Chapter

The Role of the Receptor for Advanced Glycation Endproducts (RAGE) in Type 1 Diabetes: An Immune Cell Perspective

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Abstract

Type 1 diabetes (T1DM) is an autoimmune disorder resulting in destruction of the insulin producing pancreatic β-cells that reside in the Islets of Langerhans. Despite significant progress in the understanding of T1DM pathogenesis, some fundamental contributing mechanisms remain to be fully elucidated. The receptor for advanced glycation end products (RAGE) and its ligands are increasingly believed to play a role in the development of T1DM, but this is not well understood. The location of RAGE gene is shared with major T1DM genetic susceptibility loci on chromosome 6 and polymorphism of this region confers risk for T1DM. Furthermore, changes in RAGE expression on and ligand binding by immune cells, in particular T cells, are associated with pro-inflammatory and autoimmune profiles key for T1DM development. Indeed, in murine models for T1DM, targeting of RAGE or its ligands decreased onset and severity of disease including favorable immune cell profiles and infiltration and improved beta cell insulin secretory function. Further understanding of RAGE expression and signaling in immune cells in T1DM will provide valuable insights into disease pathogenesis and therapy development. This chapter will discuss what is currently known about RAGE in the immune cells integral for the pathogenesis of T1DM.

Keywords: type 1 diabetes, RAGE, RAGE ligands, advanced glycation Endproducts, T cells, APCs, NK cells, neutrophils

1. Introduction

Type 1 diabetes (T1DM) is a complex autoimmune disorder resulting from the destruction of pancreatic insulin producing β-cells due to the loss of self-tolerance. There has been a rapid increase in disease incidence worldwide and T1DM is increasingly being diagnosed in even younger individuals and in those from diverse cultural backgrounds. Insulin replacement therapy remains the only viable option for individuals with T1DM. Ensuring any new therapies are meeting a very high safety bar and are superior to insulin replacement therapy remains a challenge. Indeed, treatment with global immunosuppression, while somewhat effective, has significant side

effects and is not a practical solution to prevent T1DM which commonly develops in early life. Also, despite insulin's excellent safety profile, management of insulin replacement therapy involves multiple daily injections, pumps and continuous blood glucose monitoring or closed loop systems which are costly and complex to maintain, particularly in younger children. These therapies also bring increased risk for life-threatening high (hyperglycemia) or low (hypoglycemia) blood glucose concentrations as well as increased risk for chronic complications and shortened lifespan. Despite significant progress made in the development of therapeutics for T1DM and some remarkable results in the pre-clinical models, a successful translation into clinic is yet to occur. Due to the T1DM's multifactorial nature, the necessity to find links between genetic predisposition, immune system abnormalities and environmental triggers is becoming increasingly apparent. Therefore, exploring relationships between these contributing factors is necessary for better understanding of the disease progression and for design of the best therapeutic approaches. More recently, multiple studies have focused on the complex biology and involvement of the receptor for advanced glycation end products (RAGE) and its ligands in inflammation, autoimmunity, diabetes complications, apoptosis, and endoplasmic reticulum stress including the exploration of various ways to alleviate these. This presents an exciting new avenue for the development of targeted RAGE-related therapeutics and their translational potential from pre-clinical models to humans.

2. Preclinical murine models of T1DM

The non-obese diabetic (NOD) mouse model has been a useful tool to examine pathological mechanisms which contribute to and may be targeted in T1DM. Although there are several fundamental differences between murine and human disease [1, 2], the late timing of human disease manifestation, pancreas inaccessibility and lack of biomarkers in the peripheral blood continue to pose a significant challenge. Therefore, NOD mouse models remain instrumental in studying the disease pathophysiology and aids efforts to improve clinical translational potential of identified pathways and a first-line screening for effective therapies. Humanized mouse models, which are mice with a "human-like" immune system have proven to be an excellent platform to bridge the gap between preclinical mouse models and clinical studies by enabling researchers to assess the efficacy of treatments on human immune cells in a more physiological context than provided by cell culture [3]. In the T1DM field several excellent preclinical experimental approaches have also been proposed [4–6], although spontaneous T1DM disease development and sufficient similarity to human disease are still challenges.

