Negative regulation of human mononuclear phagocyte function

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At mucosal surfaces, phagocytes such as macrophages coexist with microbial communities; highly controlled regulation of these interactions is essential for immune homeostasis. Pattern-recognition receptors (PRRs) are critical in recognizing and responding to microbial products, and they are subject to negative regulation through various mechanisms, including downregulation of PRR-activating components or induction of inhibitors. Insights into these regulatory mechanisms have been gained through human genetic disease–association studies, *in vivo* mouse studies utilizing disease models or targeted gene perturbations, and *in vitro* and *ex vivo* human cellular studies examining phagocytic cell functions. Although mouse models provide an important approach to study macrophage regulation, human and mouse macrophages exhibit differences, which must be considered when extrapolating mouse findings to human physiology. This review discusses inhibitory regulation of PRR-induced macrophage functions and the consequences of dysregulation of these functions and highlights mechanisms that have a role in intestinal macrophages and in human macrophage studies.

INTRODUCTION

Following microbial exposure, the peripheral immune system must mount responses to limit infection and clear microbes. Mucosal surfaces, such as the intestine, continually interact with microbes[1] and therefore must balance the mechanisms defending against pathogens or excessive entry of resident microbiota with mechanisms maintaining tolerance to resident microbiota. To maintain this balance, anti-microbial responses undergo tight regulation. Uncontrolled inflammation can lead to the development of autoimmune and inflammatory diseases. However, overactive inhibitory mechanisms can increase susceptibility to infections and decrease microbial clearance, which can lead to persistent inflammation. Initial microbial recognition and responses occur through innate cells, particularly phagocytes. Consistently, depleting or altering the proportion and phenotype of these cells modulates severity of multiple autoimmune and inflammatory diseases, including at mucosal surfaces.[2–5] Phagocytes are comprised of myeloid-derived cells such as macrophages, dendritic cells (DCs) and neutrophils;[6] this review will focus on regulation in macrophages, although the regulation in DCs can be similar[7] and will occasionally be highlighted.

Macrophages regulate microbes at multiple levels, including through immune mediator secretion, microbial killing, pyroptosis, adaptive immune instruction, and wound healing.[6] Macrophages recognize and respond to microbial components through pattern-recognition receptors (PRRs), including Toll-like receptors (TLRs), nucleotide-binding-domain containing receptors (NLRs), retinoic-acid-inducible-gene I-like receptors, and C-type lectins.[8] Distinct PRR can share specific signaling pathways but can also signal through diverse intermediates. For example, TLR2, TLR5, TLR7, and TLR9 utilize the myeloid differentiation primary response gene 88 (MyD88)-dependent pathway, which activates nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK).[9] Interleukin-1 receptor (IL-1R) and IL-18R also utilize this pathway. By contrast, TLR3 signals through MyD88-independent or Toll/interleukin1-domain-containing adaptor-inducing interferon-β (TRIF) pathways.[9] TLR4 utilizes both MyD88-dependent and -independent pathways.[9] Although multiple PRR share pathways, the outcomes can vary dramatically.[9] Contributions to this variability include different adaptor molecule combinations, subcellular PRR localization and strength of signaling; however, many mechanisms accounting for the distinct outcomes are unclear.[9]

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PRR engagement in macrophages induces pro-inflammatory and anti-inflammatory cytokines; the balance between these cytokines influences outcomes.[9] Pro- and anti-inflammatory cytokines can be distinctly regulated, as can pro-inflammatory cytokines themselves (e.g., distinct inflammasome-mediated regulation of IL-1 and IL-18 secretion). Importantly, PRR-induced cytokine regulation varies over time. For example, initial pro-inflammatory cytokine induction can be followed by anti-inflammatory cytokines and other inhibitory mechanisms, thereby contributing to inflammation resolution. Notably, altered expression of various PRR-pathway inhibitors is observed in tissue and sera in many human inflammatory diseases. However, the role of these alterations is often unknown and may be either secondary to direct dysregulation or to compensatory regulation.

Human polymorphisms in regions containing PRR-pathway genes and their regulators in macrophages have been associated with multiple autoimmune/inflammatory diseases, including inflammatory bowel disease (IBD). Polymorphisms in regions involving PRR or PRR-initiated signaling pathways (e.g., *NOD2*, *IRF5*, *NFKB1*, *RELA*, *REL*, *RIPK2*, *CARD9*, *PTPN22*), paracrine/autocrine cytokine pathways modifying PRR signaling (e.g., *IL-23R*, *IL-12*, *IL-10*, *IL-18RAP/IL1R1*, *IFNGR2/IFNAR1*, *JAK2*, *STAT3*, *TYK2*), and autophagy pathways (e.g., *ATG16L1* and *IRGM*) are associated with IBD.[10] Mouse studies provide essential mechanistic insight into how pathways in phagocytic cells mediate dysregulation *in vivo*; conditional ablation of pertinent genes in myeloid cells further elucidates select myeloid cell functions.[7] While sharing similarities, mouse and human phagocytic cells also exhibit differences in microbial responses, cytokine induction and differentiation patterns.[6,11–13] These differences must be considered when extrapolating mouse results to human studies.[12] Consequently, parallel primary human macrophage studies are necessary. This review will focus on the inhibitory mechanisms regulating phagocytic cell functions and will particularly emphasize human studies and relevance to human inflammatory diseases. Where applicable, we will highlight the relevance of such mechanisms to mucosal surfaces, particularly the intestine.

MONOCYTE MIGRATION AND TISSUE-DEPENDENT DIFFERENTIATION

Monocytes are derived from a granulocyte monocyte-forming unit that differentiates in the bone marrow through cytokines, including monocyte-stimulating factor and granulocyte/monocyte-stimulating factor;[13] the transcription factor PU.1 regulates this differentiation.[13] Differentiated monocytes exit the bone marrow, enter the blood, and then migrate into tissues. In humans, circulating blood monocytes undergo apoptosis after 3–4 days,[14] and must differentiate to prolong survival.[15] Under homeostasis, monocytes enter tissues and become macrophages where they acquire functions integral to and characteristic of the tissues in which they reside.[13] Macrophages are larger, more effective phagocytes, and survive longer than monocytes, living for up to several months.[14]

PRR-INDUCED MACROPHAGE FUNCTIONS MUST BE TIGHTLY CONTROLLED

Initial PRR-induced responses are critical in controlling microbial insults;[1] however, these responses must be tightly regulated to prevent excessive cytokine secretion leading to systemic inflammatory response syndromes, including "endotoxin shock" or "sepsis," which can result in tissue injury and death. Upon chronic microbial stimulation, PRR-induced cytokines, chemokines, and activation markers are downregulated.[16–20] This process, termed "endotoxin tolerance", has been most commonly described with chronic TLR4 stimulation.[19] Mucosal macrophages encounter ongoing microbial exposure, and may be particularly subject to this type of regulation; multiple mechanisms contribute to reducing responsiveness to microbial products. Although some mechanisms limit acute PRR-mediated outcomes, these and other inhibitory mechanisms can be enhanced or induced following chronic microbial product stimulation. These mechanisms include downregulation of expression and/or function of PRR or critical intermediates in PRR-initiated pathways, modulation of strength of signaling, regulation by microRNAs, epigenetic regulation of gene promoters, as well as regulation by the inflammasome and autophagy (**Figure 1** and **Table 1**). Below, we will emphasize select inhibitory mechanisms and highlight where appropriate their relevance to homeostasis at mucosal surfaces, including the intestine.

