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REVIEW | *Adaptive Immunity in Cardiovascular Disease*

The adaptive immune role of metallothioneins in the pathogenesis of diabetic cardiomyopathy: good or bad

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Ge T, Yu Y, Cui J, Cai L. The adaptive immune role of metallothioneins in the pathogenesis of diabetic cardiomyopathy: good or bad. *Am J Physiol Heart Circ Physiol* 317: H264–H275, 2019. First published May 17, 2019; doi:10.1152/ajpheart.00123.2019.—Diabetes is a metabolic disorder characterized by hyperglycemia, resulting in low-grade systemic inflammation. Diabetic cardiomyopathy (DCM) is a common complication among diabetic patients, and the mechanism underlying its induction of cardiac remodeling and dysfunction remains unclear. Numerous experimental and clinical studies have suggested that adaptive immunity, especially T lymphocyte-mediated immunity, plays a potentially important role in the pathogenesis of diabetes and DCM. Metallothioneins (MTs), cysteine-rich, metal-binding proteins, have antioxidant properties. Some potential mechanisms underlying the cardioprotective effects of MTs include the role of MTs in calcium regulation, zinc homeostasis, insulin sensitization, and antioxidant activity. Moreover, metal homeostasis, especially MT-regulated zinc homeostasis, is essential for immune function. This review discusses aberrant immune regulation in diabetic heart disease with respect to endothelial insulin resistance and the effects of hyperglycemia and hyperlipidemia on tissues and the different effects of intracellular and extracellular MTs on adaptive immunity. This review shows that intracellular MTs are involved in naïve T-cell activation and reduce regulatory T-cell (Treg) polarization, whereas extracellular MTs promote proliferation and survival in naïve T cells and Treg polarization but inhibit their activation, thus revealing potential therapeutic strategies targeting the regulation of immune cell function by MTs.

adaptive immunity; diabetic cardiomyopathy; metallothioneins; zinc

INTRODUCTION

Diabetes is a metabolic disorder characterized by hyperglycemia and can result in low-grade systemic inflammation (19). In the United States, 1 in 10 individuals has diabetes (13). Diabetic cardiomyopathy (DCM) is a common complication among diabetic patients clinically manifesting as left ventricular hypertrophy, decreasing cardiac diastolic and systolic function (at late stages). The final clinical outcomes are myocardial fibrosis and heart failure, in the absence of coronary artery disease, valvular disease, and hypertension (52, 84). Epidemiological studies have reported that individuals with diabetes are at a two- to fivefold higher risk of mortality from heart failure than healthy people of the same age (55). In 2014, the cost of hospitalization of adult patients with diabetes aged over 18 years in the United States was USD 7.2 million, of which USD 1.5 million was primarily used for patients with DCM (70.4 per 1,000 persons with diabetes) (13). Clearly, DCM aggravates the socioeconomic burden among patients; hence, understanding DCM pathogenesis is crucial to accelerate the development of therapeutic strategies.

The pathomechanism of DCM is complex. Diabetes-associated hyperglycemia and hyperlipidemia affect the biomechanical properties of cardiac cells (83). In particular, hyperglycemia exerts negative effects at the cellular level, directly or indirectly deterring the function of cardiac progenitor cells (114), cardiomyocytes (8, 128), fibroblasts (111), and immune cells (9). In the past few years, numerous experimental and clinical studies have reported that adaptive immunity, especially T-lymphocyte-mediated immunity, also plays an important role in the pathogenesis of diabetes (27, 149). Endothelial insulin resistance, tissue exposure to hyperglycemia, and hyperlipidemia also elevate levels of advanced glycosylation end-products (AGEs) and lead to the generation of excess

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reactive oxygen or nitrogen species (ROS or RNS), leading to oxidative stress (96). The latter stimulates NF-κB to upregulate several cytokines such as IL-1, TNF-α, chemokines such as monocyte chemoattractant protein-1 (MCP-1), and adhesion molecules such as ICAM-1 and VCAM-1, thus resulting in the recruitment of immune cells (33, 67, 68, 115) (Fig. 1).

These immune cells infiltrate tissues and promote DCM progression through multiple aspects: *1*) induction of the fibrotic response by secreting profibrotic mediators, including transforming growth factor-β (TGF-β), contributing to fibroblast proliferation in diabetic hearts (111); *2*) further stimulation of the secretion of proinflammatory cytokines and adhesion molecules, not only able to trigger the recruitment and compensation of immune cells (45, 97), but also able to suppress the major metabolic insulin signaling cascade, thus further suppressing glucose homeostasis in cardiac tissue (48, 70); *3*) immune cells recruited to local tissues can only promote local inflammation but cannot eliminate bacteria, thus reducing resistance to infections (92, 97). However, whether all these phenomena occur in the heart depends on several systemic and tissue environmental factors.

