INFLAMMATION

Endothelial cells instruct macrophages how to Rspond to lung injury

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The lung endothelial cell-derived angiocrine Rspondin3 activates Wnt-β-catenin signaling in interstitial macrophages (IMs), leading to a metabolic-epigenetic reprogramming of IMs that drives anti-inflammatory responses and attenuates endotoxin-induced lung injury.

Like the skin and the gut, mammalian lungs are at the interface between the host and the external world and are continuously exposed to microorganisms, microbial products, allergens and other noxious stimuli. In order to allow efficient gas diffusion and to support life, the air-blood barrier must therefore be very thin and tightly regulated in order to avoid excessive inflammatory damage. In the current issue of *Nature Immunology*, Zhou *et al.*[1] have identified an anti-inflammatory angiocrine-macrophage axis that contributes to the attenuation of lung inflammatory injury in endotoxemic mice.

Using multiple strains of genetically modified mice in combination with bone-marrow derived macrophages (BMDMs) stimulation experiments *ex vivo*, Zhou et al. provide convincing experimental evidence that the endothelial cell (EC)-derived angiocrine Rspondin3 is triggered by lipopolysaccharide (LPS) and drives the expansion of anti-inflammatory and tissue-protective interstitial macrophages (IMs) through macrophage-intrinsic leucine-rich repeat-containing G protein receptor 4 (LGR4)-β-catenin-mediated metabolic-epigenetic reprogramming (**Fig. 1a**).

Macrophages are present in most mammalian tissues and exhibit a remarkable microenvironment-driven heterogeneity and plasticity to fulfill tissue-specific and context-specific functions[2,3]. Besides the well-known alveolar macrophages (AMs), the steady-state lung is populated by parenchymal IMs encompassing distinct subpopulations in specific niches[4–6]. During inflammation, infection or tissue damage, monocytes can enter the tissue, be imprinted by stress and microenvironmental signals provided by the local niche and undergo differentiation into IMs that are thought to regulate immune, inflammatory and tissue repair responses[7,8]. Yet, the identification of niche-derived signals imprinting lung IMs during homeostasis and inflammation, as well as the IM-intrinsic molecular pathways

involved, remain largely unknown. Since lung ECs are located in close proximity to extravasating monocytes and IMs[4,5], they represent a potential cellular source of signals tailoring IM identity. To test this hypothesis, Zhou *et al.* performed a secretome assay on conditioned medium from LPS-activated mouse lung ECs and found the Wnt signaling activator Rspondin3 to be one of the top secreted proteins. *In vitro*, the authors first showed that stimulation of BMDMs with conditioned medium from LPS-stimulated mouse lung ECs triggered an anti-inflammatory macrophage phenotype characterized by elevated expression of CD206, CD301, Arginase 1 and interleukin(IL)-10 and reduced expression of the pro-inflammatory markers CD86, CD80, TNF and iNOS as compared to unstimulated BMDMs. Second, they showed that such anti-inflammatory macrophage programming, including the production of IL-10, a cytokine important for IM homeostatic functions[8], was also induced by stimulation of BMDMs with recombinant Rspondin3 in the presence or absence of LPS stimulation. Third, they performed BMDMs stimulation experiments using conditioned medium from mouse lung ECs isolated either from wild-type (WT) mice or from mice conditionally deficient in Rspondin3 in ECs (*Rspo3*$^{EC-/-}$) that were previously treated intraperitoneally (i.p.) with LPS (i.e., endotoxemic mice) to demonstrate that the anti-inflammatory features of BMDMs were dependent on EC-intrinsic Rspondin3 via contact-independent mechanisms[1].

To gain insights into the relevance of these findings for lung IMs *in vivo*, the authors performed elaborate lung myeloid cell profiling using mass cytometry in *Rspo3*$^{EC-/-}$ and WT mice, at baseline and after i.p. LPS challenge, with or without intravenous (i.v.) treatment with Rspondin3. While EC-intrinsic Rspondin3 deficiency did not affect the numbers and the phenotype of IMs at baseline, the expansion of IMs (defined as CD45^{+}Ly-6G/C^{-} CD64^{+}MerTK^{+}CD11b^{+}Siglec-F^{-} cells) and their shift towards an anti-inflammatory phenotype after LPS challenge was entirely absent in *Rspo3*$^{EC-/-}$ mice and could be restored

by Rspondin3 i.v. treatment. Instead, IMs displayed a pro-inflammatory phenotype after LPS in *Rspo3*$^{EC-/-}$ mice, which was associated with exacerbated signs of LPS-induced acute lung injury, including increased lung neutrophil counts and vascular permeability as well as lower survival as compared to WT mice (**Fig. 1b**). Of note, the abnormalities observed in endotoxemic *Rspo3*$^{EC-/-}$ mice could be restored by Rspondin3 i.v. treatment[1]. The expansion and the anti-inflammatory phenotype of IMs was also shown to be dependent on EC-intrinsic Rspondin3 five days after intratracheal bleomycin treatment, a model in which Lyve1^{+} IMs have previously been shown to restrain inflammation and fibrosis[4]. Altogether, these data support that lung ECs release Rspondin3 in response to lung-damaging stimuli in order to imprint IMs with an anti-inflammatory identity that mitigates the extent of lung injury (**Fig. 1a**).