3. RAGE in T1DM

RAGE is a member of the immunoglobulin superfamily It is postulated to play a role in host-pathogen defense and its expression increases during inflammation and in chronic inflammatory conditions including T1DM. The location of the RAGE gene (*AGER*) in humans is within the major histocompatibility complex on chromosome 6p21.3, a region implicated in various autoimmune disorders such as T1DM [7–9]. Similarly in mice, the *AGER* gene is located on chromosome 17 where several quantitative trait loci for T1DM have been reported [10]. RAGE is a multifunctional and promiscuous-ligand receptor, binding a wide array of ligands such as such as

advanced glycation endproducts (AGEs), high mobility group box-1 (HMGB1), S100 proteins, β-amyloid fibrils, and others [11]. Upon binding to its ligands, the cytoplasmic domain associates with adaptor proteins including TIRAP and MyD88 followed by activation of downstream signaling of various pathways including NF-κB and STAT3 (**Figure 1**) [12].

Advanced Glycation Endproducts (AGEs) are canonical RAGE ligands but are low affinity and likely only bind RAGE when increased in concentration [13]. AGEs are formed as the result of non-enzymatic modifications to amino groups on lipids, proteins, peptides, and nucleic acids. Modern industrialization of food, flavor, and color enhancement as well as increased emulsification contribute significantly to formation of AGEs [9, 14, 15]. Prolonged exposure to AGEs has detrimental effects on β-cell function, insulin secretion and sensitivity and disease development in healthy humans and rodents [16]. Moreover, accumulation of AGEs and RAGE is associated with macro and micro vascular complications in diabetic patients [14, 17–19]. In the islet autoantibody positive individuals, the levels of circulating AGEs served as independent predictor for T1DM progression [20]. Chronic exposure of rodents to AGEs led to defects in insulin secretion and beta- cell death as well as defects in mitochondrial function supported by studies in isolated islets and beta cell lines such as MIN6N8. Following the treatment with AGE-lowering agent, the incidence of autoimmune diabetes was reduced in NOD mice [21].

HMGB1 is a non-histone chromosome protein present in all cells and may serve as a transcription factor in proinflammatory conditions [22]. Both RAGE and toll-like receptor 4 (TLR4) have been reported to serve as HMGB1 receptors, although SPR binding studies suggest that HMGB1 only binds to RAGE if associated with DNA fragments. Therapeutic potential of HMGB1 blockade has been shown in NOD mice, reducing T1DM incidence and autoimmunity [23, 24]. In newly diagnosed children

