Recent advances in dead cell clearance during acute lung injury and repair

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Abstract

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are clinical syndromes that cause significant mortality in clinical settings and morbidity among survivors accompanied by huge healthcare costs. Lung-resident cell dysfunction/death and neutrophil alveolitis accompanied by proteinous edema are the main pathological features of ALI/ARDS. While understanding of the mechanisms underlying ALI/ARDS pathogenesis is progressing and potential treatments such as statin therapy, nutritional strategies, and mesenchymal cell therapy are emerging, poor clinical outcomes in ALI/ARDS patients persist. Thus, a better understanding of lung-resident cell death and neutrophil alveolitis and their mitigation and clearance mechanisms may provide new therapeutic strategies to accelerate lung repair and improve outcomes in critically ill patients. Macrophages are required for normal tissue development and homeostasis as well as regulating tissue injury and repair through modulation of inflammation and other cellular processes. While macrophages mediate various functions, here we review recent dead cell clearance (efferocytosis) mechanisms mediated by these immune cells for maintaining tissue homeostasis after infectious and non-infectious lung injury.

Keywords

Acute lung injury, Lung repair, Macrophages, Alveoli, Efferocytosis

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Acute lung injury

Acute lung injury (ALI) and its most severe form, acute respiratory distress syndrome (ARDS), together affect approximately 200,000 patients per year in the United States alone, with reported mortality rates of about 30–40%6. Oxygen supplementation (hyperoxia) by mechanical ventilation remains the primary therapy used for supporting critically ill patients with ALI/ARDS proven to decrease mortality7, but as many as 9–27% of patients undergoing this therapy contract nosocomial pneumonia, leading to excess morbidity and mortality8. Nosocomial pneumonia poses a substantial cost burden9 and accounts for approximately 27% of hospital-borne infections in American ICUs, of which 86% of cases were associated with mechanical ventilation10. Thus, identification of novel mechanisms underlying abnormal lung repair and microbial susceptibility may provide a basis for new therapeutic strategies that can improve clinical outcomes and decrease healthcare costs associated with ALI/ARDS.

Understanding of the mechanisms underlying ALI/ARDS is evolving, but, aside from ventilation, limited therapies of significant clinical benefit are available for intervening in lung injury progression5,6. Currently, treatments include statin therapy11, nutritional strategies12, and mesenchymal cell therapy13,14, but persistence of high mortality rates demonstrates their limitations and warrants exploration of alternative approaches15,16. Strategies for promoting lung repair that show favorable in vitro and in vivo results include plasma membrane repair via amphiphilic macromolecules17, administration of growth factors18, selective blockade of matrix metalloproteinases19,20, and modulation of proliferation-regulating transcription factors21,22. Additionally, gene therapy studies using viral and non-viral vector delivery, gene expression strategies, or enhanced therapeutic targeting offer promising evidence of restoring lung function, clearing inflammation, and enhancing repair mechanisms in vitro and in vivo23. However, clinical use of these techniques requires extensive progress to be made in terms of basic science and its translational approach. Perhaps most importantly, there are still immense gaps in our knowledge of molecular targets involved in the pathogenesis of ALI/ARDS. Therefore, better characterization of cellular mechanisms involved in heightened inflammation resolution and repair is necessary to develop novel therapies for ALI/ARDS patients.

Alveolar macrophages (AMΦs) account for approximately 95% of airspace leukocytes24. They are major regulators of the lung inflammatory microenvironment and the first line of defense against infectious and non-infectious stimuli25. The course of systemic inflammation and progression to ALI/ARDS is heavily dependent on signaling from these cells, and their defective functioning is associated with multiple acute and chronic inflammatory conditions26,27. This regulatory capability is in part due to the phagocytic role AMΦs play in clearing dead cells from the alveolar space, which facilitates injury resolution and prevents necrosis of apoptotic cells and release of pro-inflammatory mediators28. AMΦs are highly functionally heterogeneous and phenotypically variable, which allows them to use intracellular signals to switch between pro-inflammatory and anti-inflammatory states as well as several further subdivisions and hybrid states. It is known that AMΦ subtype populations vary between healthy individuals and patients with ALI/ARDS29, and by further investigating the cellular mechanisms by which this variation occurs it is likely we may discover new immunomodulatory targets that have the potential to mitigate the devastating effects of ALI/ARDS.

Role of apoptosis in amplifying lung inflammatory responses and injury

The main pathological features of ALI/ARDS include alveolar epithelial and endothelial cell death, neutrophil alveolitis, and destruction of epithelial capillary barriers, leading to vascular permeability and edema infiltration5,6. Furthermore, hyperoxic ventilation causes excess epithelial and endothelial cell death, exacerabates pre-existing lung injury and inflammation, and impairs alveolar fluid clearance30. Unchecked inflammation and cell death can promote tissue scarring, organ damage, and the development of autoimmune and chronic inflammatory disorders31, and impaired management of these insults can have severe long-term consequences32. Pro-apoptotic members of the tumor necrosis factor (TNF) family, Fas/FasL, are known to facilitate cell death, and increased concentrations of these mediators have been detected in bronchoalveolar lavage (BAL) samples of ARDS patients33,34. Distillation of Fas/FasL induced lung injury and inflammation35,36, while inhibition of Fas/FasL signaling or apoptosis attenuated lung injury in animals subjected to endotoxemia and mechanical ventilation37,38. This suggests that apoptosis, to some extent, affects the severity of ALI/ARDS progression and how well patients recover. Apoptotic cells may undergo secondary necrosis, or unprogrammed cell death, if not removed, leading to the release of endogenous ligands called damage-associated molecular patterns (DAMPs). High-mobility group box 1 (HMGB1) and other DAMPs resemble pathogen-associated molecular patterns (PAMPs) such as lipopolysaccharide (LPS)39. These molecules exacerbate tissue inflammation and contribute to injury observed in ALI/ARDS, COPD, pneumonia, asthma, and pulmonary fibrosis40. As expected, elevated HMGB1 levels were found in BAL obtained from peripheral airways of COPD patients41. Many patients at risk for ALI require medical attention well into the course of their initial systemic inflammatory illness, which means that blocking late-acting DAMPs may have greater clinical relevance than more rapidly released mediators. However, it appears that while ALI may resolve entirely in some patients, along with lung function recovery, other patients are more susceptible to the development of chronic disorders32.

