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Review Article

HMGB1, an innate alarmin, in the pathogenesis of type 1 diabetes

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Abstract: HMGB1, an evolutionarily conserved chromosomal protein, was recently re-discovered to act as a "danger signal" (alarmin) to alert the innate immune system for the initiation of host defense or tissue repair. Extracellular HMGB1 can be either passively released from damaged/necrotic cells or secreted by activated immune cells. Upon stimulation, dendritic cells (DCs), macrophages and natural killer (NK) cells secrete high levels of HMGB1 into the intercellular milieu. HMGB1 is potent to target DCs, macrophages, neutrophils and $CD4^+$ T cells. It also upregulates the expression of BCL-XL by which it may prevent the elimination of activated immune cells. As a result, HMGB1 has been suggested to be implicated in the pathogenesis of autoimmune disorders such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and experimental allergic encephalomyelitis (EAE). Given the similarities of autoimmune response against beta cell self-antigens in type 1 diabetes (T1D), in this view we will discuss the possible implications of HMGB1 in T1D pathogenesis. Specifically, we will summarize and update the advancement of HMGB1 in the pathogenesis of autoimmune initiation and progression during T1D development, as well as islet allograft rejection of diabetic patients after islet transplantation. Elucidation of the role for HMGB1 in T1D pathogenesis would not only enhance the understanding of disease etiology, but also have the potential to shed new insight into the development of therapeutic strategies for prevention or intervention of this disorder.

Key words: HMGB1, innate alarmin, pathogenesis type 1 diabetes, review

Introduction

Type 1 diabetes (T1D) is an autoimmune disorder characterized by the specific destruction of the insulin secreting beta cells of the pancreatic islets by a certain population of autoreactive immune cells [1-4]. The early stages of the disease process leading to the development of diabetes are characterized by insulitis, the infiltration of the pancreatic islets by mononuclear cells such as dendritic cells (DCs), macrophages and T cells [5]. The autoimmune etiology of T1D is also manifested by the presence of circulating autoantibodies, specific for beta cell proteins including insulin, glutamic acid decarboxylase 65 (GAD65), and protein tyrosine phosphatase-like protein IA2 (IA-2). These autoantibodies are present in 85-90% of subjects with diabetes at the time of diagnosis [6, 7]. Although it is unclear whether they participate directly in beta cell destruction, they can be served as markers to monitor disease progression [8, 9]. Since autoimmune response progresses many years before the onset of clinical diabetes, studies in T1D pathogenesis for the past several decades have been mainly focused on the role of adaptive immunity. T cells are believed to be the major effector cells responsible for beta cell destruction. CD8+ T cells have been found to be critical for disease pathogenesis in both T1D patients and NOD mice, a mouse model for spontaneous autoimmune diabetes [10, 11]. Autoreactive T cells target beta cell specific self-antigens including

insulin and glucose 6-phosphatase catalytic subunit-related protein and consequently mediate beta cell destruction [12, 13]. In addition, CD4+ T cells activated against self-antigens can also promote B cell to produce autoantibodies against those self-antigens. Therefore, T cell mediated adaptive immunity was emphasized so much that the role of innate immunity in disease etiology was overshadowed and ignored.

During evolution from unicellular to multicellular organisms, the immune system has developed a set of mechanisms to sense either non-self component invaded into hosts or damaged self tissues, so that organisms can initiate a defensive response and repair damaged tissue to restore tissue homeostasis, which are also called "inflammatory response". The inflammatory response is begun with the recognition of those life-threatening events called "danger signals", which then alert the innate immune system and trigger defensive immune responses. Those "danger signals" can be categorized into pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). PAMPs are defined as exogenous molecules that can alert innate immune system of life-threatening pathogens, such as lipopolysacharide (LPS) and viral RNA. The receptors on immune cells that can recognize PAMPs is so-called pattern recognition receptors (PRRs). A well-known family of PRRs is the toll-like receptor (TLR) family in which each member recognizes a specific set of PAMPs [14-16]. On ligation with PAMPs, PRRs transduce activation signals that lead to the production of proinflammatory molecules such as tumour necrosis factor (TNF) to enhance immune response [17, 18]. DAMPs are endogenous molecules now called as alarmins, and once they have released from the damaged cells, they can initiate and promote host inflammatory response and tissue repair [19].

Along with the realization of PAMPs (innate alarmins) that can be recognized by the PPRs expressed on immune cells, there is now compelling evidence suggesting the implication of innate immunity in T1D pathogenesis. Cells infiltrated into insulitis lesion derive from both innate and adaptive immune system such as T lymphocytes, B lymphocytes, DCs, macrophages and natural killer (NK) cells [20-23]. Abnormalities in some of the innate cells have been addressed both in animal model and human T1D patients as well as those subjects at risk to the disease [24]. The observation that LPS, viral infections, or generalized activation of APCs delays or prevents the establishment of peripheral tolerance further underscored the importance of innate immunity in the development of autoimmune diabetes [25-27]. In this review, we will focus on high-mobility group box 1 (HMGB1), a recently identified endogenous alarmin, in the pathogenesis of autoimmune initiation and progression during T1D development, as well as islet allograft rejection in diabetic patients after islet transplantation. Understanding of these advancements could shed new insight into the possibilities for developing novel therapeutic strategies to mitigate or prevent type 1 diabetes.