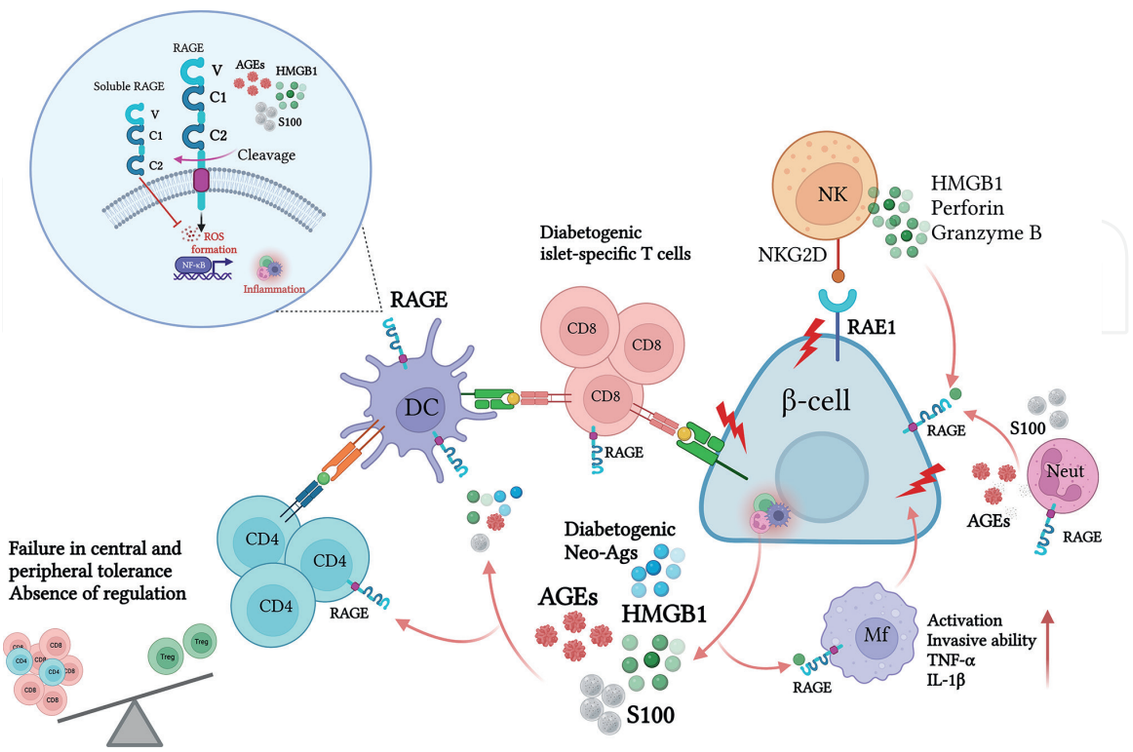

Figure 1.
RAGE cellular expression in immune cells important in T1DM development.

with T1DM, HMGB1 serum concentrations were significantly higher compared to controls suggesting its potential use as an inflammatory biomarker in the disease progression [25]. More recently, it was proposed that increases in HMGB1 impairs the stability of regulatory T cells (Tregs) in NOD mice and increases production of interferon-γ (IFN-γ). In individuals with T1DM increased levels of serum HMGB1 were directly correlated with increases in IFN-γ production by Tregs. Neutralizing HMGB1 antibody rescued Treg function and suppressed autoimmunity [23]. Furthermore, hyperglycemia may contribute to the release of HMGB1 by antigen presenting, natural killer and endothelial cells as well as necrotic and apoptotic cells leading to augmented autoimmunity [26].

Another set of proteins identified as RAGE ligands is S100 calgranulin family containing over 20 members with S100A8/9 and S100B binding RAGE [27]. Although their major site of manufacture is believed to be endothelial cells, they are known to be expressed by myeloid cells such as neutrophils, but expression by lymphocytes has not been reported. This proinflammatory heterodimer is implicated in several conditions including inflammatory bowel disease and rheumatoid arthritis. Furthermore, S100A8/A9 expression is associated with tumorigenesis, suppression of DC function and accumulation of myeloid-derived suppressor cells (MDSCs) [28].

RAGE can exist as both transmembrane protein and a truncated soluble form (sRAGE). The latter is present in serum and is postulated to act as a decoy receptor by competitively binding RAGE ligands and preventing downstream signaling [11, 12]. RAGE expression tends to be low in most tissues except for the skin and alveolar epithelial cells in the lung [29]. RAGE is expressed on a number of immune cells such as T lymphocytes, neutrophils, antigen presenting cells (APCs) including macrophages, dendritic cells (DCs) and B cells as well as endothelial cells [30]. The precise role of RAGE in these cell populations as well its ability to influence cell-cell interactions and behavior still eludes us. However, RAGE continues to serve as one of the major players in inflammatory and autoimmune conditions creating a perfect opportunity to investigate these links.

