An alloy of zinc and innate immunity: Galvanising host defence against infection

Jessica B von Pein, Claudia J Stocks, Mark A Schembri, Ronan Kapetanovic, Matthew J Sweet

Received: 30 June 2020 | Revised: 31 August 2020 | Accepted: 3 September 2020

DOI: 10.1111/cmi.13268

MICROREVIEW

WILEY

An alloy of zinc and innate immunity: Galvanising host defence against infection

Jessica B. von Pein[1,2,3] | Claudia J. Stocks[1,2,3] | Mark A. Schembri[3,4] | Ronan Kapetanovic[1,2,3] | Matthew J. Sweet[1,2,3]

[1]Institute for Molecular Bioscience (IMB), The University of Queensland, St. Lucia, Queensland, Australia

[2]IMB Centre for Inflammation and Disease Research, The University of Queensland, St. Lucia, Queensland, Australia

[3]Australian Infectious Diseases Research Centre, The University of Queensland, St. Lucia, Queensland, Australia

[4]School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, Queensland, Australia

Correspondence
Matthew J. Sweet, Institute for Molecular Bioscience (IMB), The University of Queensland, St. Lucia, QLD 4072, Australia.
Email: m.sweet@imb.uq.edu.au

Funding information
National Health and Medical Research Council, Grant/Award Numbers: APP1106930, APP1107914, APP1144967

Abstract

Innate immune cells such as macrophages and neutrophils initiate protective inflammatory responses and engage antimicrobial responses to provide frontline defence against invading pathogens. These cells can both restrict the availability of certain transition metals that are essential for microbial growth and direct toxic concentrations of metals towards pathogens as antimicrobial responses. Zinc is important for the structure and function of many proteins, however excess zinc can be cytotoxic. In recent years, several studies have revealed that innate immune cells can deliver toxic concentrations of zinc to intracellular pathogens. In this review, we discuss the importance of zinc status during infectious disease and the evidence for zinc intoxication as an innate immune antimicrobial response. Evidence for pathogen subversion of this response is also examined. The likely mechanisms, including the involvement of specific zinc transporters that facilitate delivery of zinc by innate immune cells for metal ion poisoning of pathogens are also considered. Precise mechanisms by which excess levels of zinc can be toxic to microorganisms are then discussed, particularly in the context of synergy with other antimicrobial responses. Finally, we highlight key unanswered questions in this emerging field, which may offer new opportunities for exploiting innate immune responses for anti-infective development.

KEYWORDS

antimicrobial, bacterial pathogens, host, innate immunity, pathogen, zinc toxicity, zinc transporters

1 | INTRODUCTION

The innate immune system provides the first line of defence against infection. Innate immune cells, including monocytes, macrophages, dendritic cells and neutrophils, initiate both inflammatory and antimicrobial responses to clear pathogens. During infection, pathogens require essential nutrients, including transition metals, for growth and survival. Innate immune cells thus restrict the availability of specific transition metals to limit microbial growth, in a process termed nutritional immunity (Hood & Skaar, 2012). Conversely, phagocytes can also traffic toxic concentrations of metal ions as an antimicrobial response against intracellular pathogens (Sheldon & Skaar, 2019).

Zinc is a key component of enzymes across all domains of life. This metal is essential for the structure and/or function of many proteins; for example, the catalytic activity of numerous enzymes (Andreini & Bertini, 2012). Zinc also has roles in several signal transduction pathways, including in inflammatory and immune signalling (Haase & Rink, 2007; Pyle et al., 2017; Yamasaki et al., 2007). Despite these essential roles, excessive zinc can be cytotoxic and can cause

Abbreviations: CDF, cation diffusion facilitator; IL, interleukin; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MT/Mt, metallothionein (HUMAN/murine); NO, nitric oxide; ROS, reactive oxygen species; SLC/Slc, solute carrier (HUMAN/Murine); SPI-1, Salmonella pathogenicity island-1; S. Typhimurium, Salmonella enterica serovar Typhimurium; UPEC, uropathogenic E. coli.

tissue damage, organ failure and death (Bennett et al., 1997; Lee & Koh, 2010). Therefore, zinc homeostasis needs to be tightly regulated (Blencowe & Morby, 2003; Eide, 2006). Innate immune cells can mobilise zinc during infection to achieve either zinc sequestration or to deliver toxic concentrations of zinc to intracellular pathogens (Lonergan & Skaar, 2019; Sheldon & Skaar, 2019). Zinc sequestration has previously been extensively reviewed (Haase, 2013; Hood & Skaar, 2012; Jordan, Wang, Capdevila, & Giedroc, 2020; Lonergan & Skaar, 2019; Sheldon & Skaar, 2019). This review will discuss and highlight key unanswered questions about the emerging role of zinc toxicity as an antimicrobial response deployed by the innate immune system during infection.

2 | ZINC DEFICIENCY AND SUPPLEMENTATION IN INFECTIOUS DISEASE

While zinc toxicity in humans is very rare, approximately 20% of the world population is estimated to be at risk of inadequate zinc intake (Wuehler, Peerson, & Brown, 2005). Zinc deficiency is of particular concern for susceptibility to infectious diseases, due to the established role of zinc in immune functions (Gammoh & Rink, 2017). Indeed, sepsis severity is associated with low plasma zinc concentrations in adults (Besecker et al., 2011; Hoeger et al., 2017). Low plasma zinc levels are also linked to immunosenescence, an age-related decline in immune function accompanied by recurring infections and chronic inflammation (Maywald & Rink, 2015; Rink, Cakman, & Kirchner, 1998). Consistent with this, long-term zinc supplementation reduced infection incidence in healthy elderly subjects (Prasad et al., 2007). Furthermore, zinc supplementation in humans improves outcomes for multiple types of infectious disease, such as respiratory tract infections (Brooks et al., 2005; Sazawal et al., 1998), pneumonia (Bhutta et al., 1999; Valavi, Hakimzadeh, Shamsizadeh, Aminzadeh, & Alghasi, 2011), diarrhoeal disease (Ahmadipour, Mohsenzadeh, Alimadadi, Salehnia, & Fallahi, 2019; Al Tarique et al., 2010; Bhutta et al., 2000; Castillo-Duran, Vial, & Uauy, 1988; Sazawal et al., 1998) and viral infections (Matsumura et al., 2012; Varadinova et al., 1993). Of particular note, the WHO and UNICEF recommend zinc supplementation for the clinical management of acute diarrhoea in children, as a consequence of multiple studies demonstrating its efficacy in reducing both disease severity and duration (Penny, 2013).

In keeping with the clinical evidence above, the importance of zinc in host defence has been confirmed by modulating dietary intake of zinc in animal models of infection (summarised in Table S1). For example, zinc deficiency enhanced organ damage and mortality in a murine model of polymicrobial sepsis, with these effects being rescued by zinc supplementation (Knoell et al., 2009). Furthermore, zinc restriction in mice enhanced *Streptococcus pneumoniae* infection, likely due to impaired pathogen clearance by phagocytes (Eijkelkamp et al., 2019). Such studies are consistent with zinc having important roles in facilitating innate immune cell functions during infection.

3 | EVIDENCE FOR ZINC TOXICITY AS AN INNATE IMMUNE ANTIMICROBIAL RESPONSE

3.1 | Mobilisation of zinc by innate immune cells during infection

Zinc is mobilised and concentrated in specific tissues during inflammation and infection. For example, infection of mice with *S. pneumoniae* resulted in increased zinc concentrations in the lungs, brain, nasopharynx and blood (McDevitt et al., 2011), with this pathogen subsequently shown to co-localise with zinc-enriched regions in murine lungs (Eijkelkamp et al., 2019). Several studies, utilising either fluorescent zinc-binding dyes or direct elemental analysis, have also documented fluctuations in zinc concentrations within immune cells in response to infection or microbial products (Table 1). Zinc accumulation in phagosomes during infection has been observed in multiple cellular systems. Macrophages mobilise and traffic zinc to phagosomes and intracellular compartments that co-localise with various bacterial pathogens, including *Mycobacterium tuberculosis*, *Salmonella enterica* serovar Typhimurium and *Escherichia coli* (Table 1). Subsequent studies have confirmed that zinc vesicles co-localised with an *E. coli* strain that specifically reports zinc stress (Stocks et al., 2019). Zinc accumulation in lysosomes and azurophilic granules has also been observed in neutrophils, implicating zinc delivery to phagocytosed *Streptococcus pyogenes* (Ong, Berking, Walker, & McEwan, 2018). Interestingly, in the phagocytic soil amoeba *Dictyostelium discoideum* that employs phagocytosis to feed on bacteria, zinc was also delivered towards *Mycobacterium smegmatis*-containing vacuoles (Barisch et al., 2018). Combined with evidence below linking such effects to zinc stress, these studies suggest innate immune cells can deliver zinc to poison intracellular bacteria.

