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Ferroptosis: an emerging player in immune cells

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Ferroptosis is an iron-dependent form of cell death characterized by an accumulation of lipid peroxides. A growing body of recent evidence supports the notion that ferroptosis plays an important role in mediating a wide variety of cellular processes in diseases. Notably, ferroptosis can play a significant role in mediating various functions in immune cells and immunotherapies. Here, we discuss our current understanding regarding the regulatory role of ferroptosis in immune cells, including T cells, B cells, granulocytes, monocytes, and macrophages. In addition, we discuss the general effect of immune cell ferroptosis on human pathophysiology and immunotherapies, thereby suggesting new strategies for targeting ferroptosis in order to modulate the immune system and unravel the mechanisms that underlie ferroptosis in the immune response.

The term "ferroptosis" was first coined by Brent Stockwell's group in 2012 [1], in which they described a form of cell death distinct from other forms such as apoptosis, autophagy, and necroptosis. Ferroptosis is regulated by multiple layers of metabolic signaling pathways and processes, including cellular oxidative-redox status, iron metabolism, lipid metabolism, energy metabolism, and mitochondrial function [2]. A major feature of ferroptosis is iron-dependent lipid peroxidation, which is normally suppressed by either glutathione peroxidase 4 (GPX4) and the recently identified ferroptosis suppressor protein 1 (FSP1). GPX4 suppresses phospholipid peroxidation using glutathione as its substrate, while FSP1 blocks ferroptosis via the nicotinamide adenine dinucleotide (NADH)-FSP1-coenzyme Q10 (CoQ10) pathway and does not require glutathione. In addition, several iron transporters and regulators that affect iron accumulation, including ferroportin-1 (FPN1, also known as SLC40A1), divalent metal transporter 1 (DMT1), transferrin receptor 1 (TfR1), ferritin, ZRT/IRT-like protein 14 (ZIP14, also known as SLC39A14), and heme, also play a role in ferroptosis. Recently, ferroptosis has been shown to play an important role in a wide variety of diseases and pathologies, including cardiomyopathy, neurodegeneration, cancer, stroke, kidney and liver injury [3]. In addition, both activators and inhibitors of ferroptosis have been identified and used to study the molecular mechanisms that underlie ferroptosis, thus paving the way toward designing new therapeutic strategies for treating ferroptosis-related diseases [4].

The mammalian immune system is comprised of innate and adaptive components, both of which are vital for maintaining health. The innate immune system consists of protective barriers mediated by certain tissues, as well as specific immune cells such as monocytes, macrophages, dendritic cells, granulocytes, and natural killer (NK) cells. Compared to the adaptive immune response, the innate immune response is rapid but relatively nonspecific. Working in concert with the innate immune system, the adaptive immune system mainly provides protection via lymphocytes, including T cells and B cells. The adaptive immune response is antigen-specific and generates an advanced immunological memory. These two immune processes interact with each other and form a complex, highly efficient immune network; moreover, optimizing the immune response not only protects the host, but also ensures that the body's immune cells do not attack normal tissues. Most importantly, targeted immunoregulators have been used to treat a variety of diseases, including cancer; thus, understanding the mechanisms that underlie the immune response has high clinical relevance.

Even before the discovery of ferroptosis, lipid peroxidation was linked to a variety of immune responses [5], and iron metabolism has long been known to regulate immune function and maintain immune system homeostasis; thus, the existence of a putative link between ferroptosis and the immune system was not surprising. Indeed, after ferroptosis was first identified, studies revealed that ferroptosis plays a direct role in the immune response. On one hand, just like other cell types immune cells undergo ferroptosis under specific conditions, causing impaired immune activity. On the other hand, cytokines released by immune cells can induce ferroptosis in tumor immunotherapy, thereby enhancing the immune effect. These findings have offered novel insights into immunological research, and summarizing these findings can draw attention to the relationship between ferroptosis and immunity. Below, we discuss recent progress regarding the role of ferroptosis in the immune system, with a focus on immune cells.

