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REVIEW ARTICLE

Role of macrophages in fetal development and perinatal disorders

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In the fetus and the neonate, altered macrophage function has been implicated not only in inflammatory disorders but also in developmental abnormalities marked by altered onset, interruption, or imbalance of key structural changes. The developmental role of macrophages were first noted nearly a century ago, at about the same time when these cells were being identified as central effectors in phagocytosis and elimination of microbes. Since that time, we have made considerable progress in understanding the diverse roles that these cells play in both physiology and disease. Here, we review the role of fetal and neonatal macrophages in immune surveillance, innate immunity, homeostasis, tissue remodeling, angiogenesis, and repair of damaged tissues. We also discuss the possibility of therapeutic manipulation of the relative abundance and activation status of macrophage subsets in various diseases. This article combines peer-reviewed evidence from our own studies with results of an extensive literature search in the databases PubMed, EMBASE, and Scopus.

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IMPACT:

- We have reviewed the structure, differentiation, and classification of macrophages in the neonatal period.
- Neonatal macrophages are derived from embryonic, hepatic, and bone marrow precursors.
- Macrophages play major roles in tissue homeostasis, innate immunity, inflammation, tissue repair, angiogenesis, and apoptosis of various cellular lineages in various infectious and inflammatory disorders.
- Macrophages and related inflammatory mediators could be important therapeutic targets in several neonatal diseases.

INTRODUCTION

Macrophages are resident myeloid immune cells seen in various tissues.[1] These cells are vital mediators of host immunity, inflammation, cellular homeostasis and turnover, and tissue remodeling needed for host defense, development, and repair of damaged tissues.[2] Altered macrophage function has been noted in neonatal disorders such as hypoxic–ischemic encephalopathy (HIE), bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), retinopathy of prematurity (ROP), and renal failure.[3–6] Macrophage markers may provide prognostic markers in several neonatal conditions, and targeted modifications in the composition and function of macrophages could possibly facilitate immunomodulatory treatments for neonatal diseases. This article combines peer-reviewed evidence from our own studies with results of an extensive literature search in the databases PubMed, EMBASE, and Scopus.

ORIGIN OF MACROPHAGES

Macrophages were first described in detail in 1908 by the Nobel laureate Elie Metchnikoff, who described these phagocytes as important mediators of innate immunity.[7] The name “macrophages” or “big eaters” came from the Greek words, “makros” or large, and “phagein” or eat. Macrophages are large, round cells with a central nucleus and abundant, clear, and often vacuolated cytoplasm. These cells are highly phagocytic, motile, and modulate immune responses by releasing various immune mediators. There are several sources:

- *Macrophage differentiation directly from lineage-restricted progenitors in the yolk sac (YS):* Hemocytoblasts resembling myeloblasts are first seen in blood sinuses in the wall of the secondary YS (Fig. 1a) on day 18.[8] On day 19, some of these hemocytoblasts, particularly those in the primordial vascular structures in the distal YS, differentiate directly into embryonic macrophages without passing through a monocyte phase. Similar macrophage differentiation from similar YS progenitors can be seen in the pancreatic mesenchyme at 6–12 weeks and in the skin at 9 weeks.[9]
- *Macrophage differentiation from erythro-myeloid progenitors (EMPs) in the YS*: On day 25, the YS and the developing embryo (Fig. 1a) show multiple EMPs closely associated with the capillary endothelium.[10] These cells continue to proliferate and differentiate into macrophages until day 30. At this time, some cluster of differentiation (CD) 45^+ $CD34^+$myeloid cells located near the dorsal aorta migrate into the central nervous system (CNS) and differentiate into microglial precursors.
- *Macrophage differentiation in the aorta-gonad-mesonephros (AGM) zone*: The vascular endothelium in the AGM zone (Fig. 1b) produces $CD34^+$ $CD45^+$ hematopoietic stem cells

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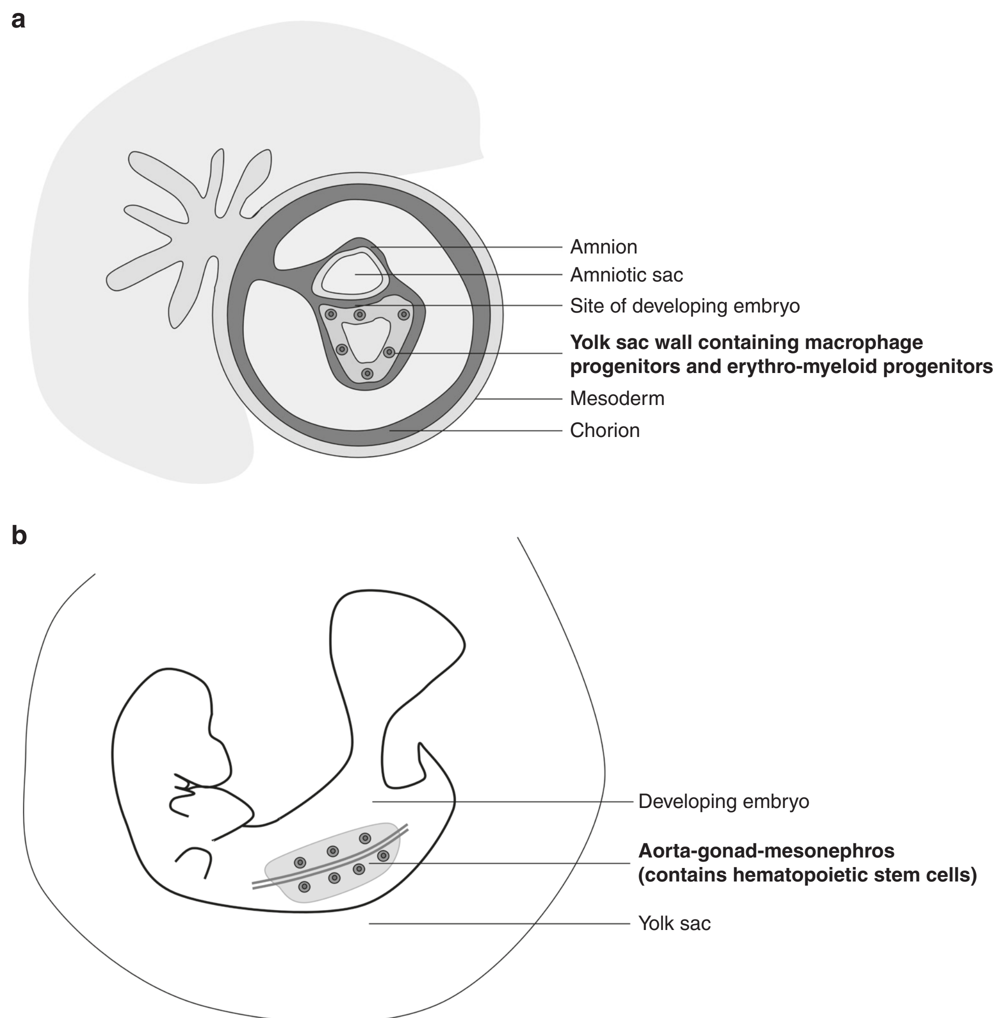