3.2 | Pathogens resist zinc toxicity by detoxification during infection

Much of the evidence for innate immune zinc toxicity is based on the necessity of bacterial zinc export systems for survival within innate immune cells. As for mammals, bacteria maintain zinc homeostasis by expression of zinc importers and exporters. Zinc export is achieved via P-type ATPases including ZntA (Beard, Hashim, Membrillo-Hernández, Hughes, & Poole, 1997; Rensing, Mitra, & Rosen, 1997), as well as the putative zinc exporters CtpC and CtpG (Botella et al., 2011) (Figure 1). In addition, cation diffusion facilitators (CDF) including ZitB (Grass et al., 2001; Wang, Hosteen, & Fierke, 2012) and heavy metal efflux systems such as CzcCBA promote zinc efflux in bacteria (Blencowe & Morby, 2003; Figure 1). Another CDF, CzcD, also mediates zinc export in both Gram-negative bacteria such as *Acinetobacter baumannii* (Alquethamy et al., 2019) and Gram-positive bacteria such as *S. pyogenes* (Ong, Gillen, Walker, Barnett, & McEwan, 2014). CzcD performs this function via an antiport mechanism (Guffanti, Wei, Rood, & Krulwich, 2002). When zinc levels are

TABLE 1 Innate immune cells mobilise zinc in response to specific stimuli and/or infection

Species	Cell type	Stimulation	Evidence	References
Human	Monocyte-derived macrophages	Chronic NOD2 stimulation	↑ Intracellular Zn^{2+}. Upregulated *MT* expression.	Lahiri and Abraham, (2014)
		M. tuberculosis infection	↑ Zn^{2+}. Lysosomal/endosomal Zn^{2+} accumulation.	Botella et al. (2011)
		E. coli infection	↑ Intracellular Zn^{2+}. Zn^{2+} co-localised with phagolysosomal *E. coli*.	Botella et al. (2011)
			Zn^{2+} co-localised with Zn^{2+}-stressed *E. coli*.	Stocks et al. (2019)
			Zn^{2+} vesicles co-localised with *E. coli*.	Kapetanovic et al. (2016)
		LPS	Vesicular Zn^{2+} accumulation.	Kapetanovic et al. (2016)
		S. Typhimurium infection	Infection induced Zn^{2+} vesicle formation. Zn^{2+} co-localised with ΔSPI-1 mutant strain.	Kapetanovic et al. (2016)
	THP-1	LPS	Vesicular Zn^{2+} accumulation.	Kapetanovic et al. (2016)
Mouse	Peritoneal macrophages	TNF (pre/post infection)	↑ Zn^{2+} in *M. avium*-containing vacuoles.	Wagner et al. (2005)
		IFNγ (post-infection)	↑ Zn^{2+} in *M. avium*-containing vacuoles.	Wagner et al. (2005)
		M. tuberculosis/*M. smegmatis* infection	↑ Phagosomal Zn^{2+}.	Wagner et al. (2005)
	Bone marrow-derived macrophages	IL-4	Mt-3 and Slc30a4-dependent ↑ of labile Zn^{2+} stores.	Subramanian Vignesh et al. (2016))
		E. coli infection	Intramacrophage *E. coli* reported Zn^{2+} stress.	Stocks et al. (2019))
		C. glabrata infection	Zn^{2+} accumulation in fungal vacuole.	Riedelberger et al. (2020)
	RAW 264.7	*S*. Typhimurium infection	↑ Intracellular Zn^{2+}.	Wu et al. (2017)
		C. glabrata infection	Zn^{2+} accumulation in fungal vacuole.	Riedelberger et al. (2020)

elevated, the cytosolic regulators ZntR (Brocklehurst et al., 1999) and GczA/SczA (Kloosterman, van der Kooi-Pol, Bijlsma, & Kuipers, 2007; Martin et al., 2017) induce *zntA* and *czcD* expression, respectively (Figure 1).

Several zinc exporters and detoxification systems are induced in various pathogens during infection of innate immune cells and/or in in vivo models of infection (Table 2). For example, zinc export was upregulated by *S. pneumoniae* following infection of human neutrophils, the macrophage-like cell line THP-1, and within an intranasal murine infection model (Eijkelkamp et al., 2019). In addition, zinc efflux systems were upregulated by *M. tuberculosis* (Botella et al., 2011), *S*. Typhimurium (Kapetanovic et al., 2016) and *E. coli* (Stocks et al., 2019) upon infection of macrophages. Several studies have also highlighted the necessity of pathogen zinc export systems for their survival during infection (Table 2). Human macrophages were able to more efficiently clear *M. tuberculosis* and *E. coli* that lack zinc export systems by comparison to isogenic wild-type strains (Botella et al., 2011; Stocks et al., 2019). Additionally, zinc-sensitive mutants of *S. pyogenes* (Ong et al., 2014; Ong et al., 2018), *S. pneumoniae* (Eijkelkamp et al., 2019) and *E. coli* (Kapetanovic et al., 2016) were more susceptible to neutrophil killing. Similarly, *S. pneumoniae*, *S. pyogenes* and *E. coli* which lack regulators required for zinc exporter expression exhibited reduced intracellular survival in THP-1 cells (Martin et al., 2017), neutrophils (Ong et al., 2014) and macrophages (Stocks et al., 2019), respectively. Recent evidence suggests that the innate immune zinc toxicity response is not just limited to bacterial infection. In murine macrophages, the fungal pathogen *Candida glabrata* required the zinc transporter *ZRC1* for survival (Riedelberger et al., 2020). This study implicated a role for *ZRC1* in the sequestration of excess zinc into intracellular vacuoles during infection. Together, this literature implies that a variety of pathogens are subjected to zinc toxicity during infection of innate immune cells and employ zinc detoxification systems to resist this response.

3.3 | Pathogen evasion of zinc toxicity responses during infection

Pathogens require strategies to resist or evade host innate immune responses to enable survival during infection. In addition to resistance by zinc detoxification systems (described above), some pathogens can also evade macrophage zinc-toxicity responses. Moreover, *Salmonella* actually employs both resistance and evasion to subvert zinc toxicity responses deployed by macrophages. While wild-type *S*. Typhimurium did not co-localise with zinc vesicles in primary human macrophages, a *Salmonella* pathogenicity island-1 (ΔSPI-1) mutant strain co-localised with zinc vesicles, upregulated *zntA* and was compromised for intramacrophage survival (Kapetanovic et al., 2016). However, a *S*. Typhimurium ΔSPI-1/Δ*zntA* mutant was no more sensitive to killing by human macrophages than an isogenic ΔSPI-1 mutant strain, suggesting that *Salmonella* also has SPI-1- and ZntA-independent mechanisms to resist macrophage-mediated zinc toxicity (Kapetanovic

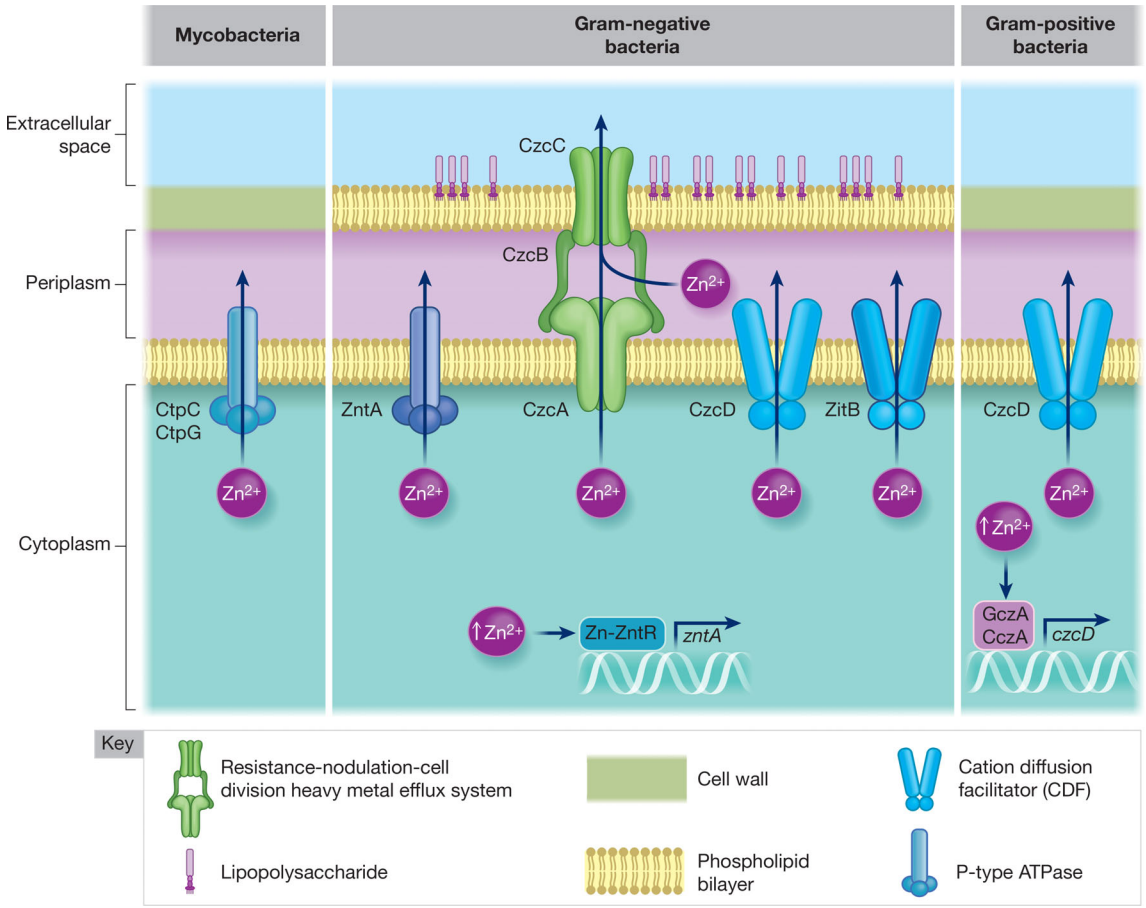

FIGURE 1 Bacterial zinc efflux systems. Bacteria express various exporters which transport zinc from the cytosol to maintain homeostasis in conditions of high zinc availability. Expression of some exporters are regulated by zinc-sensing transcription factors, thus enabling their selective induction under conditions of zinc stress