Pyroptosis (pyro meaning “fear” or “fire”) is a pro-inflammatory process of pre-programmed cell death distinct from apoptosis that results from activation of inflammatory proteases belonging to the caspase family, particularly caspase-1, -4, and -542. Inflammasomes are multiprotein complexes assembled by pattern recognition receptors (PRRs) in response to bacterial or viral PAMPs (e.g. LPS, bacterial flagella, viral DNA and RNA) and, or damaged host-cell derived DAMPs. These
complexes recruit either caspase-1 in the canonical inflammasome or caspase-4 and -5 in the non-canonical inflammasome. Recruitment occurs either directly or indirectly through a caspase activation and recruitment domain (CARD) containing adapter protein called an apoptosis-associated speck-like protein containing a CARD (ASC). The activated caspase molecules serve two crucial functions: 1) proteolytic cleavage of perforin inducing protein Gasdermin D, which creates pores in the cell membrane to induce pyroptosis, and 2) cleavage of IL-1β and IL-18 into their active forms, which are then released by the pyroptotic cell and initiate a pro-inflammatory response. Furthermore, pyroptosis also promotes HMGB1 release, which is mentioned as highly expressed in inflammatory lung conditions. Pyroptosis works in accordance with apoptosis and is necessary for clearing pathogen-infected cells but when unrestricted can induce inflammation and can lead to organ failure, sepsis, and death. In addition to pyroptosis, necrosis and necroptosis, ferroptosis, and autophagy-dependent cell death are all distinct from apoptosis in their activating stimuli but nonetheless must be cleared from the alveolar space to prevent ALI/ARDS.

The phagocytic machinery that recognizes dead cells is regulated by signaling cascades and selective upregulation of anti-inflammatory genes coordinated by communication between apoptotic cells and phagocytes. Engulfment of apoptotic cells by phagocytosis results in an abundance of reactive oxygen species (ROS), which stimulates macrophage apoptosis and inflammation persistence. Regarding the role phagocytes play in mitigating ALI/ARDS progression, AMΦs are recognized as initiators of pro-repair and pro-resolution processes necessary for restoring lung function following injury. However, AMΦs also recruit inflammatory cells, produce pro-inflammatory cytokines, and mediate pro-fibrotic processes. This functional dynamic makes AMΦs influential during both acute and resolution/recovery phases of lung injury. Studies performed with serial BAL in humans with ARDS determined that increased AMΦ numbers and matured cellular phenotype correlated with favorable clinical outcomes. Currently, there is increasing evidence suggesting that macrophages, including resident AMΦs and recruited AMΦs derived from blood-circulating monocytes, are key regulators of ALI/ARDS pathogenesis. These macrophages are phenotypically flexible and functionally heterogeneous, suggesting a key regulatory role in inflammation, injury, and repair throughout the course of ALI/ARDS. This dual functionality AMΦs play in both resolving and inducing inflammation demonstrates their unique and evasive role in maintaining lung homeostasis. Since these processes remain incompletely understood, further investigating the role of AMΦs in ALI/ARDS pathogenesis is of clinical interest.

**Macrophage plasticity and lung injury resolution**

Phagocytes are classified as either “professional” or “non-professional”. Professional phagocytes (e.g. monocytes, macrophages, neutrophils, etc.) are more abundant, secrete more cytokines, and display a wider range of phagocytic receptors than their non-professional counterparts (e.g. epithelial cells, fibroblasts, etc.). They act as first responders to infection in the steady state by recognizing and removing bacteria and promote adaptive immunity by displaying antigens of digested pathogens for T and B cell recognition. Phagocytes use PRRs including Toll-like receptors (TLRs), Nod-like receptors (NLRs), and RIG-I-like receptors (RLRs) to initiate phagocytosis or inflammatory signal transduction in response to microbial infection. In the lung, macrophages comprise two subtypes: resident and recruited. The former and more prevalent population is found within the alveoli themselves, whereas the latter are derived from circulating monocytes recruited from the interstitial space to infection or injury sites. Resident AMΦs originate from progenitor yolk sac and fetal liver monocytes and become functionally active as soon as the first week after birth, continuously repopulating alveoli by auto-regeneration. Resident AMΦs act as sentinels providing the first line of defense against respiratory infection and injury by clearing pathogens and debris. However, severe enough insult can induce circulating (bone marrow-derived) monocyte migration to the periphery to the inflamed tissue, where they differentiate into monocyte-derived AMΦs and initiate a pro-inflammatory and profibrotic response.

AMΦs are particularly unique in their phenotypic plasticity, which refers to polarization between two distinct phenotypes depending on inflammatory microenvironment conditions. Classically activated (M1) AMΦs (AMΦs) are cytotoxic and pro-inflammatory mediators that protect against pathogens by secreting pro-inflammatory cytokines and promoting Th1-type immunity. Macrophage stimulation with interferon gamma (IFNγ) or TNF alpha (TNF-α) in accordance with TLR agonists (e.g. LPS) induces M1 polarization. AMΦs produce cytotoxic and bactericidal ROS, reactive nitrogen species (RNS), and Th1 pro-inflammatory cytokines (e.g. IL-1, IL-6, IL-12, IL-23, and TNF-α) and strongly express major histocompatibility complex (MHC) II, CD80, CD86, and iNOS surface markers. (Figure 1A.) The classically activated phenotype promotes inflammation and assists in opsonization, antibody-dependent cytotoxicity, and phagocyte-dependent defense functions. Enhanced clearance of dead cells performed by pro-resolution AMΦs is a key process in tissue repair and resolution of AMΦ-promoted inflammation. This process limits the production of pro-inflammatory cytokines (e.g. IFNγ, TNF-α, IL-1, IL-6, IL-8, and LTB4) and increases the production of anti-inflammatory/regenerative cytokines (e.g. M-CSF, IL-4, IL-10, IL-13, and transforming growth factor beta [TGF-β]).

Alternatively activated (M2) AMΦs (AMΦs) are anti-inflammatory and promote tissue repair, fibrotic remodeling, and Th2-type immunity. IL-4 and IL-13 play an important role in resolving inflammation and aid in lung regeneration by facilitating wound healing through suppression of inflammatory signaling (Figure 1B). Macrophage stimulation with IL-4 and IL-13 induces AMΦ polarization, leading to the
resolution of lung inflammation. AMφ phenotypic changes are largely regulated by signal transducer and activator of transcription (STAT) transcription factors and suppressors of cytokine signaling (SOCS). STAT1/SOCS2 activation promotes the AMφM1 phenotype while activation of STAT3/6 and SOCS3 promotes the AMφM2 phenotype. AMφM1 and AMφM2 have been shown to resolve inflammation and initiate wound healing via the production of immunosuppressive cytokines (e.g. IL-10, TGF-β, CCL18, and CCL22) and angiogenesis mediators (e.g. VEGF and EGF) as well as express high levels of scavenger receptors (e.g. CD163 and CD206). AMφM1 also release immunosuppressive cytokines such as TGF-β, an inhibitor of NO production, and Arginase I, which neutralizes reactive nitrogen intermediates. AMφM2 are highly heterogeneous, with several further subdivisions (i.e. M2a, M2b, and M2c). Distinct functional roles between AMφM1 and AMφM2 suggest that a counterbalance between their pro- and anti-inflammatory responsibilities must be maintained to promote lung homeostasis in response to infection and injury.

