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REVIEW ARTICLE

Copper homeostasis; A rapier between mycobacteria and macrophages

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Abstract

Copper is a vital trace element crucial for mediating interactions between *Mycobacterium* and macrophages. Within these immune cells, copper modulates oxidative stress responses and signaling pathways, enhancing macrophage immune functions and facilitating *Mycobacterium* clearance. Conversely, copper may promote *Mycobacterium* escape from macrophages through various mechanisms: inhibiting macrophage activity, diminishing phagocytic and bactericidal capacities, and supporting *Mycobacterium* survival and proliferation. This paradox has intensified research focus on the regulatory role of copper in immune cell–pathogen interactions. Interactions among metal ions can affect *Mycobacterium* concentration, distribution, and activity within an organism. In this review, we have elucidated the role of copper in these interactions, focusing on the mechanisms by which this metal influences both the immune defense mechanisms of macrophages and the survival strategies of *Mycobacterium*. The findings suggest that manipulating copper levels could enhance macrophage bactericidal functions and potentially limit *Mycobacterium* resistance. Therefore, elucidating the regulatory role of copper is pivotal for advancing our understanding of metal homeostasis in immune cell–pathogen dynamics and TB pathogenesis. Furthermore, we recommend further investigation into the role of copper in TB pathogenesis to advance tuberculosis diagnosis and treatment and gain comprehensive insights into metal homeostasis in infectious disease contexts.

KEYWORDS

copper homeostasis, immune evasion, macrophage, *mycobacterium tuberculosis*, polarization

Abbreviations: 2,3,2-tet, 2,3,2-tetramine; BCG, Bacillus Calmette-Guérin; BMDM, bone marrow-derived macrophages; CRGs, copper death-related genes; E. coli, Escherichia coli; H. Felis, Helicobacter pylori; INH, isoniazid; iNOS, inducible nitric oxide synthase; M. bovis, Mycoplasma bovis; M. marinum, Mycobacterium marinum; MAP, *Mycobacterium avium* subspecies paratuberculosis; MDR/RR-TB, multidrug/drug-resistant TB; MFI, mean fluorescence intensity; mROS, mitochondrial reactive oxygen species; Mtb, Mycobacterium tuberculosis; NO, Nitric Oxide; PMA, phorbol 12-myristate 13-acetate; RCD, regulated cell death; ROS, reactive oxygen species; S. typhimurium, Salmonella typhimurium; TB, Tuberculosis; TCA, tricarboxylic acid; TM, tetrathiomolybdate; TM, tetrathiomolybdate; WHO, World Health Organization.

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1 | INTRODUCTION

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (Mtb). The control measures against this infection faced setbacks owing to the coronavirus disease 2019 (COVID-19) pandemic. In 2022, the World Health Organization (WHO) reported approximately 10.6 million TB infections, an increase from 10.1 million in 2020.[1] Of these, approximately 410,000 were cases of multidrug/rifampicin-resistant TB (MDR/RR-TB), which were linked to an estimated 160,000 global deaths in 2022.[2] This highlights Mtb as both a persistent public health crisis and a health security threat, highlighting the critical need for research into the molecular mechanisms of the immune response to TB, particularly macrophage anti-tuberculosis immunity, to develop targeted therapies and control drug resistance.

During bacteria–macrophage interactions, transition metal concentrations are meticulously regulated to preserve critical biological processes while limiting toxicity. Copper, vital for the growth of almost all organisms, becomes toxic when overabundant.[3] It is recognized for its antimicrobial properties in innate immunity, accumulating in macrophage phagosomes containing infected bacteria, suggesting a role in combating pathogens.[4]

Macrophages, pivotal in anti-tuberculosis defense, phagocytose, and digest pathogens. Post-phagocytosis, they restrict and eliminate Mtb by generating a high-copper environment. The interplay of copper metabolism in macrophages involves cytokine release to activate immune responses against bacterial invasion.[5] After phagocytosing *Mycobacterium tuberculosis*, macrophages inhibit and eradicate the bacterium by accumulating substantial copper concentrations, thereby establishing a high-copper microenvironment. Influenced by copper metabolism, these cells secrete specific cytokines to stimulate both autocrine and paracrine immune responses integral to the antimicrobial process.[6] Conversely, *Mycobacterium* can counteract elevated copper levels through various adaptations, including the sequestration of intracellular copper and enhancing resistance to both oxidative stress and host immune tactics, thereby facilitating its persistence and proliferation.[7] Consequently, maintaining copper homeostasis is essential for managing the interaction between macrophages and *Mycobacterium*.