The characterization and rediscovery of proinflammatory properties for HMGB1

HMGB1 is a member of high mobility group (HMG) nuclear proteins. This family of non-histone, chromatin associated nuclear proteins was discovered as specific regulators of gene expression more than 35 years ago [28]. HMG proteins are constitutively expressed in the nucleus of eukaryotic cells. They were confirmed to be involved in DNA organization and regulation of transcription. They share functional motifs that bind specific DNA structures and induce conformational changes without specificity for target sequences. They have such structural characteristics as transcripts with long AT-rich 3' untranslated regions and highly negatively charged carboxy-terminals [29].

HMGB1 is probably originated more than 500 million years ago before the split between the animal and plant kingdoms (**Figure 1A**). It is among the most evolutionarily conserved proteins in the eukaryotic kingdom and shares 100% amino acid (AA) identity between mice and rats, and 99% AA identity between rodents and humans (**Figure 1B**). HMGB1 has a concentration about 10^6 molecules per cell and is constitutively expressed in quiescent cells, and a large "pool" of performed HMGB1 is stored in the nucleus [29]. As a nuclear protein, HMGB1 is implicated in diverse cellular functions, including the regulation of nucleosomal structure and stability, and transcription factors binding to their cognate DNA sequences [29-34]. The binding activity of HMGB1 to DNA is regulated by the two 80-amino acid DNA