T cells. As an important component of the adaptive immune system, T cells originate in the bone marrow and mature in the thymus. Following a series of developmental steps, double-negative ($CD4^-CD8^-$) T cells develop into naïve single-positive ($CD4^+$ or $CD8^+$) T cells, which then enter the periphery. In the periphery, naïve $CD4^+$ T cells differentiate further into various T cell subsets, including Th (helper T (Th) cells, follicular helper T (Tfh) cells, and regulatory T (Treg) cells. The principal role of $CD8^+$ T cells is

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to directly kill target cells via their cytotoxic function; thus, $CD8^+$ T cells play an essential role in providing immunosurveillance against tumor cells and infection.

In cancer immunotherapy, $CD8^+$ T cells are believed to induce the death of cancer cells by activating the perforin-granzyme and Fas-Fas ligand pathway. Weiping Zou's group recently reported that regulating ferroptosis can increase the anti-tumor effects of $CD8^+$ T cells [6]; specifically, treating tumor-bearing mice with an antibody to block programmed death-ligand 1 (PD-L1) induced ferroptosis and increased lipid peroxidation in tumor cells. Mechanistically, the release of interferon gamma (IFNγ) by $CD8^+$ T cells significantly downregulated SLC3A2 and SLC7A11, two components of the glutamate-cystine antiporter system x_c^-, in tumor cells; the resulting reduce in cystine uptake induces lipid peroxidation and ferroptosis in the tumor cells (Fig. 1a). In addition, depriving tumor cells of cystine combined with the PD-L1 antibody