In this review, we have elucidated the role of copper in the interaction between macrophages and *Mycobacterium*, emphasizing the importance of metal homeostasis and interplay. We also discuss new diagnostic methods and research directions for Mtb (Table 1).

2 | A TRANSITION METAL: COPPER AND ITS STEADY STATE

Copper plays a vital role in living organisms and can affect the immune function of the body by influencing the activation and phagocytosis of immune cells. It can also significantly inhibit the growth of *Mycobacterium*. by reducing their virulence, affecting their drug resistance, or killing them directly, thus helping to control the spread of infection.

2.1 | Function of copper in immune cells

Copper deficiency impairs the proliferative activity of immune cells and the production and secretion of immune response mediators. It reduces IL-2 expression, adversely affecting lymphocyte growth and differentiation. Treating human T-lymphocyte cell lines with low concentrations of the copper chelator 2,3,2-tetramine (2,3,2-tet) results in a 60%–70% reduction in IL-2 production. *IL2* mRNA levels in chelator-treated cells are 40%–70% lower than those in untreated cells. The presence of copper counteracts the chelator-induced decrease in *IL2* mRNA levels following exposure to 2,3,2-tet.[8] The influence of copper on lymphocytes mirrors its effects in macrophages, particularly in regulating immune responses and tumor immunity.[9]

Copper deficiency also leads to a decrease in neutrophil quantity and functionality, impacting intrinsic immunity. Weanling rats on diets with ≤2.7 mg Cu/kg for 5 weeks exhibit significantly lower copper levels in neutrophils than that in controls, with increased survival of *Candida albicans* in copper-deficient rats.[10] Phagocytes, macrophages, and neutrophils share many similarities and can participate in natural immunity, recognizing and responding to pathogen-associated and injury-associated molecular patterns through receptors.[11] Copper similarly affects both cell types in immunomodulation, inflammatory responses, and tumor immunity.

Copper modulation of the immune system also extends to influencing the inflammatory response. Treating BV cells (immune cells with partial macrophage marker similarity) with 60 μM copper substantially increases intracellular NO production after 6, 12, and 24 h. NO production was more pronounced after 12 h of exposure than at 6 and 24 h, with the supernatant concentration of NO and secretion of inflammatory cytokines TNF-α and IL-6 also significantly higher after 12 h of exposure to 30 and 60 μM copper.[12] These observations suggest that in abnormal copper homeostasis, such phagocytes foster a local inflammatory response microenvironment, promoting the M1 phenotype polarization within a macrophage interactive environment.

TABLE 1 Essential copper related proteins in mycobacteria macrophage interactions.