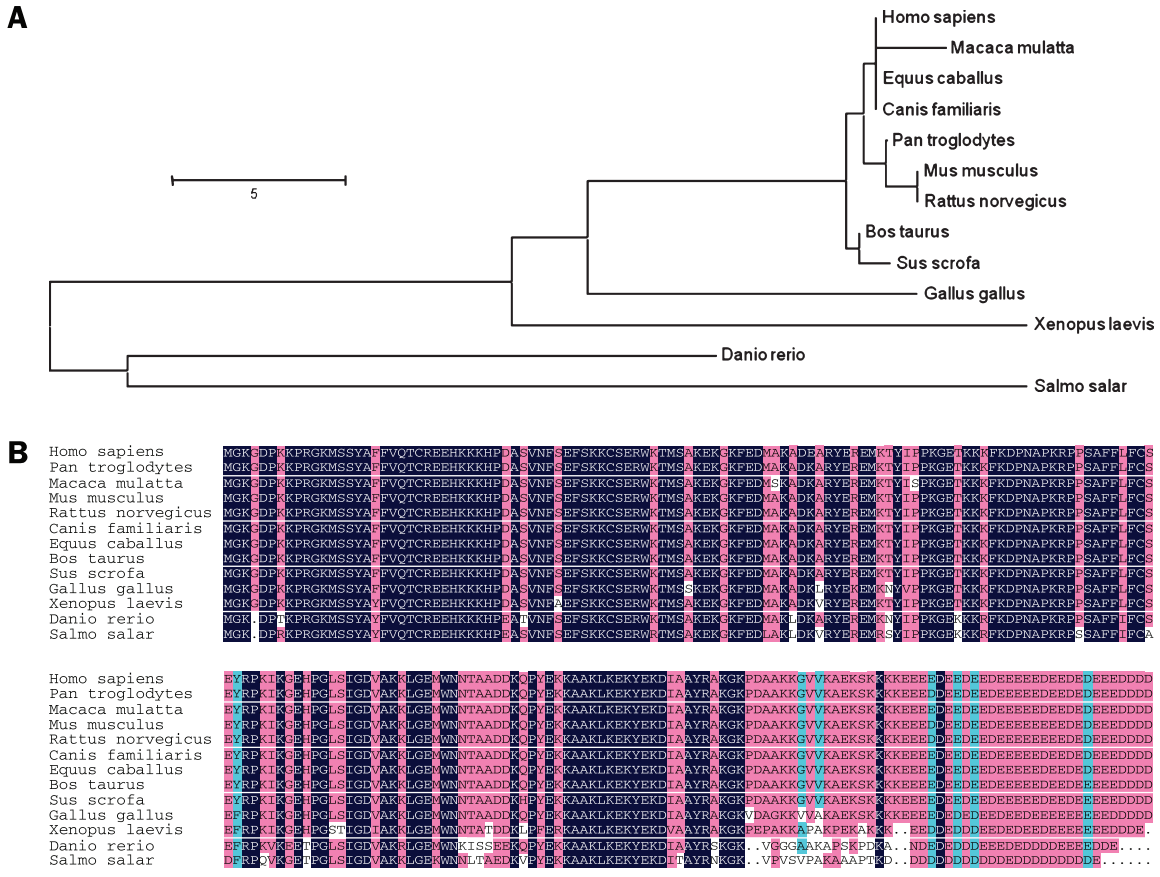


Figure 1. Sequence analysis showing evolutionary conservation of HMGB1 between diverse species. A: A phylogenetic tree showing evolutionary relationships of the *HMGB1* gene between different species. The phylogenetic tree was constructed according to the calculation of the best match for the selected sequences. B: HMGB1 amino acid sequence alignment showing evolutionary conservation between diverse species. Sequence homology: black, 100% identical; pink, >75% identical; blue, >50% identical; and white, 0% identical.

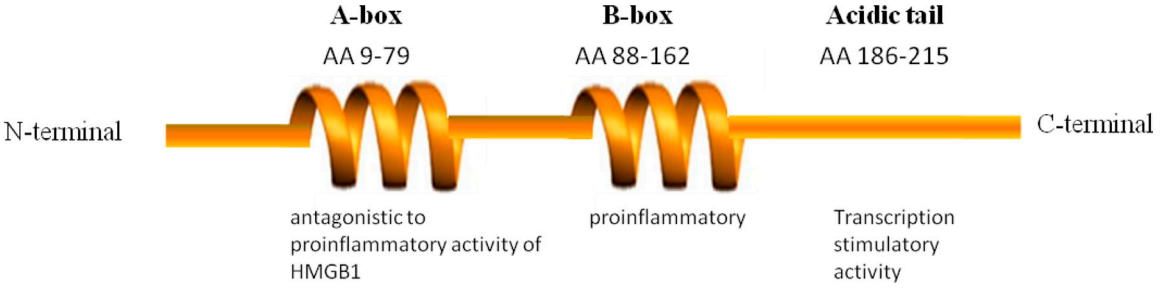


Figure 2. Functional domains within the HMGB1 amino acid sequence. The full-length HMGB1 contains 2 homogenous domains (A- and B-box) and an acidic C-terminal tail. The B-box is associated with its properties relevant to proinflammatory activity and RAGE binding, while the A-box is a specific antagonist by which it inhibits the proinflammatory properties of HMGB1. The C-terminal acidic tail is required for transcription stimulatory function of HMGB1.

binding domains, the A-box and B-box, with each structurally represented as three α-helices in a characteristic L-shaped fold [35] (**Figure 2**). In addition to A- and B-box, there is an acidic tail in the C-terminal of HMGB1. The C-terminal acidic tail is important for the transcription stimulatory function of HMGB1 [35-38]. The two boxes bind to the minor groove of chromatin thus modifying the DNA architecture. This facilitates the binding of regulatory proteins of various transcription factors to their cognate sequences, including the steroid/nuclear hormones progesterone [39] and estrogen [40, 41], HOX proteins [42], p53, homeobox-containing proteins, recombination activating gene 1/2 (RAG1/2) proteins and transcription factor II B [43]. Mice deficient for HMGB1 are viable for several days if given glucose parenterally, then waste away probably due to the inactivation of glucocorticoid receptor (GR) transcribed genes [44]. Phenotypic features include small size, ruffled and disorganized fur, long hind paws, and absence of fat. Cell lines lacking HMGB1 grow normally, but the activation of gene expression by different factors including glucocorticoid receptor, is impaired [44].

In late 1990s, in the search for a broader therapeutic window for the treatment of sepsis and endotoxemia, Wang and colleagues re-discovered HMGB1 as a late mediator of endotoxin lethality in a murine model [45], and blockade of HMGB1 by the administration of neutralizing antibodies protects against LPS lethality in mice. Follow-up studies demonstrated that extracellular HGMB1 acts as a potent innate "danger signal" to alert the innate immune system for the exogenous invasive microorganisms, the endogenous tissue injury or the presence of intercellular inflammatory mediators, which then initiates host defense or tissue repair. B-box domain is important for the proinflammatory properties of HMGB1 including cytokine release [46, 47]. Instead of possessing proinflammatory properties, the A-box competes with full length HMGB1 for binding sites leading to attenuation of the inflammatory cascade [48] (**Figure 2**).

Two distinct mechanisms have subsequently been proposed for cells to liberate HMGB1 into the extracellular milieu. The damaged/necrotic cells can passively release HMGB1 into extracellular milieu, which represents an intracellular marker selected by the innate immune system to recognize tissue damage and initiate reparative responses [49, 50]. Caspase-dependent oxidization of HMGB1 by reactive oxygen species (ROS) has been suggested to be critical for tolerance induction by damaged or apoptotic cells [51]. The second mechanism is the "active secretion" of HMGB1 by activated immune cells to mimic the necrotic process and activate innate immune response during an immunological challenge. Unlike passive release, HMGB1 active secretion has been shown to be associated with extensive acetylation of lysine residues [52]. Extracellular HMGB1 exerts not only paracrine activity, but also autocrine activity on the cells from which it is secreted to enhance chemotaxis and innate immune response, and subsequently to initiate and promote adaptive immune response [53].