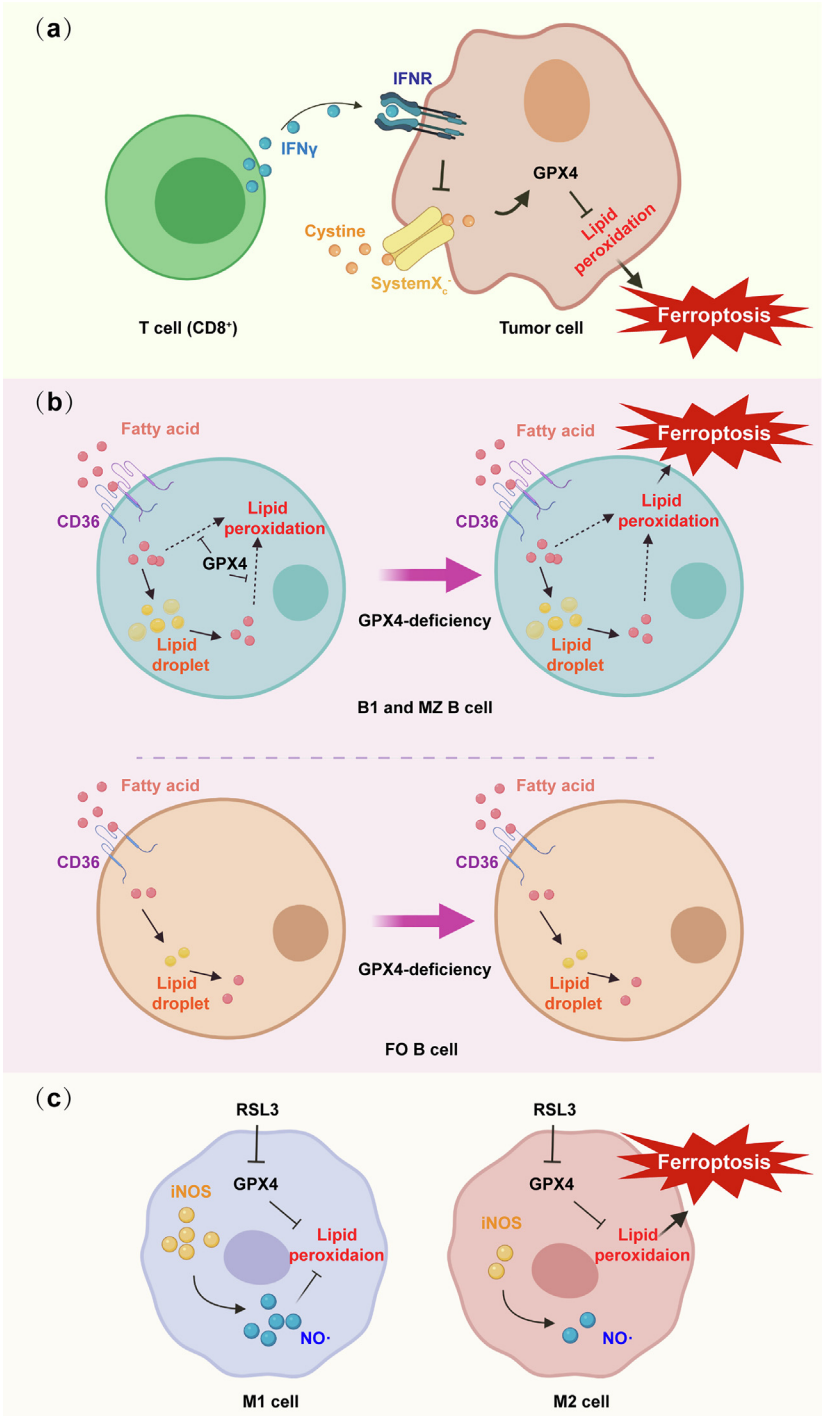

Fig. 1. Overview of the regulatory functions of ferroptosis in various immune cells. (a) In cancer immunotherapy, the release of IFNγ by T cells induces ferroptosis in tumor cells by downregulating the glutamate-cystine antiporter system x_c^-. (b) Ferroptosis induces different responses in specific B cell subtypes. Top, B1 cells and MZ B cells express high levels of the fatty acid transporter CD36, resulting in increased uptake of fatty acids and increased lipid peroxidation; thus, GPX4-deficient B1 and MZ B cells are more sensitive to ferroptosis. Bottom, in contrast, GPX4-deficient follicular (FO) B cells are less susceptible to ferroptosis due to reduced levels of intracellular fatty acids. (c) M1 and M2 macrophages differ with respect to their susceptibility to ferroptosis. M1 macrophages are less susceptible to ferroptosis induced by the GPX4 inhibitor RSL3 due to increased levels of nitric oxide free radicals (NO·), which inhibit lipid peroxidation. In contrast, M2 macrophages have less expression of iNOS and are more susceptible to RSL3-induced ferroptosis.

synergistically induced tumor ferroptosis and increased T cell immunity [6]. This study was the first to characterize ferroptosis as a novel anti-tumor mechanism, suggesting that this process could be targeted in cancer immunotherapy. Recently, Xu et al. [7] found that oxygen-boosted photodynamic therapy with a nanoplatform of ferrous hemoglobin can induce T cells to release IFNγ, which sensitizes cancer cells to ferroptosis.

As mentioned above, T cells can induce ferroptosis in tumor cells. Interestingly, T cells themselves can undergo ferroptosis, thereby preventing their immune response to infection. For example, $CD4^+$ T cells and $CD8^+$ T cells lacking GPX4 undergo ferroptosis with an accumulation of lipid peroxides in LOXs-independent manner [8]. Thus, these T cells fail to expand and do not suppress infection with the acute lymphocytic choriomeningitis virus or the parasite *Leishmania major*, indicating that GPX4-dependent ferroptosis plays an essential role in T cell–mediated immunity. Moreover, Drijvers et al. recently found that ferroptosis-resistant $CD8^+$ T cells overexpressing either FSP1 or GPX4 have normal immune functions; in contrast, deleting the ferroptosis sensitivity–promoting enzyme acyl-CoA synthetase long-chain family member 4 (ACSL4) also protects $CD8^+$ T cells from ferroptosis, but impairs their immune response [9]. Thus, understanding ferroptosis in T cells would not only unravel the molecular mechanisms underlying the immune response, but would also help guide the design of new immune-related treatments.