3.1 RAGE and T cells

Immune tolerance involves a diverse range of processes that prevent potentially harmful immune responses against self-antigens. Given that T1DM is a T cell mediated autoimmune disease, a key event in its development is the failure in the mechanisms of central tolerance, which allows for self-reactive T lymphocytes to escape deletion by the immune system. Once in the periphery, these effector T lymphocytes can exert deleterious effects with $CD4^+$ T lymphocytes believed to be important initiators and progressors of autoimmunity. Upon encounter of islet-antigen presented by dendritic cells (DCs), $CD4^+$ T lymphocytes become activated which promotes and perpetuates the diabetogenic process. The reasons as to why islet autoantigens are specifically presented to the immune system by DCs to amplify immune responses remains to be fully elucidated. Migration of DCs to pancreatic lymph nodes and islet-antigen presentation there amplifies recruitment and activation of $CD4^+$ and $CD8^+$ T lymphocytes.

An extensive pancreatic β- cell loss or loss of function is hallmark of T1DM. It is now well appreciated that autoreactive T cell are amongst key players in this process. $CD4^+$ T cells are thought to be initiators of the disease providing help for B cells in "auto"antibody production as well as enhancing effector activity of $CD8^+$ T cells and islet-resident macrophages. $CD8^+$ effector T cells are considered to cause pancreatic islet destruction and commonly dominate islet infiltrates [31–33].

Tregs are crucial for the maintenance of the peripheral tolerance where they dampen the effects of any self-reactive cells which have escaped deletion by central tolerance. In T1DM, there is impairment in function of and/or the numbers of Tregs present. This imbalance contributes to greater pathogenic activity of effector T lymphocytes and leads to a loss of peripheral tolerance. Peripheral tolerance is the local backstop to prevent self-antigen production and delete T cells with antigen specificity for self-antigens. Thymic derived Tregs (tTregs) defined as $CD4^+CD25^+Foxp3^+$ Tregs are responsible for suppression of effector T lymphocytes through secretion of anti-inflammatory cytokines and competition for IL-2. Both function and frequencies of this subset of Tregs is impaired in both humans and NOD mice [34]. Therefore, restoring their functionality and numbers appears to be an attractive option for preventing T1DM development and is certainly widely under investigation. Currently, there are a number of T cell-centered therapeutic approaches for treatment and prevention of T1DM under development. These include Treg enhancement and antigen-specific strategies, as well as strategies that dampen activation of T cells using CTLA-4-Ig (Abatacept) or anti-CD3 monoclonal antibody (Teplizumab) (reviewed here [35]). The latter has produced promising results in at risk antibody positive individuals significantly improving β-cell function [36, 37]. If this therapy progresses to clinic in the near future, it will make an excellent candidate for combination therapies.

RAGE expression is elevated on T cells from "at-risk" islet autoantibody positive (IAb+) individuals and is associated with progression to T1DM and increased effector function of T cells [38, 39]. The soluble isoform of RAGE (sRAGE) can competitively bind RAGE ligands and inhibit RAGE signaling. Previous studies have shown that RAGE gene (AGER) polymorphisms result in reduced sRAGE in the circulation which correlates with increased risk of T1DM and seroconversion to islet autoantibodies [9, 40]. Murine studies have demonstrated a role for RAGE in T cell activation, priming and effector function, where RAGE-deficient T cells showed reduced proliferation and production of pro-inflammatory cytokines such as IFN-γ [41]. RAGE also plays a role in DC maturation, migration, and function as well as T cell priming. In children with acute Kawasaki disease and juvenile idiopathic arthritis, RAGE facilitates recruitment and activation of leukocytes and sRAGE is reduced [42].

Another study explored the effects of dietary AGEs in NOD mice. Here, a T cell receptor (TCR) transgenic NOD 8.3 males with $CD8^+$ T cells specific for $IGRP_{206–214}$, one of the main diabetogenic antigens [43], and NOD/ShiLt females and their NOD8.3 female offspring were fed low or high AGEs containing diets from conception to weaning of the offspring. The low AGEs diet resulted in improvements in insulin, proinsulin, and glucagon secretion by the islets as well as reduction in AGEs and RAGE expression in offspring islets. Furthermore, reduced level of immune cell infiltration was seen in the infants whose parents were fed with low AGEs diet in the perinatal period [16]. This is consistent with another previous intergenerational study of low AGE feeding where decreasing rates of T1DM were seen in subsequent generations during feeding with a low AGE diet [44] In the NOD model RAGE antagonism using sRAGE, significantly decreases progression to overt diabetes onset in NOD mice and preserves β-cell mass and insulin secretory function and that sRAGE therapy did not work following specific depletion of Tregs. This was evidenced through reduced islet infiltration, preservation of islet integrity and numbers as well as insulin expression. Moreover, an increased proportion of Tregs in pancreata, pancreatic lymph nodes and spleens of treated animals was demonstrated. sRAGE-induced improvements in human Tregs proliferative and suppressive ability *ex vivo* [45],

