The last-minute redemption of inflammatory cells in lung repair

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Take home message: Inflammatory cells play a key role in lung repair after acute injury. These cells switch to a pro-repair phenotype over time in response to extracellular signals. Among these, VEGF-C promotes neutrophil clearance (efferocytosis) in macrophages.

Inflammatory cells play a central role in the pathogenesis of acute lung injury. Danger signals within the alveolar structures trigger a stereotypical response that results in the activation of an inflammatory response within alveoli and interstitium. In addition to resident cells, the release of proinflammatory mediators, including chemoattractants, results in the recruitment of inflammatory cells from the circulation. Activated macrophages and neutrophils participate in the early phase of acute injury, mainly by promoting tissue disruption [1]. This acute response aims to remove pathogens and damaged cells in an expedite way, and, if the severity of injury is mild or moderate, may result in effective repair or even regeneration. However, in case of severe damage, the intraalveolar inflammatory response may impair lung function, worsening gas exchange and respiratory mechanics.

Virtually all the pathogenetic mechanisms of severe acute lung injury are triggered by these inflammatory cells, including the release of proinflammatory cytokines, proteases and reactive oxygen species that cause organ damage. By these reasons, prophylactic inhibition of the cell-mediated acute response has yielded beneficial results in almost all the experimental models tested [2, 3]. However, these findings contrast with the clinical evidence coming from patients with abnormal cell immunity, who often show worse outcomes than their control population [4]. This poor prognosis comes not only from the delayed clearance of the triggering event, but also from an impaired resolution and repair of the injured tissue, thus revealing a role of these cells in tissue repair [5].

Over the last years, the involvement of different neutrophil and monocyte/macrophage subclasses in regulation of the inflammatory response and lung repair has emerged, revealing a complex, cell-mediated mechanism to preserve and restore tissue homeostasis. In this issue of the European Respiratory Journal, Yamashita *et al.* [6]

describe a novel mechanism of macrophage-mediated resolution of lung damage driven by activation of vascular-endothelial growth factor (VEGF) type 3 receptors by VEGF-C. To put this new information in context, we will briefly review how macrophages and neutrophils orchestrate lung repair.

The lung at steady state contains alveolar (AM) and interstitial macrophages (IM). These populations may be augmented by monocyte-derived macrophages recruited from the circulation under inflammatory conditions. AMs are the main immune cell population present in the healthy lung. They are located in the luminal surface of the alveolar space and have a key role in keeping pulmonary homeostasis, mainly by clearing cellular debris and excessive surfactant [7]. As the alveoli are constantly exposed to antigens, AMs must keep a suppressive phenotype in order to prevent overreaction. The alveolar epithelium promotes tolerance to innocuous antigens by both secretion of soluble factors like interleukin (IL)-10 [8] and cell-cell interactions [9]. Thus, AM responsiveness to a harmful antigen is strongly related to the integrity of the respiratory epithelium, as damage of the epithelium leads to lack of immunosuppressive signals and a shift towards a proinflammatory response by AMs. Therefore, alveolar macrophages carry out an important gate-keeping role in the defense of the respiratory tract [10]. Interstitial Macrophages (IM), also referred as lung tissue macrophages, can be observed in multiple locations, from the bronchial interstitium, to the vicinity of lymphatic vessels and on the pleural surface [11]. These cells are considered a second line of defense against invading microorganisms and have mainly an antigen-presenting and immunoregulatory activity [12]. Finally, monocyte-derived macrophages are recruited from the circulating pools in cases of severe injury and retain a more pro-inflammatory and pro-fibrotic phenotype [13].

In acute inflammation, neutrophils are quickly recruited from the circulation into the airway space and play a dual role in the development and resolution of lung injury [14]. Mature neutrophils recruited at the sites of inflammation have a strong chemotactic and proinflammatory response. Even at this early phase, neutrophils may play pro-repair roles. Migration of neutrophils along the epithelium results in activation of the Wnt pathway, that triggers epithelial proliferation [15]. In addition, release of neutrophil extracellular traps (NETs) and anti-inflammatory lipidic mediators such as lipoxin A4 contribute to mitigate the acute proinflammatory response. Neutrophils also phagocyte cell debris to provide a clean environment that facilitates repair [16].

It has recently been proposed that, rather than acting as different pre-defined cell subsets, the evolving nature of the inflammatory response induces a phenotypic switch in both macrophages and neutrophils from the pro-inflammatory roles previously described towards a pro-repair program. Anti-inflammatory macrophages are induced by the exposure of AMs to transforming growth factor(TGF)-β or type II cytokines (IL-4 and IL-13) secreted by Th2 cells and type 2 innate lymphoid cells [17, 18]. One of the most important strategies carried on by these activated macrophages is efferocytosis, the phagocytosis of apoptotic cells (mainly neutrophils), which prevents the release of pro-inflammatory and toxic components to the environment [19]. This mechanism further induces macrophage reprogramming towards the secretion of anti-inflammatory cytokines (IL-10, TGF-β) and growth factors (including keratinocyte growth factor, VEGF variants, fibroblast growth factors 2 and 10 and insulin-like growth factor-1) that promote the proliferation of parenchymal cells [20, 21].