Regulating expression of PRR signaling pathway genes

PRR-initiated macrophage responses can be inhibited by downregulating the expression and/or function of PRR complexes or PRR-initiated signaling pathways (**Figure 1** and **Table 1**). Although these mechanisms may decrease excessive inflammation and sepsis, they may also adversely affect bacterial clearance. PRR expression downregulation or receptor complex affinity alterations for microbial ligands during endotoxin tolerance have been observed in some, but not all, human and mouse macrophage studies.[21–23] However, overexpressing TLR4 or TLR adaptor molecules fails to reverse downregulated cytokine secretion in certain situations,[24–26] indicating that redundant endotoxin tolerance-inducing mechanisms can compensate for dysregulated modulation of receptor expression. Consistent with the requirement for TLR downregulation to limit intestinal PRR responses, epithelial cells and colitis-associated tumors from IBD patients and lamina propria macrophages from ulcerative colitis (UC) patients show increased TLR4 expression.[27,28] This may increase inflammation, as mice overexpressing TLR4 in epithelial cells are more susceptible to dextran sodium sulfate (DSS)-colitis and colitis-associated cancer.[29]

Downregulation or limited activation of PRR signaling intermediates, such as interleukin-1 receptor-associated kinase (IRAK)-1 and NF-κB (**Figures 1** and **2** and **Tables 1** and **2**), further attenuates PRR-initiated responses. **IRAK-1** participates in MyD88-dependent[9,30] and some MyD88-independent pathways.[30,31] IRAK-1 degradation following PRR stimulation limits subsequent inflammation in human monocytic cells.[20,31]

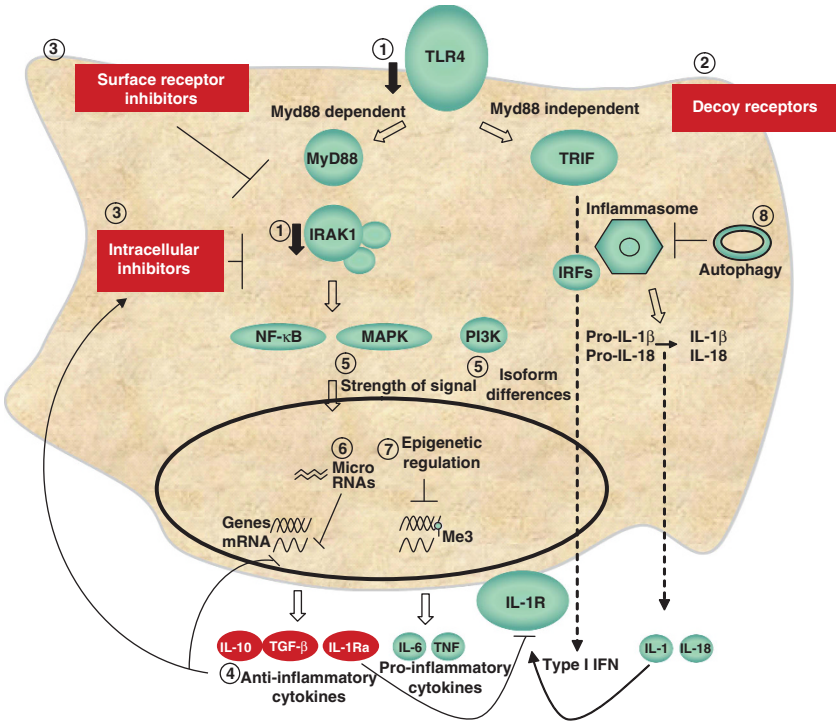

Figure 1 Mechanisms downregulating pattern-recognition receptor (PRR)-mediated macrophage responses. Signaling through PRR (e.g., Toll-like receptor 4 (TLR4)) activates distinct inflammatory pathways. The myeloid differentiation primary response gene 88 (MyD88)-dependent pathway signals through IRAK-1, nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK) pathways, while the MyD88-independent TRIF pathway signals through interferon regulatory factors (IRFs). Multiple mechanisms limit excessive macrophage responses induced by these pathways. They include: (1) downregulation of receptors and signaling molecules (e.g., PRRs, IRAK-1); (2) induction of decoy receptors (e.g., SIGIRR); (3) induction of intracellular or surface molecules that inhibit MyD88-dependent or -independent pathways; (4) induction of secretory molecules, including anti-inflammatory mediators (interleukin (IL)-10, transforming growth factor (TGF)-β, IL-1Ra), which suppress inflammation through multiple mechanisms; (5) signaling through alternative subunits or altering strength of signaling of molecules; (6) targeting of inflammatory RNAs by microRNAs thereby decreasing protein expression; (7) epigenetic regulation, including chromatin modification leading to inhibition of pro-inflammatory genes and/or upregulation of inhibitory genes; and (8) autophagy, which limits autocrine IL-1 secretion and therefore IL-1 contributions to secretion of additional pro-inflammatory cytokines. Inflammatory intermediates are indicated in green and inhibitory molecules/mechanisms in red. IFN, interferon; PI3K, phosphatidylinositol 3-kinase; TNF, tumor necrosis factor.

Furthermore, in non-monocytic cells, such as mouse enterocytes, failure to downregulate IRAK-1 upon postnatal PRR ligand exposure may contribute to necrotizing enterocolitis.[32] Consistent with IRAK-1 signaling downregulation preventing excessive inflammation, mice deficient in IRAK-4, a kinase that activates IRAK-1, are protected against endotoxin shock but are more susceptible to bacterial infections.[33] IRAK-1 polymorphisms resulting in increased expression and/or kinase activity are associated with more severe sepsis.[34,35] By contrast, human IRAK-4 loss-of-expression mutations increase invasive pneumococcal disease risk.[36] Downstream **NF-κB** signaling is critical for PRR-induced responses and its modulation changes PRR-initiated outcomes. For example, decreased phosphorylation and nuclear translocation of NF-κB subunits downregulates TLR4-mediated signaling in human intestinal macrophages.[26] Although NF-κB activation is generally associated with inflammation, its outcomes are more complex; for example, NF-κB directly contributes to anti-microbial responses. Moreover, NF-κB has various regulatory roles, which vary in different intestinal cell subsets, and different NF-κB subunits have distinct functional roles. For example, while p50/p65 NF-κB heterodimers generally induce inflammatory pathways and these individual subunits are essential for proper anti-microbial responses,[37,38] p50/p50 homodimers inhibit inflammatory responses,[39,40] and contribute to endotoxin tolerance in mouse macrophages.[41,42] Similarly, mice deficient in IKKβ (Ikappa B kinase beta) in myeloid cells are more susceptible to endotoxin shock.[43] Moreover, mice deficient in p50 and heterozygous for p65 develop spontaneous typhlocolitis.[44] However, p65 knock down attenuates experimental colitis,[45] and increased PRR-induced NF-κB activation in epithelial cells exacerbates colitis but only if accompanied with MAPK activation and tumor necrosis factor (TNF)-α production.[46] Another complexity is that p50 is processed from the *NFKB1* gene product p105,[39] which exhibits additional regulatory functions, as p105-deficient mice expressing p50 still show increased lung and liver inflammation.[47] Importantly, *NFKB1* polymorphisms are associated with UC[10] and necrotizing enterocolitis,[48] and polymorphisms in the NF-κB subunits *REL* and *RELA* are associated with IBD;[10] the functional consequences of these polymorphisms are unclear. In IBD patients, increased p65 expression and NF-κB-binding activity is seen in intestinal macrophages and epithelial cells.[49,50] Therefore, regulating the expression and function

Table 1 Mechanisms limiting PRR response and their relationship to inflammatory disease

