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A no-Wnt situation for alveolar macrophage self-renewal

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Alveolar macrophages (AMs) are central to defense against respiratory pathogens. Impediments in restoring AMs after infection increase the risk for superinfection, which is associated with significant morbidity and mortality worldwide. In this issue of Immunity, Zhu et al. report a Wnt-β-catenin-HIF-1α axis in AMs that promotes an inflammatory phenotype while restricting proliferation and self-renewal.

Alveolar macrophages (AMs) are the first responders in the lung during infections (Byrne et al., 2015). AMs have high phagocytic capacity and rapidly produce oxidants, proteases, antimicrobial mediators, and an array of cytokines and chemokines to kill the phagocytosed pathogen and initiate a well-orchestrated program that recruits other immune cells to help eliminate the invaders. At homeostasis, AMs remove small inocula of pathogens as well as toxic products, such as inhaled environmental particles and allergens. In mice, influenza infection rapidly depletes AMs, although the extent of depletion may be strain dependent. These lost cells need to be replenished to maintain the homeostatic pool. Depletion of AMs after viral infection has physiological ramifications, since it increases the risk for superinfection by bacteria that can precipitate pneumonia (Figure 1).

AMs begin as fetal liver monocytes that seed the alveolar tissue and differentiate into macrophages (Guilliams et al., 2013). After depletion, the cellular niche is restored by in situ proliferation of the residual pool rather than by differentiation of recruited monocytes, although there may be exceptions to the rule (Misharin et al., 2017). Since attrition of cells also occurs under homeostatic conditions, the process of self-renewal ensures maintenance of steady-state numbers. Signals from the local microenvironment are important in maintaining AM identity and functionality. Wnt signaling has a central role in development and tissue organization (Clevers and Nusse, 2012). At mucosal sites, a network of interactions between subepithelial mesenchyme, which secretes Wnt ligands, and overlying epithelial stem cell populations are crucial for maintaining tissue integrity during steady-state and injury conditions. Wnt pathway signaling is linked to the regenerative capacity of lung resident stem cell populations, which may become activated following injury, such as catastrophic infection. In this issue of Immunity, Zhu et al. (2021) show that this process may be intrinsically coupled to regulation of self-renewal of AMs during pneumonia induced by influenza A virus (IAV).

The authors initiated the study by examining the effect of Wnt3a on mouse AMs in vitro. Single-cell RNA sequencing (scRNA-seq) identified a signature of blocked cell-cycle progression in the AMs upon Wnt3a stimulation and downstream β-catenin activation. Concomitant with reduced cell proliferation, the authors identified an increase in the production of inflammatory cytokines in Wnt3a-treated AMs. These findings prompted a series of
in vivo interrogations using genetically manipulated mice subjected to a model of infection by IAV. A role for β-catenin specifically in myeloid cells in promotion of inflammation and loss of body temperature and weight was established by myeloid cell-specific deletion of β-catenin using Ctnnb1lox/lox mice, with these mice showing diminished adverse effects upon viral infection including morbidity and loss of body temperature. Conversely, targeted expression of the constitutively active mutant of β-catenin in Ctnnb1lox/lox mice aggravated morbidity in the mice following IAV infection.

Zhu et al. next studied the mechanism by which β-catenin interfered with AM proliferation taking cues from their scRNA-seq data of Wnt3a-treated AMs that showed enrichment of hypoxia-related genes, including increased expression of HIF-1α in Wnt3a-treated AMs. Notably, HIF-1α-deficient AMs phenocopied β-catenin-deficient AMs with reduced expression of pro-inflammatory cytokine genes suggesting a partnership between the two molecules. Also, unaltered expression of Wnt ligands following IAV infection in HIF-1α-deficient AMs suggested Wnt as a trigger upstream of HIF-1α that induced collaboration between β-catenin and HIF-1α. Indeed, β-catenin and HIF-1α coimmunoprecipitated in AMs stimulated with Wnt3a. No interaction between β-catenin and TCF-4, a common downstream interacting partner of β-catenin, was detected in Wnt3a-treated cells, although it was evident in the AMs in the absence of Wnt3a. Not only was HIF-1α implicated in expression of proinflammatory cytokine genes, increased glycolysis was also evident in Wnt3a-treated cells, with HIF-1α being a central regulator of glycolysis. Reconstitution of Ctnnb1lox/lox mice with AMs and use of mice deficient in β-catenin in CD11c+ cells demonstrated a role for β-catenin in AM cell proliferation and repopulation following IAV infection. Parabiosis experiments showed a minimal role for circulating monocytes in regeneration of the AM pool in the acute stage of infection. Disruption of glycolysis in vivo following IAV infection using 2 deoxyxylulose (2DG) did not restore AM proliferation. However, in line with defective mitochondrial morphology in AMs upon viral infection, treatment of infected mice with inhibitors of the mitochondrial respiratory chain, such as rotenone and antimycin A, impaired AM proliferation in vivo. Collectively, these data implicate increased glycolysis in the heightened inflammatory response of AMs following activation of the Wnt-β-catenin axis and concordantly disrupted mitochondrial fitness that suppresses cell proliferation.