whilst exposure to RAGE ligands, AGEs impairs Treg suppressive capacity [45]. These observations suggest that RAGE inhibition may offer protection against future T1DM development [46].

3.2 RAGE and NK cells

Natural Killer (NK) cells are cytotoxic innate lymphocytes that bridge innate and adaptive immune systems. NK cells' importance is firmly cemented in the field of cancer immunology due to their unique ability to recognize and destroy tumors and virus infected cells. Their killing capacity is driven by the activating and inhibitory receptor-ligand interactions as well as cytolytic granules containing perforin and Granzyme B similar to the $CD8^+$ T cells. Some important lectin-like activating receptors include NKG2D and KLRG1 whilst inhibitory include NKG2A and KIRs in humans and Ly49 in mice [47]. Unsurprisingly, NK cells are coming into focus in the context of T1DM as an important effector population for the disease pathogenesis. Their interaction with and ability to suppress other effector cells such as CD8+ T cells is of vital importance, particularly in the setting of autoimmunity [48].

During early human studies it was shown that proportion of NK cells was significantly lower in the peripheral blood of individuals with T1DM compared to controls. This was further linked to reduced lytic and cytotoxic capacity of NK cells and more frequent occurrence of tumors [49–51]. The dysregulation in NKG2D signaling as well as reduction in NK cell proportion was suggested to be a contributing factor to the development of T1DM [52].

HMGB1 a known RAGE ligand plays an important role in NK cell killing ability upon activation. HMGB1 released from NK cells' cytotoxic granules is very effective against oxygen-dependent cancer cells whilst those cells with anaerobic energy metabolism were resistant to HMGB1 mediated killing [53]. These observations are of importance with respect to pancreatic inflammation and associated pathologies. Narumi et al. proposed an NK cell-RAGE dependent suppression mechanism of S100A8/A9 expressing tumors It was proposed that NK cells express RAGE but not TLR4 which is also known to bind S100 family of proteins [54]. The ligation of S100A8/A9 to RAGE led to activation of NK cells, increasing their cytotoxic ability evidenced by elevated IFN-γ production, increased NKG2D activating receptor activity and amelioration of tumor growth. RAGE blockade reversed these effects, highlighting the importance of RAGE-S100 axis for suppression of tumor growth and NK cell cytotoxic ability [55]. Whilst there is as yet no direct evidence linking T1DM, NK cells and RAGE, the clear involvement of RAGE axis and NK cells in T1DM and other autoimmune and proinflammatory disorders warrants future investigation.

3.3 RAGE and neutrophils

Neutrophils are phagocytic leukocytes of innate immune system circulating in the blood in a dormant state. Their activation is initiated in the early stages of inflammation and mechanisms for pathogen clearance include release of cytotoxic granules, cytokines, and reactive oxygen species (ROS). Neutrophils possess a unique ability to form neutrophil extracellular traps (NET) when undergoing altered cell death which have unique antimicrobial properties. Moreover, neutrophils can engage with and modulate activity of other immune cells such as T and B lymphocytes, NK cells and DCs. This can lead to exacerbation of autoimmune disorders such as systemic lupus erythematosus (SLE), multiple sclerosis and autoimmune diabetes [56–58].