Fig. 1 Origin of tissue macrophages. Schematic representation of a human embryo **a** in the 3rd week of gestation. Lineage-specific macrophage progenitors and erythroid–myeloid progenitors develop in the yolk sac (marked in emboldened font); **b** at 4–5 weeks gestation. Hematopoietic stem cells develop in the aorta-gonad-mesonephros region.

(HSCs).[11] These cells differentiate into common myeloid progenitors (CMPs) and then into tissue macrophages either directly or via a monocyte stage. These macrophages migrate to all the embryonic organs except the CNS. These cells express angiotensin-converting enzyme (CD143), T cell acute lymphocytic leukemia 1/stem cell leukemia gene, and the myeloblastosis oncogene (c-Myb), indicating the origin in the subaortic mesenchyme.[12]

Most macrophages in fetal organs develop from EMP and AGM progenitors (Figs. 1b, 2).[13] The CNS is an exception, where embryonic microglia derived from YS progenitors are the dominant population (Fig. 2a).[14] Some EMPs in the skin and liver also differentiate directly into Langerhans cells and Kupffer cells, respectively, without passing through an intermediate monocyte stage (Fig. 2b). However, both EMP-derived lineages are eventually replaced by bone marrow-derived macrophages.

EMP and AGM lineage macrophages differentiate into several subsets with distinct functional properties (Fig. 2b, c):[15] (a) a classical ($CD14^{++}$, 90%); (b) a non-classical ($CD16^{++}$, 10%); and possibly, (c) an intermediate population that expresses both CD14 and CD16. $CD14^{++}$ monocytes show strong phagocytic activity, express inflammatory cytokines and reactive oxygen species (ROS), and react to Toll-like receptor (TLR) ligands. $CD16^{++}$ cells produce some inflammatory cytokines, but not much ROS. These cells patrol and assess endothelial integrity and infiltrate normal but more in inflamed tissues. The role of the third, intermediate monocyte population is not clear. These cells express major histocompatibility complex class II (MHC-II), and participate in antigen presentation and contribute to T lymphocyte activation.

- *Macrophage differentiation in the liver*: On day 32, $CD34^+$ $CD45^+$ HSCs migrate from the AGM zone to the liver.[13] These HSCs differentiate into monocytes and macrophage precursors from 8 to 20 weeks gestation, but then gradually involute during the 20–23-week period. Some cells of this lineage may arise from EMPs. Unlike in mice, the human embryonic liver may also contain some $CD34^+$ $CD45^-$ hemogenic endothelial cells that produce $CD33^+$ macrophage precursors.
- *Macrophage differentiation in the bone marrow*: Some $CD34^+$ $CD45^+$ HSCs migrate from the AGM zone into the bone marrow on day 32. The macrophage lineage differentiates into CMPs, granulocyte–monocyte precursors, common monocyte and dendritic cell (DC) precursors, pre-monocytes (committed monocyte progenitors), monocytes, and then into macrophage precursors by the seventh week of gestation (Fig. 2c). Human fetal bone marrow also contains $CD34^+$ $CD45^-$ endothelial cells that mature first into $CD33^+$ myeloid cells and then into monocytes and macrophages. These cells remain detectable at 1 in 60 $CD34^+$ $CD45^-$ cells even at

