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REVIEW

Ryanodine receptor and immune-related molecules in diabetic cardiomyopathy

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Abstract

Hyperglycaemia is a major aetiological factor in the development of diabetic cardiomyopathy. Excessive hyperglycaemia increases the levels of reactive carbonyl species (RCS), reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the heart and causes derangements in calcium homeostasis, inflammation and immune-system disorders. Ryanodine receptor 2 (RyR2) plays a key role in excitation–contraction coupling during heart contractions, including rhythmic contraction and relaxation of the heart. Cardiac inflammation has been indicated in part though interleukin 1 (IL-1) signals, supporting a role for B and T lymphocytes in diabetic cardiomyopathy. Some of the post-translational modifications of the ryanodine receptor (RyR) by RCS, ROS and RNS stress are known to affect its gating and Ca^{2+} sensitivity, which contributes to RyR dysregulation in diabetic cardiomyopathy. RyRs and immune-related molecules are important signalling species in many physiological and pathophysiological processes in various heart and cardiovascular diseases. However, little is known regarding the mechanistic relationship between RyRs and immune-related molecules in diabetes, as well as the mechanisms mediating complex communication among cardiomyocytes, fibroblasts and immune cells. This review highlights new findings on the complex cellular communications in the pathogenesis and progression of diabetic cardiomyopathy. We discuss potential therapeutic applications targeting RyRs and immune-related molecules in diabetic complications.

Keywords Ryanodine receptors; Immune-related molecules; Fibroblasts; Immune cells; Cardiomyocytes

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Introduction

Diabetic cardiomyopathy (DCM) is defined as the existence of abnormal myocardial structure and performance in the absence of other cardiac risk factors, such as dilated cardiomyopathy and congestive heart failure, in individuals with diabetes mellitus.[1–3] Excessive hyperglycaemia leads to an imbalance between oxidative and antioxidative processes within cells and tissues, which leads to an increase in reactive carbonyl species (RCS), reactive oxygen species (ROS) and advanced glycation end products (AGEs).[4–6] A substantial amount of data has demonstrated that ryanodine receptor 2 (RyR2) plays a key role in heart contractions, including rhythmic contraction and relaxation of the heart. Ryanodine receptor 1 (RyR1) is also expressed at low levels in smooth muscle cells, affecting vessel constriction, which, in turn, affects heart function.[7–10] Protein carbonylation represents the most common type of non-enzymatic post-translational modification (PTM) in a rat model of Type 1 diabetes. PTMs by RCS, AGEs and ROS contribute to the heterogeneity in RyR2 activity that is observed in experimental models of diabetes.[11,12] These findings have identified PTMs of RyR1 and RyR2 as a novel mechanism that contributes to RyR dysregulation in DCM.

Inflammation increases the risk of ischaemic and non-ischaemic cardiomyopathy. Both innate and adaptive immune responses are activated in the heart in response to inflammation-related tissue injury that results from the pathogenesis of DCM. Additionally, activation of immune responses in the heart provokes deleterious cardiac remodelling and causes left ventricular dysfunction.[13,14] A better understanding of the relationship of the pathophysiological

mechanisms between immune responses and dysfunction of RyR1/RyR2 in DCM may lead to medical progress and therapy for those with chronic DCM. We provide an updated discussion on the pathophysiological relationship between immune-related molecules and RyR1/RyR2, which involves crosstalk among myocytes, fibroblasts and immune cells, leading to different pathological processes relevant to the cardiovascular complications of DCM.