Inhibition category	Mechanism of inhibition	Animal disease models	Human disease
Regulation of PRR signaling pathways			
PRR complex downregulation	↓ PRR-ligand binding and signal transduction	● TLR4 overexpression in epithelial cells→ ↑DSS-colitis and colitis-associated cancer[29]	UC patients express↑TLR4 on intestinal macrophages potentially contributing to inflammation[29]
IRAK-1, IRAK-4, MyD88 expression downregulation	↓ signaling by PRR-initiated pathways	● IRAK-4 $^{-/-}$: protected from endotoxin shock, but decreased survival with bacterial infections[33]	● a haplotype with putative↑IRAK-1 activity associated with↑sepsis[35] ● downregulated MyD88 and IRAK-4→ ↑invasive pneumococcal disease[36]
NF-κB	Multiple outcomes depending on context and on the subunits within the complexes	*Increases inflammation*: ● IKKβ deletion in myeloid cells→ ↑endotoxin shock[43] ● ↑IKKβ in epithelial cells→ colitis if accompanied with MAPK activation[46] ● p50 $^{-/-}$p65 $^{+/-}$→ typhlocolitis[44] ● p105 $^{-/-}$p50 $^{+/+}$→lung and liver inflammation[47] *Decreases inflammation*: ● p65 knockdown → ↓ experimental colitis[45]	● ↑p65 activation in macrophages from IBD patients[49,50] ● SNP leading to decreased promoter activity of *NFKB1* associated with UC[310] ● SNPs in regions containing *NFKB1*, *REL*, and *RELA* associated with IBD[10]
Decoy receptors			
SIGIRR	Inhibits MyD88-dependent signaling by preventing IRAK1/TRAF6 recruitment[311]	● enhanced expression protects mice from LPS-induced lung injury[312] ● deficiency ↑'s endotoxin shock, lupus and colitis[313–315]	● SNPs in a region, including *SIGIRR*, are associated with TB[316] ● ↑in monocytes during sepsis[317]
ST2	● inhibits NF-κB signaling in specific cell types[52–54] ● soluble ST2 decoy receptor for IL-33[55]	Deficiency results in endotoxin tolerance defect[52]	● ↓ST2L expression on epithelia but↑soluble ST2 expression in sera from UC patients[56] ● SNP in a region containing *ST2 (IL-1RL1)* associated with CD[59]
Soluble PRR complex (sTLR2,sTLR4)	Prevents bacterial ligand binding to PRR complexes	sTLR2/sTLR4 treatment ↓'s lung and peritoneal inflammation[318,319]	SNP increasing sCD14 associated with CD;[320]↑sCD14 levels associated with CD activity[321]
Secretory mediator upregulation			
IL-10	● ↓'s inflammatory gene transcripts[117] ● induces inhibitors[123]	IL-10 $^{-/-}$ and IL10RB $^{-/-}$ develop colitis[132,322]	SNPs in the *IL10* and *IL10R* region associated with IBD[10,137]
TGF-β	● ↓'s inflammatory gene transcripts and translation[115] ● inhibits NF-κB[26] ● induces SHIP-1[107] ● ↓'s CD40[116]	● TGF-β $^{-/-}$ and TGFβRII $^{-/-}$ develop colitis[132] ● TGFBR truncation in myeloid cells → delayed DSS-colitis resolution[135]	SNPs in a region that includes *SMAD3* associated with IBD[10]
IL-1Ra	binds to the IL-1R and inhibits IL-1R signaling and IL-1 autocrine loop	*Deficiency/blockade:* ● ↑experimental colitis[141] ● ↑endotoxin shock[142] ● ↑bacterial clearance[142]	loss-of-function *IL-1Ra* SNPs associated with autoimmune skin and bone diseases[143]
*Intracellular and surface receptor inhibitors (see **Table 2**)*			
MicroRNA			
Epigenetic modification			
Autophagy			

Abbreviations: CD, Crohn's disease; DSS, dextran sodium sulfate; IBD, inflammatory bowel disease; IKK, IκB kinase; IL, interleukin; MAPK, mitogen-activated protein kinase; MyD88, myeloid differentiation primary response gene 88; NF, nuclear factor; PRR, pattern-recognition receptor; SHIP, SH2-containing inositol 5'-phosphatase; SNP, single nucleotide polymorphism; TB, tuberculosis; TGF, transforming growth factor; TLR, Toll-like receptor; UC, ulcerative colitis.

of PRR-signaling pathway intermediates in macrophages occurs on multiple levels. As both loss- and gain-of-function of these molecules can lead to inflammation, fine tuning their regulation is crucial for proper immune homeostasis.

Inhibitory molecules regulate PRR signaling by various mechanisms

PRR-induced inflammation can be downregulated through inhibitors targeting PRR-initiated pathways (**Figure 2** and **Table 2**). Some inhibitors are constitutively expressed and inhibit basal cytokine expression and/or initial PRR signaling modulation, whereas others are upregulated after PRR stimulation. Furthermore, inflammation can be regulated by several inhibitory waves following PRR stimulation.[51] Inhibitors are often increased in inflamed tissues, as the immune system attempts to control the inflammation. Below, we will discuss examples of inhibition of PRR-initiated responses by surface receptor and intracellular inhibitory molecules. A summary of these and other inhibitors is shown in **Figure 2**, **Tables 1** and **2**.

Inhibitory surface receptors or secreted decoy receptors regulate PRR-induced inflammation. For example, initial studies found that **ST2** sequesters MyD88 and Mal to prevent TLR4-dependent NF-κB signaling, cytokine induction, and endotoxin tolerance *in vivo* in mice,[52,53] as well as in human monocytes.[54] Subsequently, membrane-bound ST2 (ST2L) was identified as a receptor for IL-33,[55] and controversy ensued as to the composite effects of IL-33/ST2L interactions in inhibiting or activating cytokine-inducing pathways. These interactions may differentially affect inflammation in distinct cell types and tissues, including in the intestine.[56,57] In mice, IL-33 binds to ST2 on Th2 (T helper type 2) cells,[57] and this interaction promotes Th2-mediated colitis.[56] ST2 also exists as a soluble isoform, which is a decoy receptor that binds to IL-33 and inhibits its signaling,[58] adding further complexity to the ST2-mediated regulation of inflammatory responses. Polymorphisms in a region containing *ST2 (IL1RL1)* are associated with Crohn's disease (CD).[59] In UC patients, inflamed mucosa and sera express increased soluble ST2, whereas ST2L expression on the surface of epithelial cells is downregulated, adding further complexity to elucidating the ultimate role of ST2 in IBD.[56]

IRAK-1 inhibition regulates not only PRR-, but also IL-1R- and IL-18R-induced pathways.[9] Basal expression of the IRAK-1-activation inhibitor, **IRAK-M**, in mouse and human macrophages controls initial PRR-mediated inflammation.[31,60,61] Chronic infection or PRR stimulation further upregulates IRAK-M in macrophages,[31,60,62,63] which, in turn, downregulates cytokine induction following PRR restimulation.[31,60,62] This partially occurs by preventing IRAK-1 and IRAK-4 dissociation from MyD88, thereby abrogating further IRAK-1-dependent signaling.[60] IRAK-M deficiency increases severity of endotoxin shock[60] and of mucosal injury models in mice, including in the lung[64] and colon.[65] Loss-of-function polymorphisms have been associated with inflammatory/autoimmune diseases such as asthma,[66] and dysregulated IRAK-M levels are seen in various diseases, including asthma,[67] necrotizing enterocolitis,[68] tuberculosis,[69] and cystic fibrosis.[62]

