Alveolar macrophage-expressed Plet1 is a driver of lung epithelial repair after viral pneumonia

Introduction

Tissue-resident alveolar macrophages (TR-AM) are long-lived cells (1) localized to the alveolar space, where they exert key functions in maintaining tissue homeostasis and in immediate host defense. Several studies revealed that TR-AM are depleted upon viral pneumonia and gradually replaced by BMDM (CD11c+CD11b+Ly6C+CX3CR1+) during the infection course, eventually resulting in reprogramming of the TRAM pool (1–4). BMDM are recruited to sites of infection via CCR2 ligation (5). and are considered to reveal high functional plasticity. Polarization of BMDM into different macrophage phenotypes in the infected lung is thought to be driven by integration of spatially and timely resolved signals from the inflamed microenvironment or tissue niche in a

disease-specific manner (6,7). Whereas pro-inflammatory macrophages were found to contribute to the severity of viral pneumonia by directly damaging the lung parenchyma, and to aberrant lung remodeling (8–11). However, the role of distinct macrophage subsets in lung repair, and in particular their effector molecules mediating cross-talk with lung epithelial (stem/progenitor) cells during such processes are poorly understood.

Placenta-expressed transcript 1 (Plet1) is a 207 amino acid glycophosphatidylinositol (GPI)-anchored protein with unknown receptor, expressed in proliferating epithelia and in stem cell niches, such as differentiating trophoblasts (15,16), follicular and thymic stem cells (17–19). It is involved in keratinocyte migration (20) and gut epithelial wound healing from the Lgr5+ colon stem cell niche (21). We and others recently identified its expression in myeloid cells and its role in dendritic cell migration and in the instruction of innate lymphoid cells to release IL- 22 (22–24) (data accessible at NCBI GEO database, GEO accession GDS1874). However, its expression pattern and function in the context of lung injury and regeneration have been unknown.

This study first time revealed that, during the resolution phase of viral pneumonia a transitional subpopulation of BMDM and the re-emerging TRAM pool constitute key components of epithelial stem/progenitor cell niches and contribute to lung regeneration after injury through

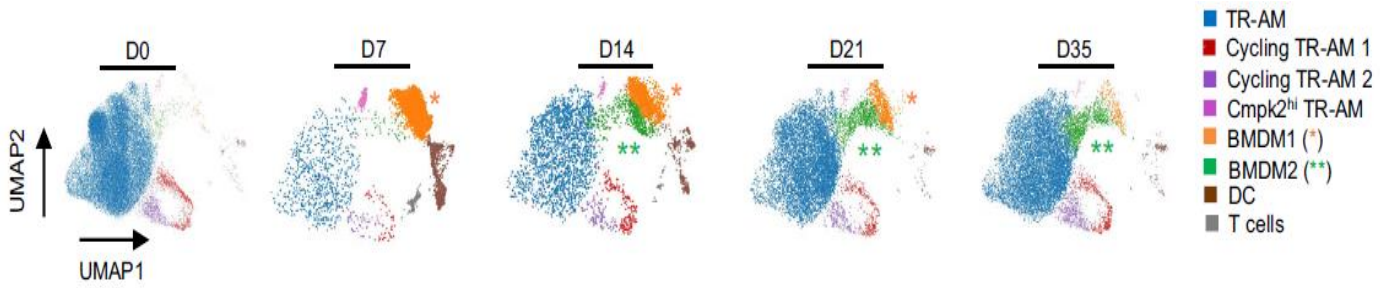
the expression of Plet1. Bone marrow chimeric mice and single-cell transcriptome data, confirmed the spatiotemporal kinetics of the BMDM-to-TR-AM transition, and captured that this trajectory is associated with distinct functional phenotypes from inflammatory towards tissue-regenerative, characterized by gradual increase of Plet1 expression. Adoptive transfer of Plet1+ macrophage populations sampled along this differentiation trajectory, into Ccr2-/- mice, combined with conditional CX3CR1$^{iCRE\text{-}Plet1flox/tom}$ transgenic mouse and lung organoid assays modeling the macrophage-epithelial niche during alveologenesis, revealed that Plet1+ macrophages efficiently attenuate influenza A virus (IAV)-induced epithelial injury and foster stem/progenitor cell-driven alveolarization and barrier repair. Finally, intraalveolar administration of recombinant Plet1 rescued mice from fatal viral pneumonia and the negatively corelated Plet1 expression in co-horts of virus induced acute respiratory distress syndrome (ARDS) patients highlighted its potential as a therapeutic to combat severe inflammatory lung injury in viral pneumonia.

Key Results

1. Alveolar macrophages cluster into TR-AM and CD40^{high} inflammatory versus CD206^{high} transitional BMDM subsets

In an effort to correlate transcriptional heterogeneity of BMDM with unique functional phenotypes in the course of IAV infection, we aimed to establish a robust protocol to flow-sort BMDM subsets for further analyses, using distinct surface markers for identification. Out of several surface antigens differentially expressed in BMDM 1 and 2 according to the single cell transcriptome dataset, we identified CD40 (Cd40) and CD206 (Mrc1) to be most suitable to identify BMDM 1 and 2 by flow-cytometry, respectively. BMDM sub-phenotyping from BALF of IAV-infected mice according to CD40 and CD206 reproduced the BMDM1/2 kinetics identified in the scRNA-Seq dataset, with a peak accumulation of CD40high BMDM 1 at d7, and alveolar presence of CD206high BMDM 2 by d14 to d21. In addition the transcriptome analysis on sorted BMDM 1 and 2 subsets of alveolar macrophages showed a contrast defrentially expressed genes. These data also demonstarted the depletion of TR-AM after IAV infection and has been replinished by BMDM 2 in later phase D21 and D35pi.

a.