Immune dysfunction in diabetes mellitus

Diabetic patients show an altered number and function of immune cells, in terms of both innate and acquired immunity. Over the past few decades, it has been suggested that immune responses play an important role in the mechanisms of diabetes mellitus. Innate immune responses represent the first line of defence against non-self pathogens. Interleukin 1 (IL-1) (IL-1α and IL-1β) stimulates insulin secretion and biosynthesis, as well as β-cell proliferation.[15,16] These findings indicate an important physiological role of innate immune signalling in priming insulin secretion under conditions of stress and increased insulin demand.[17] Diabetes is generally long-lasting and difficult to control, and adaptive immune responses should be specific to the pathogen presented. A hallmark of the adaptive immune system is clonal expansion of lymphocytes. Recent evidence has indicated that B cells play a critical role in diabetes and its complications; high rates of B-cell depletion delay progression of disease-related pathology in non-obese diabetic mice and in new-onset patients.[18] B-cell receptor (BCR) specificity to islet autoantigens also contributes to islet-antigen-reactive B-cell function in antigen presentation to diabetogenic $CD4^+$ T cells of Type 1 diabetes.[18,19] T cells also play a critical role in diabetes. Experiments have suggested a pathological role for an increase in $CD4^+$ T cells in obesity and insulin resistance; activated $CD4^+$ T cells are increased in visceral adipose tissue of obese mice.[20] It has also been shown that obesity induces MHC Class II (MHCII) expression in adipocytes and thus activates $CD4^+$ T cells to initiate inflammation in adipose tissue.[21] $CD4^+$ T cells can be further divided into pro-inflammatory Th1 and Th17 cells, as well as anti-inflammatory Th2 and $Foxp3^+$ regulatory T cells (Tregs), based on their functionalities and levels of cytokine production. Th1 cells produce interferon α (IFN-α), IL-2 and tumour necrosis factor β (TNF-β) to trigger cell-mediated immunity and phagocyte-dependent inflammation.[22,23] Th1 cells secrete IFN-? and enhance pro-inflammatory functions by inducing the release of IL-1 and IL-6. In contrast, Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13 to regulate antibody responses.[23,24] Collectively, these findings highlight that both innate and acquired immunity play a key functional role in regulating inflammatory processes underlying chronic hyperglycaemia in diabetes, as well as long-term diabetes-related health problems.

Immune cell populations in the heart

Healthy adult mouse heart contains all the major leucocyte classes, including mononuclear phagocytes, neutrophils, dendritic cells (DCs), macrophages, mast cells and eosinophils. Resident macrophages form an immune network that is interspersed with other immune cells throughout the healthy heart and lodged between cardiomyocytes. Macrophages are a type of white blood cell and are classified by their expression of MHCII molecules, CC-chemokine receptor 2 (CCR2) and lymphocyte antigen 6C (Ly6C).[25,26] CCR2− macrophages are grouped into high and low MHCII subsets, whereas CCR2+ macrophages, which express MHCII, are a minor population. $Ly6C^+$ macrophages are an even smaller population and are the only macrophage subset that expresses Ly6C.[27,28] T-cell immunoglobulin and mucin domain containing 4 (Tim-4) has emerged as a key marker of resident macrophages in various tissues. In cardiac muscle tissue, $Tim\text{-}4^+$ resident cardiac macrophages, which are lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE1) + MHCII low CCR2−, maintain self-renewal despite low input of blood monocytes, and play a non-redundant, cardioprotective role by limiting adverse remodelling.[29–31] Resident cardiac DCs represent an extremely small population that comprises less than 1% of all cardiac leucocytes. Two major subsets of DCs are CD103 + CD11b− and CD103 − CD11b+ subsets, which express zinc finger and BTB domain-containing protein 46 (ZBTB46).[32] Numerous studies have shown that leucocytes and their subsets, such as neutrophils, monocytes and lymphocytes, are involved in inflammation, which plays an important role in both the initiation and progression of cardiovascular diseases.[33]

Immune activation in DCM

Abnormal chemical reactions in hyperglycaemia alter normal metabolic processes in diabetes. This is a key process in subcellular inflammation in the early to mid-stages of diabetes, which can lead to heart dysfunction, with patients clinically exhibiting symptoms of myocardial fibrosis, diastolic and systolic dysfunction and clinical heart failure.[3] The failing heart contains an increased number of macrophages that expand by both local proliferation of both resident and recruited monocyte-derived macrophages.[25] Cardiac macrophages affect other non-myocyte cells including inflammatory cells, vascular cells and fibroblasts and lead to further heart dysfunction. Cardiac macrophages also secrete an extensive array of inflammatory cytokines, chemokines,

