$* mice were infected with *P. aeruginosa*, and expression of CCL2 was assessed by RT-PCR. *$p < 0.05$ between genotypes by unpaired t-test.

A. 6 h post Pa

WT   *Mmp10⁻/⁻*

2386   **10,286**   4878

D. **Enriched Pathway**

Regulation of response to stimulus
Regulation of response to stress
Regulation of signal transduction
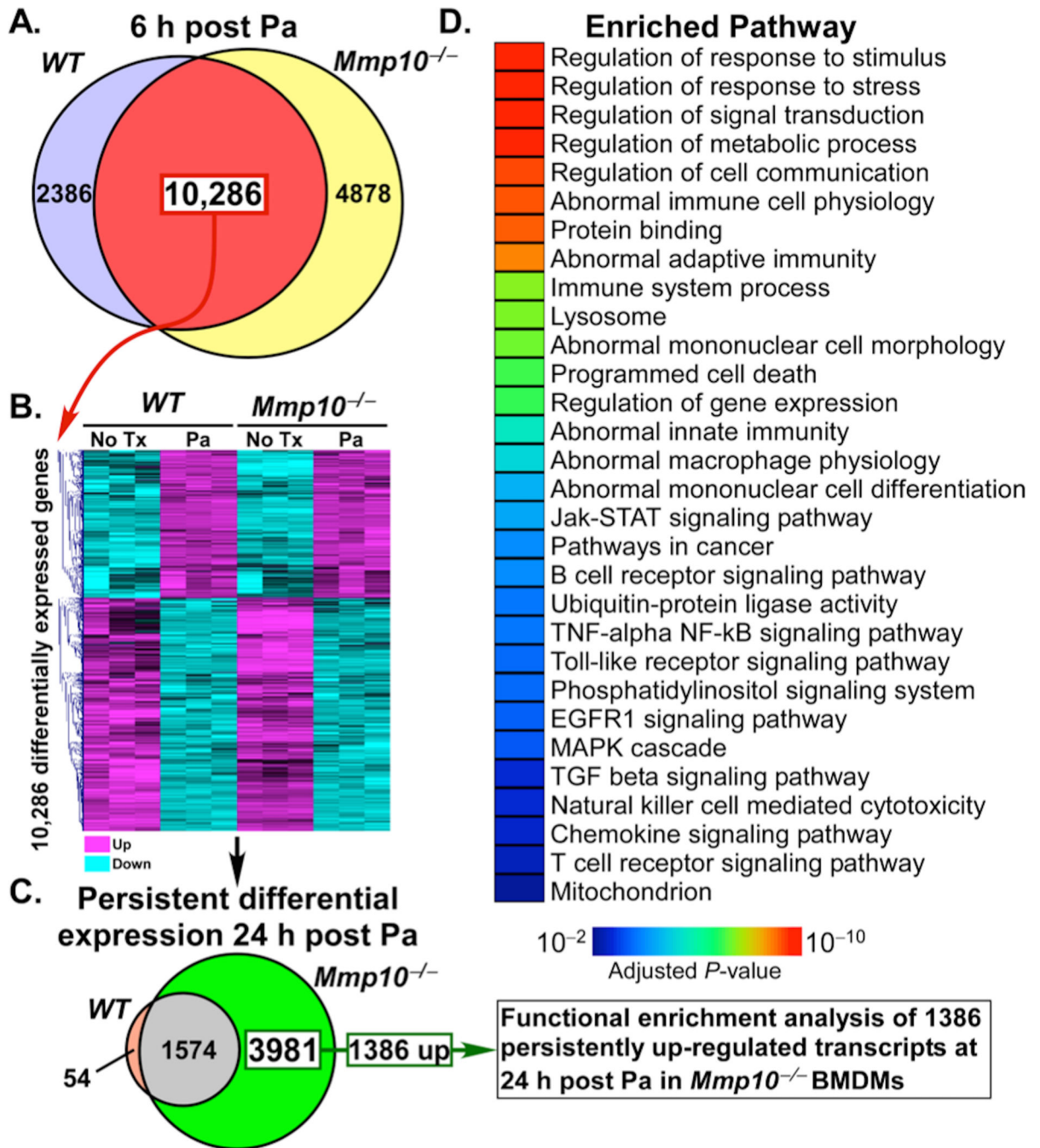Regulation of metabolic process
Regulation of cell communication
Abnormal immune cell physiology
Protein binding
Abnormal adaptive immunity
Immune system process
Lysosome
Abnormal mononuclear cell morphology
Programmed cell death
Regulation of gene expression
Abnormal innate immunity
Abnormal macrophage physiology
Abnormal mononuclear cell differentiation
Jak-STAT signaling pathway
Pathways in cancer
B cell receptor signaling pathway
Ubiquitin-protein ligase activity
TNF-alpha NF-kB signaling pathway
Toll-like receptor signaling pathway
Phosphatidylinositol signaling system
EGFR1 signaling pathway
MAPK cascade
TGF beta signaling pathway
Natural killer cell mediated cytotoxicity
Chemokine signaling pathway
T cell receptor signaling pathway
Mitochondrion