Similarly, extracellular signals such as TGF-β may polarize neutrophils towards a pro-repair phenotype characterized by the release of growth factors, including VEGF, and

matrix metalloproteinases (MMPs) [22]. Several MMPs are essential for the normal collagen deposition during the acute phase, and its subsequent digestion during repair [23]. This remodeled matrix facilitates epithelial migration and proliferation. Gelatinases (MMP-2 and MMP-9) have been involved in alveolar epithelial repair in several experimental models [24]. Furthermore, MMPs can cleave cyto- and chemokines, contributing to resolution of inflammation [25].

In this issue of the European Respiratory Journal, Yamashita and coworkers [6] add another piece of knowledge on the role of inflammatory cells in acute lung injury and repair. Using a smart combination of cell and animal models, the authors study the role of VEGF-C signaling in lung injury. During the acute phase, VEGF-C is involved in IL-10 secretion, thus contributing to attenuate inflammation, possibly by preserving the anti-inflammatory phenotype of resident cells. In a late phase, the study provides sound evidence on how VEGF-C signaling through VEGFR-3 receptor activates efferocytosis in AMs, contributing to tissue repair. Results using samples from patients with acute respiratory distress syndrome (ARDS) show a decreased activity of this pathway in monocyte-derived macrophages, reinforcing the translational nature of the study. These findings correlate with recent studies showing impaired efferocytosis in patients with the acute respiratory distress syndrome [26].

The lipopolysaccharide model of lung injury results in a severe proinflammatory response that vanishes over time due to endogenous toxin clearance. Future studies should perform a careful dissection of these mechanisms in models with ongoing lung injury (i.e. with live pathogens), making a distinction among the existing macrophage subpopulations, and probably different patient endotypes, as up half of ARDS cases do not show a marked proinflammatory response to injury [27]. Despite these limitations,

the beneficial effects observed suggest that, in opposite to the conflicting results observed with VEGFR1 and VEGFR2 stimulation [28], restoration of VEGF-C/VEGFR3 signaling could be a novel therapeutic target aimed to improve repair.

The article by Yamashita *et al.* also highlights the complex crosstalk between the acute phase of injury and the subsequent repair. In the specific case of VEGF-C, its upregulation ameliorates early lung injury and promotes repair in a macrophage-mediated manner. Therefore, VEGF-C might qualify as a time-independent target. However, this is not always the case. Some molecules released during the acute phase cause tissue disruption, but are also required in later phases. As an example, TGF-β, which, as previously described, is a key regulator of the inflammatory cell reprogramming [18, 22] towards an anti-inflammatory, pro-repair phenotype (and also pro-fibrotic), facilitates lung edema in the early phase [29]. Finally, other inflammatory mediators, such as IL-6 are released from macrophages and may decrease damage when released early [30, 31] but also be profibrotic during repair [32]. Collectively, these pleiotropic, time-dependent effects, make difficult to identify viable therapeutic targets without considering their window of opportunity (Figure 1). Indeed, targeting a specific cell population during the acute phase may have deleterious consequences for repair.

Therapeutic interventions aimed to repair such a complex organ as the lung will probably require multiple supportive and regenerative strategies over time. Identification of viable targets and timings is essential to later combine them in a therapeutic regime. Ultimately, hacking endogenous repair mechanisms that have been evolutionary optimized without disturbing some steps of the process is a challenge that probably requires exogenous (bio)technological support to both foster endogenous rebuild and overcome the resulting

side effects. The work by Yamashita et al. provides sound evidence that VEGF-C can be part of these strategies.

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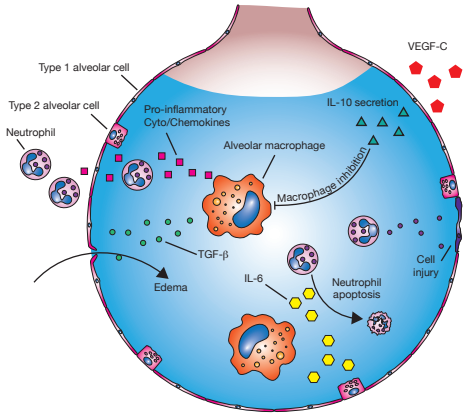
Figure legend

Figure 1. Time-dependent effects of cytokines on cell populations in acute lung injury (left) and repair (right). During the acute phase, macrophages release chemokines (among other mediators), that recruit neutrophils from the circulation. Degranulation of neutrophils release a large variety of inflammatory mediators and cytotoxic molecules that promote pathogen clearance, but also cell death. Transforming growth factor (TFG)-β is also released, promoting alveolar edema. Interleukin (IL)-6 released from inflammatory cells facilitate neutrophil apoptosis. VEGF-C stimulates IL-10 secretion, thus inhibiting macrophage response that ameliorates lung injury. During the repair phase, VEGF-C activates macrophage efferocytosis, that in turn polarizes macrophages towards an anti-inflammatory phenotype, that includes secretion of TGF-β, growth factors and anti-inflammatory cytokines. TGF-β and Th2 cytokines also polarize macrophages and neutrophils. Reprogrammed neutrophils release VEGF and matrix metalloproteinases (MMPs) involved in epithelial proliferation and matrix remodelling. In this late phase, IL-6 and TGF-β may also have pro-fibrotic effects. The pleiotropic effects of all the mediators involved in the lung response to injury make difficult to identify viable therapeutic targets outside specific time windows.

Acute phase



Repair