Protein / Enzyme	Function
Ms2173, AraC protein	A transcription regulator that modulates gene transcription through selective and non-covalent binding to specific double-stranded genomic DNA (sometimes referred to as a motif) sequences within *cis*-regulatory regions
Arg1, Arginase-1	A critical enzyme in the urea cycle that converts L-arginine into urea and L-ornithine, subsequently metabolized into proline and polyamines, which are vital for collagen synthesis and cellular bioenergetics, respectively. This cycle primarily occurs in the liver and, to a lesser extent, in the kidneys
PPE2	Inhibits nitric oxide (NO) production in activated macrophages by suppressing the expression of inducible nitric oxide synthase (iNOS). PPE2 is translocated into the host macrophage nucleus, where it interacts with a GATA-binding site overlapping the TATA box of the NOS2 (iNOS) promoter, strongly inhibiting *NOS2* transcription
JAK2, Tyrosine-protein kinase JAK2	A non-receptor tyrosine kinase that plays roles in cell growth, development, differentiation, and histone modifications. It mediates key signaling events in both innate and adaptive immunity
STAT1, Signal transducer and activator of transcription 1	Mediates cellular responses to interferons (IFNs), cytokine KITLG/SCF, other cytokines, and other growth factors.
Bcl-2, Apoptosis regulator Bcl-2	Regulates apoptosis across various cell systems, including factor-dependent lymphohematopoietic and neural cells, primarily by controlling mitochondrial membrane permeability
CpnT, outer membrane channel protein CpnT	Serves a dual role in nutrient uptake and induction of host cell death, with its N-terminal domain (NTD) forming an outer membrane channel for nutrient transport
Mrc1, Macrophage mannose receptor 1	Facilitates the endocytosis of glycoproteins by macrophages and acts as a phagocytic receptor for bacteria, fungi, and other pathogens. It binds both sulfated and non-sulfated polysaccharide chains
Zmp1, Zinc metalloprotease	Catalyzes the hydrolysis of internal, alpha-peptide bonds in polypeptides, where water acts as a nucleophile, and one or two metal ions stabilize the water molecule, with charged amino acid side chains as metal ion ligands
SLC7A11, Cystine/glutamate transporter	Functions as an antiporter in a heterodimeric complex with SLC3A2, mediating the exchange of extracellular anionic L-cystine for intracellular L-glutamate across the plasma membrane
STAT3, Signal transducer and activator of transcription 3	Acts as a signal transducer and transcription activator in response to interleukins, KITLG/SCF, LEP, and other growth factors. Once activated, it recruits coactivators such as NCOA1 or MED1 to the promoter region of target genes
STAT6, Signal transducer and transcription activator 6	Performs dual functions in signal transduction and transcription activation, involved in IL4/interleukin-4- and IL3/interleukin-3-mediated signaling
NFE2L2, Nuclear factor erythroid 2-related factor 2	A transcription factor crucial in the oxidative stress response, binds to antioxidant response (ARE) elements in the promoter regions of cytoprotective genes, such as phase 2 detoxifying enzymes, and promotes their expression, thereby neutralizing reactive electrophiles
ATP7B, P-type Cu($^{+}$) transporter	Facilitates the directed movement of copper ions out of cells or organelles
SLC31A1, High-affinity copper uptake protein 1	Mobilizes Cu^{1+} out of the endosomal compartment, making it available for export from cells
RicR, Copper-sensing transcriptional repressor	Under low copper conditions, it represses the expression of genes, including *lpqS*, *Rv2963*, *mymT*, *socA*, *socB*, *mmcO*, and its expression
CueR, HTH-type transcriptional regulator	Detects cytoplasmic copper stress and regulates transcription of the CopA and CueO genes in response to increasing copper concentrations
CueO, Multicopper oxidase	Involved in copper homeostasis and tolerance under aerobic conditions by oxidizing Cu^{+} to the less harmful Cu^{2+} in the periplasm, preventing its entry into the cytoplasm
CopA, Coatomer subunit alpha	A cytosolic protein complex component that binds to dilysine motifs and reversibly associates with Golgi non-clathrin-coated vesicles, mediating protein transport from the ER via the Golgi to the trans-Golgi network

Note: Information on proteins and their functions is provided by the online protein database, UniProt.

2.2 | Function of copper in *Mycobacterium*

The antimicrobial properties of copper are related to its capacity to disrupt cell membranes. In experiments, *Staphylococcus haemolyticus* was subjected to contact killing using copper (type C11000, 99.9% copper) and stainless steel (AISI 304, comprising approximately 67%–72% iron, 17%–19.5% chromium, and 8%–10.5% nickel). The cells remained largely unaffected by exposure to stainless steel

throughout the experimental period. In contrast, exposure to copper resulted in the complete eradication of all cells within 7 min. At this juncture, the intracellular concentration of copper had diminished from its level at 5 min, indicative of membrane leakage; subsequent cell staining confirmed membrane damage.[13] In response to elevated copper levels during bacterial assault, Mycobacterium tuberculosis enhances its survival mechanisms by upregulating efflux pumps and expressing copper chaperones and multicopper oxidases, such as CtpV and MmcO, to swiftly restore and maintain metal homeostasis.[14]

Copper kills *Mycobacterium* by mediating the production of reactive oxygen species (ROS) and causing DNA degradation. In a study, *Mycobacterium avium subspecies paratuberculosis* (MAP) was exposed to copper ions, with DNA extracted and analyzed at various intervals. Partial disintegration of DNA was observed 15 min post-exposure, with more extensive damage noted at 30 min. Concurrently, ROS production escalated significantly both with prolonged exposure to copper ions and with increased copper concentration; notably, ROS levels were higher after 30 min than at 15 min.[15] The ROS generated in response to copper ions led to substantial DNA damage, severely compromising the structural integrity of MAP at both nucleic acid and protein levels.