Receptors associated with HMGB1 signaling pathways

There are several important receptors have thus far been characterized to be implicated in HMGB1 signaling, including the receptor for advanced glycation end products (RAGE) and some members of the Toll-like family of receptors. RAGE is thought to be one of the primary receptors for HMGB1 [54]. RAGE is a transmembrane protein expressed at low levels in normal tissues, but it is upregulated at sites where its ligands accumulate [55]. RAGE expression is detected on monocytes, macrophages, neurons, and endothelial cells, as well as on a variety of tumor cells [56, 57]. Binding of RAGE by HMGB1 can activate both CDC42/Rac pathway and MAPKs-NFκB pathway [56, 58, 59]. These two HMGB1 signaling pathways then through RAGE promotes chemotaxis, production of cytokines relevant to NFκB activation [60, 61], activation of endothelial cells [62, 63], maturation and migration of immune cells [47, 64-70].

Despite the characterization of RAGE as the receptor for HMGB1, RAGE alone, however, could not explain all the observed effects of HMGB1, suggesting the existence of additional receptor(s) relevant to HMGB1 signaling. Consistent with this assumption, follow up studies further characterized that toll-like receptors 2 (TLR2) and 4 (TLR4) are involved in HMGB1 signaling [71-73]. TLR4 is the main receptor for endotoxin, whereas TLR2 responds to Gram-positive components and fungi [74]. HMGB1 signaling through TLR2 and TLR4 is

mediated by Rac1/PI3K/CDC42 pathway and MyD88 dependent NFκB activation pathway [61, 71-76]. More recently, Ivanov and colleagues further demonstrated that HMGB1 acts as a CpG-ODN–binding protein, by which it interacts and preassociates with TLR9 in the endoplasmic reticulum-Golgi intermediate compartment (ERGIC), and as a result, forms a complex within specialized vesicles [77]. CpG-ODN stimulates macrophages and DCs to secrete high levels of HMGB1, which in turn accelerates the delivery of CpG-ODNs to its receptor, leading to a TLR9-dependent augmentation of IL-6, IL-12, and TNFα secretion. It seems that HMGB1 does not affect the uptake of CpG DNA or its entry into early endosomes, but rather accelerates the redistribution of TLR9 from the endoplasmic reticulum to the early endosomes in response to CpG DNA [77].

HMGB1 in the pathogenesis of autoimmune initiation during T1D development

The initiation of autoimmune response during T1D development is a multifactorial and complex process that requires genetic predisposition to synergize with unknown exogenous and/or endogenous triggers [78-83]. Once triggered by those factors, macrophages and DCs infiltrated into islets and followed shortly thereafter by the recruitment of T and B lymphocytes [20-23, 84, 85]. Apoptosis is a normal physiological process contributing to tissue turnover during the development and regulation of tissue homeostasis in the adult [86]. Unlike necrosis, apoptosis is usually considered to be a non-inflammatory process. However, recent investigations ranging from animal models to human pathology lend support to the view that apoptosis plays a pivotal role in the development of autoimmunity [87-93]. Apoptotic cells are a source of autoantigens, and apoptosis has been suggested in certain conditions, contributing to autoimmune response in multiple autoimmune disorders including systemic lupus erythematosus (SLE) [89, 91, 94], rheumatoid arthritis (RA) [95, 96], and experimental allergic encephalomyelitis (EAE) [97, 98]. A number of recent studies indicate that HMGB1 can be passively released from late apoptotic cells in a cell type dependent manner [99, 100], probably through a mechanism by binding to some nuclear molecules such as DNA that can be leaked out from apoptotic cells [99, 101, 102].

To address whether HMGB1 can be passively released from late apoptotic beta cells, we first induced NIT-1 cell (a NOD-derived β cell line) apoptosis by combination of cytokines (100U/ml INFY, 10U/ml IL-1β and 100U/ml TNFα) [103]. After 72h of treatment, around 73% of NIT-1 cells became apoptotic, and 79% of which were undergoing secondary necrosis as determined by propidium iodide (PI) and annexin V staining. Western blot analysis of culture supernatants derived from cells treated with cytokines detected high levels of HMGB1, while HMGB1 was absent from control culture supernatants, indicating passive release of HMGB1 from late apoptotic beta cells [103]. To further address the question, we performed follow-up studies to examine the effect of late apoptotic beta cells on NOD DC activation and functionality. NIT-1 cells were first induced apoptosis by UV treatment and the resulting pro-apoptotic cells were subsequently labeled with carboxyfluorescein succinimidyl ester (CFSE). Immature NOD bone marrow-derived DCs (BMDCs) were then co-cultured with CSFE-labeled apoptotic NIT-1 cells at 1:20 ratio for 4h, followed by staining DCs with a PE-labeled anti-CD11c antibody. Flow cytometry analysis indicated that approximately 81% of DCs were double positive for CD11c and CFSE, indicating that NOD DCs have high potency for uptake of apoptotic NIT-1 cells (unpublished data). Confocal microscopic analysis of DCs in different layers further confirmed that some DCs can actually ingest more than one apoptotic NIT-1 cells. More interestingly, NOD DCs after internalizing CSFE-labeled NIT-1 cells show a matured phenotype as manifested by high levels of surface MHC II expressions (unpublished data). Together, all of these observations provide an overall support for the scenario that HMGB1 originated from late apoptotic beta cells has high potency to stimulate NOD DC activation, which could then initiate autoimmune response against beta cell self antigens leading to beta cell destruction.