growth factors and matrix metalloproteinases (MMPs) to regulate inflammation that directly or indirectly alter the cardiac extracellular matrix (ECM) network.[34] In local immune responses, macrophages receive assistance from other immune cells, such as Toll-like receptor (TLR)-proficient mast cells. TLR activation also induces the production of TNF-α from cardiac macrophages that cause ECM deposition, where transforming growth factor β (TGF-β) is frequently present during hypertrophy in the heart.[35,36] Inflammatory signals induce release of IL-6, IL-1, IL-10 and IL-18 by macrophages and exacerbate cardiac dysfunction as well as fibrosis in infarcted and non-infarcted hearts; among these signals, IL-18 precipitates adverse cardiac remodelling by reducing capillary density in ischaemic heart tissues.[37] In addition, the role of T cells is clearly documented in tissue fibrosis in cardiac injury. Left ventricular function is protected by T-cell depletion, and Th and Treg subsets significantly participate in inflammation, insulin resistance and vascular alterations in diabetes mellitus. For example, multiple studies have shown that Th1, Th2, Th17 and Th22 cells exhibit many significant signs of pro-inflammatory phenotypes upon the onset of coronary artery disease, which has been reported in DCM patients.[14,33,35] Chemokines are small molecules originating from one ancestral gene, C-X-C motif chemokine ligand 10 (CXCL10), which is secreted by leucocytes and some types of tissue cells under inflammatory stimuli, including human endothelial cells, fibroblasts and cardiomyocytes. CXCL10 increases the migration of inflammatory cells through C-X-C chemokine receptor 3 (CXCR3), which is mainly expressed by T cells, natural killer (NK) cells and monocytes. These data suggest a pivotal role of the CXCL10–CXCR3 axis in early Th1-driven responses, as well as multifaceted functions of CXCL10 in several cardiovascular diseases, which has been previously shown.[38–40] The discussion above suggests that immune-related molecules may contribute to communication among myocytes, fibroblasts and immune cells in DCM and elucidation of such mechanisms may aid in the development of therapeutic targets for ameliorating diabetic complications. The detailed mechanisms of immune responses in DCM are summarized in *Figures 1* and *2C*.

RyR1 and RyR2 in the heart

RyRs are a family of Ca^{2+} release channels found on endoplasmic reticulum (ER) and sarcoplasmic reticulum (SR) membranes; they mediate Ca^{2+} release and excitation–contraction (E-C) coupling in both cardiac and skeletal muscles. Three different isoforms of RyRs have been found in mammalian organisms: RyR1, RyR2 and RyR3. RyR1 is expressed in skeletal muscle and smooth muscle, as well as at low levels in cardiac muscle. RyR2 is expressed in cardiac muscle, whereas RyR3 is expressed in the brain.[10,41] Cryo-electron microscopy (cryo-EM) reconstructions have shown that RyRs have an overall mushroom shape, with the stalk crossing the ER/SR membrane and the large cap being located entirely in the cytosol; the closed-pore and open-pore structures of both RyR1s and RyR2s have been determined to have a resolution of 3.6–6 Å.[42–47] Structural comparison of open- and closed-pore RyRs has revealed a small armadillo intradomain rearrangement of the

Figure 1 Schematic diagram of the major pathways involved in a model of RCS and AGE that affect Ca^{2+} dynamics and immune responses in fibroblasts in diabetes. RCS and AGEs induce release of important immune factors from many different immune cell types, including factors such as TNF-α, interleukin and IFN-γ. In diabetic heart tissue, Ca^{2+} alterations may further contribute to inflammatory responses. Activated immune cells, cytokines and fibroblasts then promote pro-hypertrophic and pro-fibrotic signalling, which induces cardiac hypertrophy and triggers cardiac fibrosis and remodelling. The red arrow denotes an increase in function or concentration.[18] RCS, reactive carbonyl species; AGEs, advanced glycation end products; ROS, reactive oxygen species; Cav1.2, L-type Ca^{2+} channels; TNF-α, tumour necrosis factor alpha; TRL9, Toll-like receptor 9; IFN-γ, interferon gamma; IL, interleukin; TFG-β, transforming growth factor beta.