et al., 2016). Other bacterial pathogens such as uropathogenic *E. coli* (UPEC) also evade the macrophage zinc toxicity response. In contrast to non-pathogenic *E. coli*, the majority of intramacrophage UPEC did not report zinc stress, as indicated by a novel zinc-stress reporter system based on zinc-inducible *zntA* expression (Stocks et al., 2019). Consistent with this, intramacrophage survival of zinc-sensitive *zntA*- and *zntR*-deficient UPEC mutants was not reduced, in contrast to observations with corresponding mutants of a non-pathogenic *E. coli* strain (Stocks et al., 2019). This suggests that UPEC effectively evades macrophage-mediated zinc toxicity. What remains to be determined are the specific zinc toxicity evasion strategies employed by *S.* Typhimurium and UPEC.

4 | HOST CELL MECHANISMS UNDERPINNING ANTIMICROBIAL ZINC DELIVERY

Innate immune delivery of zinc for microbial intoxication is likely to be driven by multiple, interconnected mechanisms, including zinc import, zinc export and interactions with metallothioneins (MTs; Figure 2). Mammalian cells utilise 14 highly conserved solute carrier (SLC) 39A family members [also referred to as Zrt-, Irt-like proteins (ZIP)] for the import of zinc and/or other metal ions from the extracellular space and the lumen of organelles into the cytoplasm (Eide, 2006; Hara et al., 2017). In addition, mammals possess 10 CDF zinc exporters of the SLC30A family (also referred to as ZnT) that can export zinc and/or other metal ions from the cytoplasm into luminal compartments or the extracellular space (Eide, 2006; Huang & Tepaamorndech, 2013). It is likely that both SLC39A and SLC30A zinc transporters are involved in the coordinated shuttling of zinc pools towards engulfed bacteria. In this manner, zinc importers may take up zinc from the extracellular environment and/or cellular compartments to maintain adequate cytosol zinc concentrations, while zinc exporters may concentrate zinc ions into vesicles or bacteria-containing compartments.

Various innate immune stimuli induce zinc mobilisation in macrophages. For example, agonists of several Toll-like receptors (TLRs) increased available zinc as determined by intracellular staining with Fluozin-3 in RAW 264.7 cells and human monocytes (Brieger, Rink, & Haase, 2013). In addition, TLR 3, 4 and 7 stimulation promoted vesicular zinc accumulation in primary human macrophages and THP-1

TABLE 2 Evidence for innate immune-mediated zinc toxicity against intracellular pathogens

Pathogen	Infection model	Phenotype	References
Zinc transporters			
M. tuberculosis	Human macrophage	↑ *ctpC*, *ctpG*, *ctpV* expression. Growth defect in Δ*ctpC* mutant.	Botella et al. (2011)
S. Typhimurium	Mouse infection model (intraperitoneal)	Δ*zntA*Δ*zitB* strain out-competed by wild-type strain in liver and spleen.	Frawley et al. (2018)
	Human macrophage	↑ *zntA* expression and ↑ susceptibility to killing in ΔSPI-1 mutant strain. SPI-1 prevents Zn^{2+} vesicle co-localisation with bacteria.	Kapetanovic et al. (2016)
E. coli	Human neutrophil	Δ*zntA* mutant strain more susceptible to killing.	Kapetanovic et al. (2016)
	Human macrophage	↑ *zntA* expression.	Kapetanovic et al. (2016)
		↓ Survival Δ*zntA* mutant strain.	Botella et al. (2011)
		↑ *zntA* expression. ↓ survival Δ*zntA* mutant strain. Intramacrophage *E. coli* reported Zn^{2+} stress (*zntA*-driven fluorescence).	Stocks et al. (2019)
	Murine macrophage	Intramacrophage *E. coli* reported Zn^{2+} stress (*zntA*-driven fluorescence).	Stocks et al. (2019)
	Mouse infection model (intraperitoneal)	↓ Dissemination of Δ*zntA* mutant strain in liver and spleen.	Stocks et al. (2019)
S. pneumoniae	Human neutrophil	↑ *czcD* and ↓ *phtE* (Zn^{2+} importer) expression. Zn^{2+} supplementation ↓ wild-type and Δ*czcD* mutant survival.	Eijkelkamp et al. (2019)
	Mouse infection model (intranasal)	Zn^{2+} co-localised with bacteria in lung cavity. ↑ *czcD* and ↓ *phtE* expression. ↓ Δ*czcD* mutant abundance in lungs.	Eijkelkamp et al. (2019)
	THP-1	↑ *czcD* expression. Zn^{2+} supplementation ↓ wild-type and Δ*czcD* mutant survival.	Eijkelkamp et al. (2019)
S. pyogenes	Human neutrophil	Δ*czcD* mutant more susceptible to killing.	Ong et al. (2014); Ong et al. (2018)
	Mouse infection model (invasive subcutaneous)	Δ*czcD* mutants ↓ mortality of mice. Δ*czcD* mutants had ↓ survival relative to wild-type during competitive infection.	Ong et al. (2014)
	Mouse infection model (invasive subcutaneous)	Δ*czcD* mutants ↓ initial lesion size, dissemination in blood and mortality of mice.	Ong et al. (2018)
C. glabrata	Murine macrophage	Survival defect in Δ*ZRC1* mutants.	Riedelberger et al. (2020)
Pseudomonas aeruginosa	THP-1	↑ *cadA* and *czcC* expression during infection.	Ducret, Gonzalez, Leoni, Valentini, and Perron (2020)
Regulators of zinc exporters			
E. coli	Human macrophage	↓ Survival Δ*zntR* mutant strain.	Stocks et al. (2019)
S. pneumoniae	THP-1	↓ Survival of Δ*sczA* mutant.	Martin et al. (2017)
S. pyogenes	Human neutrophil	Δ*gczA* mutant more susceptible to killing.	Ong et al. (2014)
	Mouse infection model (subcutaneous)	↓ Virulence and ↓ survival of Δ*gczA* mutants relative to wild-type strains.	Ong et al. (2014)

cells that had been differentiated into macrophage-like cells (Kapetanovic et al., 2016). As TLRs initiate late-stage antimicrobial responses (Stocks, Schembri, Sweet, & Kapetanovic, 2018), zinc toxicity may be engaged by innate immune cells to defend against persistent intracellular pathogens. It is therefore likely that these receptors, and potentially other receptors that sense infections and/or danger, regulate the expression and/or functions of SLC39A, SLC30A and/or MT family members to initiate and/or sustain the zinc toxicity response (Figure 2).

Although it is currently unknown if specific importers are involved in zinc-mediated antimicrobial responses, roles for some SLC39A transporters appear likely. For example, SLC39A8 is a manganese and zinc importer (Begum et al., 2002; Lin et al., 2017) that is induced by LPS in monocytes (Pyle et al., 2017) and macrophages (Liu