Previous studies including ours have consistently demonstrated that NOD neonates show abnormal beta cell apoptosis during beta mass turnover after birth, probably due to defective clearance of apoptotic cells in NOD mice [104-107]. In situ TUNEL assays of pancreatic sections originated from NOD and B6 neonates indicate that NOD newborns have at

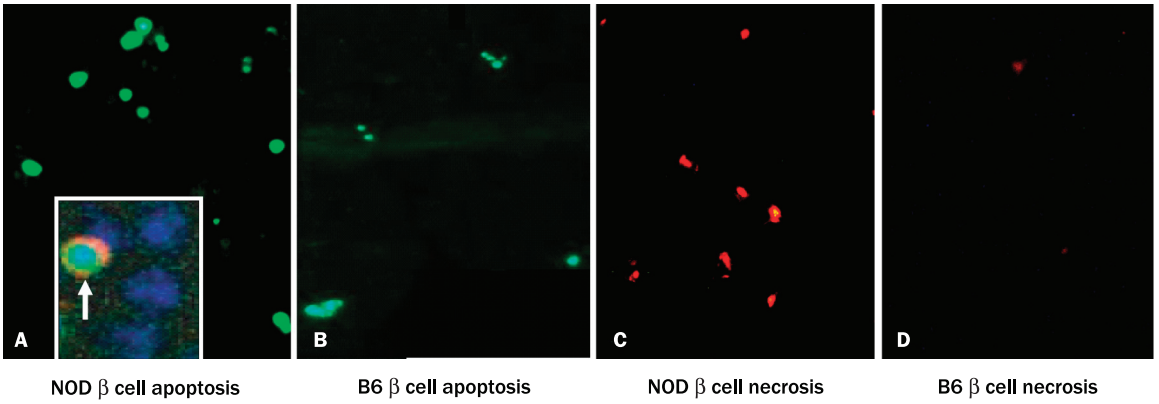


Figure 3. Altered beta-cell apoptosis during NOD physiological beta mass turnover after birth. A & B: TUNEL assay of pancreatic beta cell apoptosis during NOD and B6 neonate beta mass turnover. Insulin costaining indicates that the apoptotic cells are beta cells (A, the apoptotic cell is indicated by an arrow). C & D: Propidium Iodide (PI) staining of secondary necrotic beta cells in pancreatic sections originated from neonates undergoing beta mass turnover.

least 2-fold higher apoptosis as compared to that of B6 newborns (**Figure 3 A vs. B**). Insulin co-staining further confirmed that these apoptotic cells were actually insulin-producing beta cells (**Figure 3A**, inset figure, where insulin stained blue, apoptotic cell stained red, and the apoptotic cell was indicated by an arrow). Since excessive apoptosis is usually associated with secondary necrosis [108], *in vivo* propidium iodide (PI) staining was then carried out to check beta cell necrosis by injection of 15μl PI (20μg per gram of body weight) into 2wk-old NOD and control newborns *via* tail vein. Pancreatic frozen sections were prepared after 3h injection. In line with previous observations, PI positive cells (necrotic cells) were observed in the sections of NOD neonates, while necrosis was almost undetectable in B6 neonates (**Figure 3, C vs. D**). Since HMGB1 passive release is associated with secondary necrosis, in situ HMGB1 immunostaining was then conducted in pancreatic sections of NOD neonates. Unlike B6 neonate islets in which HMGB1 is solely localized in the nucleus of islet cells, some islet cells in NOD neonate show condensed nuclei, plasma membrane blebbings, and displayed positive nuclear and cytoplasmic staining of HMGB1 (unpublished data), indicating HMGB1 passive release from secondary necrotic beta cells. These passively released HMGB1 in turn, may function as an innate alarmin to activate DCs for the presentation of beta cell self-antigens, predisposing to the development of autoimmune diabetes. Although the experimental evidence is currently lacking, it would not be difficult to extrapolate that blockade of HMGB1 during NOD neonate beta mass turnover would prevent or delay insulitis, a typical characteristic of autoimmune response during T1D development.