M1 and M2 classification is generally useful for describing functional differences in AMφs throughout inflammatory processes but as a dichotomy neglects what appears to be a continuum of activation states that exists in vivo. AMφs are constantly altered by extrinsic factors, with M1 and M2 phenotypes representing the extreme sides of an expression spectrum. In fact, most AMφs in the steady state display markers of both M1 and M2 phenotypes simultaneously, which is thought to allow quick switching between M1 and M2 functions. Due to this flexible programming, AMφs
have shown critical activity at all stages of alveolar repair and fibrosis and phenotype-dependent roles at distinct inflammatory and resolving phases. Transcriptomic datasets have provided immense amounts of information regarding macrophage integration and computation of local inflammatory signals, and understanding of AMΦ transcriptional regulation can potentially be used to locate macrophage subset-specific therapeutic targets. Flexibility in AMΦ programming and their adaptability to environmental changes suggests modulating these processes may provide therapeutics for ALI/ARDS patients.

**Neutrophil death contributions to ALI/ARDS**

Neutrophils are specialized leukocytes with life cycles ranging from only a few hours to days. Due to these cells’ short lifespans, neutrophil death is highly concerted and can occur via several mechanisms, including apoptosis, necrosis/necroptosis, and release of neutrophil extracellular traps (NETs). Neutrophil alveolitis and cell death contribute to inflammatory injury observed in ALI/ARDS patients, and their activating stimuli leading to efferocytosis influence the course of systemic inflammation. Distinct modes of neutrophil cell death have been implicated in several pathologies, including cancer, neurodegenerative disease, and autoimmune disorders. Neutrophils are recruited as first responders to microbial infection to fight off invading pathogens, where they participate in phagocytosis, degranulation, ROS release, and NET release.

NETs are DNA–protein complexes released by neutrophils to neutralize pathogens in a process called NETosis. NETs are increasingly being investigated as contributors towards ALI/ARDS. In *in vitro* and *in vivo*, and clinical studies have confirmed that NETs promote ARDS inflammation by inducing AMΦ polarization and pro-inflammatory cytokine release, and increased M1 markers and decreased M2 markers were found in ARDS BAL fluid and lung tissue. Furthermore, ARDS patients experience increased NET formation accompanied by decreased levels of AMΦ engulfment of NETs and apoptotic neutrophils. Neutralization of HMGB1 in the BAL fluid was shown to improve efferocytosis and NET clearance, and engulfment of apoptotic neutrophils by phagocytes was found to promote anti-inflammatory signaling and homeostasis maintenance. These results demonstrate that neutrophil contributions to ALI/ARDS are at least in part due to their influence on AMΦ phenotype switching and in part due to the effectiveness of efferocytic clearance following cell death.

**Efferocytosis and resolution of inflammatory lung injury**

Host defense and the protective roles leukocyte recruitment and phagocytosis play in acute inflammatory injury were first described in 1908 by Nobel Prize laureate Elie Metchnikoff. However, much remains unclear as to how cellular communication facilitates apoptotic cell clearance and promotes homeostasis. As many as 150 billion cells, representing 0.4% of the body’s cellular mass, are known to be turned over via apoptosis every day in the average adult. Apoptotic cells are rarely observed, even in tissues with frequent cell turnover, which suggests an efficient framework for clearing dead cells.

Removal of apoptotic cells and debris by phagocytosis, a term coined “efferocytosis” by Henson, Gardai *et al.* (from the Latin *effero* meaning “to carry to the grave” or “to bury”), appears to serve a crucial protective role against inflammatory injury. The process by which dead cells are identified, taken up, and disposed of by phagocytes is a highly regulated and concerted series of coordinated signaling (see below).

**Activation of efferocytosis machinery**

Efferocytic signaling refers to phagocyte recruitment (“find me”), engulfment (“eat me”), and “post-engulfment” signals (Figure 2), and communication of these signals depends on phagocyte/apoptotic cell type, apoptotic stimuli, and stage of apoptosis. Apoptotic cells release “find me” signals to initiate phagocytic uptake. Four different apoptotic “find me” signals have been identified: lysophosphatidylcholine (LPC), sphingosine-1-phosphate (SIP), nucleotides ATP and UTP, and CX3CL1 or fractalkine. The first three mechanisms are caspase-3 dependent: 1) phosphatidylcholine is converted into LPC by apoptotic cells and subsequently released and recognized by G2A receptors on proximal macrophages; 2) SIP, produced by the sphingosine kinase-catalyzed conversion of sphingosine, is released from apoptotic cells and recognized by SIP receptors on macrophages; and 3) apoptotic release of nucleotides ATP and UTP induces monocyte recruitment through recognition by phagocytic purinergic receptors. Furthermore, ATP and UTP receptor P2Y<sub>12</sub> deficiency in mice showed a significant decrease in monocyte and macrophage recruitment, and nucleotide deficiency/P2Y<sub>12</sub> interference also resulted in inadequate clearance of apoptotic thymocytes. In a caspase-3-independent mechanism, CX3CL1, or fractalkine, a membrane-associated protein released by apoptotic cells, binds to CX3C motif chemokine receptor 1 (CX3CR1) on phagocytes to promote recruitment. Additionally, upregulation of several solute carrier (SLC) proteins was found to take place at distinct “find-me” and “eat-me” stages of efferocytosis, suggesting a complex and incompletely understood regulatory system that warrants further investigation. After recognition of these apoptotic “find-me” signals, phagocytes use additional cell signaling mechanisms to dispose of marked cells.

