Immunological Reviews

Günter Weiss
Ulrich E. Schaible

Macrophage defense mechanisms against intracellular bacteria

Authors' addresses
Günter Weiss[1], *Ulrich E. Schaible*[2,3,4]
[1]Department of Internal Medicine VI, Infectious Disease, Immunology, Rheumatology, Pneumology, Medical University of Innsbruck, Innsbruck, Austria.
[2]Cellular Microbiology, Priority Area Infections, Research Center Borstel, Borstel, Germany.
[3]Department of Immunology, London School of Hygiene and Tropical Medicine, London, UK.
[4]German Centre of Infection Research, TTU-TB, Borstel, Germany.

Correspondence to:
Guenter Weiss
Department of Internal Medicine VI
Infectious Disease, Immunology, Rheumatology, Pneumology
Medical University of Innsbruck
Anichstr. 35, A-6020 Innsbruck, Austria
Tel.: +43 512 504 23251
e-mail: guenter.weiss@i-med.ac.at

Acknowledgements
Continuous support by the Austrian research Funds (FWF) to G. W. and by the German Science Foundation, the Federal Ministry of Research and Education of Germany and the German Center for Infection Research to U.S. is gratefully acknowledged. The authors have no conflicts of interest to declare.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

This article is part of a series of reviews covering Tuberculosis appearing in Volume 264 of *Immunological Reviews*.

Video podcast available
Go to www.immunologicalreviews.com to watch an interview with Guest Editor Carl Nathan.

Immunological Reviews 2015
Vol. 264: 182–203

© 2015 *The Authors. Immunological Reviews published by John Wiley & Sons Ltd.*
Immunological Reviews
0105-2896

Summary: Macrophages and neutrophils play a decisive role in host responses to intracellular bacteria including the agent of tuberculosis (TB), *Mycobacterium tuberculosis* as they represent the forefront of innate immune defense against bacterial invaders. At the same time, these phagocytes are also primary targets of intracellular bacteria to be abused as host cells. Their efficacy to contain and eliminate intracellular *M. tuberculosis* decides whether a patient initially becomes infected or not. However, when the infection becomes chronic or even latent (as in the case of TB) despite development of specific immune activation, phagocytes have also important effector functions. Macrophages have evolved a myriad of defense strategies to combat infection with intracellular bacteria such as *M. tuberculosis*. These include induction of toxic anti-microbial effectors such as nitric oxide and reactive oxygen intermediates, the stimulation of microbe intoxication mechanisms via acidification or metal accumulation in the phagolysosome, the restriction of the microbe's access to essential nutrients such as iron, fatty acids, or amino acids, the production of anti-microbial peptides and cytokines, along with induction of autophagy and efferocytosis to eliminate the pathogen. On the other hand, *M. tuberculosis*, as a prime example of a well-adapted facultative intracellular bacterium, has learned during evolution to counter-balance the host's immune defense strategies to secure survival or multiplication within this otherwise hostile environment. This review provides an overview of innate immune defense of macrophages directed against intracellular bacteria with a focus on *M. tuberculosis*. Gaining more insights and knowledge into this complex network of host-pathogen interaction will identify novel target sites of intervention to successfully clear infection at a time of rapidly emerging multi-resistance of *M. tuberculosis* against conventional antibiotics.

Keywords: *macrophages, phagolysosome, radicals, iron, vitamin D, efferocytosis*

Introduction

Phagocytic cells such as macrophages (MΦs) and polymorphonuclear neutrophilic granulocytes (PMNs) represent the first line of defense against invading bacterial pathogens. In addition to their functions in tissue homeostasis and removal of dying cells by efferocytosis, tissue-resident MΦs patrol epithelia of barrier organs, which represent putative entry and colonization sites for pathogens and the first location for controlling infectious invaders. The prime examples are alveolar MΦs, which keep the pulmonary surface under surveillance for inhaled pathogens.

Once encountered, MΦs recognize bacteria via their microbe-associated molecular patterns (MAMPs) by surface

exposed, vesicular, or cytoplasmic pattern recognition receptors (PRRs). Surface PRRs include C-type lectins, mannose receptor (MR), dectin 1, dectin 2, Mincle, MCL, DC-SIGN, and scavenger receptors such as SR-A and MARCO, which not only recognize but also bind bacteria to initiate phagocytosis. Mannose-capped lipoarabinomannan (manLAM), phosphatidyl inositol mannosides (PIMs), as well as trehalose dimycolate (TDM) are mycobacterial ligands for MR, DC-SIGN, Mincle, and MCL, respectively. The Toll-like receptors (TLRs) 1/2, 2/6, 4, 5, and 9, are specific for lipopeptides, lipopolysaccharides, flagellin, and low-methylated DNA sugar backbone, respectively, with TLR2/6 and 9 as the pivotal PRRs for mycobacteria. In the cytoplasm, NOD1, NOD2, and Galectin 3 sense DAP, muramyl-dipeptide, and β-galactosides, respectively, while STING is the recently described receptor for cyclic GMP-AMP generated from microbial DNA, which is delivered into the cytoplasm by cGAMP synthase, which triggers type 1 interferon production (1, 2). Non-cell associated molecules of the humoral host defense system are also able to recognize and decorate the bacterial surface, thereby opsonizing the pathogens to facilitate indirect recognition and binding by MΦs through receptors for these molecules. Opsonization of invading bacteria is facilitated by soluble PRRs including surfactant proteins (SP-A, SP-D), and the mannose-binding lectin (MBL), as well as by the complement components C1q, C3, C3b, and C4. In case, a specific immune reaction against a pathogen has preceded a recurrent invasion by the same germ, specific antibodies generated during the first encounter are essential for antigen-specific recognition and opsonization of a recurrent infectious agent, and provide the basis of successful vaccines. Opsonized bacteria are subsequently bound by receptors for soluble PRR, C receptors (C1qR, CR3, CR4), and, in the presence of specific antibodies, Fcγ receptors (FcγR I-III). Downstream signaling cascades linked to these receptors induce receptor-mediated phagocytosis, a hallmark in host defense, in addition to a variety of pro-inflammatory responses executed by expression and/or secretion of chemokines and cytokines and release of anti-microbial effectors.

Upon phagocytosis, epithelia-associated MΦs and other phagocytes such as dendritic cells (DCs) carry engulfed bacteria to deeper tissues and further to draining lymph nodes. There, antigens are presented to T cells by professional antigen-presenting cells (APCs), such as DCs and MΦs (as well as B cells), to initiate specific cellular immunity and generate specific T cells.

Upon interaction of bacterial invaders with tissue MΦs and epithelial cells, initial inflammatory responses are triggered which comprises secretion of cytokines, chemokines, small lipid mediators (SLM) as well as anti-microbial peptides (AMPs). Interleukin-8 (IL-8) in humans (KC in mice) as well as prostaglandins and leukotrienes attract and/or activate PMNs, which further perpetuate inflammation by secretion of additional IL-8 but also the chemokines MCP-1, MIP1α/β, and IP10 that attract monocyte-derived MΦs and other immune cells to the site of infection. Although tremendously differing in their anti-microbial armamentarium, both phagocyte populations cooperate with tissue MΦs in eliminating the bacterial invaders and maintaining inflammation including generation of a myriad of pro-inflammatory cytokines such as tumor necrosis α (TNF-α), interleukin-1 β (IL-1β), IL-6, IL-12, IL-18, or IL-23 until elimination of the pathogens and/ or anti-inflammatory regulatory mechanisms kick in. Macrophages are thus in the center of infection with *M. tuberculosis* and other intracellular bacteria and use multiple strategies to eliminate this pathogens. Sophisticated analyses of the network of activities of macrophages against mycobacteria along with an elucidation of counter-strategies of *M. tuberculosis* to escape from or to neutralize such anti-mycobacterial host effects have demonstrated that the fight of the host against the pathogen in *M. tuberculosis* infection involves several levels including containment of the microbe, generation of radicals and a hostile, acidic environment, deprivation from essential nutrients, formation of anti-mycobacterial peptides and cytokines which strengthen host responses by attracting other immune cells, down to cell damage and suicidal activities of MO and PMN, such as autophagy, necroptosis, or efferocytosis to prevent spread of infection (3–5).

Come in and get killed: phagocytes' elimination of bacterial pathogens

Phagocytosis and phagosome maturation

Phagocytosis is a hallmark of anti-bacterial host defense. Upon binding and recognition of bacterial invaders, intracellular signaling pathways triggered by PRR, CR3, or FcRs engaged by their respective ligands induce actin polymerization and formation of the phagocytic cup. This process involves the GTPases, Rac1, Rac2, and Cdc42. The latter one can directly interact with WASP (Wiskott-Aldrich syndrome protein), which subsequently activates Arp2/3 as a direct initiator of actin polymerization. The process described is primarily associated with FcγR-mediated phagocytosis, whereas different players have been suggested to function

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

during uptake via other receptors such as DIAPH1 (diaphanous-related formin) in CR3-mediated engulfment. Of note, *M. tuberculosis* can evade immune recognition by macrophages upon masking their PRRs via cell surface-associated phthiocerol dimycocerosate (PDIM) lipids. In addition, phenolic glycolipids promote the recruitment of *M. tuberculosis* permissive macrophages via stimulation of host chemokine receptor 2 expression, whereas the invasion of TLR responsive microbicidal macrophages producing reactive nitrogen and oxygen species is impaired (6).

Subsequent enclosure of the phagocytic cup leads to formation of the phagosome and initiates its highly choreographed biogenesis driven by subsequent fusion and fission events. During this process, the maturing phagosome acquires and loses molecules functioning at but also characterizing individual maturation stages and diversion routes (7). Through fusion with endosomal or trans-Golgi-derived transport vesicles and fission of vesicles, which then can be transported to the plasma membrane, the protein and lipid composition of a phagosome is constantly changing (*Fig. 1*).

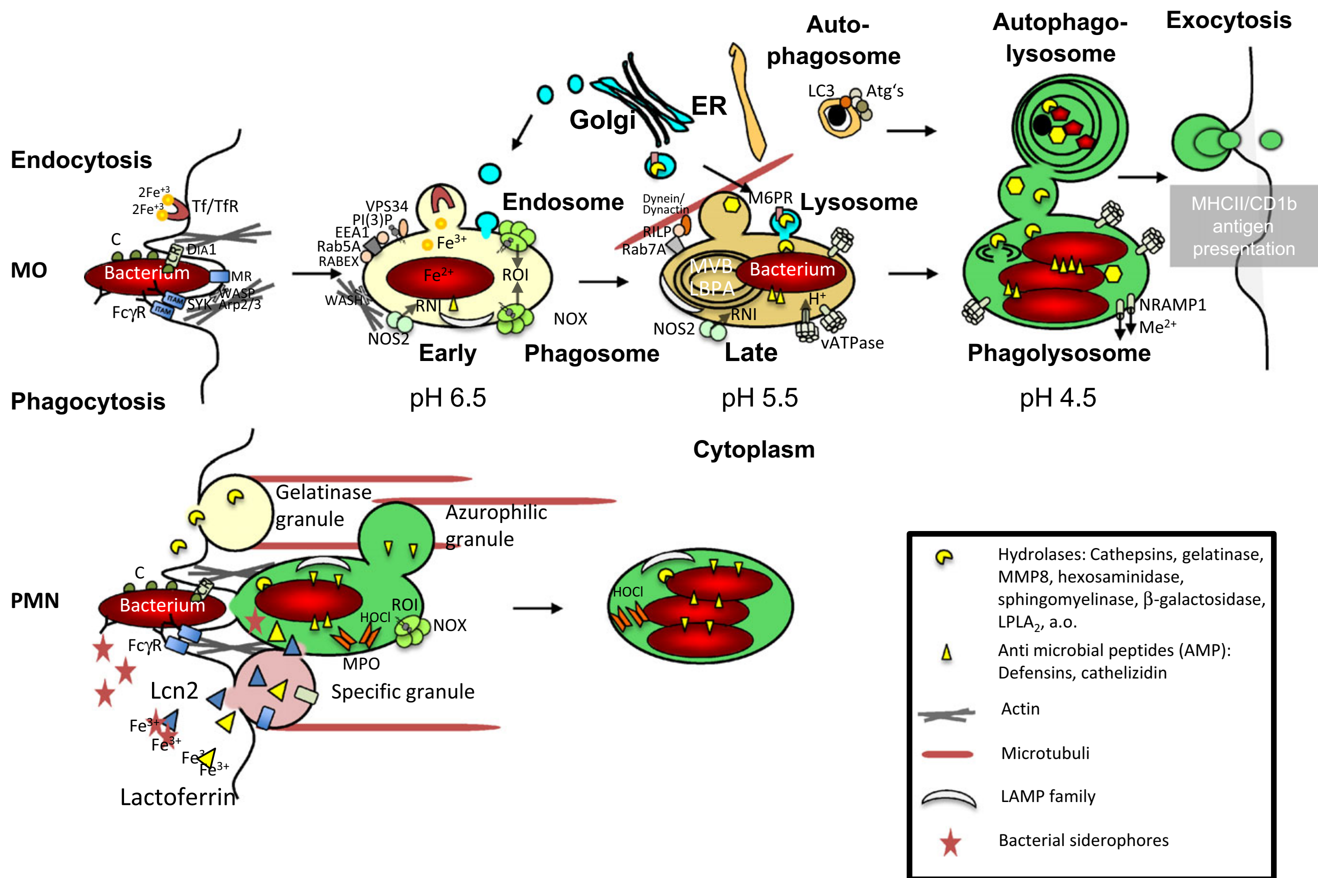

Fig. 1. Intracellular trafficking of bacteria in phagocytes and anti-microbial responses in macrophages and polymorphonuclear neutrophils. Schematic drawing depicts the phagocytic responses of macrophages (MΦs) and neutrophils (PMNs) against bacterial invaders, and the intracellular fate of the engulfed particles. Non-opsonized or antibody-/complement (C)-opsonized bacteria are recognized and bound by surface receptors for bacterial compounds or the respective opsonins, which triggers signaling cascades involving Syk or DIA1 and leads to actin polymerization and phagocytic cup formation. In MΦs, upon phagosome closure, the maturing phagosome traverses an early and late phagosomal and a phagolysosomal stage paralleling endosomal maturation. Phagosome biogenesis is accompanied by continuous fusion and fission events, including fusion with trans-Golgi transport vesicles, endosomes, lysosomes, and autophagosomes. These interactions cause acquisition and loss of different stage-specific markers. A hallmark of phagosome biogenesis is acidification of the phagosomal lumen by the proton pumping vATPase. A low pH is a prerequisite for optimal enzymatic activity of most late endosomal/lysosomal hydrolases, which are delivered to the nascent phagosome bound to the M6PR from the trans-Golgi. The stepwise succession of phagosomal maturation in macrophages is strikingly different from phagosome formation in PMNs. Phagocytosis and fusion with the lysosomal azurophilic granules is often happening simultaneously. At the same time, specific granules discharge iron sequestering lactoferrin and Lcn2 into both, the phagosomal lumen and the extracellular space. These granules also deliver phagocytic receptors to the PMN surface for recognition and uptake of bacteria. Finally, the gelatinase granules spill out proteases and other enzymes to degrade extracellular matrix proteins, leading to tissue disruption to allow PMN evasion into infection site but ultimately to pathogenesis.

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

The phagosomal stages parallel those of endosomal maturation and can therefore be roughly divided into early (around 10 min after uptake) and late phagosomes (10–30 min) and phagolysosomes (later than 30 min). However, the receptors involved in uptake determine the speed by which a particle is delivered to the phagolysosome. FcγR-mediated phagocytosis accelerates phagosome maturation when compared to CR-mediated uptake, and engaging the MR for engulfment has even been associated with particle delivery into early phagosomes without immediate phagosome maturation. The latter mechanism probably involving MR interaction with the mycobacterial cell wall lipids, man-LAM or PIMs, has been suggested to contribute to the generation of the early phagosomal niche of *M. tuberculosis* (8). Similarly, the mycobacterial cord factor TDM has been shown to delay phagosome maturation when coated onto beads (9). Importantly, mycobacteria and TDM induced impairment of phagosome maturation can be overcome by IFN-γ (10). Acceleration of phagosome maturation by engaging and clustering FcγR by antibody-opsonized bacteria is caused by Src-family kinase-mediated phosphorylation of the ITAM (immunoreceptor tyrosine-based activation motif) on the cytoplasmic part of the FcγR. Upon ITAM phosphorylation, the SYK tyrosine kinase is recruited to the receptor and its activation leads to downstream phosphorylation events including activation of SHP-1 and RAC (11, 12) and inhibition of SYK blocks phagosome maturation (13).

