Diverse macrophage populations mediate acute lung inflammation and resolution

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ALVEOLAR MACROPHAGES form the first line of defense against airborne particles and microbes and use a variety of pattern recognition and scavenger receptors to sense and phagocytose pathogens. Macrophages remain in constant exposure to the outside world with close contact to both air- and blood-borne materials (98). Since the introduction of the macrophage system by Metchnikoff in the late 19th century when it was first described as a phagocytic cell, dynamic factors have been uncovered to define a heterogeneous cell population identified by differences in maturation, tissue migration, phenotypes, and cellular interaction (35, 160, 179). The presence and severity of tissue inflammation is paramount in defining macrophage sub-populations.

In the lung and alveolar space, inflammation is intimately tied to macrophage phenotype and function, a process well described during the acute inflammatory stage of acute respiratory distress syndrome (ARDS) and now increasingly being recognized during resolution and recovery from ARDS (63). Studies performed over 10 years ago in humans with ARDS using serial bronchoalveolar lavages (BAL) determined that increased alveolar macrophage number and a mature cellular phenotype were associated with a favorable outcome (156, 172); however, a causative role for macrophages in enhancing resolution and repair from human ARDS remains unknown. Using diverse experimental acute lung injury (ALI) and resolution models, investigators have identified important cellular mechanisms and molecular pathways of macrophage response to help define their roles in lung homeostasis, acute inflammation, resolution, and repair (Fig. 1). We will focus on key aspects of lung macrophage biology (phenotype, differentiation, function, and fate) within the context of acute inflammation and resolution responses. We will identify areas of controversy and those with therapeutic potential and will summarize our understanding in humans.

Macrophage Phenotype at Steady State

Murine. At a portal of entry to the body, alveolar and lung macrophages encounter constant exposure to foreign particles and infectious agents (182). During steady state, lung macrophages express relatively high levels of pathogen-associated molecular pattern (PAMP) and danger-associated molecular pattern (DAMP) receptors. Macrophages express molecular pattern (PAMP) and danger-associated molecular pattern (DAMP) receptors. Macrophages express mannose receptor (CD206) and β-glucan specific receptor (Dectin-1), potent scavenger receptors that also help define a M2, alternative-activated, anti-inflammatory macrophage phenotype.
**Macrophage Immunobiology**

**Steady-state**
- Microbe phagocytosis
- Debris removal
- Local proliferation
- Danger/scaevenger repressor expression

**Inflammation**
- Inflammatory cytokine secretion
- Neutrophil recruitment
- Monocyte/inflammatory macrophage recruitment
- T-effector cell interaction

**Resolution**
- Cessation of cell recruitment
- Neutrophil/macrophage apoptosis
- Treg interaction
- Epithelial repair

**Alveolar macrophage (M1)**
- IFN-γ
- TNF-α
- IL-1β
- IL-12

**Alveolar macrophage (M2)**
- IL-4
- IL-13
- IL-10

**Cytokines and Chemokines**
- TNF-α
- MIP-2/KC
- TGF-β
- IL-10
- IL-1ra
- TRAIL
- CCL2
- CCR2
- CD86
- CD28
- CD11b
- CD11c
- M-CSF
- MHCII
- CD28
- CD86

**Macrophage Activation**
- TLRs
- Dectin-1
- CD11b
- M-CSF
- GM-CSF
- MCP-1
- CCL2
- CCR2
- CD11b
- MIP-2/KC

**Phagocytosis**
- Integrin αβ6
- TLRs
- IFN-γ
- TNF-α
- IL-1β
- IL-12

**Apoptosis**
- IL-1ra
- TRAIL
- MMPs

**Recruitment**
- CCL2
- CCR2
- CD11b

**Epithelial Repair**
- Treg
- IL-1ra
- Lipoxins
- Resolvins
- Growth factors

**PAMPs**
- PAMPs

**DAMPs**
- DAMPs

**Stimulus**
- Stimulus

**Alveolus**
- Alveolar macrophage
- Alveolar macrophage (M1)
- Alveolar macrophage (M2)

**Capillary**
- PMN
- T effector
- Treg
- TRAIL

**Steady-state Inflammation Resolution**
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inflammatory macrophage cell activation state (35, 182). Quiescent alveolar macrophages express high levels of CD11c, a marker not typically expressed by other tissue macrophages (60, 195), and express low levels of CD11b, a marker with higher expression on most other tissue macrophages (60). Kasahara et al. (84) demonstrated that newborn mice exposed to microbial extracts increased CD11c+ cells in the airway, introducing a possible mechanism for induction of alveolar macrophage CD11c expression due to persistent airway pathogen exposure into adulthood. Using adoptive transfer experiments, Guth et al. (60) demonstrated the influential effects of the lung and airway microenvironment on macrophage phenotype, including local granulocyte-macrophage colony-stimulating factor (GM-CSF) secretion.

Morphologically, alveolar macrophages resemble other tissue macrophages with their large size and increased cytoplasm/nucleus ratio. Lung interstitial macrophages are smaller, with reduced granularity and autofluorescence (98, 128), and may represent an intermediate cell population between blood monocytes and terminally differentiated alveolar macrophages (17, 68, 98). Even though interstitial and alveolar macrophages can both express CD11c and F4–80 with high intensity (76, 128, 203), CD11b expression is generally higher on interstitial macrophages (128, 203). Macrophage form and function are also related, as McWhorter et al. (120) recently demonstrated that M1-polarizing stimuli (endotoxin, IFN-γ) induced a rounded cell shape whereas M2-polarizing stimuli (IL-4, IL-13) induced an elongated cell shape. Furthermore, regulating cell shape by a micropatterning technique demonstrated that cell elongation, without other stimuli, enhanced a M2 phenotype and anti-inflammatory cytokine production in an actin-dependent manner.

DEFINING SUBPOPULATIONS. Concise definitions of different lung macrophage subpopulations need optimization and should include a standardized list of cell surface flow markers that accurately distinguish dendritic cells from macrophages as well. With the advancement of immunological tools including multiparameter flow cytometry, knowledge and quantification of membrane glycoproteins to immunophenotype macrophages is attainable, especially when verified by gene assessment of traditional macrophage markers (Table 1). Flow cytometric analysis is limited by macrophage autofluorescence, necessitating techniques to minimize its interference with other exogenously applied fluorophores. Approaches to circumvent this problem include avoidance of the fluorescein or FITC fluorophores that exhibit similar wavelength spectra (43), or, as demonstrated by Soroosh et al. (168), using relative cell autofluorescence to help identify macrophages and dendritic cells. Most studies suggest a combination of cell surface markers F4–80+, CD11b+/-, CD11c+, and major histocompatibility complex (MHC) II+ to identify mature or interstitial macrophages in the lung and alveolar space (58, 81, 103, 182, 189). Although macrophages and dendritic cells both express CD11c, dendritic cells express higher levels of MHC II, and macrophages express higher levels of F4–80. Recently, Zaynagedinov et al. (203) demonstrated that CD68, an intracellular marker that requires cell permeabilization, was more specific for alveolar macrophages (CD68hi, F4–80+, CD11b–) and helped to distinguish them from dendritic cells (CD68hi, CD11c+, F4–80–), interstitial macrophages (CD68low, F4–80+, CD11b+, CD11c+, CD14low), or monocytes (CD68low, F4–80+, CD11b+, CD11c–, CD14–) during steady state.