B cells. B cells produce and release antibodies against specific antigens and are therefore an essential component of the humoral immune response. Conventional B cells (commonly referred to as B2 cells) terminally differentiate into plasma B cells and memory B cells upon activation. Other B cell subtypes include B1 cells, marginal zone (MZ) B cells, follicular B cells, and regulatory B (Breg) cells. Follicular B cells are the largest population of B cells and are the principal B cell type involved in humoral immunity.

B cell subtypes differ with respect to their sensitivity to ferroptosis [10]. Interestingly, the ferroptosis regulator GPX4 is not required for the development and immune responses of follicular B cells, but is required for B1 and MZ B cells, as loss of *GPX4* expression in B1 and MZ B cells increases lipid peroxidation and ferroptosis (Fig. 1b); importantly, these B cells do not generate an efficient antibody response against *Streptococcus pneumoniae*. This difference between B cell subtypes can be explained by the fact that compared to follicular B cells, both B1 and MZ B cells express higher levels of the fatty acid transporter CD36 and therefore take up higher levels of fatty acids.

Neutrophils and eosinophils. Granulocytes are generally considered to be an essential part of the innate immune system by eliminating invading pathogens. Based on Wright's staining, granulocytes are classified as neutrophils (the most abundant granulocytes), eosinophils, and basophils. Single-cell RNA sequencing has shown that neutrophils in liver metastases from colorectal cancer have abnormal ferroptosis, providing the first evidence that ferroptosis occurs in immunocytes in the tumor microenvironment [11]. In allergic inflammation of the airway, inducing ferroptosis in eosinophils has a synergistic effect with glucocorticoid therapy, thereby alleviating the underlying symptoms [12]. Nevertheless, many questions remain with respect to the role of ferroptosis in granulocytes.

Mononuclear phagocytes. The mononuclear phagocyte system is comprised of progenitor cells, circulating monocytes, tissue-resident macrophages, and dendritic cells (DCs). Monocytes are derived from hematopoietic stem cells in the bone marrow, giving rise to DCs and macrophages, providing the principal source of tissue macrophages. The primary function of macrophages is to eliminate pathogens in order to maintain immune homeostasis. Traditionally, macrophages are classified as pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages.

The GPX4 inhibitor Ras-selective lethal small molecule 3 (RSL3) has been widely used to induce ferroptosis. Interestingly, M1 and M2 macrophages differ with respect to their susceptibility to RSL3-induced ferroptosis [13]. M1 macrophages express higher levels of the enzyme inducible nitric oxide synthase (iNOS) compared to M2 macrophages; as a result, M1 macrophages are more resistant to ferroptosis. Downregulating iNOS in M1 macrophages increases their sensitivity to ferroptosis, while treating M2 with nitric oxide free radicals ($NO\cdot$) reduces their susceptibility to ferroptosis (Fig. 1c). Furthermore, iNOS/$NO\cdot$ regulates ferroptosis in macrophages by reprogramming redox lipids, providing a novel mechanism by which ferroptosis controls the immune responses. Interestingly, ferroptotic tumor cells can cause the conversion of M2 macrophages to M1 macrophages during radiotherapy and immunotherapy [14]; moreover, the ferroptosis-induced release of mutant Kirsten rat sarcoma 2 viral oncogene homolog $(KRAS)^{G12D}$ from tumor cells can cause macrophages to polarize into the pro-tumor M2 phenotype [15]. These findings provide compelling evidence that ferroptosis may serve as a promising therapeutic target by driving immune reprogramming.