Exchange of incoming and outgoing membrane molecules and cargo is either delivered by a 'kiss and run' process or by complete fusion with the maturing phagosome, or most likely by a mixture of both types of vesicular interaction. Consequently, vesicle fusion is essential for phagosome maturation. The fusion between vesicles requires a closely adjacent position, which is facilitated by motor proteins. For example, dynein and dynactin bring endosomes and phagosomes close together to promote fusion most probably involving the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, VAMP7, and VAMP8 (vesicle associated membrane proteins). SNAREs are essential for vesicle fusion by forming fusogenic protein complexes at the cytoplasmic side of phagosomes comprising SNARE, NSF (N-ethylmaleimide sensitive factor), and SNAP (NSF-attachment protein). Fusion competence between two types of vesicles requires the presence of a vesicular (v) on one and a target (t) SNARE on the other vesicle such as the VAMPs and syntaxins, respectively. Together, v- and t-SNARE establish a protein complex, which facilitates membrane fusion. As the fusion process requires energy, GTPases, in this case of the Rab family, are also important for vesicle fusion during phagosome biogenesis. A number of Rab proteins have been associated with pathogen-containing phagosomes including Rab 3, 4, 5, 7, 9, 10, 11, and 14 (14, 15) and allocated to individual maturation stages. In addition to stage-specific v-, t-SNAREs and Rab proteins, phosphatidylinositide (PI) lipids synthesized by stage-specific phosphatidyl inositol kinases (PIK) at the cytoplasmic sheet of phagosomal membranes are specific for different phagosome stages and functionally contribute to maturation-associated vesicle fusion and actin-polymerization associated processes (16). The PIs phosphatidyl inositol (4,5)-biphosphate [$PI(4.5)P_2$] and $PI(3,4,5)P_3$ participate in phagocytic cup formation and enclosure, probably in synergy with the SNAREs, VAMP3, and VAMP7, otherwise associated with recycling endosomes or exocytosis, respectively. These VAMPs are likely involved when more membranes are required for phagocytosis of larger cargo. In this context, the Ca^{2+}-binding synaptotagmin VII, which is linked to VAMP7 function in exocytosis, as well as the synaptotagmins II and XI have also been associated with phagosome formation and biogenesis including the link between phagosomes and lysosomes (17–19).

The early endosome-like phagosome represents the first stage upon closure of the phagocytic cup. Scission of the newly formed phagosome from the plasma membrane seems to involve myosins. *M. tuberculosis* phagosomes have also been described to acquire a coat protein, coronin or TACO. Coronin then activates the calcium-dependent phosphatase calcineurin, which inhibits phagosome-lysosome fusion (20). This process can be blocked by calcineurin-inhibitors cyclosporin or FK506, resulting in reduction of mycobacterial proliferation within macrophages (20). The early phagosome lacks the proton pumping vesicular ATPase (vATPase) and is therefore characterized by a mildly acidic pH of 6–6.5 (21). The early endosomal PI(3)P, which is synthesized by the PI3K VPS34, recruits proteins to the early endosome or phagosome essential for subsequent maturation. The small GTPase Rab5A is a marker of early phagosomes and facilitates downstream maturation by recruiting early endosomal antigen 1 (EEA1), which then binds NSF, Rabex5, and Rabaptin-5 and subsequently syntaxin13 to form a fusogenic complex with endosomes (22–24). PI(3)P also contributes to the recruitment of proteins containing PX or FYVE motifs including EEA1 and the NADPH-oxidase subunits $p40^{phox}$ and $p47^{phox}$ (25–27). Mycobacteria interfere with phagosome maturation and consequently, these phagosomes are characterized by early endosomal properties, i.e. low in vATPase,

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

an almost neutral pH and association with VPS34, PI(3)P and Rab5A (28, 29). Non-maturing *M. tuberculosis* phagosomes have been associated with actin nucleation by the multi-subunit complex, WASP, and SCAR homolog (WASH), which drives actin polymerization on endosomal membranes by activating the Arp2/3 complex (30). Removal of this actin 'coat' drives mycobacteria into phagolysosomes and limits mycobacterial growth. The early endosome recycles certain receptors back to the plasma membrane such as the TfR, which carries iron-loaded Tf to the early endosome. There, it is released from the TfR to get rid of the two Fe^{3+} ions for transfer to the cytoplasm. Rab11A is involved in recycling vesicles between the early endosome and plasma membrane, and probably also early phagosomes. The coat protein I and the GTPase Arf have also been suggested to contribute to this recycling process, which seems also to maintain the small size of early endosomes and tight adjunction to particles of early phagosomal membranes. Early phagosomes recruit Tf via TfR and have therefore been suggested as an ideal niche for microbes, which require iron for growth such as *M. tuberculosis* (31, 32).

Upon subsequent maturation, the late endosome-like phagosome is the transient stage toward an oxidative and hydrolytic compartment, where the phagosomal cargo is degraded, and associated with the loss of Rab5A and acquisition of the late endosomal Rab7A through the HOPS (VpsC-homotypic protein sorting) complex. Downstream effector proteins of Rab7A include the Rab-interacting lysosomal protein (RILP), which links the phagosomal membrane to a motor complex consisting of dynein and dynactin through stepwise recruitment of p150Glued, ORP1L, and the receptor βlll spectrin (33, 34). This complex brings endosomes/phagosomes in close proximity as a prerequisite for fusion (probably involving VAMP7 and 8) (35, 36). The late phagosomal stage is characterized by the acquisition of the lysosome-associated membrane proteins (LAMP1 and 2) from endosomes as well as newly synthesized lysosomal enzymes delivered in trans-Golgi transport vesicles bound to the mannose-6-phosphate receptors (M6PR), which are transiently enriched in late phagosomes. The enzymes delivered to late endosomes include cystein-, serine, and aspartate-proteases (such as the cathepsins B, L, and D), peptidases, glycosidases (such as N-acetylglycosaminidase, β-glucuronidase, β-galactosidase) as well as (phospho-) lipases. Anti-bacterial activity has been described for a number of these hydrolases, which probably synergize with other microbicidal molecules such as defensins, cathelizidin, and ubiquitin-derived AMPs. The two latter ones require processing to their active components through proteolytic cleavage by PMN elastase, proteinase 3, or cathepsins, respectively (37, 38).

A hallmark of the late phagosome is its luminal acidification (pH of around 5) facilitated by the vATPase, which is assembled to function in the late phagosomal/endosomal membrane. This process is controlled by Abl tyrosine kinase, which negatively affects the expression of vATPase. Inhibition of Abl tyrosine kinase by the clinically used drug imatinib resulted in increased acidification of the lysosome and improved control of *M. tuberculosis* (39). Acidification is required for the function of most lysosomal hydrolases with a low pH optimum. Some of those such as cathepsin D depend on further processing by other proteases and/or autocatalysis to generate the enzymatically active forms (40).

The late endosome/phagosome is the intracellular compartment for membrane degradation. Lysosomal lipid transfer proteins (LTPs), such as the four active saposins generated from pro-saposin by proteolytic cleavage, are essential for glyco-lipid degradation in lysosomes and glycolipid homeostasis, in addition to their immunologically relevant function in processing and presentation of lipid antigens by CD1b molecules in DC (41, 42). Inherited lack of saposins or pharmacological inhibition of certain lysosomal hydrolases leads to severe lysosomal storage diseases. LTPs have been shown to have anti-microbial activity. Lysosomal phospholipase A_2 ($LPLA_2$) plays a unique role in degrading exogenous and endogenous membranes, including surfactant by alveolar MΦs as well as of engulfed bacteria. Its lack causes enhanced susceptibility to *M. tuberculosis* (43) and *S. typhimurium* infection (S. Renk, G. Graßl, P. Rausch, J. Shayman, BE. Schneider, UE. Schaible, unpublished data), although a direct microbicidal effect has not yet been found. Lyso-biphosphatidic acids (LBPA) are key lipids of late endosomes/phagosomes and terminal phospholipid degradation products (44). LBPA is also functionally involved in membrane degradation by recruiting the programmed cell death-6-interacting protein (PDCD6IP) (45, 46). Overexpression PDCD6IP promotes vacuolarization. PDCD6IP also binds in a Ca^{2+}-dependent manner to endophilins, which influence the endosomal membrane shape (bending) probably through interaction with dynamin via SH3 domains (46). Incorporation of membranes into maturing endosomes/phagosomes requires an inward budding process. ESCORT proteins I and III have been shown to facilitate inward budding from phagosomal/endosomal membranes thereby generating multivesicular bodies (MVB). The phagolysosome is the final product of late phagosome to lysosome

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

fusion and is characterized by a pH of 4–4.5, high hydrolase activities and low amounts of M6PR, LBPA and PI(3)P. Non-degradable material is removed from the cell by exocytosis, i.e. outward budding of lysosome-derived vesicles. An acidic pH has been thought to be a prerequisite for mycobacterial killing. However, *M. tuberculosis* can survive at pH of 4.5, which has been linked to the expression of a specific resistance gene named Rv3671c (47, 48). Of note, acidification of the phagolysosome decreases mycobacterial metabolism, which becomes highly dependent on the availability of specific carbon sources such as pyruvate, acetate, or cholesterol, otherwise resulting in growth arrest (48). Bacteria-containing phagosomes not only interact with vesicles of various origins during biogenesis but also with lipid storage compartments, i.e. lipid bodies (or droplets) (49). Formation of lipid bodies requires the structural proteins, perilipins 1–3, which are inserted in the body's rim. GTPases involved in vesicle trafficking such as Rab5, Rab7, and IRGM3 have been found in lipid bodies, though their presence has not yet been functionally linked to phagosome-lipid body interactions. A number of bacterial infections induce lipid body formation including those by *Chlamydia* ssp., *S. aureus*, *M. leprae*, and *M. tuberculosis*, and foamy MΦs are found in infectious lesions. Both mycobacterial phagosomes and chlamydiophorous vacuoles interact with lipid bodies during bacterial dormancy (50, 51). Access to lipid droplets allows phagosomal bacteria usage of TAG and other lipids as carbon sources. However, these compartments contain the enzymatic machinery including the arachidonic acid precursor for synthesis of eicosanoid SLM such as prostaglandins (PGs) and leukotrienes (LTs) involved in inflammation and PMN attraction. PGE_2 and PMN influx can limit protective T-helper 1 (Th1) cell responses and will therefore be beneficial for mycobacterial survival and growth. Consequently, non-steroidal drugs including COX inhibitors (aspirin, ibuprofen) ameliorate PMN-driven necrotic granulomas and *M. tuberculosis* growth in mice but also abolish mycobacteria-induced lipid body formation and PGE_2 production (52, 53). These observations point out modulation of SLM synthesis as a novel therapeutic strategy to accompany conventional antibiotic therapy in TB.

The activated macrophage and its anti-mycobacterial defense armature

Radical formation

T-cell activation in the context of pro-inflammatory cytokines, such as IL-12, IL-18, and IL-23 secreted by MΦs and DCs, leads to interferon-γ (IFN-γ)-producing Th1 cells and subsequent activation of MΦs and enhanced TNF-α secretion. Murine MΦs rely on IFN-γ plus PRR signals and/or TNF-α for full activation, whereas human MDMΦs additionally require vitamin D2 (VD) as a cofactor, which facilitates expression of AMPs such as cathelicidin (LL-37) through binding to the VD receptor (VDR). The activated MO has widely strengthened anti-bacterial capacities, including generation of reactive nitrogen intermediates such as nitric oxide (NO) through nitric oxide synthase activity (NOS2) and indoleamine 2,3-dioxygenase (IDO)-mediated tryptophan depletion through generation of N-formyl-kynurenine and its subsequent degradation products. Activated MΦs can limit growth of intracellular bacteria including *M. tuberculosis* and drive phagosome maturation toward phagolysosomes and auto-phagolysosomes (54, 55). The activated MO is also better equipped with the antigen-presenting and co-stimulating molecules MHC I, II, CD80, and CD86, respectively, for T-cell activation, though not exceeding the optimal APC function of DCs.

Using arginine and oxygen, the homodimeric NOS2 generates NO and citrulline, as one subunit transfers electrons between NADPH, FAD, flavin mononucleotide to the heme iron of the second subunit (56, 57). NO must diffuse through the phagosomal membranes, since NOS2 is located at the cytoplasmic side of the phagosomal membrane. In parallel, activated macrophages produce reactive oxygen intermediates (ROI) via activation of NOX2 (58). NOX2 consists of five catalytic subunits, which assemble after phagocytosis thereby forming an active enzymatic complex producing superoxide anions and hydrogen peroxide along with the formation of hydroxyl radicals, the latter pathway being catalyzed by iron via the Fenton reaction (56, 59–61). Upon stimulation with IFN-γ, macrophages activate GTPases, which are responsible for the recruitment of NOX2 to the mycobacterial phagosome (62, 63). In addition, macrophages can produce ROI by mitochondria in response to stimulation with TNF-α. However, excess TNF-α may have the opposite effect by inducing apoptosis and necrosis of infected macrophages via stimulation of receptor-interacting serine-threonine kinases 1 and 3 (RIP1 and RIP3) and excessive radical formation (64–66). This results in release of mycobacteria into the extracellular medium, which promotes bacterial growth. Of interest, blocking of the TNF-α-mediated apoptotic pathway by inhibition of mitochondrial cyclophilin and blockade of acid sphingomyelinase prevented necroptosis while preserving TNF-α-mediated anti-mycobacterial activity (67).

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

After crossing the phagosomal membrane, NO and ROI can spontaneously react in the phagosomal lumen to generate nitrogen dioxide (NO_2), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), nitrosothiols, nitroxyl (HNO), and dinitrosyl-iron complexes (58). Of interest, recent evidence obtained in models of *S. typhiumrium* infection indicate specific compartmentalization of nitrosative and oxidative stress in macrophages and thus divergent effects on the fate and propagation of bacteria, extending previous work on cycle specific susceptibility of these bacteria to either of these stress responses (61, 68, 69). Whether this also applies to mycobacteria remains to be shown. However, the *M. tuberculosis* proteasome provides protection against killing by RNI in activated. Nonetheless, these highly reactive intermediates execute their microbicidal activity by oxidative destruction of membrane lipids, DNA, thiol and tyrosine residues, whereas NO can exert toxicity by directly targeting iron sulfur cluster of central metabolic enzymes (57, 58, 70–72). However, to exert anti-bacterial activity by this pathway, NO has to be constantly produced. Limitation of L-arginine availability thus blocks NO formation. Therefore, the end product of the NO pathway, citrulline, has to be re-utilized to fuel arginine synthesis, which is managed by the enzymes arginine-succinate synthase (Ass1) and argininosuccinate lyase (Asl). Accordingly, Ass1-deficient macrophages fail to control mycobacterial infection (73).

Although many of these observations originate from mouse models, the role of both RNI and ROI as central mediators of innate immune defense in human *M. tuberculosis* infection is well established. It has also been observed that both mutations or polymorphisms in the humans NOS2 or NOX2 genes are associated with an increased susceptibility to or a more severe course of tuberculosis (74, 75).