Cell immunophenotyping in other tissues may be more refined. Meredith and coworkers (126, 127) utilized expression of zinc finger transcription factor to distinguish bone marrow-derived classical dendritic cells from plasmacytoid dendritic cells and monocyte/macrophages, and Rivollier et al. (155) distinguished dendritic cells (CD103+) from macrophages (CX3CR1hi) in colonic lamina propria using surface markers. For the majority of macrophage surface markers (Table 1, top), the functional significance is not well known. Exceptions include CD11b, which is required for monocyte migration to sites of inflammation (152), and CX3CR1, which is required for patrolling and monocyte survival (8, 96). Additionally, CD115 is important for a number of functions as the macrophage colony-stimulating factor (M-CSF) receptor, including macrophage proliferation and differentiation.

Human. AUTOFLORESCENCE. Phenotypic characterization of human lung macrophages at steady state is hindered by a lack of accessibility to samples. In addition, the intensity of autofluorescence in lung macrophages is comparable to exogenously applied fluorophores and is only partially overcome with advances in flow cytometry. Edelson et al. (43) described the autofluorescence spectrum: excitation at wavelengths 370 and 490 nm, and maximum emission at 541 nm with a shoulder at 580 nm; heterogeneity in intensity and autofluorescent wavelengths between subjects further complicates the assessment (199). Intracellular storage of fluorescent flavoprotein, lipofuscin, cigarette smoke hydrocarbons, and other factors prevalent because of the unique position of lung macrophages at the

Fig. 1. A schematic displaying major alveolar macrophage phenotypes and functions during steady state, inflammation, and resolution. During quiescence, macrophages are tethered to epithelial cells, with persistent expression of pattern recognition (TLRs) and scavenger (Dectin-1) receptors. Turnover is slow and driven primarily by M-CSF/GM-CSF induced local proliferation, with minimal recruitment from circulating monocytes. With increased foreign antigen stimulation (PAMPs, DAMPs), macrophage activation occurs, skewing resident macrophages toward an M1 activation state (transcription factors NF-κB, STAT1, IRF-5; cytokines IFN-γ, TNF-α, IL-1β, IL-12) hallmarkied by secretion of proinflammatory cytokines capable of neutrophil (MIP-2/KC) and inflammatory monocyte/macrophage recruitment (MCP-1/CCL2), as well as stimulation of alveolar epithelial cells (TNF-α) and effector T cells (CD86/CD28) to help promote and sustain the inflammatory response. With sufficient control of foreign antigen, macrophages reprogram toward an M2 activation state (transcription factors STAT6, STAT3, IRF-4, 15-LO; cytokines IL-4, IL-13, IL-10) and coordinate a series of regulated responses to abrogate inflammation, and enhance resolution/repair. Key processes include cessation of inflammatory cell recruitment (IL-1ra, MMPs); apoptosis of inflammatory cells (IL-1ra, TRAIL, IL-10, TGF-β); interaction with Tregs; and secretion of lipoxins, resolvins, and growth factors to promote epithelial repair and type II to type I AEC transition. AEC, alveolar epithelial cell; CCL2, chemokine (C-C motif) ligand 2; CCR2, chemokine (C-C motif) receptor 2; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN-γ, interferon-γ; IL, interleukin; INF-γ, interferon-gamma; M-CSF, macrophage colony-stimulating factor; MCP-1, monocyte chemotactic protein-1; MMP-2, macrophage inflammatory protein-2; MPPs, matrix metalloproteinases; MR, mannose receptor (MRC1); NF-κB, nuclear factor-k-light-chain-enhancer of activated B cells; PMN, polymorphonuclear leukocyte (neutrophil); STAT, signal transducer and activator of transcription; TGF-β, transforming growth factor β; TLRs, Toll-like receptors; TNF-α, tumor necrosis factor-α; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; Treg, regulatory T cell.
### Table 1 Macrophage/monocyte activation markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>Classification</th>
<th>Primary Function(s)</th>
<th>Stimulus/Receptor</th>
<th>Human Expression</th>
<th>Murine Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11b (Mac-1, Itgam)</td>
<td>+/+ IM/MO, ± AM, + DC</td>
<td>cell adhesion/migration, complement-opsonized phagocytosis</td>
<td>ICAM-1 (CD54)</td>
<td>nonresolving ARDS (156)</td>
<td>lower in alveolar space; increased in migrated cells</td>
</tr>
<tr>
<td>CD11c (Itgax)</td>
<td>± IM/MO, +/+ AM, +/+ + DC</td>
<td>cell adhesion, associates with CD18</td>
<td>ICAM-1 fibrinogen and IC3b</td>
<td>++ iMO, + rMO</td>
<td>BAL expression higher than most tissues</td>
</tr>
<tr>
<td>CD68 (macrosialin)</td>
<td>+/IM/MO, +/+ AM</td>
<td>IC glycoprotein, specific macrophage interaction (203), cell-cell interaction</td>
<td>+ MO, IM, AM</td>
<td>+ MO, IM, AM</td>
<td>good marker, but not as alveolar macrophage-specific</td>
</tr>
<tr>
<td>F4-80 (EMR1)</td>
<td>+/IM/MO, +/+/+ AM</td>
<td>glycoprotein coupling EC to IC signaling, signaling during tolerance/priming</td>
<td>downregulation by IFN-γ</td>
<td>70% murine homolog, gene level only, +/+ Eos</td>
<td>does not readily define macrophage-specific</td>
</tr>
<tr>
<td>CD14</td>
<td>+/MO, + IM, ± AM</td>
<td>LPS-binding protein, TLR signaling, apototic cell receptor (182)</td>
<td>apoptotic cells</td>
<td>++ (+CD16) = murine ++ Ly6c MO, + acute LPS</td>
<td>does not readily define macrophage-specific</td>
</tr>
<tr>
<td>CD16</td>
<td>+/AM, + iMO, + PMN</td>
<td>Fc receptor (FcγII), NK cell activation, ADC</td>
<td>IgG, IgG-antigen complex</td>
<td>++ (and + CD14) = IM/MO murine + Ly6c MO</td>
<td>BM cells, MO, AM, NK cells, PMN</td>
</tr>
<tr>
<td>Siglec F</td>
<td>+/+ + IM, + + Eos</td>
<td>inhibits Eos proliferation (30), virus/bacteria cell entry</td>
<td>Sialic acid</td>
<td>convergent paralog to Siglec-8 (human) (30)</td>
<td>primarily on PMNs</td>
</tr>
<tr>
<td>CD33 (Siglec-3)</td>
<td>+ MO (human), + PMN (murine)</td>
<td>regulates fcR via glycan recognition, pathogen uptake recognition/phagocytosis of bacteria (182)</td>
<td>Sialic acid</td>
<td>MO and myeloid precursors</td>
<td></td>
</tr>
<tr>
<td>Siglec 1 (Sialoadhesin, CD169)</td>
<td>+ + AM</td>
<td>phagocytosis, Ag capture, ADC, signals through ITAM motif</td>
<td>Sialic acid</td>
<td>macrophages, ?AM</td>
<td></td>
</tr>
<tr>
<td>FcγR1 (CD64)</td>
<td>+/+ IM/AM, + MO</td>
<td>phagocytosis, Ag capture, ADC, signals through ITAM motif</td>
<td>IgG immune complex</td>
<td>3 distinct genes (CD64A, B, C)</td>
<td></td>
</tr>
<tr>
<td>CD115 (M-CSFR, CSF1R)</td>
<td>++ MO (human iMO, rMO), +/AM.IM</td>
<td>cell production, maturation, and fcR via M-CSF</td>
<td>M-CSF</td>
<td>++ on iMO and rMO (58)</td>
<td>++ MO, useful for microbead isolation</td>
</tr>
<tr>
<td>Gr-1 (Ly6g, Ly6c)</td>
<td>++ MO, ± IM (Ly6c, +/PMN (Ly6g)</td>
<td>precursor for IM (Ly6c +/ MO) and AM, AAM? (Ly6c+/−)</td>
<td>Ly6c+ = monocyte; Ly6g− neutrophil; Gr-1 both</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/4</td>
<td>+/MO (+/CCR2, + CX3CR1), + PMN</td>
<td>? inflammatory response</td>
<td>Ly6c+ = rMO human; Ly6c− = iMO human</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD62L (L-selectin)</td>
<td>+/IM (also + Ly6c), +/PMN</td>
<td>monocyte recruitment, tethering and rolling</td>
<td>glycoproteins (CD34, GYLCAM1, MADCAM1)</td>
<td>present on iMO</td>
<td></td>
</tr>
<tr>
<td>CX-CR1</td>
<td>+/MO, + AM/MIM</td>
<td>migration, survival signal (96), patrolling (8)</td>
<td>CXCL1</td>
<td>Ly6C+ = rMO human; Ly6c− = iMO human</td>
<td></td>
</tr>
</tbody>
</table>