In NOD mice activated neutrophils are recruited to the pancreatic islets initiating development of autoimmune diabetes and facilitating recruitment of $CD8^+$ T cells and DCs. Studies in NOD mice shown that migration of neutrophils plays pivotal role in disease progression and preventing it halts or reverses disease development [56, 59]. Similarly, neutrophil infiltrates were found in human pancreata prior to T1DM onset and in individuals with overt disease where there was an evidence of NET formation highlighting pathogenicity of the infiltrating neutrophils. Furthermore, neutrophils from the peripheral blood of autoantibody negative at-risk individuals, displayed a unique molecular signature with overexpression of interferon-related genes [60]. These data reinforce the importance of neutrophils in the initiation and progression of T1DM.

It was previously demonstrated that RAGE expression on human neutrophils and binding to AGEs was associated with impaired neutrophil function, in particular bacterial killing [61]. In the murine streptozotocin induced T1DM model the RAGE ligand S100 calcium-binding proteins A8/A9 (S100A8/A9) are released by neutrophils binding to RAGE on hepatic Kupffer cells leading to increase of IL-6 thrombopoietin production associated with atheroprogression in humans [62]. Albeit limited, these data support the importance of RAGE axis in effector immune populations which may be important for the development of T1DM.

3.4 RAGE and antigen presenting cells

Professional antigen presenting cells (APCs) include dendritic cells (DCs), macrophages and B cells. These cell populations have been extensively studied in T1D due to their tolerogenic and immunogenic properties and importance in therapy development [63].

DCs are categorized into conventional (cDCs), plasmacytoid (pDCs), monocyte-derived (moDCs) and Langerhans cells (LCs). They are important regulators of immune tolerance, can select specific T cell subsets for anergy or deletion and therefore play pivotal roles in the pathogenesis of T1DM. Their immunomodulatory abilities have been widely explored and are targets for various therapeutic strategies in development including antigen specific immunotherapy [64]. Within the pancreata of NOD mice, DCs and macrophages can be detected as early as 3 weeks of age [65, 66]. Interestingly, studies of chronic high AGE feeding of healthy rats also show the appearance of an islet specific infiltrate that is comprised primarily of macrophages [21]. Other studies suggested that plasmacytoid DCs (pDCs) are recruited to the pancreata of NOD mice where they promote diabetogenic T cell activity and initiate T1DM [56]. Many other studies highlighted the importance of DC function in disease initiation and progression (reviewed here [67, 68]). Their maturation status dictates the level and type of response exerted. The immature or tolerogenic DCs usually exhibit low levels of MHC class II and costimulatory molecules expression such as CD80 and CD86, reduced ability to stimulate T cells and produce proinflammatory cytokines, however their phagocytic and antigen processing and presentation capacity is not affected [68]. Several strategies explored immunomodulatory potential of tolerogenic DCs and maintaining them in their immature state for treatment and prevention of autoimmune disorders including T1DM (reviewed in [64, 68, 69]. DCs will continue to be in the T1DM therapy development spotlight due to their proven ability to induce T cell anergy or deletion while promoting and increasing regulatory T and B cell populations.

The exposure of immature DCs from human PBMCs to a high glucose environment and AGEs results in the upregulation of costimulatory markers, increases

production of reactive oxygen species (ROS) and proinflammatory cytokines such as IL-12 and IL-6, decrease in regulatory cytokines such IL-10 and enhances expression of AGEs scavenger receptors SR-A and CD36 and RAGE [70, 71]. In another study by Ge et al., AGE-BSA treatment increased DC expression of both SR-A and RAGE but pre-treatment of DCs with RAGE neutralizing antibody halted maturation by impairing upregulation of costimulatory markers and expression of IL-12 [72]. Another study described the effects of AGEs-stimulation in the presence of the antioxidant resveratrol in immature DCs derived from healthy donor PBMCs. Pre-treatment of DCs with resveratrol prior to AGE exposure prevented their maturation, MAPK and NK-κB activation and production of inflammatory cytokines and reduced RAGE expression [73]. This once more highlights that delineating the AGE-RAGE axis in inflammatory or aging processes may have future benefit in understanding T1DM pathogenesis.