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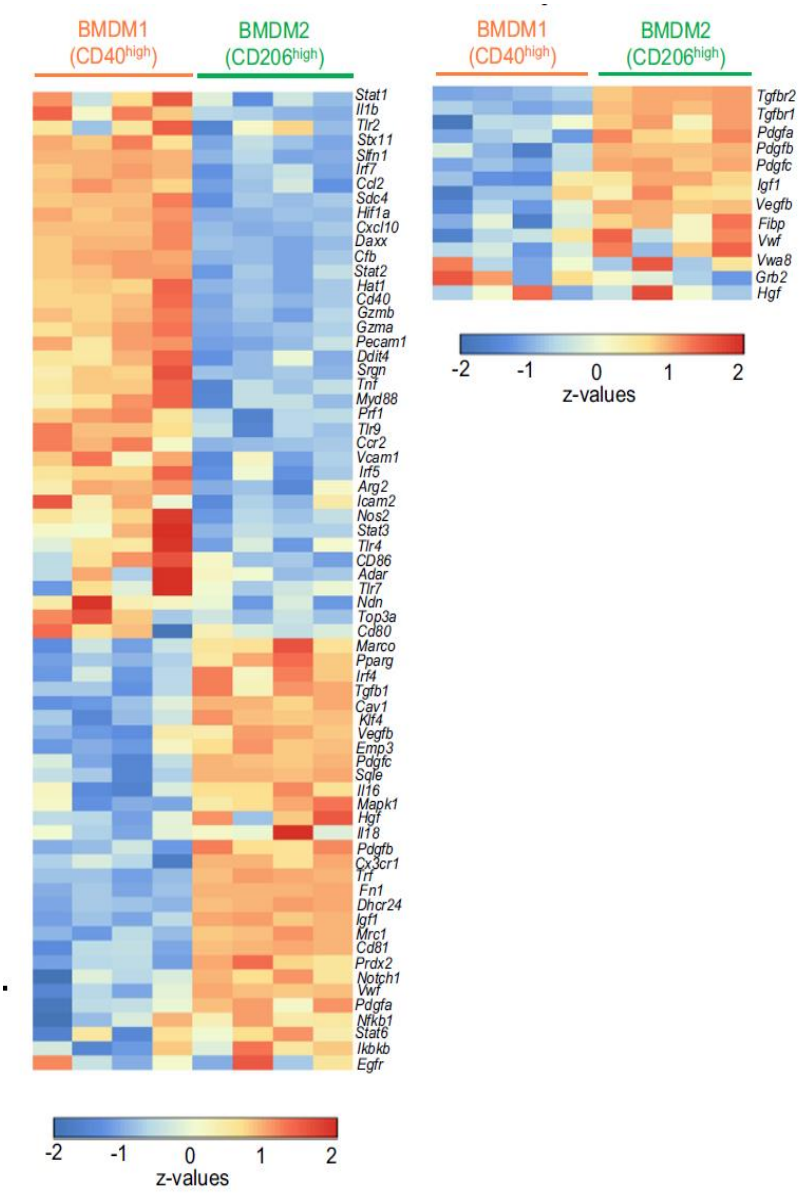
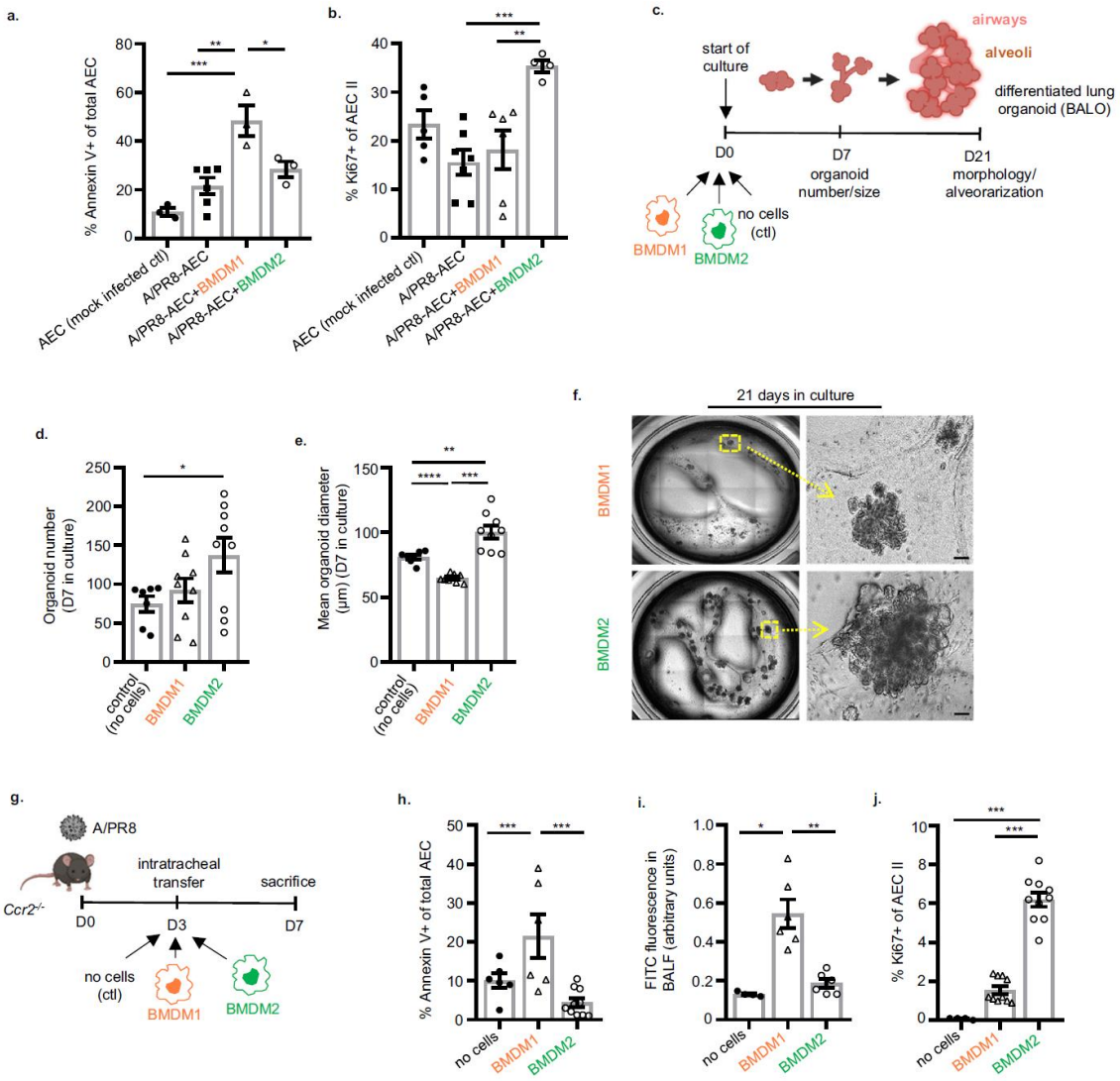


In line with these data, the data from bone marrow transplantation experiements with CD45.1/2 chimeric mice showed that the depleted TR-AM of recipient (CD45.1) phenotype were

gradually replenished between D14 and D21 by CD45.2+ BM-derived precursors, confirming that BMDM are able to replenish the TR-AM pool in this model.

2. BMDM2 in contrast to BMDM1 exert proliferative and barrierprotective effects on alveolar epithelial cells in vitro and in vivo.

