Immunity

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Review

Ironing out the role of ferroptosis in immunity

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SUMMARY

Ferroptosis is a type of regulated cell death that drives the pathophysiology of many diseases. Oxidative stress is detectable in many types of regulated cell death, but only ferroptosis involves lipid peroxidation and iron dependency. Ferroptosis originates and propagates from several organelles, including the mitochondria, endoplasmic reticulum, Golgi, and lysosomes. Recent data have revealed that immune cells can both induce and undergo ferroptosis. A mechanistic understanding of how ferroptosis regulates immunity is critical to understanding how ferroptosis controls immune responses and how this is dysregulated in disease. Translationally, more work is needed to produce ferroptosis-modulating immunotherapeutics. This review focuses on the role of ferroptosis in immune-related diseases, including infection, autoimmune diseases, and cancer. We discuss how ferroptosis is regulated in immunity, how this regulation contributes to disease pathogenesis, and how targeting ferroptosis may lead to novel therapies.

INTRODUCTION

Cell death is a fundamental biological process essential for survival that regulates development, tissue homeostasis, and disease. Regulated cell death eliminates unneeded cells and cells damaged by infection, mutation, or old age.[1] Spatial, temporal, and mechanistic regulation of cell death are critical checkpoints for preventing human illness. Regulated cell death can be either immunostimulatory or immunoinhibitory, but the overall goal is the systematic removal of cells targeted to die without harming nearby neighbors.[2,3] Immunogenic cell death can be mediated by changes in membrane proteins on the cell surface alongside the release of soluble molecules. Together, these signals stimulate antigen-presenting cells (APCs), such as macrophages and dendritic cells (DCs), to present antigens to T cells and induce adaptive immunity.[3] How different cell death stimuli contribute to divergent cell surface markers and secreted proteomes is an active area of exploration.[4,5] Regardless of immunogenicity, failure of the balance between pro-survival and death-inducing signals is a hallmark of infection, autoimmune disease, cancer, and organismal development. This dynamic and responsive balance is also critical in the physiological regulation of the immune system. Regulated cell death of non-immune cells modulates the immune response to pathogens, controls damage inflicted by autoimmunity, and regulates anti-tumor immunity.[2,6–11] Regulated cell death of immune cells regulates the enrichment and specificity of T cell responses, stimulates antiviral defense, regulates the inflammatory response, mediates immune tolerance, and generates protective immunity to tumor and pathogens.[2,6–8,11]

Ferroptosis is a form of regulated cell death driven by iron, reactive oxygen species (ROS), and lipid peroxidation of cell membrane lipids.[12] Although many forms of cell death have oxidative features, ferroptosis is morphologically and molecularly distinct. Ferroptotic cells have shrunken mitochondria with fewer cristae, reduced cellular volume with normal nuclear volume, and an accumulation of membrane lipid peroxides.[13,14] The lipid peroxidation of the plasma membrane is a final event in ferroptosis.[15] In contrast to other forms of regulated cell death, there are no discovered overarching ferroptotic "execution genes." Ferroptosis is instead regulated by a complex interplay between death-promoting signals, which are often metabolic, and defense systems, some of which rely on metabolites, such as cystine and glutathione (GSH). Although ferroptosis still needs additional specific "smoking gun" markers, molecular *in vitro* studies and *in vivo* use of inhibitors and inducers have begun to reveal its biological role. A missing piece of ferroptosis research—endogenous ferroptosis inducers that do not require synthetic chemical compounds—has recently been identified. Ferroptosis can be induced by pro-inflammatory cytokines, such as interferon-γ (IFNγ), and some fatty acids, such as arachidonic acid, in the tumor microenvironment (TME); in addition, polyunsaturated fatty acids (PUFAs) or iron can trigger ferroptosis in some contexts.[16] Endogenous glutamate accumulation after system xc inhibition can determine ferroptosis sensitivity by suppressing the yes-associated protein (YAP) pathway.[17] The search for other endogenous mediators of ferroptosis is ongoing.

Regulated cell death is essential for organ and tissue development. In development, regulated cell death has been viewed as

predominantly executed through apoptosis. Nonetheless, the emerging understanding of ferroptosis highlights that although ferroptosis lacks the characteristic biochemical and genetic executioners of apoptosis, it plays a role in development. The emerging roles of ferroptosis in tissue homeostasis and the increasing evidence supporting a role for ferroptosis in many pathologies have driven a flurry of recent research.

Ferroptosis is regulated by the accumulation of iron, ROS, and lipids with susceptibility to peroxidation. Defense against ferroptosis is primarily controlled by the activity and protein level of the glutathione peroxidase (GPX)4.[18] GPX4 is a selenoprotein that is the only GPX family member that can detoxify lipid hydroperoxides embedded into the cellular membrane.[19,20] Inactivation of GPX4 leads to lipid peroxidation and cell death in many cell types.[21,22] GPX4 itself is essential in preventing lipid oxidation-induced renal failure and death in non-transformed murine models.[23] GPX4 is the primary protein responsible for preventing the final execution of ferroptotic cell death.[18–20] Lipid peroxidation can originate from damage to mitochondria, the lysosome, or the endoplasmic reticulum (ER).[24]

We highlight here the mitochondria as an essential hub for the terminal execution of ferroptosis. The mitochondria play an essential role in many forms of regulated cell death, including ferroptosis, apoptosis, and pyroptosis. In pyroptosis, gasdermin D permeabilizes the mitochondria.[25] In apoptosis, mitochondrial outer membrane permeabilization releases cytochrome *c* and initiates caspase activation.[26] In ferroptosis, cystine deprivation results in mitochondrial lipid peroxide accumulation, while inhibiting the electron transport chain prevents lipid peroxidation and ferroptosis.[27] Potentially, the mitochondria are an underappreciated node for modulation and crosstalk between different types of regulated cell death.[15]

Ferroptosis has been identified in cultured cell lines and mice as death that is inhibited by iron chelation, lipophilic antioxidants, lipid peroxidation inhibitors, GPX4, or the depletion of PUFAs.[12,14,21,28] Ferroptosis was initially defined through the study of synthetic small-molecule cell death inducers, such as erastin and Ras-selective-lethal 3 (RSL3). Erastin, sulfasalazine, quisqualic acid, and (S)-4-carboxyphenylglycine selectively inhibit the cystine importer system xc-, while RSL3 inhibits GPX4.[18,29,30] It has also been suggested that RSL3 acts through inhibition of another selenoprotein, thioredoxin reductase 1 (TXNRD1), or that it requires 14-3-3ε activity.[31,32] Other work suggests that the activity of RSL3 is not specific but that RSL3 can target a variety of selenoproteins.[33] This screen reveals that RSL3 may function through many mediators, including voltage-dependent anion-selective channel protein 2 (VDAC2).[33] Ferroptosis plays a role in several diseases.[9,16,23,34–41] In this review, we focus on the interplay between ferroptosis and immunity to better understand the implications of ferroptosis in infectious diseases, autoimmunity, and cancer.