Metallothioneins (MTs), initially isolated from equine kidney in 1957 (77), are ubiquitous low-molecular-weight proteins that have antioxidant properties. They have a high-cysteine content and form metal-thiolate clusters (12). Concurrent with previous studies, we reported that cardiac MT overexpression in mice significantly protected the heart against diabetes (10, 37, 69, 127, 131). Several mechanisms are potentially responsible for MT-mediated cardiac protection from diabetes, including the important roles of MTs in antioxidant action, Zn homeostasis, calcium regulation, and insulin sensitivity (30, 59). We previously reported that oxidative stress-mediated TRB3 upregulation inhibits insulin-induced Akt2 and GSK-3β phosphorylation and GS phosphorylation, eventually inhibiting glucose metabolism and inducing oxidative stress and inflammation in cardiac cells, resulting in cardiac damage (cardiomyopathy) during diabetes. However, mice with cardiomyocyte-specific overexpression of MT gene (MT-TG) or Zn induction of MT can prevent these pathological and functional changes (38). Nonetheless, MT-mediated metal homeostasis, especially Zn homeostasis, is reportedly essential for immune function (66, 86–89, 123). However, its potential role in the immune response is yet unclear. Zn deficiency is common among patients with diabetes (130, 134). Zn deficiency also inhibits intracellular signal pathways in immune cells (40, 60, 73, 79, 99). Zn is an important metal bound by MT under normal physiological conditions, such that, simultaneously, MTs are the "master" regulators of Zn homeostasis most probably via Zn storage. Therefore, it is conceivable that MTs are important regulators of immune function. Immune cells regulate MTs during stress stimulation, cytokine signaling, and microbial challenges (75, 123, 139). In these cells, MTs in turn regulate the release, transport, and distribution of metal ions including Zn and the cellular redox status, cell signaling, and enzyme activity (139). Hence, the use of MTs to coordinate

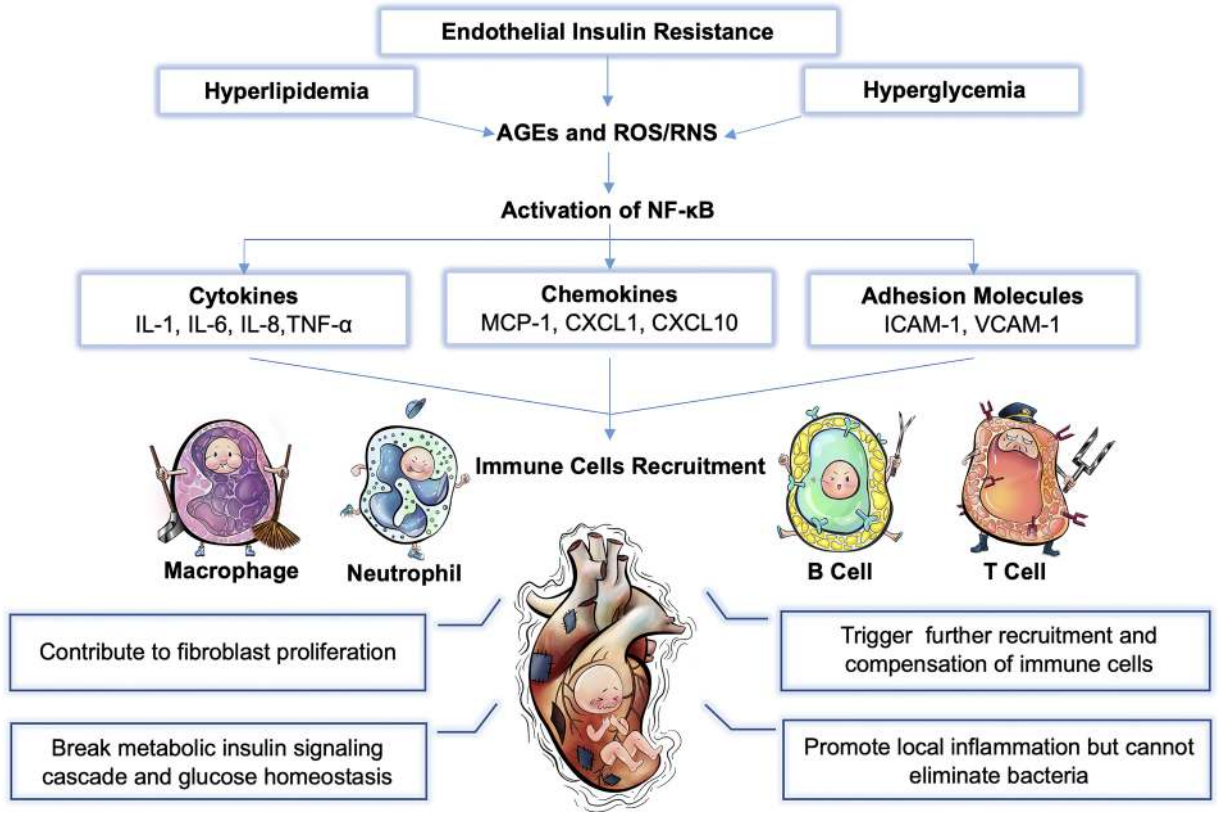

Fig. 1. Endothelial insulin resistance and tissue exposure to hyperglycemia and hyperlipidemia affect immune cell function in the heart. They elevate levels of advanced glycosylation end-products (AGEs) and generate excess reactive oxygen species (ROS) or reactive nitrogen species (RNS). RNS stimulates the upregulation of several cytokines, chemokines, and adhesion molecules via NF-κB, thus promoting the recruitment of immune cells, which infiltrate tissues and promote diabetic cardiomyopathy progression. MCP-1, monocyte chemoattractant protein-1; CXCL, chemokine (C-X-C motif) ligand.

inflammatory responses is a potential therapeutic target for chronic inflammatory diseases, including DCM.

Furthermore, numerous studies have indirectly indicated the immunoprotective effects of MTs in DCM pathogenesis. A study reported that in a mouse model of T2D generated via administration of a high-fat diet followed by a minor dose of streptozotocin, IL-6 and MCP-1 mRNA in the heart of MT-knockout (KO) mice were significantly upregulated compared with wild-type mice (37). In contrast, TNF-α, ICAM-1, and PAI-1 mRNA in the heart of (MT-TG) were significantly downregulated compared with the wild-type mouse model of T1D, which were induced via five daily injections of a minor dose of streptozotocin (131). Moreover, Zn supplementation reduces the intensity of the inflammatory response and prevents DCM by inducing cardiac MTs upon administration of a single dose of streptozotocin (62).