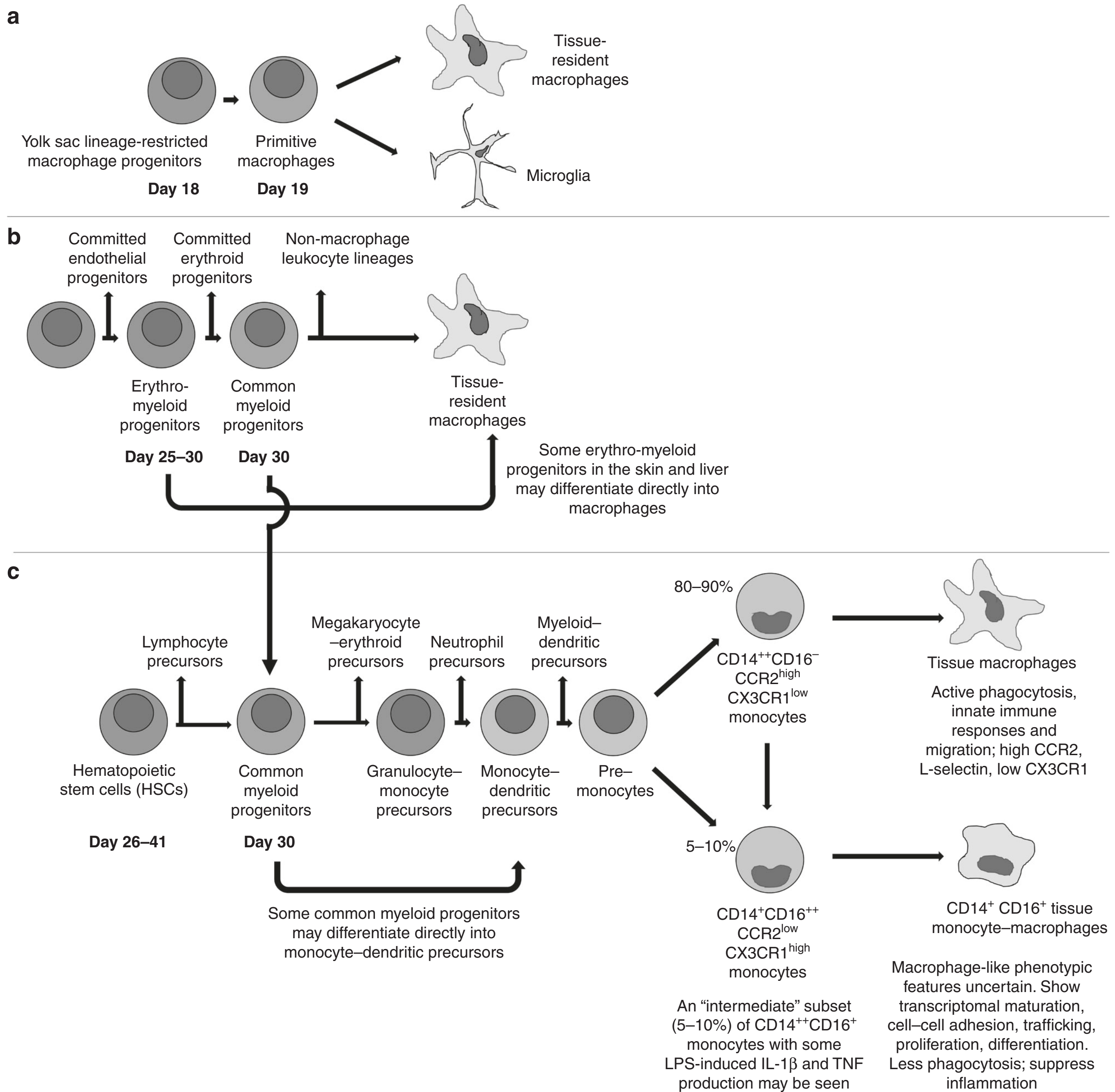

Fig. 2 Macrophage differentiation. Schematic shows macrophage development from **a** lineage-restricted macrophage progenitors; **b** yolk sac endothelium, which differentiates into erythroid–myeloid progenitors and then into common myeloid progenitors (CMPs). Some CMPs differentiate into macrophages and other primitive leukocytes, whereas others differentiate into granulocyte–monocyte precursors and then in sequential steps into macrophages as shown in **c**; **c** hematopoietic stem cells in sequential stages of common myeloid progenitors, granulocyte–monocyte precursors, monocyte–dendritic precursors, pre-monocytes, M1 or M2 (and possibly an intermediate subtype), and then into corresponding macrophages.

24 weeks gestation.[16] After birth, the HSCs migrate from the liver to the bone marrow and mature as part of the “definitive” hematopoiesis.[17] These hematopoietic lineages can also be detected in other tissues such as the brain, heart, liver, and skeletal muscle (Fig. 1).

Blood monocyte counts increase linearly between 22 and 42 weeks of gestation and make up 3–7% of all hematopoietic cells with absolute monocyte counts (AMCs) of 300–3300/μL (mean 1400/μL) at birth. The AMCs decline to ~700/μL by the third week.[18,19] After leaving the bone marrow, monocytes circulate in the bloodstream for 1–3 days and then move into the tissues to differentiate either into macrophages or into myeloid DCs (mDCs). Both macrophages and mDCs are involved in a variety of immune functions, such as phagocytosis, antigen presentation, and cytokine production.[20] In neonates and infants, macrophages are of particular interest as determinants of innate immunity before the development of adaptive immune mechanisms.

Besides differentiation into macrophage precursors, circulating monocytes serve as a first-line defense against pathogens, in tissue development, and in homeostasis. Some subsets also participate in reparative changes.[1] Monocytes migrate to the sites of infection, differentiate in situ, and become polarized (sections “Classification of macrophages” and “Macrophages in neonatal

inflammatory disorders") as needed.[21] The three subgroups of circulating monocytes have been described in the section on EMP and AGM lineages above.

MACROPHAGE BIOLOGY

Macrophages are seen in all tissues, and with their functional diversity, are one of the most pliable cells in the hematopoietic system. Functionally, macrophages are actively motile, phagocytic, and modulate immune responses.[22] Some antigenic profiles are prominent in premature and young infants, particularly the high expression levels of CD11b, chemokine receptors CCR1, CCR2, CCR5, CXCR1, CXCR2 (CXC-receptor 2), and other molecules such as CD115 and 6-sulfo *N*-acetyllactosamine glycan structures, and triggering receptors expressed on myeloid cells. In some microenvironments, the immaturity of neonatal macrophages becomes evident with ongoing phenotypic differentiation and still emerging efficiency in movement, phagocytosis, and regulation of inflammation. These cells can be stimulated by a panoply of endogenous triggers, such as cytokines; oxidized lipids; ROS and reactive nitrogen species (RNS); metabolic products; and debris released from dying cells, such as heat-shock proteins and damage-associated molecular patterns (DAMPs).[23] There are also multiple well-known exogenous activators, such as microbial products, microparticles, and chemicals.[23]

Circulating monocytes and differentiated macrophages play a critical role in host defense. Monocytes make up 5–10% of blood leukocytes, most of which (90%) comprise the "classical" $CD14^{+}$ $CD16^{-}$ subgroup. Some (<10%) are "non-classical" $CD14^{low}$ $CD16^{+}$ monocytes.[24] In mice, the classical inflammatory monocytes express Ly6C (a monocyte differentiation antigen that was named initially as the lymphocyte antigen 6 complex, locus C) and CCR2, but show only limited expression of the C–X3–C motif chemokine receptor 1 (CX_3CR1). The $Ly6C^{+}$ $CCR2^{+}$ CX_3CR1^{low} monocytes show an inflammatory profile[25] unlike the non-classical $Ly6C^{-}$ CX_3CR1^{high} $CCR2^{-}$ hypoinflammatory, healing/scavenging "resident" monocytes.[26,27] This functional polarization of monocytes and the lineage-specific, differentiated macrophages derived from these cells reflects the summated effect of a wide range of stimuli on these cells at a given point in space and time,[28] emphasizing their reversible and dynamic plasticity. Broadly, the major regulators of macrophage polarization include (a) epigenetic and cell survival pathways, which can shorten or prolong macrophage development and survival; (b) extrinsic factors, such as the physical microenvironment and microbial products; and (c) the inflammatory factors described above.[28]

