Review

Are mast cells important in diabetes?

Duraisamy Kempuraj1, Alessandro Caraffa2, Gianpaolo Ronconi3, Gianfranco Lessiani4, Pio Conti5

1Department of Neurology, Carver College of Medicine, University of Iowa, Iowa, USA
2Department of Pharmacology, University of Perugia, Perugia, Italy
3Policlinico Gemelli, Rome, Italy
4Villa Serena Hospital, Città Sant’Angelo, Italy
5Postgraduate Medical School, Università G. d’Annunzio, Chieti, Italy

Diabetes is a metabolic disorder characterized by hyperglycemia and associated with microvascular and macrovascular syndromes mediated by mast cells. Mast cells are activated through cross-linking of their surface high affinity receptors for IgE (FceRI) or other antigens, leading to degranulation and release of stored inflammatory mediators, and cytokines/chemokines without degranulation. Mast cells are implicated in innate and acquired immunity, inflammation and metabolic disorders such as diabetes. Histamine and tryptase genes in mast cells are overexpressed in pancreatic tissue of type 2 diabetes mellitus (T2DM) patients. Histamine is a classic inflammatory mediator generated by activated receptors of mast cells from the histamine-forming enzyme histidine decarboxylase (HDC), which can be activated by two inflammatory chemokines, RANTES and MPC1, when injected intramuscularly or intradermally in mice. This activation is inhibited in genetically mast cell-deficient W/Wv mice, which show higher insulin sensitivity and glucose tolerance. This study contributes to understanding the mechanism by which mast cells profoundly affect diabetes, and their manipulation could represent a new therapeutic strategy. However, further studies are needed to clarify the role of mast cells in inflammation and metabolic disorders such as diabetes.

Key words: type 1 diabetes, type 2 diabetes, mast cells, autoimmunity, immune modulation, peripheral vascular disease.

Introduction

This article reviews the literature and our experience accumulated over the past twenty years on the pathophysiology of mast cells (MCs).

Autoimmune disease occurs when the body tissues are attacked by its own immune system [1], and it is generally accepted that type 1 diabetes mellitus (T1DM) is an autoimmune disease triggered in susceptible individuals by an unknown agent(s) [2]. Often autoimmune diseases are caused by delayed-type hypersensitivity (DTH) reactions induced by auto-reactive T lymphocytes which cross-talk with MCs and provoke tissue injury by triggering a DTH reaction [3]. Mast cells release considerable amounts of interleukin (IL)-4 upon stimulation, and participate in the induction of T-cell differentiation [4]. Apart from MCs, also basophils, Th2 cells, NK1.1+ cells, γδ+ T cells, and eosinophils have been found to produce IL-4 [5].

After activation, T cells, macrophages, endothelial cells and MCs participate in DTH by generating and releasing a sequential cascade of inflammatory products such as reactive oxygen intermediates, nitric
oxide, hydrolytic enzymes, and inflammatory cytokines, including IL-1β, tumor necrosis factor (TNF), IL-18, IL-32 and IL-33 [6].

Type 1 diabetes mellitus is an autoimmune disorder which affects about 0.2% of the population in western countries, mostly boys aged 11 to 12 years [7].

Type 1 diabetes mellitus was previously called insulin-dependent diabetes mellitus (IDDM) and is a complex metabolic disease with impaired insulin production. It is a progressive and persistent autoimmune complex metabolic disease with impaired insulin sensitivity [8]. Diabetes is a metabolic disease characterized by hyperglycemia and associated with microvascular and macrovascular syndromes [9].

Type 1 diabetes mellitus can occur for genetic and environmental reasons, and presents with destruction of pancreatic β cells due to the infiltration of immune and inflammatory cells, including MCs, in the pancreas (an effect called insulitis) [10]. Therefore, inhibition of activated MCs and their degranulation and/or inflammatory cytokine/chemokine generation might act to protect pancreatic β cells in T1DM [11].

Cytokines/chemokines are low-molecular-weight soluble polypeptides released by immune cells as well as other cell types. They are produced in response to microbes and other antigens, regulate immune and inflammatory responses, and act in paracrine and autocrine manners [12]. Cytokines/chemokines play an important role in the pathogenesis of several infectious and non-infectious diseases including autoimmune disorders [13, 14]. An increase in pro-inflammatory cytokines/chemokines has been associated with tissue lesions and clinical dysfunction in patients with autoimmune diseases [15]. The stimulation of multiple cytokines/chemokines probably reflects the complex pathology of autoimmune disorders [16].