In addition, patients with type 2 diabetes (T2D) often have obesity, characterized by upregulation of B-cell lymphoma/leukemia 10 (BCL10)/caspase-recruitment domain 9 (CARD9) proteins, further activating p38 MAPK and promoting the occurrence of myocardial hypertrophy. In this process, Zn deficiency further enhanced the activation of BCL10/CARD9/p38 MAPK, whereas Zn supplementation suppressed them via upregulation of MTs to protect the heart (129). Moreover, CARD9 is a caspase recruitment domain-containing signaling protein that plays a critical role in innate and adaptive immunity (148). TNF-α, IL-6, chemokine (C-X-C motif) ligand, and MCP-1 were significantly downregulated in cardiac tissue and serum from CARD9$^{-/-}$ mice compared with wild-type mice (100). Furthermore, BCL10 is a critical regulator of the activation and termination of immune cell signaling (28). BCL10 is recruited to different CARMA/CARD scaffolds to mediate T-cell receptor (TCR)/B-cell receptor (BCR) signaling to activate immune and proinflammatory genes (28). However, whether MTs can regulate the immune response through BCL10/CARD9 and affect the occurrence of myocardial hypertrophy remains unclear. Future studies are required to elucidate the applications of MTs as a therapeutic target to regulate the inflammatory response in DCM pathogenesis. This review discusses aberrant immune regulation in DCM pathogenesis and the effects of intracellular and extracellular MTs on adaptive immunity, thus elucidating therapeutic strategies for the targeted regulation of immune cell function by MTs.

ROLE OF INNATE AND ADAPTIVE IMMUNITY IN DCM

Several types of cardiac resident immune cells exist under physiological conditions, namely macrophages, localized near endothelial cells or within the interstitial space (21, 93, 98, 124); mast cells, responsible for early triggers of the immune response (26); a small number of adaptive immune cells, including B cells, against foreign antigen; regulatory T (Treg) cell subsets, preventing an overactive immune response, leading to an autoimmune attack (21, 117, 150); and dendritic cells (DCs), responsible for antigen presentation (15, 21). Metabolic disruption by nutrients, e.g., hyperglycemia and hyperlipidemia, initially results in chronic low-grade inflammation in primary insulin targets, including the liver and adipose tissue (49). Proinflammatory cytokines, released by immune cells infiltrating the adipose tissue, further promote the production and secretion of proinflammatory mediators, leading to local and systemic inflammatory responses, which can aggravate systemic insulin resistance and eventually lead to cardiac metabolic disorder and immune dysregulation, further remodeling inflammatory tissue with time (36, 94, 124). Consequently, resident fibroblasts are activated under inflammatory pathological conditions, which lead to stiffened cardiac walls and decreased contractility in cardiac fibrosis, possibly leading to diabetes-related heart failure (111). Together, metabolic disruption and inflammatory signaling pathways during DCM progression are associated with alterations in immune-cell activation and enhanced cardiac inflammation. The following sections will discuss the aberrant changes in innate and adaptive immunity in DCM.

INNATE IMMUNITY IN DCM

Innate immunity, especially macrophage- and neutrophil-mediated innate immunity, modulates and influences the pathogenesis of diabetes (46). Macrophages are a type of phagocytes that can effectively eliminate apoptotic and necrotic cells. Owing to their persistent phenotypes, they are difficult to classify, with all types being present simultaneously (34); however, presently, the most widely used classification is proinflammatory (M1) vs. proreparative (M2) macrophages. Certainly, the balance between M1 and M2 subtypes is important for the homeostasis of inflammation (4, 91, 93). In diabetes, macrophage-mediated phagocytosis is impaired (57), lysosomal enzyme release is decreased (81), chemotaxis is reduced (57, 101), and the balance of M1 and M2 tends toward the M1 phenotype (103). M1 macrophages secrete more TNF-α and MCP-1 to induce the chronic low-grade inflammation (103). In addition, M1 macrophages release resistin, an adipokine contributing to insulin resistance (61). Moreover, M1 macrophages are more numerous in cardiac tissue before myocardial dysfunction (93). Depletion of early nonselective macrophages with clodronate encapsulated within liposomes (clodrolip) reportedly reduced cardiac inflammation and improved cardiac function in a transgenic mouse model of lipotoxic cardiomyopathy (MHC-ACS) (118). On the contrary, M2 macrophages can antagonize M1 macrophages, thus potentially inhibiting inflammation and reducing insulin resistance. M2 macrophages, not only express proinflammatory cytokines (usually expressed in M1 macrophages) at baseline levels, but also secrete higher levels of inflammatory inhibitors, such as IL-10 (24). The differentiation of M2 macrophages significantly influences cardiac tissue repair depending on IL-4 secretion (120) and is associated with a reduction in cardiac inflammation in Zucker diabetic fatty rats (51). However, these findings are preliminary, and further evidence is needed to elucidate the effect of macrophage depletion or activation with different phenotypic specificity on DCM. In future research, we should refine the classification of macrophages, and the previous M1 and M2 macrophage classification systems are oversimplified, such that they do not meet the current requirements. Nonetheless, one study stimulated macrophages with different activation signals and obtained transcriptomic data sets reflecting the activation status spectrum of macrophages, extending the present M1 and M2 polarization classification models (138). This may further elucidate the effects of macrophage expression profiles on DCM.