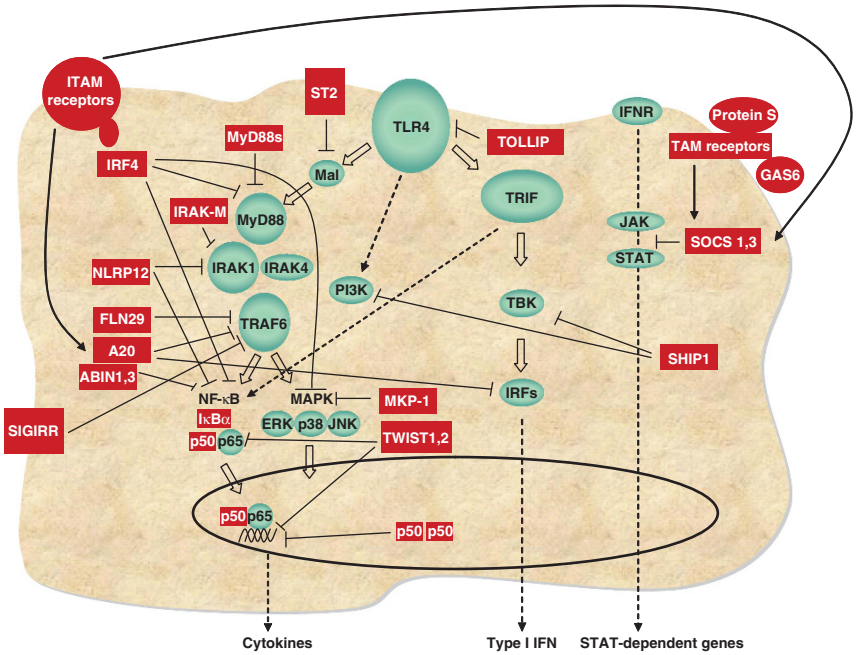

Figure 2 Inhibitory molecules operate by numerous mechanisms to limit pattern-recognition receptor (PRR)-induced macrophage functions. Intracellular inhibitors regulate inflammatory responses on multiple levels by interfering with PRR-initiated signaling. Selected mechanisms of action of intracellular inhibitors are also summarized in **Table 2**. Inflammatory intermediates are indicated in green and inhibitory molecules in red. ABIN, A20 binding inhibitor of NF-κB; ERK, extracellular signal–regulated kinase; GAS, growth-arrest specific; IFNR, interferon receptor; IRF, interferon regulatory factor; ITAM, immunoreceptor tyrosine activation motif; JAK, Janus tyrosine kinase; MKP, mitogen-activated protein kinase phosphatase; MyD88, myeloid differentiation primary response gene 88; NF-κB, nuclear factor κB; NLR, nucleotide oligomerization domain-like receptor; PI3K, phosphatidylinositol 3-kinase; SHIP, SH2-containing inositol 5′-phosphatase; SOCS, suppressor of cytokine signaling; TLR, Toll-like receptor; TRAF, TNF receptor–associated factor.

Table 2 Intracellular inhibitors regulating PRR function

Inhibitory molecule	Mechanism of inhibition	Animal disease models	Human disease
A20	Ubiquitinates/deubiquitinates molecules in PRR and TNFR pathways[92–95]	*Deficiency*: ● death due to severe multi-organ inflammation[91] ● ↑endotoxin shock[91] ● deficiency in DCs → colitis[96]	SNPs in the *A20* region associated with CD,[97] SLE[98,99] and RA[100,101]
ABIN1	Recruits A20[323,324]	● dysfunction→ SLE and autoimmunity in mice[325,326] ● ↑expression protects from TNF-induced death and allergic airway inflammation[327,328]	SNPs in *ABIN1* region associated with SLE[329,330]
ABIN3	● inhibits NF-κB activation[331] ● binds A20[331]	adenoviral expression in mice protects from LPS -induced death[331]	
FLN29	● inhibits TRAF6-mediated NF-κB signaling[332] ● inhibits the Rig I pathway[333]	FLN29$^{-/-}$ →↑endotoxin shock[333]	
IRAK-M	Inhibits IRAK-1 activation[60]	*Deficiency*: ● ↑inflammation to microbes[60] ● ↑lung injury[64] ● ↑IL-10$^{-/-}$ colitis[65] ● endotoxin tolerance defect[60]	Mutations↑risk for asthma;[66,67] dysregulated levels seen in necrotizing enterocolitis,[68] TB[69] and cystic fibrosis[62]
IRF4	↓'s signaling through RIP2[102] and MyD88[103]	● IRF4$^{-/-}$ →↑DNA shock[103] ● IRF4 knockdown *in vivo* →↑experimental colitis[102]	↑ expression in mucosa of IBD patients[105]
ITAM	Induces inhibitors, including SOCS-1, ABIN-3, A20, and IL-10[112]	ITAM-containing receptors suppress inflammatory diseases, including colitis[113,114]	
MKP-1	Inhibits MAPK signaling[334]	*Deficiency*: ● IL-10$^{-/-}$ colitis[335] ● sepsis due to impaired bacterial killing[334,336]	Impaired induction in: ● alveolar macrophages from asthma patients[337] ● LPS-treated BAL cells from sarcoidosis patients[338]
MyD88s	Prevents MyD88 signaling by failure to recruit IRAK-4[339]		● ↑in monocytes during sepsis[317]
NLRP12	● inhibits IRAK1 phosphorylation[195] ● ↓'s NIK expression[194]	*Deficiency*: ● ↑DSS colitis and colitis-associated cancer[340] ● ↑*Y. pestis* susceptibility[193]	Mutations → auto-inflammatory diseases[198,199] and atopic dermatitis[200]
PGE	● induces IRAK-M[341] ● ↓phagocytosis, bacterial killing[342,343]	Mice with prostaglandin pathway deficiency →↑colitis and colonic injury[344,345]	SNPs in a region that includes *PTGER4* associated with IBD[10]
SHIP-1	● dephosphorylates TBK1 and inhibits IFNβ induction[106] ● inhibits PI3K signaling[107]	*Deficiency*: ● ileitis[109] ● endotoxin tolerance defect[107] ● macrophage infiltration in bone marrow and spleen[110] ● ↓life span[110]	↑ SHIP-1 expression in the intestinal mucosa of IBD patients[111]
SOCS-1	Inhibits JAK/STAT signaling[70–72]	Deficiency ↑'s inflammatory cytokine production and endotoxin shock[72,346]	Loss-of-function/expression SNPs:↑serum IgE[78] and↑asthma[79] and lymphoma[347]
SOCS-3	Inhibits STAT1 and STAT3 activation[348,349]	● deficiency → embryonic lethality[350] ● deficiency in macrophages→↑endotoxin shock[351]	↑ SOCS-3 expression in mucosal biopsies from CD patients[352]
TOLLIP	● associates with TLR2 and TLR4 to limit signaling[353,354] ● inhibits IRAK-1 signaling[354,355]	Deficiency→↑inflammation following sublethal endotoxin[356]	SNPs associated with atopic dermatitis[357] and SNPs with decreased Tollip mRNA associated with TB[358]
TWIST family	● Twist1 and Twist2 inhibit NF-κB p65 transactivation[359] ● Twist1 and Twist2 repress the TNF-α gene promoter[82] ● Twist2 induces IL-10 through c-Maf activation[360]	● Twist1$^{-/-}$: embryonic death[361] ● Twist2$^{-/-}$: →perinatal death with cachexia and↑cytokines[359] →endotoxin tolerance defect in macrophages[360]	● ↑Twist1 expression in Th1 lymphocytes in inflamed tissue from IBD and RA patients[362]
TAM receptors	● SOCS-1 and -3 induction[80] ● apoptotic cell clearance[85–87]	*Deficiency/functional defect*: ● SLE and autoimmunity[81,85] ● ↑endotoxin shock[83]	TAM receptor ligand levels dysregulated in SLE and UC[89,90]

Abbreviations: BAL, bronchoalveolar lavage; CD, Crohn's disease; DC, dendritic cell; DSS, dextran sodium sulfate; IBD, inflammatory bowel disease; IFN, interferon; IgE, immunoglobulin E; IL, interleukin; IRF, interferon regulatory factor; JAK, Janus tyrosine kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NF, nuclear factor; NLR, nucleotide-binding-domain containing receptor; PI3K, phosphatidylinositol 3-kinase; PRR, pattern-recognition receptor; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SNP, single nucleotide polymorphism; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription factor; TB, tuberculosis; TLR, Toll-like receptor; TNFR, tumor necrosis factor receptor; TRAF, TNF receptor–associated factor; UC, ulcerative colitis.