Autophagy, vitamin D, and nutritional imbalances

Activated macrophages can use macro-autophagy, the cellular intrinsic degradation and recycling system for senescent organelles and compartments, to eliminate intracellular bacteria such as *L. monocytogenes*, *Shigella* spp., and *M. tuberculosis* (55). Infection-induced autophagy by IFN-γ activation or by starvation requires activation of the small GTPase LRG47 (IRGM in humans) (76, 77). Autophagosomes are generated from PI(3)P carrying ER sheets (and other intracellular membranes including the Golgi) through recruitment of the lipid-binding proteins LC3A, LC3B, LC3C, and γ-aminobutyric acid receptor-associated proteins (Atg8). This process is controlled by beclin 1 (Atg6) complexed to the serine/threonine kinases ULK1, ULK2 (Atg1), as well as VPS34. In a downstream conjugation cascade, the Atg5/Atg12/Atg16 complex supports linking LC3s to membrane phosphoethanolamine (55). Autophagy targets ubiquitinylated organelles/proteins to the autophagosome, which matures in similar steps as phagosomes and eventually fuses with lysosomes through recruitment of the SNARE syntaxin 17 to generate an auto-phagolysosome. Killing of bacteria within this compartment has been attributed in part to ubiquitin-derived AMP generated by cathepsin D-mediated proteolysis (78). Autophagy is further accelerated by microRNA-155, resulting in maturation of phagosomes and reduced survival of intracellular mycobacteria. This effect is exerted upon binding of miR-155 to the 3′-untranslated region of RAS homolog enriched in brain (Rheb), which is a negative regulator of autophagy (79). Of interest, a recent chemical screen study to identify new compounds, which affect the intracellular survival of *M. tuberculosis*, identified two compounds, which exert beneficial effects by promoting autophagy. Specifically, fluoxetine, a selective serotoine re-uptake inhibitor, and gefitinib, an inhibitor of epidermal growth factor receptor (EGFR) activity stimulated autophagy and reduced intra-macrophage survival of *M. tuberculosis*. The effect of gefitinib could be traced back to blockade of EGFR mediated p38-MAPK activation, a known inhibitory pathway of autophagy (80).

TLR2 and TLR4 signals as well as vitamin D2 have been linked to induction autophagy as an anti-microbial effector mechanism (81). Several lines of evidence indicated that vitamin D metabolism plays an important role in human macrophage host responses to infection with mycobacteria. The active form of vitamin D 1,25-dihydroxyvitamin D3 exerts its activity upon binding to a specific vitamin D receptor (VDR). The expression of this latter receptor as well as the vitamin D converting hydroxylases, mainly vitamin D-1 hydroxylase CYP27b1, is induced after TLR2 activation of human monocytes/macrophages (82). This results in increased expression of target genes containing vitamin D response elements within their promoter regions, such as the anti-microbial peptide cathelicidin (LL37) (83). Part of the anti-mycobacterial activity of cathelicidin can be traced back to augmentation of NOX2-mediated ROI formation and modulation of cytokine expression (84) as well as to induction of autophagy (4, 85). Accordingly, macrophages from diabetic patients with low vitamin D2 levels were shown to have an impaired control of *M. tuberculosis* infection *ex vivo*, which can be significantly augmented upon vitamin D supplementation (86). Of note, recent evidence suggests that vitamin D impacts *M. tuberculosis* infection also by modulating lipid composition within the mycobacterial phago-

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

some. By inhibiting peroxisome proliferator-activated receptor γ (PPARγ), vitamin D prevented *M. tuberculosis*-induced lipid droplet accumulation, which is essential for mycobacterial multiplication (87). That points to the importance of modulating the availability of nutrients to pathogens as part of the host response (88). Glucose and lipids are essential for *M. tuberculosis*, and MΦs aim at restricting the microbial access to this source. However, *M. tuberculosis* tries to reprogram the MO to exploit lipids derived from triacyclglycerol and cholesterol, which accumulate in the bacteria-containing phagosome and lead to the formation of foamy cell macrophages (89). This is done by diversion of the host metabolism from a glycolytic pathway toward ketone biosynthesis, which is mediated by the mycobacterial virulence factor ESAT-6 (90). Of interest, breakdown products of fatty acids, such as propionyl–coenzyme A, can exert toxic effects toward mycobacteria, which thus try to detoxify this intermediate by converting it to acetyl-CoA that can be then used by bacteria as a central metabolic compound (91). In terms of glucose consumption, the enzyme fructose-1,6-bis-phosphanate aldolase (FBA) is essential for mycobacteria to get access to carbon sources derived from carbohydrates, and the inhibition of FBA impairs *M. tuberculosis* proliferation and increases the susceptibility to mycobacteria to host responses (92). However, we are only beginning to understand the mechanisms by which MO counter-balance the mycobacterial interference with host metabolism and limit the access of these nutrients and carbon sources to intra-phagosomal bacteria which may involve lysosomal lipid transfer proteins (LTP) and $LPLA_2$ to restrict modulation of lipolytic pathways (43, 93).

Whether cellular starvation and nutrient deprivation, which triggers autophagy through AMPK (AMP-activated protein kinase) and abrogates inhibition of autophagy by mTOR (mammalian target of rapamycin) is also involved in autophagous elimination of bacteria is not clear, although recent data suggest that AMPK in association with PPARγ-co-activator1α strengthen anti-microbial immune effector mechanisms of infected macrophages by ameliorating mitochondrial function (94).

Another defense strategy to limit bacterial growth is amino acid deprivation by NOS2 and IDO upon MO activation (95). This cannot only affect microbial energy consumption and therefore metabolic activity but also is envisaged to cause autophagy and subsequent elimination of intracellular bacteria. However, in the setting of *M. tuberculosis* infection, IDO activation appears to be ineffective in controlling the infection. IDO induces the degradation of the essential amino acid tryptophan to kynurenine and subsequent breakdown products. Tryptophan is essential for *M. tuberculosis* growth, however, IDO deficient mice were able to control *M. tuberculosis* infection as good as wildtype animals and lack of IDO did not affect mycobacterial specific T-cell responses (96). These observations could be recently traced back to the fact that *M. tuberculosis* is able to synthesize tryptophan, and thus IFN-γ-inducible activation of IDO and subsequent reduction in tryptophan availability fails to exert an anti-mycobacterial effect, whereas blockage of mycobacterial tryptophan synthesis increased the efficacy of this host defense mechanism (97).

Cytokines

Whereas the central roles of IFN-γ and TNF-α to mount a protective immune response in *M. tuberculosis* infection is well appreciated as discussed herein, the functions of other cytokines origination from MΦs or T cells in this infection are less clear. IL-1β is massively induced upon infection of macrophages with *M. tuberculosis*, and it appears to play important roles in anti-mycobacterial immune defenses because knock-out mice are more susceptible to this infection (98), and human polymorphisms of IL-1 genes were associated with susceptibility to tuberculosis (99). The formation of IL-1β as well as of IL-18 from pro- IL-1β and pro-Il18β, respectively, is dependent on the activation of the inflammasome and of caspase-1 (100). During *M. tuberculosis* infection inflammasome activation is controlled by the nucleotide binding and oligomerization domain, leucine rich-containing proteins 3 (NLRP3). *M. tuberculosis* is capable of reducing this activation pathway by inducing the formation of IFN-β, thereby contributing to immune evasion of the bacterium (100). Therefore, it is no surprise that IFN-β transcriptome signatures were found to be associated with active but not latent TB (101). Similarly, NO directly blocks inflammasome activation via nitrosylation of NLRP3, thereby preventing excessive tissue damage in the course of *M. tuberculosis* infection (102, 103). In a similar fashion, but via another pathway, the enzyme heme oxygenase-1 (HO-1), which is induced in activated macrophages, exerts tissue protection by limiting the accumulation of pro-oxidant and cytotoxic heme molecules (104). However, the mechanism underlying the protective effects of IL-1β in *M. tuberculosis* infection is far from being clear but may partly relate to synergistic activity of the cytokine to induce anti-mycobacterial host response such as formation of ROI and RNI whereas induction of IL-17 does not appear to play a major role in this setting. On the other hand, IL-17 is important to mount a

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

protective T cell-mediated immune response against *M. tuberculosis* (105), whereas another Th17-derived cytokine, IL-22, exerts direct anti-mycobacterial activity in macrophages by stimulating phagolysosomal fusion. This effect could be traced back to IL-22-mediated activation of calgranulin A with subsequent induction of Rab7 and inhibition of Rab14 expression (106). Similarly to IL-1β, IL-18 appears to be of importance to mount an efficient immune response because IL-18 knockout mice are more susceptible to *M. tuberculosis* infection than wildtype ones. The underlying mechanisms have not been elucidated fully but may be related partly to a reduced IFN-γ activity and impaired activation of its downstream anti-mycobacterial pathways (54).

For the sake of completeness, additional populations of MΦs need to be mentioned. Alternatively activated MΦs are induced by a Th2 cell cytokine environment, i.e. IL-4 and IL-13. As a hallmark enzyme, alternatively activated MO express arginase 1 (Arg1) depleting the inflamed tissue of arginine by generating L-ornithine and urea, and the downstream products of ornithine decarboxylase (ODC) and ornithine aminotransferase (OAT) activities, polyamines, and proline (important for induction of collagen deposits and fibrosis), respectively. Thereby Arg1 reduces the potential of activated MΦs to produce NO and generally limits tissue nitrogen sources important for T-cell activation and proliferation (57, 107). Consequently, mice deficient in Arg1 are better protected against *M. tuberculosis* infection (108). Recent evidence also suggests that in the absence of functional NOS2, Arg1 exerts tissue protective effects and limits exacerbated granuloma formation and mycobacterial proliferation, which could be traced back to the function of Arg1 in controlling overwhelming T-cell activation (109). Macrophages, which suppress T-cell responses through secretion of IL-10 and TGF-β, have been grouped together with a certain PMN population showing similar suppressor and tissue protective functions and were termed myeloid suppressor cells (MDSCs). Although mostly described in the context of tumor maintenance limiting anti-tumor T-cell responses, recent studies indicate their appearance as counter regulators in a wide variety of inflammatory immune responses including those to pathogenic microbes. Interestingly, natural suppressor cells have first been described in the context of *M. bovis* BCG infection (110) and MDSC, as these cells are termed today, of the $GR1^{intermediate}/CD11b^{+}$ phenotype expressing IL-17 and arg1 accumulate at the rim of *M. tuberculosis*-induced lung granulomas, especially in the absence of NOS2, where they may be protective (111). These studies indicate that different MO populations are important to keep the tight balance between exacerbated pathology and control of bacterial growth.

Granulocytes assisting macrophages in *M. tuberculosis* infection

Whereas macrophages comprise the first line of defense against *M. tuberculosis* by interacting with the pathogen at the primary site of infection, neutrophils are recruited following macrophage apoptosis and eventual release of mycobacteria to the extracellular space. The aim of these neutrophils is to take up such mycobacteria and to rapidly kill them (112). In contrast to the successive process of phagosome maturation in MΦs, which takes several minutes, it is a matter of seconds in PMNs. Actually, phagosome maturation is almost coinciding with phagocytosis, and fusion of preformed PMN granules happens during closure of the phagocytic cup. Thereby, granule content is also released into the environment (113).

PMNs carry three different types of granules (*Fig. 1*): primary azurophilic, secondary specific, and tertiary gelatinase granules. These granules contain distinct anti-microbial effector molecules. Whereas azurophilic and specific granules primarily contain AMP, lactoferrin (LF), lysozyme, myeloperoxidase (MPO), elastase, cathepsins, and proteinase-3 (114–116), tertiary ones carry enzymes facilitating disruption of the extracellular matrix including matrix metalloproteinase 9 (MMP-9), collagenase, and gelatinase. The iron-sequestrating proteins, lipocalin 2 (Lcn2) and LF, either recapture iron from loaded mycobacterial siderophores or directly bind Fe^{3+} (*Fig. 1*) (see below). The vesicular NADPH-oxidase generates microbicidal ROIs such as oxygen radicals and, through dismutation, H_2O_2, reacting with oxygen radicals (O_2^{-}) hydroxyl radicals, and singlet oxygen. H_2O_2 is further metabolized by MPO to hyperchlorous acid and chloramines. Non-oxidative effectors comprise AMP such as cathelicidins, β-defensins, and the human neutrophil peptide 1 (HNP-1).

Azurophilic granules release their contents into the forming phagosome, whereas specific granules fuse with both the phagosome as well as with the plasma membrane to release their contents into the extracellular space to deliver enzymes, which facilitate degradation of extracellular matrix proteins. Upon encountering microbes, PMNs release DNA forming NET (neutrophil extracellular traps), which bind microbes and contribute to their killing. NET release has been described as a targeted process, which seems to be associated with a certain type of cell death termed NETosis requiring elastase, ROI, and MPO activity (117).

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

PMNs are of importance in containment of mycobacteria following their release from apoptotic/necrotic macrophages (118, 119). Upon co-culture with *M. tuberculosis*, PMNs actively migrate toward the mycobacteria in an *in vitro* assay and become activated upon phagocytosis as indicated by ROI production, IL-8 release and generation of active cathelicidin, MPO and NETs (120). Upon uptake by PMNs, opsonized mycobacteria quickly become associated with MPO- and cathelizidin-containing phagosomes. However, infected PMNs quickly succumb to necrotic cell death induced by the PMN's own NADPH-oxidase/MPO generated ROI. In contrast, attenuated *M. tuberculosis* strains lacking the RD1 genomic virulence region not only extend the survival of PMNs before apoptotic suicide but also eventually kills those attenuated strains in a ROI (MPO, NOX2)-dependent manner. Interestingly, PMNs from chronic granulomatous disease patients lacking a functional NADPH-oxidase fail to succumb to necrotic cell death by wildtype *M. tuberculosis* but are also unable to kill attenuated strains (120). These data suggest that virulent *M. tuberculosis* strains use ROI-mediated necrosis as an escape mechanism from killing by PMNs. The RD1 region of *M. tuberculosis* encodes the secretion apparatus ESX1 as well as secreted small proteins such as ESAT-6 and CFP-10. ESAT-6 is membrane active and was made responsible for host cell apoptosis and cell-to-cell spread by several authors, as comprehensively reviewed by Aguilo *et al.* (121, 122). Whether ESAT-6 is involved in PMN necrosis is yet not shown. Virulence-associated escape of mycobacteria from killing by PMNs targets them to PMN- or MO-derived efferocytes.

Efferocytosis: a second instance of defense

Efferocytosis is the guarding mechanism to remove dying/dead cells from tissues during growth and remodeling and is executed primarily by tissue MΦs and upon onset of inflammation by monocyte-derived MΦ as well as PMN. During infection, large numbers of cells involved in host defense succumb to cell death. These cells have to be removed to limit tissue damage and inflammation. As these cells are also parasitized by intracellular pathogens, which now lose their niches to cell death, the need to contain the infection makes efferocytosis an essential process during the host response to intracellular bacteria. However, its function in infection is not well studied yet.

During ontogeny of complex organisms, cell death is a natural process facilitating growth and tissue remodeling. Removal of senescent and dead cells is therefore essential to maintain tissue homeostasis and integrity and to promote healing. Infection and subsequent inflammation is often accompanied by massive turnover of immune cells, the life time thereof is limited by apoptotic and/or necrotic cell death. Intracellular bacteria induce different types of cell death in their host cells. Apoptosis of host MΦs and PMNs has been associated with *Salmonella* spp., *L. monocytogenes*, as well as attenuated *M. tuberculosis* strains. In contrast, virulent *M. tuberculosis* avoids MO apoptosis by inhibiting annexin I cross-linking and membrane repair, which ultimately leads to necrosis (123). Certain virulent *M. tuberculosis* strains trigger necrosis by inner mitochondrial membrane rupture (124–127). High burden infection with or high intracellular growth rates of virulent *M. tuberculosis* strains also trigger a caspase-independent type of cell death, probably caused by metabolic exhaustion (126). Notably, upon IFN-γ-mediated activation and *M. tuberculosis* infection, murine MΦs succumb to caspase- and NO-dependent apoptosis with a lethal outcome for the mycobacteria (128).

Under homeostatic conditions within the body, macrophages are the prime cells to clear out apoptotic bodies and remnants thereof as well as necrotic material. These cells are functionally termed efferocytes (129). Efferocytosis is essential in tissue remodeling during growth and development as well as in wound healing. During immune reactions and inflammatory responses however, efferocytosis becomes essential to remove dying/dead cells from the tissue. Under these pathological conditions, phagocytes otherwise not present in healthy tissue such as monocyte-derived MΦs and PMNs become the prime efferocytes. PMNs are essential to take over the job when massive cell death occurs (130).