**M1 activation**

**M1 (CAM)**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Classification</th>
<th>Primary Function(s)</th>
<th>Stimulus/Receptor</th>
<th>Human Expression</th>
<th>Murine Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC II (I-AI-E, HLA-DR)</td>
<td>+/+ rMO, + iMO, also + + DC</td>
<td>Antigen presentation</td>
<td>IFN-γ, but also IL-4/13</td>
<td>HLA-DR: + iMO, ++ rMO, + resolve ARDS (156)</td>
<td>IM, stimulated AM expression may be distinct than human effect</td>
</tr>
<tr>
<td>CD80</td>
<td>also + DC</td>
<td>produce NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD86</td>
<td>also + DC</td>
<td>produce NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nNOS (Nos2)</td>
<td></td>
<td>induce NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cox2 (Pgs2)</td>
<td>IL-12</td>
<td>induce NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCL15</td>
<td></td>
<td>induce NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
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<tr>
<td>CCL20</td>
<td></td>
<td>induces NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
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<tr>
<td>CXCL9</td>
<td></td>
<td>induces NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
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<tr>
<td>CXCL10 (IP-10)</td>
<td></td>
<td>induce NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
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<tr>
<td>IL23a (IL23p19)</td>
<td></td>
<td>induce NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
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<tr>
<td>CXCL11</td>
<td></td>
<td>induce NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
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<tr>
<td>CCR2</td>
<td></td>
<td>induce NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
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<tr>
<td>CCR5</td>
<td></td>
<td>induce NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
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<tr>
<td>27E10</td>
<td></td>
<td>induce NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
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<tr>
<td>MARCO</td>
<td></td>
<td>induce NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
<td></td>
<td></td>
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<tr>
<td>CD36</td>
<td></td>
<td>induce NO, kill bacteria inflammation and pain</td>
<td>IFN-γ</td>
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**Continued**
Identification of macrophage/monocyte markers and activation states, including primary function, key stimuli/receptor, and known details about human or murine expression. Top: identification of macrophage and monocyte subpopulations in mice and humans are based on single or multiple combinations of surface and intracellular markers. We present correlative data on human leukocyte expression for each marker. Middle: surface and intracellular markers of M2 macrophage activation, along with primary function in addition to general characteristics including microbiocidal activity, tissue damage, and cellular immunity. Transcription factors that promote an M1 activated phenotype include NF-κB, IRF5, STAT1. Stimuli common to the group include IFN-γ, TNF-α, and IL-12. Bottom: surface and intracellular markers of M2 macrophage activation, along with primary function in addition to general characteristics including wound healing, tissue repair, humoral immunity, and allergic response. Transcription factors that promote an M2-activated phenotype include IRF4, KLF4, PPAR, and STAT6. Stimuli common to the group include IL-4, IL-13, and IL-10, although some consider IL-10 antagonistic; STAT6/STAT1-binding (56) and IL-10, TGF-β mainly IL-10, TGF-β. Not upregulated by IFN-γ STAT3 too (56). Not expressed by Gr-1 + CCR2-ex. Macs (64).

### Table 1—Continued

<table>
<thead>
<tr>
<th>Marker</th>
<th>Classification</th>
<th>Tissue Function(s)</th>
<th>Stimulus / Receptor</th>
<th>Human Expression</th>
<th>Murine Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mannose receptor (Mrc1,CD206)</td>
<td>also + DC (myeloid), − M0/MPN</td>
<td>phagocytosis of ligands (MPO, HIV Ag, bacteria (56), present Ag apoptotic cell clearance</td>
<td>IL-4, IL-13, IL-10</td>
<td>downregulated by IFN-γ</td>
<td></td>
</tr>
<tr>
<td>Scavenger receptor A (CD204)</td>
<td>− MO and + DC subsets</td>
<td>β-glucan receptor, TLR2 interaction (182), phagocytosis, proliferate T cells</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Dectin-1</td>
<td></td>
<td></td>
<td>IL-4/IL-13 signaling</td>
<td>IL-10 (56), + feedback with IL-4/13 induced by IFN-γ STAT3 too (56)</td>
<td>Not upregulated</td>
</tr>
<tr>
<td>IL-4Ra</td>
<td></td>
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<td>Arg1</td>
<td></td>
<td></td>
<td></td>
<td>M-CSF</td>
<td></td>
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<tr>
<td>12.15-lipoxygenase (LOX)</td>
<td></td>
<td>mediates PPARγ upregulation by IL-4</td>
<td></td>
<td></td>
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<tr>
<td>TIR (CD71)</td>
<td>(79)</td>
<td>carrier protein for transferrin, iron uptake, cell proliferation</td>
<td>mainly IL-10, TGF-β</td>
<td></td>
<td></td>
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<tr>
<td>OX2R</td>
<td>(CD200R)</td>
<td>bind epithelial CD200 to dampen AM inflammation, deactivate resp. burst and TNF-α, ? fibrogenesis</td>
<td></td>
<td>TLR activation</td>
<td></td>
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<tr>
<td>IL-10</td>
<td>not specific, ?Mreg subpopulation</td>
<td>key role in fibrosis</td>
<td></td>
<td>IL-13 (56)</td>
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<tr>
<td>TGF-b decoy receptor</td>
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<tr>
<td>Cxc13</td>
<td></td>
<td>B cell chemoattractant monocyte chemoattractant, recruitment of fibrocytes</td>
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<tr>
<td>CCL12</td>
<td></td>
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<tr>
<td>CCL17 (TARC)</td>
<td>binds CCR4, ++ MO, ++ DC</td>
<td>drives fibrogenesis with IL-10, acts on CCR4+ subset of CD4 T cells (56)</td>
<td>IL-4/IFN-γ antagonistic; STAT6/1-binding (56)</td>
<td></td>
<td></td>
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<tr>
<td>CCL18 (AMAC1)</td>
<td>also + DC</td>
<td>attracts lymphocytes, monocytes</td>
<td>IL-10</td>
<td></td>
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<tr>
<td>CCL24 (MDC)</td>
<td></td>
<td>attracts CCR4+ Th cells to polarize Th2 response chemokineattract for eosinophils, mast cells and basophils</td>
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<tr>
<td>YM1 (Ch33L)</td>
<td>IC staining by flow cytometry</td>
<td>chitinase-like protein, binds to ECM, heparan sulfate</td>
<td>IL-4/IFN-γ antagonistic; STAT6/1-binding (56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RELMα (Fizz1)</td>
<td>IC staining by flow cytometry</td>
<td>resistin-like secreted protein (56), promote ECM deposition</td>
<td>IL-4/IFN-γ antagonistic; STAT6/1-binding (56)</td>
<td></td>
<td></td>
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<tr>
<td>Mac-2 (galactin-3)</td>
<td>++ MO, AM</td>
<td>CD98 binding activates PI3K, + feedback loop with IL-4</td>
<td>Not expressed</td>
<td>+ Eos, epithelial cells</td>
<td></td>
</tr>
<tr>
<td>25F9</td>
<td>resident macs</td>
<td></td>
<td></td>
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<tr>
<td>RM3/1</td>
<td>++ MO, AM, CD163 family</td>
<td>membrane glycoprotein, late inflammatory macrophage</td>
<td></td>
<td>++ resolving ARDS (156)</td>
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Identification of macrophage/monocyte markers and activation states, including primary function, key stimuli/receptor, and known details about human or murine expression. Top: identification of macrophage and monocyte subpopulations in mice and humans are based on single or multiple combinations of surface and intracellular markers. We present correlative data on human leukocyte expression for each marker. Middle: surface and intracellular markers of M2 macrophage activation, along with primary function in addition to general characteristics including microbiocidal activity, tissue damage, and cellular immunity. Transcription factors that promote an M1 activated phenotype include NF-κB, IRF5, STAT1. Stimuli common to the group include IFN-γ, TNF-α, and IL-12. Bottom: surface and intracellular markers of M2 macrophage activation, along with primary function in addition to general characteristics including wound healing, tissue repair, humoral immunity, and allergic response. Transcription factors that promote an M2-activated phenotype include IRF4, KLF4, PPAR, and STAT6. Stimuli common to the group include IL-4, IL-13, and IL-10, although some consider IL-10 antagonistic; STAT6/STAT1-binding (56) and IL-10, TGF-β mainly IL-10, TGF-β. Not upregulated by IFN-γ STAT3 too (56). Not expressed by Gr-1 + CCR2-ex. Macs (64).
interface of the external environment can all explain the increased autofluorescence. Techniques to minimize its effects include use of dyes exciting at and/or emitting light of higher wavelengths (allophycocyanin, cyanine5/cyanine7, or Alexa Fluor 647), quenching of autofluorescence using eta-octyl-β-D-galactopyranoside or crystal violet (requires cell permeabilization), and either enzymatic or immunological signal amplification.