TLR3 and TLR4 activate the MyD88-independent TRIF pathway, which induces Type I interferons (IFNs) and IFN-γ. Autocrine interferon-mediated inflammation is, in turn, regulated by the suppressor of cytokine signaling (SOCS) proteins, in particular **SOCS-1**, which primarily inhibits interferon-initiated JAK/STAT (Janus tyrosine kinase/signal transducer and activator of transcription factor) pathways.[70–74] SOCS-1-deficient mice show early death due to multi-organ inflammation,[73] and decreased survival to sublethal TLR4 stimulation.[72] Initial papers described SOCS-1 as down-regulating TLR4-induced NF-κB;[72,75] however, subsequent studies found the SOCS-1-deficient mouse phenotype was mostly attributable to excessive type I IFN inflammatory effects, rather than defects in direct TLR4 signaling inhibition by SOCS-1.[76,77] Mutations decreasing SOCS-1 expression are associated with immune dysregulation, including increased serum Immunoglobulin E[78] and asthma.[79] SOCS-1 upregulation can be mediated by the three TAM receptors expressed primarily in myeloid cells: Tyro3, Axl, and Mer.[80,81] Axl also upregulates the transcriptional repressor Twist1 in human macrophages.[82] Mer-deficient mice are more susceptible to endotoxin shock.[83] Deficiency in all the three TAM receptors and the subsequent lack of SOCS-1 induction results in uncontrolled macrophage-mediated pro-inflammatory cytokine secretion and autoimmunity.[81] Besides inhibiting PRR-mediated inflammation,[80] TAM receptors regulate DC chemotaxis[84] and apoptotic cell phagocytosis and clearance by macrophages.[85–87] Defects in TAM receptor-induced phagocytosis contribute to autoimmunity in TAM-deficient mice.[85] Consistently, apoptotic cell ingestion is associated with anti-inflammatory cytokine production and tolerance.[88] Increased levels of Protein S, a TAM receptor ligand, are seen in inflammatory diseases such as SLE (systemic lupus erythematosus) and UC;[89,90] the role of this elevation in the disease process is unclear.

An important inhibitor targeting multiple pathways, **A20**, was initially implicated in terminating TNF-induced NF-κB activation in mice[91] through ubiquitinating a TNF signaling intermediate receptor-interacting protein (RIP) and targeting it for degradation.[92] Subsequent studies demonstrated that A20 also inhibits PRR-initiated pathways such as the TLR4 pathway through ubiquitinating TRAF6 (TNF receptor–associated factor 6),[93] the TLR3 pathway by preventing IRF3 (interferon regulatory factor 3) dimerization,[94] and the nucleotide-binding oligomerization domain-containing protein 2 (NOD2) pathway by ubiquitinating and degrading the NOD2 downstream effector RIP2.[95] A20-deficient mice die prematurely due to severe multi-organ inflammation.[91] Moreover, selective A20 deficiency in DCs induces spontaneous colitis.[96] Polymorphisms in the A20 region, including loss-of function mutations, are associated with multiple autoimmune/inflammatory diseases, including CD,[97] SLE,[98,99] and rheumatoid arthritis (RA).[100,101] **IRF4** inhibits PRR pathways in mouse and human monocytic cells by preventing RIP2[102] and MyD88 signaling,[103] and decreasing JNK and NF-κB activation.[104] Consistently, IRF4$^{-/-}$ mice demonstrate more severe endotoxin shock.[103] Furthermore, IRF4 upregulation upon chronic NOD2 stimulation contributes to protection from experimentally induced colitis,[102] and IRF4 expression is increased in the mucosa of IBD patients.[105]

SH2-containing inositol 5'-phosphatase (**SHIP-1**) inhibits MyD88-independent pathways by dephosphorylating and inhibiting tank binding kinase 1 (TBK1), thereby modulating IFNβ induction.[106] SHIP-1 also limits phosphatidylinositol 3-kinase (PI3K) signaling.[107] SHIP-1-deficient mice are hyper-responsive to lipopolysaccharide (LPS) stimulation and defective in endotoxin tolerance.[107] However, over time SHIP-1 deficiency promotes anti-inflammatory M2 macrophage polarization, possibly to counteract the inflammatory phenotype.[108] SHIP-1$^{-/-}$ mice exhibit ileitis[109] and increased macrophage infiltration into bone marrow and spleen, which ultimately decreases survival.[110] Increased SHIP-1 expression is observed in the intestinal mucosa of IBD patients;[111] it is unclear if this increase reflects a compensatory mechanism to counteract the inflammation.

FCγ receptors and β2-integrins containing **ITAM** domains downregulate multiple PRR pathways by inducing PRR signaling inhibitors, including SOCS-1, A20, and IL-10 in primary human macrophages.[112] Consistently, ITAM receptor induction protects from inflammation, including experimental colitis.[113,114] Which specific ITAM-containing receptors mediate inhibitory effects and whether ITAM domain-containing peptides can be used therapeutically in inflammatory diseases has yet to be defined.

Secreted inhibitory mediators downregulate PRR-induced pro-inflammatory pathways in an autocrine and/or paracrine fashion

PRR stimulation results in secretion of autocrine/paracrine factors that can feed back to inhibit PRR pathways directly or indirectly, thereby suppressing inflammatory outcomes. For example, PRR-induced transforming growth factor (TGF)-β can inhibit pro-inflammatory cytokines by degrading their transcripts and suppressing their translation.[115] TGF-β also inhibits NF-κB signaling,[26] downregulates CD40[116] and induces SHIP-1.[107] IL-10 also regulates inflammation through multiple mechanisms. IL-10 decreases transcript and protein expression of numerous genes, including pro-inflammatory cytokines,[115,117–119] and leads to degradation of the MyD88-dependent signaling intermediates IRAK-1 and IRAK-4.[120] IL-10 also upregulates inhibitors such as soluble TNFR, IL-1Ra and SOCS-3[121] and upregulates STAT3 and PI3K pathways,[122] which can inhibit inflammatory pathways.[123–130] Moreover, IL-10 inhibits additional myeloid cell functions, including phagocytosis and antigen presentation.[123] Autocrine IL-10 and TGF-β signaling frequently combine to effectively suppress inflammation, including in human macrophages and at mucosal sites.[18,126,131] Mouse studies have long confirmed the importance of these secretory mediators in homeostasis at mucosal surfaces: IL-10 and TGF-β-deficient mice develop spontaneous colitis.[132] Depleting macrophages in IL-10-deficient mice attenuates the colitis,[133] demonstrating that

dysregulated macrophage function contributes to disease. Besides downregulating inflammation in macrophages proper, IL-10 production by gut macrophages suppresses inflammation by modulating other cell subsets, such as intestinal FoxP3 + T cells.[134] Mice with deletion or loss-of-function of the TGF-β receptor in myeloid cells show impaired DSS-colitis resolution,[135] indicating that besides producing TGF-β myeloid cells must also respond to TGF-β to limit intestinal inflammation. Consistently, TGF-β is required for downregulating pro-inflammatory cytokines in human lamina propria macrophages.[136] Importantly, human polymorphisms in regions including *IL10*, *IL10R*, and *SMAD3* are associated with autoimmune/inflammatory diseases, including IBD.[10,137,138] An additional PRR-induced secretory mediator, IL-1Ra, inhibits IL-1R signaling.[139] As autocrine/paracrine IL-1 dramatically enhances overall cytokine secretion by human macrophages, IL-1Ra ultimately downregulates multiple cytokines (**Figure 1**).[140] Consistently, IL-1Ra deficiency or blockade worsens experimental colitis[141] and increases endotoxin susceptibility in mice,[142] and loss-of-function *IL-1Ra* polymorphisms are associated with autoimmune diseases[143] (**Table 1**).