Dying/ dead cells attract and are recognized by efferocytes through distinct signals. Apoptotic cells release 'find me' and expose 'eat me' signals such as PS, lysoPS, cardiolipin, calreticulin, and CD31, as well as the chemokine fractalkine (CX3CL1 as recognized by CX3CR1) and small amounts of nucleotides (ATP, UTP). These ligands are recognized by phagocytes promoting efferocytosis (131, 132). Soluble receptors can opsonize apoptotic cells to bridge them to respective phagocyte receptors. These include collectins [surfactant proteins A/D (SP-A/D), complement component 1q (C1q)], mannose-binding lectin (MBL), pentraxin 3 (PTX3), which binds ficolin 1 on PMNs, ficolins 2 and 3, and thrombospondin (133). For direct recognition of apoptotic material by efferocytes an array of receptors, which mediate tethering and/or engulfment, include CR3 and CR4, CD36, CD91 [low density lipoprotein

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

receptor-related protein (LRP)], and other scavenging receptors (SR-A), vitronectin receptor ($\alpha_v\beta_3$) and $\alpha_v\beta_5$integrin as well as Mincle, which also recognizes the *M. tuberculosis* cell wall lipid TDM (134). TIM4 and G2A recognize PS and lysoPS, respectively. P2Y$_2$ senses free nucleotides and the sphingosine-1 phosphate receptor (S1P$_{1-5}$) sphingosin-1-phosphate (S1P). The redundancy of opsonins and receptors involved in removal of apoptotic material indicate a strategy to make sure that any phagocyte is able to participate in efferocytosis but the full repertoire of ligands and receptors required for efficient apoptotic cell removal is not well defined. It has also been shown that CD44 cross-linking on MΦs enhances efferocytosis (135).

The signaling pathways governing apoptotic cell removal comprises either the RhoG, ELMO, Dock180, TRIO, CrkII or the GULP, LRP, ABC-A1, and 7 pathway. Both pathways finally converge in the activation of Rho GTPases such as Rac1 to promote actin polymerization, membrane ruffling, and phagocytosis (131, 132). Efferosomes containing apoptotic material follow a similar succession as phagosomes going from an early to late endosomal to phagolysosomal stages as indicated by differential association with marker proteins such as the small GTPases Rab5 and Rab7, Vsp34 and dynamin, Lamp-1, and finally lysosomal hydrolases (136) (*Fig.* 1). Cell corpses are degraded in phagolysosomes by cathepsins, phospholipases, and other acid hydrolases supported by lipid transfer proteins such as saposins for recycling (137).

There are also regulators of efferocytosis in place. Binding of lactadherin (MFG-E8) to PS on apoptotic corpses blocks efferocytosis (138). An inflammatory environment can also hinder efferocytosis of apoptotic corpses limited by TLR4 signals and TNF-α (139–141). Myeloid cells can interfere with apoptotic cell clearance by elastase-mediated cleavage of receptors for apoptotic corpses (142). In contrast, the anti-inflammatory mediators IL-4, IL-10, and the peroxidase proliferator-activated receptor γ (PPAR-γ) promote removal of apoptotic cells (143). Interaction between efferocytes and apoptotic corpses primarily triggers TGF-β, IL-10, PG, and PPAR-γ putatively generating an anti-inflammatory tissue environment promoting efferocytosis but also pathogen persistence. However, mycobacterial pathogen-associated molecular patterns binding PRRs, such as TLR2 and TLR9, may have an opposite effect (144, 145).

In contrast to factors involved in the removal of apoptotic corpses, our knowledge on the efferocytic process of necrotic cell material is yet rather limited, probably due to its less defined properties and the concomitant occurrence of both processes in inflammation. However, our recent finding on induction of necrosis, especially of PMNs, upon *M. tuberculosis* infection indicates analyses of this process are warranted. In the course of necrosis, the plasma membrane ruptures causing spillage of cytoplasmic material. Necrotic cell death is therefore accompanied by release of damage-associated molecular patterns (DAMPs) such as cytoplasmic heat shock proteins (HSP) and high mobility group box 1 (HMGB-1), which trigger pro-inflammatory signals (145). Interestingly, LF released by dying PMNs is a 'keep out' signal for efferocytes.

Compared to apoptosis, necrosis appears a less coordinated process of cell death and is often following an initial apoptotic stage with PS exposition. This so-called secondary necrosis can result from insufficient removal of apoptotic cells. Cathelizidin/LL37 causes secondary necrosis of apoptotic PMNs, probably by its membrane-permeabilizing properties. However, LL37 spares intracellular membranes and prohibits release of (potentially harmful) granule proteases such as elastase. LL37-induced necrotic PMNs do not induce pro-inflammatory cytokines (146, 147). We observed decoration of *M. tuberculosis*-infected PMNs by cathelicidin, suggesting that this AMP can contribute to PMN necrosis (B. Corleis, UE. Schaible, unpublished results). Pyroptosis is a special case of necrosis, as it is initiated by caspase-1 and leads to release of the pro-inflammatory cytokines IL-1β and IL-18. Pyroptotic material is also recognized and removed in a PS-dependent manner including recognition and uptake via TIM4 (T-cell immunoglobulin and mucin 4) (138).

'Find' and 'eat me' signals of necrotic material resemble to some extent those of apoptotic cells including nucleotides (ATP, UDP), which however are released in larger amounts, as well as oxidized ox-lysoPS, lysoPC, and lysosomal S1P. Oxidation of phospholipids is mediated through ROI generated by the NADPH-oxidase (148). Receptors recognizing and/or engulfing necrotic material include P2Y$_2$ (nucleotides), G2A (lysoPS), and S1P$_{1-5}$ (S1P) (149, 150). Although a certain redundancy with respect to receptors and signals for recognition of necrotic cells probably facilitates quick detection and removal, the relevance of some of these signals in efferocytosis of necrotic cells as well as the existence of other ligands/receptors is not yet clear. In the case of cells sent into cell death by a pathogen antigens decorating dying/dead cells, specific antibodies may facilitate FcR-mediated uptake of dead cell material. Expression of the FcγRs, CD32 and CD64, by MΦs is boosted by the presence of the PMN granule factors heparin-binding protein (HBP) (acting via β2 integrins) and human neutrophil peptides

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

1–3 (HNP1-3), which may also be relevant during efferocytosis of infected PMNs (151).

Efferocytosis can pose a health problem for efferocytes as uptake of large amounts of lipids such as cholesterol can damage the cells leading to apoptosis, which may start a vicious cycle of cell death and tissue damage. There are protective mechanisms in place to avoid this scenario. Efferocytosis enhances cholesterol efflux via the ATP-binding cassette transporters A1 and G1 (ABCA-1, ABCG1) upon apoptosis (but not necrotic cell uptake), which protects efferocytes from cholesterol-induced ER stress and apoptosis upon massive uptake of dead cells (152, 153).

Although MΦs killed by this cell death pathway are not efficiently phagocytosed by naive MΦs, this interaction still reduces the number of mycobacteria (154). In another study however, apoptosis of infected MΦs and subsequent efferocytosis by naive ones have been described as efficient way to eliminate mycobacteria (155). In this study, *M. tuberculosis*-associated apoptotic corpses were engulfed by the receptors for PS on apoptotic cells, TIM4, and transported into phagolysosomes. In another study, co-uptake of apoptotic PMN granules or HNP-1 enhanced killing of *M. tuberculosis* by MΦs (156). As reported previously, apoptotic blebs from infected MΦs can also promote cross presentation and protective T-cell immunity (137, 157). Apoptotic material released from infected cells carries an antigenic cargo including proteins and glycolipids. Upon efferocytosis by non-infected APCs such as MΦs and DCs, the apoptotic material is transported into the lysosomal pathway for degradation. However, the co-delivered antigens are processed and cross-presented to both $CD4^+$ and $CD8^+$ T cells. Vaccination with apoptotic material from BCG-infected MΦs protected mice against aerosol challenge with *M. tuberculosis* in a similar manner as BCG alone. Therefore, avoiding apoptosis in general appears as a strategy of virulent mycobacteria to escape from detrimental T-cell responses. This is in contrast to another intracellular pathogen, *Leishmania major*, which infects PMNs to abuse their apoptotic camouflage to silently enter MΦs (158). Excessive necrosis caused by virulent tubercle bacilli can promote tissue damage and rupture of granulomas to secure transmission (159). The quick turnover of infected PMNs indicates that removal of dying/dead cells by efferocytes is important in TB but is either disturbed or cannot keep up with PMN influx, death, and bacterial growth. Whether necrotic cell materials from infected MΦs or PMNs can also serve as shuttles for antigens to cross-prime T cells is not known. In addition, whether nutritional sources including intracellular iron stores in apoptotic or necrotic cells, which are co-delivered with intracellular bacteria into efferocytes, provide an energy source for the pathogens is an interesting question to follow up.

Metals in macrophage host responses to *M. tuberculosis*

Metal ions are at the crossroads of host-pathogen interactions. On one hand, microbes need metals such as iron, zinc, copper, or manganese for important metabolic processes, pathogen proliferation, or as central components for defenses against host-mediated radical formation (160, 161). The basis for that can be traced back to the metals' ability to accept or donate electrons needed during metabolic processes; however, metal accumulation can become toxic due the metals' ability to catalyze the formation of toxic oxygen and nitrogen radicals that can intoxicate microbes. However, microbes such as *M. tuberculosis* take up transition metals by multiple pathways, and a sufficient acquisition of these metals by pathogens is linked to their pathogenicity and proliferation. On the other hand, transient metals play important roles in anti-microbial host responses, first by synergistic effects toward anti-microbial radical formation but second, by directly affecting immune cell proliferation and anti-microbial immune effector pathways. Thus, the host immune system affects the metabolism of these metals and/or their availability for microbes via the action of cytokines, cellular proteins, or hormones, for which the term 'nutritional immunity' has been coined. The importance of metal ion composition on the course of *M. tuberculosis* infection was underlined by X-ray fluorescence analyses demonstrating significant changes of ionic and divalent metal composition within the mycobacteria containing phagosome over time (162).

Iron

It is well-established that increased availability of iron promotes the growth of *M. tuberculosis* and exacerbates TB (163–166). Based on the decisive role of iron for both host immune system and microbe function, it is obvious that iron metabolism is significantly changed during the course of an infection (32, 167, 168). These alterations of iron traffic are thought to result from defense strategy of the body to limit the availability of iron for invading pathogens (167–169), which lead to retention of iron in MΦs and an impaired iron absorption from the diet (170). Iron restriction in monocytes and MΦs is achieved by the activity of

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

different cytokines, such as TNF-α, IL-1, IL-6, and IL-10, on iron transport and storage proteins of macrophages and most importantly by the action of the liver-derived acute phase protein hepcidin, which binds to the only known cellular iron exporter ferroportin, resulting in its internalization and degradation, thereby blocking macrophage iron egress (170, 171). However, these alterations of iron homeostasis result also in a limited availability of iron for erythropoiesis, which is a cornerstone for the development of anemia, termed anemia of infection or anemia of chronic disease (170). In a recent study (172), 86% of patients with pulmonary tuberculosis were found to be anemic, and the well-documented association between anemia and severity of an infection (173) was confirmed in this study (172) by the fact that anemic patients were three times more likely to have positive results on sputum smear tests.

Iron retention in MΦs via the action of hepcidin and cytokines is a good strategy to limit the availability of the metal for circulating, extracellular pathogens whereas it may be detrimental in case of an infection with a intracellular microbe, such as *M. tuberculosis* (161). Of interest, hepcidin-deficient mice were as susceptible to aerosol infection with low dose *M. tuberculosis*, as wildtype littermates indicated that hepcidin-triggered changes in murine iron metabolism is not affecting mycobacterial growth (M. Podinovskaya, P. Masaratana, RJ. Simpson, S. Vaulont, AT. McKie, UE. Schaible, unpublished data).

It is well established that the growth and pathogenicity of this bacterium is highly dependent on a sufficient supply of iron (174). *Mycobacteria* acquire iron through multiple avenues including the acquisition of the metal from transferrin via the endocytic pathway, by directly binding iron-loaded transferrin via specific receptors, by uptake of cytoplasmic iron or heme iron by specific transporters, or by the production of siderophores such as mycobactin, which can bind iron and re-utilize the metal (174–177). Accordingly, transcriptional analysis of macrophages and *M. tuberculosis* has demonstrated that *Mycobacteria* regulate a myriad of genes to secure a sufficient supply of iron within the cell, whereas macrophages aim to restrict the availability of this metal by different mechanisms (5).

The Th1-derived cytokine IFN-γ induces the transcription but also affects the translation of the major iron storage protein ferritin, the latter being due to modulation of iron regulatory protein (IRP) binding affinity by the cytokine. IFN-γ stimulates NO formation, which then activates IRP-1 binding to the ferritin IRE, leading to inhibition of ferritin translation, whereas IRP-2 activity is affected depending on the type of cell and NO product (178). Moreover, hydrogen peroxide and superoxide anion modulate IRP-1 activity by a rapidly inducible process involving kinase/phosphatase signal transduction pathways resulting in divergent posttranscriptional regulation of IRE-regulated target genes such as transferrin receptor (TfR) and ferritin (178, 179). The net effects of these partly controversial changes in iron gene expression by these radicals have not been systemically studied; however, a recent investigation in BCG-infected splenocytes indicated a NO-dependent reduction in the expression of ferritin and TfR (180). The latter may be also referred to a direct inhibitory effect of IFN-γ on TfR expression in monocytes, thereby decreasing iron concentration in the mycobacteria containing phagosome (174, 181) (Fig. 2). In an attempt to limit the availability of iron for intracellular bacteria, such as *Salmonella typhimurium*, a Gram-negative intracellular bacterium, which like *M. tuberculosis* also resides in the phagosome, macrophages stimulate iron export via induction of ferroportin transcription (182). This mechanism has also proven to be effective in macrophages infected with *M. tuberculosis* (183). In addition, IFN-γ further stimulates MO iron egress by this pathway and reduces the iron availability for intracellular bacteria (184). Recently, new link between the NO pathway and iron regulation has been characterized. Mice lacking NOS2 presented with MO iron loading, and intra-macrophage *Salmonella* were able to acquire more iron, which positively impacted on their proliferation. Conversely, the induction of NOS2 expression and the subsequent formation of NO resulted in the activation of the transcription factor nuclear factor erythroid 2-related factor-2 (Nrf2), which stimulated the expression of ferroportin and induced iron export from macrophages (185) (Fig. 2). An endemic form of secondary iron overload has been linked to a mutation in the ferroportin gene (186) and is associated with an increased incidence and mortality from TB.