HMGB1 in autoimmune progression during T1D development

Upon the presence of activation signals derived from PRR transduction, beta cell-specific antigens would be recognized by APCs which then become activated to initiate autoimmune response. Once autoimmune response initiated, disease undergoes a progression stage in which adaptive immunity is activated by self-reactive APCs and plays a major role in beta cell destruction. The subsequent beta cell destruction would further increase the amount of self-antigens for APCs which then activate additional autoreactive T and B cells. Therefore, as T1D progresses in severity, so does the number of autoreactive T and B cell clones, known as epitope spreading [109]. Once the disease enters the progression stage of autoimmune-mediated beta cell destruction, the difficulty of therapy will be dramatically increased. Unfortunately, at the time of clinical diagnosis all diabetic patients have already been in the progression stage. Therefore, understanding the mechanism of autoimmune process in diabetes progression stage is pivotal for the development of novel therapeutic strategies.

As aforementioned, in addition to passive release from damaged beta cells, HMGB1 can also be secreted by activated immune cells such as DCs [64, 66]. By examining HMGB1 subcellular localization in DCs we have clearly demonstrated that NOD DCs have high potency to actively secrete HMGB1 upon stimulation. Before stimulation, HMGB1 was solely localized in the nucleus, while a major proportion of HMGB1 translocated into the cytoplasm upon LPS or TNFα/IFNγ stimulation. In line with this observation, Western blot analysis of culture supernatants detected high levels of HMGB1 derived from stimulated NOD DCs in a time-dependent manner [103]. To demonstrate the evidence of HMGB1 active secretion by NOD islet infiltrated DCs, *in situ* HMGB1 immunostaining was carried out using pancreatic sections originated from NOD mice with insulitis (14wk-old). It was found that a large proportion of HMGB1 had translocated into the cytoplasm in the islet infiltrated DCs as they were also positive for CD11c staining, demonstrating HMGB1 active secretion by activated autoreactive DCs, which could then enhance autoimmune progression [103].

Two sets of experiments were carried out to demonstrate the direct evidence for HMGB1 in the pathogenesis of autoimmune progression during T1D development. An endotoxin-free HMGB1 neutralizing antibody was used for the purpose. The study was first carried out in NOD mice at the early stage of autoimmunity (8wk-old). The blocking antibody was administered into those mice once a week (600μg/mouse) for 18 consecutive weeks and then monitored for diabetes onset up to 35wk-old. Remarkably, HMGB1 antibody treatment reduced diabetes incidence by almost 2-fold. Furthermore, the treatment also significantly delayed the onset of diabetes. In average, the age for onset of diabetes in HMGB1 antibody treated mice was 28.7 ± 3.4wk, while the control IgG treated mice was only 18.4 ± 3.1wk [103]. Examination of insulitis severity by histological analysis of pancreatic sections further confirmed that the extent of insulitis in HMGB1 antibody treated mice was significantly less severe than the control IgG treated mice at 12wk, 15wk and 18wk time point examined. To demonstrate the implication of HMGB1 in late stage of autoimmunity, HMGB1 blocking antibody was then administered into 12wk-old NOD mice by then autoimmune infiltration in these mice had already progressed for at least 5wk [110]. Similar as above, the mice were administered with HMGB1 blocking antibody once a week for 13 consecutive weeks and then monitored for diabetes onset. Consistently, blockade of HMGB1 significantly reduced diabetes incidence, but unlike animals in the early stage of autoimmunity, the treatment failed to delay the onset of diabetes [103].

Given the critical role of DCs in T1D associated autoimmunity, we first checked the impact of HMGB1 blocking antibody on DCs, the most potent APCs known today. It was found that antibody treatment reduced the number and maturation of a subset of DCs probably associated with auto-antigen presentation to naïve T cells in the pancreatic lymph nodes (PLN). We also noticed a skewed population of $CD11c^+CD8a^+$ DCs in mice after blockade of HMGB1. As those cells showed less potency to stimulate alloimmune response, they were probably responsible for inducing peripheral tolerance to tissue associated antigens [111]. In line with this assumption, there was a significant increase for the $CD4^+Foxp3^+$ regulatory T cells (Tregs) in the PLN of mice treated with neutralizing antibody [103]. Taken together, these data demonstrated strong evidence indicating that HMGB1 is implicated in the progression of autoimmune response during T1D development.