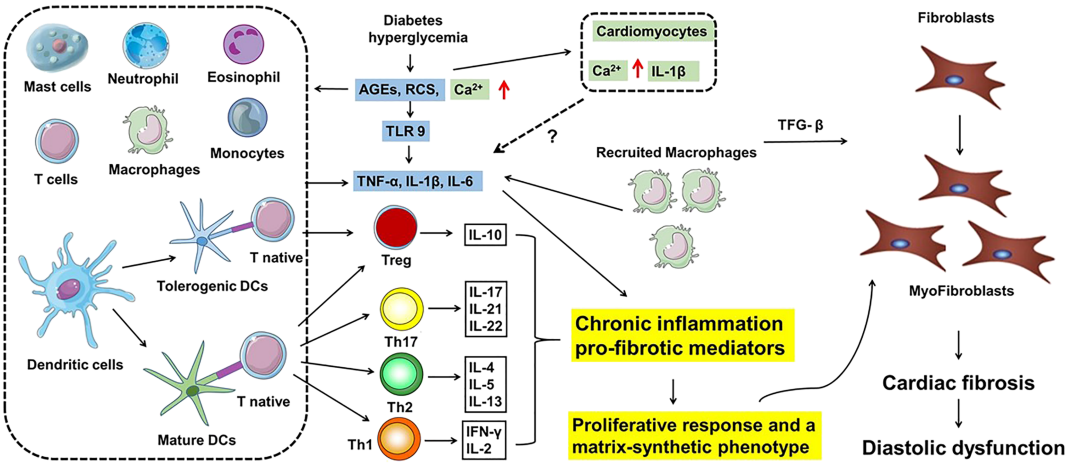

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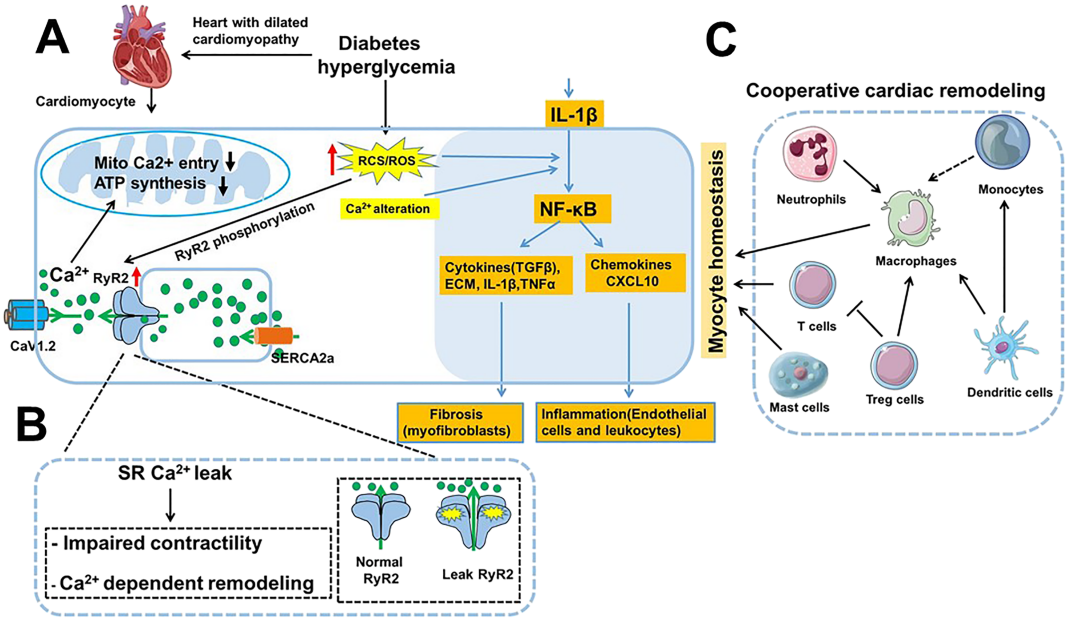
Figure 2 Schematic representation of the mechanism by which RCS and AGE concentrations modify RyRs and immune cells in cardiomyocytes in diabetes. Increased RCS and AGE concentrations modify RyRs. (A) A possible mechanism is that accumulation of RCS/AGEs in diabetes leads to modified RyRs, resulting in the activation of RyRs and Ca^{2+} leakage from the ER. In addition, altered intracellular Ca^{2+} signalling occurs as a consequence of mitochondrial Ca^{2+} uptake. Dysfunction of mitochondria is a cause or consequence of RCS/ROS overproduction. This may represent a feedback loop between the endoplasmic reticulum–mitochondrial interface.[43,44] The key determinants of RCS affect excitation–contraction coupling in cardiomyocytes. Physiologically, Ca^{2+} entry through CaV1.2 triggers SR Ca^{2+} release through RyR2. The systolic transient Ca^{2+} activates myofilaments, initiating contraction. (B) In the diabetic heart, RCS and AGEs alter RyR2 phosphorylation, increase SR Ca^{2+} leakage, promote Ca^{2+}-dependent remodelling and impair contractility. These are consequences of the diabetic heart.[43,53] In addition, cytokines and chemokines released from innate immune cells by stimulation of RCS and AGEs play key roles in the regulation of the immune response between cardiomyocytes. Interleukin-1β is induced by inflammatory signals of RCS, AGEs and Ca^{2+} alterations in a broad number of immune cells types (C) and also stimulates the production and release of multiple cytokines and chemokines from immune cells. These immune responses may affect fibrosis function or connections between cardiomyocytes in diabetic heart disease.[75] The red arrow denotes an increase in function or concentration. RyR, ryanodine receptor; RCS, reactive carbonyl species; AGEs, advanced glycation end products; ROS, reactive oxygen species; Cav1.2, L-type Ca^{2+} channels.