Emerging evidence shows that in specific circumstances, cytokines with pro-inflammatory roles such as IL-1β,[126] interferons,[82,144] and TNF-α,[61,145,146] can, in fact, also downregulate PRR- and FCγR-mediated inflammation in human macrophages. Mechanisms mediating these anti-inflammatory responses can include induction of certain inhibitory proteins and inhibitory signaling pathways (e.g., GSK3 (glycogen synthase kinase-3) signaling).[61]

Distinct isoforms and strength of signaling modulate PRR-induced macrophage outcomes

Targeting of MAPK and PI3K signaling pathways is being studied in therapeutic trials.[147,148] As modulating the quality and/or quantity of these pathways determines whether they activate or inhibit PRR-initiated pathways (**Figure 1**), an improved understanding of their signaling would more accurately predict therapeutic outcomes.

Extracellular signal–regulated kinase (ERK) can inhibit LPS-induced pro-inflammatory cytokines in mouse macrophages.[149,150] However, this inhibition has not generally been observed in human studies.[151,152] More recent studies in human macrophages identified that PRR-induced ERK indeed inhibits pro-inflammatory cytokines but that this inhibition is masked by autocrine IL-1, which then increases the strength of MAPK signaling and modifies the downstream outcomes.[153] Differential susceptibility to lowering ERK signaling can be observed between different pro-inflammatory cytokines as well, such that IL-1β secretion decreases upon ERK inhibition more readily than TNF-α in LPS-stimulated mouse macrophages.[154] Therefore, the strength of MAPK signaling can dramatically influence whether pro-inflammatory cytokine secretion is inhibited or enhanced in macrophages.

PI3K can both downregulate[124–130] and upregulate[155,156] pro-inflammatory signaling. PI3K consists of multiple subunits,[157] which could contribute to these observed differences,[124,130,158,159] as could differential strength of PI3K signaling induced by distinct PRR. Furthermore, the subunits targeted vary between different pharmacological PI3K inhibitors and the inhibitor concentrations used. In human disease, targeting the PI3K substrate mTOR (mammalian target of rapamycin) induces tolerance during transplantation by upregulating regulatory T cells.[148] However, rapamycin inhibits PRR-mediated tolerance in human macrophages,[126] and mTORC1 deficiency in DCs exacerbates DSS-induced colitis.[160] Therefore, although PI3K and mTOR signaling can negatively regulate PRR-initiated inflammatory pathways in macrophages, this signaling in T cells can lead to distinct outcomes.

microRNAs inhibit PRR signaling on a transcriptional level

A rapidly developing field of PRR-signaling regulation is microRNAs (**Figure 1**). MicroRNAs regulate inflammation through targeting the 3' untranslated region of transcripts leading to either their stabilization or degradation.[161] Such regulation is central to fine-tuning inflammatory responses. Interestingly, microRNAs can modify expression levels of both the positive and negative PRR-signaling regulators, including MyD88 signaling intermediates, transcription factors, cytokines, and inhibitors. Multiple microRNAs have been associated with TLR signaling,[161] with miR-146 and miR-155 being prominent in regulating inflammation.[161] For example, miR-146 inhibits IRAK-1 expression in human macrophages, thereby decreasing NF-κB activation and pro-inflammatory cytokine induction.[162] Polymorphisms altering miR-146 expression are associated with SLE and RA.[163] Similarly, miR-23b, which suppresses multiple pro-inflammatory molecules, ameliorates mouse lupus, RA, and MS models and is downregulated in human autoimmune diseases, including RA.[164] By contrast, microRNAs such as miR-155 that downregulate PRR-signaling inhibitors are increased in macrophages from RA patients. Consistently, miR-155-deficient mice are protected against experimental arthritis.[165] Interestingly, microRNAs can act as TLR7 and TLR9 agonists, inducing paracrine inflammatory responses.[166] Multiple human inflammatory diseases, including UC[167] and RA,[168] show dysregulated microRNA profiles. Expression and function of microRNAs can vary in human and mouse immune cells;[169] elucidating these differences is crucial to understanding the mechanisms through which distinct microRNAs regulate separate human macrophage functions.

Epigenetic regulation of PRR signaling

Immune cell phenotypes can be broadly modulated by epigenetic modifications (**Figure 1**). Upon PRR restimulation of mouse and human macrophages following chronic LPS stimulation, some genes are repressed ("tolerant"), whereas others remain transcribed ("non-tolerant");[16,17] such outcomes are partially regulated through epigenetic modifications. The tolerant genes largely include inflammatory genes, while the non-tolerant genes mediate microbial killing and inhibit inflammation.[16] Importantly, in mucosal tissues such as the intestine, which chronically encounter microbial products,

the epigenetic regulation mediating this dual transcriptional and functional regulation may ultimately enhance antimicrobial function while minimizing tissue inflammation and injury. In mouse lamina propria myeloid cells, bacterial-induced IL-10 was found to downregulate IL-12p40 through histone deacetylase 3 (HDAC3)-mediated histone deacetylation.[170] However, overall epigenetic regulation of intestinal macrophages is poorly defined. In primary human DCs and THP-1 macrophages, acute LPS stimulation results in histone acetylation and H3K4 methylation (both activating transcription) in pro-inflammatory cytokine and activation marker genes.[171,172] However, during endotoxin tolerance, inhibitory histone methylation (e.g., H3K27) is observed on pro-inflammatory genes in these cells.[172–174] Interestingly, a polymorphism in *TLE1*, a HDAC-interacting transcription factor, is associated with CD.[175] On the other hand, HDAC inhibitors improve experimental colitis and kidney disease in mice[176,177] and are undergoing evaluation as therapy for inflammatory diseases, such as systemic onset juvenile idiopathic arthritis[178] and IBD.[179] The therapeutic properties of HDAC inhibitors are incompletely understood and may involve mechanisms independent of inflammatory gene regulation, including protein acetylation and the induction of apoptosis.[180]

Inflammasomes regulate PRR-induced inflammation and are associated with human inflammatory diseases