Furthermore, Cu^{2+} impacts bacterial drug resistance and virulence. A Mycobacterium tuberculosis (Mtb) mutant lacking the outer membrane channel protein Rv1698 accumulated 100-fold more Cu and was more susceptible to Cu toxicity than WT Mtb.[16] The protein Ms2173, a copper ion-responsive transcription factor from the GntR/FadR family, suppresses the expression of numerous membrane-associated drug efflux pumps implicated in *Mycobacterium* resistance. *Mycobacterium* strains overexpressing Ms2173 exhibited markedly slowed growth in Middlebrook 7H9 medium supplemented with 30 μg/mL isoniazid, whereas Ms2173-deficient mutants demonstrated accelerated growth under identical conditions. Cu^{2+} inhibits the DNA-binding capacity of Ms2173, with 2.5 μM Cu^{2+} significantly mitigating the inhibitory effects of 30 μM/mL isoniazid (INH) on the growth of these overexpressing strains.[17] These observations suggest that Cu^{2+} plays a crucial role in modulating drug resistance in *Mycobacterium*, indicating potential research avenues targeting copper mechanisms to combat *Mycobacterium* drug resistance.

2.3 | Role of copper homeostasis in infectious diseases

During infections with bacteria or fungi, the invading microorganisms must acquire copper from their host. Exposure of mice infected with *Helicobacter felis* to the copper chelator tetrathiomolybdate (TM) results in a reduction of copper levels in the stomachs of the infected mice by approximately 50% compared with those in the stomachs of uninfected mice, indicating competition for copper between the host and the bacteria. Moreover, the gastric mucosa in the infected group is thicker than that in the uninfected group. TM-treated *H. felis*-infected mice exhibit signs of chronic gastritis with mucosal hyperplasia and altered tissue structure characterized by inflammatory infiltration.[18] This suggests that chelated copper exacerbates the pathological features following *Helicobacter* infection.

The nonspecific antimicrobial properties of copper allow it to kill a wide range of pathogenic microorganisms with diverse morphologies. Viruses and bacteria, when directly inoculated onto copper surfaces, are inactivated at differing rates; *Escherichia coli* and the bacteriophage MS2 are inactivated at an exponential rate (0.01 $\log_{10}$ PFU min^{-1}). A positive correlation exists between the inactivation rate of the hepatitis A virus on copper surfaces (0.17 $\log_{10}$ PFU min^{-1}) and contact time, although this rate is significantly slower than that for MS2 (0.98 $\log_{10}$ PFU min^{-1}).[19] These results confirm the direct bactericidal effect of copper.

The intracellular concentration of transition metals, including copper, must be carefully regulated to balance between deficiency and toxicity, a task requiring coordination by both mammals and microorganisms. Disruption of this balance can detrimentally affect the survival of both host and pathogen.[20] For instance, *Salmonella typhimurium* in a medium supplemented with 100 μM copper sulfate and 200 μM bathocuproine disulfonate (BCS), which do not compromise the viability of mouse bone marrow-derived macrophages (BMDMs), demonstrates that reducing intracellular macrophage activity with BCS significantly enhances the survival of *S. typhimurium* after 24 h of infection. These findings imply that the endogenous copper levels in intracellular or extracellular media are adequate to support the antimicrobial response of macrophages against *S. typhimurium*.[21]

3 | INTERACTIONS BETWEEN *MYCOBACTERIUM* AND MACROPHAGES

Mycobacterium infection, particularly that caused by Mtb, typically exists in a state of equilibrium characterized by immune control and bacterial persistence. The capacity of these bacteria to infect and propagate is contingent upon their ability to evade and exploit the host immune response. Throughout the course of an infection, both the immune cells responding to the infection and the infected cells themselves undergo dynamic changes. Mtb

manipulates the pathogenic process by secreting proteins and lipids that impair macrophage functionality and modulate inflammatory responses.