Furthermore, neutrophils, as the first line of defense at inflammatory sites, play an important role in cardiac tissue repair by polarizing macrophages toward repair phenotypes (47). Compared with healthy people, neutrophils displayed higher response and secretion levels to cytokines and growth factors, such as IL-8, IL-1β, TNF-α, and IL-1ra, in individuals with diabetes, further contributing to migration of neutrophils toward inflammatory sites, phagocytosis, release of lytic proteases, production of ROS, and apoptosis (6, 42, 58, 133).

ADAPTIVE IMMUNITY IN DCM

The adaptive immune system, also called acquired immunity, relies on fewer cell types, i.e., only T and B cells, to carry out its functions. Recently, numerous studies have suggested that adaptive immune cells, especially T lymphocytes, play a pivotal role in diabetes (5). Studies have reported that T-cell infiltration is associated with an increased risk of DCM, whereas circulatory T-cell depletion can exert cardioprotective effects in streptozotocin-induced DCM (1). Actually, the T-lymphocyte subtype is abnormally altered in DCM, resulting in a proinflammatory and anti-inflammatory immune imbalance (Fig. 2), thereby regulating inflammatory responses and insulin resistance. Similar to macrophages, on the basis of the function and cytokine production, $CD4^+$ effector T cells can be classified into proinflammatory T helper cells (Th1, Th17, and anti-inflammatory Th2 and $Foxp3^+$ Treg subtypes) (104). Increased frequency of Th1 and Th17 subsets reportedly contributes to DCM after adjusting for age, sex, and duration of diabetes in patients (147). The immune Th1/Th2 ratio tends toward Th1, whereas the Th17-to-Treg ratio tends toward Th17 in diabetes, suggesting that the role of each T-lymphocyte subset would be worth exploring, especially given the present background of DCM.

Th1 cells primarily produce IFN-γ, IL-2, TNF-β, triggering cell-mediated immunity and phagocyte-dependent inflammation (104), whereas Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 to regulate antibody responses (53). Some clinical studies have reported that Th1-associated cytokines are more numerous in peripheral blood in prediabetes or individuals with T2D (80, 145), whereas the activation of Th2 cell-mediated immunity is decreased and impaired in diabetes (137). Furthermore, an increase in Th1-associated cytokines (IL-12 and IFN-γ) with strong suppression of Th2-associated cytokines (IL-4, IL-5) in peripheral circulation was reportedly associated with DCM (74). Th17 cells, including the important proinflammatory $CD4^+$ T-cell subtype, secrete IL-17 and IL-22 and are more numerous in diabetes (146, 151). However, Treg cells, primarily secreting anti-inflammatory cytokines IL-10, are another subset of $CD4^+$ lymphocytes that suppress activation, proliferation, and immune responses of both innate and adaptive immunity (110, 112, 113). In patients with idiopathic dilated cardiomyopathy, a significant reduction in peripheral IL-10 with decreased Treg cells disrupted the Treg/Th17 balance (63, 64, 125). Another study reported that Treg cells can suppress Th1 and Th17 responses through various pathways, including the suppression of cytokine secretion, modulation of the microenvironment, and alteration of the expression of surface receptors to improve insulin resistance in diabetes (7). Therefore, an appropriate balance of proinflammatory (Th17 or Th1) and regulatory (Treg) subset T cells is probably warranted to maintain T-cell homeostasis and prevent chronic inflammation (5).

Furthermore, B-cell-mediated immunity contributes to cardiac dysfunction in mice with autoimmune diabetes (17). Mice with B-cell deficiency showed less inflammation and better glucose tolerance (135). However, the exact mechanism underlying B-cell-mediated regulation of cardiac function during DCM development is still unclear. Because B cells are the earliest cells to infiltrate the islets in mice with diabetes during islet destruction and can directly regulate T-cell infiltration of the islets, targeted B-cell therapy can effectively prevent diabetes in a mouse model of T1D induced with five repeated minor doses of streptozotocin (116). However, further studies are required to investigate the role of B cells in DCM pathogenesis.

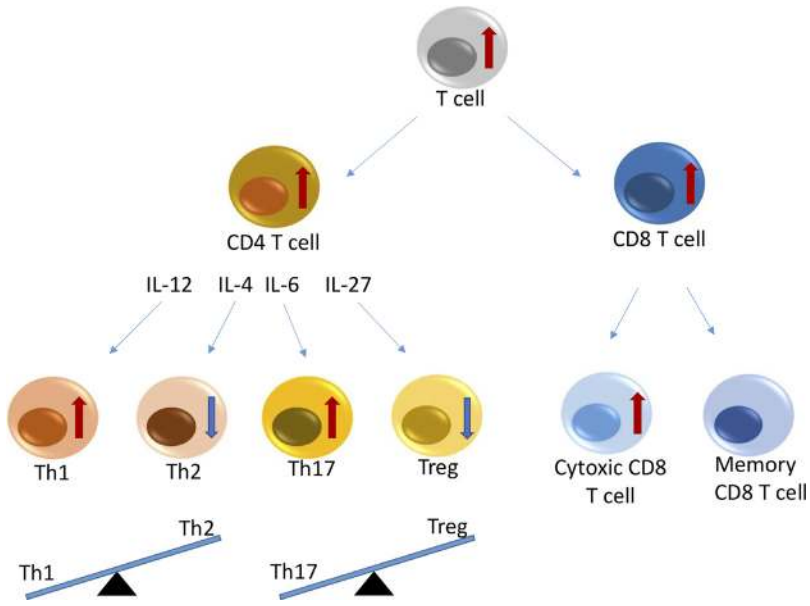

Fig. 2. Aberrant changes in T-cell subsets in diabetic cardiomyopathy. Proinflammatory and anti-inflammatory immune imbalance under diabetic conditions are as follows: proinflammatory $CD4^+$ T cells, including Th1 and Th17, and cytotoxic $CD8^+$ T cells are increased, whereas anti-inflammatory $CD4T^+$ cells, including Th2 and regulatory T cells (Tregs) are decreased. Th1/Th2 balance and Th17/Treg balance tend toward Th1 and Th17, respectively.