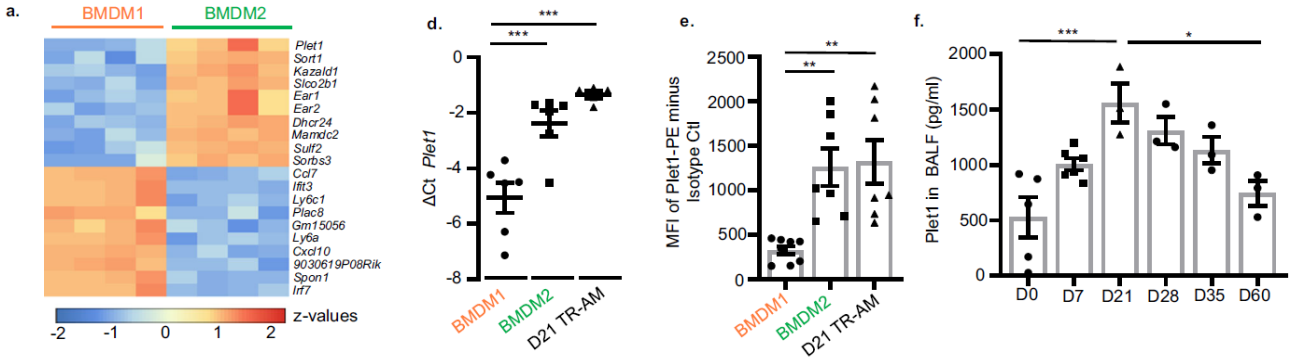
To investigate whether BMDM transition towards BMDM2 endowed the latter with an epithelial-regenerative phenotype, as suggested by the transcriptome analysis, flow-sorted BALF BMDM subsets were cocultured with ex vivo IAV-infected murine alveolar epithelial cells (AEC). While BMDM1 (collected at d7 p.i.) increased IAV-induced AEC apoptosis, BMDM2 (collected at d21 p.i.) induced AEC proliferation after infection (Fig. 3a, b). To confirm the latter, we used bronchoalveolar lung organoid (BALO) cultures that reveal proximo-distal patterning with branched airways and alveoli allowing modeling of lung development and epithelial cell differentiation, and profiling of macrophage-epithelial cell interactions. BMDM2 but not BMDM1 supported the generation of BALO (in terms of numbers and size), suggesting that the presence of BMDM2 promoted epithelial stem cell proliferation and organoid differentiation. To confirm the robustness of the BMDM2 phenotype outside of its local (inflammation-resolving) microenvironment, we developed a short-term infection model and applied in vivo-generated BMDM2 (vs. BMDM1) by intrapulmonary transfer into IAV-infected Ccr2-/- mice) lacking BM-monocyte mobilization and BMDMs in inflamed tissues, in an effort to prevent severe injury peaking at D7. BMDM2 were far less injury-promoting than BMDM1 (as quantified by AEC apoptosis), and induced proliferation of CD45/31^{neg}EpCamlowT1-α^{neg} alveolar epithelial progenitor cells (AEC II)37, resulting in improved barrier function at D7 p.i..Notably, the tissue-protective features of BMDM2 were conserved in the “experienced” TR-AM (i.e., partially BMDM-replenished) in this model, as opposed to naive, fetal monocyte-derived TR-AM that were sampled before depletion by the infection. Together, these data confirm the alveolar epithelial-regenerative function of transitional BMDM2 (and of experienced TR-AM) implied by their transcriptomic profile in ex vivo and in vivo models, and confirm functional heterogeneity of macrophage subsets identified by scRNA-seq.