CLASSIFICATION OF MACROPHAGES

MDMs are generally viewed in a binary classification with a classically activated M1 and an alternatively activated, immunoregulatory M2 phenotype that are believed to be derived from the two monocyte subsets described above (Fig. 3). These two subgroups show differences in surface antigens, tissue localization, intracellular signaling, and function. M1 macrophages are regulated by cytokines, such as tumor necrosis factor (TNF) and interferon-γ (IFN-γ), bacterial lipopolysaccharide (LPS), granulocyte–macrophage colony-stimulating factor (GM-CSF), and express cell surface proteins, such as CD54, CD80, CD86, and CD197.[29,30] M1 macrophages may be quicker to "inhibit" and kill pathogens as a primary host defense mechanism.[31] In contrast, M2 macrophages may respond more strongly to interleukin-4 (IL-4), IL-10, IL-13, and IL-21, and to glucocorticoids. M2 macrophages express higher levels of surface scavenger receptors, such as CD163, CD204, and the mannose receptor, CD206.[29,30] These macrophages are active in immunoregulation, maintain tissue integrity following injuries and in chronic infections, and promote angiogenesis.[31] The M2 macrophages are a relatively heterogeneous group, comprising of four subcategories (M2a, M2b, M2c, and M2d; Fig. 3).

MDMs need to be carefully differentiated from mDCs.[32] Ex vivo, monocytes differentiate into macrophages upon exposure to M-CSF, and into mDCs following treatment with GM-CSF and IL-4. Both macrophages and DCs share phenotypic markers CD11b, CD11c, CD80, CD86, CD163, CD209, and MHC-II. Macrophages typically express CD68 (macrosialin) and CD33 (siglec-3) with some specificity; DCs may express intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN/CD209), CD1c, and CD141.[33] mDCs have a stellate appearance and act as sentinels, migrate into lymphoid tissues upon antigen encounter, and present antigen to activate native T lymphocytes.[33] The mDCs are classified into two subsets: mDC1, which express CD141; and mDC2, which express CD1c. DCs express CD103 (integrin αE), CD11b (integrin αM), CX_3CR1, F4/80, CD8α, CD24, CD172a, XC-chemokine receptor 11, C-type lectin domain family 9 member A, E-cadherin (cadherin 1), and CD64 (also known as FcγRI).

MACROPHAGES IN NEONATAL INFLAMMATORY DISORDERS

In infants, macrophage production and activation is well regulated and maintained at low levels in the physiological state. However, during stress, infection, and inflammation, the macrophage population may be expanded with altered proportion of various subsets. The macrophage subsets may also be altered when inflammation begins to resolve and during restoration of tissue architecture or scarring.[28] In other instances, immature macrophages recruited during resolving inflammation may trigger apoptosis,[34,35] persistent low-grade inflammation,[34–36] or atypical inflammatory and microvascular changes.[36] Table 1 lists macrophage surface markers and inflammatory mediators that are known to be involved in various neonatal inflammatory conditions and may emerge as biomarkers in these conditions.

Circulating monocyte-derived macrophages in healthy infants and during neonatal sepsis

Blood monocytes serve as a critical first-line defense against invading pathogens.[37] These cells detect, phagocytose, and kill pathogens, and recruit other leukocytes by expressing inflammatory mediators, such as cytokines, chemokines, complement components, coagulation factors, and extracellular matrix (ECM) proteins.[38] Some macrophages may also present foreign antigens to the adaptive immune system (Table 2).

Neonatal macrophages show efficiency similar to those in adults in some,[39] although not all,[38] host defense functions. Pathogens are recognized by four types of PRRs located on the cell surface, within intracellular vesicles, and in the cytoplasm:[40] (a) TLRs; (b) nucleotide-binding oligomerization domain-like receptors (NLRs); (c) retinoic-acid-inducible protein I (RIG-I)-like receptors (RLRs); and (d) the integrins. These PRRs recognize pathogen-associated molecular patterns in bacterial cell walls, flagellin, and nucleic acids.[41] TLR activation induces the production of inflammatory mediators. TLR expression in neonatal macrophages resembles that in adults, and increases during bacteremia. NLRs detect peptidoglycans in the cytosol, including those released from intracellular bacteria such as *Listeria monocytogenes*.[41] The RLRs sense double-stranded viral RNA.[42] The β_2-integrin complement receptor 3 (CR3; Mac1, CD11b/CD18) on macrophage surface also serves as a pathogen sensor.[43] CR3 binds LPS and activates inducible NOS (iNOS).

In TLR signaling, MyD88 (myeloid differentiation factor 88), NEMO (nuclear factor-κB (NF-κB) essential modulator), and IRAK-4 (IL-1-receptor-associated kinase 4) are critical mediators.[44] The TLR pathway is most important early in life; the risk of infection in infants with IRAK-4 deficiency may decrease with increasing age. Some, but not other, reports suggest that endotoxin stimulation of neonatal macrophages may suppress MyD88, IRF5 (interferon