FERROPTOTIC MECHANISMS OF ACTION

The mechanisms and regulators of ferroptosis have been discussed extensively in previous reviews.[24,42] We present an abbreviated description of its basic biology and mechanisms of regulation. Ferroptosis is defined as an oxidative and iron-dependent form of cell death.[12,28,43] Genetic inactivation of GPX4 is similarly found to induce a non-apoptotic form of oxidative cell death.[21] GPX4 and system xc^- are the primary ferroptosis defense proteins.[42] As the only enzyme that can detoxify lipid peroxides embedded in cellular membranes, GPX4 is critical for cellular defense against lipid peroxidation and cell death.[20,44] Genetic deletion or chemical inhibition of GPX4 induces ferroptosis (Figure 1A).[21,28] System xc^- is the predominant amino acid antiporter that imports L-cystine through the transporter subunit solute carrier family 7 member 11 (SLC7a11) in exchange for glutamate (Figure 1A).[45,46] Cystine is converted to cysteine intracellularly through a nicotinamide-adenine-dinucleotide-phosphate-(NADPH)-consuming reduction reaction and can then be used to synthesize GSH, an important cellular cofactor for antioxidant systems.[47] Depleting cystine availability or blocking SLC7a11 through erastin induces ferroptosis.[21,28,48] Ferroptosis is executed by the peroxidation of PUFA moieties in phospholipids and ether lipids, which results in membrane permeabilization and ferroptotic death. Acyl-coenzyme A (acyl-CoA) synthase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3) enrich cellular membranes with PUFAs.[49] Genetic loss of *Acsl4* or pharmacological inhibition of ACSL4 induces resistance to ferroptosis (Figure 1A).[49] Treatment of cells or animals with PUFAs containing deuterium in place of hydrogen at reactive bisallylic sites can also protect against ferroptosis by preventing lipid peroxidation.[50,51]

High iron concentrations or low concentrations of cystine or GSH tip the cells to a pro-ferroptotic state and can then lead to multiorgan failure.[24,52] Increased ACSL4 or decreased expression of GPX4 or SLC7a11 favors ferroptosis.[24] Selenium drives GPX4 translation and is protective against ferroptosis, as is iron chelation through deferoxamine.[53,54] Selenium augments the transcription of GPX4 and other genes in a transcriptional program, the selenome, through the activation of transcription factors TFAP2c and Sp1.[53] Selenome regulation is critical in neurons during hemorrhagic stroke, but the role of the selenome in immunity is currently underexplored.[53]

Nuclear receptor coactivator 4 (NCOA4) regulates ferroptosis through ferritinophagy, the delivery of ferritin to lysosomes for iron recycling.[55] NCOA4-deficient cells cannot degrade ferritin and therefore have decreased intracellular iron.[55] Modulating NCOA4-mediated ferritonophagic flux regulates ferroptosis.[56] Other aspects of cancer metabolism also control sensitivity to ferroptosis. For example, glutamate oxaloacetate transaminase 1 (GOT1) inhibition promotes pancreatic cancer cell death via ferroptosis, highlighting a potential targetable vulnerability of tumor cells.[57] Inactivation of dihydroorotate dehydrogenase (DHODH) was suggested to induce mitochondrial lipid peroxidation and ferroptosis in GPX4-low cancer cells and to synergize with small-molecule ferroptosis inducers in GPX4-high cancer cells (Figure 1A).[58] The role of DHODH has recently been questioned.[59] DHODH likely operates alongside mitochondrial GPX4 to inhibit ferroptosis in the mitochondrial inner membrane by reducing ubiquinone to ubiquinol, a powerful antioxidant.[58] Ferroptosis suppressor protein 1 (FSP1) protects against ferroptosis induced by GPX4 deletion.[60,61] FSP1 catalyzes the regeneration of ubiquinone using NADPH. FSP1 inhibition synergizes with GPX4 inhibitors, suggesting that the FSP1-coenzyme-Q_{10}-NADPH axis is an independent system to prevent ferroptosis.[61]

This pathway provides a potential explanation for why pharmacological targeting of GPX4 may not achieve major anti-tumor effects due to FSP1-mediated protection.

The mitochondrial role of ferroptosis was recently strengthened by the identification of hyperoxidized peroxiredoxin 3 (PRDX3) as a ferroptotic marker *in vivo* and *in vitro*.[62] PRDX3 hyperoxidation is triggered by the accumulation of lipid peroxides in the mitochondria.[62] Once hyperoxidized, PRDX3 translocates from the mitochondria to the plasma membrane, where it inhibits cystine uptake and promotes ferroptosis.[62] Perhaps hyperoxidized PRDX3 facilitates the execution of ferroptosis, supporting the idea that the mitochondria are a key hub for ferroptosis. However, how hyperoxidized PRDX3 is translocated to the plasma membrane and whether hyperoxidized PRDX3 is present and functionally critical in endogenous ferroptosis in different tissue types remain poorly understood.

We highlight several synthetic inducers and inhibitors of ferroptosis that are commonly used in research (Figure 1A) and endogenous ferroptosis defense systems (highlighted in Figure 1B). Ferroptosis inhibitors, such as ferrostatin-1 and liproxstatin-1 and related compounds, prevent ferroptosis by acting as radical-trapping antioxidants and, in some cases, as inhibitors of lipoxygenase.[53,54,63] Ferroptosis can be defined as cell death that is inhibited by ferrostatin-1 or liproxstatin-1, as well as by iron chelators. This dependence on synthetic chemical inhibition and induction of ferroptosis has led to questions of whether these aspects of ferroptosis are non-physiological, despite the similarity to pan-caspase inhibitors being used to identify apoptotic cell death. This debate may be partly eased by the identification of endogenous ferroptosis inducers, such as activated T cell-derived cell IFNγ plus fatty acids, in the TME.[16,39] As early as the mid-1990s, it had been observed that cystine transport activity in murine macrophages was repressed by IFNγ, but only recently has the biological impact of this regulation in tumor immunology been explored.[16,39,64] Understanding how ferroptosis is regulated *in vivo* is critical for characterizing the physiological and pathological functions of this cell death process. Ferroptosis seems to be a natural consequence of iron metabolism and oxidation that is prevented by a network of overlapping defense proteins and factors.

Ferroptosis can be regulated through cell-cell contacts. E-cadherin induced by cell-cell contact suppresses ferroptosis by activating intracellular neurofibromatosis type 2 (NF2) and the Hippo signaling pathway.[65] Removal of the downstream protein, tafazzin, confers ferroptotic resistance, suggesting that this is the end-mediator of cellular-density-regulated ferroptosis.[66] Genetically inactivating NF2 increases ferroptosis sensitivity, revealing another potential intervention point to translationally drive ferroptosis, particularly in the case of cancer.[65]

The final stage of ferroptosis is the peroxidation of membrane PUFA-containing lipids, which leads to membrane permeabilization (Figure 1C) and generates a wealth of lipid-derived electrophiles (LDEs). Ferroptosis may also transfer between cells through the interaction of oxidized lipids with adjacent cell plasma membranes, creating wave-like propagation of cell death.[15,67] According to this model, ferroptotic cell rupture is mediated by plasma membrane pores, and intercellular propagation of ferroptotic death is induced by erastin but not by direct inhibition of GPX4.[15,67] Propagation of ferroptotic death between cells is upstream of cell rupture and is spread through cellular swelling dependent on lipid peroxide and iron.[15] However, this mechanism of cell killing has only been demonstrated in cell culture and limited *ex vivo* settings, so the relevance to *in vivo* ferroptosis is uncertain. Potentially, a small fraction of tumor cell ferroptotic death induced by immunotherapy could initiate a wave of killing that might induce tumor regression, but this has yet to be experimentally demonstrated. Another possible mechanism for the spread of ferroptosis cell-to-cell is through metabolic adaptation to counteract phospholipid oxidation. Accumulation of 7-dehydrocholesterol shields phospholipids from peroxidation and prevents membrane fragmentation by preferentially reacting with peroxyl radicals.[68] How the intercellular propagation of cell death and eventual rupture impacts immunity in response to different pathogenic insults is not clear.

A variety of cellular organelles regulate ferroptotic cell death (Figure 1C). The ER is a central site of lipid peroxidation.[50] The ER membranes have abundant PUFA-PLs, likely explaining their centrality in driving lipid peroxidation during ferroptosis (Figure 1C).[50] This suggests an ordered progression of membrane peroxidation from initial peroxidation in the ER and later accumulation in the plasma membrane.[50] The mitochondria house a likely ferroptosis-inhibiting pathway discussed above mediated by DHODH.[58] Mitochondria also drive ferroptosis by providing oxidation through the electron transport chain (Figure 1C).[58] In fact, inhibiting the electron transport chain or depletion of mitochondria prevents ferroptosis but not ferroptosis induced by inhibiting GPX4.[69,70]

The peroxisome has also been implicated in ferroptosis regulation. A genome-wide CRISPR suppressor screen has determined that peroxisomes contribute to ferroptosis by synthesizing polyunsaturated ether phospholipids, a ferroptosis substrate.[71] Ferroptosis resistance *in vivo* is driven by the downregulation of polyunsaturated ether phospholipid metabolism.[71] Targeting peroxisomal lipid biosynthesis may therefore induce or inhibit ferroptosis.