Stimulation of ferroportin expression results in iron limitation to intra-macrophage bacteria and an improved control of the infection, which is also due to stimulation of MO effector functions as a consequence of intracellular iron deficiency. Mechanistically, the latter observation can be referred to the fact that iron exerts inhibitory effects toward IFN-γ-mediated immune pathways such as the formation of TNF-α, IL-6, IL-12 MHC class II, of IDO or NOS2 (185, 187–189), whereas iron excess stimulates the formation of the macrophage de-activating cytokine IL-10 (190). The negative effects of iron on NOS2 transcription, which can be referred to a reduced binding affinity of the

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

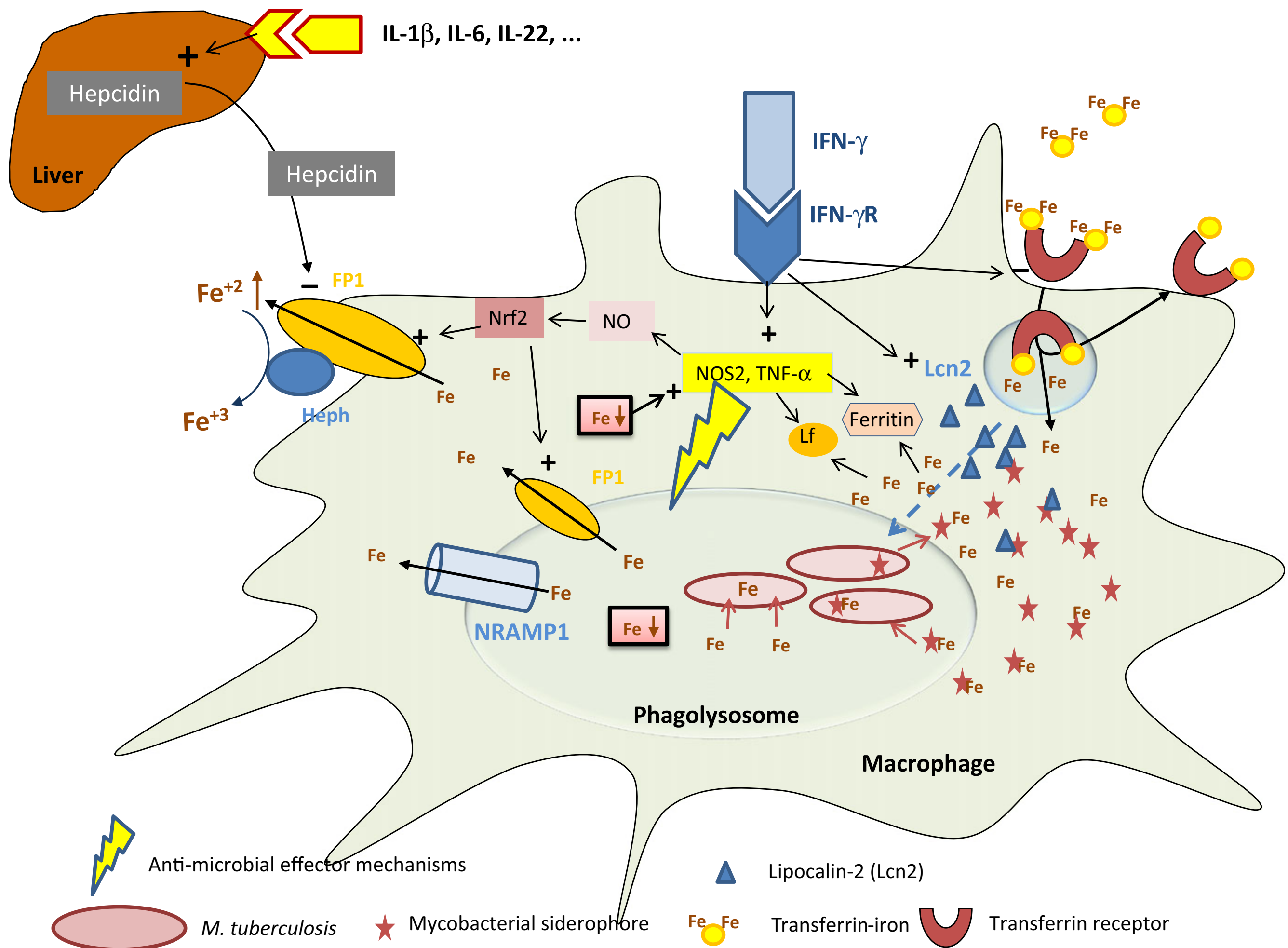

Fig. 2. The battle for iron. Macrophages use multiple pathways to restrict the essential growth factor iron for intracellular mycobacteria. First, cytokines such as IFN-γ inhibit the transcriptional expression of transferrin receptor (TfR). TfR is a major source of iron for mycobacteria, because the bacteria can utilize its ligand, transferrin iron, following its endosomal transfer. Macrophages produce lipocalin-2 (Lcn2), which binds and neutralizes siderophores produced by *M. tuberculosis* to scavenge and re-utilize cytoplasmic iron. Furthermore, macrophage-derived cytokines such as TNF-α induce the formation of the iron-binding protein ferritin, which incorporates iron into its core rendering it unavailable for intracellular bacteria as well as the iron binding protein lactoferrin (Lf), which also scavenges this metal. Activated macrophages express the phagolysomal protein Nramp1, which among other effects pumps iron out of macrophages, thereby reducing the availability of the metal in the phagosome for mycobacteria. Finally, upon formation of nitric oxide, the transcription factor Nrf2 is activated, stimulating the expression of the major iron export protein ferroportin (FP1) pumping iron out of the phagolyosome and of the cytoplasma of macrophages. By mechanisms that remain elusive, the stimulation of hepcidin expression in the liver, which is a major mechanism for iron restriction to extracellular pathogens, is circumvented. All these events result in reduction in intracellular iron levels and a limited availability of iron for intra-macrophage bacteria. Based on the negative regulatory effects of iron on IFN-γ activity, the reduction in this metal's availability results in strengthened innate anti-microbial immune responses. Importantly, some of the pathways shown in this figure have been investigated for other intracellular bacteria such as *S. typhimurium*, and their importance for *M. tuberculosis* remains to be shown.

transcription factors NF-IL6 (C/EBP-β) and hypoxia inducible factor 1 (HIF-1) to the NOS2 promoter (167, 189, 191), is part of a regulatory feedback loop, by which NO modulates the IRE-binding function of IRPs, thereby affecting ferritin translation and intracellular iron availability and linking maintenance of iron homeostasis to NO formation for host defense. It will be of interest to study whether NO can also modulate ferritin translation via IRE/IRP interaction in *M. tuberculosis*, because functional IRPs have been detected within these bacteria (192). NO-mediated modulation of bacterial ferritin expression could be an effective anti-microbial strategy, because mycobacterial ferritin expression is associated with resistance to oxidative stress and reduced susceptibility to antibiotics (177).

Higher dietary iron intake was associated with an increased risk of pulmonary tuberculosis in humans (166) and reduced MO effector function, as reflected by lower circulating levels of IL-12 and nitrate, a stable end product of

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

the NO pathway (193). This was confirmed by another study indicating that imbalances of iron homeostasis, both iron deficiency and iron overload, are associated with a poor outcome from tuberculosis (194). This outcome may be linked to impaired lymphocyte proliferation in association with iron deficiency and impaired macrophage effector function in association with iron loading (161, 169).

M. tuberculosis tries to counter-balance these iron-depleting strategies of macrophages by several strategies. Ferroportin was found in the membrane of the *M. tuberculosis*-containing phagosome, which can be referred to a direct stimulatory effect of *M. tuberculosis* on ferroportin transcription. Although the direction of iron transport in this setting has not been elucidated, it is suggested that ferroportin may provide intraphagosomal iron in favor of the pathogen (172). Moreover, MΦs produce minute amounts of hepcidin in response to challenge with LPS or IL-6, which targets in an autocrine fashion ferroportin exposed on the cell surface, thereby resulting in blockage of iron egress to rapidly limit iron access to extracellular microbes (195, 196). However, *M. tuberculosis* can also stimulate the formation of hepcidin in MΦs and subvert the stimulation of ferroportin-mediated iron excess and ensure iron retention within the MO (197). Most recently, it was demonstrated that *Salmonella typhimurium* ensures a sufficient supply of iron within MΦs by inducing the formation of estrogen-related receptor-γ, which stimulates hepcidin expression in hepatocytes and results in ferroportin degradation and iron retention in MΦs (198). Whether or not similar endocrine feedback loops also apply to *M. tuberculosis* infection needs to be shown. *M. tuberculosis* growth in infected MΦs did not differ, whether cells were deficient for hepcidin or it was added exogenously (M. Podinovskaya, P. Masaratana, RJ. Simpson, S. Vaulont, AT. McKie, UE. Schaible, unpublished results). Of note, the host immune system and MΦs have evolved divergent strategies to limit iron availability of microbes, depending on their primary cellular localization (32, 161, 169, 199). This goes along with the observation in systemic *M. avium* infection demonstrating that alterations of iron traffic were paralleled by increased expression of ferroportin whereas hepcidin formation in the liver remained unchanged (200).

Investigations regarding the susceptibility toward and the course of infections in individuals suffering from primary iron overload, hereditary hemochromatosis (HH), which is mostly due to a mutation within the non-classical MHC class I gene HFE (201), are of great interest. The HFE mutation leads to an impaired formation of hepcidin and thus to an iron deficiency phenotype of MΦs, whereas excessively absorbed iron is stored in parenchymal organs such as the liver, heart, or pancreas. While subjects with HFE-related HH are more susceptible to infections with pathogens such as *Yersinia* spp. or *Vibrio* spp., they appear to be protected from infection with the intracellular bacteria *M. tuberculosis* and *S. typhimurium* (161, 202). Supporting evidence derives from *in vitro* investigations using MΦs from human subjects with HH (203). The improved control of experimental *M. tuberculosis* infection by MΦs was based on a reduced availability of iron for the pathogens. While this is partly based on the reduced cytoplasmic iron concentrations in macrophages from HH subjects, studies obtained with $Hfe^{-/-}$ mice, a model of HH, offer an additional explanation. These mice were able to control invasive infection with *S. typhimurium* significantly better than wildtype littermates, which translated into reduced bacterial burden in spleen and liver and improved survival of $Hfe^{-/-}$ mice (204). This beneficial effect was linked to increased formation of lipocalin-2 (Lcn2) by $Hfe^{-/-}$ mice, whereas the improved control of infection was abolished in $Hfe^{-/-}$ and $Lcn2^{-/-}$ double knockout mice (204).

Lcn2 is an anti-microbial peptide which exerts its effect upon binding of bacterial siderophores. Siderophores are synthesized and secreted by bacteria into their microenvironment to acquire soluble iron or to steal the metal from host iron proteins (205). *M. tuberculosis* produces two classes of such siderophores, namely mycobactins and carboxymycobactins. These mycobacterial siderophores can diffuse out of the phagloysome into the cytoplasm and are re-introduced by the bacteria to increase their access to iron, which is essential for the pathogenicity of *M. tuberculosis* (168, 206). Genetic mutations affecting siderophore utilization by the bacteria resulted in significant reduction in bacterial virulence or bacterial numbers in the lung and absent mortality in mice with tuberculosis (207, 208). Recent evidence suggest that *M. tuberculosis* recycle siderophores to enable efficient iron use, whereas disruption of this process results in iron-mediated self-poisoning of the bacteria (209). Lcn2 has been shown to bind mycobacterial siderophores and to limit the growth of *M. tuberculosis* (210). In addition, Lcn2 may contribute to this effect by shuttling iron out of macrophages, which is supposed to result from binding of a mammalian siderophore (211, 212). However, such siderophores can be also utilized by bacteria as a source for iron. Recent evidence suggests that macrophages reduce the synthesis of the siderophore 2,5-dihydroxybenzoic acid while increasing Lcn2 formation upon bacterial infection to limit microbial iron access (213), the relevance of this finding in *M. tuberculosis* infection remains to be shown. Of interest, while Lcn2

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

formation is increased in macrophages infected with *M. avium*, these bacteria avoided population of Lcn2-expressing phagosomes but resided within Rab11$^+$ recycling endosomes, where they have excess to transferrin (31). Importantly, Lcn2$^{-/-}$ mice are more susceptible to mycobacterial infections (214), which might be different from the iron-sequestrating role of Lcn2 (215) but rather due to its function as efficient chemoattractant for neutrophils to sites of infection (216–218). Iron homeostasis and Lnc2 formation are also linked to the activity of natural resistance associated macrophage protein 1 (NRAMP1 or SLC11A1), conferring resistance to infections with intracellular pathogens, such as *Leishmania*, *Salmonella*, or *Mycobacteria* (219, 220).

NRAMP1 is expressed in the late phagolysosome and has been characterized as a transporter for divalent metals and protons (221–225). Although the importance of NRAMP1 functionality for resistance to infection against several mycobacteria but not *M. tuberculosis* has been shown in mice, an increased susceptibility to tuberculosis in high endemic areas has a mild association with specific polymorphisms in the NRAMP1 gene (226, 227). Upon *M. tuberculosis* aerosol infection, NRAMP1-susceptible C57BL/6 mice complemented by transgenic expression of a functional NRAMP1 gene showed similar mycobacterial loads as their susceptible littermates aside from the first 2 weeks after infection, when transgenic mice carrying a functional NRAMP1 had one log less mycobacteria in lung and spleen (M. Podinovskaya, P. Masaratana, RJ. Simpson, S. Vaulont, AT. McKie, UE. Schaible, unpublished results).

Investigations of RAW264.7 MO cell line stably transfected with functional or non-functional Nramp1 demonstrated that MΦs expressing functional Nramp1 exhibited a lower iron uptake via TfR and an increased iron release mediated via increased ferroportin expression, resulting in reduced cellular iron content (228). This fits into the concept of iron efflux from MΦs and iron deprivation for intraphagosomal bacteria including mycobacteria (32, 160, 222, 225, 229, 230). In addition, Nramp1 expression increases Lcn2 formation, which contributes to the control of infection at least with intra-macrophage *Salmonella* (228). In addition, Nramp1-mediated alterations of iron homeostasis stimulate anti-microbial immune effector function in murine macrophages as reflected by increased formation of NO or TNF-α whereas the expression of the anti-inflammatory cytokine IL-10 is significantly reduced (167, 190). In conclusion, control over iron homeostasis appears to be decisive for the course of *M. tuberculosis* infection and attempt to modulate macrophage iron status or bacterial iron acquisition may be attractive strategies for novel treatment approach of this devastating infection in an era of rapidly emerging drug resistance (231, 232).

Alterations of copper, zinc, and manganese homeostasis

Copper homeostasis is closely linked to iron metabolism. The ferroxidases hephaestin and ceruloplasmin, which mediate the oxidation of ferrous to ferric iron and thus its incorporation into transferrin, are copper-containing enzymes (178). Thus, copper deficiency leads to iron overload and subsequent iron-mediated tissue damage. In addition, copper plays important roles as a prostethic group for many enzymes such as cytochromes, proteins involved in oxidative phosphorylation or copper/zinc-superoxide dismutase (233).

As with iron, copper is a redox-active metal able to catalyze the formation of toxic hydroxyl radicals, and copper accumulation is associated with increased anti-microbial toxicity also termed as metal poisoning (234). However, evidence also suggests that copper kills microbes by mechanisms independent from radical formation, e.g. by displacing iron from iron-sulfer clusters within enzymes. However, these functions of copper await further investigation. Copper and zinc have been shown to accumulate in phagosomes of macrophages infected with *M. tuberculosis* (235). Pro-inflammatory cytokines such as IFN-γ induce the expression of the copper permease Ctr1 in macrophages, which results in increased uptake of copper into macrophages and translocation of the P-type ATPase ATP7A to phagolysosomes, thereby mediating copper influx into these vesicles and subsequent metal poisoning of bacteria (236). On the other hand, *M. tuberculosis* expresses the mycobacterial copper transport protein B (MctB) and the ATPase CtpV, which play an important role for pathogen proliferation by avoiding copper intoxication within the phagolysome and maintaining low copper levels in this environment (160, 237–239).

The transition metal zinc plays central roles for the function of structural proteins and is essential for immune cell proliferation and differentiation (240). However, zinc may also be used by MΦs as an anti-microbial weapon to intoxicate microbes. Recent evidence suggests that granulocyte macrophage colony-stimulating factor (GM-CSF) induces the sequestration of zinc in MΦs. Specifically, GM-CSF induced the expression of two zinc transport proteins leading to accumulation of the metal in the Golgi apparatus, which triggered the formation of toxic radicals by NADPH-oxidase and thereby exerting anti-fungal activity against *Histoplasma capsulatum* (241). As with copper, *M. tuberculosis* express p-type

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

ATPase to promote zinc efflux and to resist metal intoxication (239, 242). Such ATPases may also transport other divalent metals such as manganese, thereby contributing to strengthening of bacterial resistance to oxidative stress (243).

Bacterial periplasmic superoxide-dismutases (SOD) contain zinc or manganese to elaborate their defense against host-mediated oxidative stress, and bacteria thus have a certain need for these metals (233). Components of the S100 protein family bind Zn^{+2}, Cu^{+2}, and Mn^{+2}, which thereby exert anti-microbial activity (233). Two of these proteins, S100A8 and S100A9, form a heterodimeric complex named calprotectin and are mainly expressed by PMN. In addition, S100 proteins can be induced by IL-17 and IL-22, and S100 proteins exert pro-inflammatory activity and may promote chemotaxis of PMN (233, 244). However metal depletion strategies of the host not only affect pathogenic bacteria but also the commensal/protective flora, and thus bacteria that have evolved strategies to outcompete these metal restrictions benefit from a developmental advantage and may become more pathogenic (240).