SUBPOPULATIONS. Use of flow cytometry to rigorously define human lung macrophage subpopulations is underutilized. Macrophage markers CD68, CD163, and Mac2 (Galectin 3) are expressed in most tissues based on immunohistochemistry but have not been well defined with use of flow cytometry. Monocyte populations are better characterized and are typically divided into “inflammatory” and “resident.” Inflammatory (classical) monocytes are CD14\(^+\)CD16\(^{-}\)CCR2\(^+\) and resident monocytes are CD14\(^-\)CD16\(^+\)CCR5\(^+\). A comparison of gene expression profiles between human and murine monocyte populations revealed similarity between human inflammatory monocytes (CD14\(^+\)CD16\(^^+\)) with murine CD115\(^+\)Ly6c\(^{hi}\) monocytes, and between human resident monocytes (CD14\(^+\)CD16\(^-\)) with murine CD115\(^+\)Ly6c\(^{low}\) monocytes (73). Among what is known about human lung macrophages, important differences in phenotypic markers defining subpopulations exist compared with murine lung. Compared with murine macrophages, human macrophages produce relatively little arginase (Arg1) following IL-4 stimulation and little nitric oxide synthase [inducible nitric oxide synthase (iNOS), Nos2] following IFN-γ stimulation.

Macrophage Phenotype with Inflammation

Murine. The presence of lung inflammation complicates the characterization of macrophage subpopulations. Using bleomycin as a murine lung injury model, Misharin et al. (128) found that F4–80 was less specific for alveolar macrophages since it was also expressed on interstitial macrophages, monocytes, eosinophils, or CD11b\(^+\) DCs. Instead, Siglec F (a member of the family of sialic-acid-binding immunoglobulin-like lectins) (30), in combination with CD11c or CD64, accurately identified alveolar macrophages when verified using morphological analysis. During lung inflammation, two main states of differentiation exist, characterized by classically activated macrophages (CAM) and alternatively activated macrophages (AAM). CAMs display the M1 macrophage phenotype and produce high levels of proinflammatory cytokines IL-1β, TNF-α, IL-12, and iNOS in response to paracrine signaling from the T helper 1 (Th1) cytokine IFN-γ and also in response to autocrine signaling by IFN-β (99, 191). AAMs display the M2 macrophage phenotype and produce anti-inflammatory cytokines IL-10 and IL-1ra in response to signaling from Th2 cytokines IL-4 and IL-13. Macrophages skewed toward an M1 activation state express surface markers including but not limited to MHC II (Ia/IE), CD80, CD86, and CCR2. M2-skewed macrophages express markers including but not limited to mannose receptor (MR), Dectin-1, TIR (transferrin receptor) (81), and CD200R. Expression of different STAT transcription factors or suppressors of cytokine signaling (SOCS) proteins drives M1 (STAT1, SOCS2) vs. M2 (STAT6/STAT3, SOCS3) expression (169).

LIMITATIONS OF PHENOTYPIC MARKERS. Relying on one or even several markers to define the presence of M1 or M2 macrophages creates problems, often magnified during inflammation. Macrophages demonstrate significant plasticity, allowing them to transition between distinct phenotypes or even to a hybrid phenotype based on environmental signals. For example, M1- to M2 macrophage transition occurs after phagocytosis of apoptotic neutrophils or in the presence of IL-4 (50, 81, 181). However, when macrophages were stimulated with immune complexes in the presence of IL-4, they produced high levels of IL-10, a prominent feature of regulatory macrophages (Mregs). Mregs are a distinct macrophage subtype, yet in this case the cells also retained features of M2 macrophages (44). Another example in which phenotypic markers don’t necessarily reflect true cell function is the high expression of the M2 markers Dectin-1 and mannose receptor on macrophages in the alveolar space at baseline, in the setting of persistent, albeit low-level, antigen exposure. The functions of an unchallenged macrophage are likely quite different from an alveolar macrophage expressing the same M2 markers during the resolution phase of experimental lung injury. Therefore, it is probably more effective to define macrophage subpopulations during disease states on the basis of function including cytokine profiles, phagocytosis, and Th1 lymphocyte skewing and proliferation.

Human. Extensive characterization of human alveolar macrophage differentiation and phenotypes has not been performed, but there are a few noteworthy studies. Rosseau performed serial BALs in ventilated ARDS patients compared with one BAL in ventilated cardiogenic pulmonary edema (CPE) patients or in nonventilated healthy volunteers (156). In ARDS patients, total alveolar macrophage number and expression of CD14 and CD11b increased, and expression of CD71, human leukocyte antigen (HLA)-DR, and CD16 (mature tissue macrophage markers) decreased compared with healthy volunteers or CPE patients. Furthermore, alveolar macrophages from ARDS patients resembled blood monocytes with smaller size and less autofluorescence compared with mature alveolar macrophages. With increased 27E10, a marker that recognizes acute-phase monocyte/macrophages (15), and decreased R3M1/3, a marker denoting dampened inflammation (205), alveolar macrophages from ARDS patients skewed toward a more proinflammatory, M1 activation phenotype compared with other groups. Interestingly, persistent expression of M1 proinflammatory macrophage surface markers as well as increased BAL monocyte chemoattractant protein-1 (MCP-1), a proinflammatory cytokine that is also a monocyte chemoattractant, portended a worse prognosis (156).