B. *WT*   *Mmp10⁻/⁻*

No Tx   Pa   No Tx   Pa

10,286 differentially expressed genes

■ Up
■ Down

10^{-2} ■■■■■■■■ 10^{-10}
Adjusted *P*-value

C. **Persistent differential expression 24 h post Pa**

Mmp10⁻/⁻

WT

54   1574   **3981** → **1386 up** →

Functional enrichment analysis of 1386 persistently up-regulated transcripts at 24 h post Pa in *Mmp10⁻/⁻* BMDMs

Figure 7. Global gene expression analysis of wildtype and *Mmp10⁻/⁻* BMDM in response to *P. aeruginosa* exposure

BMDM from wildtype and *Mmp10⁻/⁻* mice were exposed to *P. aeruginosa* for 1 h at MOI 5:1. Infection medium was removed, and cells were cultured in medium containing antibiotics until cells were harvested for RNA collection 6 or 24 h later. (A) Bacteria exposure induced significant transcriptional changes in both wildtype and *Mmp10⁻/⁻* BMDM 6 h post challenge. (B) Heatmap of the genes commonly differentially expressed in both wildtype and *Mmp10⁻/⁻* BMDM shows similar patterns of up- and down-regulation 6 h post-infection. (C) Of the genes that were commonly differentially expressed at 6 h, many

genes remained significantly changed at 24 h post infection exclusively in *Mmp10*$^{-/-}$ BMDM. (D) Functional analysis of genes exclusively up-regulated in *Mmp10*$^{-/-}$ at 24 h reveals significant enrichment in immune and inflammatory signaling pathways.

Table I

Summary of *In Vivo* Phenotypes

Process/Factors		Response in *Mmp10*$^{-/-}$ Mice Relative to Wildtypes
Neutrophil Influx		No
Bacterial Clearance		No
Lethality, Morbidity		↑
Macrophage Influx		↑
M1 Markers	iNOS, IFNγ, CCL2[1]	↑
	IL-12a	↓
	CCL3[1], IL-1β[2], TNFα[2]	No
M2 Markers	IL-10, Arg1	↓
	Retnla	No

[1] Not necessarily a consensus M1 marker.

[2] Assessed by protein levels in BAL (Fig. 2S)

No : no significance difference between genotypes.

Table II

Relative Expression of Macrophage Activation Markers

Pathway	Factor	Response in *Mmp10*^{−/−} Macrophages[1]		
		M0[2]	M1 Activated[3]	M2 Activated[4]
M1 Markers or Function	IFNγ	↑	↑	↑
	iNOS, CCL5	nd	↑	nd
	CCL2	No or nd	↑	No
	Phagocytosis		↑	
	IL-1β, IL-12a	No or nd	↓	No
	IL-6, TNFα	No	No	No
M2 Markers	Arg1, CD206, IL-10	No	↓	No
	CCR2, Retnla	No	No	No

[1] Relative to expression levels in wildtype BMDM. Arrow indicates about a 2-fold or greater difference between genotypes ($P < 0.01$).
No : no difference between genotypes.
nd: not detected in either genotype.

[2] BMDM 7 d in culture after marrow harvest.

[3] BMDM were exposed to live *P. aeruginosa* for 1 h, and RNA was isolated 24 h later.

[4] BMDM were treated with IL-4 and IL-13 for 24 h.