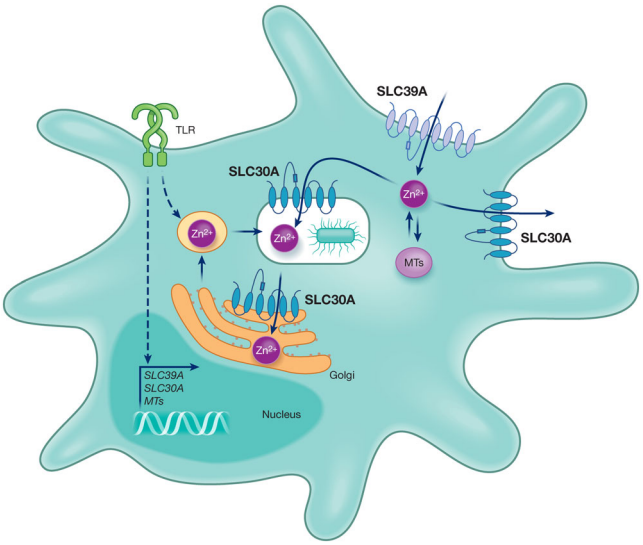

FIGURE 2 Innate immune cells mobilise zinc during infection. Innate immune cells traffic zinc towards pathogen-containing intracellular compartments, likely through the coordinated actions of zinc importers, zinc exporters and metallothioneins (MTs). SLC39A family members import zinc from extracellular environments into the cytoplasm, while members of the SLC30A family export zinc from the cytoplasm for its delivery to pathogens and/or to maintain zinc homeostasis. MTs regulate cytosolic zinc homeostasis and may control the delivery of zinc to zinc transporters in response to infection. Innate immune signalling systems, including Toll-like receptors, induce the expression of various zinc transporters and MTs, as well as zinc vesicle accumulation in immune cells

et al., 2013), with defined roles in regulating inflammatory responses (Hall, Smith, Katafiasz, Bailey, & Knoell, 2019; Liu et al., 2013; Pyle et al., 2017). *SLC39A14*, which is closely related to *SLC39A8* (Girijashanker et al., 2008), is also LPS-inducible and regulates cytokine production in human macrophages (Sayadi, Nguyen, Bard, & Bard-Chapeau, 2013). As SLC39A8 and SLC39A14 are both induced by LPS in macrophages, these transporters may be involved in not only inflammatory responses, but also LPS-induced zinc mobilisation for metal ion poisoning. This possibility remains to be investigated.

With respect to SLC30A family members, SLC30A1 expression is induced by LPS in primary human macrophages, with its ectopic expression in monocytic THP-1 cells being sufficient to drive the formation of zinc vesicles and to subject intracellular *E. coli* to zinc stress (Stocks et al., 2020). Moreover, this study demonstrated that SLC30A1 localised to compartments containing zinc-stressed *E. coli*. However, silencing of SLC30A1 in primary human macrophages did not affect bacterial clearance, suggesting that other zinc exporters may also contribute to this response. One candidate that may play a role in the absence of SLC30A1 is SLC30A4. Interleukin (IL)-4 treatment upregulated *Slc30a4* in murine macrophages, resulting in an increase in labile zinc within the phagolysosome (Subramanian Vignesh et al., 2016). *Slc30a4*, along with *Slc30a7*, was also implicated in granulocyte macrophage colony stimulating factor-mediated zinc sequestration in the Golgi in murine macrophages (Subramanian Vignesh, Landero Figueroa, Porollo, Caruso, & Deepe, 2013). This sequestration was associated with a zinc starvation response against an intramacrophage fungal pathogen, but it is possible that these transporters may additionally be involved in the delivery of zinc toxicity to different pathogens and/or in different microenvironments. Consistent with a role for one or more SLC30A family members in the zinc toxicity response in human and mouse macrophages, zinc was rapidly delivered towards *M. smegmatis*-containing vacuoles via SLC30A homologues in *D. discoideum* (Barisch et al., 2018). The predicted mechanism of action of SLC30A family zinc transporters has been modelled using the *E. coli* CDF proteins ZitB (Chao & Fu, 2004) and YiiP (Lu & Fu, 2007), which export zinc via a H^+ antiport mechanism (Chao & Fu, 2004; Grass et al., 2005). Indeed, SLC30A1 homodimers utilise a H^+ electrochemical gradient to drive Zn^{2+} efflux in exchange for H^+ in a pH-driven, sodium-independent and calcium-sensitive manner (Shusterman et al., 2014). Thus, the acidic environment of phagolysosomes might enable the delivery of zinc to pathogens by one or more SLC30A transporters.

Another important factor influencing zinc transport in mammalian cells is control by the MT family. MTs are a family of monomeric metal-binding proteins that regulate cytosolic zinc homeostasis under both steady and non-steady state conditions (Colvin, Holmes, Fontaine, & Maret, 2010). The MTs are encoded by 10–12 genes (chromosome 16) and 4 genes (chromosome 8) in humans and mice, respectively (Cox & Palmiter, 1983; Vašák & Meloni, 2011). Some studies have demonstrated that MTs are regulated in innate immune cells during infection (Botella et al., 2011; Subramanian Vignesh et al., 2013). Moreover, silencing of the transcription factor metal-regulatory factor-1, which controls cellular responses to metals, disrupted MT expression, prevented intracellular zinc accumulation and impaired bacterial clearance in human macrophages chronically stimulated with the microbial product muramyl dipeptide (Lahiri & Abraham, 2014), thus implicating MTs in antimicrobial responses. More recently, *Mt-1* and *Mt-2* were shown to be required for zinc intoxication against phagocytosed *C. glabrata* in murine macrophages (Riedelberger et al., 2020). This study demonstrated that Mts did not co-localise with the pathogen during infection, suggesting that Mts are involved in zinc mobilisation rather than direct delivery of zinc to intracellular pathogens. It is likely that MTs cooperate with zinc transporters, providing a sophisticated network of effector proteins and zinc chaperones to control the distribution of intracellular metal ions for effective inflammatory and antimicrobial responses.

5 | ZINC AS AN ANTIMICROBIAL EFFECTOR MOLECULE

5.1 | Direct antimicrobial impacts of zinc

High concentrations of zinc are likely toxic to pathogens through multiple mechanisms. Zinc is predicted to preferentially bind to proteins in place of other first-row transition metal ions, resulting in mismetallation of essential proteins (Braymer & Giedroc, 2014; Stafford et al., 2013; Xu &

Imlay, 2012). For example, zinc irreversibly binds to manganese-acquisition proteins in *S. pneumoniae* (Couñago et al., 2014; McDevitt et al., 2011), *Bacillus anthracis* (Vigonsky et al., 2015) and *Staphylococcus pseudintermedius* (Abate et al., 2014). Thus, zinc excess can induce manganese deficiency in bacteria, leading to increased sensitivity to oxidative stress (Eijkelkamp et al., 2014; McDevitt et al., 2011). This concept has been recently confirmed in a study whereby ionophores that induced zinc accumulation in *Streptococcus uberis* disabled oxidative stress protection through inhibition of manganese-dependent superoxide dismutase (Harbison-Price et al., 2020). Zinc excess can also dysregulate homeostasis of other trace metal ions. Excessive zinc can induce copper depletion, which may be driven by dysregulation of copper transporters and detoxification systems (Hassan et al., 2017; Xu et al., 2019). In addition, zinc excess increases the concentration of iron in *E. coli* by transiently inducing expression of iron-uptake genes and downregulating iron storage genes (Xu et al., 2019). Zinc may also have direct impacts on key cellular processes required for microbial survival. For example, excessive intracellular zinc can disrupt iron-sulfur (4Fe-S) complex biogenesis in *E. coli*, thereby arresting key metabolic and growth pathways (Li et al., 2019; Xu & Imlay, 2012). In addition, excess zinc impaired capsule synthesis and perturbed glycolysis through inhibition of glycolytic enzymes in *S. pyogenes* (Ong, Walker, & McEwan, 2015). Thus, high concentrations of zinc can dysregulate many facets of homeostasis in microorganisms to exert its antimicrobial effects.

5.2 | Cooperation between zinc and other innate immune antimicrobial pathways

In addition to direct toxicity, zinc may also promote bacterial clearance through indirect mechanisms, particularly through enhancement of, or synergism with, other innate immune antimicrobial pathways. For example, basal and inducible autophagy is dependent on zinc in various cell types (Hung, Huang, & Pan, 2013; Hwang et al., 2010; Lee & Koh, 2010; Liuzzi & Yoo, 2013). In human macrophages, signalling via the pattern recognition receptor NOD2 increased intracellular zinc levels to promote autophagy and intracellular clearance of *S.* Typhimurium, *S. aureus* and adherent invasive *E. coli* (Lahiri & Abraham, 2014). Therefore, regulated zinc trafficking in innate immune cells may contribute to autophagy-mediated control of intracellular pathogens.

Innate immune cells use reactive oxygen species (ROS), generated either through the NADPH oxidase system or through mitochondrial activity, as an antimicrobial weapon. Some evidence suggests that zinc may act cooperatively with ROS to exert antimicrobial effects. The growth of zinc-sensitive *E. coli* mutants was synergistically inhibited when bacteria were cultured in the presence of both zinc and the superoxide anion generator paraquat (Stocks et al., 2019). In contrast, zinc chelation by MTs was required for robust ROS generation upon challenge with *C. glabrata* (Riedelberger et al., 2020), *S.* Typhimurium (Wu et al., 2017) and *Histoplasma capsulatum* (Subramanian Vignesh et al., 2013) in murine or human macrophages. Therefore, the precise interplay between zinc and ROS during antimicrobial responses is likely to be context dependent.