The Golgi's role in mediating ferroptosis has been considerably less studied. One report demonstrates that Golgi-dispersing compounds, such as brefeldin A and golgicide A, induce ferroptosis.[72] Inhibitors of ferroptosis prevent cell death induced by these compounds and maintain Golgi function. However, it is unclear how Golgi damage triggers ferroptosis or if ferroptosis affects the integrity of the Golgi. The endosomal recycling compartment has also been implicated in ferroptosis, as transferrin receptor 1 (also known as CD71) localizes to this Golgi-adjacent compartment and is mobilized to the plasma membrane during ferroptosis in many cell types, and thus it serves as a marker of ferroptosis.[73,74]

The lysosome can also mediate ferroptosis in response to specific compounds, such as salinomycin, ironomycin, and related compounds.[75,76] Inhibition of the lysosome by bafilomycin A1 prevents erastin-induced ferroptosis, although lysosomes do not seem to be needed for ferrostatin-1 rescue of ferroptosis (Figure 1C).[77,78] Thus, several cellular organelle membranes are implicated in the initiation, execution, and completion of ferroptosis.

The organellar initiator of ferroptosis is up for debate, but the conclusion that lipid peroxidation is essential for ferroptotic death remains clear. Lipidomics, especially of peroxidized lipids,

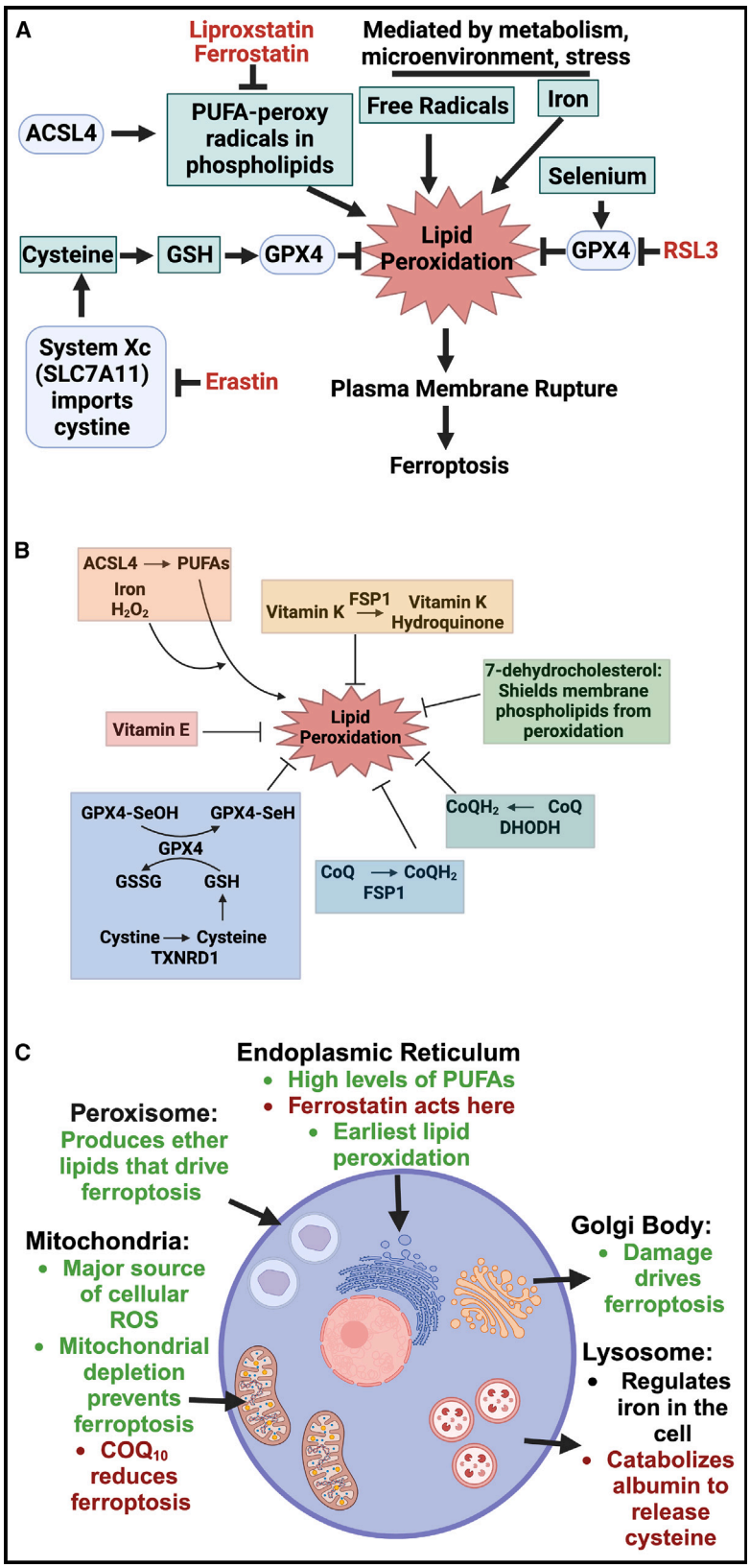

Figure 1. Ferroptosis mechanisms and sites

(A) An overview of ferroptosis.

(B) Schematic depicting the predominant endogenous lipid peroxidation defense systems.

(C) Schematic illustrating the function of various organelles in ferroptosis.

is technically challenging compared with proteomics or genomics.[79] This is due to the almost limitless plethora of peroxidation products, the lack of off-the-shelf reagents, the necessity of expensive and technically demanding equipment to quantify and analyze these lipid changes, and the lack of availability of robust, non-chemical ferroptosis models. Only recently have commercial kits to detect lipid peroxidation become available, and these have limited utility due to an inability to identify many peroxidized lipid species. Only a few groups can measure the oxidized lipidome.[80,81] Lipidomics is just reaching the single-cell level but remains challenging due to the impossibility of amplifying lipids.[82,83]

PUFAs are highly vulnerable to peroxidation and are critical for ferroptotic death.[49,84,85] By contrast, monounsaturated fatty acids are protective against ferroptosis.[18,49,84–86] Recent data suggest that ferroptosis involves increased oxidation of a myriad of phospholipid species.[87] The relative contribution of individual phospholipids is undetermined, providing novel opportunities to counteract ferroptosis, such as through treatment with vitamin K.[87] Therefore, fatty acid, ether lipid biosynthesis, and phospholipid remodeling are essential components to fully understand the steps involved in ferroptosis. Understanding lipid metabolism could also help reveal ferroptotic biomarkers. Phosphatidylethanolamine-(18:0/20:4-OOH) and phosphatidylethanolamine-(18:0/22:4-OOH) are possible ferroptosis markers.[81] These can be oxidatively truncated to PE-(18:0/hydroxy-8-oxo-oct-6-enoic acid), another molecule enriched in cells treated with RSL3.[81] Integrating biological manipulation with lipidomics would be required to understand the biochemical underpinnings of ferroptosis.

Ferroptosis is regulated by lipid composition, iron and cystine availability, and the activity and expression of ferroptosis defense proteins. Ferroptosis requires endogenous metabolites, such as cystine, GSH, and iron. It is not fully understood what *in vivo* factors determine whether a cell dies through ferroptosis or a different type of regulated cell death. In addition, the stage at which ferroptosis is irreversible and how ferroptotic priming impacts other regulated cell death types through crosstalk are underexplored. Furthermore, cell-type differences in ferroptosis sensitivity need more exploration, as does how to modulate microenvironmental factors to therapeutically induce or inhibit ferroptosis.[88] The specific species of PUFAs that drive ferroptosis are also not fully characterized. This area of research is challenged by the difficulty of characterizing ferroptosis mechanisms at the single-cell level. Despite these technical challenges, there are important advances in the immune aspect of this dynamic and growing research field.