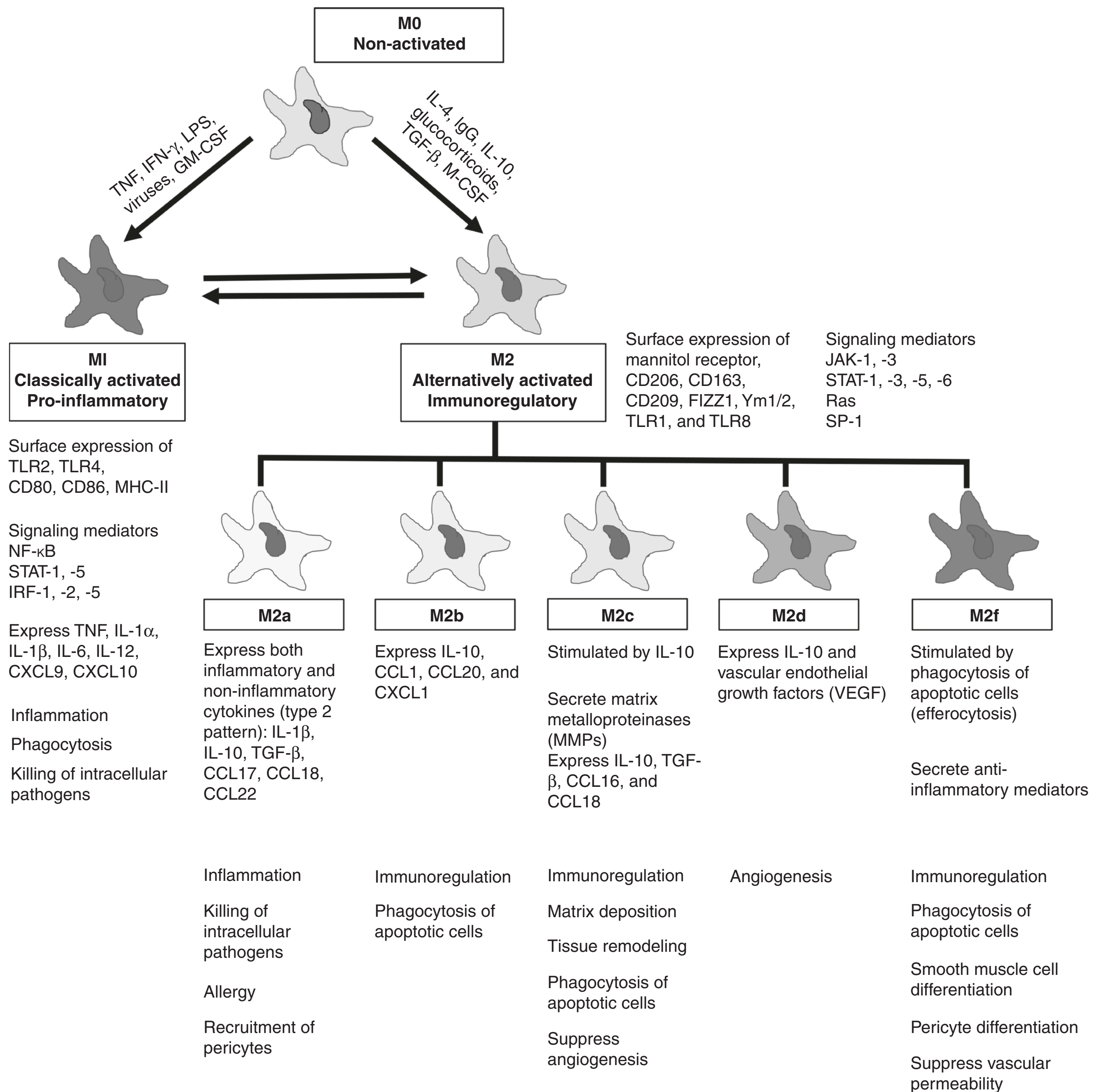

Fig. 3 Differentiation of monocyte-derived macrophages. Schematic shows differentiation of naive macrophages into classically activated M1 and alternatively activated M2 subclasses. The surface markers and key signaling mediators are depicted with each group. The M2 macrophages may comprise four subgroups with distinct inflammatory functions and physiological roles.

regulatory factor 5), and p38 phosphorylation. Some endogenous DAMPs, including cytokines, intracellular proteins, and those released from damaged cells, can increase inflammation.[45]

Compared to adults, neonatal macrophages produce less T-helper type 1 (T_H1)-polarizing cytokines, such as TNF, IFN-γ, and IL-12p70, and more T_H2/T_H17 anti-inflammatory cytokines, such as IL-6, IL-10, IL-17, and IL-23.[46] Less T_H1 cytokines may explain the impaired defenses against intracellular pathogens such as *Listeria*, *Mycobacteria*, and Herpes simplex virus. Neonatal macrophages express lower levels of MHC-II and costimulatory molecules, such as CD40 and CD86, and may also be efficient at stimulating the adaptive immune system.[46]

Preterm macrophages may show a gestational age-dependent weakness in the uptake of bacterial pathogens, such as group B streptococci.[37] Compared to macrophages from term infants, these cells show less TNF secretion, bactericidal function, and adherence receptor expression. In microarray analysis, neonatal macrophages show less expression of genes associated with antigen processing and presentation, such as the MHC class II (H2-Ab1, B2m, H2-K1, and H2-Q10). Costimulatory molecules CD80 and CD86 may also be lower,[22] and phagocytosis and chemotaxis may be less efficient.[37]

Circulating monocyte-derived macrophages become larger in size when activated in neonatal sepsis, a sign that can also be easily monitored.[47] Macrophage migration inhibitory factor (MIF) activates macrophages during neonatal sepsis, increases cytokine expression, and promotes bacteriocidal action against Gram-negative bacteria.[48] Infants with sepsis show

Table 1. Important signaling changes in neonatal inflammatory conditions that could emerge as clinically relevant biomarkers.