Apoptotic cells expose phosphatidylserine (Ptd-Ser) as an “eat-me” signal that can be recognized by several receptors (Figure 2). Remarkably, many molecules have been shown to act as Ptd-Ser receptors, including scavenger receptors (CD36 and SRA-1), αβ integrins, MerTK, Tim-4, BA11, and stabilin-1 and -2. Many Ptd-Sers recognize multiple ligands and likely have roles other than apoptotic cell clearance. Among the known Ptd-Ser receptors, Tim-4 and BA11 and stabilin-2 directly bind to Ptd-Ser on apoptotic cells. Tim-4 is exclusively expressed on professional phagocytes and the main receptor mediating the phagocytosis of apoptotic cells. Tim-4 is thought to act as a tethering receptor rather than directly transmitting engulfment signals, and Tim-4-dependent efferocytosis depends on the activation of integrins, focal adhesion kinase (FAK), and phosphoinositol-3-kinases. Dysregulation of Tim-4 expression has been found in autoimmune
conditions\(^{95,96}\), and expression of Tim-4 decreased in response to oxidative stress\(^8\). Increased expression of Ptd-Ser receptor MerTK was found in airway macrophages of cigarette smokers and has been implicated in apoptotic cell bud buildup in the lungs of patients with COPD\(^9\). The MerTK–ERK pathway is also known to play a role in the resolution of inflammation\(^9\). Ptd-Ser receptor Axl is thought to play a role in apoptotic cell removal and was found to be expressed in mouse airway macrophages but not in interstitial macrophages or other lung leukocytes\(^{100}\). Receptor for advanced glycation end products (RAGE) is a recently characterized Ptd-Ser receptor highly expressed in AM\(^{+}\)s. RAGE-deficient macrophages showed impaired phagocytic uptake of apoptotic thymocytes and neutrophils and led to increased alveolar accumulation of inflammatory cells following LPS stimulation\(^{101}\). Additionally, calreticulin (CRT) is thought to act as a Ptd-Ser-binding bridging molecule that can behave as an “eat-me” signal in cell death induced by endoplasmic reticulum (ER) stress. Protein kinase RNA-like ER kinase (PERK) phosphorylates eIF2\(\alpha\), promoting Bap31 cleavage and Bax activation in a caspase-8-dependent manner. CRT then moves from the ER to the Golgi apparatus and is displayed by apoptotic cells through SNARE-mediated exocytosis, where recognition of low-density lipoprotein receptor-related protein (CD91) by local phagocyte receptors leads to dead cell engulfment\(^{102}\). Hodge \textit{et al}. have done extensive work investigating efferocytic deficiency in COPD patients and have found reduced CD31, CD91, CD44, and CD71 expression and enhanced Ki-67 expression in the lungs of smokers compared with non-smokers\(^{103}\).

In contrast to “eat me” signals, “don’t eat me” signals are displayed by healthy cells to prevent uptake by phagocytosis. Some of these signals have recently been characterized and are now of clinical interest as potential therapeutic targets in ALI/ARDS recovery. One of these “don’t eat me” signals is integrin-associated protein (CD47), a surface membrane protein activated by activator protein 1 (AP-1) transcription factor c-Jun in fibroblasts, overexpression of which is associated with fibrotic injury\(^{104}\). Interestingly, antibody-mediated blockade of CD47 was found to be sufficient for reversing fibrosis and improving lung function in mice by increasing phagocytosis of profibrotic fibroblasts\(^{104}\). CD47 is an anti-phagocytic molecule that was found to be constitutively expressed in certain myeloid leukemias, indicating its role in assisting
cancer cells by evading phagocytic recognition\textsuperscript{[165]}. Preclinical data on anti-CD47 cancer therapy is promising\textsuperscript{[166,167]}, and clinical trials have shown optimistic results in ameliorating tumor growth\textsuperscript{[166,168]}. Similarly, platelet endothelial cell adhesion molecule (CD31) is another surface membrane protein that plays a role in preventing engulfment by a repulsive CD31–CD31 interaction between healthy cells and phagocytes\textsuperscript{[169]}. Many “don’t eat me” signals with therapeutic potential for alleviating ALI/ARDS remain undiscovered or are not fully understood, so continuous investigation into these molecules is of clinical interest.

**Efferocytosis-induced intracellular signaling**

Following engulfment, phagocytic cytoskeletons must adapt to internalize dead cells. The Rho family of GTPases is an established regulatory factor in cellular movement and cytoskeletal changes\textsuperscript{[171]} and is involved in virtually all actin-dependent processes including mobility, adhesion, and phagocytosis\textsuperscript{[172]}. Rho GTPases use guanine nucleotide exchange factors (GEFs) to switch between inactive, or GDP-bound, and active, or GTP-bound, states\textsuperscript{[173]}. One of these Rho family proteins highly expressed in macrophages\textsuperscript{[174]}, Rac1, induces plasma membrane remodeling to allow phagosome internalization of dead cell particles by stimulating actin polymerization via the Rac-WAVE-Arp2/3 pathway\textsuperscript{[174,175]}. Rac1 activation occurs when engulfment and cell motility protein 1 (ELMO1) and dock of cytokinesis protein 1 (Dock180) work coactively as a GEF for Rac1, promoting the cytoskeletal changes required for internalization\textsuperscript{[175]}. Additionally, the intracellular domain of BAI1 was found to interact with ELMO1 and Dock180 GEF processes\textsuperscript{[176]}. The role of ELMO1/Rac1 signaling in proper inflammatory functions is becoming particularly clear. For example, ELMO1 and Rac1 were found to be necessary for internalization processes and promoting inflammatory signaling, and inhibition of ELMO1 led to a sixfold decrease in *Salmonella* internalization\textsuperscript{[176]}. Furthermore, ELMO1-deficient macrophages experienced reduced in TNF-α and monocyte chemoattractant protein 1 (MCP-1) pathway\textsuperscript{[174,175]}. Rac1 activation requires GEFs, such as Dock180, to promote the cytoskeletal changes required for internalization\textsuperscript{[175]}. Rac1 activation signals in proper inflammatory functions are becoming particularly clear. For example, ELMO1 and Rac1 were found to be necessary for internalization processes and promoting inflammatory signaling, and inhibition of ELMO1 led to a sixfold decrease in *Salmonella* internalization\textsuperscript{[176]}. Furthermore, ELMO1-deficient macrophages experienced reduced TNF-α and monocyte chemoattractant protein 1 (MCP-1) signal pathway\textsuperscript{[174,175]}. Rac1 actin polymerization abilities likely involve interplay with ERK, FAK, AKT, and STAT6 as well\textsuperscript{[177]}. Other members of the Rho GTPase family including Rac2, Rho, and Cdc42 showed involvement in macrophage efferocytosis\textsuperscript{[178]}. Rho A is known to antagonize Rac1-mediated actin reorganization\textsuperscript{[179,180]}, and its suppression assisted in apoptotic engulfment, whereas its overexpression inhibited phagocytic uptake\textsuperscript{[179]}. Cigarette smoke has been found to inhibit efferocytosis through oxidant-dependent activation of RhoA, but antioxidants supplementation prevented this effect, leading to the reversal of efferocytic impairment\textsuperscript{[181]}. RhoA was found to assist in actomyosin cytoskeleton contractions via the Rho-associated coiled-coil-containing protein kinase (ROCK) pathway, as well as in other processes including cell proliferation and migration\textsuperscript{[182]}. C-type lectins like mannose-binding lectin (MBL) have also shown therapeutic potential. MBL promotes apoptotic cell uptake by increasing Rac1/2/3 expression and is reduced in airways of COPD patients\textsuperscript{[183]}.