Microbial stimuli can activate inflammasomes, macromolecular complexes containing NLR family members activating caspase-1, which cleaves pro-IL-1β and -IL-18 into their active forms (**Figure 1**).[181] Multiple NLRs contribute to inflammasome diversity,[181] although not all NLR proteins form inflammasomes. Tight balance of inflammasome regulation is crucial; inhibition or deletion of inflammasome components can either ameliorate or exacerbate animal inflammatory disease models. For example, ASC-deficient mice are more resistant to endotoxin shock,[182] and caspase-1 deficiency attenuates DSS colitis.[183] However, NLRC4 and NLRP6 deficiency exacerbates experimental mouse colitis, partially through dysregulated inflammatory cytokines, including IL-18, and altered intestinal microbiota.[184,185] Furthermore, polymorphisms in NLRs and molecules regulating inflammasome activation are associated with the autoinflammatory diseases.[186–188] Inflammasome-associated pathways also mediate mucosal-associated inflammatory diseases as evidenced by the association of *NLRP1* to vitiligo and systemic sclerosis,[189,190] *NOD2* to CD,[191,192] and *CARD9*, *IL-18RAP/IL12RL2/IL18R1/IL1RL1* and *IL1R2* regions to IBD.[10,59] Unlike the activating role of many NLRs, most, but not all,[193] reports find that NLRP12 inhibits inflammation.[194,195] Consistently, NLRP12-deficient mice are more susceptible to DSS-induced colitis and colitis-associated tumorigenesis,[196] although not to airway hypersensitivity.[197] *NLRP12* loss-of-function mutations are associated with inflammatory diseases,[198,199] including atopic dermatitis.[200] Studies defining diseases that benefit from targeting either the inflammasome directly or the products of inflammasome activation (e.g., IL-1, IL-18) are ongoing.[201]

Autophagy regulates multiple macrophage functions

PRR stimulation induces autophagy, which facilitates cellular organelle and bacterial clearance.[202] *In vivo* mouse studies and human genetic association studies have demonstrated that autophagy-associated genes and pathways are essential for intestinal homeostasis;[203–207] loss-of-function polymorphisms in the autophagy genes *ATG16L1* and *IRGM* are associated with CD.[10,208] These polymorphisms impair bacterial killing in some,[203,207,209] but not all, situations.[207,210] ATG16L1 hypomorphic mice have dysregulated Paneth cell morphology and exhibit microbiota-driven intestinal inflammation.[205,211] Moreover, ATG16L1 can contribute to anti-viral activity in macrophages in an autophagy-independent manner.[212] Interestingly, besides mediating bacterial killing, autophagy downregulates cytokine production from myeloid cells. Recent mouse studies demonstrate that autophagy promotes absent in melanoma 2 (AIM2) and NLRP3 inflammasome degradation[213] and decreases IL-1 secretion.[214,215] Human peripheral blood mononuclear cells studies show that autophagy also downregulates IL-1 through degrading IL-l transcripts.[216] Therefore, autophagy regulates at least two distinct and critical PRR-mediated functions: (1) microbial clearance and (2) cytokine downregulation (**Figure 1**). These dual functions are crucial in the intestine, thereby highlighting fundamental mechanisms through which autophagy can contribute to intestinal immune homeostasis.

PRR SIGNALING IN INTESTINAL MACROPHAGES IS MEDIATED BY DIVERSE LOCAL FACTORS

In previous sections, we discussed mechanisms inhibiting PRR-mediated functions and emphasized in select places how some of these mechanisms contribute to intestinal macrophage function and intestinal homeostasis; here we focus specifically on aspects of the unique phenotype observed in intestinal macrophages and on additional factors and mechanisms contributing to this phenotype. Intestinal macrophages constitute one of the largest reservoirs of myeloid cells.[25,217] Macrophages are located throughout the intestinal tract,[25,218] but most prominently in the lamina propria, beneath the protective epithelial layer, making macrophages particularly important in bacterial recognition following bacterial translocation during events such as epithelial injury.[25] Relative to peripheral monocyte-derived cells, intestinal macrophages secrete low levels of pro-inflammatory cytokines upon PRR stimulation but upregulate bacterial killing.[136,219,220] This limits unnecessary inflammation and tissue damage while simultaneously protecting against overgrowth of resident microbiota and pathogenic bacteria. As peripheral monocytes enter the intestinal lamina propria, multiple local mechanisms contribute to their differentiation into intestinal macrophages.[25] Contributing factors include microbial components (e.g., PRR ligands, polysaccharide A[221]), anti-inflammatory mediators (e.g., TGF-β, IL-10), nutrients, and apoptotic cells. Notably, during acute infection or tissue injury, intestinal macrophages can mount inflammatory responses.[220] This inflammation can be mediated by peripheral monocyte

recruitment to the intestine; the intestinal microenvironment can then influence subsequent differentiation patterns.[222–227] Consistently, altered proportions, functions, and/or differentiation of intestinal myeloid cells can lead to intestinal inflammation in mice,[222,228–236] and myeloid cell dysregulation in human intestine is observed in IBD.[237,238]

Consistent with decreased pro-inflammatory cytokine secretion, intestinal macrophages demonstrate downregulated CD14, MD2, TLR2, TLR4, MyD88, and IRAK-1, and decreased NF-κB activation, although certain PRR (e.g., TLR3, 5–9) are expressed.[26,136] Chronic PRR stimulation could contribute to downregulated inflammation in intestinal macrophages. For example, chronic NOD2 stimulation downregulates cytokines in myeloid-derived cells[31,102,126] and protects mice from experimental colitis.[102] Interestingly, intestinal myeloid cells from germ-free mice show downregulated PRR-mediated cytokine secretion,[170,220] although these mice were exposed to PRR ligands through food and bedding. To clearly dissect the role of chronic PRR stimulation in the downregulated cytokines observed in intestinal macrophages, germ-free mice will need to be examined under conditions of food and bedding devoid of microbial products, combined with recurrent intestinal injury. Stromal and epithelial cell secretions (e.g., TGF-β, IL-10, retinoic acid, and thymic stromal lymphopoietin) can also downregulate intestinal myeloid cell responses.[26,136,239–242] For example, TGF-β signaling in DCs regulates intestinal inflammation in mice,[243] and intestinal stromal cell-derived TGF-β downregulates CD14 expression, NF-κB activation, and PRR-induced cytokine secretion in peripheral human monocytes.[26,136,240] Nutrients, including vitamin D and retinoic acid that are abundant in the intestine, also contribute to intestinal macrophage tolerance. Vitamin D downregulates PRR-induced pro-inflammatory cytokines from human monocytes.[244] Consistently, vitamin D administration attenuates experimental mouse colitis.[245,246] Furthermore, higher vitamin D plasma levels correlate to decreased CD risk.[247] Conversely, compared with healthy controls, CD patients exhibit vitamin D deficiency,[248] which is multi-factorial in etiology, and correlates to disease severity.[249] Similarly, in addition to its immunoregulatory roles in T cells,[250] retinoic acid downregulates pro-inflammatory cytokines in PRR-stimulated human DC.[251] Finally, intestinal macrophages ingest apoptotic cells, which leads to TGF-β and prostaglandin production.[252–254] Importantly, mice lacking intermediates in apoptosis-inducing pathways, such as TAM receptors, C1q, MFG-E8, and TIM-4, develop autoimmunity.[86,255–257] Moreover, autophagy clears apoptotic debris,[258] highlighting one mechanism through which autophagy dysfunction may contribute to IBD susceptibility.[203–207]

Despite downregulated cytokines, human intestinal macrophages upregulate bactericidal activity. Intestinal factors can mediate both processes. PRR[16,17] and Vitamin D[259,260] stimulation of human and mouse macrophages upregulates multiple anti-microbial pathways. Interestingly, some studies show bactericidal defects in CD patient macrophages.[261] As heterogeneous mechanisms lead to CD, bactericidal defects likely exist in a subset of CD patients, such as those carrying polymorphisms in bactericidal pathways (e.g., *NOD2*, *ATG16L1*, *IRGM*, and *NCF-2*).[10,262] Targeting mechanisms mediating the dichotomy of downregulated inflammatory and upregulated bactericidal pathways in human intestinal macrophages might be particularly beneficial in IBD therapy.