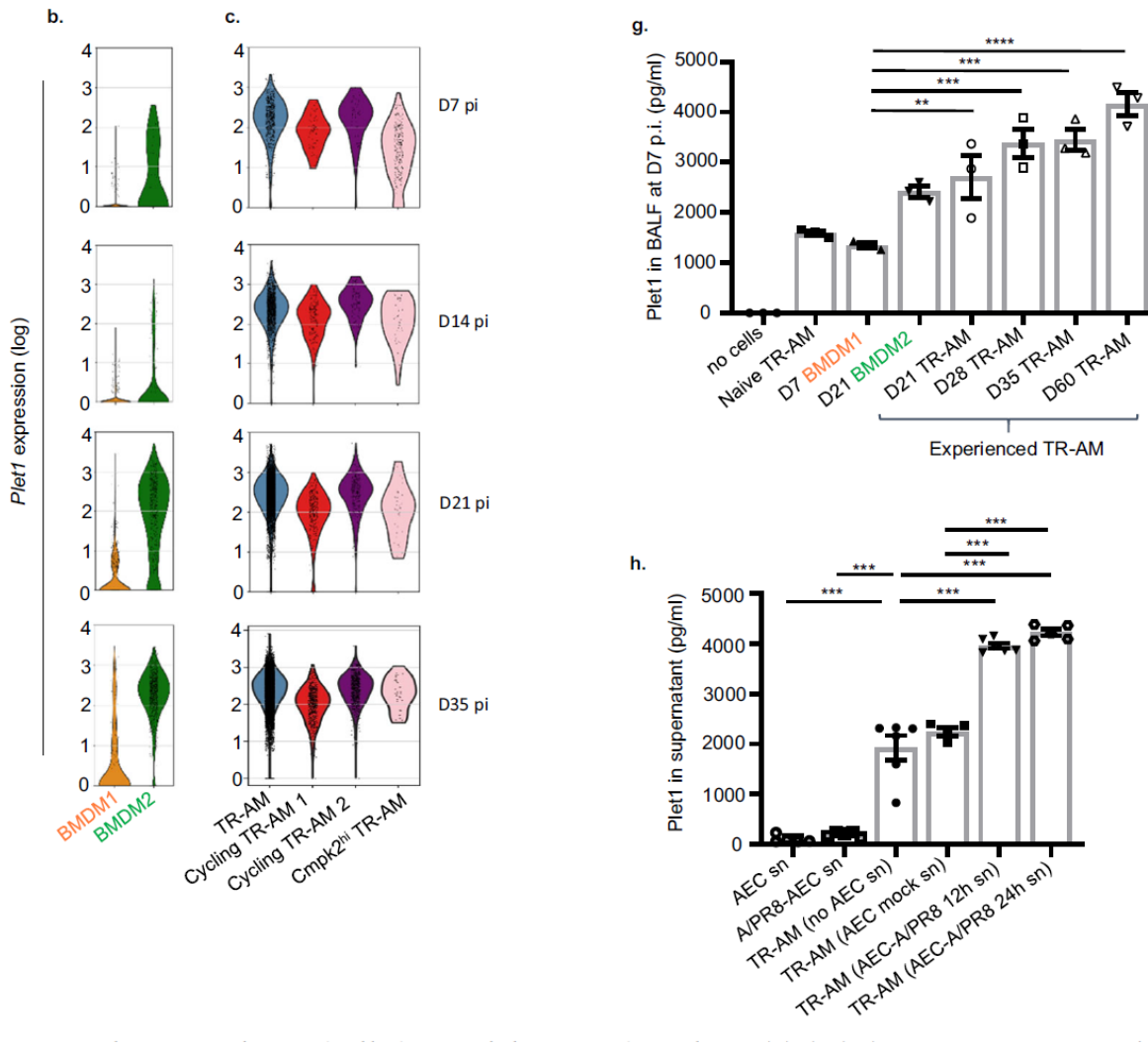


3. Tissue-regenerative BMDM 2 macrophage clusters are characterized by the expression of Plet1

To define putative key regulators of epithelial-regenerative macrophage functions, we compared the top 10 DEGs in the transcriptome data of flow-sorted BMDM subsets. We identified Plet1 (Placentaexpressed transcript 1), a gene that has been previously associated with wound healing in skin and gut (20,21) and encoding a protein with a GPI (glycosylphosphatidylinositol) anchor localized to cell membranes (20), as the top gene expressed in BMDM2 versus BMDM1. In line with the BMDM2 transitional state towards TR-AM differentiation, Plet1 has been reported to be a marker gene of TRAM identity (34,38). Correspondingly, we found Plet1 highly expressed in the BMDM2 and TR-AM clusters in the scRNA-seq dataset across all time points, whereas BMDM1 showed low expression. Plet1

expression was confirmed by qPCR and corresponding cell surface expression was quantified by FACS in D7 BMDM1, D21 BMDM2 and in D21, experienced, TR-AM. As a GPI anchored membrane protein, Plet1 can be shed from the cell surface. Quantification of soluble Plet1 in BALF of IAV-infected WTmice revealed a significant increase starting in the injury phase and peaking at D21 throughout the resolution phase until D35, declining at D60.We next quantified Plet1 in BALF of Ccr2-/-mice at D7 p.i. after adoptive transfer of naive TR-AM, BMDM1, BMDM2 or experienced TR-AMs. In this model where macrophages are exposed to acutely infected/injured lung tissue (from D3 to D7 p.i.), we observed that BMDM2 and experienced TR-AMs are major sources of soluble Plet1 in BALF. These data suggest that Plet1 surface expression on, or release from, regenerative macrophages was induced by an inflamed or injured alveolar microenvironment. To identify whether a soluble mediator released from the injured AEC during infection was involved, Plet1+ macrophages were stimulated with IAV-infected versus non-infected AEC conditioned medium. In fact, whereas no Plet1 could be detected in supernatants of infected and non-infected AEC in the absence of macrophages, conditioned medium of 12 or 24 h infected AEC but not of non-infected AEC significantly increased Plet1 release by TR-AM compared to baseline, indicating that Plet1 surface expression and/or secretion/ shedding are significantly increased by AEC signals induced by IAV infection.



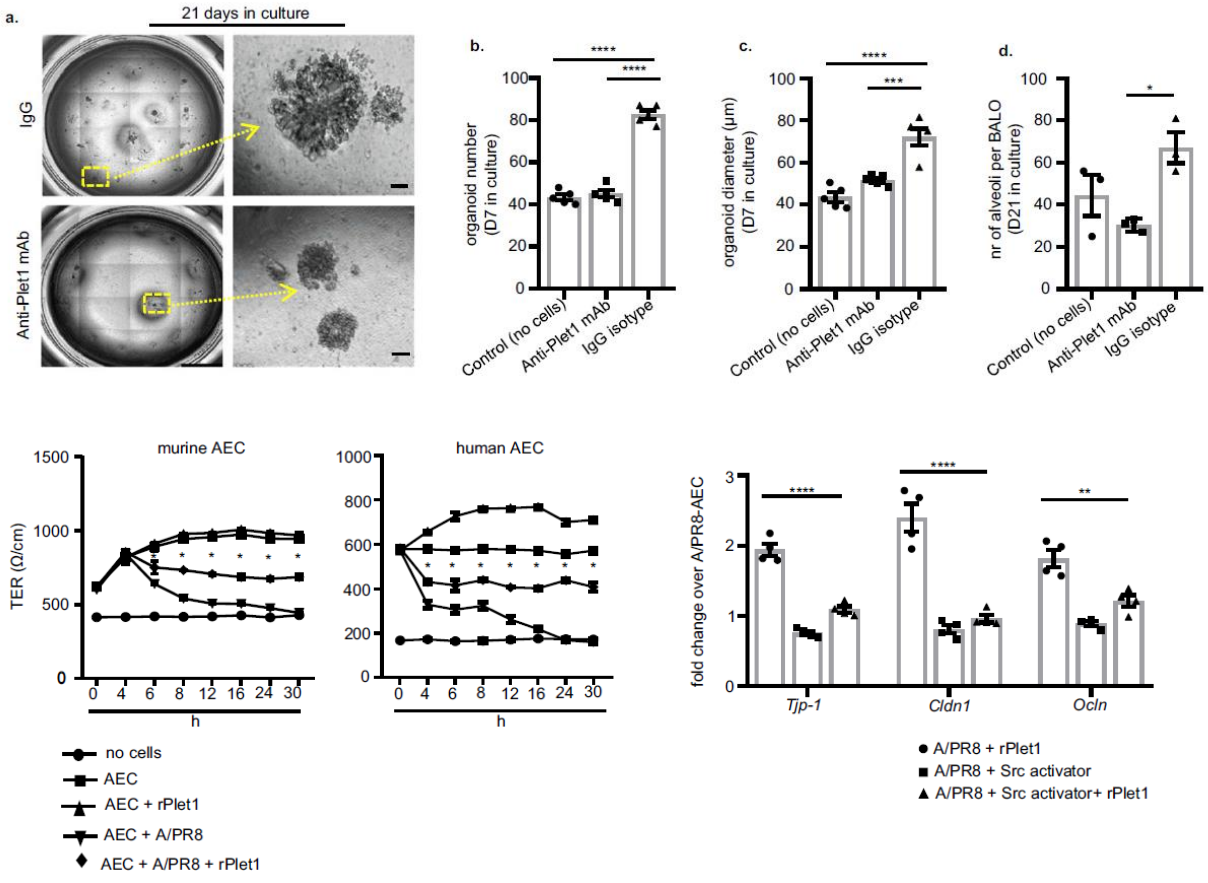


4. Soluble Plet1 drives AEC expansion and promotes alveolar epithelial barrier function in ex vivo models

To corroborate our hypothesis that BMDM2-expressed Plet1 supported AEC (re-)generation, we co-cultured BALO with BMDM2 in the presence of a neutralizing anti-Plet mAb or isotype control (ctl), or without BMDM. Both organoid numbers and size were significantly increased in the presence of Plet1^{high}BMDM2 (ctl), and this effect was abolished with anti-Plet1mAb at D7 of culture and correspondingly, the alveoli number in fully developed BALO was reduced.