**Oxidant stress and efferocytosis impairment**

The accumulation of ROS inflicts intracellular destruction and initiates enhanced pro-inflammatory gene expression and cell death\textsuperscript{[184]}. Engulfment of apoptotic cells creates an abundance of harmful ROS, and macrophage functions are heavily regulated by ROS production\textsuperscript{[185,186]}. ROS are involved in macrophage polarization, functional and phenotypic regulation, and cell death, proliferation, and phagocytic ability\textsuperscript{[187]}. ALI/ARDS patients exhibit significant oxidative stress on the lungs due to ventilation therapy-induced ROS accumulation\textsuperscript{[188]}. Mitigation of excessive ROS and maintenance of intracellular redox homeostasis is crucial for ALI resolution. Cells have intrinsic antioxidant defense mechanisms for maintaining equilibrium in response to excess ROS generation\textsuperscript{[189,190]}. A better understanding of the host defense response, such as antioxidant enzyme functions and deregulation in ARDS patients with and without hyperoxic ventilation, can help detect therapeutic targets that may prove useful for alleviating lung injury.

Hyperoxic stress and ROS accumulation can lead to functional changes in AMΦs. For example, ROS production upregulates the expression of AMΦ-associated pro-inflammatory transcription factors AP-1 and NF-κB\textsuperscript{[191]}. Additionally, TNF-α is known to induce activation of NF-κB and AP-1, both of which in accordance produce inflammatory signals used by AMΦs for communicating the inflammatory response to other cells in the lung\textsuperscript{[192]}. The TNF-α promoter region was found to contain both NF-κB and AP-1 binding sites, allowing for autoregulation\textsuperscript{[193]}. Glucocorticoids inhibit NF-κB and impair binding of AP-1, leading to a decrease in pro-inflammatory cytokine production, but administration of these drugs can detrimentally dampen the immune response to acute injury, making them oftentimes more harmful than useful to ALI/ARDS patients\textsuperscript{[194]}. Another transcription factor, IFN regulatory factor 5 (IRF5), polarizes macrophages toward the pro-inflammatory phenotype (M1)\textsuperscript{[195]} and was found in neutrophils and other myeloid cells\textsuperscript{[196]}. Blockage of IRF5 is being investigated as a therapeutic measure for alleviating inflammation\textsuperscript{[197]} and promoting efferocytosis\textsuperscript{[198]}. Another IFN-regulated transcription factor is STAT1, a member of the STAT protein family. STAT1 is known to activate quickly in response to IFNs and other pro-inflammatory cytokines and leads to mitochondrial stress, ROS accumulation, and apoptosis\textsuperscript{[199]}. STAT1 has been shown to modulate intracellular oxidative stress in macrophages through a p38 MAPK/STAT1/ROS positive feedback loop\textsuperscript{[200]} and the absence of NADPH oxidase (NOX)-derived superoxide in AMΦs\textsuperscript{[201]} was shown to reduce both STAT1 and IRF5 expression as well as increase AMΦ transcriptional profile\textsuperscript{[202]}. ROS accumulation and inflammatory signaling are intimately related, and a better understanding of their genetic basis and how it relates to the AMΦ protective role in efferocytosis can potentially reveal therapeutic targets to assist recovery in ALI/ARDS patients.
Activation of efferocytosis to accelerate lung injury resolution

Imbalance between apoptotic cell death and efferocytosis can promote pathological conditions such as ALI/ARDS, COPD, cystic fibrosis, and asthma\(^2,14\), and impaired efferocytosis is implicated in complications associated with these conditions\(^14\). Inflammation in these lung diseases appears to worsen with inefficient removal of dead cells and debris, thus prolonging inflammation and impeding tissue repair. Delayed efferocytosis can cause apoptotic cells to undergo secondary necrosis and release DAMPs, which further promote inflammation by stimulating both innate and adaptive immune responses\(^6\). Efferocytotic impairment of airway macrophages leads to apoptotic and necrotic cell buildup, DAMPs release, upregulation of pro-inflammatory genes, and production of autoreactive T cells and B cells, all of which contribute to autoimmunity and chronic inflammation\(^15\). Prolonged inflammation can weaken resolution and potentially develop into fibrosis or chronic inflammatory conditions such as COPD\(^15\). Efferocytosis promotes lung homeostasis, facilitates resolution of apoptotic cell-induced inflammation\(^16\), and corresponds with improved clinical outcomes in ALI/ARDS patients\(^20\).

Although defective clearance of apoptotic cells in the development of ALI/ARDS has been proposed\(^20,142\) and oxidant stress affects efferocytotic activity of macrophages in vitro\(^46,147\) and in vivo\(^6\), the exact mechanisms contributing to dysfunctional efferocytosis are not completely understood. Further characterizing the molecular mechanisms used by AM\(^{\uparrow}\)s to perform efferocytosis and resolve excessive inflammation in the lung, and how and which efferocytosis machinery is activated or affected/impaired in acute clinical syndromes resulting in chronic lung diseases, may offer better clinical prognosis and therapeutic treatment strategies. Identifying both the activators and the effectors of efferocytosis that can be easily and preferentially targetable with fewer off-target effects, perhaps by administration of small molecules/drugs (protein or non-protein), is necessary to optimally accelerate lung tissue repair in ALI/ARDS patients and for improving clinical outcomes and reducing huge healthcare costs associated with microbial- and non-microbial-induced lung injury.