3.1 | Recognition and immune response to *Mycobacterium* by macrophages

The polarization of macrophages during an infection influences the ability of the host to manage mycobacterial infections. Macrophages can adopt one of two primary activation states: the classically activated M1 phenotype and the alternatively activated M2 phenotype (Figure 1).[22] M1 macrophages, induced by factors such as interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and lipopolysaccharide (LPS), exhibit a pro-inflammatory phenotype with capabilities to eradicate pathogens. Conversely, M2 macrophages, induced by interleukin-4 (IL-4), interleukin-13 (IL-13), and interleukin-10 (IL-10), tend to promote cellular proliferation and tissue repair.[23–25] Experiments with peripheral blood mononuclear cell-derived macrophages and granulocyte-macrophage colony-stimulating factor-cultured macrophages, polarized to M1 and M2 phenotypes using IFN-γ or IL-4, respectively, demonstrated a 19% reduction in colony-forming units (CFUs) in M1 macrophages compared with that in M2 macrophages 7 days post-Mtb infection. Additionally, M1 macrophages also significantly limited the growth of Bacillus Calmette-Guérin (BCG).[26]

Macrophages exert cytotoxic effects by engulfing pathogens and releasing molecules such as ROS. This action, mediated by mitochondrial ROS (mROS), is regulated by TNF. Administering exogenous TNF to zebrafish larvae phenotypically similar to those infected with *Mycobacterium marinum* leads to TNF overdose, resulting in a 1.7–2.2-fold increase in mROS in infected macrophages compared with that in the uninfected group on day 5 post-infection, and a subsequent increase of 3.6–6.6-fold in macrophages that overdosed with TNF.[27] Excessive TNF also triggers programmed necrosis in infected macrophages through mROS production.[28,29]

New forms of programmed necrotic cell death also affect *Mycobacterium*-macrophage interactions.[30] Exposing mouse BMDMs to the *Mycobacterium* H37Rv strain at varying multiplicities of infection (MOIs) leads to a 22.6% reduction in viable cell count in the infected group versus the uninfected group, accompanied by erratic increases in intracellular iron levels and mitochondrial superoxide mean fluorescence intensity of 10^3 at an MOI of 10, which was 5-fold higher than that in the control group, demonstrating that iron overload exacerbates cell death.[31] A strong correlation exists between metal-induced cell death and the progression of Mtb-induced infectious disease.

3.2 | Immune escape of *Mycobacterium* from macrophages

Mycobacterium pubescens evades the immune system by affecting host cell activation and differentiation. A Mce2D knockout strain of *M. pubescens*, after 48 h, harbors fewer intracellular bacteria than its wild-type counterpart. Furthermore, the pro-inflammatory cytokine TNF-α is elevated by 20%, while the anti-inflammatory cytokine IL-10 is reduced by approximately 13% in the culture supernatant relative to control levels. Levels of the M2 markers CD206 and ARG1 significantly decrease

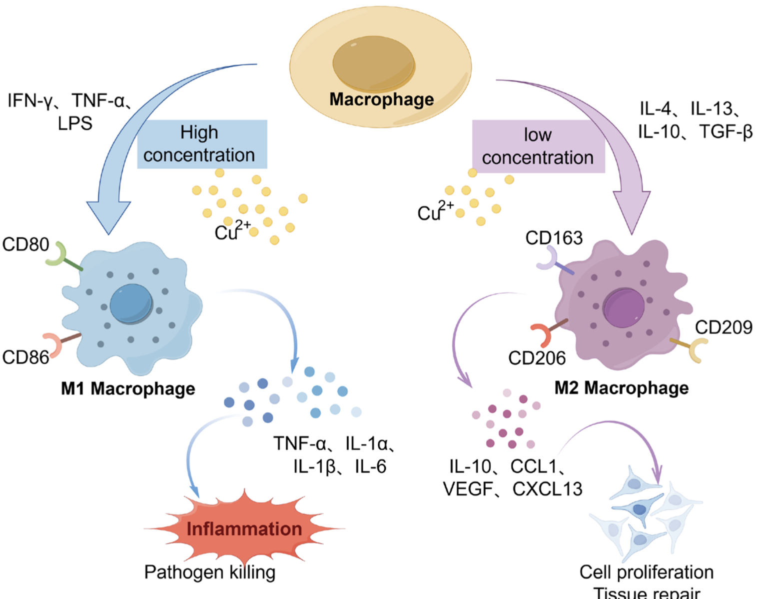

FIGURE 1 Macrophages of different polarization types. Macrophages can be polarized based on inducing factors and release different factors resulting in different effects. LPS, lipopolysaccharides; VEGF, vascular endothelial growth factor.

following infection of THP-1 cells with the *M. pubescens* knockout strain, whereas expression of the M1 marker iNOS significantly increases.[32] Mce2D is implicated in facilitating immune escape by inhibiting M1 polarization in macrophages.