Sequestration of zinc is often paralleled by capturing of manganese, which is also needed by microbes as part of the anti-oxidant defense protein manganese/zinc-superoxide dismutase and as a catalytic component of several central proteins, where it can also replace the more redox active metal iron. Like iron, zinc, and manganese are transported by NRAMP1, and limited manganese availability within the phagolysosome is considered to be an important mechanism by which MΦs confer resistance toward infection with intracellular pathogens like *M. tuberculosis*. In addition, NRAMP2, also known as divalent metal transporter 1 (DMT1), transports a myriad of divalent metal ions across membranes in an ATP and proton-dependent process. DMT1 expression and iron transport capacity are increased in inflammatory MΦs (245); however, it has not been investigated whether this is also paralleled by increased accumulation of copper, zinc, or manganese in macrophages and whether or not this strengthens anti-microbial activities against *M. tuberculosis*. The central role of manganese starvation for anti-microbial activity has recently been underpinned by the finding that calprotectin-mediated manganese restriction causes maximum growth inhibition of bacteria (246).

Conclusion

Efforts to better understand host immune responses including novel mechanism such as autophagy and efferocytosis along with nutritional immunity pathways, such as metal trafficking between the host and the microbe, and to disentangle the multiple roles of e.g. transition metals, radicals, and nutritional breakdown products for innate and adaptive anti-microbial immune responses as well as for microbes will pave the ground to generate new knowledge regarding novel therapeutic targets. We also need to get insights on how modulation of nutrient availability and metabolic cascades on either the host or the pathogen side will positively impact the control of *M. tuberculosis* infection. This approach also applies to our attempt to better understand the mechanisms by which *M. tuberculosis* succumb to anti-microbial effector mechanisms of macrophages and how the mutual interference can strengthen innate immune function and outcompete anti-mycobacterial responses.

References

1. Watson RO, Manzanillo PS, Cox JS. Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. Cell 2012;**150**:803–815.
2. Sun L, Wu J, Du F, Chen X, Chen ZJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 2013;**339**:786–791.
3. Sambarey A, Prashanthi K, Chandra N. Mining large-scale response networks reveals 'topmost activities' in Mycobacterium tuberculosis infection. Sci Rep 2013;**3**:2302.
4. Bruns H, Stenger S. New insights into the interaction of Mycobacterium tuberculosis and human macrophages. Future Microbiol 2014;**9**:327–341.
5. Schnappinger D, et al. Transcriptional adaptation of Mycobacterium tuberculosis within macrophages: insights into the phagosomal environment. J Exp Med 2003;**198**:693–704.
6. Cambier CJ, et al. Mycobacteria manipulate macrophage recruitment through coordinated use of membrane lipids. Nature 2014;**505**:218–222.
7. Flannagan RS, Cosio G, Grinstein S. Antimicrobial mechanisms of phagocytes and bacterial evasion strategies. Nat Rev Microbiol 2009;**7**:355–366.
8. Kang PB, et al. The human macrophage mannose receptor directs Mycobacterium tuberculosis lipoarabinomannan-mediated phagosome biogenesis. J Exp Med 2005;**202**:987–999.
9. Axelrod S, et al. Delay of phagosome maturation by a mycobacterial lipid is reversed by nitric oxide. Cell Microbiol 2008;**10**:1530–1545.
10. Schaible UE, Sturgill-Koszycki S, Schlesinger PH, Russell DG. Cytokine activation leads to acidification and increases maturation of Mycobacterium avium-containing phagosomes in murine macrophages. J Immunol 1998;**160**:1290–1296.
11. Mocsai A, Ruland J, Tybulewicz VL. The SYK tyrosine kinase: a crucial player in diverse biological functions. Nat Rev Immunol 2010;**10**:387–402.
12. Kant AM, et al. SHP-1 regulates Fcgamma receptor-mediated phagocytosis and the activation of RAC. Blood 2002;**100**:1852–1859.
13. Majeed M, Caveggion E, Lowell CA, Berton G. Role of Src kinases and Syk in Fcgamma receptor-mediated phagocytosis and phagosome-lysosome fusion. J Leukoc Biol 2001;**70**:801–811.
14. Galvez T, Gilleron J, Zerial M, O'Sullivan GA. SnapShot: Mammalian Rab proteins in endocytic trafficking. Cell 2012;**151**:234–e2.

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

15. Hoffmann C, et al. Functional analysis of novel Rab GTPases identified in the proteome of purified Legionella-containing vacuoles from macrophages. Cell Microbiol 2014;**16**:1034–1052.
16. Yeung T, et al. Receptor activation alters inner surface potential during phagocytosis. Science 2006;**313**:347–351.
17. Arango Duque G, Fukuda M, Descoteaux A. Synaptotagmin XI regulates phagocytosis and cytokine secretion in macrophages. J Immunol 2013;**190**:1737–1745.
18. Lindmark IM, et al. Synaptotagmin II could confer Ca(2^+) sensitivity to phagocytosis in human neutrophils. Biochim Biophys Acta 2002;**1590**:159–166.
19. Czibener C, et al. $Ca2^+$ and synaptotagmin VII-dependent delivery of lysosomal membrane to nascent phagosomes. J Cell Biol 2006;**174**:997–1007.
20. Jayachandran R, et al. Survival of mycobacteria in macrophages is mediated by coronin 1-dependent activation of calcineurin. Cell 2007;**130**:37–50.
21. Sturgill-Koszycki S, et al. Lack of acidification in Mycobacterium phagosomes produced by exclusion of the vesicular proton-ATPase. Science 1994;**263**:678–681.
22. McBride HM, Rybin V, Murphy C, Giner A, Teasdale R, Zerial M. Oligomeric complexes link Rab5 effectors with NSF and drive membrane fusion via interactions between EEA1 and syntaxin 13. Cell 1999;**98**:377–386.
23. Christoforidis S, McBride HM, Burgoyne RD, Zerial M. The Rab5 effector EEA1 is a core component of endosome docking. Nature 1999;**397**:621–625.
24. Lawe DC, et al. Sequential roles for phosphatidylinositol 3-phosphate and Rab5 in tethering and fusion of early endosomes via their interaction with EEA1. J Biol Chem 2002;**277**:8611–8617.
25. Simonsen A, et al. EEA1 links PI(3)K function to Rab5 regulation of endosome fusion. Nature 1998;**394**:494–498.
26. Gaullier JM, Simonsen A, D'Arrigo A, Bremnes B, Stenmark H, Aasland R. FYVE fingers bind PtdIns (3)P. Nature 1998;**394**:432–433.
27. Kanai F, et al. The PX domains of p47phox and p40phox bind to lipid products of PI(3)K. Nat Cell Biol 2001;**3**:675–678.
28. Roberts EA, Deretic V. The Mycobacterium tuberculosis phagosome. Methods Mol Biol 2008;**445**:439–449.
29. Rohde K, Yates RM, Purdy GE, Russell DG. Mycobacterium tuberculosis and the environment within the phagosome. Immunol Rev 2007;**219**:37–54.
30. Kolonko M, Geffken AC, Blumer T, Hagens K, Schaible UE, Hagedorn M. WASH-driven actin polymerization is required for efficient mycobacterial phagosome maturation arrest. Cell Microbiol 2014;**16**:232–246.
31. Halaas O, et al. Intracellular Mycobacterium avium intersect transferrin in the Rab11(+) recycling endocytic pathway and avoid lipocalin 2 trafficking to the lysosomal pathway. J Infect Dis 2010;**201**:783–792.
32. Schaible UE, Kaufmann SH. Iron and microbial infection. Nat Rev Microbiol 2004;**2**:946–953.
33. Johansson M, et al. Activation of endosomal dynein motors by stepwise assembly of Rab7-RILP-p150Glued, ORP1L, and the receptor betalll spectrin. J Cell Biol 2007;**176**:459–471.
34. Jordens I, et al. The Rab7 effector protein RILP controls lysosomal transport by inducing the recruitment of dynein-dynactin motors. Curr Biol 2001;**11**:1680–1685.
35. Wade N, et al. Syntaxin 7 complexes with mouse Vps10p tail interactor 1b, syntaxin 6, vesicle-associated membrane protein (VAMP)8, and VAMP7 in b16 melanoma cells. J Biol Chem 2001;**276**:19820–19827.
36. Antonin W, Holroyd C, Tikkanen R, Honing S, Jahn R. The R-SNARE endobrevin/VAMP-8 mediates homotypic fusion of early endosomes and late endosomes. Mol Biol Cell 2000;**11**:3289–3298.
37. Sorensen OE, et al. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. Blood 2001;**97**:3951–3959.
38. Cole AM, Shi J, Ceccarelli A, Kim YH, Park A, Ganz T. Inhibition of neutrophil elastase prevents cathelicidin activation and impairs clearance of bacteria from wounds. Blood 2001;**97**:297–304.
39. Bruns H, et al. Abelson tyrosine kinase controls phagosomal acidification required for killing of Mycobacterium tuberculosis in human macrophages. J Immunol 2012;**189**:4069–4078.
40. Sturgill-Koszycki S, Schaible UE, Russell DG. Mycobacterium-containing phagosomes are accessible to early endosomes and reflect a transitional state in normal phagosome biogenesis. EMBO J 1996;**15**:6960–6968.
41. Kolter T, Winau F, Schaible UE, Leippe M, Sandhoff K. Lipid-binding proteins in membrane digestion, antigen presentation, and antimicrobial defense. J Biol Chem 2005;**280**:41125–41128.
42. Winau F, et al. Saposin C is required for lipid presentation by human CD1b. Nat Immunol 2004;**5**:169–174.
43. Schneider BE, Behrends J, Hagens K, Harmel N, Shayman JA, Schaible UE. Lysosomal phospholipase A2: a novel player in host immunity to Mycobacterium tuberculosis. Eur J Immunol 2014;**44**:2394–2404.
44. Kobayashi T, Stang E, Fang KS, de Moerloose P, Parton RG, Gruenberg J. A lipid associated with the antiphospholipid syndrome regulates endosome structure and function. Nature 1998;**392**:193–197.
45. Matsuo H, et al. Role of LBPA and Alix in multivesicular liposome formation and endosome organization. Science 2004;**303**:531–534.
46. Bissig C, Gruenberg J. ALIX and the multivesicular endosome: ALIX in Wonderland. Trends Cell Biol 2014;**24**:19–25.
47. Vandal OH, Pierini LM, Schnappinger D, Nathan CF, Ehrt S. A membrane protein preserves intrabacterial pH in intraphagosomal Mycobacterium tuberculosis. Nat Med 2008;**14**:849–854.
48. Baker JJ, Johnson BK, Abramovitch RB. Slow growth of Mycobacterium tuberculosis at acidic pH is regulated by phoPR and host-associated carbon sources. Mol Microbiol 2014;**94**:56–69.
49. Melo RC, Dvorak AM. Lipid body-phagosome interaction in macrophages during infectious diseases: host defense or pathogen survival strategy? PLoS Pathog 2012;**8**:e1002729.
50. Peyron P, et al. Foamy macrophages from tuberculous patients' granulomas constitute a nutrient-rich reservoir for M. tuberculosis persistence. PLoS Pathog 2008;**4**:e1000204.
51. Cao C, Laporte J, Backer JM, Wandinger-Ness A, Stein MP. Myotubularin lipid phosphatase binds the hVPS15/hVPS34 lipid kinase complex on endosomes. Traffic 2007;**8**:1052–1067.
52. Vilaplana C, Marzo E, Tapia G, Diaz J, Garcia V, Cardona PJ. Ibuprofen therapy resulted in significantly decreased tissue bacillary loads and increased survival in a new murine experimental model of active tuberculosis. J Infect Dis 2013;**208**:199–202.
53. D'Avila H, Melo RC, Parreira GG, Werneck-Barroso E, Castro-Faria-Neto HC, Bozza PT. Mycobacterium bovis bacillus Calmette-Guerin induces TLR2-mediated formation of lipid bodies: intracellular domains for eicosanoid synthesis in vivo. J Immunol 2006;**176**:3087–3097.
54. Schneider BE, et al. A role for IL-18 in protective immunity against Mycobacterium tuberculosis. Eur J Immunol 2010;**40**:396–405.
55. Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. Nat Rev Immunol 2013;**13**:722–737.
56. Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc Natl Acad Sci USA 2000;**97**:8841–8848.
57. Bogdan C. Nitric oxide and the immune response. Nat Immunol 2001;**2**:907–916.
58. Fang FC. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. Nat Rev Microbiol 2004;**2**:820–832.
59. Klebanoff SJ, Waltersdorph AM, Michel BR, Rosen H. Oxygen-based free radical generation by ferrous ions and deferoxamine. J Biol Chem 1989;**264**:19765–19771.
60. Rosen GM, Pou S, Ramos CL, Cohen MS, Britigan BE. Free radicals and phagocytic cells. FASEB J 1995;**9**:200–209.
61. Mastroeni P, et al. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. II. Effects on microbial proliferation and host survival in vivo. J Exp Med 2000;**192**:237–248.
62. Kim BH, Shenoy AR, Kumar P, Das R, Tiwari S, MacMicking JD. A family of IFN-gamma-inducible 65-kD GTPases protects against bacterial infection. Science 2011;**332**:717–721.
63. MacMicking JD, Taylor GA, McKinney JD. Immune control of tuberculosis by IFN-gamma-inducible LRG-47. Science 2003;**302**:654–659.