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References

1. Rubenfeld GD, Caldwell E, Peabody E, et al.: Incidence and outcomes of acute lung injury. N Engl J Med. 2005; 353(16): 1685–93. Published Abstract | Publisher Full Text | Faculty Opinions Recommendation

2. Young M, Dilliwio B, Rao S, et al.: Mechanical Ventilation in ARDS. Crit Care Nurs Q. 2019; 42(4): 392–9. Published Abstract | Publisher Full Text | Faculty Opinions Recommendation

3. Hunter JD: Ventilator associated pneumonia. BMJ. 2012; 344: e3325. Published Abstract | Publisher Full Text

4. Dietrich ES, Demmler M, Schulgen G, et al.: Nosocomial pneumonia: A cost-of-illness analysis. Infection. 2002; 30(2): 61–7. Published Abstract | Publisher Full Text

5. Huppert LA, Matthay MA, Ware LB: Pathogenesis of Acute Respiratory Distress Syndrome. Semin Respir Crit Care Med. 2019; 40(1): 31–9. Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

6. Matthay MA, Zemans RL, Zimmerman GA, et al.: Acute respiratory distress syndrome. Nat Rev Dis Primers. 2019; 5(1): 18. Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

7. Cafche C, Delucchi KL, Sinha P, et al.: Acute respiratory distress syndrome subphenotypes and differential response to simvastatin: Secondary analysis of a randomised controlled trial. Lancet Respir Med. 2018; 6(9): 691–. Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

8. Kzak A, Pleva M, Napolitano LM: Nutrition therapy for ALI and ARDS. Crit Care Clin. 2011; 27(3): 647–59. Published Abstract | Publisher Full Text

9. Laffey JG, Matthay MA: Fifty Years of Research in ARDS. Cell-based Therapy for Acute Respiratory Distress Syndrome. Biology and Potential Therapeutic Value. Am J Respir Crit Care Med. 2017; 196(3): 266–73. Published Abstract | Publisher Full Text | Free Full Text

10. Matthay MA, Cafche CS, Zhuo H, et al.: Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): A randomised phase 2a safety trial. Lancet Respir Med. 2019; 7(2): 154–62. Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

11. Piaiaik M, Lee YD, Rasmussen DL, et al.: Poloxamer 188 facilitates the repair of alveolar resident cells in ventilator-injured lungs. Am J Respir Crit Care Med. 2011; 184(8): 939–47. Published Abstract | Publisher Full Text | Free Full Text

12. Lindsay CD: Novel therapeutic strategies for acute lung injury induced by lung...
damaging agents: The potential role of growth factors as treatment options. 

_Hum Exp Toxicol._ 2011; 30(7): 701–24. 

_Published Abstract | Publisher Full Text_

13. Álvaraceta GM, Gutiérrez-Fernández A, Parra D, et al.: Lack of matrix metalloproteinase-9 worsens ventilator-induced lung injury. _Am J Physiol Lung Cell Mol Physiol._ 2008; 294(3): L535–43. 

_Published Abstract | Publisher Full Text | Faculty Opinions Recommendation_

14. Álvaraceta GM, Gutiérrez-Fernández A, García-Prieto E, et al.: Absence or inhibition of matrix metalloproteinase-9 decreases ventilator-induced lung injury. _Am J Respir Cell Mol Biol._ 2010; 43(5): 555–63. 

_Published Abstract | Publisher Full Text_

15. González-López A, Álvaraceta GM: Repair after acute lung injury: Molecular mechanisms and therapeutic opportunities. _Crit Care._ 2012; 16(2): 209. 

_Published Abstract | Publisher Full Text | Free Full Text_

16. Lee JM, Kwon HJ, Bae SC, et al.: Lung tissue regeneration after induced injury in Rnu/N mice. _Cell Tissue Res._ 2010; 341(3): 469–79. 

_Published Abstract | Publisher Full Text | Free Full Text_

17. Martin TR, Frevert CW: Innate immunity in the lungs. _Proc Am Thorac Soc._ 2005; 2(6): 403–9. 

_Published Abstract | Publisher Full Text | Free Full Text_

18. Fan EKY, Fan J: Regulation of alveolar macrophage death in acute lung inflammation. _Respir Res._ 2018; 19(1): 50. 

_Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation_

19. Roh JS, Sohn DH: Damage-Associated Molecular Patterns in Inflammatory Diseases. _Immun Netw._ 2018; 18(4): e27. 

_Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation_

20. Schmidt EP, Tudor RM: Role of Apoptosis in Amplifying Inflammatory Responses in Lung Diseases. _J Cell Death._ 2010; 3: 41–53. 

_Published Abstract | Free Full Text_

21. Yurdagul A, Subramanian M, Wang X, et al.: Macrophage Metabolism of Apoptotic Cell-Derived Arginine Promotes Continual Efferocytosis and Resolution of Injury. _Cell Metab._ 2020; 31(3): 518–533.e10. 

_Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation_

22. Morrell ED, Wiedeman A, Long SA, et al.: Cytometry TOF identifies alveolar macrophage subtypes in acute respiratory distress syndrome. _JCI Insight._ 2018; 3(10): e99281. 

_Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation_

23. Matthay MA, Ware LB, Zimmerman GA: The acute respiratory distress syndrome. _J Clin Invest._ 2012; 122(8): 2731–40. 

_Published Abstract | Publisher Full Text | Free Full Text_

24. Elliott MR, Koster KM, Murphy PB: Efferocytosis Signaling in the Regulation of Macrophage Inflammatory Responses. _J Immunol._ 2017; 198(4): 1387–94. 

_Published Abstract | Publisher Full Text | Free Full Text_

25. Chimello D, Coppola S, Friso S, et al.: What’s Next After ARDS: Long-Term Outcomes. _Respir Care._ 2016; 61(5): 689–99. 

_Published Abstract | Publisher Full Text_

26. Albetime KH, Soulier MF, Wang Z, et al.: Fas and Fas Ligand Are Up-Regulated in Pulmonary Edema Fluid and Lung Tissue of Patients with Acute Lung Injury and the Acute Respiratory Distress Syndrome. _Am J Pathol._ 2002; 161(5): 1783–96. 

_Published Abstract | Publisher Full Text | Free Full Text_

27. Matute-Bello G, Liles WC, Steinberg KP, et al.: Soluble Fas ligand induces epithelial cell apoptosis in humans with acute lung injury (ARDS). _J Immunol._ 1999; 163(4): 2217–25. 

_Published Abstract | Publisher Full Text | Free Full Text_

28. Lopez AD, Avasariil S, Grewe S, et al.: Differential role of the Fas/Fas ligand apoptotic pathway in inflammation and lung fibrosis associated with reovirus 1/L-induced bronchiolitis obliterans organizing pneumonia and acute respiratory distress syndrome. _J Immunol._ 2005; 165(3): 8244–57. 

_Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation_

29. Kawasuki M, Kusakino K, Hagimoto N, et al.: Protection from Lethal Apoptosis in Lipopolysaccharide-Induced Acute Lung Injury in Mice by a Caspase Inhibitor. _Am J Pathol._ 2000; 157(2): 597–603. 