cytoplasmic domains,[42–44,48] which are modulated directly or indirectly by L-type Ca^{2+} channels (Ca1.1/1.2), various ions (Ca^{2+}, Mg^{2+}) and protein kinase A (PKA) and have been shown to fine-tune intracellular Ca^{2+} homeostasis.[11,48] Physiologically, Ca^{2+} entry through L-type Ca^{2+} channels (ICa, L) triggers SR Ca^{2+} release through RyR2 at the junction of the SR. During each heartbeat, these RyR clusters are activated by a relatively small Ca^{2+} influx via L-type Ca channels and provides the trigger to induce Ca^{2+}-induced Ca^{2+} release (CICR) from juxtaposed ryanodine receptors on the SR, resulting in cardiac muscle contraction.[49] RyR2 channels contain several phosphorylation sites. In DCM, PKA and Ca^{2+}/calmodulin-independent protein kinase II (CaMKII) mediate RyR2 phosphorylation and control RyR2 channel activity by RCS and ROS.[7,50,51] In general, low cytosolic diastolic Ca^{2+} concentrations (<10 μM) increase the open probability (Po) of RyR2 channels. Po is maximal at a Ca^{2+} concentration of approximately 10 μM, and elevating Ca^{2+} concentrations beyond this point (1 mM) leads to a reduction in Po.[51,52] Western blot analysis of subcellular fractions indicate that in the heart, RyR2 is localized in the SR, whereas RyR1 is localized in mitochondria (mRyR1), suggesting that mRyR1 becomes activated as soon as Ca^{2+} is released from the SR and is maximally activated at a rise in $[Ca^{2+}]i$. Further increases in the local Ca^{2+} concentration lead to inactivation of mRyR1 to prevent mitochondrial Ca^{2+} overload and opening of permeability transition pores.[10] RyR1 may be related to immune response in heart failure and DCM.[53] SR/ER Ca^{2+} ATPase 2a (SERCA2a) is the major isoform of SERCA expressed in cardiomyocytes and plays a crucial role in the regulation of contraction and relaxation during cardiac contractility. SERCA2a transports cytoplasmic Ca^{2+} into the SR. Ca^{2+} stored within the SR is released with each action potential to induce contraction, and muscle contraction is terminated when this Ca^{2+} is transported back into the SR.[54,55]

RyR in DCM

During the past decade, numerous studies have suggested that increased RyR2 expression is involved in the

pathogenesis of cardiac diastolic dysfunction, systolic dysfunction, heart failure and arrhythmias.[9,12,51,56] However, the role of RyR2 in DCM remains disputed. Hyperglycaemia alters normal metabolic biochemical pathways, resulting in production of RCS and AGEs that accumulate on select basic residues. Mutations of these residues have a physiochemical impact on carbonyl/oxidative stress via RyR channels, with enhanced or reduced cytoplasmic Ca^{2+} responsiveness. Multiple studies have shown that methylglyoxal (one kind of RCS) increases and then decreases the RyR2 Po in experimental models of Type 1 diabetes.[11,48,57] Diabetes mellitus impairs mitochondrial Ca^{2+} handling in the diabetic heart.[10,58] Pathological levels of RyR2 phosphorylation lead to heart failure and DCM. Numerous human cardiac tissue studies show that RyR2 phosphorylation and/or mutations can cause deadly ventricular arrhythmias and atrial fibrillation, sinoatrial node and atrioventricular node dysfunction, atrial fibrillation, atrial standstill, dilated cardiomyopathy, heart failure and sudden cardiac death.[59–61] Diabetic complications are accompanied by increased oxidative stress resulting from the formation of ROS, including superoxide anions, hydrogen peroxide and hydroxyl radicals. These compounds are capable of reacting with reactive cysteines, which cause RyR2 dysfunction. Sulfhydryl oxidation by various oxidizing agents has been shown to increase RyR2 Po.[6,62,63] In addition, reduced activity and expression of SERCA2 protein have been described in heart failure and DCM, resulting in altered Ca^{2+} handling and cardiac contractility.[55,56] These studies suggest that the dysfunction of RyR may lead to the pathology of DCM. The detailed mechanisms of immune responses in DCM are summarized in *Figure 2A,B*.