Failure of peripheral and central tolerance not only results in autoreactive T cells but also autoreactive B cells and their migration to pancreas and pancreatic lymph nodes. B cells can present autoantigens to islet specific $CD4^+$ and $CD8^+$ effector T cells, which in turn causes destruction of pancreatic β-cells. B cells are necessary for the development of autoimmune diabetes in NOD mice in particular autoantibody production and B cell depletion prevents development of the disease [74–76]. In newly diagnosed individuals with T1DM, B cell depletion using anti-CD20 monoclonal antibody rituximab showed initial preservation of β-cell function and the need for exogenous insulin was reduced for up to 1 year post treatment. However, following a two-year follow up period, initial improvements in C-peptide (a marker of beta cell insulin secretion/function) were diminished and the clinical trial was terminated [77, 78]. Nonetheless, despite disappointing therapeutic results, B cell importance in T1DM progression is well appreciated and several improvements to the B cell depletion therapeutic approaches have since been proposed [79].

In murine models of antibody mediated autoimmune disorders such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) the absence of RAGE tended to cause reduction in germinal center B cells along with decreased anti-dsDNA autoantibody titers and increases in follicular B cells [80]. Germinal centers play important role in B cell maturation, clonal expansion, and class-switching as well an antibody production? [81]. Though these modest changes in B cells in RAGE−/− animals may not be sufficient to prevent autoimmunity, they are indicative that RAGE signaling is an important contributor to these processes [80]. Another study explored the effects of RAGE/HMGB1 interactions on activation of autoreactive B cells. In that particular study, it was concluded that HMGB1 binding promotes activation of autoreactive B cells through TLR9 rather than RAGE [82].

Macrophages act alongside DCs for antigen presentation [83, 84]. Though human pancreatic islets have macrophages present, it is unclear whether there are changes between resident and infiltrating populations in T1DM [85]. The phenotype of macrophages can in general be divided into M1/pro-inflammatory cytotoxic and M2/anti-inflammatory, alternatively activated cells. However, the local microenvironment plays important role in the process of monocyte to macrophage differentiation. Human macrophages represent less than half of the APC population seen in T1DM and form a mixture of both M1 and M2 phenotypes [86]. In NOD mice APCs are represented almost entirely by macrophages and they have an intricate relationship with lymphocytes, although this may be due to limitations in assessing APC phenotypes in mice. Some studied observed that the absence of resident macrophages arrested T1DM development in NOD mice [87]. Depletion of islet-resident APCs caused complete elimination

of pancreatic lymphocyte infiltration which was restored upon re-introduction of DCs and macrophages [88, 89]. Following the depletion of macrophages, lymphocytes are also unable to initiate T1DM in the NOD-SCID adoptive transfer mouse model [90].

Monocytes, macrophages as well as RAGE- ligands axis are known players in the diabetic vascular complications. RAGE expression is associated with activation of both monocytes and macrophages which has been well explored in diabetes complications such as atherosclerosis [91, 92] and kidney disease [93–95]. Furthermore hypoxic environments can enhance monocyte adhesion and chemotaxis as well as induction of macrophage proinflammatory phenotype mediated by RAGE activity [96]. Furthermore, HMGB1 signaling through RAGE promotes secretion of IL-10 by M2 macrophages is of particular significance in the hypoxic environments of certain metastatic tumors [97]. In the individuals with T1DM suffering from retinopathy and nephropathy, the mRNA RAGE expression in monocytes was significantly reduced compared to controls. Upon exposure of monocyte cultures to glyceraldehyde-derived AGEs both mRNA and protein levels of RAGE were decreased [98]. This was a surprising result considering that upregulation of RAGE is associated with proinflammatory processes. Another study highlighted increased RAGE expression and activation in the M2 macrophages necessary for tumor vascularization and invasion [99]. Despite significant gaps in our knowledge there is unequivocal evidence that upregulation of RAGE upon binding its ligands leads to activation of proinflammatory cascades which likely impart detrimental effects in autoimmunity.