_Published Abstract | Publisher Full Text | Free Full Text_

30. Le A, Damico R, Saria M, et al.: Alveolar epithelial cell apoptosis is dependent on p38 MAP kinase-mediated activation of xanthine oxidoreductase in ventilator-induced lung injury. _J Appl Physiol._ 1988; 2005; 105(4): 1282–90. 

_Published Abstract | Publisher Full Text | Free Full Text_

31. Gambini S, Casciaro M, Trapani G, et al.: Association between HMGB1 and COPD: A Systematic Review. _Mediators Inflamm._ 2015; 2015: 164913. 

_Published Abstract | Publisher Full Text | Free Full Text_

32. Vande Walle L, Lamkarfi M: Pyroptosis. _Curr Biol._ 2016; 26(13): R568–R572. 

_Published Abstract | Publisher Full Text | Free Full Text_

33. He W, Wan H, Hu L, et al.: Gasdermin D is an executor of pyroptosis and required for interleukin-1β secretion. _Cell Res._ 2015; 25(12): 1285–98. 

_Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation_

34. Nada-Romero E, Martínez J, Heckmann BL, et al.: The clearance of dead cells by efferocytosis. _Nat Rev Mol Cell Biol._ 2020; 21(7): 398–414. 

_Published Abstract | Publisher Full Text | Free Full Text_

35. Frank D, Vinc JE: Pyroptosis versus necroptosis: Similarities, differences, and crosstalk. _Cell Death Dis._ 2019; 2019; 26(1): 1–114. 

_Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation_

36. Newton K, Manning G: Necroptosis and Inflammation. _Annu Rev Biochem._ 2016; 85: 743–63. 

_Published Abstract | Publisher Full Text | Free Full Text_

37. Denton D, Kumar S: Autophagy-dependent cell death. _Cell Death Differ._ 2019; 26(4): 605–16. 

_Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation_

38. Li J, Cao F, Yin HL, et al.: Ferroptosis: Past, present and future. _Cell Death Dis._ 2020; 11(2): 88. 

_Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation_

39. Park SY, Kim JS: Engagement signals and the phagcicrypr machinery for apoptotic cell clearance. _Exp Mol Med._ 2017; 49(5): e331. 

_Published Abstract | Publisher Full Text | Free Full Text_

40. Tan HY, Wang N, Li S, et al.: The Reactive Oxygen Species in Macrophage Polarization: Reflecting Its Dual Role in Progression and Treatment of Human Diseases. _Oxid Med Cell Longev._ 2016; 2016: 2795090. 

_Published Abstract | Publisher Full Text | Free Full Text_

41. Brune B, Dehne N, Grossmann N, et al.: Redox control of inflammation in macrophages. _Antioxid Redox Signal._ 2013; 19(6): 595–637. 

_Published Abstract | Publisher Full Text | Free Full Text_

42. Robb CT, Regan KH, Doward DA, et al.: Key mechanisms governing resolution of lung inflammation. _Semim Immunopathol._ 2016; 38(4): 425–48. 

_Published Abstract | Publisher Full Text | Free Full Text_

43. Wynn TA, Vannella KM: Macrophages in Tissue Repair, Regeneration, and Fibrosis. _Immunol._ 2016; 44(3): 450–62. 

_Published Abstract | Publisher Full Text | Free Full Text_

44. Puttar F, Gregory LG, Lloyd CM: Airway macrophages as the guardians of tissue repair in the lung. _Immunol Cell Biol._ 2019; 97(3): 246–57. 

_Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation_

45. Steinberg KP, Milberg JA, Martin TR, et al.: Evolution of bronchoalveolar cell populations in the adult respiratory distress syndrome. _Am J Respir Crit Care Med._ 1994; 150(1): 113–22. 

_Published Abstract | Publisher Full Text | Free Full Text_

46. Rosseau S, Hammal P, Mauss U, et al.: Phenotypic characterization of alveolar monocye recruitment in acute respiratory distress syndrome. _Am J Physiol Lung Cell Mol Physiol._ 2000; 278(1): L25–35. 

_Published Abstract | Publisher Full Text | Free Full Text_

47. Johnston LK, Rims CR, Gill SE, et al.: The clearance of dead cells by efferocytosis. _J Immunol._ 2017; 199(6): 2709–18. 

_Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation_

48. Aggarwal NR, King LS, D’ Alessio FR: Diverse macrophage populations mediate acute lung inflammation and resolution. _Am J Physiol Lung Cell Mol Physiol._ 2014; 306(8): L709–25. 

_Published Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation_

49. Rabinovitch M: Professional and non-professional phagocytes: An introduction. _Trends Cell Biol._ 1995; 5(3): 85–7. 

_Published Abstract | Publisher Full Text | Free Full Text_

50. Hayama D, Iida T, Nakase H: The Phagocytic Function of
96. Rodriguez-Manzanet R, Sanjuan MA, Wu HY, et al.: T and B cell hyperactivity and autoimmunity associated with nongenetic defects in apoptotic body clearance in TIM-4-deficient mice. Proc Natl Acad Sci U S A. 2010; 107(19): 8790–11.

97. Fraser ST, Miederer RG, Coupland LA, et al.: Heme oxygenase-1 deficiency alters erythroblastic island formation, steady-state erythropoiesis and red blood cell lifespan in mice. Haematologica. 2015; 100(5): 601–10.

98. Kazerios A, Harvey BG, Carolan BJ, et al.: Mechanisms and Implications for Disease Pathogenesis. Turning on the switch. J Clin Oncol. 2020; 38(18): 2151–39.

99. Liu J, Wang L, Zhao F, et al.: Pre-Clinical Development of a Humanized Anti-CD47 Antibody with Anti-Cancer Therapeutic Potential. PLoS One. 2015; 10(9): e0137345.

100. Li W, Chen SY, Lerro T, et al.: Activation of JUN in fibroblasts promotes pro-fibrotic program and modulates protective immunity. Nat Commun. 2020; 11(1): 2795.

101. Jin J, Wang L, Zhao F, et al.: Pre-Clinical Development of a Humanized Anti-CD47 Antibody with Anti-Cancer Therapeutic Potential. PLoS One. 2015; 10(9): e0137345.

102. Willingham SB, Volkmer JP, Gentles AJ, et al.: The CD47-signal regulatory protein alpha (SIRPα) interaction is a therapeutic target for human solid tumors. Proc Natl Acad Sci U S A. 2012; 109(17): 6662–7.

103. Advani R, Flinn I, Popplewell L, et al.: Mechanisms and Implications for Disease Pathogenesis. 2016; 8: 427–31.

104. Castellano F, Montourier P, Chavrier P: Membrane recruitment of Rac1 triggers phagocytosis. J Cell Sci. 2000; 113(Pt 17): 2955–61.