Mycobacteria deploy defense mechanisms against ROS-mediated cytotoxicity in macrophages. Rv2617c, a key protein in the antioxidant activity of *Mycobacterium* spp., was tested by incubating a *Mycobacterium* mutant strain with the wild-type in 10 mM H_2O_2 for 3 and 16 h. After 3 h, the CFUs of the wild-type strain were nearly five times higher than those of the mutant.[33] Rv2617c is an integral membrane-bound protein, and its absence may compromise normal membrane structure and protein secretion, increasing stress susceptibility.

Mtb induces necrosis to facilitate bacterial dissemination. By inhibiting apoptosis and inducing necrosis, Mtb may evade or delay antigen presentation.[34] Infection of THP-1 cells with virulent wild-type *Mycoplasma bovis* and its attenuated counterpart, BCG, demonstrates that BCG induces significant nucleosome fragmentation in THP-1 cells, a response not observed with wild-type *M. bovis*. Twelve hour post-infection, BCG activates cysteine asparaginases 8, 9, and 10, whereas pathogenic *M. bovis* does not trigger cysteoaspartase activation at the same infectious dose.[34,35] This suggests that the strategy of *Mycobacterium* to avoid triggering apoptosis in *M. bovis* is related to an early phase before the activation of the cysteoaspartase promoter.

3.3 | Role of metal metabolism in *Mycobacterium* and macrophages

Iron is crucial for most organisms, and macrophage polarization is closely related to iron metabolism. Glia maturation factor gamma (GMFG) was knocked down in mouse BMDM and stimulated with M1 (LPS/IFN-γ) or M2 (IL-4/IL-13) macrophage inducers for 24 h. This resulted in mRNA expression of *Arg1*, *Mrc1*, *IL10*, and *Fizz1* were all elevated over two-fold in IL-4/IL-13-induced M2 macrophages compared with that in the controls, whereas that of IL-6 was reduced by 50% in LPS/IFN-γ-induced M1 macrophages.[36] GMFG knockdown altered the expression of iron-metabolizing proteins and increased iron levels in mouse macrophages while promoting their polarization toward an anti-inflammatory M2 phenotype.

Nutrient metal availability similarly modulates immune cell functions during infection. Zinc can regulate inflammation through different Toll-like receptor signaling pathways. Zinc metalloproteinase-1 (Zmp1) from Mtb, a virulence factor involved in inactivating inflammatory vesicles and arresting phagosome maturation, induces the release of chemokines MCP-1, MIP-1β, and IL-8, which further aid in inflammatory cell migration and aggregation, thus favoring bactericidal and inflammatory responses.[36,37]

Given the critical role of transition metals in biological processes, their metabolism and homeostasis are considered double-edged swords. BCG-infected macrophages treated with low- (10 mg/L) and high-dose (40 mg/L) zinc oxide nanoparticles (ZnONPs) exhibited a dose-dependent decrease in the number of bacteria phagocytosed. Analysis of differential gene expression between the two groups identified 479 genes, including those encoding GPX4, ASLC4, and SLC7A11, which are closely associated with ferroptosis. ZnONPs thus enhance BCG-induced ferroptosis in high-dose macrophages, impacting both the physiological state of the organism and the bacteria.

4 | COPPER HOMEOSTASIS, MYCOBACTERIA, AND MACROPHAGE INTERACTIONS

Copper homeostasis is vital for interactions between *Mycobacterium* and macrophages. Mycobacteria utilize specific copper homeostasis mechanisms to resist ROS and other immune responses from macrophages, enabling their survival and replication within macrophages. Conversely, alterations in copper homeostasis may influence macrophage polarization. Since the metabolic balance of other metal elements also significantly affects macrophage responses to intracellular bacterial infections, potential crosstalk between the metabolic effects of each metal element exists.