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

64. He S, et al. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. Cell 2009;**137**:1100–1111.
65. Zhang DW, et al. RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science 2009;**325**:332–336.
66. Cho YS, et al. Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell 2009;**137**:1112–1123.
67. Roca FJ, Ramakrishnan L. TNF dually mediates resistance and susceptibility to mycobacteria via mitochondrial reactive oxygen species. Cell 2013;**153**:521–534.
68. Vazquez-Torres A, et al. Toll-like receptor 4 dependence of innate and adaptive immunity to Salmonella: importance of the Kupffer cell network. J Immunol 2004;**172**: 6202–6208.
69. Burton NA, et al. Disparate impact of oxidative host defenses determines the fate of Salmonella during systemic infection in mice. Cell Host Microbe 2014;**15**:72–83.
70. Nathan CF, Hibbs JB Jr. Role of nitric oxide synthesis in macrophage antimicrobial activity. Curr Opin Immunol 1991;**3**:65–70.
71. Weiss G, et al. Translational regulation via iron-responsive elements by the nitric oxide/NO-synthase pathway. EMBO J 1993;**12**:3651–3657.
72. MacMicking JD, North RJ, LaCourse R, Mudgett JS, Shah SK, Nathan CF. Identification of nitric oxide synthase as a protective locus against tuberculosis. Proc Natl Acad Sci USA 1997;**94**:5243–5248.
73. Qualls JE, et al. Sustained generation of nitric oxide and control of mycobacterial infection requires argininosuccinate synthase 1. Cell Host Microbe 2012;**12**:313–323.
74. Ehrt S, Schnappinger D. Mycobacterial survival strategies in the phagosome: defence against host stresses. Cell Microbiol 2009;**11**:1170–1178.
75. Bustamante J, et al. Germline CYBB mutations that selectively affect macrophages in kindreds with X-linked predisposition to tuberculous mycobacterial disease. Nat Immunol 2011;**12**:213–221.
76. Singh SB, Davis AS, Taylor GA, Deretic V. Human IRGM induces autophagy to eliminate intracellular mycobacteria. Science 2006;**313**:1438–1441.
77. Kim YM, et al. SH3BP4 is a negative regulator of amino acid-Rag GTPase-mTORC1 signaling. Mol Cell 2012;**46**:833–846.
78. Alonso S, Pethe K, Russell DG, Purdy GE. Lysosomal killing of Mycobacterium mediated by ubiquitin-derived peptides is enhanced by autophagy. Proc Natl Acad Sci USA 2007;**104**:6031–6036.
79. Wang J, et al. MicroRNA-155 promotes autophagy to eliminate intracellular mycobacteria by targeting Rheb. PLoS Pathog 2013;**9**: e1003697.
80. Stanley SA, et al. Identification of host-targeted small molecules that restrict intracellular Mycobacterium tuberculosis growth. PLoS Pathog 2014;**10**:e1003946.
81. Medzhitov R, Janeway CA Jr. An ancient system of host defense. Curr Opin Immunol 1998;**10**:12–15.
82. Liu PT, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006;**311**:1770–1773.
83. Martineau AR, et al. IFN-gamma- and TNF-independent vitamin D-inducible human suppression of mycobacteria: the role of cathelicidin LL-37. J Immunol 2007;**178**:7190–7198.
84. Sonawane A, et al. Cathelicidin is involved in the intracellular killing of mycobacteria in macrophages. Cell Microbiol 2011;**13**:1601–1617.
85. Jo EK. Innate immunity to mycobacteria: vitamin D and autophagy. Cell Microbiol 2010;**12**:1026–1035.
86. Lopez-Lopez N, et al. Vitamin D supplementation promotes macrophages' anti-mycobacterial activity in type 2 diabetes mellitus patients with low vitamin D receptor expression. Microbes Infect 2014;**16**:755–761.
87. Salamon H, et al. Cutting edge: vitamin D regulates lipid metabolism in Mycobacterium tuberculosis infection. J Immunol 2014;**193**:30–34.
88. Ehrt S, Rhee K. Mycobacterium tuberculosis metabolism and host interaction: mysteries and paradoxes. Curr Top Microbiol Immunol 2013;**374**:163–188.
89. Vromman F, Subtil A. Exploitation of host lipids by bacteria. Curr Opin Microbiol 2014;**17**:38–45.
90. Singh V, Jamwal S, Jain R, Verma P, Gokhale R, Rao KV. Mycobacterium tuberculosis-driven targeted recalibration of macrophage lipid homeostasis promotes the foamy phenotype. Cell Host Microbe 2012;**12**:669–681.
91. Lee W, VanderVen BC, Fahey RJ, Russell DG. Intracellular Mycobacterium tuberculosis exploits host-derived fatty acids to limit metabolic stress. J Biol Chem 2013;**288**:6788–6800.
92. Puckett S, et al. Inactivation of fructose-1,6-bisphosphate aldolase prevents optimal co-catabolism of glycolytic and gluconeogenic carbon substrates in Mycobacterium tuberculosis. PLoS Pathog 2014;**10**:e1004144.
93. Podinovskaia M, Lee W, Caldwell S, Russell DG. Infection of macrophages with Mycobacterium tuberculosis induces global modifications to phagosomal function. Cell Microbiol 2013;**15**:843–859.
94. Yang CS, et al. The AMPK-PPARGC1A pathway is required for antimicrobial host defense through activation of autophagy. Autophagy 2014;**10**:785–802.
95. Schroecksnadel K, Zangerle R, Bellmann-Weiler R, Garimorth K, Weiss G, Fuchs D. Indoleamine-2, 3-dioxygenase and other interferon-gamma-mediated pathways in patients with human immunodeficiency virus infection. Curr Drug Metab 2007;**8**:225–236.
96. Blumenthal A, et al. M. tuberculosis induces potent activation of IDO-1, but this is not essential for the immunological control of infection. PLoS ONE 2012;**7**:e37314.
97. Zhang YJ, et al. Tryptophan biosynthesis protects mycobacteria from CD4 T-cell-mediated killing. Cell 2013;**155**:1296–1308.
98. Mayer-Barber KD, et al. Innate and adaptive interferons suppress IL-1alpha and IL-1beta production by distinct pulmonary myeloid subsets during Mycobacterium tuberculosis infection. Immunity 2011;**35**:1023–1034.
99. Wilkinson RJ, et al. Influence of polymorphism in the genes for the interleukin (IL)-1 receptor antagonist and IL-1beta on tuberculosis. J Exp Med 1999;**189**:1863–1874.
100. Briken V, Ahlbrand SE, Shah S. Mycobacterium tuberculosis and the host cell inflammasome: a complex relationship. Front Cell Infect Microbiol 2013;**3**:62.
101. Berry MP, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature 2010;**466**:973–977.
102. Hernandez-Cuellar E, et al. Cutting edge: nitric oxide inhibits the NLRP3 inflammasome. J Immunol 2012;**189**:5113–5117.
103. Mishra BB, et al. Nitric oxide controls the immunopathology of tuberculosis by inhibiting NLRP3 inflammasome-dependent processing of IL-1beta. Nat Immunol 2013;**14**:52–60.
104. Silva-Gomes S, Appelberg R, Larsen R, Soares MP, Gomes MS. Heme catabolism by heme oxygenase-1 confers host resistance to Mycobacterium infection. Infect Immun 2013;**81**:2536–2545.
105. Chatterjee S, et al. Early secreted antigen ESAT-6 of Mycobacterium tuberculosis promotes protective T helper 17 cell responses in a toll-like receptor-2-dependent manner. PLoS Pathog 2011;**7**:e1002378.
106. Dhiman R, Venkatasubramanian S, Paidipally P, Barnes PF, Tvinnereim A, Vankayalapati R. Interleukin 22 inhibits intracellular growth of Mycobacterium tuberculosis by enhancing calgranulin A expression. J Infect Dis 2014;**209**:578–587.
107. Das P, Lahiri A, Lahiri A, Chakravortty D. Modulation of the arginase pathway in the context of microbial pathogenesis: a metabolic enzyme moonlighting as an immune modulator. PLoS Pathog 2010;**6**:e1000899.
108. El Kasmi KC, et al. Toll-like receptor-induced arginase 1 in macrophages thwarts effective immunity against intracellular pathogens. Nat Immunol 2008;**9**:1399–1406.
109. Duque-Correa MA, et al. Macrophage arginase-1 controls bacterial growth and pathology in hypoxic tuberculosis granulomas. Proc Natl Acad Sci USA 2014;**111**:E4024–E4032.
110. Bennett JA, Rao VS, Mitchell MS. Systemic bacillus Calmette-Guerin (BCG) activates natural suppressor cells. Proc Natl Acad Sci USA 1978;**75**:5142–5144.
111. Obregon-Henao A, Henao-Tamayo M, Orme IM, Ordway DJ. Gr1(int)CD11b+ myeloid-derived suppressor cells in Mycobacterium tuberculosis infection. PLoS ONE 2013;**8**:e80669.
112. Yang CT, Cambier CJ, Davis JM, Hall CJ, Crosier PS, Ramakrishnan L. Neutrophils exert protection in the early tuberculous granuloma by oxidative

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

killing of mycobacteria phagocytosed from infected macrophages. Cell Host Microbe 2012;**12**:301–312.
113. Nordenfelt P, Tapper H. Phagosome dynamics during phagocytosis by neutrophils. J Leukoc Biol 2011;**90**:271–284.
114. Faurschou M, Borregaard N. Neutrophil granules and secretory vesicles in inflammation. Microbes Infect 2003;**5**:1317–1327.
115. McLeish KR, Uriarte SM, Tandon S, Creed TM, Le J, Ward RA. Exocytosis of neutrophil granule subsets and activation of prolyl isomerase 1 are required for respiratory burst priming. J Innate Immun 2013;**5**:277–289.
116. Rorvig S, Ostergaard O, Heegaard NH, Borregaard N. Proteome profiling of human neutrophil granule subsets, secretory vesicles, and cell membrane: correlation with transcriptome profiling of neutrophil precursors. J Leukoc Biol 2013;**94**:711–721.
117. Brinkmann V, Goosmann C, Kuhn LI, Zychlinsky A. Automatic quantification of in vitro NET formation. Front Immunol 2012;**3**:413.
118. Eum SY, et al. Neutrophils are the predominant infected phagocytic cells in the airways of patients with active pulmonary TB. Chest 2010;**137**:122–128.
119. Lowe DM, Redford PS, Wilkinson RJ, O'Garra A, Martineau AR. Neutrophils in tuberculosis: friend or foe? Trends Immunol 2012;**33**:14–25.
120. Corleis B, Korbel D, Wilson R, Bylund J, Chee R, Schaible UE. Escape of Mycobacterium tuberculosis from oxidative killing by neutrophils. Cell Microbiol 2012;**14**:1109–1121.
121. Aguilo JI, et al. ESX-1-induced apoptosis is involved in cell-to-cell spread of Mycobacterium tuberculosis. Cell Microbiol 2013;**15**:1994–2005.
122. Aguilo N, Marinova D, Martin C, Pardo J. ESX-1-induced apoptosis during mycobacterial infection: to be or not to be, that is the question. Front Cell Infect Microbiol 2013;**3**:88.
123. Gan H, Lee J, Ren F, Chen M, Kornfeld H, Remold HG. Mycobacterium tuberculosis blocks crosslinking of annexin-1 and apoptotic envelope formation on infected macrophages to maintain virulence. Nat Immunol 2008;**9**:1189–1197.
124. Chen M, Gan H, Remold HG. A mechanism of virulence: virulent Mycobacterium tuberculosis strain H37Rv, but not attenuated H37Ra, causes significant mitochondrial inner membrane disruption in macrophages leading to necrosis. J Immunol 2006;**176**:3707–3716.
125. Lee J, Remold HG, Ieong MH, Kornfeld H. Macrophage apoptosis in response to high intracellular burden of Mycobacterium tuberculosis is mediated by a novel caspase-independent pathway. J Immunol 2006;**176**:4267–4274.
126. O'Sullivan MP, O'Leary S, Kelly DM, Keane J. A caspase-independent pathway mediates macrophage cell death in response to Mycobacterium tuberculosis infection. Infect Immun 2007;**75**:1984–1993.
127. Park JS, Tamayo MH, Gonzalez-Juarrero M, Orme IM, Ordway DJ. Virulent clinical isolates of Mycobacterium tuberculosis grow rapidly and induce cellular necrosis but minimal apoptosis in murine macrophages. J Leukoc Biol 2006;**79**:80–86.
128. Herbst S, Schaible UE, Schneider BE. Interferon gamma activated macrophages kill mycobacteria by nitric oxide induced apoptosis. PLoS ONE 2011;**6**:e19105.
129. Erwig LP, Henson PM. Clearance of apoptotic cells by phagocytes. Cell Death Differ 2008;**15**:243–250.
130. Rydell-Tormanen K, Uller L, Erjefalt JS. Neutrophil cannibalism – a back up when the macrophage clearance system is insufficient. Respir Res 2006;**7**:143.
131. Fullard JF, Kale A, Baker NE. Clearance of apoptotic corpses. Apoptosis 2009;**14**:1029–1037.
132. Poon IK, Lucas CD, Rossi AG, Ravichandran KS. Apoptotic cell clearance: basic biology and therapeutic potential. Nat Rev Immunol 2014;**14**:166–180.
133. Ma YJ, et al. Ficolin-1-PTX3 complex formation promotes clearance of altered self-cells and modulates IL-8 production. J Immunol 2013;**191**:1324–1333.
134. Richardson MB, Williams SJ. MCL and mincle: C-type lectin receptors that sense damaged self and pathogen-associated molecular patterns. Front Immunol 2014;**5**:288.
135. Hart SP, Rossi AG, Haslett C, Dransfield I. Characterization of the effects of cross-linking of macrophage CD44 associated with increased phagocytosis of apoptotic PMN. PLoS ONE 2012;**7**:e33142.
136. Kinchen JM, et al. A pathway for phagosome maturation during engulfment of apoptotic cells. Nat Cell Biol 2008;**10**:556–566.
137. Winau F, et al. Apoptotic vesicles crossprime CD8 T cells and protect against tuberculosis. Immunity 2006;**24**:105–117.
138. Wang Q, Imamura R, Motani K, Kushiyama H, Nagata S, Suda T. Pyroptotic cells externalize eat-me and release find-me signals and are efficiently engulfed by macrophages. Int Immunol 2013;**25**:363–372.
139. Borges VM, et al. TNFalpha inhibits apoptotic cell clearance in the lung, exacerbating acute inflammation. Am J Physiol Lung Cell Mol Physiol 2009;**297**:L586–L595.
140. Fernandez-Boyanapalli R, et al. Impaired phagocytosis of apoptotic cells by macrophages in chronic granulomatous disease is reversed by IFN-gamma in a nitric oxide-dependent manner. J Immunol 2010;**185**:4030–4041.
141. Shiratsuchi A, Watanabe I, Takeuchi O, Akira S, Nakanishi Y. Inhibitory effect of Toll-like receptor 4 on fusion between phagosomes and endosomes/lysosomes in macrophages. J Immunol 2004;**172**:2039–2047.
142. Vandivier RW, et al. Elastase-mediated phosphatidylserine receptor cleavage impairs apoptotic cell clearance in cystic fibrosis and bronchiectasis. J Clin Invest 2002;**109**:661–670.
143. Fernandez-Boyanapalli R, Frasch SC, Riches DW, Vandivier RW, Henson PM, Bratton DL. PPARgamma activation normalizes resolution of acute sterile inflammation in murine chronic granulomatous disease. Blood 2010;**116**:4512–4522.
144. Ravichandran KS. Find-me and eat-me signals in apoptotic cell clearance: progress and conundrums. J Exp Med 2010;**207**:1807–1817.
145. Ravichandran KS. Beginnings of a good apoptotic meal: the find-me and eat-me signaling pathways. Immunity 2011;**35**:445–455.
146. Li HN, et al. Secondary necrosis of apoptotic neutrophils induced by the human cathelicidin LL-37 is not proinflammatory to phagocytosing macrophages. J Leukoc Biol 2009;**86**:891–902.
147. Brown KL, et al. Host defense peptide LL-37 selectively reduces proinflammatory macrophage responses. J Immunol 2011;**186**:5497–5505.
148. Frasch SC, Bratton DL. Emerging roles for lysophosphatidylserine in resolution of inflammation. Prog Lipid Res 2012;**51**:199–207.
149. Natarajan V, et al. Sphingosine-1-phosphate, FTY720, and sphingosine-1-phosphate receptors in the pathobiology of acute lung injury. Am J Respir Cell Mol Biol 2013;**49**:6–17.
150. Hochreiter-Hufford A, Ravichandran KS. Clearing the dead: apoptotic cell sensing, recognition, engulfment, and digestion. Cold Spring Harb Perspect Biol 2013;**5**:a008748.
151. Soehnlein O, et al. Neutrophil primary granule proteins HBP and HNP1-3 boost bacterial phagocytosis by human and murine macrophages. J Clin Invest 2008;**118**:3491–3502.
152. Yvan-Charvet L, et al. ABCA1 and ABCG1 protect against oxidative stress-induced macrophage apoptosis during efferocytosis. Circ Res 2010;**106**:1861–1869.
153. Cui D, et al. Pivotal advance: macrophages become resistant to cholesterol-induced death after phagocytosis of apoptotic cells. J Leukoc Biol 2007;**82**:1040–1050.
154. Hartman ML, Kornfeld H. Interactions between naive and infected macrophages reduce Mycobacterium tuberculosis viability. PLoS ONE 2011;**6**:e27972.
155. Martin CJ, et al. Efferocytosis is an innate antibacterial mechanism. Cell Host Microbe 2012;**12**:289–300.
156. Tan BH, et al. Macrophages acquire neutrophil granules for antimicrobial activity against intracellular pathogens. J Immunol 2006;**177**:1864–1871.
157. Schaible UE, et al. Apoptosis facilitates antigen presentation to T lymphocytes through MHC-I and CD1 in tuberculosis. Nat Med 2003;**9**:1039–1046.
158. van Zandbergen G, et al. Cutting edge: neutrophil granulocyte serves as a vector for Leishmania entry into macrophages. J Immunol 2004;**173**:6521–6525.
159. Ehlers S, Schaible UE. The granuloma in tuberculosis: dynamics of a host-pathogen collusion. Front Immunol 2012;**3**:411.
160. Botella H, Stadthagen G, Lugo-Villarino G, de Chastellier C, Neyrolles O. Metallobiology of host-pathogen interactions: an intoxicating