THE ROLES OF FERROPTOSIS DURING HOMEOSTASIS

How ferroptosis contributes to the development and homeostasis of the immune system is understudied. A recent report shows that human hematopoietic stem cells (HSCs) are vulnerable to ferroptosis.[89] This observation began through the study of a rare, genetic cause of bone marrow failure caused by the loss of the histone deubiquitinase MYSM1 (myb-like SWIRM and MPN domains 1). Loss of MYSM1 results in reduced global protein translation and increased labile iron through an unknown mechanism, thereby inducing lipid peroxidation and ferroptosis in the HSC population.[89] Inhibiting ferroptosis rescues the HSC depletion seen in *Mysm1*−/− mice.[89] However, it is unknown whether increased ferroptosis is evident in other models of stem cell failure, or how MYSM1 loss mechanistically sensitizes cells to ferroptosis. Nonetheless, this work suggests that hematopoietic homeostasis may be regulated through ferroptosis. In line with this, a previous report demonstrates that iron overload impairs the proliferation of HSCs mediated by increased ROS, while iron chelation or ferroptosis inhibition may help to promote HSC expansion.[90] Hence, ferroptosis inhibition may be valuable in the treatment of bone marrow failure caused by radiation exposure, somatic mutations, or acquired diseases, such as aplastic anemia and myelodysplastic syndrome.[89] In addition, ferroptosis, as detected by a marker of lipid peroxidation, is present in nucleated erythrocytes at embryonic day 13.5 in Fischer-344 rats, but ferroptosis disappears in enucleated erythrocytes at embryonic day 18.5.[91] Preventing ferroptosis using liproxstatin 1 significantly delays erythrocyte developmental enucleation.[91] These data suggest that ferroptosis may control the development of red blood cells.

In addition to HSC development, the role of ferroptosis in modulating HSC function is not yet clear. The genetic ablation of *Gpx4* in HSCs does not inhibit HSC function in healthy adult mice. HSCs are only dependent on GPX4 when their diet is depleted of vitamin E, a lipophilic antioxidant.[92] This suggests that the sensitivity of HSCs to ferroptosis is context dependent, or at least that vitamin E can replace GPX4 in protecting HSCs from ferroptosis. Monitoring HSCs may be beneficial when using ferroptosis inducers as a therapy. How ferroptosis regulates the stem cells that produce immune cells is unknown.

Ferroptosis also contributes to aging. There is an increase in ferroptosis and labile iron in most major organs as rats age.[91] A high-fat diet increases ferroptosis in aged tissues, potentially contributing to aging. Ferrous iron also accumulates over time in *Caenorhabditis elegans* (*C. elegans*).[93] This, coupled with age-related decreases in GSH, primes older cells for ferroptosis in nematodes. Blocking ferroptosis in *C. elegans* prevents age-related cell death and increases both lifespan and health span.[93] Perhaps, inhibiting ferroptosis may support healthy aging. This provides the first hint that ferroptosis may play a role in physiological processes, such as development and aging, although the precise mechanisms remain unclear.

Ferroptosis impacts macrophage physiology. Erythrophagocytosis results in ferroptosis in splenic red pulp macrophages, accompanied by an increase in ROS, lipid peroxidation, and monocyte migration from the bone marrow to spleen.[94] As the lifespan of erythrocytes is controlled by macrophage-mediated erythrophagocytosis, it is plausible that this process regulates ferroptosis in macrophages physiologically.

Genetic or pharmacologic depletion of inducible nitric oxide synthase (iNOS) increases the sensitivity of classically activated phagocytes to ferroptosis.[95] Classically activated macrophages are more resistant to ferroptosis *in vivo* than alternatively activated macrophages, but this resistance is reduced in iNOS-deficient cells in the TME. This suggests the involvement of iNOS in macrophage ferroptosis in the TME. How ferroptosis physiologically regulates macrophage subset development, function, and survival during homeostasis is unknown.[95]

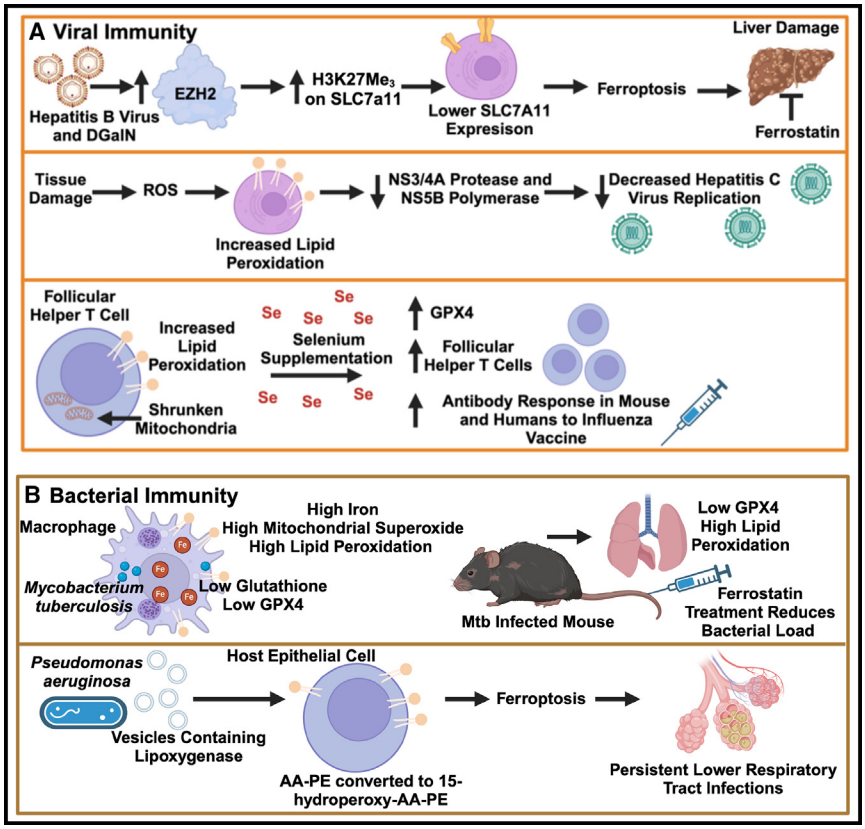

Figure 2. The role of ferroptosis in viral and bacterial immunity

(A) Hepatitis B and C virus use different approaches to manipulate ferroptosis to promote viral infection.

(B) Bacteria induce ferroptosis in mammalian cells to facilitate bacterial replication and dissemination throughout the host. AA-PE, arachidonic acid phosphatidylethanolamine.

Evidence is emerging that ferroptosis regulates aging, hematopoiesis, and macrophage function. However, these results remain an isolated island in a sea of ferroptosis papers (more than 10,000 as of the end of 2023) using a disease-based model focused on cancer or infection. In both physiology and pathology, ferroptosis is microenvironmentally and metabolically modulated. Understanding how ferroptosis contributes to immune homeostasis may reveal new ways to intervene in disease or even improve organismal lifespan.

THE ROLES OF FERROPTOSIS IN IMMUNITY TO INFECTION

Pathogens commandeer regulated cell death for their own propagation and survival. For instance, hepatitis B virus (HBV) induces ferroptosis in hepatocytes by suppressing SLC7a11 expression through H3K27Me$_3$ modification, while overexpression of SLC7a11 or treatment with ferrostatin-1 rescues hepatocytes from ferroptosis and prevents acute liver failure.[96] Thus, inducing ferroptosis may be a major mechanism of hepatocyte death in HBV infection (Figure 2A).[96] Interestingly, hepatitis C virus (HCV) itself is sensitive to lipid peroxidation (Figure 2A).[97] As the viral protease is directly regulated by lipid peroxides, lipid peroxidation restricts HCV replication, thereby potentially preventing viral replication in oxidative tissues and allowing for viral persistence.[97] In line with this observation, inducing ferroptosis chemically prevents HCV replication and increases sensitivity to antiviral agents.[97] Whether preventing or inducing ferroptosis alters the therapeutic course of either HBV or HCV in humans remains unexplored. In addition, SARS-CoV-2 infection causes ferroptosis and pyroptosis gene signatures in COVID-19 patients.[98] Whether the genetic or epigenetic reduction in SLC7a11 expression or cystine transport capability mediates susceptibility to viral-induced tissue damage or sensitivity to viral infection has not been investigated.