To model the early phase of ARDS, Brittan et al. (19) exposed healthy volunteers to inhaled lipopolysaccharide (LPS, 60 µg, vehicle saline), followed by blood and BAL collection up to 8 h later. In contrast to similar monocyte subpopulations in the blood, BAL from LPS-challenged patients revealed a significant increase in total alveolar macrophages (CD14\(^+\)), consisting primarily of pulmonary monocyte-like cells (PMLC) defined by smaller size and less granularity compared with mature alveolar macrophages. PMLCs were predominantly recruited (CD16\(^-\)), not resident (CD16\(^+\)) monocytes (204). Brittan et al. defined recruited monocytes by differences in forward/side scatter and CD14\(^+\) expression, and determined that these cells expressed HLA-DR at higher levels.
compared with resident monocytes. In contrast, Rosseau et al.’s study (157) suggested an increase in HLA-DR expression on more mature, proresolution macrophages. Expression and abundance of a broader array of human lung macrophage markers needs is necessary during homeostasis and inflammatory responses.

**Macrophage Recruitment and Proliferation**

**Murine.** Baseline conditions. Since the identification of macrophage systems, scientists have debated the relative importance of local proliferation vs. recruitment from blood monocytes to help maintain tissue homeostasis. Evidence in humans and mice supports dual and perhaps complementary mechanisms to repopulate the alveolar macrophage pool, skewed by the presence and severity of inflammation (35, 36, 201). A recent fate-mapping study using mice with Cre recombinase in their CX3CR1 gene loci confirmed alveolar macrophages to be of prenatal origin, devoid of blood monocyte input for tissue renewal at baseline (201). During unchallenged states, alveolar macrophage turnover is slow. Local proliferation regulates cell replacement and is primarily mediated by GM-CSF and M-CSF (CSF-1) signaling through macrophage CSF-1R (58, 71, 77). Recently, however, Jenkins et al. (77) showed that IL-4 can induce pleural macrophage proliferation in a CSF-1-independent manner.

Studying macrophage turnover under true baseline conditions is difficult, since even mild inflammation can skew lung and alveolar macrophage cell replacement toward repopulation from the bone marrow (1, 58, 97, 114, 146). Chimera studies offer some insight into the influence of inflammation on macrophage turnover. Whole body irradiated mice undergo donor alveolar macrophage replacement within a few weeks (53, 80, 97, 110, 114, 177). In contrast, mice irradiated with a lung shield had stable host alveolar macrophage presence even at 1 year (176, 177), demonstrating that tissue damage and inflammation from irradiation accelerate cell turnover. To try and study cell turnover without irradiation, Landsman and Jung (97) used systemic diptheria toxin (DT) to deplete CD11c+ alveolar/lung macrophages and CD11c+ dendritic cells in CD11c-DTR mice. In doing so, they observed an intermediate lung macrophage population derived from blood monocytes that proliferated in the lung and migrated to the alveolar space to replace alveolar macrophages. Repeated injections of DT have been shown to induce injury (159), however, and likely also increase lung inflammation compared with baseline. Furthermore, rapid depletion of resident macrophages may enhance bone marrow recruitment signals.

**Inflammation.** More significant inflammation due to stimuli such as intratracheal LPS increases alveolar macrophage numbers, and bone marrow recipient mice demonstrate accelerated replacement of a significant percentage of lung and alveolar macrophages by donor cells (1, 114, 146). With inflammation, alveolar macrophage recruitment and differentiation occur due to local secretion of growth factors (GM-CSF and M-CSF) and other cytokines (CCL2-CCR2 axis), and through communication with activated resident lung macrophages and epithelial cells (65, 170). During the inflammatory response, local cytokine profiles influence transition toward either an M1 or M2 macrophage phenotype as follows: secreted IFN-γ or GM-CSF (M1, CAM), IL-4 or IL-13 (M2, AAM), or cell-based IL-25 or IL-33 (M2) (56, 65).

**Human.** Inflammation alters recruitment. As is the case in murine models, regulation of human alveolar macrophage replacement may be based on the severity of inflammation. In patients with ARDS, Rosseau et al. (156) observed an increase in total BAL macrophages without a concomitant increase in macrophage proliferation assessed by staining of a nuclear antigen, Ki67, and suggested that MCP-1 may mediate macrophage recruitment. Studies in human recipients of allogeneic bone-marrow transplants (allo-BMT) demonstrate recruitment of blood monocyte precursors, followed by local proliferation to repopulate lung leukocytes (137, 186). Alveolar macrophage size and numbers were similar to nonsmoking controls until day 50 after allo-BMT; after day 50, a threefold increase in alveolar macrophage numbers occurred in the transplant recipients, along with a significant increase in expression of proliferating cell nuclear antigen (PNCA) (137). The importance of local proliferation in vivo is supported by in vitro studies demonstrating GM-CSF-enhanced proliferation of alveolar macrophages (136). Despite utilizing recruitment and local proliferation to replace host macrophages, patients remain susceptible to infection after transplantation. Donor macrophages express less HLA-DR and mount an inadequate response to inflammatory stimuli, similar to endotoxin tolerance (16, 97, 129, 130). However, susceptibility to infection may be related to pharmacological immunosuppression or decreased presence of alveolar neutrophils rather than inadequate numbers or function of macrophages.

**Implications of a GM-CSF trial.** Based on a study observing higher levels of BAL GM-CSF in patients with improved outcome following ARDS (111), as well as the effects of GM-CSF on macrophage proliferation and maturation, alveolar surfactant removal, epithelial cell survival, and protection in experimental lung injury models (171), GM-CSF was proposed as therapy in ARDS patients. However, a randomized clinical trial of systemic GM-CSF in patients with ARDS did not demonstrate a benefit on the primary outcome of ventilator-free days or on mortality (21); GM-CSF did increase blood leukocytes, but alveolar leukocytes were not enumerated or phenotyped. GM-CSF, along with granulocyte colony-stimulating factor (G-CSF), can inhibit neutrophil apoptosis in the lungs of patients with ARDS, which may have blunted any positive effects on macrophage maturation or epithelial survival in the study (111). Other therapeutic options to consider include localized tissue delivery and use of a growth factor such as M-CSF (CSF-1) capable of inducing macrophage proliferation and shifting the balance toward anti-inflammatory, proresolving M2 macrophages (70). In addition, a more rigorous assessment of inflammatory cells in the alveolar space during ARDS may help identify biomarkers critical for resolution and help determine which patients would benefit most from therapy.

**Macrophage Function: Murine**

**Homeostasis.** During homeostasis, alveolar macrophages phagocytose dust, allergens, and senescent cells and perform other trophic duties (56). Compared with other tissue macrophages, alveolar macrophages possess a reduced capacity to present antigen (190). Expression of M2, anti-inflammatory...
proteins, and a reduced capacity to present antigen may be adaptive responses to limit deleterious inflammation in the presence of constant low-level stimuli (185). However, alveolar macrophages also express higher levels of proinflammatory genes even at baseline and are thus prepared to initiate a prompt innate immune response when necessary (56, 57).