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

new insight. Trends Microbiol 2012;**20**:106–112.
161. Nairz M, Haschka D, Demetz E, Weiss G. Iron at the interface of immunity and infection. Front Pharmacol 2014;**5**:152.
162. Wagner D, et al. Elemental analysis of Mycobacterium avium-, Mycobacterium tuberculosis-, and Mycobacterium smegmatis-containing phagosomes indicates pathogen-induced microenvironments within the host cell's endosomal system. J Immunol 2005;**174**:1491–1500.
163. Schaible UE, Collins HL, Priem F, Kaufmann SH. Correction of the iron overload defect in beta-2-microglobulin knockout mice by lactoferrin abolishes their increased susceptibility to tuberculosis. J Exp Med 2002;**196**:1507–1513.
164. Lounis N, Truffot-Pernot C, Grosset J, Gordeuk VR, Boelaert JR. Iron and Mycobacterium tuberculosis infection. J Clin Virol 2001;**20**:123–126.
165. McDermid JM, et al. Host iron redistribution as a risk factor for incident tuberculosis in HIV infection: an 11-year retrospective cohort study. BMC Infect Dis 2013;**13**:48.
166. Gangaidzo IT, et al. Association of pulmonary tuberculosis with increased dietary iron. J Infect Dis 2001;**184**:936–939.
167. Nairz M, Schroll A, Sonnweber T, Weiss G. The struggle for iron – a metal at the host-pathogen interface. Cell Microbiol 2010;**12**:1691–1702.
168. Cassat JE, Skaar EP. Iron in infection and immunity. Cell Host Microbe 2013;**13**:509–519.
169. Drakesmith H, Prentice AM. Hepcidin and the iron-infection axis. Science 2012;**338**:768–772.
170. Weiss G, Schett G. Anaemia in inflammatory rheumatic diseases. Nat Rev Rheumatol 2013;**9**:205–215.
171. Ganz T, Nemeth E. Hepcidin and disorders of iron metabolism. Annu Rev Med 2011;**62**:347–360.
172. Van Zandt KE, et al. The iron export protein ferroportin 1 is differentially expressed in mouse macrophage populations and is present in the mycobacterial-containing phagosome. J Leukoc Biol 2008;**84**:689–700.
173. Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med 2005;**352**:1011–1023.
174. Olakanmi O, Schlesinger LS, Ahmed A, Britigan BE. Intraphagosomal Mycobacterium tuberculosis acquires iron from both extracellular transferrin and intracellular iron pools. Impact of interferon-gamma and hemochromatosis. J Biol Chem 2002;**277**:49727–49734.
175. Madigan CA, et al. Lipidomic discovery of deoxysiderophores reveals a revised mycobactin biosynthesis pathway in Mycobacterium tuberculosis. Proc Natl Acad Sci USA 2012;**109**:1257–1262.
176. Boradia VM, et al. Mycobacterium tuberculosis acquires iron by cell-surface sequestration and internalization of human holo-transferrin. Nat Commun 2014;**5**:4730.
177. Pandey R, Rodriguez GM. A ferritin mutant of Mycobacterium tuberculosis is highly susceptible to killing by antibiotics and is unable to establish a chronic infection in mice. Infect Immun 2012;**80**:3650–3659.
178. Pantopoulos K, Porwal SK, Tartakoff A, Devireddy L. Mechanisms of mammalian iron homeostasis. Biochemistry 2012;**51**:5705–5724.
179. Recalcati S, et al. Differential regulation of iron homeostasis during human macrophage polarized activation. Eur J Immunol 2010;**40**:824–835.
180. Thom RE, et al. The expression of ferritin, lactoferrin, transferrin receptor and solute carrier family 11A1 in the host response to BCG-vaccination and Mycobacterium tuberculosis challenge. Vaccine 2012;**30**:3159–3168.
181. Byrd TF, Horwitz MA. Regulation of transferrin receptor expression and ferritin content in human mononuclear phagocytes. Coordinate upregulation by iron transferrin and downregulation by interferon gamma. J Clin Invest 1993;**91**:969–976.
182. Nairz M, et al. The co-ordinated regulation of iron homeostasis in murine macrophages limits the availability of iron for intracellular Salmonella typhimurium. Cell Microbiol 2007;**9**:2126–2140.
183. Johnson EE, Sandgren A, Cherayil BJ, Murray M, Wessling-Resnick M. Role of ferroportin in macrophage-mediated immunity. Infect Immun 2010;**78**:5099–5106.
184. Nairz M, Fritsche G, Brunner P, Talasz H, Hantke K, Weiss G. Interferon-gamma limits the availability of iron for intramacrophage Salmonella typhimurium. Eur J Immunol 2008;**38**:1923–1936.
185. Nairz M, et al. Nitric oxide-mediated regulation of ferroportin-1 controls macrophage iron homeostasis and immune function in Salmonella infection. J Exp Med 2013;**210**:855–873.
186. Gordeuk VR, et al. Iron overload in Africans and African-Americans and a common mutation in the SCL40A1 (ferroportin 1) gene small star, filled. Blood Cells Mol Dis 2003;**31**:299–304.
187. Oexle H, et al. Pathways for the regulation of interferon-gamma-inducible genes by iron in human monocytic cells. J Leukoc Biol 2003;**74**:287–294.
188. Weiss G, Werner-Felmayer G, Werner ER, Grunewald K, Wachter H, Hentze MW. Iron regulates nitric oxide synthase activity by controlling nuclear transcription. J Exp Med 1994;**180**:969–976.
189. Melillo G, Taylor LS, Brooks A, Musso T, Cox GW, Varesio L. Functional requirement of the hypoxia-responsive element in the activation of the inducible nitric oxide synthase promoter by the iron chelator desferrioxamine. J Biol Chem 1997;**272**:12236–12243.
190. Fritsche G, Nairz M, Werner ER, Barton HC, Weiss G. Nramp1-functionality increases iNOS expression via repression of IL-10 formation. Eur J Immunol 2008;**38**:3060–3067.
191. Dlaska M, Weiss G. Central role of transcription factor NF-IL6 for cytokine and iron-mediated regulation of murine inducible nitric oxide synthase expression. J Immunol 1999;**162**:6171–6177.
192. Banerjee S, Nandyala AK, Raviprasad P, Ahmed N, Hasnain SE. Iron-dependent RNA-binding activity of Mycobacterium tuberculosis aconitase. J Bacteriol 2007;**189**:4046–4052.
193. Gordeuk VR, et al. Circulating cytokines in pulmonary tuberculosis according to HIV status and dietary iron content. Int J Tuberc Lung Dis 2009;**13**:1267–1273.
194. Isanaka S, et al. Iron status predicts treatment failure and mortality in tuberculosis patients: a prospective cohort study from Dar es Salaam. Tanzania. PLoS One 2012;**7**:e37350.
195. Theurl I, et al. Autocrine formation of hepcidin induces iron retention in human monocytes. Blood 2008;**111**:2392–2399.
196. Peyssonnaux C, Zinkernagel AS, Datta V, Lauth X, Johnson RS, Nizet V. TLR4-dependent hepcidin expression by myeloid cells in response to bacterial pathogens. Blood 2006;**107**:3727–3732.
197. Sow FB, Florence WC, Satoskar AR, Schlesinger LS, Zwilling BS, Lafuse WP. Expression and localization of hepcidin in macrophages: a role in host defense against tuberculosis. J Leukoc Biol 2007;**82**:934–945.
198. Kim DK, et al. Inverse agonist of estrogen-related receptor gamma controls Salmonella typhimurium infection by modulating host iron homeostasis. Nat Med 2014;**20**:419–424.
199. Fang FC, Weiss G. Iron ERRs with Salmonella. Cell Host Microbe 2014;**15**:515–516.
200. Rodrigues PN, et al. Mycobacteria-induced anaemia revisited: a molecular approach reveals the involvement of NRAMP1 and lipocalin-2, but not of hepcidin. Immunobiology 2011;**216**:1127–1134.
201. Pietrangelo A. Hereditary hemochromatosis – a new look at an old disease. N Engl J Med 2004;**350**:2383–2397.
202. Silva-Gomes S, Vale-Costa S, Appelberg R, Gomes MS. Iron in intracellular infection: to provide or to deprive? Front Cell Infect Microbiol 2013;**3**:96.
203. Olakanmi O, Schlesinger LS, Britigan BE. Hereditary hemochromatosis results in decreased iron acquisition and growth by Mycobacterium tuberculosis within human macrophages. J Leukoc Biol 2007;**81**:195–204.
204. Nairz M, et al. Absence of functional Hfe protects mice from invasive Salmonella enterica serovar Typhimurium infection via induction of lipocalin-2. Blood 2009;**114**:3642–3651.
205. Chakraborty S, Kaur S, Guha S, Batra SK. The multifaceted roles of neutrophil gelatinase associated lipocalin (NGAL) in inflammation and cancer. Biochim Biophys Acta 2012;**1826**:129–169.
206. Luo M, Fadeev EA, Groves JT. Mycobactin-mediated iron acquisition within macrophages. Nat Chem Biol 2005;**1**:149–153.
207. Wells RM, et al. Discovery of a siderophore export system essential for virulence of Mycobacterium tuberculosis. PLoS Pathog 2013;**9**:e1003120.
208. Reddy PV, et al. Disruption of mycobactin biosynthesis leads to attenuation of Mycobacterium tuberculosis for growth and virulence. J Infect Dis 2013;**208**:1255–1265.

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.

209. Jones CM, et al. Self-poisoning of Mycobacterium tuberculosis by interrupting siderophore recycling. Proc Natl Acad Sci USA 2014;**111**:1945–1950.
210. Holmes MA, Paulsene W, Jide X, Ratledge C, Strong RK. Siderocalin (Lcn 2) also binds carboxymycobactins, potentially defending against mycobacterial infections through iron sequestration. Structure 2005;**13**:29–41.
211. Devireddy LR, Hart DO, Goetz DH, Green MR. A mammalian siderophore synthesized by an enzyme with a bacterial homolog involved in enterobactin production. Cell 2010;**141**:1006–1017.
212. Bao G, et al. Iron traffics in circulation bound to a siderocalin (Ngal)-catechol complex. Nat Chem Biol 2010;**6**:602–609.
213. Liu Z, et al. Regulation of mammalian siderophore 2,5-DHBA in the innate immune response to infection. J Exp Med 2014;**211**:1197–1213.
214. Saiga H, et al. Lipocalin 2-dependent inhibition of mycobacterial growth in alveolar epithelium. J Immunol 2008;**181**:8521–8527.
215. Martineau AR, et al. Neutrophil-mediated innate immune resistance to mycobacteria. J Clin Invest 2007;**117**:1988–1994.
216. Guglani L, et al. Lipocalin 2 regulates inflammation during pulmonary mycobacterial infections. PLoS ONE 2012;**7**:e50052.
217. Schroll A, et al. Lipocalin-2 ameliorates granulocyte functionality. Eur J Immunol 2012;**42**:3346–3357.
218. Liu Z, Petersen R, Devireddy L. Impaired neutrophil function in 24p3 null mice contributes to enhanced susceptibility to bacterial infections. J Immunol 2013;**190**:4692–4706.
219. Forbes JR, Gros P. Divalent-metal transport by NRAMP proteins at the interface of host-pathogen interactions. Trends Microbiol 2001;**9**:397–403.
220. Blackwell JM, Searle S, Goswami T, Miller EN. Understanding the multiple functions of Nramp1. Microbes Infect 2000;**2**:317–321.
221. Jabado N, Jankowski A, Dougaparsad S, Picard V, Grinstein S, Gros P. Natural resistance to intracellular infections: natural resistance-associated macrophage protein 1 (Nramp1) functions as a pH-dependent manganese transporter at the phagosomal membrane. J Exp Med 2000;**192**:1237–1248.
222. Mulero V, Searle S, Blackwell JM, Brock JH. Solute carrier 11a1 (Slc11a1; formerly Nramp1) regulates metabolism and release of iron acquired by phagocytic, but not transferrin-receptor-mediated, iron uptake. Biochem J 2002;**363**:89–94.
223. Barton CH, Biggs TE, Baker ST, Bowen H, Atkinson PG. Nramp1: a link between intracellular iron transport and innate resistance to intracellular pathogens. J Leukoc Biol 1999;**66**:757–762.
224. Hackam DJ, Rotstein OD, Zhang W, Gruenheid S, Gros P, Grinstein S. Host resistance to intracellular infection: mutation of natural resistance-associated macrophage protein 1 (Nramp1) impairs phagosomal acidification. J Exp Med 1998;**188**:351–364.
225. Cellier MF. Nramp: from sequence to structure and mechanism of divalent metal import. Curr Top Membr 2012;**69**:249–293.
226. Bellamy R. The natural resistance-associated macrophage protein and susceptibility to intracellular pathogens. Microbes Infect 1999;**1**:23–27.
227. van Crevel R, et al. Infection with Mycobacterium tuberculosis Beijing genotype strains is associated with polymorphisms in SLC11A1/NRAMP1 in Indonesian patients with tuberculosis. J Infect Dis 2009;**200**:1671–1674.
228. Fritsche G, Nairz M, Libby SJ, Fang FC, Weiss G. Slc11a1 (Nramp1) impairs growth of Salmonella enterica serovar typhimurium in macrophages via stimulation of lipocalin-2 expression. J Leukoc Biol 2012;**92**:353–359.
229. Nairz M, Fritsche G, Crouch ML, Barton HC, Fang FC, Weiss G. Slc11a1 limits intracellular growth of Salmonella enterica sv. Typhimurium by promoting macrophage immune effector functions and impairing bacterial iron acquisition. Cell Microbiol 2009;**11**:1365–1381.
230. Appelberg R. Macrophage nutriprive antimicrobial mechanisms. J Leukoc Biol 2006;**79**:1117–1128.
231. Olakanmi O, Kesavalu B, Pasula R, Abdalla MY, Schlesinger LS, Britigan BE. Gallium nitrate is efficacious in murine models of tuberculosis and inhibits key bacterial fe-dependent enzymes. Antimicrob Agents Chemother 2013;**57**:6074–6080.
232. Mair SM, et al. Nifedipine affects the course of Salmonella enterica serovar Typhimurium infection by modulating macrophage iron homeostasis. J Infect Dis 2011;**204**: 685–694.
233. Hood MI, Skaar EP. Nutritional immunity: transition metals at the pathogen-host interface. Nat Rev Microbiol 2012;**10**:525–537.
234. Samanovic MI, Ding C, Thiele DJ, Darwin KH. Copper in microbial pathogenesis: meddling with the metal. Cell Host Microbe 2012;**11**: 106–115.
235. Kim A, et al. A mouse model of anemia of inflammation: complex pathogenesis with partial dependence on hepcidin. Blood 2014;**123**:1129–1136.
236. White C, Lee J, Kambe T, Fritsche K, Petris MJ. A role for the ATP7A copper-transporting ATPase in macrophage bactericidal activity. J Biol Chem 2009;**284**:33949–33956.
237. Hodgkinson V, Petris MJ. Copper homeostasis at the host-pathogen interface. J Biol Chem 2012;**287**:13549–13555.
238. Wolschendorf F, et al. Copper resistance is essential for virulence of Mycobacterium tuberculosis. Proc Natl Acad Sci USA 2011;**108**:1621–1626.
239. Neyrolles O, Mintz E, Catty P. Zinc and copper toxicity in host defense against pathogens: Mycobacterium tuberculosis as a model example of an emerging paradigm. Front Cell Infect Microbiol 2013;**3**:89.
240. Liu JZ, et al. Zinc sequestration by the neutrophil protein calprotectin enhances Salmonella growth in the inflamed gut. Cell Host Microbe 2012;**11**:227–239.
241. Subramanian Vignesh K, Landero Figueroa JA, Porollo A, Caruso JA, Deepe GS Jr. Granulocyte macrophage-colony stimulating factor induced zn sequestration enhances macrophage superoxide and limits intracellular pathogen survival. Immunity 2013;**39**:697–710.
242. Botella H, et al. Mycobacterial p(1)-type ATPases mediate resistance to zinc poisoning in human macrophages. Cell Host Microbe 2011;**10**:248–259.
243. Padilla-Benavides T, Long JE, Raimunda D, Sassetti CM, Arguello JM. A novel P(1B)-type $Mn2^{+}$-transporting ATPase is required for secreted protein metallation in mycobacteria. J Biol Chem 2013;**288**:11334–11347.
244. Diaz-Ochoa VE, Jellbauer S, Klaus S, Raffatellu M. Transition metal ions at the crossroads of mucosal immunity and microbial pathogenesis. Front Cell Infect Microbiol 2014;**4**:2.
245. Ludwiczek S, Aigner E, Theurl I, Weiss G. Cytokine-mediated regulation of iron transport in human monocytic cells. Blood 2003;**101**:4148–4154.
246. Damo SM, et al. Molecular basis for manganese sequestration by calprotectin and roles in the innate immune response to invading bacterial pathogens. Proc Natl Acad Sci USA 2013;**110**:3841–3846.

© 2015 The Authors. *Immunological Reviews* published by John Wiley & Sons Ltd.