Sepsis
Increased TLRs, MyD88 (myeloid differentiation factor 88), NEMO (NF-κB essential modulator), and IRAK-4 (IL-1-receptor-associated kinase 4)
Decreased phosphorylation of MyD88, interferon regulatory factor 5 (IRF5), and p38 MAPK
Increased damage/danger-associated molecular patterns (DAMPs)
Decreased expression of MHC class II (H2-Ab1, B2m, H2-K1, H2-Q10).
Increased cellular size of circulating monocyte-derived macrophages
Increased serum levels of macrophage migration inhibitory factor (MIF)
Increased macrophage microRNA (miR)-141
Increased monocyte CD64
Increased monocyte CD163
Hypoxic–ischemic encephalopathy
Activation of microglia
Decreased umbilical cord blood derived $CD34^{+}$ HPC monocytes
Increased macrophage-derived inflammatory cytokines and chemokines
Increased nitric oxide synthase (NOS)
Increased reactive oxygen (ROS) and nitrogen species (RNS)
Increased excitatory amino acid agonists
Increased matrix metalloproteinases
Increased bcl-2 and bcl-XL
Increased neurotrophins such as nerve growth factor
Increased death receptor agonists
Bronchopulmonary dysplasia
Increased monocyte-derived $CD14^{+}$ (single-positive) and $CD14^{+}$ $CD16^{+}$ (double-positive) macrophages
Increased $CD206^{+}$ macrophages
Increased macrophage surface expression of HLA-DR, CD11b, CD11c, and CD64
Depletion of anti-inflammatory alveolar macrophage pools
Increased macrophage migration inhibitory factor (MIF)
Increased inflammatory cytokines IL-1α, IL-1β, IL-6, IL-8, and TNF and chemokines CXCL5, CXCL6, CCL3, and CCL20
Increased IL-1 receptor antagonist
Necrotizing enterocolitis
Decreased TGF-β1 in plasma, TGF-β2 in affected tissue
Macrophage TLR4 expression
Increased IL-1α, IL-1β, TNF, CXCL2, CXCL5, CCL3, CCL4, IL-17a
Increased smad7 in affected tissues
Increased monocyte-derived macrophages in affected intestine
Decreased blood monocyte counts
Intestinal epithelial cell apoptosis
Retinopathy of prematurity
Increased macrophage marker CD86
Increased MMP2, MMP-9
Increased IL-1 receptor antagonist
Retinal microglia bearing ionized calcium-binding adaptor molecule 1 (Iba1)
Acute kidney injury
Increased plasma TGF-β and IL-20
Increased urinary monocyte chemoattractant protein-1 (MCP-1/CCL2)

CD cluster differentiation, *SIRS* systemic inflammatory response syndrome, *MMP* matrix metalloproteinase, *CXCL* chemokine (C–X–C motif) ligand, *CCL* chemokine (C–C motif) ligand, *TNF* tumor necrosis factor, *IL-1ra* interleukin-1 receptor antagonist, *TLR* Toll-like receptor, *TGF* transforming growth factor, *MCP* monocyte chemoattractant protein.

lower serum and macrophage levels of microRNA-141 than controls, which explains increased LPS-induced inflammation in these infants.[49] Infants with sepsis may have higher plasma MIF concentrations than controls,[50–52] and these levels may have some diagnostic value as a biomarker in combination with other findings.

Macrophages in the neonatal brain and in HIE

In the healthy neonatal brain, resting microglia "survey" the local microenvironment with thin, ramified cytoplasmic processes.[53] In perinatal HIE, activated microglia and newly recruited macrophages express inflammatory cytokines, chemokines, NOS, ROS, and RNS, excitatory amino acid agonists, and death receptor

Table 2. Macrophage subpopulations.

Macrophage subpopulation	Stimulators	Function	Biological processes and signaling
M0	Naive, unstimulated macrophages		
M1	Inflammatory macrophages		
	Lipopolysaccharide (LPS) and interferon-γ (IFN-γ) Macrophage-produced inducible nitric oxide synthase (162) Macrophage-produced IL-12, IL-18, and IL-23 (163)	Proinflammatory, antimicrobial Initiate angiogenesis by producing vascular endothelial growth factor A (VEGFA) (164) May inhibit angiogenesis under certain conditions (149, 165) Express MMP-1, MMP-3, and MMP-10 (166)	Activate tie signaling Promote endothelial cell chemotaxis, and migration of other cells involved in angiogenesis (167)
M2	Anti-inflammatory, prohealing macrophages		
M2a	Cytokines such as interleukin-4 (IL-4) and IL-13 (168)	Platelet-derived growth factor-BB (PDGF-BB) (164) Transforming growth factor-β_1 (167)	Support pericyte and smooth muscle cell differentiation (169) Stabilize new blood vessels (167)
M2b	Immune complexes, IL-1β and pathogen-associated molecular pattern molecules (170) Immune complex plus Toll-like receptor (TLR) ligands (30)	Inhibit proliferation, migration, and apoptosis resistance of pulmonary artery smooth muscle cells (171) Produce proinflammatory cytokines IL-1, IL-6, TNF, and anti-inflammatory IL-10 (172, 173)	Dysregulation of PI3K/Akt/FoxO3a pathway (171)
M2c	IL-10, TGF-β, and glucocorticoids (172, 173)	Secrete matrix metalloproteinases (MMPs) (167) Secrete IL-10, TGF-β, and pentraxin-3 (PTX3) (172, 173)	Negative regulation of sprouting angiogenesis, endothelial cell apoptosis, and blood vessel branching morphogenesis (167)
M2d	TLR signals (172, 173) Costimulated by TLR and adenosine A2A receptor agonists (174) IL-6	Promotes tumor progression, metastasis Immunosuppression (175)	Expression of IL-10 and VEGF (176)
M2f	Phagocytosis of apoptotic cells (efferocytosis) (177) Upregulates TGF-β_1 (167)	Secretion of anti-inflammatory mediators (167)	Stimulate endothelial cells to suppress vascular permeability (167)

agonists.[54] Many of these signal transduction pathways directly disrupt the neuronal axons, the myelin sheath, and precursor and mature oligodendrocytes.[55]

In early HIE, activated microglia look “hypertrophic” with short and thick processes. Later, these cells often acquire an enlarged, amoeboid appearance with few to no cytoplasmic processes.[53] These cells show M1 characteristics with increased phagocytosis, antigen presentation, and production of inflammatory cytokines and matrix metalloproteinases (MMPs).[56] Activated microglia produce glutamate, NO, and ROS to promote axonal and oligodendrocyte degeneration and disrupt the immature blood–brain barrier (BBB).[57] Compared to adults, neonatal microglia accumulate quickly and survive for longer periods in ischemic lesions, particularly in the hippocampal dentate gyrus and in periventricular and subcortical white matter.[58] CNS also contains M1 tissue macrophages in the perivascular areas in the meninges and the cerebellar choroid plexus.[59] This is a heterogeneous population of macrophages; most are derived from YS progenitors, but some choroid plexus macrophages are continuously replenished from circulating monocytes. The damaged BBB is further breached by peripheral leukocytes, and the normally immune-privileged brain environment is exposed to systemic inflammatory responses. During resolution of the acute responses, M2 activation of microglia and macrophages promotes anti-inflammatory signaling (M2a), clearance of ROS and RNS (M2b), and healing of injured tissues (M2c).[6] Interestingly, HIE may be marked by a biphasic role of NF-κB; early post-insult NF-κB activation may contribute to brain damage, whereas it may promote neuronal survival in later stages through anti-apoptotic pathways. In these later stages, NF-κB may upregulate bcl-2 and bcl-XL, and increase neurotrophins such as the nerve growth factor to promote cell survival.[60]