**Inflammation.** With pattern recognition of sufficient foreign substances, alveolar macrophages initiate a proinflammatory cascade to clear airway and alveolar pathogens and propagate the innate immune response. Early response cytokines including IFN-γ, TNF-α, and IL-1β potentiate chemokine secretion to recruit neutrophils, exudative macrophages, and lymphocytes (63). Along with neutrophils, recruited macrophages are essential for pathogen clearance through release of toxic mediators, reactive species, and phagocytosis of infected particles or other inflammatory debris. In the absence of intrinsic anti-inflammatory pathways (3) or with persistent inflammatory stimuli, tissue resident and recruited proinflammatory, M1 macrophages can directly induce tissue damage through excessive release of toxic species including nitric oxide (NO), superoxide, and matrix metalloproteinases (MMPs) and also indirectly augment tissue damage through excessive recruitment of neutrophils and T<sub>H</sub>17 cells. A chronic venous ulcer skin model demonstrated that excessive iron taken up by macrophages induced a persistent M1 macrophage phenotype and worse tissue injury (166). Recruited macrophages express proinflammatory genes (97, 170) but also express high levels of immunosuppressive genes (56, 57) and thus exhibit significant reprogramming potential as an adaptive response to mitigate ongoing inflammation.

**Resolution and repair.** Resolution of lung inflammation and injury is an active, coordinated process dependent on pathogen clearance, reduced proinflammatory signaling, and removal of proinflammatory neutrophils and macrophages as well as transition to an anti-inflammatory and reparative milieu. Resident and recruited macrophages are central to the resolution of lung inflammation with their ability to transition from proinflammatory to anti-inflammatory, tissue-reparative phenotypes (63, 76). Specific recruited macrophage populations have been shown to be critical for resolution. Following direct LPS or bacterial stimulus, Gr-1<sup>high</sup>CCR2<sup>high</sup> exudative macrophages migrate to the lung and express high levels of IL-1ra (IL-1 receptor antagonist) (14), a M2 marker that antagonizes the M1 cytokine IL-1β and may also induce neutrophil removal (64).

**Effercytosis and Macrophage Phenotype.** Engulfment of apoptotic inflammatory cells, or effercytosis, shifts macrophages toward a M2, anti-inflammatory phenotype by abrogating release of proinflammatory mediators (88, 150, 194) and augmenting TGF-β (47, 72) and IL-10 production (23) to promote resolution and wound repair. Effercytosis reduces NO by increasing the arginase-to-iNOS ratio (50, 54) via PPAR-β and PPAR-γ signaling (50, 79, 134). Additionally, M2-derived cytokines including IL-4, IL-13, and IL-10 increase mannose receptor expression to further augment effercytosis (7, 90). Inflammatory macrophage clearance is also important for lung injury resolution. In direct lung injury models using streptococcus (108), LPS (76), or influenza (76), inflammatory (exudative) macrophage-dependent caspase or Fas-mediated apoptosis and clearance was critical for resolution of inflammation. This is particularly important work, and a potential effect of inflammatory macrophage apoptosis on clearance of other inflammatory cell populations warrants further study.

**Lipid mediators.** Macrophages secrete many classes of lipid mediators, i.e., lipoxins, resolvins, and protectins, generally via 15-lipoxygenase (15-LO)-mediated signaling to actively promote resolution of inflammation (7). Efferocytosis upregulates 15-LO expression and activity (50, 161), as do cytokines IL-4 and IL-13. By signaling through the ALX/FPR2 receptor expressed on alveolar macrophages and epithelial cells, exogenous aspirin-triggered resolvin D1 reduced murine lung injury induced by hydrochloric acid or LPS; the mechanism appears to involve dampening NF-κB activation (45, 104).

**Prolonged M1 or M2 Macrophage Activation.** Persistent activation of either M1 or M2 macrophages appears to be detrimental to wound healing and tissue repair (165, 166). Production of lipid mediators is anti-inflammatory, however, and can balance the profibrotic effects of a sustained M2 macrophage phenotype that can occur with prolonged IL-10 or IL-13 exposure (125, 135, 173, 174). We found that macrophage-derived iNOS (a marker for CAM or M1 activation state) was critical for resolution of experimental lung injury (33). iNOS<sup>−/−</sup> mice had unremitting lung inflammation and injury following intratracheal LPS, and alveolar macrophages demonstrated a sustained M1 phenotype. In addition to iNOS, exploration of transcription factors or pathways responsible for this transition could reveal important targets to accelerate resolution of lung inflammation and promote lung repair after injury (81). Sufficient inflammation may be necessary to trigger pathways responsible for the transition from M1 to an M2 phenotype, and further investigation is warranted to determine whether macrophage transition from M1 to M2 phenotype is critical for resolution of lung inflammation and injury.

**Macrophage Function During Human ARDS**

**Acute, inflammatory phase.** Human studies characterizing macrophage function in ARDS are limited and primarily conjectural. Existing knowledge centers on the contribution of macrophage-derived cytokines to the injury and resolution phases of ARDS. With onset of inflammation, resident alveolar macrophages are thought to be the primary source of IL-1β and TNF-α, which then stimulate neighboring cells to propagate the inflammatory response characteristic of acute-phase ARDS. Jacobs et al. (74) found that total IL-1 and subtype IL-1β were secreted in increased amounts by alveolar macrophages from ARDS patients than from healthy patients or patients with pneumonia without ARDS; a similar pattern was observed when those alveolar macrophages were isolated and rechallenged with LPS and IFN-γ. Donnelly et al. (41) noted that increased BAL IL-8 secretion and increased alveolar macrophage IL-8 expression was associated with progression to ARDS among at-risk patients with significant systemic inflammation. Additionally, increased BAL IL-8 was associated with increased mortality in patients with established ARDS (31, 116, 121).

**Resolution phase.** Any role for alveolar macrophages in resolution of ARDS remains quite speculative. Steinberg et al. (172) demonstrated that among patients with sepsis-induced ARDS, a persistent increase in alveolar neutrophils and a persistent decrease in alveolar macrophages (both by percentage of total BAL cells and by absolute number) at days 7 and 14.
14 were associated with worse outcome. Interestingly, Matute-Bello et al. (112) found that rates of neutrophil apoptosis were low throughout phases of ARDS and did not predict survival in established ARDS patients. To our knowledge, macrophage efferocytosis or rates of macrophage apoptosis have not been assessed during any stage of human ARDS, investigation of which is limited by the fleeting presence of apoptotic inflammatory cells in animals and humans. In addition to efferocytosis, another potential mechanism for macrophage-derived resolution is through secretion of epithelial growth factors including KGF, HGF, and GM-CSF to promote epithelial repair. The cellular source(s) of these growth factors remain unknown in ARDS. Human fibroblasts have been shown to be a source of KGF in response to inflammatory stimuli (18, 149). Human macrophages may secrete HGF to help regenerate lung epithelium as was shown in a mouse influenza model (138). To enhance repair, human lung epithelial cells may be a source of GM-CSF when stimulated by macrophage-derived TNF-α as shown in murine cells by Carakova et al. (24).

Macrophage Fate

Murine. As is the case with differentiation, phenotype, and function of alveolar macrophages, inflammation regulates macrophage cell fate. Alveolar macrophages either become apoptotic or they migrate to draining lymph nodes. Although both may occur in mice (89, 180), only macrophage apoptosis appears to be critical for resolution of acute inflammation (76).