HMGB1 in islet allograft rejection of diabetic patients after islet transplantation

Current therapeutic strategy for T1D is lifelong commitment of exogenous insulin, monitoring blood glucose, taking healthy foods plus maintaining a healthy weight. However, insulin therapy and healthy lifestyle cannot provide adequate glycemic control for some patients with severe type 1 diabetes, or with complications that cannot be efficiently managed by insulin. Furthermore, insulin administration cannot control blood glucose accurately enough to maintain at a perfect level even with careful insulin administration, and thus would result in chronic complications. It has been shown that even transient hyperglycemia can lead to persistent epigenetic changes predisposing to the development of diabetic complications. In battle against type 1 diabetes, pancreatic transplantation has been considered to offer a

successful therapeutic approach for many years [112, 113]. However, similar as other solid organ transplantation, lifelong immunosuppression is required and donor organs are in short supply [79]. An alternative approach, transplantation of donor islets, was later established, which seems to be a promising therapeutic approach to tightly maintain blood glucose levels for patients with type 1 diabetes [114]. Some centers have actually achieved high rate for long-term independence of exogenous insulin. Nevertheless, the total success rate is still not high enough [115, 116].

The major problem hampering islet transplantation is islet allograft dysfunction caused by rejection although it is less severe than solid organ transplantation. Similar as the study field of autoimmunity, the role of adaptive immunity in allograft rejection has been extensively studied for decades. It has been suggested that acute allograft rejection is principally a T cell-mediated adaptive immune response [117]. However, the contribution of innate immunity to allograft rejection has generally been ignored until the discovery of the TLR system and the subsequent realization of innate immunity in driving and shaping adaptive immunity. Early islet loss is considered to be responsible for islet allograft dysfunction, and it is believed to be attributable to inflammatory events in which innate immunity plays an important role [118]. Early loss of islet results in the release of alloantigen and some endogenous alarmins such as HMGB1 which then initiate alloimmune response against islet allograft. Therefore, similar as its role in autoimmunity relevant to T1D pathogenesis, HMGB1 could be also implicated in the initiation and progression of islet allograft rejection.

It has been suggested that ischemia/reperfusion is a major pathophysiological component of acute allograft malfunction after transplantation, which includes direct cellular damage and acute allograft rejection. To demonstrate the implication of HMGB1 in this process, we examined HMGB1 passive release from allograft damaged cells and active secretion by allograft infiltrated immune cells. By analysis of cytoplasmic proteins originated from cardiac allografts that underwent around one hour of cold ischemic insult, we have obtained strong evidence supporting that HMGB1 can be passively released from allograft damaged cells [117]. Consistent with this observation, HMGB1 has been found to be critical in mediating hepatic injury after murine ischemia/reperfusion [119]. We further confirmed that allograft infiltrated immune cells actively secrete high levels of HMGB1 into the intercellular milieu through immuno histochemical analysis of cardiac allograft sections that underwent acute rejection [117]. A steady increase for HMGB1 expression in the allografts after transplantation was noticed by comparative analysis of HMGB1 temporal expression changes between syngeneic and allogeneic cardiac grafts, and the increase of HMGB1 expression in allografts is accompanied by the interstitial infiltration and active secretion of HMGB1 by infiltrated immune cells. In sharp contrast, the increase for HMGB1 expression in syngenic grafts was only noticed after first 3-day of transplantation, and after which, the expression of HMGB1 dropped back to the normal levels. Together, these data indicate that HMGB1 is implicated in acute rejection of allografts and tissue repair of syngeneic grafts. Based on these observations, an endotoxin-free recombinant peptide encoding HMGB1 A-box (rA-box) was used to treat recipients after cardiac allograft transplantation. Notably, rA-box treatment significantly prolonged allograft survival (12.5 ± 1.87 days vs. 6.5 ± 1.04 days) [117]. In line with this result, recipient mice received rA-box displayed well preserved myocardium and significant reduced inflammatory infiltration after day 7 of transplantation. This protective effect was associated with suppressed production of pathogenic $CD8^+IFN\gamma^+$ (Tc1) and $CD4^+IFN\gamma^+$ (Th1) cells. Similarly, blockade of RAGE, a primary receptor for HMGB1, prevented cardiac allograft rejection [120].

The implication of TLRs in the pathogenesis of allograft rejection further supports a role for HMGB1 in islet allograft rejection. Chen *et al.* revealed that inhibition of TLR signaling promoted the acceptance of allografts that are resistant to tolerance induction such as skin, whereas administration of TLR agonist during perioperative period abrogated tolerance [121, 122]. MyD88 deficiency inhibited the activation, migration of macrophages in mice transplanted with porcine islet xenografts [123]. Loss of MyD88 signaling reduced mature DCs in the draining lymph nodes and prevented the development of T cell response against graft, and thus induced acceptance of allograft [124, 125]. More interestingly,