Given the beneficial effects of Plet1^{high} BMDM2 compared to BMDM1 on alveolar barrier function in vivo, we next addressed further putative mechanisms of rPlet1-mediated improvement of AEC barrier function in the context of IAV injury and analyzed tight junction gene expression. IAV infection of ex vivo culturedmurine AEC reduced the mRNA expression of

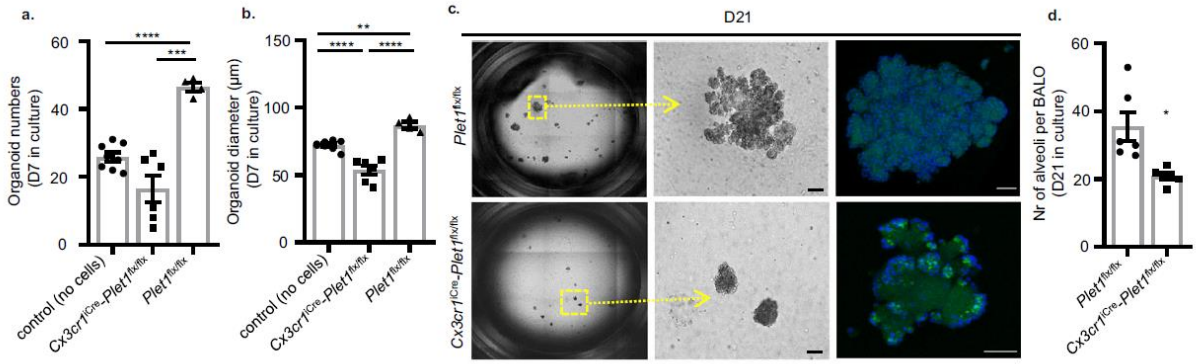
Tjp-1 (encoding zonula occludens-1, ZO-1), but not of tight junction genes Cldn1 or Ocln (encoding claudin-1 and occludin). Treatment with rPlet1 increased expression of all three genes compared to non-treated controls, and increased protein levels of ZO-1 as revealed by immunofluorescence. Correspondingly, transepithelial resistance of primary murine and human AEC monolayers was significantly impaired by IAV infection, and this was rescued at least in part by rPlet1 treatment. These data indicate that, BMDM2-expressed Plet1 drives AEC proliferation, resulting in alveolarization in lung organoids, and that Plet1 restores alveolar epithelial barrier function after infectious challenge ex vivo. Given that the putative receptor of Plet1 and its downstream signaling events are unknown, our data suggest that a Raf-MEK-ERK signaling pathway might be activated in response to rPlet1 stimulation in AEC II, confirmed by the phosphorylation of c-Raf and ERK1/2 in response to rPlet1 stimulation in AEC and an increase in AEC proliferation in absence, but not in presence of the MEK inhibitor U0126.

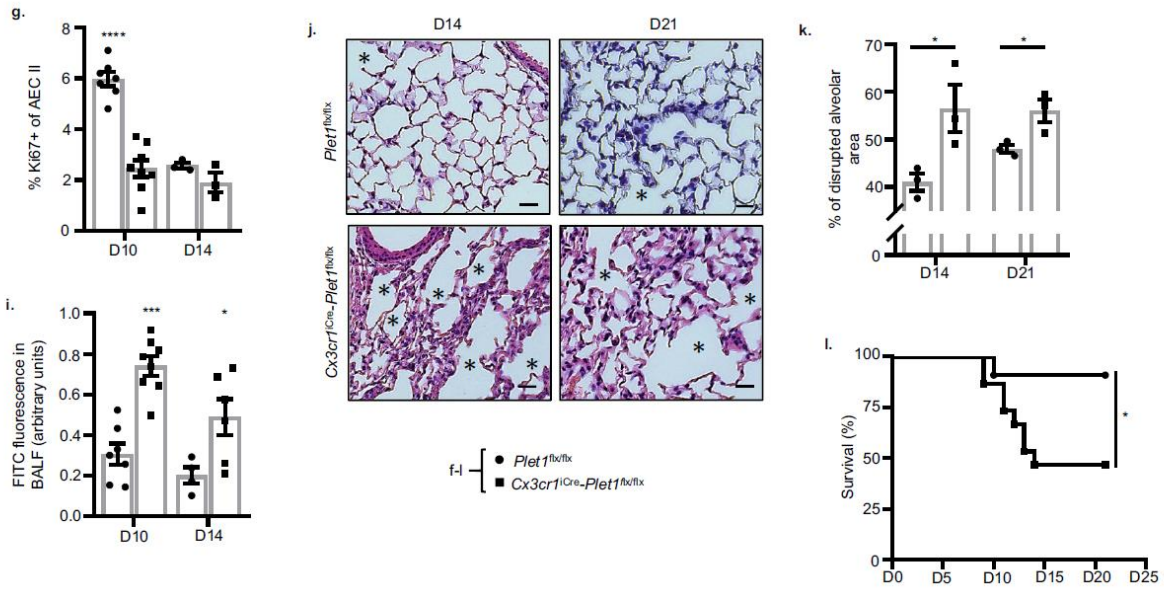


5. BMDM2-expressed Plet1 drives epithelial progenitor (AEC II) cell expansion and improves lung barrier function in IAV-induced lung injury in vivo