COMPLEX REGULATION OF MTs IN ADAPTIVE IMMUNITY

Zn homeostasis is essential for the development and optimal functioning of innate and adaptive immunity. Zn deficiency has adverse effects on antibody production, cytokine production, chemotaxis, cell signal transduction, proliferation, and function of B and T helper cells, whereas excess Zn exerts toxic effects on immune cells (14, 25, 40, 99, 108, 119). MTs can calibrate Zn availability; therefore, it is an important regulator of immune cell function. Moreover, MTs are expressed both in central immune organs (such as the thymus and bone marrow) and peripheral immune organs (such as lymph nodes and the spleen) and can also be detected in macrophages, lymphocytes and granulocytes, simultaneously involved in immune regulation (3, 123). Studies have reported that MTs were downregulated in leukocytes with Zn depletion and upregulated with Zn supplementation in a dose-dependent manner, suggesting that MTs in leukocyte subtypes may be a component determining Zn status (44). Another study reported that naive CD4 T cells from MT-KO mice differentiated to produce significantly less IL-10 compared with wild-type mice. Conversely, treatment with exogenous MTs during the priming phase drove naive wild-type CD4 T cells to differentiate into cells producing more IL-10 than untreated cells. These results suggest that MTs promote IL-10 production and suppress autoimmune disorders (50). Although MTs reportedly regulate the immune system in a complex manner, limited information is available regarding its role in cardiac inflammation of the diabetic heart.

Although MTs have been considered intracellular proteins, numerous studies have reported its presence in the serum, urine (22), bronchoalveolar spaces (41), liver sinusoids (18), and other extracellular sites. These extracellular MTs may either be secreted by live cells or released from dead cells. Intracellular and extracellular MTs reportedly exert different effects on immune regulation, especially for T-cell activation and regulation of Treg differentiation; hence, further studies are required to assess MT-mediated complex regulation of adaptive immunity in modulating immune cell function by targeting intracellular and extracellular MTs to potentially prevent or treat DCM.

DIFFERENT EFFECTS OF INTRACELLULAR AND EXTRACELLULAR MTs ON T-CELL ACTIVATION

$CD4^+$ T-cell stimulation is reportedly associated with significant upregulation of MT family members. Furthermore, bioavailable cytoplasmic Zn levels in activated $CD4^+$ T cells are increased during the initial 48–72 h and then gradually reverted to baseline/prestimulation levels (60). In addition, the peak duration of MT transcriptional activity is subsequent to that of traditional T-cell activation markers, thereby increasing the possibility of an intermediary mechanism regulating T-cell activation-induced transcription (60). MT synthesis is primarily regulated by Zn and is an important redox system in activated T cells. Alternatively, Zn transport mechanisms may be involved in MT synthesis.

Zn transport systems are of the following two types (20, 54, 95): *1*) ZnT (SLC30A) family members that decrease intracellular Zn levels by exporting Zn to extracellular fluid or intracellular vesicles (95); *2*) Zip proteins of the SLC39A family that transport Zn from extracellular or intracellular vesicles into the cytoplasm (20). The increase in cytoplasmic Zn levels in activated T cells (at least partially) depends on the expression and transport of the Zip6 transporter. Silencing of Zip6 reportedly significantly downregulated MTs after T-cell stimulation, indicating that MT upregulation in activated T cells resulted from Zn influx (60). Metal regulatory transcription factor 1, which stimulates MT expression, is regulated by intracellular influx of Zn ions. At this time, MTs are upregulated to neutralize ROS produced during T-cell activation (Fig. 3). Interestingly, Zn signaling and MT expression during primary $CD4^+$ T-cell activation are altered with age (60). For instance, more attention must be paid to individuals aged 60–75 years because naive $CD4^+$ T-cell populations display significant alterations in this age group, after which their naive immune cells become increasingly deficient (35). Naive $CD4^+$ T cells from young adults display a less sustained labile cytoplasmic Zn elevation and consequently MT downregulation after stimulation compared with $CD4^+$ T cells from older individuals (60). This is a potential protective mechanism because aging T cells produce more ROS during activation, thus increasing the intracellular influx of Zn ions, and MTs may be upregulated to eliminate the potentially damaging effects of excessive ROS production.

Limited information is presently available regarding the effect of extracellular MTs on immune regulation. Extracellular MTs are reportedly potent inducers of lymphocyte proliferation (71). Extracellular MTs may promote the beneficial migration of leukocytes to the site of inflammation (143); however, they reduce the T-cell-dependent humoral response (72) and suppress cytotoxic T-lymphocyte function (144). The potential mechanism is outlined in Fig. 4, showing that extracellular MTs bind to Zn, sequestering Zn ions entering the cytoplasm. Zn ions cannot enter the cytoplasm with Zip6, thus preventing T-cell activation. Simultaneously, potentially uncertain MT receptors may be present on the surface of T cells, which bind to the MT-Zn complex and upregulate IL-2 through an uncertain signaling pathway to enhance T-cell proliferation and survival (85, 123).