Both pathogen-specific and host defense mechanisms can alter rates of macrophage apoptosis during the acute infectious or inflammatory period. In direct bacteria models, alveolar macrophage apoptosis may be a pathogen-derived mechanism utilized primarily by intracellular organisms (Shigella, Salmonella) to evade the immune system (66, 206). Alternatively, the host can promote alveolar macrophage apoptosis to limit intracellular bacterial replication by Mycobacterium or Chlamydia species (85, 95, 145), or to enhance clearance of extracellular bacteria such as Streptococcus (39). Recruited macrophages initially behave similarly to neutrophils in their ability to kill pathogens and in their susceptibility to apoptosis. Apoptosis of recruited macrophages can also enhance killing of engulfed bacterial pathogens and help terminate proinflammatory responses (47, 95, 107, 198). Additionally, as inflammation resolves, recruited macrophages can further differentiate into a mature phenotype to increase their half-life far beyond resolution of lung inflammation (40).

Migration of alveolar macrophages to draining lung lymph nodes has been suggested in the context of pathogen removal and antigen transport, but not during resolution of significant inflammation; this contrasts the fate of peritoneal macrophages following resolution of inflammatory peritonitis (13). Kirby et al. (89) delivered low-dose fluorescently labeled Streptococcus (10^4 CFUs, intratracheal) to mice, which caused mild lung inflammation without neutrophil alveolitis, and demonstrated increased migration of alveolar macrophages to draining lymph nodes within 6–12 h. In contrast, a model using an intratracheal LPS dose sufficient to induce prominent neutrophil alveolitis did not demonstrate significant alveolar macrophage migration to draining lymph nodes when assessed 6 days later (76); notably, markers to identify macrophages were different between the two studies. Wissinger et al. (200) suggested that limiting alveolar macrophage migration to lymph nodes may shield the immune system from the constant barrage of inhaled antigens. Some controversy exists as to whether alveolar macrophages migrate to lymph nodes in any circumstance, or rather do cells with a similar phenotype arise in the lymph nodes by alternate means (35, 75).

Human. To our knowledge, there are no human studies assessing alveolar macrophage fate during resolution phases of ARDS. The study by Steinberg et al. (172) suggested that an insufficient number of alveolar macrophages impaired ARDS survival, implying either protracted recruitment or accelerated apoptosis and removal; neither mechanism has been investigated in human studies.

Macrophage Cellular Interactions During Inflammation and Resolution

Alveolar macrophages interact with other macrophage subtypes and several other cell types during inflammation, resolution, and tissue repair. We will highlight four main interactions: macrophage-inflammatory cell (neutrophil and recruited macrophages), macrophage-epithelial, macrophage-lymphocyte, and macrophage-mesenchymal stem cell. Macrophages do interact with additional cell types during inflammation and resolution, such as macrophage-derived reactive oxygen species causing damage to endothelial cells (48); however, we will focus on those four interactions.

Macrophage-Neutrophil/Recruited Macrophage

Murine. To initiate the immune response, binding of pattern recognition receptors activates alveolar macrophage NF-κB and IRF-5 transcription factors leading to secretion of early response cytokines type 1 interferon, TNF-α, and IL-1β to stimulate chemokine secretion from neighboring macrophages and other cells (63). To potentiate the innate immune response, macrophages secrete chemokines macrophage inflammatory protein-2 (MIP-2) and keratinocyte-derived chemokine (KC) to recruit neutrophils to the alveolar space (42, 158, 178, 196, 202), and secrete MCP-1 (CCL2) to recruit monocytes (113, 115). Multiple pathways within macrophages downstream of Toll-like receptor signaling appear to be involved in this response. Hasan et al. (62) and Cornell et al. (29) recently demonstrated that the macrophage proteins geranylgeranyl transferase and mitogen-activated protein kinase phosphatase 2, respectively, mediate neutrophil recruitment in experimental ARDS models. After rapid decline of MIP-2 and KC, persistent, or second-phase, neutrophil alveolitis can be maintained by inflammatory macrophages (38, 113) and by SDF-1 (CXCL12) secreted primarily by alveolar epithelial cells (AECs) (151).

Transition to the resolution phase involves a series of coordinated events primarily regulated by resident and recruited macrophage populations (6, 63, 64). Macrophage-derived MMPs 1, 3, 8, and 12 cleave CXC chemokines (MIP-2) and CC chemokines (CCL2, 3, 7, 8, 9, and 12) to mitigate neutrophil and exudate macrophage recruitment in various lung injury models (37, 118, 119, 139, 153).

A cornerstone stage in resolution of inflammation is apoptosis of neutrophils and clearance by macrophages (20, 160, 179). Studies have demonstrated the importance of neutrophil apoptosis for resolution in experimental lung injury models
(34, 117). During acute inflammation, survival signals G-CSF and IL-1β enhance the half-life of neutrophils (83); however, IL-1ra, a prominent source of which is recruited macrophages, helps modulate neutrophil influx and augment neutrophil apoptosis. High levels of macrophage-derived TNF-α can also induce neutrophil apoptosis (192), as can alveolar macrophage death ligand TRAIL expression (117). Rapid recognition of apoptotic neutrophils by macrophages hastens removal to limit tissue damage from contents released by necrotic neutrophils. Even though impaired clearance of apoptotic neutrophils can exacerbate inflammation and worsens mortality (183), studies have yet to definitively demonstrate that enhancing clearance of apoptotic neutrophils can improve outcome in ALI.

**Human.** ARDS studies investigating important macrophage communication with other inflammatory cells have focused primarily on the acute phase, where alveolar neutrophil recruitment is predicated largely on IL-8 secretion via alveolar macrophages (41). To attempt to assess the contribution of circulating monocytes toward alveolar neutrophil recruitment, Barr et al. (10) randomized patients to receive leukapheresis of mononuclear cells at intervals after inhaled LPS, followed by a BAL at 9 h. Leukapheresis was successful in significantly reducing, but not eliminating, monocytes (and lymphocytes, platelets) in the blood and BAL but did not significantly reduce circulating or alveolar neutrophil numbers. BAL and serum levels of IL-8, the predominant neutrophil chemokine, and MCP-1, the predominant monocyte chemokine, were similar in the sham and leukapheresis groups (10). The study was limited by a short exposure interval, relatively mild inflammation, incomplete elimination of monocytes, and inability to assess the contribution of resident alveolar macrophages.

Differences in the alveolar milieu ARDS during acute vs. resolution phases may alter clearance of inflammatory cells. Compared with BAL fluid from patients in late-phase ARDS when inflammation was resolving, only BAL fluid from patients in early-phase ARDS inhibited neutrophil apoptosis in vitro (111), thought to be mediated by IL-8 or G-CSF (2). Interestingly, rates of alveolar neutrophil apoptosis were not predictive of outcome in human ARDS subjects; however, a direct assessment of macrophage efferocytosis, or actual clearance of apoptotic neutrophils, was not performed (111).