RAGE is an immunoglobulin type receptor comprised of a ligand binding V-type domain, C1 and C2 domains, transmembrane and cytoplasmic domains. C-truncated soluble RAGE can result from proteolytic cleavage or endogenous splicing of the RAGE gene, *AGER*. RAGE as a pattern recognition receptor can bind a wide range of ligands including AGEs, HMGB1, S100 calgranulins. Commonly, upon ligation downstream signaling via Diaphenous-1 and JAK-STAT pathways ROS production and NF-κB activation occurs stimulating inflammatory processes [11]. In T1DM, RAGE is postulated as important for various mechanisms of central and peripheral tolerance which fail to suppress the escape and activation of autoreactive T cells. This occurs in the presence of already dampened regulatory mechanisms where both function and number of Tregs are reduced This leads to further activation and expansion of pathogenic islet specific T cells aided by antigen presentation by DCs. $CD8^+$ T cells migrate to pancreatic islets facilitating immune destruction and, pancreatic β-cell death and further formation and release of diabetogenic antigens. Pancreatic β-cells may also act as APCs by presenting MHC class I molecules and engaging with cytotoxic $CD8^+$ T cells [100]. Inflamed and apoptotic β-cells can also release molecules that act as RAGE ligands further perpetuating the inflammatory cascade by maturation and activation of DCs and macrophages leading to pancreatic injury. NK cells can directly engage with β-cells through NKG2D-RAE1 interaction known to cause pancreatic β-cells death [101]. Furthermore, NK cells can release large quantities of cytotoxic granules and HMGB1 that may directly interact with RAGE expressed on β-cells [53]. Similar to NK cells, neutrophils can also release RAGE ligands and interact with other RAGE expressing cells including pancreatic β-cells [61].

3.5 RAGE, diabetes and COVID-19

The severe acute respiratory syndrome coronavirus (SARS-CoV)-2 pandemic has affected millions of people globally in recent years [102]. Life threatening severe lung inflammation and infections of cardiovascular and central nervous systems as well as the gastrointestinal tract have been reported during SARS-CoV-2 infection [103].

Emerging evidence suggests that individuals with diabetes have poorer prognosis when infected with SARS-CoV-2. Given that the greatest basal expression of RAGE in the body is within the lungs, it is highly likely that RAGE mediated immune processes contribute to these poorer outcomes. In hyperglycemia priming of neutrophils may result in the uncontrolled formation of NETs and release of HMGB1 further increasing vascular permeability. RAGE levels were elevated in both rodent models and humans with acute lung injury and associated with inflammasome formation and IL-1β release [104–106] Whilst most recent SARS-CoV-2 studies focused on comorbidities associated with type 2 diabetes, a common theme of imbalanced immune responses, upregulation of RAGE ligands such as HMGB1 and S100 proteins, insulin resistance, hyperglycemia and release of pro-inflammatory cytokines is seen [107, 108]. Furthermore, several studies reported a significant increase in diabetic ketoacidosis (DKA) in recent onset T1DM during the pandemic [109–111]. These findings have important implications on future treatment and management of patients with dysglycaemia and severe respiratory conditions.

4. Conclusions

The robust evidence pointing towards involvement of RAGE and its ligands in inflammation and autoimmunity paves a new pathway towards understanding the pathophysiology of T1DM. Valuable lessons can also be learned about approaches to undertake when designing new therapies to target this axis from previous findings. Further understanding of the role of greater RAGE expression on immune cells and pancreatic islets during T1DM pathogenesis is required and is likely to be multifaceted given the myriad of inflammatory diseases which involve RAGE mediated processes. T1DM is incredibly complex and heterogeneous disorder, and it is unlikely for a 'silver bullet' therapy to emerge. This highlights the importance of exploring alternative mechanistic pathways for therapy which have not been previously considered. Indeed, it is likely that the best approach to T1DM may be combination therapies which are targeted towards various aspects of disease and provide greater coverage of the vast immune dysfunction which may be present.

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Conflict of interest

The authors declare no conflict of interest.

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