In addition to inhibiting GPX4, other stimuli can also induce ferroptosis in mononuclear phagocytes. For example, ferric citrate has been shown to potently induce ferroptosis in bone marrow–derived macrophages (BMDMs), and loss of SLC7A11 promotes iron overload–induced ferroptosis in BMDMs [16]. Moreover, macrophages can undergo ferroptosis induced by robust erythrophagocytosis, and macrophage homeostasis can be restored by self-maintenance and by differentiation of circulating monocytes [17]. Recently, Zhang et al. [18] found that radiation-induced hemorrhage causes iron overload in the bone marrow and subsequently triggers ferroptosis in macrophages derived from hematopoietic progenitor cells.

On the other hand, the monocyte-phagocyte system can be less susceptible to ferroptosis under certain circumstances. For example, human peripheral blood mononuclear cells (PBMCs) are resistant to ferroptosis induced by the system x_c^- inhibitor erastin; in contrast, erastin promotes the proliferation and differentiation of human PBMCs into B cells and NK cells by modulating bone morphogenetic proteins (BMPs) [19]. Similarly, macrophages that express p53 with the P47S mutation ($p53^{P47S}$) are resistant to treatment with ferroptosis inducers [20]. In this case, iron accumulates in the macrophages, altering the cytokine profile, increasing arginase levels, and decreasing NOS activity; thus, introducing the $p53^{P47S}$ mutation in mice increases their susceptibility to bacterial infection but improves their response to malarial toxins. In contrast, treating $p53^{P47S}$ macrophages and mice with the iron chelator deferoxamine reduces iron concentrations and suppresses bacterial infection. Despite these promising findings, however, the precise mechanisms by which the $p53^{P47S}$ mutation regulates ferroptosis, iron content, and bacterial infection are poorly understood and warrant further study.

In summary, a variety of immune cells—including T cells, B cells, granulocytes, monocytes, and macrophages—can undergo ferroptosis, thereby affecting the immune response. From a clinical perspective, T cells can promote tumor ferroptosis in cancer immunotherapy, providing a novel anti-tumor mechanism. Notably, distinct populations of B cells and macrophages have a wide range of sensitivity to ferroptosis; indeed, some immune cells can be resistant to ferroptosis under certain circumstances. Moreover, loss of GPX4 in myeloid cells causes pyroptosis (an inflammatory form of programmed cell death commonly associated with infection with intracellular pathogens), but not ferroptosis, during polymicrobial sepsis [21]. Therefore, in addition to GPX4, other molecules such as FSP1 may also play a role in ferroptosis-resistant immune cells. Nevertheless, additional research is needed in order to identify the molecular mechanisms that underlie ferroptosis in specific immune cells. Moreover, strikingly little is currently known regarding the role of ferroptosis in immunity, as ferroptosis reduces immune cells.

Although the precise role of mitochondria in ferroptosis is currently under debate, morphological change in the mitochondria is a specific characteristic of ferroptosis. Given the importance of the role that mitochondria plays in the immune system, this organelle may serve as a site of convergence between ferroptosis and immunity.

Clinically, the involvement of ferroptosis in cancer immunotherapy suggests a new therapeutic strategy against invading pathogens. Importantly, both ferroptosis and immunity are closely related to a variety of diseases and conditions, including cancer, neurodegeneration, and organ damage. Recently, Efimova et al. [22] found that early ferroptotic cancer cells are immunogenic and may promote the phenotypic maturation of bone marrow–derived dendritic cells and activation of the adaptive immune system. Perhaps even more interesting, oxidized phospholipids present on the surface of ferroptotic cells are recognized by toll like receptor 2 (TLR2) on macrophages, leading to the phagocytosis of ferroptotic cells [23].

It is reasonable to speculate that ferroptosis and the immune response can mutually affect each other under specific circumstances. For example, several ferroptosis-related metal transporters such as ZIP14 may also play a regulatory role in the immune system [24]. Thus, unraveling the molecular mechanisms that underlie ferroptosis in immune cells will facilitate the development of novel therapeutic strategies designed to treat a wide range of diseases.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary materials

Supplementary materials to this article can be found online at https://doi.org/10.1016/j.scib.2021.02.026.

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