4.1 | Copper homeostasis in macrophage recognition and immune response

Copper homeostasis influences macrophage polarization. Macrophages stimulated with Cu^{2+} at concentrations of 1, 10, and 100 μM displayed induction of an M2-like phenotype at 24 h. The low (1 μM) and medium (10 μM) concentrations maintained expression of M2-associated markers IL-10, TGF-β, and CD206 at 48 h. Higher Cu^{2+} concentrations (100 μM) led to a significant decrease in CD206 while maintaining elevated levels of the M1 marker TNF-α, indicating a shift toward an M1 phenotype.[39] This suggests that Cu^{2+} influences macrophage polarization in a dose-dependent manner. Various non-toxic concentrations of copper differentially stimulate macrophage polarization, affecting their response to *Mycobacterium*.

Copper promotes bactericidal activity in macrophages through a ROS-dependent mechanism. Experiments were conducted on mouse macrophages, applying the peptide DAB-10 to *Mycobacterium*, including Mtb, under

copper-enriched (50 μM) and copper-deficient conditions showed that DAB-10-treated bacteria experienced a 10^2-fold reduction in CFUs in the presence of excess copper compared with that in the controls.[40] DAB-10 enhances macrophage bactericidal activity by inducing oxidative stress in *Mycobacterium* in a copper-dependent manner.

Copper also affects the expression of cytokines and chemokines secreted by macrophages via the NF-κB pathway, thereby influencing the regulation of other immune cells. After 49 days on diets with varying copper concentrations (11, 110, 220, or 330 mg Cu/kg), excessive copper intake (330 mg Cu/kg) increased TNF-α by 75%, IFN-γ by 20%, and NF-κB by 50% in the thymus compared to controls.[41] Thus, excessive copper intake significantly upregulates inflammatory factor mRNA levels in immune organs.

4.2 | Copper homeostasis in the immune escape of *Mycobacterium*

Mtb has developed strategies to evade killing by M1-activated macrophages and induce a switch to an M2-polarized state, facilitating chronic infection and suppressing host immune defenses.[5] Infection of BMDMs with virulent Mtb H37Rv or attenuated Mtb H37Ra increases the production of M2-associated molecules (STAT3 and STAT6) in H37Rv-infected macrophages; post-infection, M1-type cells exhibit more than a two-fold increase in IL-12 p40 expression compared with that of M2-type cells, which show no increase.[42] *M. avium toxicum* can skew macrophage functionality toward an M2 phenotype, particularly through ESAT-6 production, to alter the host survival environment.

Copper ions modulate cellular responses to oxidative stress.[43] When THP-1 cells, induced with phorbol 12-myristate 13-acetate (PMA), are exposed to purified MmcO protein, ROS levels decrease in a dose-dependent manner. The presence of 500 ng MmcO reduces ROS to 59% of levels observed in the unexposed group,[44] suggesting that MmcO protein acts as an anti-copper agent protecting Mtb from ROS during infection.

The toxicity of excess copper can also damage macrophages or organisms. Copper-dependent regulated cell death (RCD) relies on mitochondrial respiration.[45] Analysis of copper death-related gene (*CRG*) expression in healthy controls and patients with TB led to the identification of 11 differentially expressed genes. Among them, *NFE2L2*, *NLRP3*, *ATP7B*, *SLC31A1*, *MTF1*, and *DLD* are upregulated, whereas *LIAS*, *LIPT1*, *DLAT*, *GLS*, and *DBT* are downregulated in patients with TB.[46] Deficiency in copper down-regulated the transcriptional level of the virulence factor CFP-10 in M. marinum, suppressed cytosolic signaling via the macrophage STING pathway, leading to decreased production of IFN-β and reduced cell apoptosis.[47] This suggests that *Mycobacterium* may evade immune attack by altering intracellular copper concentrations and inducing copper-dependent RCD in host immune cells.[48]

4.3 | Metal metabolism, macrophages, and intracellular bacterial infections

Transition metals, critical in both eukaryotic and prokaryotic organisms, regulate host-pathogen interactions through their bioavailability. Copper resistance is critical for Mtb virulence; inhibition of the RicR regulator by copper sensitizes Mtb to copper, reducing its growth in mice.[49] Transition metals enhance the evolutionary fitness of bacteria and often regulate bacterial virulence, impacting macrophage heterogeneity and their response to mycobacteria.[5]

Interactions among different metals within the human body can affect the distribution of these elements, impacting the human microenvironment. For instance, varying dietary iron levels (45 and 180 mg/kg) significantly alter copper levels in the liver and kidneys of female Wistar rats, with excess iron intake decreasing liver and kidney copper levels but increasing splenic copper levels.[50] These findings suggest that excessive iron intake decreases copper levels in the liver and kidneys but increases copper levels in the spleen.