Bacterial pathogens can also exploit ferroptosis to induce host cell damage and facilitate bacterial replication (Figure 2B). Intracellular pathogens, such as *Mycobacterium tuberculosis* (*Mtb*), reduce the expression of GPX4 and increase free iron, mitochondrial superoxide, and lipid peroxidation in infected host cells, causing ferroptotic cell death (Figure 2B).[99] Moreover, gram-negative bacteria, such as *Pseudomonas aeruginosa*, express lipoxygenase that directly oxidizes host arachidonic acid-phosphatidylethanolamines and triggers ferroptosis in human bronchial epithelia (Figure 2B).[100] This bacterium often causes deadly infection in cystic fibrosis patients. Fungi can also promote ferroptosis: the rice (*Oryza sativa*) fungal pathogen *Magnaporthe oryzae* promotes ferroptosis in host plants but leads to protection from infection, and ferroptosis functions as a defense mechanism in this case.[101] Given that pathogens induce ferroptosis in host cells, combining ferroptosis inhibitors with antibiotics could allow the immune system to effectively restrain this infection.[102]

Ferroptosis also regulates the immune response to pathogens. For example, selenium supplementation increases GPX4 expression in follicular helper T (Tfh) cells and reduces their ferroptosis potential, thereby improving antibody responses in influenza-immunized mice (Figure 2B).[54] In line with this, T cell-specific GPX4-deficient mice cannot fight against infections with acute lymphocytic choriomeningitis virus or *Leishmania* parasite.[54] This immune deficiency can be prevented by high-dose supplementation with vitamin E, a lipophilic antioxidant, which protects GPX4$^{-/-}$ T cells from ferroptosis.[103] Nonetheless, when, and how T cell ferroptosis occurs *in vivo* during the immune response to different pathogen infections is largely unknown.

How pathogens coopt ferroptosis to facilitate their own survival and how host organisms use ferroptosis to prevent infection needs further investigation. How the different forms of cell death regulate each other and communicate between execution pathways in infection is unknown. Understanding what pathogen

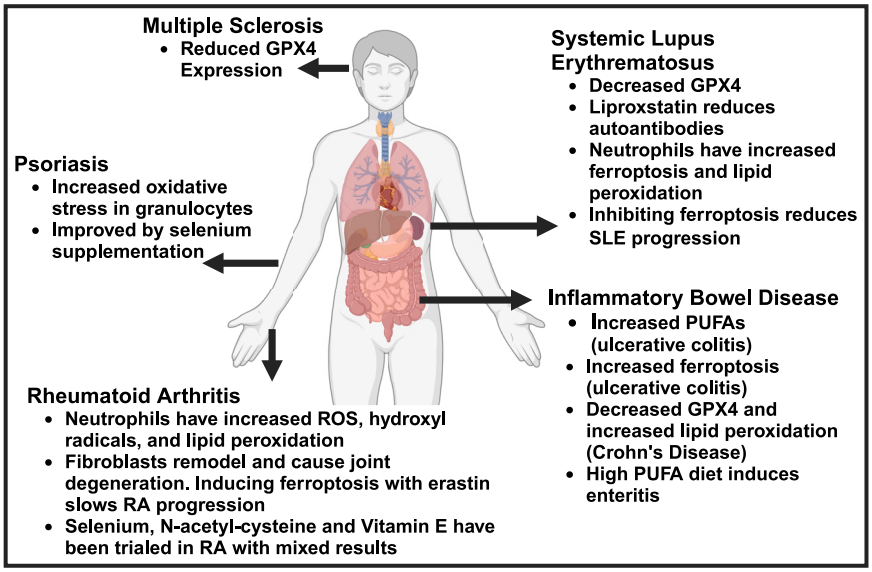

Figure 3. Ferroptosis induces damage in autoimmune diseases

Autoimmune diseases frequently have reduced GPX4 expression and increased lipid peroxidation, but the mechanisms of ferroptosis in this sphere are poorly understood.

factors direct a target cell toward a certain cell death fate could lead to the development of novel classes of antibiotics or antivirals. Ferroptosis may be a double-edged sword in infectious immunity, as different pathogens can either be limited or promoted by ferroptotic death. How both the pathogens themselves and the host immune system react to ferroptosis-modulating agents must be carefully studied for effective clinical translation.

THE ROLES OF FERROPTOSIS IN AUTOIMMUNITY

Ferroptosis can affect the pathology of several autoimmune diseases, including inflammatory bowel disease (IBD), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and multiple sclerosis (MS) (Figure 3).

Ferroptosis contributes to the pathogenesis of IBD (Figure 3). As early as the 1980s, far before the conceptual establishment of ferroptosis, it was reported that patients with ulcerative colitis (UC) had increased PUFAs, suggesting that inflammation alters the lipid composition in the colon.[104] We now appreciate that this lipid alteration may increase the sensitivity of intestinal epithelial cells to ferroptosis.[105] In addition to UC, intestinal epithelial cells in Crohn's disease (CD) have decreased GPX4 activity and increased lipid peroxidation.[106] Treatment with a diet enriched in PUFAs results in neutrophilic granulomas and enteritis in mice lacking one allele of GPX4.[106] Potentially, a high PUFA diet interacts with genetic factors to modulate ferroptosis in patients with autoimmune disease.

RA consists of progressive deterioration of joints mediated by the predominant cellular components of the synovial fluid, such as activated neutrophils and fibroblasts. Neutrophils in the synovial fluid in RA patients exhibit increased oxidative stress, manifested by high ROS, hydroxyl radicals, and lipid peroxidation (Figure 3).[107] Treatment with imidazole ketone erastin, a ferroptosis inducer, reduces the fibroblast number in the synovial fluid in an RA mouse model, and its combinatory therapy with a tumor necrosis factor (TNF) antagonist, etanercept, slows down arthritis progression.[108] Nonetheless, research is lacking on how to target specific cell types with ferroptosis-modulating drugs. This becomes even more important in a disease like RA, where one cell type is damaging another. The published literature does not yet elucidate the mechanism of how oxidation alters RA progression.

The dysregulation of ferroptosis in autoimmune diseases is not limited to IBD and RA. In a mouse model of experimental autoimmune encephalomyelitis (EAE), a murine model of MS, the amounts of GPX4 and SLC7a11 are reduced, along with increased lipid peroxidation, in the gray matter and spinal cord.[109] EAE mice have an abnormal neuronal mitochondrial morphology characteristic of ferroptosis.[109] Inhibiting ferroptosis or reducing ACSL4 expression in EAE mice improves the phenotype, reduces neuronal death, and reduces neuroinflammation.[110] Potentially, ferroptosis occurs early in EAE pathology, and this may promote T cell activation and autoimmunity.[110] This suggests a role for ferroptosis in mediating central nervous system damage in MS.[111]

Few studies have investigated how ferroptosis is induced in SLE. Kidneys isolated from patients or mice with lupus nephritis demonstrate increased lipid peroxidation and increased expression of ACSL4.[112] Serum from patients with lupus nephritis increases the sensitivity of human proximal tubular cells to ferroptosis, suggesting that a serum factor may propagate lupus nephritis.[112] SLE damage may also be mediated through ferroptosis of neutrophils. Neutrophils isolated from an SLE mouse model or patients with SLE undergo increased ferroptosis (Figure 3).[113] Autoantibodies and IFNα enhance the binding of a transcriptional repressor to the GPX4 promoter, which leads to reduced expression of GPX4 and eventual elevation of lipid peroxidation. In addition, mice with neutrophil deletion of one allele of GPX4 exhibit autoantibodies, neutropenia, skin lesions, and proteinuria, which are symptoms of human SLE.[113] Treating SLE-prone mice with a ferroptosis inhibitor reduces disease severity.[113] Thus, neutrophil ferroptosis may play a role in SLE pathology. In psoriasis, an autoimmune skin disease, ACSL4 is upregulated in psoriatic lesions in the epithelium, driving the production of lipid substrates for peroxidation.[114] This promotes an inflammatory response by activating ferroptosis.[114] Inhibiting ferroptosis with ferrostatin suppresses ferroptosis-related changes in erastin-treated keratinocytes and prevents dermatitis in murine models. Together, these data suggest that ferroptosis plays a role in psoriasis.