RyR and immune responses in fibroblasts associated with DCM

Studies by Zak et al. and Nag et al. have confirmed that the adult rat heart consists of 30% myocytes and 70% non-myocytes.[64,65] Cardiac fibroblasts respond to a wide range of different stimuli during cardiac development and disease, including abnormal glucose metabolism in diabetes mellitus, as well as changes in chemical and mechanical signals.[38] Various studies have shown that stimulation of fibroblasts could lead to a marked upregulation of cardiac ECM components, including proteins and polysaccharides secreted by cardiac cell types, ECM-specific receptors and expression of various cytokines and growth factors.[66] Cardiac fibroblasts are also the main source of the pro-inflammatory cytokine IL-1β in the heart following injury.[67] A number of studies have found a close connection between plasma TNF-α levels and the progression of left ventricular remodelling and heart failure,[68] and animal studies have demonstrated that infusion of TNF-α results in cardiac dysfunction and heart failure.[37,69,70] It has been shown that TNF-α increases expression levels of IL-1β and IL-6 in cardiac fibroblasts *in vitro*.[71] TGF-β is another cytokine that is produced by many immune and non-immune cells. TGF-β signalling is typically upregulated during heart failure and interacts with its cell surface receptors, with cardiac fibroblasts being the primary producers; thus, the role of TGF-β is involved cellular proliferation, cellular differentiation, migration, apoptosis and ECM production to form myofibroblast differentiation.[66,72] Taken together, these data suggest that immune-related molecules are a key factor in cardiac structure and function in DCM and Ca^{2+} signalling in cardiomyocytes also activates myofibroblasts. In human cardiac myofibroblasts, the expression of Cav1.2 has been detected at the RNA level in human cardiac fibroblasts and ventricular myocytes.[73,74] Influx of Ca^{2+} through Cav1.2 initiates a much larger Ca^{2+} release from the SR through RyR2 via CICR; the process contributes to the electrical properties of cardiomyocytes. Unlike cardiac myocytes, whose electrical properties, mechanisms of Ca^{2+} signalling and RyRs function in cardiac fibroblasts and myofibroblasts are unclear. For example, RyR1 signalling does not arise from human cardiac fibroblasts; additionally, Ca^{2+} oscillations observed in cultured human cardiac fibroblasts are not influenced by ryanodine or caffeine[74]; however, functional Cav1.2 currents cannot be obtained via patch-clamp recordings.[73,75] These results indicate that RyRs and Cav1.2 may indirectly be involved in Ca^{2+} signalling and electrophysiological conditions. Multiple immune cytokines and interleukins, such as TNF-α, IL-1β and IL-6, have similar effects on intracellular Ca^{2+} handling via modification of RyRs and Cav1.2, thereby influencing fibrosis formation.[75] The discussion above suggests a number of scenarios and indicate that immune-related molecules, RyRs and Ca^{2+} signalling may be linked to myocyte–fibroblast communication in DCM and may also be functionally relevant to electrical coupling in cardiac myocytes, fibroblasts and immune cells. *Figure 1* shows the detailed mechanisms of immune responses and Ca^{2+} signalling in DCM.

Relevance of mechanisms between RyRs and immune-related molecules in DCM

As discussed above, the pathophysiology of DCM and heart failure consists of dysfunction of cardiomyocytes, fibroblasts and immune cells. Leaky RyR2 channels contribute to defective cardiac E-C coupling and abnormal properties in fibroblasts that modify the output of the ECM, resulting in abnormal immune responses. TGF-β is an important mediator in the differentiation of cardiac fibroblasts to myofibroblast and has been shown to have direct effects on cardiomyocyte function; there is also evidence for the involvement of IL-6 in the communication processes.[76–78] Evidence for their direct

and indirect effects on communications between cardiac fibroblasts and cardiomyocytes remains unclear. RyRs and immune-related molecules may contribute to mechanisms of cardiomyocyte–fibroblast communication. Studies have shown that inflammatory cytokines impact myocardial function and increase diastolic Ca^{2+} release, which may contribute to arrhythmias. This may, in turn, also induce structural alterations such as myocarditis.[79] TNF-α and IL-1β increase Ca^{2+} leakage from the SR, which contributes to depressed systolic Ca^{2} transients, resulting in the cardiac contractile function in arrhythmia in rat ventricular myocytes.[67,80] One possible explanation by which immune-related molecules, such as TNF-α and IL-1β, increase mimic detrimental effects on triggered SR Ca^{2+} release from the SR is that RyR2 clusters involved in the calcium sparks and calcium transients may be refractory to triggered CICR.[80] It has been shown that RyR1 is expressed in lymphocytes, DCs and macrophages.[18,53,81,82] Studies on the role of RyR1 have suggested that activation of B lymphocytes is coupled to inflammatory cytokine release[83] and leads to enhanced DCs, release of pro-inflammatory cytokines and enhanced priming of T cells.[81] Kho et al.[55] have shown that B lymphocytes exhibit leaky RyR1 channels, leading to reduced ER Ca^{2+} stores and Ca^{2+} dysregulation in chronic heart failure. Expression of RyR1/RyR2 mRNA has additionally been reported in human T cells, and RyR1/RyR2 protein immunoreactivity using primary antibody was seen in activated but not in resting T cells. The RyR agonist caffeine evokes $[Ca^{2+}]i$ transients recorded from activated T cells but not in resting T cells. These data indicate that RyR expression is functionally upregulated in activated T cells, suggesting that RyRs are an important regulator of Ca^{2+} signalling and Ca^{2+}-dependent functions in immune cells for modulating immune responses in humans.[81–87] Several reports of pharmacological inhibition of RyRs by ryanodine or dantrolene demonstrate effects on major functions of human T cells such as Ca^{2+} release via RyRs, which is not a direct result of TCR activation but instead depends on RyRs activation by Ca^{2+} originating from store-operated Ca2 + entry; inhibition of RyRs reduces T-cell proliferation and IL-2 production.[85,86] *Figure 3* shows how RyRs affect T-cell function during T-cell activation. These studies collectively indicate that alterations in Ca^{2+} signalling and abnormal immune responses may represent another source of cardiomyopathy. Evidence suggests that