We next confirmed that Plet1 is a crucial mediator of the macrophage epithelial cell protective phenotype in vivo, focusing on BMDM2 as the key transitional cell population upregulating Plet1 during the course of IAV infection. We applied the BMDM2 → Ccr2-/- mice intrapulmonary transfer model using BMDM2 from Plet1-/- mice or BMDM2 pre-incubated with anti-Plet1 mAb, and analyzed AEC parameters at D7 where we observed the peak of alveolar injury. Plet1-/- mice were obtained by generation of Plet1tdtomato-flox/flox (termed Plet1flox/flox) crossbred with Cre-deleter mice, with loss of Plet1 mRNA and concomitant loss of tdTomato reporter confirmed in BMDM2 of IAV infected Plet1-/- mice compared to Cre-negative Plet1flox/flox mice. Plet1 neutralization or knockout in BMDM2 reduced the beneficial effects of BMDM2, resulting in increased AEC apoptosis, reduced expression of tight junction genes in epithelial cells, and alveolar barrier dysfunction, as well as reduced proliferation of AEC II, as compared to controls. Finally, Ccr2-/- mice receiving Plet1-deficient or Plet1-neutralized BMDM2 revealed persistent tissue inflammation compared to controls at D7. We next generated a conditional (Tamoxifen (Txf)-dependent) BMDM-specific Plet1-knockout line by breeding Plet1flox/flox mice to Cx3cr1tm(cre/ERT2) Jung mice (Cx3cr1iCre-Plet1flx/flx). Circulating classical monocytes express high levels of Cx3cr1, while TR-AM do not.Of note, ifCx3cr1+monocytes give rise to BMDMand then to TR-AM, these will permanently remain Plet1-deficient and tdTomato^{neg} after Cre recombination by Txf administration, even after the expression of endogenous Cx3cr1 has ceased during differentiation into TRAM, allowing genetic targeting of BMDMand BMDM-derived TR-AM. In line, Cx3cr1neg (not BMDM-replenished, fetal monocyte-derived) TRAM of Txf-treated Cx3cr1iCre-Plet1flx/flx mice revealed similar levels of Plet1 mRNA and tdTomato expression as those of Txf-treated Plet1flx/flx control mice, whereas BMDM2 (analyzed at D21 p.i.) showed significantly reduced Plet1 mRNA and tdTomato expression; tdTomato-MFI of C57BL/6 WT TR-AM and D21 BMDM2 depicted for comparison). We next co-cultured Cx3cr1iCre-Plet1flx/flx versus Plet1flx/flx BMDM2 flow-sorted at D21 from IAV-infected, Txf-treated mice with BALO as described earlier. As expected, loss of Plet1 in BMDM2 resulted in reduced organoid numbers and growth at D7 and D21 of culture, with reduced BALO complexity and limited alveolarization at D21. We next analyzed parameters of persistence of alveolar

injury and induction of alveolar repair in Txf-treated Cx3cr1iCre-Plet1flx/flx versus Plet1flx/flx mice, a process starting around D10 post IAV infection. Lung histopathology reveals that Plet1flx/flx mice had widely (D10) or completely (D14, D21) resolved the injury-associated inflammation, whereas dense inflammatory infiltrates were found in Cx3cr1iCre-Plet1flx/flx mice with partial resolution until D21. Concomitantly, AEC apoptosis was still significantly increased and AEC II proliferation decreased in Cx3cr1iCre-Plet1flx/flx mice at D10 p.i., together with reduced expression of tight junction genes (Tjp-1, Cldn1, Ocln), resulting in higher alveolar barrier leak persisting until D14 p.i . Alveolar repair was incomplete in Cx3cr1iCre-Plet1flx/flx mice with evidence of disrupted alveoli (Fig. 6j, asterisks, Fig. 6k) and increased thickness of septa. Finally, after IAV infection with a virus dose that resulted in low mortality in control mice, Cx3cr1iCre-Plet1flx/flx mice revealed significantly reduced survival between D10 and D14 p.i., coinciding with BMDM2 appearance in the interstitium and airspaces that confer protection in the Plet1flx/flx control mice. Plet1 was not detected in BALF of Txf-treated Cx3cr1iCre-Plet1flx/flx mice compared to Plet1flx/flx control mice at D7 and 21 p.i., indicating that BMDM2 (and BMDM2-derived TR-AM) are a major source of Plet1 in the alveolar compartment. Of note, quantification of cytokines and chemokines, and extent of inflammatory infiltrates revealed that lack of BMDM2-Plet1 delayed the resolution of the inflammatory responses at d10-14, whereas the level of tissue fibrosis was not affected at the indicated time points. These data indicate that Plet1 expressed in BMDM2 (and likely, in BMDM-derived TR-AM arising after D14) is crucial for lung tissue recovery and to survive severe viral infection.

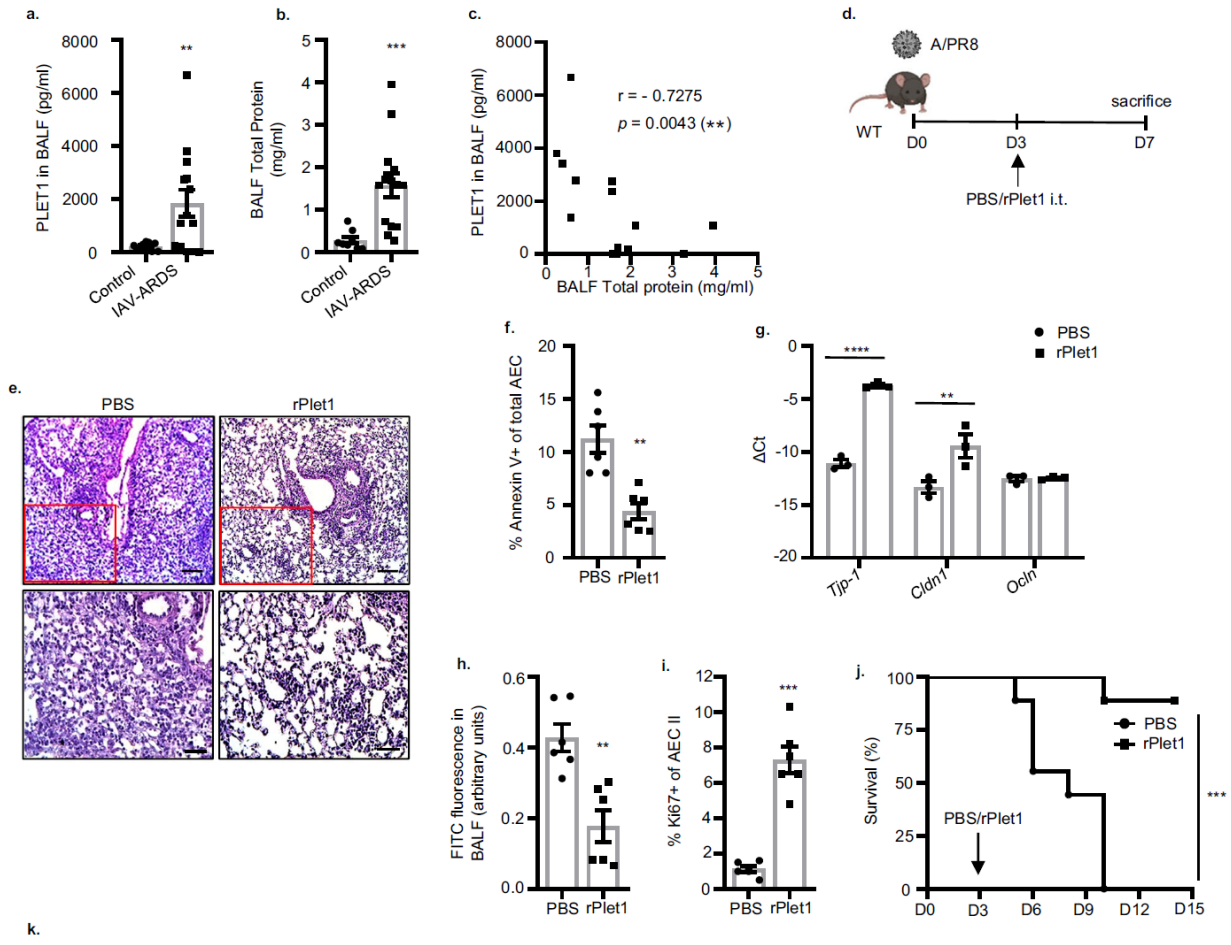




6. Plet1 in expressed in human BALF and Orotracheal administration of rPlet1 rescues mice after lethal IAV infection with therapeutic implications