In addition to ROS, innate immune cells also generate nitric oxide (NO) for free radical attack of intracellular pathogens. There is some evidence that zinc may influence NO production and/or that NO itself can initiate zinc toxicity. Zinc chelation abrogated TLR4- and TLR3-inducible nitric oxide synthase (iNOS/Nos2) expression that is required for inducible NO production in murine macrophages (Brieger et al., 2013). In bacteria, nitrosative stress may also interfere with zinc homeostasis. In *Salmonella*, NO triggers zinc release from multiple metalloproteins, including those involved in nitrosative stress sensing (Henard et al., 2014), cell metabolism, protein synthesis (Frawley et al., 2018) and DNA binding (Schapiro, Libby, & Fang, 2003). In addition, pharmacological inhibition of Nos2 in an intraperitoneal murine infection model restored bacterial loads of a *S.* Typhimurium Δ*zntA*Δ*zitB* mutant to those of wild-type levels in murine spleen and liver, suggesting that NO may induce zinc toxicity in *Salmonella* (Frawley et al., 2018). This suggests that, under nitrosative stress, effective zinc efflux is required to maintain homeostasis and survival of *Salmonella* during murine infection (Frawley et al., 2018). Of note, however, TLR signalling induces the zinc toxicity response (Kapetanovic et al., 2016), but not *NOS2* expression (Gross et al., 2014), in human macrophages. Thus, it seems unlikely that NO production contributes to the susceptibility of zinc-sensitive bacterial mutants in these cells.

Zinc toxicity may also be influenced by other metal ions during antimicrobial responses. Copper is utilised by the innate immune system as an antimicrobial weapon against various pathogens (Sheldon & Skaar, 2019). Non-toxic copper concentrations enhanced the cytotoxic effects of zinc on both *S.* Typhimurium and *E. coli* Δ*zntA* mutants, with these effects being rescued by *zntA* complementation (Kapetanovic et al., 2016). Similarly, co-treatment of *A. baumannii* with zinc and copper resulted in altered membrane composition and increased susceptibility to ROS (Hassan et al., 2017). Together, these studies suggest that copper and zinc may act cooperatively to exert an antimicrobial effect. Indeed, the concentrations of both zinc and copper were increased in Mycobacterium-containing phagosomes of murine macrophages under certain conditions (Wagner et al., 2005). In keeping with this concept, in LPS-stimulated human macrophages, vesicular zinc and copper partially co-localised (Kapetanovic et al., 2016). This raises the possibility of spatial and/or time-dependent cooperativity of these two metal ions, supporting the 'brass dagger' model of host defence (German, Doyscher, & Rensing, 2013). However, co-supplementation of THP-1 cells with zinc and copper did not affect clearance of *A. baumannii* (Hassan et al., 2017), suggesting that other exogenous factors and/or cell types may be important for combinatorial antimicrobial effects of these two metal ions.

6 | FINAL REMARKS AND FUTURE DIRECTIONS

In conclusion, zinc toxicity is emerging as an antimicrobial mechanism employed by innate immune cells to combat many

microorganisms, particularly bacterial and fungal pathogens. Significant advances have been made in our understanding of immune cell types that utilise zinc toxicity as an antimicrobial response, the pathogens which are subjected to zinc stress during infection, and the innate stimuli that drive this antimicrobial response. However, as highlighted throughout this review, key questions still remain. Specifically, the molecular mechanisms by which zinc is mobilised towards engulfed pathogens, the precise evasion mechanisms employed by pathogens, and the contributions of other antimicrobial pathways to zinc toxicity require further elucidation. Intriguing recent evidence also points to intertwining effects of the seemingly oppositional antimicrobial pathways of zinc sequestration and zinc toxicity. In a murine infection model, *A. baumannii* was subjected to zinc starvation in the respiratory tract, but experienced zinc intoxication in the spleen (Alquethamy et al., 2019). Similarly, another study showed that while neutrophils employ zinc sequestration against extracellular *S. pyogenes*, phagocytosed bacteria were subjected to zinc toxicity (Ong et al., 2018). One possibility is that the capacity of concentrated zinc to be cytotoxic to intracellular pathogens is enhanced by initial zinc starvation encountered in extracellular environments and/or in different tissues. In this way, innate immune-mediated zinc starvation may predispose microorganisms to subsequent zinc poisoning.

As increasing rates of antimicrobial resistance threaten global health worldwide, there is an urgent need for the development of novel therapies for infectious diseases. One approach under investigation is harnessing of innate immune antimicrobial responses through 'host-directed therapies'. Enhancing our understanding of the molecular mechanisms by which zinc is delivered to engulfed pathogens for metal ion poisoning, as well as the subversion mechanisms employed by pathogens to resist and/or evade zinc toxicity, could ultimately guide the development of new anti-infectives. Of note, zinc ionophores induce intracellular zinc accumulation, with one such ionophore recently passing second phase human clinical trials for the treatment of Alzheimer's disease and Huntington's disease (Huntington Study Group Reach2HD Investigators, 2015; Lannfelt et al., 2008; Villemagne et al., 2017). These zinc ionophores also have antibacterial activity against a variety of Gram-positive bacterial pathogens and can act synergistically with antibiotics (Bohlmann et al., 2018; Harbison-Price et al., 2020). Ionophores have also been effective in reversing antibiotic resistance in Gram-negative bacteria (Jen et al., 2020). Therefore, it may also be possible to mimic innate immune-mediated zinc toxicity through application of zinc ionophores. Therapeutics that target zinc homeostasis, either through host or pathogen manipulation, may thus provide new opportunities for combating antibiotic-resistant bacterial infections. Moreover, knowledge of how zinc toxicity responses are engaged by the host and subverted by pathogens will likely deliver fascinating new insights into the host-pathogen dynamic.

ACKNOWLEDGEMENTS

This work was supported by a National Health and Medical Research Council of Australia (NHMRC) project grant (APP1144967). M.J.S. and M.A.S. are supported by NHMRC Senior Research Fellowships (APP1107914 and APP1106930, respectively).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Jessica B. von Pein, **Matthew J. Sweet**, **Claudia J. Stocks**, **Ronan Kapetanovic**, **Mark A. Schembri:** Wrote the manuscript, after **Jessica B. von Pein** generated the initial draft. **Jessica B. von Pein**: Created the figures. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed in this review. This review was composed entirely from previously published data.

ORCID

Jessica B. von Pein https://orcid.org/0000-0002-7233-7177
Claudia J. Stocks https://orcid.org/0000-0001-6120-2851
Mark A. Schembri https://orcid.org/0000-0003-4863-9260
Ronan Kapetanovic https://orcid.org/0000-0002-2908-1014
Matthew J. Sweet https://orcid.org/0000-0002-0406-8139

REFERENCES

Abate, F., Malito, E., Cozzi, R., Lo Surdo, P., Maione, D., & Bottomley, M. J. (2014). Apo, Zn^{2+}-bound and Mn^{2+}-bound structures reveal ligand-binding properties of SitA from the pathogen *Staphylococcus pseudintermedius*. *Bioscience Reports*, *34*, 743–758.

Ahmadipour, S., Mohsenzadeh, A., Alimadadi, H., Salehnia, M., & Fallahi, A. (2019). Treating viral diarrhea in children by probiotic and zinc supplements. *Pediatric Gastroenterology, Hepatology & Nutrition*, *22*, 162–170.

Al Tarique, A., Sheikh, A., Saha, A., Alam, M. M., Chowdhury, M. I., Nahar, S., … Larson, C. P. (2010). Zinc influences innate immune responses in children with enterotoxigenic *Escherichia coli*-induced diarrhea. *The Journal of Nutrition*, *140*, 1049–1056.

Alquethamy, S., Adams, F. G., Naidu, V., Khorvash, M., Pederick, V. G., Zang, M., … Eijkelkamp, B. A. (2019). The role of zinc efflux during *Acinetobacter baumannii* infection. *ACS Infectious Diseases*, *6*, 150–158.

Andreini, C., & Bertini, I. (2012). A bioinformatics view of zinc enzymes. *Journal of Inorganic Biochemistry*, *111*, 150–156.

Bao, S., Liu, M.-J., Lee, B., Besecker, B., Lai, J.-P., Guttridge, D. C., & Knoell, D. L. (2010). Zinc modulates the innate immune response *in vivo* to polymicrobial sepsis through regulation of NF-κB. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *298*, L744–L754.

Barisch, C., Kalinina, V., Lefrançois, L. H., Appiah, J., López-Jiménez, A. T., & Soldati, T. (2018). Localization of all four ZnT zinc transporters in *Dictyostelium* and impact of ZntA and ZntB knockout on bacteria killing. *Journal of Cell Science*, *131*, 222000.

Beard, S. J., Hashim, R., Membrillo-Hernández, J., Hughes, M. N., & Poole, R. K. (1997). Zinc(II) tolerance in *Escherichia coli* K-12: Evidence that the zntA gene (*o732*) encodes a cation transport ATPase. *Molecular Microbiology*, *25*, 883–891.

Begum, N. A., Kobayashi, M., Moriwaki, Y., Matsumoto, M., Toyoshima, K., & Seya, T. (2002). *Mycobacterium bovis* BCG cell wall and lipopolysaccharide induce a novel gene, *BIGM103*, encoding a 7-TM protein: Identification of a new protein family having Zn-

transporter and Zn-metalloprotease signatures. *Genomics*, *80*, 630–645.