Figure 3 RCS and AGEs affect organellar Ca^{2+} channels and modulators of Ca^{2+} signalling during T-cell activation. The role of IP3 in initiating and sustaining T-cell Ca^{2+} signalling is well established. NAADP and cADPR are proposed to synergize with IP3 during T-cell Ca^{2+} signalling; NAADP activates RyR1 and causes ER Ca^{2+} release, which synergizes with IP3 to cause further Ca^{2+} release via IP3 receptors (IP3Rs). In addition, activation of RyR2 by cADPR serves to sustain Ca^{2+} signalling for T-cell function. Ca^{2+} entry through ORAI channels may activate RyR isoforms to maintain store depletion and SOCE. Mitochondrial Ca^{2+} uptake through the mitochondrial MCU at the vicinity of ORAI channels maintains CRAC channel activity by relieving its CDI. Expression of CaV1.2 and CaV1.1 at the plasma membrane may affect TCR-activated Ca^{2+} entry and nuclear factor for activated T-cell (NFAT) activity. One intriguing hypothesis in T cells is the existence of a conformational coupling between CaV1.1, CaV1.2 and RyRs. RCS and AGEs may affect RyR function and alter Ca^{2+} signalling in T cells. This intriguing possibility in T cells may further contribute to cross-communication between fibroblasts and cardiomyocytes. The red arrow denotes an increase in function or concentration. CRAC, Ca^{2+} release-activated Ca^{2+}; NAADP, nicotinic acid adenine dinucleotide phosphate; cADPR, cyclic ADP ribose; ER, endoplasmic reticulum; IP3, inositol-1,4,5-trisphosphate; IP3R, IP3 receptor; SOCE, store operated Ca^{2+} entry; MCU, Ca^{2+} uniporter; CDI, Ca^{2+}-dependent inhibition; TCR, T-cell receptor 90.