There is also a complex interplay in regulating various metals during infection. Copper disrupts the assembly of iron–sulfur clusters, diminishes iron–sulfur enzyme activity, and impairs mitochondrial function both in vivo and in vitro.[51] Zinc, iron, and copper homeostasis in *E. coli* are affected by zinc overload, which impacts iron regulatory mechanisms and poisons CueR, reducing the expression of the copper exporter CopA and the copper oxidase CueO.[52]

5 | DISCUSSION

In this review, we have highlighted the regulatory role of copper in interactions between *Mycobacterium* and macrophages, emphasizing the importance of copper homeostasis. Copper enhances the immune functionality of macrophages, as well as facilitates the immune escape of *Mycobacterium*. We focus on oxidative stress and cellular polarization, where the role of copper in generating ROS can kill Mycobacterium, whereas metal-induced oxidative stress leads to mitochondrial dysfunction, affecting cell polarization, survival, and death.

Copper toxicity and copper limitation can both be effective host defense mechanisms against pathogens.[53] Excessive or deficient cellular copper levels alter

mitochondrial function, affecting cell proliferation or differentiation.[54] Non-cytotoxic levels of copper promote complex IV assembly and ROS generation, enhancing mitochondrial biogenesis. Conversely, reduced copper levels downregulate complex IV expression, decreasing mitochondrial oxidizability.[55] Copper death, linked to mitochondrial tricarboxylic acid (TCA) cycle metabolism, involves direct binding of copper to thiooctanoylated components of the TCA cycle, resulting in proteotoxic stress and cell death.[45] Additionally, microorganisms may exploit cuproptosis as a mechanism for immune evasion.

Mitochondrial dysfunction caused by abnormal copper homeostasis also affects lipid levels. In a study of yellow catfish fed 0. 008 (adequate copper, AC) g/kg and 0. 4 (excess copper, CE) g/kg diets, yellow catfish fed CE diets had two-fold higher triglyceride levels than those in the AC group, and many vesicles and mitochondrial swelling were found in hepatocytes. Conversely, Keap1 protein expression was lower in the CE group compared with that in the AC group, but Nrf2 protein expression was unchanged.[56] Lipid deposition induced by excess copper activates Nrf2 activation via oxidative stress and mitochondrial dysfunction. The functional effects of metals on mitochondria involve crucial regulation of metabolic processes. Dysregulation of intracellular environmental homeostasis, linked to copper death, plays a role in the functional regulation of immune cells.

Typically, the diverse biological properties of copper facilitate various roles in the growth processes of living organisms. As a cofactor, copper is crucial for cellular homeostasis and is involved in the metabolism of several substances within cells, including amino acids and lipids, which are essential for cellular behavior, differentiation, and function. Copper influences the host immune response; furthermore, the regulation of copper homeostasis also plays a critical role in the survival and pathogenicity of microbial pathogens.[57] Macrophage-targeted iron oxide nanopackages enhance innate immunity and drug killing for more effective Mycobacterium tuberculosis clearance. Metallic nanodrugs are also emerging as an emerging research hotspot.[58] The homeostasis of metals in macrophage-Mycobacterium tuberculosis interactions represents a complex mechanism of intercellular communication.[58] Future research should be conducted to explore the mechanisms of copper metabolism within the context of bacterial infections and assess copper metabolism as a potential therapeutic target. Further studies aimed at fully characterizing the roles of various metals in macrophages and Mycobacterium tuberculosis, as well as understanding the metal metabolism of the organism, are vital. This knowledge will enhance our ability to identify potential diagnostic and therapeutic targets for specific pathogens and to develop metal metabolism-related drugs.

AUTHOR CONTRIBUTIONS

D.H., Z.Y., J.Z., and G.L. initiated and supervised the review and revised the final manuscript. Y.Z. and Y.W. conducted the review and drafted the manuscript. JP and JX assisted with completing the review. All authors have reviewed and approved the final version of the paper.

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CONFLICT OF INTEREST STATEMENT

All authors declare no competing interests.

DATA AVAILABILITY STATEMENT

No new data. Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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