105. Molinie N, Gautreau A: The Arg23 Regulatory System and Its Deregulation in Cancer. Physiol Rev. 2018; 98(1): 215–38.

106. Brugnera E, Haney L, Grimsley C, et al.: Unconventional Rac-GEF activity is mediated through the Dock180-ELMO complex. Nat Cell Biol. 2002; 4(8): 574–82.

107. Park D, Tosello-Trampont AC, Elliott MR, et al.: BA1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. Nature. 2007; 450(7168): 403–9.

108. Liu X, Yin S, Chen Y, et al.: Cigarette smoke impairs engulfment of alveolar macrophages in vitro: Role of Apo-1 and NF-kappaB. Am J Respir Cell Mol Biol. 2001; 25(5): L905–13.

109. Reddy SP, Hassoun PM, Broder R: Redox imbalance and ventilator-induced lung injury. Antioxid Redox Signal. 2007; 9(11): 2003–12.

110. Nishi C, Yanagihashi Y, Segawa K, et al.: MerTK tyrosine kinase receptor together with TIM4 phosphatidylserine receptor mediates distinct signal transduction pathways for efferocytosis and cell proliferation. J Biol Chem. 2015; 290(18): 12955–66.

111. Redzic-Outodorov M, Averill-Bates DA: Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta. 2016; 1863(12): 2977–92.

112. Cho HY, Reddy SP, Kleibeuger SR: NRF2 defends the lung from oxidative stress. Antioxid Redox Signal. 2006; 8(1–2): 76–87.

113. Hoppe AD, Swanson JA: Cdc42, Rac1, and Rac2 display distinct patterns of activation during phagocytosis. Mol Biol Cell. 2004; 16(6): 3509–19.

114. Li L, Chen SY, Lerro T, et al.: Activation of JUN in fibroblasts promotes pro-fibrotic program and modulates protective immunity. Nat Commun. 2020; 11(1): 2795.

115. Julan L, Olson MF: Rho-associated coiled-coil containing kinases (ROCK): Structure, regulation, and functions. Small GTPases. 2014; 5: e25846.

116. Richters TR, Linderman DJ, Horstmann SA, et al.: Cigarette smoke impairs engulfment of alveolar macrophages in vitro: Role of Apo-1 and NF-kappaB. Am J Respir Cell Mol Biol. 2001; 25(5): L905–13.

117. Bencivenga S, Berson DS, Kellett RA, et al.: Hyperoxia upregulates the NO pathway in alveolar macrophages in vitro: Role of Ap-1 and NF-kappaB. Am J Physiol Lung Cell Mol Physiol. 2001; 280(5): L505–13.

118. Li H, Sidipoulos P, Song G, et al.: TNF-alpha gene expression in macrophages: Regulation by NF-kappaB is independent of e-Jun or C/EBP beta. J Immunol. 2000; 164(8): 4277–85.

119. Chow CW, Herrera Abreu MT, Suzuki T, et al.: Oxidative stress and acute lung injury. Am J Respir Cell Mol Biol. 2003; 29(4): 427–31.
135. Krausgruber T, Blazek K, Smallie T, et al.: IRF5 promotes inflammatory macrophage polarization and T<sub>H</sub>1-T<sub>H</sub>17 responses. Nat Immunol. 2011; 12(3): 231–8. PubMed Abstract | Publisher Full Text | Faculty Opinions Recommendation

136. Weiss M, Byrne AJ, Blazek K, et al.: IRF5 controls both acute and chronic inflammation. Proc Natl Acad Sci U S A. 2015; 112(35): 11001–6. PubMed Abstract | Publisher Full Text | Free Full Text

137. Almuttagi H, Udalova IA: Advances and challenges in targeting IRFs, a key regulator of inflammation. FEBS J. 2019; 286(9): 1624–37. PubMed Abstract | Publisher Full Text | Free Full Text | Faculty Opinions Recommendation

138. Seneviratne AN, Edsfeldt A, Cole JE, et al.: Interferon Regulatory Factor 5 Controls Necrotic Core Formation in Atherosclerotic Lesions by Impairing Efferocytosis. Circulation. 2017; 136(12): 1140–54. PubMed Abstract | Publisher Full Text | Free Full Text

139. Lee HJ, Oh YK, Rhee M, et al.: The role of STAT1/IRF-1 on synergistic ROS production and loss of mitochondrial transmembrane potential during hepatic cell death induced by LPS/d-GalN. J Mol Biol. 2007; 369(4): 967–84. PubMed Abstract | Publisher Full Text

140. Kim HS, Lee MS: Essential role of STAT1 in caspase-independent cell death of activated macrophages through the p38 mitogen-activated protein kinase/STAT1/reactive oxygen species pathway. Mol Cell Biol. 2005; 25(15): 6821–33. PubMed Abstract | Publisher Full Text | Free Full Text

141. Padgett LE, Burg AR, Lei W, et al.: Loss of NADPH oxidase-derived superoxide skews macrophage phenotypes to delay type 1 diabetes. Diabetes. 2015; 64(3): 937–46. PubMed Abstract | Publisher Full Text | Free Full Text

142. Yun JH, Henson PM, Tudor RM: Phagocytic clearance of apoptotic cells: Role in lung disease. Expert Rev Respir Med. 2008; 2(6): 753–65. PubMed Abstract | Publisher Full Text | Free Full Text

143. Vandiver RW, Henson PM, Douglas IS: Burying the dead: The impact of failed apoptotic cell removal (efferocytosis) on chronic inflammatory lung disease. Chest. 2006; 129(6): 1673–82. PubMed Abstract | Publisher Full Text

144. McCubbrey AL, Curtis JL: Efferocytosis and lung disease. Chest. 2013; 143(6): 1750–7. PubMed Abstract | Publisher Full Text | Free Full Text

145. Sachet M, Liang YY, Oehler R: The immune response to secondary necrotic cells. Apoptosis. 2017; 22(10): 1189–204. PubMed Abstract | Publisher Full Text | Free Full Text

146. Raffin TA, Simon LM, Braun D, et al.: Impairment of phagocytosis by moderate hyperoxia (40 to 60 per cent oxygen) in lung macrophages. Lab Invest. 1980; 42(6): 622–6. PubMed Abstract

147. Crowell RE, Hallin G, Heaphy E, et al.: Hyperoxic suppression of Fc-gamma receptor-mediated phagocytosis by isolated murine pulmonary macrophages. Am J Respir Cell Mol Biol. 1995; 12(2): 190–5. PubMed Abstract | Publisher Full Text