Bennett, D. R., Baird, C. J., Chan, K.-M., Crookes, P. F., Bremner, C. G., Gottlieb, M. M., & Naritoku, W. Y. (1997). Zinc toxicity following massive coin ingestion. *The American Journal of Forensic Medicine and Pathology*, *18*, 148–153.

Besecker, B. Y., Exline, M. C., Hollyfield, J., Phillips, G., Disilvestro, R. A., Wewers, M. D., & Knoell, D. L. (2011). A comparison of zinc metabolism, inflammation, and disease severity in critically ill infected and noninfected adults early after intensive care unit admission. *The American Journal of Clinical Nutrition*, *93*, 1356–1364.

Bhutta, Z., Black, R. E., Brown, K., Gardner, J. M., Gore, S., Hidayat, A., … Penny, M. (1999). Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: Pooled analysis of randomized controlled trials. *The Journal of Pediatrics*, *135*, 689–697.

Bhutta, Z. A., Bird, S. M., Black, R. E., Brown, K. H., Gardner, J. M., Hidayat, A., … Shankar, A. (2000). Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: Pooled analysis of randomized controlled trials. *The American Journal of Clinical Nutrition*, *72*, 1516–1522.

Blencowe, D. K., & Morby, A. P. (2003). Zn(II) metabolism in prokaryotes. *FEMS Microbiology Reviews*, *27*, 291–311.

Bohlmann, L., De Oliveira, D. M. P., El-Deeb, I. M., Brazel, E. B., Harbison-Price, N., Ong, C. Y., … Walker, M. J. (2018). Chemical synergy between ionophore PBT2 and zinc reverses antibiotic resistance. *mBio*, *9*, e02391–e02318.

Bolick, D. T., Kolling, G. L., Moore, J. H., de Oliveira, L. A., Tung, K., Philipson, C., … Guerrant, R. L. (2014). Zinc deficiency alters host response and pathogen virulence in a mouse model of enteroaggregative *Escherichia coli*-induced diarrhea. *Gut Microbes*, *5*, 618–627.

Botella, H., Peyron, P., Levillain, F., Poincloux, R., Poquet, Y., Brandli, I., … Neyrolles, O. (2011). Mycobacterial p(1)-type ATPases mediate resistance to zinc poisoning in human macrophages. *Cell Host & Microbe*, *10*, 248–259.

Braymer, J. J., & Giedroc, D. P. (2014). Recent developments in copper and zinc homeostasis in bacterial pathogens. *Current Opinion in Chemical Biology*, *19*, 59–66.

Brieger, A., Rink, L., & Haase, H. (2013). Differential regulation of TLR-dependent MyD88 and TRIF signaling pathways by free zinc ions. *The Journal of Immunology*, *191*, 1808–1817.

Brocklehurst, K. R., Hobman, J. L., Lawley, B., Blank, L., Marshall, S. J., Brown, N. L., & Morby, A. P. (1999). ZntR is a Zn(II)-responsive MerR-like transcriptional regulator of *zntA* in *Escherichia coli*. *Molecular Microbiology*, *31*, 893–902.

Brooks, W. A., Santosham, M., Naheed, A., Goswami, D., Wahed, M. A., Diener-West, M., … Black, R. E. (2005). Effect of weekly zinc supplements on incidence of pneumonia and diarrhoea in children younger than 2 years in an urban, low-income population in Bangladesh: Randomised controlled trial. *The Lancet*, *366*, 999–1004.

Castillo-Duran, C., Vial, P., & Uauy, R. (1988). Trace mineral balance during acute diarrhea in infants. *The Journal of Pediatrics*, *113*, 452–457.

Chao, Y., & Fu, D. (2004). Kinetic Study of the Antiport Mechanism of an *Escherichia coli* Zinc Transporter, ZitB. *Journal of Biological Chemistry*, *279*, 12043–12050.

Colvin, R. A., Holmes, W. R., Fontaine, C. P., & Maret, W. (2010). Cytosolic zinc buffering and muffling: Their role in intracellular zinc homeostasis. *Metallomics*, *2*, 306–317.

Couñago, R. M., Ween, M. P., Begg, S. L., Bajaj, M., Zuegg, J., O'Mara, M. L., … McDevitt, C. A. (2014). Imperfect coordination chemistry facilitates metal ion release in the Psa permease. *Nature Chemical Biology*, *10*, 35–41.

Cox, D. R., & Palmiter, R. D. (1983). The metallothionein-I gene maps to mouse chromosome 8: Implications for human Menkes' disease. *Human Genetics*, *64*, 61–64.

Ducret, V., Gonzalez, M. R., Leoni, S., Valentini, M., & Perron, K. (2020). The CzcCBA efflux system requires the CadA P-Type ATPase for timely expression upon zinc excess in *Pseudomonas aeruginosa*. *Frontiers in Microbiology*, *11*, 911. https://doi.org/10.3389/fmicb.2020.00911

Eide, D. J. (2006). Zinc transporters and the cellular trafficking of zinc. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1763*, 711–722.

Eijkelkamp, B. A., Morey, J. R., Neville, S. L., Tan, A., Pederick, V. G., Cole, N., … McDevitt, C. A. (2019). Dietary zinc and the control of *Streptococcus pneumoniae* infection. *PLoS Pathogens*, *15*, e1007957.

Eijkelkamp, B. A., Morey, J. R., Ween, M. P., Ong, C.-L. Y., McEwan, A. G., Paton, J. C., & McDevitt, C. A. (2014). Extracellular zinc competitively inhibits manganese uptake and compromises oxidative stress management in *Streptococcus pneumoniae*. *PloS one*, *9*, e89427.

Fraker, P. J., Caruso, R., & Kierszenbaum, F. (1982). Alteration of the immune and nutritional status of mice by synergy between zinc deficiency and infection with *Trypanosoma cruzi*. *The Journal of Nutrition*, *112*, 1224–1229.

Frawley, E. R., Karlinsey, J. E., Singhal, A., Libby, S. J., Doulias, P.-T., Ischiropoulos, H., & Fang, F. C. (2018). Nitric oxide disrupts zinc homeostasis in *Salmonella enterica*; serovar Typhimurium. *mBio*, *9*, e01040–e01018.

Gammoh, N. Z., & Rink, L. (2017). Zinc in infection and inflammation. *Nutrients*, *9*, 624.

German, N., Doyscher, D., & Rensing, C. (2013). Bacterial killing in macrophages and amoeba: Do they all use a brass dagger? *Future Microbiology*, *8*, 1257–1264.

Girijashanker, K., He, L., Soleimani, M., Reed, J. M., Li, H., Liu, Z., … Nebert, D. W. (2008). *Slc39a14* gene encodes ZIP14, a metal/bicarbonate symporter: Similarities to the ZIP8 transporter. *Molecular Pharmacology*, *73*, 1413–1423.

Grass, G., Fan, B., Rosen, B. P., Franke, S., Nies, D. H., & Rensing, C. (2001). ZitB (YbgR), a member of the cation diffusion facilitator family, is an additional zinc transporter in *Escherichia coli*. *Journal of Bacteriology*, *183*, 4664–4667.

Grass, G., Otto, M., Fricke, B., Haney, C. J., Rensing, C., Nies, D. H., & Munkelt, D. (2005). FieF (YiiP) from *Escherichia coli* mediates decreased cellular accumulation of iron and relieves iron stress. *Archives of Microbiology*, *183*, 9–18.

Gross, T. J., Kremens, K., Powers, L. S., Brink, B., Knutson, T., Domann, F. E., … Monick, M. M. (2014). Epigenetic silencing of the human *NOS2* gene: Rethinking the role of nitric oxide in human macrophage inflammatory responses. *The Journal of Immunology*, *192*, 2326–2338.

Guffanti, A. A., Wei, Y., Rood, S. V., & Krulwich, T. A. (2002). An antiport mechanism for a member of the cation diffusion facilitator family: Divalent cations efflux in exchange for K^+ and H^+. *Molecular Microbiology*, *45*, 145–153.

Haase, H. (2013). An element of life: Competition for zinc in host-pathogen interaction. *Immunity*, *39*, 623–624.

Haase, H., & Rink, L. (2007). Signal transduction in monocytes: The role of zinc ions. *BioMetals*, *20*, 579–585.

Hall, S. C., Smith, D. R., Katafiasz, D. M., Bailey, K. L., & Knoell, D. L. (2019). Novel role of zinc homeostasis in IL-23 regulation and host defense following bacterial infection. *The Journal of Immunology*, *202*, 62–66.

Hara, T., Takeda, T.-A., Takagishi, T., Fukue, K., Kambe, T., & Fukada, T. (2017). Physiological roles of zinc transporters: Molecular and genetic importance in zinc homeostasis. *The Journal of Physiological Sciences*, *67*, 283–301.

Harbison-Price, N., Ferguson, S. A., Heikal, A., Taiaroa, G., Hards, K., Nakatani, Y., … Cook, G. M. (2020). Multiple bactericidal mechanisms of the zinc ionophore PBT2. *mSphere*, *5*, e00157–e00120.