Using Egln3-YFP reporter mice, Egln3 being a direct target of HIF-1α, the authors showed increased expression of HIF-1α, active β-catenin, and inflammatory cytokine genes and decreased expression of Ki67 in sorted YFPΔ20 AMs in contrast to increased Ki67 expression in YFPΔ10 AMs. The YFPΔ10 AMs also displayed less mitochondrial damage and increased expression of reparative genes. These data showed a role for HIF-1α in dissociating the proliferative capacity of AMs and at the same time consolidating its proinflammatory role. By utilizing mice expressing CD169-diphtheria toxin receptor (CD169-DTR/Ctnnb1fl/fl or CD169-DTR/Ctnnb1lox/lox mice) the authors showed that transient depletion of AMs at the recovery stage increased lung inflammation and damage, compromising recovery of the mice following IAV infection. Importantly, this axis is conserved in humans. Treatment of human AMs with Wnt3a increased β-catenin and HIF-1α expression and induction of a proinflammatory phenotype. Examination of publicly available gene expression data for human AMs infected by IAV or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) revealed enrichment of inflammatory and hypoxia-associated genes but decreased expression of genes involved in self-renewal. Pharmacological inhibition of HIF-1α in IAV-infected mice suppressed inflammation and accelerated recovery. HIF-1α inhibition also promoted AM repopulation, suggesting HIF-1α as a therapeutic target for recovery of patients with severe viral pneumonia, including those with severe coronavirus disease 2019 (COVID-19).

These findings open many questions. The source and identity of the Wnt...
ligand(s) released during viral infection remain to be determined. Furthermore, a set directionality of the Wnt-β-catenin-HIF-1α axis is not evident in all contexts. In stem-cell niches, oxygen deprivation induced HIF-1α stabilization, which engaged with the Wnt-β-catenin pathway to regulate neural stem-cell proliferation (Mazumdar et al., 2010). Unlike decreased expression of Wnt target genes in AMs during IAV infection (Zhu et al., 2021), expression of these genes was increased in the stem cells, resulting from β-catenin-HIF-1α collaboration (Mazumdar et al., 2010). It is unclear why conventional Wnt effector genes that promote cell renewal are not induced in AMs unlike those observed in neural stem cells, despite interaction between β-catenin and HIF-1α observed in both cell types. It is possible that Wnt-induced HIF-1α upregulation in AMs prompts a negative feedback response in which suppression of beneficial Wnt effector genes, such as TCF-1 and LEF-1, is a collateral damage. The findings of Zhu et al. (2021) suggest that inappropriate Wnt ligand expression during viral infection of the lung, whether because of the specific Wnt molecule that is expressed or the extent of downstream signaling in the AMs, may backfire. Instead of promoting self-renewal of the target cell that is the normal function of Wnt molecules, Wnt-activated β-catenin may prompt a sequela involving severe lung inflammation and halted AM recovery, in turn setting up a perfect storm for reinfection by other pathogens, such as bacteria. Better understanding of the cellular sources of Wnt ligands produced during viral infection and the regulatory mechanisms that control Wnt production may shed some light into these questions. Also, HIF-1αα AMs survive after viral infection and proliferate to reestablish the resident AM pool, but how these cells are distinguished from HIF-1αα counterparts at a mechanistic level is unclear, as is whether there are functional consequences to the daughter cells. Although infiltrating monocytes do not appear to contribute to the regenerated pool, at least acutely after infection, it is possible that infection-experienced resident AMs are not similar to those in naïve mice. In a recent study of pneumococcal pneumonia in mice, a profound reprogramming of AMs was observed, such that these seemingly trained macrophages were more efficient in protection against another pneumococcal serotype (Guillon et al., 2020).

Altogether, Zhu et al. add a dimension of complexity to the proinflammatory function of HIF-1α by focusing on its ability, when present at high levels during viral infection, to direct inflammation and restrict proliferation of AMs that involves interaction with β-catenin. There is intense interest in understanding the role of HIF-1α in regulating effector functions of immune cells in addition to its role in glycolysis (Cramer et al., 2003). HIF-1α appears necessary for SARS-CoV2 replication within macrophages (Codo et al., 2020). Targeting HIF-1α in patients with severe non-resolving pneumonia may not only achieve short-term benefits but also prevent long-term consequences of severe pneumonia such as lung fibrosis.

**DECLARATION OF INTERESTS**

A.R. has a research agreement with Pieris Pharmaceuticals. M.J.C. is a consultant for Pieris Pharmaceuticals.

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**PREVIEWS**

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