Next, the authors investigated the intrinsic molecular players involved in Rspondin3-mediated imprinting of IMs and focused their attention on the contribution of LGR4, highly and specifically expressed by IMs, and of the Wnt-β-catenin signaling pathway, activated in IMs after Rspondin3 i.v.[1]. Using BMDMs deficient in *Lgr4* or *Ctnnb* (encoding β-catenin) and mice conditionally deficient in *Lgr4* or *Ctnnb* in macrophages, the authors accumulated experimental evidence that LGR4 expression and the activation of the β-catenin pathway in IMs were mediating Rspondin3-induced programming of IMs towards an anti-inflammatory phenotype as well as the attenuation of LPS-induced inflammatory lung injury[1] (**Fig. 1b**). These results are concordant with a previous report showing that germline *Lgr4*-deficient mice were highly susceptible to endotoxemia and exhibited increased production of pro-inflammatory cytokines by macrophages[9]. Nevertheless, the role of LGR4 in macrophages seems to be context-specific, as it has recently been suggested that LGR4 rather promoted a pro-inflammatory macrophage program in a model of myocardial infarction[10].

Metabolic adaptations and epigenetic modifications are increasingly recognized to contribute to macrophage diversity[11,12]. In the last part of the study, Zhou *et al.* analyzed BMDMs *in vitro* and found that Rspondin-3 promoted oxidative phosphorylation via glutaminolysis and the generation of α-ketoglutarate in an LGR4- and β-catenin-dependent manner. Furthermore, Rspondin-3 also increased the activity of TET methylcytosine dioxygenase, which utilizes α-ketoglutarate as a cofactor, to catalyze DNA hydroxymethylation and activate anti-inflammatory genes, highlighting a Rspondin-3-induced metabolic-epigenetic anti-inflammatory reprogramming in BMDMs[1]. Interestingly, the authors found that mice conditionally deficient in *Tet2* in macrophages exhibited the same deficit in the expansion of anti-inflammatory IMs and in the attenuation of inflammatory lung injury as the one observed in $Rspo3^{EC-/-}$ mice and in mice conditionally deficient in *Lgr4* or *Ctnnb* in macrophages, suggesting that TET2-mediated epigenetic reprogramming also occurs in IMs *in vivo* after systemic LPS challenge (**Fig. 1b**).

The work of Zhou *et al.* makes important contributions to our understanding of the niche and identity of lung IMs during inflammation by identifying an angiocrine-macrophage axis that shapes IMs towards an anti-inflammatory phenotype and attenuates lung inflammatory injury. It would be interesting to extend such investigations to other organs where IMs are present, e.g., the fat, the heart or the dermis[4], and assess whether the Rspondin3-LGR4-Wnt-β-catenin pathway also can reprogram such IMs toward an anti-inflammatory phenotype to regulate excessive inflammation elsewhere in the body.

At baseline, the identity of IMs, as assessed in this study, was not significantly affected by Rspondin3 treatment, by EC-intrinsic *Rspo3* deficiency nor by macrophage-specific *Lgr4, Ctnnb* or *Tet2* deficiency. There is therefore no experimental evidence so far to support that endothelial-derived Rspondin3 and the corresponding pathway in IMs is involved in the maintenance of steady-state IMs. Additional investigations looking at the abundance,

phenotype, secretory profile and single-cell transcriptome of previously identified steady-state IMs subpopulations[4–6] in those transgenic animals at baseline might reveal that ECs also release similar signals imprinting the identity of IM subpopulations during homeostasis. The identification of blood vessel-associated IMs[4] and nerve-associated IMs[4,6] in lungs and across tissues is consistent with the idea that both ECs and nerves release signals that instruct IM subpopulations at baseline.

The relevance of these findings for human diseases is currently unknown. Nevertheless, on the long term, identifying the niche cells and the signals that are important to control beneficial, anti-inflammatory functions of lung IMs might open new therapeutic avenues for diseases in which lung macrophage dysregulations have been implicated, as it has been suggested for the most severe forms of COVID-19 or other acute respiratory distress syndromes[13].

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Competing financial interests

The author declares no competing interests.

Figure legend

Figure 1. A novel angiocrine-macrophage axis negatively regulates lung inflammatory injury in endotoxemic mice. a, In wild-type mice, LPS-triggered release of Rspondin3 (*Rspo3*) by lung ECs activates LGR4-β-catenin signaling in interstitial macrophages (IMs), leading to a metabolic-epigenetic reprogramming of IMs that drives their expansion and anti-inflammatory responses, including expression of anti-inflammatory surface markers such as CD206, and elevated levels of IL-10. The metabolic-epigenetic pathway involves glutaminolysis, generation of α-ketoglutarate serving as a co-factor for TET2 methylcytosine dioxygenase, which catalyzes DNA hydroxymethylation and activation of anti-inflammatory genes. Similar observations can be made in LPS-challenged mice with EC-specific deletion of *Rspo3* that are supplemented with Rspondin3 i.v. **b**, In mice conditionally deficient for *Rspo3* in ECs, or in mice with macrophage-intrinsic deficiency in *Lgr4*, *Ctnnb* (encoding β-catenin) or *Tet2*, i.p. LPS treatment does not lead to the expansion of IMs, which exhibit a pro-inflammatory profile, including high surface expression of CD86 and elevated levels of IL-1β. This is associated
with exacerbated features of lung inflammatory injury, such as elevated neutrophil activity and counts, and vascular leakage. Other immune cells are not depicted due to limited space available and for a sake of clarity.