Overall, autoimmunity is associated with increased lipid peroxidation and ferroptotic cell death in different cell types. Targeting ferroptosis to treat autoimmune diseases may be a therapeutic option.

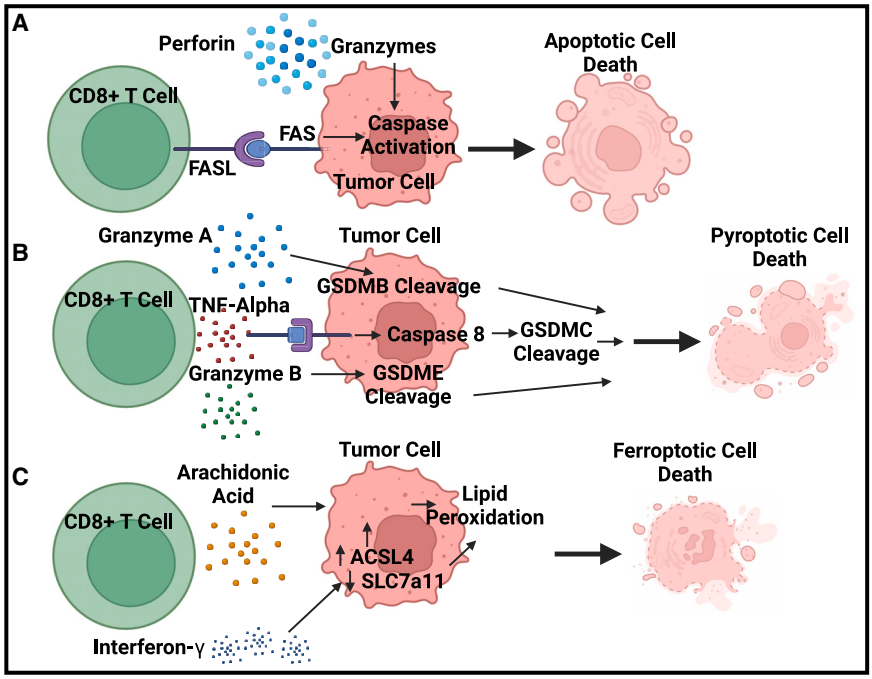

Figure 4. T cells kill tumors through apoptosis, pyroptosis, and ferroptosis

(A) T cells can kill tumors through apoptosis mediated through the perforin-granzyme pathway and the FAS/FAS ligand pathway that both activate caspases.

(B) T cells can kill tumor cells using pyroptosis mediated by the cleavage of gasdermins (GSDMs).

(C) T cell-secreted IFNγ targets system xc and ACSL4 and collaborates with arachidonic acid to induce lipid peroxidation and ferroptotic cell death.

THE ROLES OF FERROPTOSIS IN TUMOR IMMUNITY

Recent research has shed light on the role of ferroptosis in anti-tumor immunity. T cells use several mechanisms to induce apoptosis in cancer cells, including the Fas and Fas-ligand pathway and the perforin-granzyme pathway. Both pathways activate caspases, inducing apoptosis (Figure 4A). T cells can also induce a regulated cell death known as pyroptosis (Figure 4B).[115,116] Granzyme A, secreted by natural killer (NK) cells, and cytotoxic T lymphocytes (CTLs) can kill through pyroptosis.[116] Granzyme A cleaves gasdermin B, which unleashes its capability of forming pores.[116] Gasdermin B is highly expressed in some tumors derived from the digestive tract.[116] This suggests that granzyme-A-induced pyroptosis may be an important anti-tumor immunity mechanism in some tumors. Notably, killer cells can activate caspase-independent pyroptosis via granzyme B by directly cleaving gasdermin E.[115]

Interestingly, activated T cell-derived IFNγ combined with specific fatty acids in the TME can induce tumor cell ferroptosis (Figure 4C).[16,39] IFNγ can be released from several immune cell subsets, such as T helper-1 (Th1) cells, NK cells, and natural killer T (NKT) cells. Hence, IFNγ-producing immune cells can trigger cell ferroptosis in a specific lipid-enriched microenvironment. Given that most ferroptosis studies use synthetic chemical inducers, such as RSL3 and erastin, the identification of IFNγ and fatty acids as endogenous ferroptosis inducers provides information critical to understanding ferroptosis *in vivo*.[16] Other potential endogenous inducers remain to be discovered in future studies.

Tumor ferroptosis contributes to cancer immunity and immunotherapy. Immunotherapy stimulates $CD8^+$ T cells to produce IFNγ. On the one hand, IFNγ reduces the expression of system xc- (SLC3A2 and SLC7A11), thereby impairing the uptake of cystine by tumor cells, abolishing tumor cell GSH, and consequently sensitizing tumors to ferroptosis.[39] On the other hand, IFNγ stimulates ACSL4 expression and alters tumor cell lipid profiles in the presence of arachidonic acid and other fatty acids, thereby increasing incorporations of arachidonic acid into C16 and C18 acyl chain-containing phospholipids and inducing tumor cell ferroptosis.[16] Thus, tumor-infiltrating T cells can induce tumor cell ferroptosis through multiple mechanisms.[16,39] High expression of the cystine import machinery and low amounts of ACSL4 in tumors are associated with poor cancer patient outcomes. Thus, inducing ferroptosis alongside immune checkpoint blockade (ICB) is a potential therapeutic approach.[16,39] Similarly, $CD8^+$ T cells can sensitize cancer cells to cisplatin-based chemotherapy via IFNγ.[117] Cisplatin can be chelated by GSH, and the GSH-platinum complex subsequently effluxes from the tumor cells, causing tumor chemoresistance.[117] IFNγ inhibits system xc-, diminishing cystine and GSH in tumor-associated fibroblasts.[117] This results in reduced cellular GSH in tumor cells, decreased GSH-platinum complex formation, increased intracellular platinum accumulation, and increased tumor killing.[117] Ferroptosis also mediates tumor cell death induced by tumor radiotherapy.[36] Radiotherapy-activated ataxia telangiectasia mutated (ATM) synergistically interacts with IFNγ to suppress SLC7A11 expression and cystine uptake in tumor cells.[36] This leads to increased tumor cell ferroptosis and tumor control in radiation therapy.[36] Activation of the ER stress response factor X-box binding protein (XBP1) in DCs suppresses anti-tumor immunity by driving abnormal lipid accumulation.[118] This lipid accumulation inhibits DC and T cell interaction.[118] Silencing XBP1 in DCs improved their anti-tumor functions and immunostimulatory dendritic cell function.[118] Although this paper did not demonstrate directly that XBP1 activation induced ferroptosis in DCs, it suggests that targeting the ER stress response pathway may enhance anti-cancer immunity as well as directly induce tumor death. Altogether, ferroptosis is an emerging mechanism of immune killing of tumor cells.

The role of granulocyte ferroptosis in the TME is a developing area of research. In a murine glioblastoma model, neutrophils isolated from brain tumors kill tumor cells through ferroptosis.[119] Neutrophils induce lipid peroxides in tumor cells by transferring myeloperoxidase into tumor cells through granules.[119] In human glioblastoma, ferroptosis is associated with necrosis and

predicts poor survival.[119] Potentially, ferroptosis drives necrosis in brain tumors and is pro-tumorigenic. However, the crosstalk between ferroptotic, necrotic, and apoptotic pathways and how this impacts the immune response to tumors is unknown.

How phagocytes clear ferroptotic cells is also poorly understood. One key phagocytic signal on the ferroptotic cell surface is oxidized phospholipid, 1-steaoryl-2-15-HpETE-sn-glycero-3-phosphatidylethanolamine (SAPE-OOH).[120] Enriching with SAPE-OOH improves macrophage phagocytosis of ferroptotic cells mediated through toll-like receptor 2.[120] TLR2 is critical for the clearance of ferroptotic cells in an *in vivo* mammary tumor mouse model as well.[120] How SAPE-OOH is regulated and whether this TLR pathway is immunogenic or immunosuppressive must still be investigated.