Macrophages in the developing lung and in BPD

There are two major classes of lung macrophages.[61] The most abundant population comprises alveolar macrophages, which remove foreign particles through phagocytosis and promote the turnover of the surfactant lining of the alveolar surface.[62] The other major population is interstitial macrophages, which are located in close proximity to the alveolar capillaries and comprise 30–40% of all lung macrophages.[63] These macrophages promote tissue remodeling and antigen processing.[61] In the embryo, lung macrophages develop in the YS, and later from liver or bone marrow monocytes.[64] These monocyte-derived macrophages differentiate first into interstitial macrophages and then into alveolar macrophages.[65] Some alveolar macrophage pools can also be maintained through in situ division and renewal independent of the circulating precursors.[61]

In lung injury, macrophages express increased HLA-DR, CD11b, CD11c, and CD64.[66,67] In infants with severe lung disease and extensive epithelial damage, the depletion of the some anti-inflammatory alveolar macrophage pools[34,68] due to integrin-dependent cytokine expression[69] may increase inflammation. These macrophage numbers are quickly restored in some infants through recruitment of blood monocytes and local macrophage proliferation.[34,70] However, if these newly recruited or differentiated macrophages have an inflammatory profile, there is a risk of pulmonary inflammation, disrupted lung development, and BPD.[71,72] Macrophage MIF seems to be an important activator of this inflammatory response. It activates extracellular signal-regulated kinase 1 (ERK1)/ERK2-mitogen-activated protein kinase pathway, upregulates TLR4 expression, prolongs the lifespan of inflammatory macrophages by inhibiting p53-dependent apoptosis, counter-regulates the immunosuppressive effects of glucocorticoids, and promotes the expression of inflammatory

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cytokines.[73,74] Besides the changes in lung development, MIF has also been implicated in exacerbated inflammatory responses to infection.[73,75,76]

Macrophage activation appears to be an early event during BPD.[77] Premature infants may be susceptible to lung injury because of the immaturity of the inflammatory mechanisms in midgestation.[78] Increasing information on chemokine generation has generated mechanistic understanding of the recruitment of macrophages to sites of lung injury.[79] The expression of inflammatory genes and surface markers on lung macrophages suggest an alternatively activated phenotype. In murine models, depletion of specific macrophage populations may increase the severity of lung injury, suggesting that some subsets of these cells may be anti-inflammatory and protective.[80] In contrast, tracheal aspirates of extremely premature infants with evolving BPD show increased inflammatory $CD14^+$ (single-positive) and $CD14^+$ $CD16^+$ (double-positive) macrophages that express IL-1α, IL-1β, and IL-1 receptor antagonist.[79] Transcriptional gene profiling shows that infants at risk of BPD showed increased levels of inflammatory chemokines CXCL5 (CXC-motif chemokine ligand 5), CXCL6, CCL3, and CCL20 that predicted BPD and rose prior to the rise in inflammatory cytokines such as IL-1β.[81]

Macrophages in the intestinal mucosa and in NEC

In the neonatal intestine, monocyte recruitment and differentiation into mucosal macrophages contributes to mucosal immunity.[82,83] These macrophages display near normal phagocytic and bactericidal properties, but have only partially developed the tolerance to bacterial products that is characteristic of the mature intestine.[84] These changes occur under the influence of transforming growth factor-β (TGF-β), particularly the isoform TGF-$β_2$, present in the local ECM.[85] In the midgestation intestine, the ECM stores and the bioavailability of TGF-β are still in development.[86] Interestingly, low circulating TGF-β concentrations even on the first postnatal day may predict later occurrence of NEC.[87] Intestinal macrophages are also relatively resistant to the hypoinflammatory effects of TGF-β as the downstream signaling pathways are still in development.[83,84,88–90] One explanation for both the developmental deficiency of TGF-$β_2$ and the resistance to its intracellular effects is in increased levels of the signaling inhibitor smad7, which in turn, are related to low levels of the ski-like oncoprotein that normally represses the Smad7 promoter.[91,92] Besides blocking TGF-β signaling, smad7 also inhibits the autocrine expression of TGF-$β_2$ in the intestinal epithelium through increased dimethylation of lysine 9 on the histone H3 nucleosome and consequent transcriptional silencing.[93] Smad7 may also suppress TGF-β signaling by (a) binding histone deacetylase 1, silent mating type information regulation-1, and acetyltransferase p300, all of which compete with the activating Smads; (b) promoting the degradation of the TGF-β receptor I (TBRI) either through direct binding or via the formation of a ternary complex with the bone morphogenetic protein and activin membrane-bound inhibitor; (c) binding the phosphatase growth-arrest and DNA-damage-inducible protein 34-protein phosphatase 1c complex to promote TBRI dephosphorylation.[94–96]

Macrophages comprise up to two-thirds of the leukocyte infiltrates in NEC and play an important role in its pathogenesis.[88,97] Circulating monocyte-derived M1 macrophages with a proinflammatory profile are recruited to NEC lesions, but these cells do not undergo the normal TGF-β-induced hypoinflammatory differentiation.[88,98] These macrophages damage the epithelial barrier, and promote bacterial ingress and inflammation in the intestine.[99] The active recruitment of circulating monocytes to NEC lesions is reflected in decreased AMCs in peripheral blood; this acute drop in AMCs may be a useful early diagnostic marker of NEC.[100] Consistent with these findings, the severity of necrosis in NEC lesions can be reduced in animal models by inhibiting macrophage recruitment by (a) using specific antibodies against the chemokine ligands or the cognate receptors; (b) using pharmacological inhibitors; or (c) depleting macrophages in genetically modified mice.[4,88,98,101] Epithelial-derived CXCL5/epithelial-derived neutrophil-activating peptide 78 and its cognate receptor, CXCR2 on macrophages seem to be particularly important in macrophages recruitment.[102] During NEC, the TLR-mediated activation of the nuclear transcription factor NF-κB plays an important role in the recruitment, differentiation, and M1 activation of macrophages in the affected intestine.[103] IRF5 can activate M1 genes and promote the immune differentiation and TLR-mediated signal transduction.[104,105] In this context, recent findings that TGF-β and heparin-binding epidermal growth factor-like growth factor can promote an M2-like polarization in macrophages are exciting.[88,98,99]