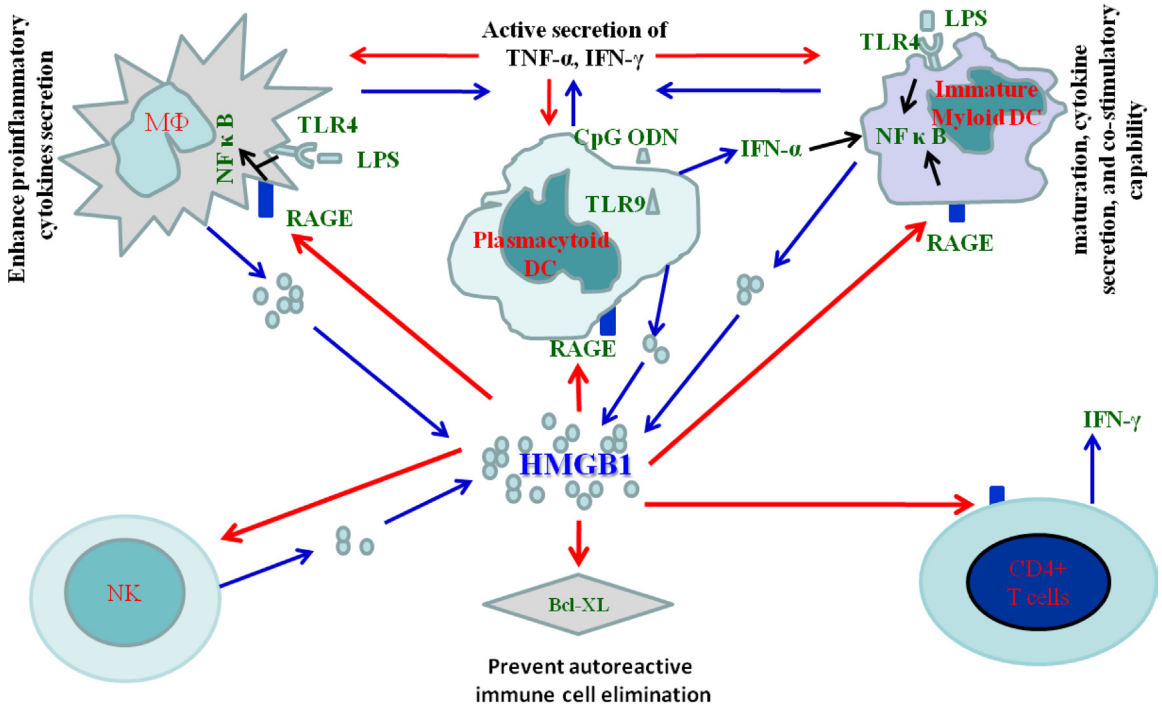


Figure 4. The immuno-regulatory properties for extracellular HMGB1. HMGB1 secretion by activated immune cells is indicated by blue arrows. Extracellular HMGB1 targets myeloid DCs, plasmacytoid DCs, macrophages, neutrophils and $CD4^+$ T cells via the corresponding receptor(s) expressed on their surface. HMGB1 has also been found to upregulate the expression of BCL-XL, which may in turn prevent the elimination of activated autoreactive immune cells.

TLRs on beta cells are also involved in islet allograft rejection. Enhanced TLR expression was observed on porcine islets exposed to macrophages [126]. Goldberg *et al.* reported that TLR4 expression was elevated on beta cells during islet isolation process, and enhanced TLR4 promoted beta cell death and graft rejection after islet transplantation [124]. In contrast, suppression of TLR4 on donor beta cells by either carbon monoxide (CO) exposure or pre-infection with a TLR4 dominant negative virus prevented the initiation of inflammation and subsequent islet allograft rejection [124]. Similarly, RAGE, another receptor for HMGB1, is also implicated in the rejection of islet allografts. Loss of RAGE or blockade of RAGE by its antagonist TTP488 in recipient mice suppressed T cell response and prolonged islet allograft survival [127]. All together, similar as its role in autoimmune response during T1D development, HMGB1 also plays a pivotal role in the initiation and progression of islet allograft rejection and contributes to islet allograft dysfunction after transplantation.

Conclusion remarks

HMGB1, an evolutionarily conserved chromosomal protein, was recently re-discovered to be an alarmin that alerts the innate immune system to initiate defensive response. Monocytes, macrophages, NK, myeloid and plasmacytoid DCs secrete high levels of HMGB1 in response to pathogen or damage associated molecules. Extracellular HMGB1 targets myeloid DCs, plasmacytoid DCs, macrophages, neutrophils and $CD4^+$ T cells. HMGB1 increases maturation and cytokine secretion from myeloid and plasmacytoid DCs that have been stimulated with TLR ligands [53]. Upon interaction, DCs secrete IL-18, causing NK cells to secrete HMGB1, which in return acts back on DCs to prevent them from NK cell mediated lysis [53] (**Figure 4**). Based on its importance in orchestrating and modulating adaptive immune responses, here we have discussed the possible implications of HMGB1 in multiple stages of autoimmunity, from innate recognition of self antigens to progression of adaptive autoimmune response against beta cells, as well as islet

allograft rejection after transplantation in diabetic patients. The challenge for future studies would be the development of high potent HMGB1 blockade to evaluate its impact on reversal of overt type 1 diabetes and recurrence of autoimmunity after islet transplantation.

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