Hassan, K. A., Pederick, V. G., Elbourne, L. D. H., Paulsen, I. T., Paton, J. C., McDevitt, C. A., & Eijkelkamp, B. A. (2017). Zinc stress induces copper depletion in *Acinetobacter baumannii*. *BMC Microbiology*, *17*, 1–15.

Henard, C. A., Tapscott, T., Crawford, M. A., Husain, M., Doulias, P.-T., Porwollik, S., … Vázquez-Torres, A. (2014). The 4-cysteine zinc-finger motif of the RNA polymerase regulator DksA serves as a thiol switch for sensing oxidative and nitrosative stress. *Molecular Microbiology*, *91*, 790–804.

Hoeger, J., Simon, T.-P., Beeker, T., Marx, G., Haase, H., & Schuerholz, T. (2017). Persistent low serum zinc is associated with recurrent sepsis in critically ill patients - A pilot study. *PloS one*, *12*, e0176069.

Hood, M. I., & Skaar, E. P. (2012). Nutritional immunity: Transition metals at the pathogen-host interface. *Nature reviews. Microbiology*, *10*, 525–537.

Huang, L., & Tepaamorndech, S. (2013). The SLC30 family of zinc transporters – A review of current understanding of their biological and pathophysiological roles. *Molecular Aspects of Medicine*, *34*, 548–560.

Hung, H.-H., Huang, W.-P., & Pan, C.-Y. (2013). Dopamine- and zinc-induced autophagosome formation facilitates PC12 cell survival. *Cell Biology and Toxicology*, *29*, 415–429.

Huntington Study Group Reach2HD Investigators. (2015). Safety, tolerability, and efficacy of PBT2 in Huntington's disease: A phase 2, randomised, double-blind, placebo-controlled trial. *The Lancet Neurology*, *14*, 39–47.

Hwang, J. J., Kim, H. N., Kim, J., Cho, D.-H., Kim, M. J., Kim, Y.-S., … Koh, J.-Y. (2010). Zinc(II) ion mediates tamoxifen-induced autophagy and cell death in MCF-7 breast cancer cell line. *BioMetals*, *23*, 997–1013.

Jen, F. E. C., Everest-Dass, A. V., El-Deeb, I. M., Singh, S., Haselhorst, T., Walker, M. J., … Jennings, M. P. (2020). *Neisseria gonorrhoeae* becomes susceptible to Polymyxin B and Colistin in the presence of PBT2. *ACS Infectious Diseases*, *6*, 50–55.

Jordan, M. R., Wang, J., Capdevila, D. A., & Giedroc, D. P. (2020). Multi-metal nutrient restriction and crosstalk in metallostasis systems in microbial pathogens. *Current Opinion in Microbiology*, *55*, 17–25.

Kapetanovic, R., Bokil, N. J., Achard, M. E. S., Ong, C.-L. Y., Peters, K. M., Stocks, C. J., … Sweet, M. J. (2016). *Salmonella* employs multiple mechanisms to subvert the TLR-inducible zinc-mediated antimicrobial response of human macrophages. *The FASEB Journal*, *30*, 1901–1912.

Kloosterman, T. G., van der Kooi-Pol, M. M., Bijlsma, J. J. E., & Kuipers, O. P. (2007). The novel transcriptional regulator SczA mediates protection against Zn^{2+} stress by activation of the Zn^{2+}-resistance gene *czcD* in Streptococcus pneumoniae. *Molecular Microbiology*, *65*, 1049–1063.

Knoell, D. L., Julian, M. W., Bao, S., Besecker, B., Macre, J. E., Leikauf, G. D., … Crouser, E. D. (2009). Zinc deficiency increases organ damage and mortality in a murine model of polymicrobial sepsis. *Critical Care Medicine*, *37*, 1380–1388.

Lahiri, A., & Abraham, C. (2014). Activation of pattern recognition receptors up-regulates metallothioneins, thereby increasing intracellular accumulation of zinc, autophagy, and bacterial clearance by macrophages. *Gastroenterology*, *147*, 835–846.

Lannfelt, L., Blennow, K., Zetterberg, H., Batsman, S., Ames, D., Harrison, J., … Ritchie, C. W. (2008). Safety, efficacy, and biomarker findings of PBT2 in targeting Aβ as a modifying therapy for Alzheimer's disease: A phase IIa, double-blind, randomised, placebo-controlled trial. *The Lancet Neurology*, *7*, 779–786.

Lee, C. M., Humphrey, P. A., & Aboko-Cole, G. F. (1983). Interaction of nutrition and infection: Effect of zinc deficiency on resistance to *Trypanosoma musculi*. *The International Journal of Biochemistry*, *15*, 841–847.

Lee, S.-J., & Koh, J.-Y. (2010). Roles of zinc and metallothionein-3 in oxidative stress-induced lysosomal dysfunction, cell death, and autophagy in neurons and astrocytes. *Molecular Brain*, *3*, 1–9.

Li, J., Ren, X., Fan, B., Huang, Z., Wang, W., Zhou, H., … Tan, G. (2019). Zinc toxicity and iron-sulfur cluster biogenesis in *Escherichia coli*. *Applied and Environmental Microbiology*, *85*, e01967–e01918.

Lin, W., Vann, D. R., Doulias, P.-T., Wang, T., Landesberg, G., Li, X., … Rader, D. J. (2017). Hepatic metal ion transporter ZIP8 regulates manganese homeostasis and manganese-dependent enzyme activity. *The Journal of Clinical Investigation*, *127*, 2407–2417.

Liu, M.-J., Bao, S., Gálvez-Peralta, M., Pyle, C. J., Rudawsky, A. C., Pavlovicz, R. E., … Knoell, D. L. (2013). ZIP8 regulates host defense through zinc-mediated inhibition of NF-κB. *Cell Reports*, *3*, 386–400.

Liu, M.-J., Bao, S., Napolitano, J. R., Burris, D. L., Yu, L., Tridandapani, S., & Knoell, D. L. (2014). Zinc regulates the acute phase response and serum amyloid A production in response to sepsis through JAK-STAT3 signaling. *PloS one*, *9*, e94934.

Liuzzi, J. P., & Yoo, C. (2013). Role of zinc in the regulation of autophagy during ethanol exposure in human hepatoma cells. *Biological Trace Element Research*, *156*, 350–356.

Lonergan, Z. R., & Skaar, E. P. (2019). Nutrient zinc at the host–pathogen interface. *Trends in Biochemical Sciences*, *44*, 1041–1056.

Lu, M., & Fu, D. (2007). Structure of the zinc transporter YiiP. *Science*, *317*, 1746–1748.

Martin, J. E., Edmonds, K. A., Bruce, K. E., Campanello, G. C., Eijkelkamp, B. A., Brazel, E. B., … Giedroc, D. P. (2017). The zinc efflux activator SczA protects *Streptococcus pneumoniae* serotype 2 D39 from intracellular zinc toxicity. *Molecular Microbiology*, *104*, 636–651.

Matsumura, H., Nirei, K., Nakamura, H., Arakawa, Y., Higuchi, T., Hayashi, J., … Moriyama, M. (2012). Zinc supplementation therapy improves the outcome of patients with chronic hepatitis C. *Journal of Clinical Biochemistry and Nutrition*, *51*, 178–184.

Maywald, M., & Rink, L. (2015). Zinc homeostasis and immunosenescence. *Journal of Trace Elements in Medicine and Biology*, *29*, 24–30.

McDevitt, C. A., Ogunniyi, A. D., Valkov, E., Lawrence, M. C., Kobe, B., McEwan, A. G., & Paton, J. C. (2011). A molecular mechanism for bacterial susceptibility to zinc. *PLoS Pathogens*, *7*, e1002357.

Ong, C.-L. Y., Berking, O., Walker, M. J., & McEwan, A. G. (2018). New insights into the role of zinc acquisition and zinc tolerance in Group A Streptococcal infection. *Infection and Immunity*, *86*, e00048–18. https://doi.org/10.1128/IAI.00048-18.

Ong, C.-L. Y., Gillen, C. M., Walker, M. J., Barnett, T. C., & McEwan, A. G. (2014). An antimicrobial role for zinc in innate immune defense against Group A *Streptococcus*. *The Journal of Infectious Diseases*, *209*, 1500–1508.

Ong, C.-L. Y., Walker, M. J., & McEwan, A. G. (2015). Zinc disrupts central carbon metabolism and capsule biosynthesis in *Streptococcus pyogenes*. *Scientific Reports*, *5*, 10799.

Penny, M. E. (2013). Zinc supplementation in public health. *Annals of Nutrition and Metabolism*, *62*, 31–42.

Prasad, A. S., Beck, F. W. J., Bao, B., Fitzgerald, J. T., Snell, D. C., Steinberg, J. D., & Cardozo, L. J. (2007). Zinc supplementation decreases incidence of infections in the elderly: Effect of zinc on generation of cytokines and oxidative stress. *The American Journal of Clinical Nutrition*, *85*, 837–844.