Macrophages in the developing kidney and in acute kidney injury (AKI)

In the developing kidney, macrophages are sequentially derived from the YS, fetal liver, HSC, and then the bone marrow.[106] YS-derived macrophages typically express the runt-related transcription factor 1.[107] The kidney shows only a few macrophages during early development; most of these cells appear during midgestation.[107]

M2 macrophages have been implicated in progression of AKI to chronic kidney injury.[108,109] TGF-β and IL-20 are known to play a role in AKI.[110] IL-20 is known to promote inflammation, fibrosis, and apoptosis,[110] and IL-20 blockage may have some therapeutic value. In animal models, IL-4- and IL-13-mediated M2a macrophage polarization seem to promote recovery from ischemia–reperfusion and curtail tubulointerstitial fibrosis.[111] Consistent with these findings, elevated urinary levels of monocyte chemoattractant protein-1 (MCP-1/CCL2) are often seen in acute renal injury.[112]

MACROPHAGES AS MODULATORS OF ANGIOGENESIS AND IN RETINOPATHY OF PREMATURITY (ROP)

Macrophages have been linked with several disorders of angiogenesis.[113] These cells express vascular endothelial growth factor (VEGF), a well-known angiogenic factor in developing tissues and organ systems.[114] VEGF has two transmembrane receptors, the fms (feline McDonough sarcoma)-related receptor tyrosine kinase 1 (Flt1; VEGFR1) and the kinase insert domain receptor (VEGFR2). There is also a soluble Flt1 receptor generated by alternative splicing of the Flt1 mRNA.[115] Macrophages promote vascularization by aligning with and stabilizing newly forming blood vessels in several body tissues including the retina.[116,117]

In ROP, the immature retina shows areas of abnormal vascular development.[118] The disease involves two phases: an initial phase of tissue hypoxia and vaso-obliteration, and the second marked by compensatory neovascularization.[119] The developmental similarity of the retinae in premature infants and newborn mice has allowed the development of murine pup models of ROP-like oxygen-induced retinopathy (OIR).[120–122] The vascular abnormalities in OIR are associated with increased microglia and macrophages.[123,124] Unlike normal microglia with extensive dendritic processes, these cells show a simplified, ameboid appearance in OIR.[124] These inflammatory myeloid lineages play an important pathophysiological role in ROP.[125]

Human ROP and murine OIR are both characterized by endothelial cell proliferation and survival, and angiogenesis.[126,127] Both disorders show increased endothelial VEGF,[121,128,129] macrophage MIF and IL-8, which recruit and activate macrophages expressing CD86, IL-1 receptor antagonist, and MMPs and create a feed-forward cycle with further recruitment of macrophages, M1 activation, and increased expression of IL-6 and IL-1β.[130] Endothelium-derived IL-17A in the neovascularized choroid and retina also promotes M1 polarization of macrophages.[131] These

microglia and macrophages express IL-6 and IL-1β,[130] Notch1, and mitogen-activated protein kinase-related pathways involving the p38 MAPK, c-Jun N-terminal kinase, and the ERKs.[125] M2 macrophages, particularly the M2a, M2c, and M2d subsets, secrete VEGF in hypoxic areas of the retina to activate the Müller glial cells and promote endothelial proliferation and survival to increase angiogenesis, and neovascularization in both the retina and the choroid.[121,125–129,132–136]

MACROPHAGES IN TISSUE REPAIR

M1 macrophages can recognize nucleic acids released from dying cells.[137] TLR3 recognizes double-stranded RNA.[138] TLR9 (CD289) can recognize double-stranded DNA, DNA:RNA hybrids, and unmethylated CpG.[138] Once activated, TLR9 moves from endoplasmic reticulum to the Golgi apparatus and lysosomes, where it induces MyD88-dependent signaling. TLR7 and the closely related TLR8 respond to purine-rich single-stranded ribonucleic acid.[139] Intracellular proteins released from dying cells such as IFN-γ, and bacterial components such as peptidoglycan and LPSs are also recognized by M1 macrophages.[140] These macrophages express CD86, ROS and proinflammatory cytokines, such as IL-1, IL-6, TNF, and IFN-γ.

MACROPHAGES IN CELL DEATH

Macrophages recognize dying cells by several mechanisms.[141] Apoptosis is marked by the activation of caspase-3, caspase-8, and caspase-9.[36] Pyroptosis is another type of programmed cell death mediated by caspase-1 and caspase-11, which also trigger a strong inflammatory response.[142] Bacterial pathogens activate caspase-1 in macrophages through the recruitment of procaspase-1 into multiprotein complexes known as inflammasomes.[36] In macrophages, pyroptosis occurs with the release of inflammatory cytokines IL-1β and IL-18.[143] Interestingly, the ingestion of apoptotic cells by macrophages can trigger active immunosuppressive and anti-inflammatory responses. Macrophages can also induce apoptosis of some cells during tissue remodeling.[141] In some tissues, the uptake of apoptotic cells can suppress further induction of apoptosis in other cells by suppressing the expression of TNF and IFN-γ and increasing that of IL-10.

CONCLUSIONS

This review highlights the development and functional importance of macrophages in physiology, immune function, inflammation, and infection in neonates. We have also summarized emerging evidence that suppression of inflammatory activation of selected macrophage subsets in specific temporal windows may be of therapeutic value and yet allow ongoing organ development. Future investigations may help elucidate the relationship between macrophage maturation and the mechanisms of injury in fetal and neonatal periods.

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AUTHOR CONTRIBUTIONS

The two authors together developed the study concept, acquired and interpreted the data, wrote, and approved the final version of the manuscript.

ADDITIONAL INFORMATION

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