DIFFERENT EFFECTS OF INTRACELLULAR AND EXTRACELLULAR MTs ON Treg POLARIZATION

$CD4^+$ $Foxp3^+$ Tregs play a crucial role in the balance between immunity and tolerance (109). IL-27 stimulates the production of type 1 regulatory T (Tr) 1 cells through the signal transducer and activator of transcription 1 (STAT1) and STAT3 signaling pathways and secretes IL-10 to drive immunosuppression. When intracellular MTs were downregulated, IL-27 significantly increased the differentiation of Tr1, indicating that intracellular MTs inhibit the differentiation of Tr1 (136). On the contrary, upregulation of intracellular MTs significantly affect IL-10 secretion and the differentiation of Tr1 cells (50), the underlying mechanism potentially involving the regulation of Zn homeostasis by MTs. Studies have reported that Zn signaling regulates kinase and phosphatase function (39, 40). Protein tyrosine phosphatase 1B (PTP1B) is a major negative regulator of the insulin and leptin signaling pathways and is an important therapeutic target for diabetes and obesity, and Zn ions inhibit its activity (102). Therefore, MTs were assumed to attenuate the inhibitory effects of Zn ions on PTP1B activity by sequestrating Zn, followed by

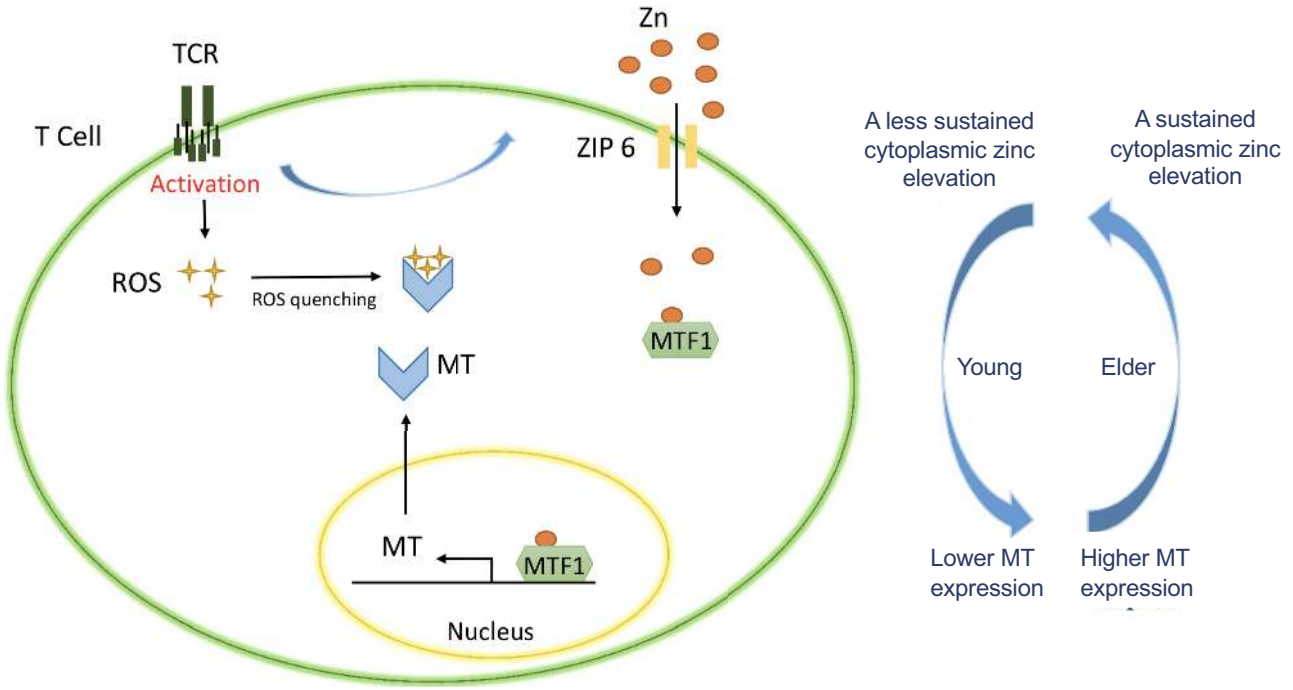

Fig. 3. Intracellular metallothioneins (MTs) are involved in naive T-cell activation. An increase in the cytoplasmic Zn concentration in activated T cells (at least partially) depends on the expression and transport of the Zip6 transporter. Metal regulatory transcription factor 1 (MTF1), the promoter of MTs, is regulated by the intracellular influx of Zn ions. Simultaneously, MTs are upregulated to neutralize the reactive oxygen species (ROS) produced during T-cell activation. The physiological process of Zn signaling and MT expression in the primary $CD4^+$ T-cell activation reactions can be increased with age. TCR, T-cell receptor.

dephosphorylation of STAT1 and STAT3 by PTP1B to inhibit IL-27-induced Tr1-cell differentiation (123). Indeed, MT-KO Tr1 cells exhibit hyperphosphorylation of STAT1 and STAT3 along with an increase in immunosuppressive IL-10 production (136) (Fig. 5).

DCs exert immune effects through professional antigen presentation and expression of costimulatory molecules. Furthermore, different types of DCs perform distinct functions, which determine the fate of adaptive T-cell immunity (121). Unlike inflammatory DCs, which promote the response of effector T cells, tolerogenic DCs can induce fork head box P3 (FoxP3) expression and promote Treg production (76, 106). Studies have reported that DCs express MTs on their cell surfaces, which, if inhibited, inhibits its tolerogenic potential and the differentiation of naive T cells into FoxP3-expressing Tregs (122). Therefore, cell-surface MT receptors in DCs bind to extracellular MTs, thus affecting the tolerance potential of DC cells. In addition, intracellular and extracellular MTs regulate the distribution of Zn ions, affect the redox state of DCs, and alter the differentiation process (Fig. 4).