Treatment of human IAV-infected AEC with rPLET1 resulted in significant improvement of barrier function. To verify the putative relevance of PLET1 in the context of human disease, we quantified PLET1 levels in BALF of patients with IAV-induced ARDS compared to a control group of patients with non-inflammatory/non-infectious lung disease (patient characteristics provided in Supplementary Table), and detected high levels in a subset of IAV-ARDS patients. To correlate PLET1 levels in IAV-ARDS BALF with the level of alveolar epithelial injury, we additionally quantified total BALF protein as a measure of alveolar barrier dysfunction in both cohorts, and PLET1 concentrations negatively correlated with total BALF protein concentrations, indicating that soluble PLET1 was present in BALF of patients with IAV-ARDS and might exert similar barrierprotective functions as observed in mice. We next evaluated the therapeutic potential of alveolar administration of rPlet1 in mice at D3 p.i., using an experimental setup aimed to attenuate the peak of lung injury at D7 to D108 in a severe IAV infection model. rPlet1 treatment reduced the injury-associated tissue inflammation, reduced AEC apoptosis, upregulated expression of tight junction genes Tjp-1 and Cldn1 together with improvement of alveolar barrier function, and induced substantial AEC II proliferation, indicative of a tissueprotective and -regenerative effect. Of note, quantification of cytokines (IL-6, KC, MIP1α,

IL10), extent of inflammatory infiltrates and collagen deposition revealed that rPlet1 did not affect inflammatory responses or levels of tissue fibrosis. In addition, viral clearance was not affected by rPlet1 treatment either. Finally, mice challenged with a lethal dose of IAV were rescued by 85.7% by rPlet1 compared to control treatment, suggesting that local administration of rPlet1 may represent a putative treatment strategy in human virus-induced ARDS.



Remarkably, in BALF of a small cohort of patients with IAV-induced ARDS, PLET1 concentrations negatively correlated with a marker of alveolar injury, underscoring Plet1 administration as a putative therapeutic approach in patients with virus-induced lung injury and beyond, that are urgently needed to date.

Discussion

Respiratory infections by endemic and emerging viruses pose a major threat to human health as currently evidenced by the COVID-19 pandemic. Lung macrophages have been attributed a crucial role in driving the severity of virus-induced lung tissue injury in IAV (8,11,44) and COVID-19 (10,45). Several studies revealed that TR-AM pools are depleted upon viral pneumonia to different extent, depending on viral strain and dose, and are gradually replaced by BMDM during the healing phase, resulting in reprogramming of the TR-AM pool that can either confer trained immunity, tolerance or resilience to subsequent challenges (1–3,25–27). The bone marrow origin of replaced, rather than training of locally persisting TR-AM by pathogen encounter or microenvironmental cues, seem to determine the net functional phenotype of the TR-AM pool at least after viral infections. To date, most of the data relating to macrophage ontology and the resulting phenotype have focused on their contribution to subsequent infections and remain conflicting; however, far less is known about their role in the resolution of inflammation and tissue repair (3,4,28–30). Our data provide evidence that a specific epithelial repair program is induced in alveolar macrophages, via a distinct trajectory of functional BMDM specification from pro-inflammatory/injury-promoting to tissue-healing, with the latter effectsmediated by Plet1. The BMDM2 Plet1-driven epithelial-protective phenotype remained upregulated in replenished, experienced TR-AM for at least 60D p.i., whereas the direct epithelial-proliferative effect declined at 35D p.i. As we could detect Plet1 expression in TR-AM at D60 p.i., other co-factors may be needed in addition to exert the pro-proliferative effect of Plet1 at later stages. Also, despite our RNA velocity data on a likely in vivo transition of BMDM1 to BMDM2phenotype, we cannot exclude that an additional wave of BMDM2 enters the lungs at later stages of the infection course, when repair of the lungs starts to be initiated. Plet1 is a GPI-anchored membrane protein, mediating epithelial repair responses such as keratinocyte migration in wound healing (20) and proliferation of Lgr5+ colonic stem cells after injury (21) in a cellautonomous manner. It was recently identified as a marker gene for alveolar macrophage specification among tissue-resident macrophages of various organs38; however, no data so far have revealed a role for Plet1 in myeloid cell-mediated tissue repair. At least two different effects were induced by Plet1 in the lung epithelium ex vivo, in organoids, and in vivo, ultimately resulting in the protection of barrier function: First, Plet1 mediated organoid outgrowth from