**Macrophage-Epithelial Cell**

**Murine.** Onset of inflammation. At baseline, cell contact between macrophages and the alveolar epithelium appears to be regulated by epithelial integrin αvβ6 (94, 164, 200) which activates macrophage TGF-β to inhibit secretion of TNF-α and IL-6. During the innate immune response, alveolar macrophages detach from alveolar epithelial cells and secrete more TNF-α to stimulate AECs (5, 26, 163). In response to TNF-α or direct Toll-like receptor binding, AECs secrete KC and LPS-induced chemokine (LIX) to recruit PMNs (9, 78), secrete MCP-1 to recruit monocytes (143, 163, 187), and express ICAM-1 and MCP-1 to promote macrophage mobility and phagocytic potential (11, 82, 100, 124, 147, 162). Viruses with direct epithelial infectivity, such as influenza A, enhance monocyte recruitment by inducing AECs to secrete CCL2 and CCL5 and by facilitating monocyte β1 and 2 integrin contact with integrin-associated protein (CD47), ICAM-1, VCAM-1, and junctional adhesion molecule-on the AEC surface (12, 63, 157). After monocytes and macrophages migrate into the alveolar space during acute inflammation, cellular interactions are not well understood. Mice deficient in epithelial integrin αvβ6 demonstrated persistent alveolar macrophage activation due to decreased lung TGF-β (132), but specific interactions occurring between inflammatory macrophages and the apical AEC surface are not known.

**Resolution and repair.** To dampen inflammation and transition toward resolution, exudative macrophages secrete IL-1ra, which competitively inhibits IL-1β binding to IL-1r1, its receptor on AECs, reducing AEC damage and decreasing ICAM-1 secretion. With reduced stimulus from ICAM-1, macrophages secrete less MIP-2; reduced ICAM-1 and MIP-2 attenuate alveolar neutrophil recruitment (22).

To repair and regenerate the airway and alveolar epithelial barriers, type II AECs and Clara cells are progenitor cells that regulate proliferation and differentiation into type I AECs. Traditionally, primary lung epithelial cells have been easier to isolate from rats, so we have included some of this important work. In murine or rat lung injury models, lung growth factors, matrix components, and other soluble mediators can contribute to epithelial repair by mitogenic and nonmitogenic pathways, but the cellular source(s) are frequently not macrophages or in some cases remain unknown. According to more definitive work in the muscle (105), gut (175), kidney (102), and heart (61), M2-predominant lung macrophages may also contribute to lung epithelial repair, but a direct effect has not been determined by use of macrophage depletion studies. Morimoto et al. (131) postulated an indirect effect of hepatocyte growth factor (HGF) based on macrophage production after efferocytosis of apoptotic neutrophils in a bacterial pneumonia model; efferocytosis can promote a M2 macrophage phenotype, but this was not specifically demonstrated. HGF is a potent mitogen for rat alveolar epithelial cells (109, 149), but specific mechanisms of alveolar repair in ALI models have not been thoroughly examined. Even macrophage-derived TNF-α, a predominantly proinflammatory cytokine, can stimulate AEC production of GM-CSF, leading to reparative downstream signaling (24), strengthening support for GM-CSF as an integral cytokine in epithelial repair (69, 148). Proliferation, M2 macrophages have also been shown to secrete EGF and IL-1ra to enhance alveolar fluid clearance by increasing active sodium transport through apical epithelial Na+ (ENaC) and basolateral Na-K-ATPase channels (63, 133, 184). In a ventilator-induced lung injury model, antibody-mediated blockade of MMP-2 impaired epithelial wound closure (MLE-12 cell line); MMP-2 was expressed by epithelial cells and intra-alveolar inflammatory cells (55).

**Human**

Macrophage contribution to epithelial repair has been described in animal models, but direct evidence in humans is incomplete and hard to interpret. KGF and HGF, which can be secreted by multiple cell types including macrophages, were present in pulmonary edema fluid in the acute phase of ALI (193). HGF was significantly higher in ALI patients compared with those with hydrostatic pulmonary edema. Somewhat surprisingly, increased HGF was associated with increased mortality; the authors hypothesized that higher HGF levels were present as a failed attempt to combat the severity of ALI, as the
mortality in this group was 73%. Geiser et al. (52) used BAL fluid from ARDS patients to test epithelial repair following scratch assay in an epithelial cell line. Interestingly, he found that IL-1β, a macrophage-derived cytokine critical for the acute phase of ALI, accelerated epithelial repair especially when the fluid source originated from BAL of early (<12 h) compared with late (12–48 h) ARDS patient samples. TGF-β and KGF have also been shown to enhance epithelial repair in vitro (86). Using BAL samples from ARDS patients, Gonzalez-Lopez et al. (55) showed that inhibition of MMP-2 abrogated epithelial wound healing in A549 cells, a human alveolar epithelial cell line.

Outside of the ARDS realm, stimulated human alveolar macrophages have been shown to enhance epithelial growth. Conditioned media from silica-exposed alveolar macrophages identified platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-1), and fibroblast-derived growth factor (FGF) as potential mitogens toward rat type II pneumocytes on the basis of depletion studies (123).

**Macrophage-Lymphocyte**

**Murine.** As antigen-presenting cells (APC), macrophages coordinate the adaptive immune response using cognate antigenic peptide presentation by MHC molecules to T cell receptors (TCR) at the immunological synapse (27, 28). APCs synergize with the TCR to form a crucial ligand-receptor cosignaling molecule interaction that regulates T cell activation, differentiation, effector function, and cell survival using an expanding list of ligand-receptor combinations (25, 28, 93). In murine models of direct lung injury including intratracheal LPS (33, 34, 67, 92), low-dose intratracheal LPS plus moderate hyperoxia (4), gram-negative or gram-positive bacterial infection (106, 165), and a sepsis model of indirect lung injury (141, 197). Proresolution Treg effects may occur through direct interactions. Extensive work in animals has helped dissect nu-

**Human.** Possible interactions between macrophages and T cells in human ARDS remain purely theoretical at present. Human Foxp3+ Tregs can induce an M2, alternatively activated macrophage phenotype and blunt their subsequent response to LPS in culture (188). In addition, alveolar Tregs are present in patients with ARDS (34), but specific function(s) remain unknown in ARDS. Somewhat limited by the practi-

**Macrophage-Mesenchymal Stem Cell**

**Murine.** Mesenchymal stem cells (MSCs), also known as multipotent mesenchymal stromal cells or bone marrow stromal cells (BMSCs), make up one class of adult stem cells with immunomodulatory properties. Immune suppression occurs primarily through modulation of other innate and adaptive immune cells (100), including macrophages and monocytes. MSCs have been shown to enhance macrophage phagocytic activity in mice (122). Using cecal ligation and puncture as a sepsis model, Nemeth et al. (140) showed that PGE2 secretion derived from MSCs reprogrammed alveolar macrophages to secrete IL-10, which enhanced murine survival and organ function (100, 140). In a direct lung injury model using endotoxin or live bacteria, MSCs improved survival and lung injury parameters. Alveolar MIP-2 and TNF-α levels were decreased, and alveolar IL-10 was increased, leaving the authors to postulate a macrophage effect mediated by paracrine MSC signaling (59).

**Human.** MSCs present potentially attractive therapy for ARDS patients according to animal work and preliminary studies in humans. Support is also generated by studies demon-

**Conclusion**

Abundant in the lung and alveolar space during steady state, macrophages are critical to the initiation and resolution of lung inflammation, during which biological signatures are defined by their differentiation, phenotype, function, and cell-cell interactions. Extensive work in animals has helped dissect numerous processes critical for the development of ARDS using experimental models. More recent work has also focused on the determinants of resolution and repair. With a myriad of potential cellular reprogramming targets, macrophages represent an attractive target for intervention at various stages of ARDS. However, our understanding of macrophage biology...
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