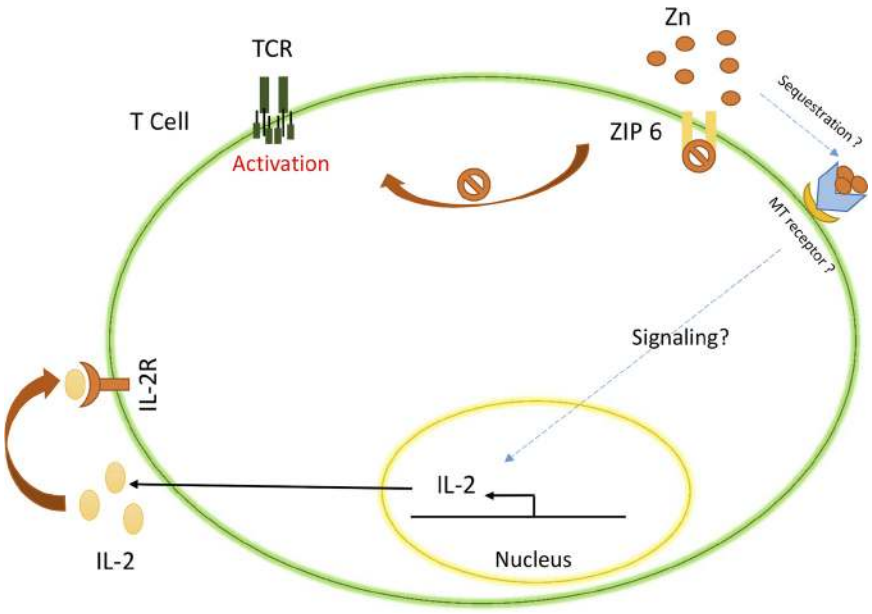

Fig. 4. Extracellular metallothioneins (MTs) promote the proliferation and survival of naive T cells but inhibit their activation. Extracellular MTs bind to Zn, sequestrating the Zn ions entering the cytoplasm. Zn ions cannot enter the cytoplasm with the transport of Zip6, thus preventing T-cell activation. Simultaneously, uncertain MT receptors may be present on the surface of T cells, which can bind to the MT-Zn complex and promote IL-2 expression through an uncertain signaling pathway to enhance T-cell proliferation and survival. TCR, T-cell receptor.

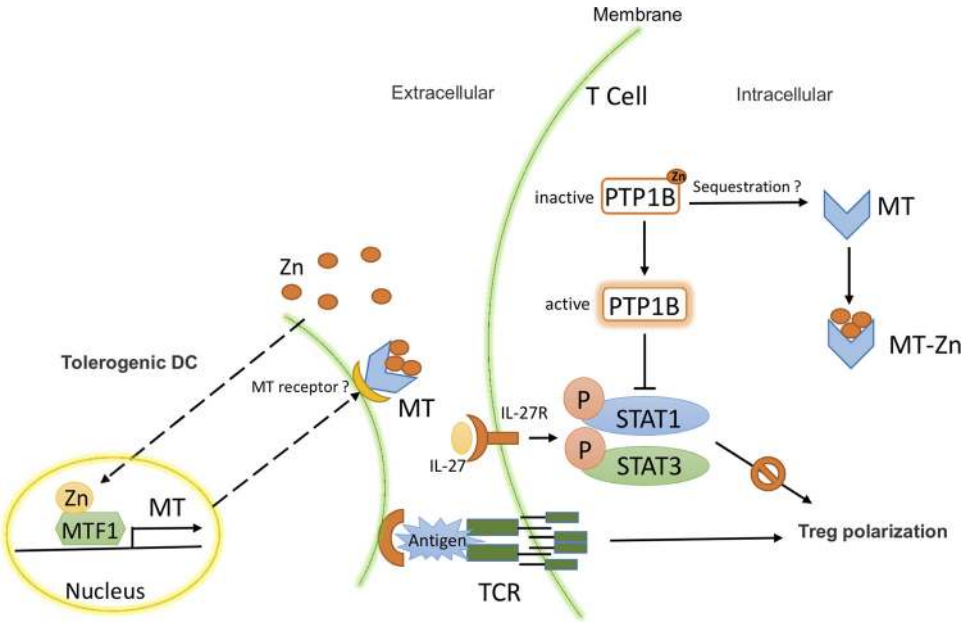

Fig. 5. Extracellular metallothionein (MT) promotes regulatory T cell (Treg) polarization; however, intracellular MTs reduce Treg polarization. MT cell-surface receptors may be present in dendritic cells (DCs), which bind to extracellular MTs to promote the tolerance potential of DCs. On the contrary, intracellular MTs attenuate the inhibition of Zn ions on protein tyrosine phosphatase 1B (PTP1B) activity by sequestrating Zn, and PTP1B induces the dephosphorylation of signal transducer and activator of transcription 1 (STAT1) and STAT3 to inhibit IL-27-induced Treg polarization. TCR, T-cell receptor.

AN IMMUNOREGULATORY STRATEGY TARGETING MTs

At present, numerous studies have reported MTs as immunoregulatory targets; however, most studies are primarily focused on autoimmune arthritis and encephalomyelitis, and no study has assessed diabetes and its complications. Studies have reported an increase in Tr1 cells upon MT-KO and suppression of IL-10 secretion upon intracellular increase in Zn^{2+} (107). Hence, Tr1 cells from MT-KO mice were adoptively transferred to autoimmune encephalomyelitis mice, and more IL-10 was produced, which effectively inhibited the progression of autoimmune diseases (136). Another study reported that naive $CD4^+$ T cells from MT-KO mice differentiated to produce significantly less IL-10 and other inhibitory cytokines. Importantly, MT-TG mice were significantly less sensitive to collagen-induced arthritis and had higher serum IL-10 levels than their control littermates (50).