Ferroptotic tumor cells may regulate immune cell infiltration and activity in the TME. DCs are professional APCs. How ferroptotic cancer cells impact DC function and tumor immune responses remains uncertain.[121,122] Wiernicki and colleagues report that co-culturing of early ferroptotic cancer cells with DCs reduces DC maturation and antigen cross-presentation.[122] Wiernicki et al. found that DCs loaded with ferroptotic, in contrast to necroptotic cancer cells, cannot protect against tumor growth and that ferroptotic cancer cells reduce the vaccination potential of apoptotic cancer cells.[122] By contrast, Efimova et al. indicate that ferroptosis is immunogenic *in vitro* and *in vivo* and that ferroptotic tumor cells promote DC maturation and elicit a vaccination-like effect in immune-competent mice.[121] However, in the Efimova et al. paper, tumor growth at the vaccination site was not considered, making it difficult to distinguish between vaccination with living tumor cells and cells undergoing the earliest stages of ferroptosis.[121] For a viable translatable vaccine, no tumor growth at the vaccination site is critical, limiting the potential of using early ferroptotic cells as an immunogen. These conflicting reports have not fully revealed the causal mechanisms linking ferroptosis and DCs. The discrepancy may be related to different experimental conditions, such as different chemical inducers *in vitro*, different types of tumor cells, different exposure times to tumor cells with these ferroptosis-inducing chemicals, and different degrees of tumor cell ferroptosis. The latter is critical, as the remaining live tumor cells post-ferroptosis inducers are ferroptosis-resistant cells, which likely possess an aggressive tumor phenotype, potentially generating conflicting results.

Ferroptosis of immune cell subsets may alter tumor immune responses in the TME. A recent study shows that granular myeloid-derived suppressor cells (G-MDSCs), which are neutrophils, are sensitive to ferroptosis in the TME.[123] These ferroptotic neutrophils release oxidized lipids and limit the activity of T.[123] By contrast, a different report demonstrates that tumor-infiltrating neutrophils are resistant to ferroptosis.[124] Mechanistically, tumor-infiltrating neutrophils produce itaconate, which prevents ferroptosis and allows neutrophils to remain live in the TME.[124] Notably, tumor-associated G-MDSCs, namely neutrophils, are well established as immunosuppressive entities, irrespective of their involvement in ferroptosis. A direct comparison regarding whether apoptotic, ferroptotic, or live neutrophils in the TME are differentially immunosuppressive is currently lacking in the literature. Hence, it is misleading to conclude that because ferroptotic neutrophils may have an immunosuppressive role, tumor microenvironmental ferroptosis is immunosuppressive and pro-tumorigenic. Rather, it is likely that the context and cellular composition of the TME are critical to whether ferroptosis is immunosuppressive or immunogenic. This provides additional complications to inducing ferroptosis to inhibit tumor growth, as other cell types are susceptible to ferroptosis, and may inhibit anti-tumor immunity. Currently, it is impossible to induce ferroptosis in a cell-type-specific manner without genetic modulation, but cell-type targeting is likely required before ferroptosis induction is adapted widely as a cancer-fighting strategy. Similar concerns can be implied in inducing cancer apoptosis and pyroptosis for therapy.

In addition to neutrophils, ferroptosis of T cell subsets has been observed in different settings, including in tumors. Interestingly, human lymphocytes have surprisingly low cystine transport activity even after activation.[125] Loss of GPX4 in regulatory T cells (Tregs) leads to lipid peroxidation and ferroptosis in Treg cells after T cell-receptor signaling.[126] As expected, ablation of Treg cell GPX4 represses tumor growth and potentiates anti-tumor immunity.[126] By contrast, genetic deletion of GPX4 in $CD8^+$ T cells led to the failure of antigen-stimulated T cell expansion and reduced pathogen immunity.[103] This is attributed to the rapid accumulation of membrane lipid peroxides and ferroptosis in $CD8^+$ T cells.[103] Genetic deletion of GPX4 in T cells also prevents the generation of Tfh cells and germinal center responses.[54] This potentially curtails antibody responses. Together, these data suggest that GPX4 is critical to protect T cells from lipid peroxidation and ferroptosis. Notably, although T cell proliferation *in vitro* is dependent on system xc-, the cystine-glutamate antiporter, it appears that system xc- is not essential for T cell proliferation *in vivo* and the generation of primary and memory immune responses to tumors.[127] It is possible that the importance of extracellular cystine in T cells is compensated by alternative mechanism(s).[127] However, the differences in ferroptosis induced by cystine depletion or xCT inhibition compared with ferroptosis induced by GPX4 depletion are not well characterized.

Tumors often have aberrantly regulated lipid production. How this dysregulation mediates ferroptosis in tumor cells or the anti-tumor immune response is poorly understood. It has been reported that tumor-infiltrating T cells accumulate lipids.[128,129] This accumulation is dependent on CD36, a scavenger receptor for oxidized lipids.[129] Knocking out CD36 allowed T cells to maintain their effector function in the tumor compared with wild-type T cells.[129] CD36 promotes the uptake of oxidized low-density lipoproteins into T cells, which induces lipid peroxidation.[129] In support of this work, ablation of CD36 in effector $CD8^+$ T cells led to an increase in cytotoxic cytokines and increased tumor clearance.[128] Thus, blocking CD36 or inhibiting ferroptosis in $CD8^+$ T cells could restore anti-tumor immunity and potentially increase the efficacy of ICB.[128,129]

The functional importance of ferroptosis-regulatory genes in different cell types in the TME is slowly being dissected. However, there are many unanswered questions: in what contexts is ferroptosis immunogenic; what damage-associated molecular patterns are present in cells undergoing ferroptosis; what TME cells initiate the response to cells dying of ferroptosis; what propagates immune response to the tumor over time; what and how potential crosstalk among different death pathways is in the TME; and how can we optimize pro- vs. anti-ferroptotic agents

to control the balance between help and harm in tumor suppression? Answering these questions will reveal the nature of ferroptosis in the TME and identify novel cancer treatment modalities.

THERAPEUTICALLY TARGETING FERROPTOSIS

No current ferroptosis-modulating drugs are used to treat infectious diseases. Proof-of-concept studies have demonstrated that inducing bacterial ferroptosis may lead to the development of a new class of antibiotics. Treatment with ferrous-sulfate-loaded hydrogel induces a variety of ROS, lipid peroxidation, and ferroptosis in *Staphylococcus aureus (S. aureus)*.[130] This iron-sulfate-based drug kills *S. aureus* persister cells and biofilms, both of which are canonically resistant to antibiotics, prevents the dissemination of *S. aureus* to the lung, and reduces systemic inflammation.[130] Moreover, arachidonic acid induces lipid peroxidation and death in *S. aureus*.[131] Mechanistically, arachidonic acid is oxidized and modifies macromolecules in the bacteria, inducing toxicity.[131] Treatment with antioxidants prevents *S. aureus* death after exposure to arachidonic acid.[131] Thus, inducing ferroptosis in bacteria may treat infections resistant to traditional antibiotics and is an approach to develop a new class of antibiotics.

Anti-ferroptotic modulation, partly via dietary supplementation, has been investigated to modulate autoimmune disease progression. Several studies report that antioxidants do not change clinical indexes of inflammation in RA.[132,133] However, N-acetylcysteine, a cysteine donor, improves the clinical presentation of RA and SLE.[132,134] Selenium supplementation improves RA patients' rating of their health compared with a placebo and leads to normalized oxidative stress markers of granulocytes and improved clinical conditions in psoriatic patients.[113,135] The current literature does not provide a consensus about how modulating ferroptosis through diet could prevent the pathology of autoimmune disease. Understanding the temporal ferroptosis involvement, cell types undergoing ferroptosis, and ferroptotic molecular pathways will be required to achieve translational impact.