Pyle, C. J., Akhter, S., Bao, S., Dodd, C. E., Schlesinger, L. S., & Knoell, D. L. (2017). Zinc modulates endotoxin-induced human macrophage inflammation through ZIP8 induction and C/EBPβ inhibition. *PloS one*, *12*, e0169531.

Rensing, C., Mitra, B., & Rosen, B. P. (1997). The *zntA* gene of *Escherichia coli* encodes a Zn(II)-translocating P-type ATPase. *Proceedings of the National Academy of Sciences of the United States of America*, *94*, 14326–14331.

Riedelberger, M., Penninger, P., Tscherner, M., Hadriga, B., Brunnhofer, C., Jenull, S., … Kuchler, K. (2020). Type I interferons ameliorate zinc intoxication of *Candida glabrata* by macrophages and promote fungal immune evasion. *iScience*, *23*, 101121.

Rink, L., Cakman, I., & Kirchner, H. (1998). Altered cytokine production in the elderly. *Mechanisms of Ageing and Development*, *102*, 199–209.

Salvin, S. B., & Rabin, B. S. (1984). Resistance and susceptibility to infection in inbred murine strains: IV. Effects of dietary zinc. *Cellular Immunology*, *87*, 546–552.

Sayadi, A., Nguyen, A.-T., Bard, F. A., & Bard-Chapeau, E. A. (2013). Zip14 expression induced by lipopolysaccharides in macrophages attenuates inflammatory response. *Inflammation Research*, *62*, 133–143.

Sazawal, S., Black, R. E., Jalla, S., Mazumdar, S., Sinha, A., & Bhan, M. K. (1998). Zinc supplementation reduces the incidence of acute lower respiratory infections in infants and preschool children: A double-blind, controlled trial. *Pediatrics*, *102*, 1–5.

Schapiro, J. M., Libby, S. J., & Fang, F. C. (2003). Inhibition of bacterial DNA replication by zinc mobilization during nitrosative stress. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 8496–8501.

Sheldon, J. R., & Skaar, E. P. (2019). Metals as phagocyte antimicrobial effectors. *Current Opinion in Immunology*, *60*, 1–9.

Shusterman, E., Beharier, O., Shiri, L., Zarivach, R., Etzion, Y., Campbell, C. R., … Moran, A. (2014). ZnT-1 extrudes zinc from mammalian cells functioning as a Zn^{2+}/H^{+} exchanger. *Metallomics*, *6*, 1656–1663.

Stafford, S. L., Bokil, N. J., Achard, M. E. S., Kapetanovic, R., Schembri, M. A., McEwan, A. G., & Sweet, M. J. (2013). Metal ions in macrophage antimicrobial pathways: Emerging roles for zinc and copper. *Bioscience Reports*, *33*, 541–554.

Stocks, C. J., Phan, M.-D., Achard, M. E. S., Nhu, N. T. K., Condon, N. D., Gawthorne, J. A., … Sweet, M. J. (2019). Uropathogenic *Escherichia coli* employs both evasion and resistance to subvert innate immune-mediated zinc toxicity for dissemination. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 6341–6350.

Stocks, C. J., Schembri, M. A., Sweet, M. J., & Kapetanovic, R. (2018). For when bacterial infections persist: Toll-like receptor-inducible direct antimicrobial pathways in macrophages. *Journal of Leukocyte Biology*, *103*, 35–51.

Stocks, C. J., von Pein, J. B., Curson, J. E. B., Rae, J., Phan, M.-D., Foo, D., … Sweet, M. J. (2020). Frontline Science: LPS-inducible SLC30A1 drives human macrophage-mediated zinc toxicity against intracellular *Escherichia coli*. *Journal of Leukocyte Biology*. https://doi.org/10.1002/JLB.1002HI0420-1160R

Subramanian Vignesh, K., Landero Figueroa, J. A., Porollo, A., Caruso, J. A., & Deepe, G. S., Jr. (2013). Granulocyte macrophage-colony stimulating factor induced Zn sequestration enhances macrophage superoxide and limits intracellular pathogen survival. *Immunity*, *39*, 697–710.

Subramanian Vignesh, K., Landero Figueroa, J. A., Porollo, A., Divanovic, S., Caruso, J. A., & Deepe, G. S., Jr. (2016). IL-4 induces metallothionein 3- and SLC30A4-dependent increase in intracellular Zn^{2+} that promotes pathogen persistence in macrophages. *Cell Reports*, *16*, 3232–3246.

Valavi, E., Hakimzadeh, M., Shamsizadeh, A., Aminzadeh, M., & Alghasi, A. (2011). The efficacy of zinc supplementation on outcome of children with severe pneumonia. A randomized double-blind placebo-controlled clinical trial. *The Indian Journal of Pediatrics*, *78*, 1079–1084.

Varadinova, T. L., Bontchev, P. R., Nachev, C. K., Shishkov, S. A., Strachilov, D., Paskalev, Z., … Panteva, M. (1993). Mode of action of Zn-complexes on herpes simplex virus type 1 infection in vitro. *Journal of Chemotherapy*, *5*, 3–9.

Vašák, M., & Meloni, G. (2011). Chemistry and biology of mammalian metallothioneins. *JBIC Journal of Biological Inorganic Chemistry*, *16*, 1067–1078.

Vigonsky, E., Fish, I., Livnat-Levanon, N., Ovcharenko, E., Ben-Tal, N., & Lewinson, O. (2015). Metal binding spectrum and model structure of the *Bacillus anthracis* virulence determinant MntA. *Metallomics*, *7*, 1407–1419.

Villemagne, V. L., Rowe, C. C., Barnham, K. J., Cherny, R., Woodward, M., Bozinosvski, S., … Masters, C. L. (2017). A randomized, exploratory molecular imaging study targeting amyloid β with a novel 8-OH quinoline in Alzheimer's disease: The PBT2-204 IMAGINE study. *Alzheimer's & Dementia: Translational Research & Clinical Interventions*, *3*, 622–635.

Wagner, D., Maser, J., Lai, B., Cai, Z., Barry, C. E., Höner zu Bentrup, K., … Bermudez, L. E. (2005). Elemental analysis of *Mycobacterium avium*, *Mycobacterium tuberculosis*, and *Mycobacterium smegmatis*-containing phagosomes indicates pathogen-induced microenvironments within the host cell's endosomal system. *The Journal of Immunology*, *174*, 1491–1500.

Wang, D., Hosteen, O., & Fierke, C. A. (2012). ZntR-mediated transcription of *zntA* responds to nanomolar intracellular free zinc. *Journal of Inorganic Biochemistry*, *111*, 173–181.

Wessels, I., Pupke, J. T., von Trotha, K.-T., Gombert, A., Himmelsbach, A., Fischer, H. J., … Grommes, J. (2020). Zinc supplementation ameliorates lung injury by reducing neutrophil recruitment and activity. *Thorax*, *75*, 253–261.

Wiegand, S., Zakrzewski, S. S., Eichner, M., Schulz, E., Günzel, D., Pieper, R., … Bücker, R. (2017). Zinc treatment is efficient against *Escherichia coli* α-haemolysin-induced intestinal leakage in mice. *Scientific Reports*, *7*, 1–13.

Wirth, J. J., Fraker, P. J., & Kierszenbaum, F. (1989). Zinc requirement for macrophage function: Effect of zinc deficiency on uptake and killing of a protozoan parasite. *Immunology*, *68*, 114–119.

Wu, A., Tymoszuk, P., Haschka, D., Heeke, S., Dichtl, S., Petzer, V., … Weiss, G. (2017). *Salmonella* utilizes zinc to subvert antimicrobial host defense of macrophages via modulation of NF-κB signaling. *Infection and Immunity*, *85*, e00418–e00417.

Wuehler, S. E., Peerson, J. M., & Brown, K. H. (2005). Use of national food balance data to estimate the adequacy of zinc in national food supplies: Methodology and regional estimates. *Public Health Nutrition*, *8*, 812–819.

Xu, F. F., & Imlay, J. A. (2012). Silver(I), mercury(II), cadmium(II), and zinc(II) target exposed enzymic iron-sulfur clusters when they toxify *Escherichia coli*. *Applied and Environmental Microbiology*, *78*, 3614–3621.

Xu, Z., Wang, P., Wang, H., Yu, Z. H., Au-Yeung, H. Y., Hirayama, T., … Yan, A. (2019). Zinc excess increases cellular demand for iron and decreases tolerance to copper in *Escherichia coli*. *Journal of Biological Chemistry*, *294*, 16978–16991.

Yamasaki, S., Sakata-Sogawa, K., Hasegawa, A., Suzuki, T., Kabu, K., Sato, E., … Hirano, T. (2007). Zinc is a novel intracellular second messenger. *The Journal of Cell Biology*, *177*, 637–645.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: von Pein JB, Stocks CJ, Schembri MA, Kapetanovic R, Sweet MJ. An alloy of zinc and innate immunity: Galvanising host defence against infection. *Cellular Microbiology*. 2021;23:e13268. https://doi.org/10.1111/cmi.13268