Furthermore, MT expression in mice and humans primarily involves four gene subfamilies: *MT1*, *MT2*, *MT3*, and *MT4*. In humans, the gene cluster is located on chromosome 16 (16q12-22) (56). *MT1* and *MT2* are ubiquitously expressed, and both comprise nine functional (*MT1A*, *MT1B*, *MT1E*, *MT1F*, *MT1G*, *MT1H*, *MT1M* also called *MT1K*, *MT1X*, and *MT2A*) and seven nonfunctional (*MT1C*, *MT1D*, *MT1I*, *MT1J*, *MT1L*, *PT1P*, and *MT2B*) paralogs (105). Therefore, present mouse models with MT upregulation or knockout are primarily focused on *MT1* and *MT2*. However, *MT3* expression is seemingly limited to the brain (126); *MT4*, squamous epithelium (82). Recent studies have reported that, in addition to changes in metal levels, MT single-nucleotide polymorphisms (SNPs), particularly those in *MT1A* and *MT2A* genes, are associated with the susceptibility to metabolic diseases including diabetes (29, 43, 105, 141). A study reported that, among 694 Italian individuals with relatively similar plasma Zn levels, a polymorphism (+647 A/C) in the human *MT-1A* gene affects intracellular Zn ion release (iZnR) from the proteins and promotes DCM (29). Furthermore, +1245 G+ *MT1A* carriers displayed increased plasma AGEs and ROS production in peripheral blood mononuclear cells at baseline and a significant improvement in intracellular labile Zn (iZnL) after Zn intervention with respect to G- individuals (32). Another study analyzed seven SNPs in *MT* genes (rs8052394 and rs11076161 in *MT1A* gene, rs8052334, rs964372, and rs7191779 in *MT1B* gene, rs708274 in *MT1E* gene, and rs10636 in *MT2A* gene) among 851 Chinese individuals of Han descent and reported that the rs8052394 SNP in the *MT1A* gene most probably predisposes individuals to T2D or altered serum superoxide dismutase activity, and diabetes with neuropathy was positively associated with SNPs rs10636 *MT2A* and rs11076161 *MT1A* (141). In addition, a Japanese study involving 749 men and 2,025 women reported that *MT2A* A-5G may be associated with the risk of chronic kidney disease (CKD) and diabetes. This polymorphism is a promising target to evaluate the risk of CKD and diabetes with the potential involvement of low-dose chronic exposure to environmental pollutants (43). In particular, polymorphisms in *MT1A* and *MT2A* genes also contribute to inflammation and immunity (105). MTs regulate Zn homeostasis by binding Zn and releasing Zn during an immune response. However, Zn release by MTs is limited in chronic inflammation and aging. A study reported that the +647 *MT1A* polymorphism significantly influences MT induction and Zn release, which are indispensable for regulating the inflammatory status (16). Therefore, it may be speculated that some MT polymorphisms, especially those in the *MT1A* subtype, may regulate the immune response in DCM by affecting Zn release.

Another study reported that the C allele in SNP rs10636 in *MT2A* is also associated with decreased NK-cell cytotoxicity and increased MCP-1 levels in patients with carotid artery stenosis (31). MCP-1 affects DCM pathogenesis and aggravates the local inflammatory response through recruitment of immune cells (2, 140); hence, it may be speculated that polymorphisms in *MT2A* may influence immunity in DCM at different degrees. In conclusion, future studies are required to investigate the effect of MT polymorphisms on DCM and its immune alterations to develop treatment strategies.

SUMMARY AND PERSPECTIVES

Together, MTs can affect immune function in different immune cell subtypes, different differentiation stages, and even different intracellular and extracellular distributions of immune cells. Numerous studies have reported that overexpression of MTs can reduce the occurrence of DCM and that the cardioprotective mechanism of MTs from diabetes is becoming clearer (11, 23, 37, 38, 65, 127, 132, 142). However, few studies have investigated the effect of MT overexpression on immune cells. With increasing clarification of the role of the immune system in DCM pathogenesis, more attention should be paid to whether MT overexpression affects the development of DCM from the perspective of immunity. Therefore, MT expression and distribution can be further used to achieve immunoregulatory treatment of DCM.

Therefore, the following points should be focused on when targeting MTs for DCM immunotherapy. First, the bioavailability of Zn is the basis of immune efficiency (89). Zn supplementation helps alleviate chronic immune responses. Studies have reported that plasma Zn deficiency and altered immune response are more evident in individuals harboring the *IL-6* −174 C− allele and the *MT1A* +647 C+ (78), suggesting that genetic screening for polymorphisms in *IL-6* and *MT1A* and a careful evaluation of Zn status might provide a strong rationale to select individuals critically requiring Zn supplementation (86, 90). Moreover, future studies are required to determine the effect of MT polymorphism on immune regulation of DCM. Second, the aforementioned evidence shows that intracellular MT expression in immune cells activates T cells and inhibits Treg differentiation. However, extracellular MTs promote T-cell proliferation but inhibit their activation and may induce Treg differentiation by affecting cell-surface MT receptors in DCs. These reports suggest that careful attention should be paid to the effect of intracellular and extracellular MTs of immune-cell surfaces on inflammatory responses in future studies. Finally, in future studies, adoptive immunotherapy for DCM may be considered. For example, MT-deficient Treg cells can be injected back into the body to secrete more inflammatory suppressors, such as IL-10, to inhibit chronic tissue inflammation during DCM. These reports provide novel insights into DCM immunotherapy.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

T.G. drafted manuscript; Y.Y. edited and revised manuscript; L.C. and J.C. approved final version of manuscript.

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