Preclinical studies have shown that targeting tumor ferroptosis pathways is a promising anti-cancer therapeutic strategy. In fact, erastin was identified as a ferroptosis inducer, in a screen for anti-cancer drugs. Treatment with imidazole ketone erastin, a potent ferroptosis inducer, induces GSH depletion, increases lipid peroxidation, and slows down tumor growth in murine models of diffuse large B cell lymphomas.[136] As cysteine is critical for the survival of some cancer cells in oxidative conditions, treatment with cysteinase, an agent that depletes cysteine and cystine, inhibited tumor growth *in vivo*.[39,136] Sorafenib is the first-line drug for advanced hepatocellular carcinoma (HCC).[137] Some studies suggest that Sorafenib induces an iron-dependent ferroptotic tumor cell death.[138,139] Solute carrier family 27 member 5 (SLC27A5) is downregulated in sorafenib-resistant HCC.[138] SLC27A5 is involved in fatty acid elongation and complex lipid synthesis, and its deficiency suppresses ferroptosis and drives sorafenib resistance. However, whether sorafenib truly induces ferroptosis has recently been up for debate. A recent study utilizing genetically engineered cell lines and canonical ferroptosis inhibitors showed that sorafenib does not appear to induce ferroptosis in a panel of tumor cell lines.[139] Whether sorafenib can induce ferroptosis in certain *in vitro* contexts or *in vivo*, and the underlying reasons for the discrepancy between these reports, is unknown.

As noted above, immune cell subsets are subjected to ferroptosis regulation in the TME. Ferroptosis-inducing drugs may indiscriminately induce ferroptosis in both cancer cells and other cells in the TME, such as immune cells. Recent drug screening has identified, N6F11, a previously unstudied small molecule, can trigger selective degradation of GPX4 in cancer cells, but not in DCs, T cells, and NK cells.[140] Treatment with N6F11 induces ferroptotic cancer cell death and sensitizes pancreatic tumor response to ICB.[140] It remains to assess how N6F11 mediates tumor-selective targeting. Given that increased microenvironmental iron can mediate ferroptosis, magnetic fields can be used to deliver iron oxide to the TME to trigger tumor cell ferroptosis and inhibit tumor progression.[141]

Combining ferroptosis induction with well-established cancer therapies, such as immunotherapy, radiotherapy, and chemotherapy, has been experimentally evaluated.[36,142,143] Several approaches to induce ferroptosis in tumors are actively being explored. Biomimetic magnetic nanoparticles generate a mild immune response and improve the efficacy of immunotherapy in a murine metastatic breast cancer model.[144] Another potential approach is to combine several approaches, including thermal energy, ferroptosis, and immunotherapy. A gel delivery therapy platform combined with embedded gold nanorods, and iron oxide nanoparticles allows for targeted photothermal therapy and ferroptosis to trigger both innate and adaptive immunity.[145] Recently, a novel approach of a six enzyme co-expressed nanoplatform, including lipoxygenase and phospholipase A2 co-loaded FeCo/Fe-Co dual metal atom nanozyme, induces initial ferroptosis and upregulates arachidonic acid expression and synergizes with IFNγ to induce immunogenic ferroptosis.[146] Inducing ferroptosis by depleting cysteine, intermittent methionine intermittent deprivation, inhibiting system xc-, or targeting GPX4 can increase the therapeutic efficacy of ICB in tumor-bearing animal models.[39,136,147,148] Interestingly, human obesity is associated with an increased response to ICB, the phenomenon termed the "obesity-immunotherapy paradox.[149]" This paradox may be partially explained because ICB-activated immune cells promote and induce cancer ferroptosis via IFNγ, in collaboration with microenvironmental fatty acids.[16] No direct evidence has yet demonstrated that obese patients with cancer have a distinctly favorable lipidome. However, fatty acids serum and feces profiles do correlate with ICB efficacy in patients with cancer.[150,151] Thus, it is possible that obese patients with cancer may manifest lipid metabolism favorable for immune-induced tumor ferroptosis, although this has not been directly demonstrated. Overall, current basic and translational studies have generated rationales for the development of ferroptosis therapeutics in infectious diseases, autoimmune diseases, and cancers.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Compelling evidence demonstrates that ferroptosis participates in the regulation of immune responses. Several molecular pathways, including system xc-, GPX4, the lipid peroxidation and detoxification system, ACSL-mediated fatty acid activation, the

FSP1-coenzme Q_{10} system, and DHODH-associated mitochondrial pathway, can regulate ferroptotic cell death.[152] Unlike apoptosis and pyroptosis, there is no identified execution gene for ferroptosis. This not only highlights mechanistic distinctions among the different types of regulated cell death but also demands the necessity of further understanding the relationship between ferroptosis-promoting and ferroptosis-inhibiting signals and environmental contexts in physiological and pathological settings.

Current studies point toward the involvement of several cellular organelles in ferroptosis. The roles of mitochondria, ER, Golgi, lysosome, and plasma membrane have been examined in ferroptotic cell death in different experimental settings, but the specific cell types, timing, instigators, microenvironmental factors, and intervention points need to be clarified.[58] We posit that different ferroptosis-modulating determinants alter different organelles, as some act through the mitochondria, some through the ER, and some through the Golgi. Nonetheless, no matter the organellar path to get there, the final and irreversible mechanism of ferroptosis may be cellular membrane damage due to lipid peroxidation (Figure 1). Potentially and arguably, the mitochondria act as a central hub for the induction of cell death and communication between different cell death pathways.

It will be important to further elucidate the precise molecular and cellular regulation used by the immune system to regulate ferroptotic cell death. In the presence of specific fatty acids, effector T cells inhibit system xc- and stimulate ACSL4 via IFNγ to promote and induce tumor cell ferroptosis.[16,39] This contributes to T cell tumor killing and ICB. Based on these discoveries, it is reasoned that activated immune cells, such as T cells, NK cells, and NKT cells, may similarly induce ferroptotic cell death in the disease microenvironments of infection and autoimmune disorders. Ferroptosis generates thousands of diverse lipid peroxidation byproducts from hydroperoxyl lipids to secondary electrophilic oxidatively truncated species. It is known that some lipid species may form covalent adducts with proteins and attach to biomolecules. On the one hand, this may generate potential neo-antigens, offering an opportunity to harness a response to these neo-antigens against cancer. On the other hand, these lipid-peroxidation-induced neo-antigens may stimulate autoimmune responses via self-antigen cross-reactivity. Thus, exploration of the interaction between oxidized lipids and proteins in ferroptosis may yield novel insight into ferroptosis biology.

Finally, oxidative stress is common to different forms of regulated cell death, including ferroptosis, apoptosis, and pyroptosis. However, phospholipid peroxidation is specific to ferroptosis. The unique feature of ferroptosis provides additional access points to modulate cell death. Mitochondrial and ER stress, induced by cellular metabolism, immune stress, and therapeutic intervention, may be a crossroad for interaction among different forms of regulated cell death. All forms of cell death end with the disintegration of the plasma membrane and dissolution of the cell, but the triggering pathways to this end differ wildly. Iron dependency and lipid peroxidation are specific to ferroptosis. Functionally, the role of apoptosis is initially and predominantly defined in the development of tissue and organ and cell differentiation, while pyroptosis is heavily involved in infection control, and ferroptosis lies somewhere in between—with developing roles in homeostasis and established roles in autoimmunity, pathogenicity, and tumor. A future research direction would be to explore whether, how, and when crosstalk among different forms of regulated cell death determines the fate of infectious diseases, autoimmune diseases, and tumors.

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AUTHOR CONTRIBUTIONS

B.R.S., W.Z., and H.N.B. conceived the idea and outlined this review article. H.N.B. wrote this review article and designed all figures. B.R.S., W.Z., and H.N.B. edited, finalized, and approved this paper.

DECLARATION OF INTERESTS

B.R.S. is an inventor on patents and patent applications involving ferroptosis; co-founded and serves as a consultant to ProJenX, Inc. and Exarta Therapeutics; holds equity in Sonata Therapeutics; and serves as a consultant to Weatherwax Biotechnologies Corporation and Akin Gump Strauss Hauer & Feld LLP. W.Z. has served as a scientific advisor or consultant for Cstone, NextCure, and HanchorBio Inc.

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