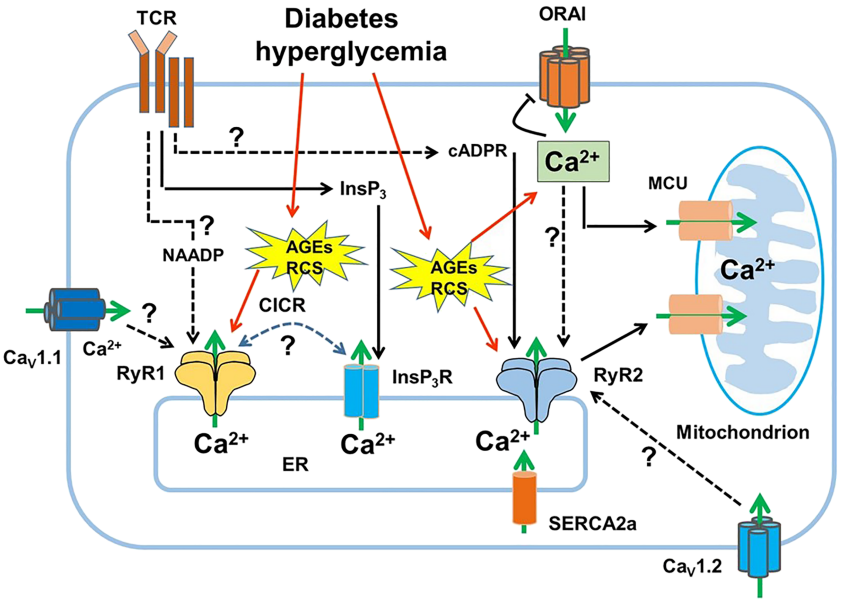
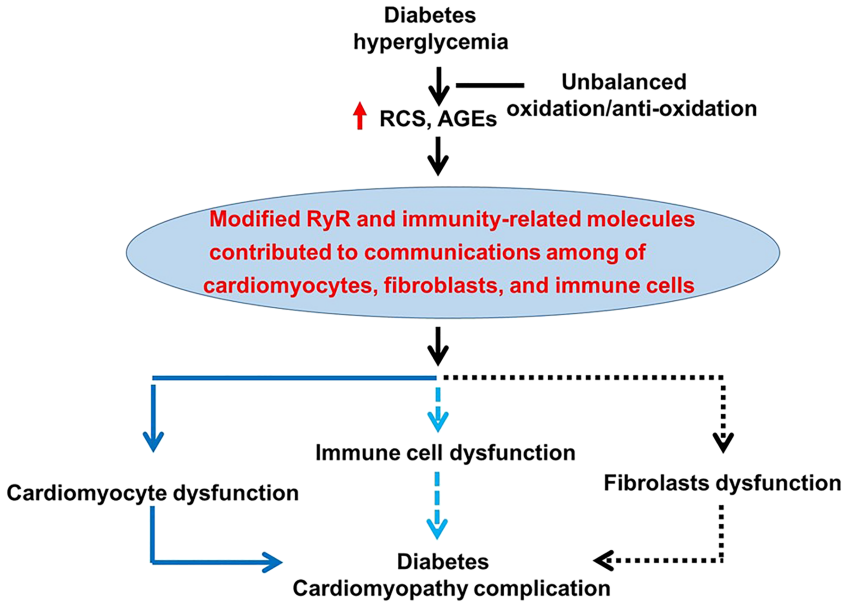


Figure 4 Pathological mechanism of diabetic cardiomyopathy and relevance of intercellular communication among cardiomyocytes, fibroblasts and immune cells in diabetic heart tissue. One major pathological mechanism of cardiomyopathy is that RyR2 PTMs by RCS and AGEs result in RyR2 dysfunction and dysfunctional Ca^{2+}-signalling pathways in cardiac muscle tissue. Additionally, immune-related molecules also contribute to pro-inflammatory signalling in cardiomyocytes, which may represent the main source of cardiomyopathy. Another pathological mechanism of cardiomyopathy is that RCS and AGEs may affect RyRs and Ca^{2+} singling in immune cells, such as T cells, resulting in dysfunction of the immune system. An alteration in Ca^{2+} singling and immune-related molecules may contribute to RyR2 dysfunction in cardiomyocytes, as well as inflammation in fibroblasts. The red arrow denotes an increase in function or concentration. RyR2, ryanodine receptor type 2; RCS, reactive carbonyl species; AGEs, advanced glycation end products.



interactions among RyR1, RyR2, Ca^{2+} signalling and immune-related molecules may directly or indirectly be affected in diabetic hearts during some pathophysiological stages in DCM. Further elucidation of the relationship between RyRs and immune-related molecules will likely improve treatments for mitigating diabetic complications.

Conclusion and future directions

The heart contains cardiomyocytes, cardiac fibroblasts and many kinds of immune cells. Initial studies mainly focused on RyR2 in cardiomyocytes because RyR2 directly reflects the contractile function of the heart. RyR1 is also expressed in cardiac tissue but at low levels. RyR1 and RyR2 collectively control cytosolic Ca^{2+} concentrations to maintain cardiomyocytic function. Physiological functions of cardiac fibroblasts are capable of eliciting an immune response to protect the function of cardiomyocytes. Contributions from immune cells, such as T lymphocytes and monocyte/macrophage-lineage cells, have also been reported in cardiac tissue. The past few decades have generated a growing recognition that the immune system provides an important contribution to cardiac development, composition and function. Evidence suggests that interactions among RyRs, Ca^{2+} signalling and the immune system may be directly or indirectly affected in diabetic hearts. Elucidation of RyRs and immune-related molecules has significantly contributed to our understanding of intercellular communication among cardiomyocytes, cardiac fibroblasts and immune cells (*Figure 4*). Our understanding of the complex communication between immune cells and RyRs in cardiac tissue remains incomplete. Future studies should focus on determining the components that intervene between individual immunity molecules and RyR function in diabetes, which will be crucial for obtaining invaluable insights into the design of therapeutic approaches to prevent and/or treat DCM in humans.

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Conflict of interest

The authors declare that they have no competing interests.

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