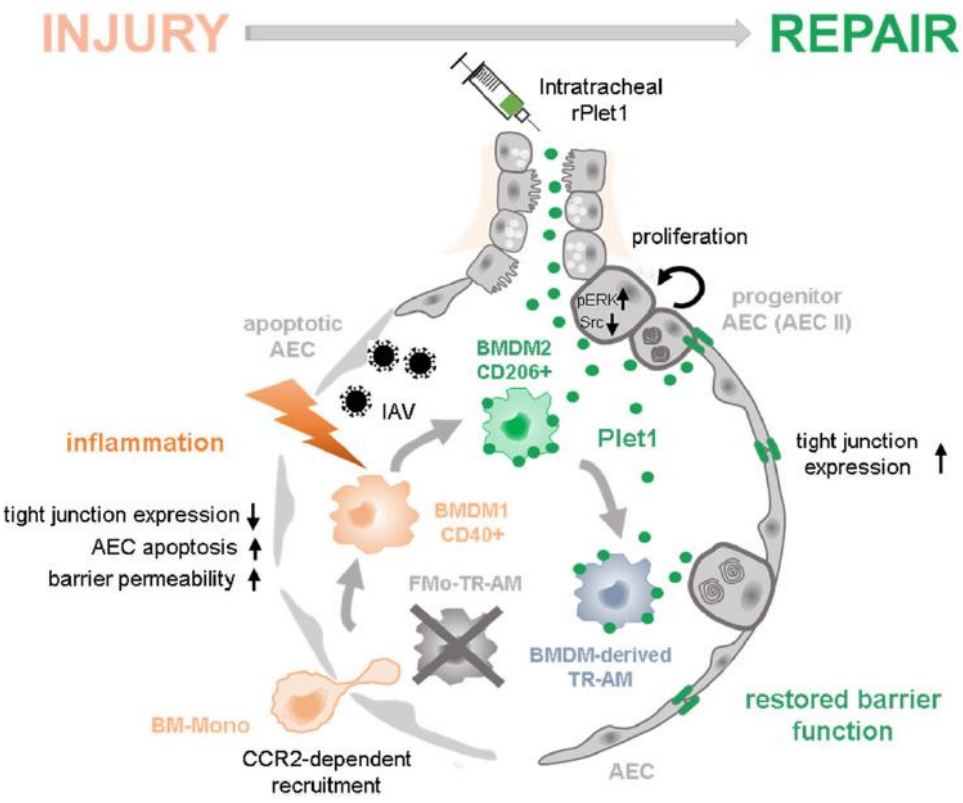
BASC and subsequent alveolarization in BALO, and increased proliferation of AEC II in vivo, indicating its pro-proliferative action on lung epithelial progenitor cells. Furthermore, Plet1 protected AEC fromapoptosis, an effect likely associated with the self-renewal program induced (31). Proliferation of progenitor cells in different niches of the distal lung is a crucial step in repair after viral lung injury. The alveolar epitheliumcan be re-established fromdifferent local stem/progenitor pools, themost important being Axin2+ subsets of AEC II, that via Krt8+ intermediates de-differentiate into AEC I to ultimately re-establish alveolar epithelial barrier function. At least in mice, alveolar repair after IAV injury is additionally driven by expansion of bronchoalveolar stem cells, the BALO cells of origin, that de-differentiate into AEC II and further into AEC I (32,33-37). In search of a putative signaling mechanism by which Plet1 would drive proliferation of alveolar epithelial cells with regenerative capacity, we identified aMAPK pathway, asMEK inhibition completely abolished Plet1-induced proliferation, suggesting that Raf-MEK-ERK signaling was involved. Similar findings were reported (21), where Plet1 induced colonic stemcell proliferation via ERK1/2.However,we cannot exclude furthermechanisms being involved, e.g. CK2a1 (a further hit in the phosphokinome screen) known to mediate cell survival and proliferation (38), or others.Ongoing experiments aimed at identification of the Plet1 receptor will likely reveal the full spectrum of Plet1-driven progenitor cell activation. A second effect elicited by Plet1 was re-establishment of epithelial barrier properties, associated with increased expression of tight junction-associated molecules ZO-1, occludin and claudin-1, the latter previously described to confer sealing properties of tight junctions in airway epithelia56. Disruption of the apical junction complex in pulmonary epithelial cells is one of the pathomechanisms driving loss of barrier integrity in IAV infection (39). Therefore, we expected that, in addition to the regenerative program elicited in lung epithelial progenitor cells, induction of tight junction proteins by Plet1 is crucial in both, protection from IAV-induced epithelial barrier disruption, and re-sealing of newly generated AEC. We speculated that this re-sealing program was mediated by pathways distinct from those driving proliferation as it was demonstrated that inhibition of Src kinases prevented inflammation-dependent loss of tight junction proteins in lung epithelial cells (40). In addition to our own phosphoproteome prediction data showing that Plet1 treatment was associated with reduction of Src kinase activity, treatment with a Src kinase activator abolished these Plet1-induced tight junction

protein upregulation. This certainly does not rule out further mechanisms involved, but indicates that, likely, two separate regeneration and repair programs are induced by Plet1 in alveolar epithelial cells, driving proliferation and re-establishment of cell-cell contacts, respectively. The mechanism by which macrophage Plet1 interacts with the epitheliumis an important question to be discussed.We speculate that it might act as a soluble protein, at least in addition to its membraneexpressed form, for several reasons. First, use of recombinant soluble Plet1 instead of Plet1+ macrophages reproducedmany of the observed effects in vitro and in vivo. In fact, Plet1 can be removed from cells by the addition of Phospholipase C, suggesting that shedding from macrophage surfaces might occur in vivo (20). In addition, shedding of Plet1 from the surface of TR-AM occurred in in vitro culture and was significantly increased upon treatment with conditioned medium of infected AEC, indicating thatmediators released fromIAV-injured AEC, possibly cytokines or danger-associated molecular patterns (DAMPS), were a major signal to increase soluble Plet1 concentrations at sites of infection or injury. Interestingly, naive, non-replenished TR-AM express Plet1, but did not release high amounts upon transfer into inflamed/injured lungs of IAV-infected Ccr2-/- mice, whereas transfer of BMDM2 or experienced TR-AMresulted in highly increased Plet1 BALF concentrations. This could explain why BMDM2/experienced TR-AM transfer, but not naïve TR-AM transfer, protected the lung epithelium of IAV-infected Ccr2-/- mice. TR-AM self-renewal has been pointed out as a major contributor to TR-AM replenishment in lung viral infection, being progressively outcompeted by BMDM-derived TR-AM following IAV infection (3,41). Although we cannot fully exclude a contribution of self-renewing fetal monocyte-derived TR-AM to Plet1-mediated repair, this seems unlikely since Cx3cr1iCre-Plet1flx/flx mice had undetectable levels of Plet1 in BALF. With regard to this transgenic model, the possibility exists that Plet1 knockout in other Cx3cr1-expressing cells might contribute to the outcome of Cx3cr1iCre-Plet1flx/flx mice. For instance, interstitial macrophages, DCs and T cells can express Cx3cr1; however, we found no significant levels of Plet1 expression in those cell populations in IAV-infected mice by flow cytometry. Together, our data reveal an innovative mechanism of macrophage mediated epithelial repair that is key to survive IAV-induced lung injury. Independently of the source; it has to be highlighted that local administration of recombinant Plet1 reproduced the epithelial-protective and -regenerative effects driven by Plet1+

macrophage populations, and rescued 85% of mice from fatal influenza. Remarkably, in BALF of a small cohort of patients with IAV-induced ARDS, PLET1 concentrations negatively correlated with a marker of alveolar injury, underscoring Plet1 administration as a putative therapeutic approach in patients with virus-induced lung injury and beyond, that are urgently needed to date.

Summary

Together, this study reveal a novel mechanism of macrophage-mediated epithelial repair that is key to survive IAV-induced lung injury. Independently of the source; it has to be highlighted that local administration of recombinant Plet1 reproduced the epithelial-protective and -regenerative effects driven by Plet1+ macrophage populations. Remarkably, in BALF of a small cohort of patients with IAV-induced ARDS PLET1 concentrations negatively correlated with a marker of alveolar injury. In conjunction, our data highlight Plet1 administration as putative therapeutic approach in patients with virus-induced lung injury and beyond that are urgently needed to date.



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Signature

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