HUMAN AND MOUSE MACROPHAGES DEMONSTRATE DISTINCT REGULATION IN VARIOUS PATHWAYS

Human and murine-based studies are essential and complementary to defining mechanisms of disease pathogenesis. There are multiple, fundamental differences between human and mouse PRR signaling outcomes,[11,12] including distinct PRR and cytokine stimulation responsivity,[31,102,263–267] and differential PRR utilization.[268] These differences are critical when extending mouse findings to human physiology and disease. Reasons for inter-species differences include distinct function and/or expression of relevant genes, dissimilar life spans, distinct microbial colonization, and altered environmental exposures. Furthermore, human genetic diversity is greater than that of experimental inbred mice, adding significant complexity, but also unique opportunities, in pursuing human immunology.

Different factors regulate mouse and human macrophage polarization

As macrophages enter or are activated in various microenvironments, they differentiate into distinct subtypes, characterized by differential surface marker, cytokine, and protein expression. Two broad categories of polarized mouse macrophages include classically activated macrophages (M1), associated with pro-inflammatory cytokine secretion, and alternatively activated macrophages (M2), associated with an anti-inflammatory phenotype.[6] Distinct factors mediate mouse and human macrophage polarization and macrophage phenotypes. M1- and M2-like human macrophages exist[269,270] but are less well defined than their mouse counterparts. IFN-γ stimulation of mouse, but not human M1 macrophages, dramatically induces NOS2, and IL-4-stimulated human macrophages produce significantly less arginase than mouse M2 macrophages.[270] Human macrophage polarization likely involves specific transcription factors. Although in one study, IRF5 mediated M1, and IRF4 mediated M2 polarization of human macrophages,[269] another study found contrasting results.[271] Notably, IRF5 is central to macrophage function as evidenced by *IRF5* polymorphism associations with numerous autoimmune/inflammatory diseases exhibiting dysregulated cytokine expression[272] and the dramatic contribution of *IRF5* polymorphisms to human variance in PRR-induced cytokine secretion.[273] Another IRF family member, IRF8, has been recently shown to promote mouse M1 polarization;[274] IRF8 effects on human macrophage polarization are still unclear. Further studies are needed to better define the regulation of human macrophage polarization and how this polarization contributes to immune homeostasis.

Mouse and human macrophages demonstrate differences in inflammasome activation

PRR stimulation induces pro-IL-1β, but a second signal, such as adenosine triphosphate (ATP), is required to activate P2×7 receptors. This results in potassium efflux and calcium influx, which activates the inflammasome and induces caspase-1-mediated processing of pro-IL-1β and -IL-18 to their active forms.[275–277] Tissue damage releases ATP,[278] thereby providing a second signal to mouse macrophages.[279] However, PRR-ligand stimulated human monocytes secrete autocrine ATP;[280] therefore, PRR-stimulation alone induces IL-1β in these cells. Some studies demonstrate that PRR-stimulated human macrophages do not secrete IL-1β,[281,282] with a second stimulus necessary for IL-1β secretion.[283] However, others detect active IL-1β in PRR-stimulated human macrophages due to the sufficiency of PRR stimulation for ATP production.[140,153,284–286] These differences might partially reflect different culture conditions used to generate macrophages; growth factor and cytokine differences can profoundly influence human macrophage responses.[287] Improved insight into differential regulation of IL-1 in human and mouse macrophages will be important, as IL-1 contributes to multiple human diseases,[201] and autocrine/paracrine IL-1β dramatically amplifies PRR-induced cytokine secretion in human macrophages.[140,153]

Human variance adds complexity to examination of PRR-induced macrophage functions

Mouse studies minimize variance in PRR-induced inflammatory outcomes through inbreeding, housing in conditions that reduce environmental differences, and utilizing age-, gender-, and littermate-matched mice. However, humans show significant variance in their genetic background,[288] environmental exposures,[289,290] and intestinal microbiota,[291–294] which translates into broad inter-individual immunological differences. For example, there is dramatic inter-individual variation in cytokine and inhibitor molecule induction upon PRR stimulation in human macrophages.[31,61,273,295–298] Such variance likely affects the balance between susceptibility to infections versus autoimmune/inflammatory diseases. Genes identified to regulate variance in human cytokine secretion include *IRF5*, *IL-1Ra*, and *TLR1*. *IRF5* polymorphisms account for up to 53% of variance in PRR-induced TNF-α secretion from human monocyte-derived cells;[273] this dramatic contribution likely results from the distinct genotypes being commonly distributed across the population and from the dramatic gene-dose-dependent regulation mediated by *IRF5* polymorphisms. *IL-1Ra* polymorphisms mediate 5% of variance in constitutive IL-1β plasma levels,[299] and *TLR1* polymorphisms contribute to variance in IL-6 secretion during sepsis.[297] Perhaps not coincidentally, these polymorphisms contribute to susceptibility and/or outcomes in multiple inflammatory/autoimmune diseases associated with dysregulated cytokine production.[272,297,299] Importantly, inhibitory mechanisms regulating PRR-initiated pathways demonstrate varying contributions in myeloid cells from different individuals (e.g., IRAK-M and SHIP-1).[31,61] Of note is that mutations modulating inflammatory pathway intermediates, such as MyD88[300] and TRIF,[301] may dramatically affect cytokine induction, but if relatively rare, will not significantly influence overall inter-individual heterogeneity. Another consideration is that some PRR-pathway polymorphisms (e.g., *NOD2*,[302,303] *TLR4*[304]), have different frequencies across distinct ancestries, which will therefore influence the inter-individual differences upon PRR stimulation between population groups. Host–microbe interactions are central in natural selection and functional variation; the inter-individual variability inherent in human immunological studies can ultimately be leveraged to define underlying mechanisms of autoimmune-mediated diseases.[305]

Another contribution to variance in human immune responses is inter-individual differences in microbial composition. Multiple mouse studies have implicated intestinal microbiota in regulating immunity and disease development, including mucosal diseases.[185,221,306–308] Altered human intestinal microbial composition is also associated with dysregulated immunity and disease;[291–294] it is unclear to what degree these microbial changes are a consequence or a cause of the inter-individual immunological differences.

Taken together, mouse models have provided tremendous insight into defining the importance of various pathways and functions in myeloid-derived cells in health and in disease development. Furthermore, humanized mice can be used to address select *in vivo* myeloid cell functions given successful reconstitution of human myeloid cells into mice.[309] However, in applying the information from mouse studies to human immune function, it is critical to understand the similarities and differences between mouse and human immune pathways.

FUTURE PERSPECTIVES

Despite significant advances in understanding human macrophage regulation and functions, multiple questions remain. What are the functional outcomes of the many disease-associated polymorphisms in macrophages? Which factors influence human macrophage polarization? How do monocytes acquire distinct resident phenotypes as they enter tissues? How do intestinal macrophages determine when to maintain tolerance and when to mount inflammatory responses to resident or pathogenic bacteria?

Human heterogeneity poses a specific challenge when conducting human macrophage studies; sampling from well-powered cohorts is essential. Moreover, limited tissue access restricts the number of functional immunological readouts; high throughput approaches minimizing sample sizes are continuously being developed and improved. Uniform sample processing, standard operating techniques and normalization of immune readouts based on criteria, including age, gender, ancestry, or specific genotypes, will be essential for future studies. Despite these challenges, elucidating the inhibitory mechanisms in primary human macrophages is essential to fully understand mechanisms mediating both health and disease.

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DISCLOSURE

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