Autoimmunity, including T1DM, is associated with apoptosis and the generation and up-regulation of several pro-inflammatory cytokines such as interferon γ (IFN-γ), interleukin 1 (IL-1) family members, extracellular matrix, integrins expressed by immune cells, and tumor necrosis factor (TNF) [17]. Therefore, innate immunity and inflammatory mediators might play a crucial role in inducing and amplifying the immune attack against the β cells of the pancreas. In addition, the arrival of inflammatory immune cells, recruited by chemokines, induces immunity against infection by microorganisms and self-tissue [18].

The over-expression of MHC class-1 antigens leads to the production of several chemokines including RANTES and MCP-1 that attract MCs and other immune cells, which can be deleterious for the pancreatic β cells [19]. We previously reported that RANTES and MCP-1, which are part of the chemokine supergene family (8- to 10-kD proteins) inducible in a number of pathophysiologic processes, intradermally injected (10 ng/50 μl subcutaneously in the abdominal skin of rat) produce a strong inflammatory reaction, due to the recruitment of mast cells, showing that these chemokines are potent pro-inflammatory proteins with specific chemoattraction on inflammatory MCs [20]. When genetically mast cell-deficient W/Wv mice were used, neither RANTES nor MCP-1 provoked any appreciable inflammatory reaction or MC extravasation, demonstrating that MCs are important for the inflammatory responses [20].

Mast cells are multifunctional immune cells implicated in the pathogenesis of allergy [21], autoimmunity [22], and neurodegenerative disorders [23]. Mast cells are the first line of defense and are located preferentially in intraepithelial locations, in connective tissue, and around blood vessels, performing important beneficial roles in host defense [24]. A number of immune molecules and a variety of pathogens contribute to triggering mast cells, which, after activation, play a crucial role in immunity and the inflammatory process [25].

Mast cells participate in innate, acquired immunity, inflammation, autoimmunity, allergy, infectious diseases and metabolic disorders [26]. Antigen stimulates B lymphocytes to produce IgE, and MCs can provide the necessary signals for IgE production such as IL-4, IL-13, and CD40L [27]. The induction of IgE synthesis is completely abrogated by neutralizing IL-4 and IL-13 (which are differentially controlled by IL-3 and protein kinase C (PKC) with monoclonal antibodies or CD40L with soluble CD40 in vitro [28].

Mast cells are activated through cross-linking of their surface high affinity receptors for IgE (FcεRI), leading in seconds to degranulation and release of stored mediators such as histamine, heparin, tryptase, chymase, kinogenases, carboxypeptidase A3 and TNF. Mast cells synthesize de novo inflammatory compounds, such as growth factors, leukotrienes, prostaglandins, NO, and cytokines/chemokines, which are selectively released later, since the proinflammatory action of the mast cells can occur through non-histamine and non-IgE-mediated routes [29] (Table I). Therefore, MCs are activated not only by IgE and specific antigens, but can also be triggered via innate mechanisms, such as Kit-ligand, stem cell factors (SCF), IL-33, IL-1, complement C3a, C5a, LPS, pathogen-associated molecular patterns (PAMPs), endothelin-1 (ET-1), and venom components [30]. Activation of MCs by cross-linking of FcεRI molecules or other antigens, such as neuropeptides, leads to the generation of PI3K, ERK, JNK, NF-κB and PKC, resulting in release of potent inflammatory mediators and to differential release of distinct mediators without degranulation [31] (Table II). PKCs, which induce signaling events, mediate diverse and important cellular functions, and their mutation and dysregulation contribute to the pathogenesis of many human diseases, including diabetes and its vascular
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Several lines of evidence suggest that MCs are implicated in diabetes, and it is likely that histamine and tryptase genes are over-expressed in pancreatic tissue of T2DM patients [33]. Type 1 diabetes mellitus is a chronic disease, which may lead to ischemic necrosis of organs, microvascular obstruction of the retina, renal glomeruli, peripheral nerve diseases, and atherosclerosis [34]. The vascular damage is caused by abnormal glucose metabolism with a still unclear mechanism. In some patients the presence of autoantibody against the islet cells without the disease may predict the development of T1DM [35].

In T1DM, MCs present pancreatic antigens to T cells, permitting pro-inflammatory cytokines/chemokines to enter and aggravate and damage the functionality and survival of the pancreatic \(\beta\) cells [36].

Tumor necrosis factor, which participates in T1DM, is a pro-inflammatory cytokine [37] involved in the acute phase reaction, in increased vascular permeability and can be generated and released by degranulated and non-degranulated activated mast cells [38].

Lymphocyte infiltration around the pancreatic tissue consists in both CD4\(^+\) and CD8\(^+\) [39]. Macrophages CD11c\(^+\) and CD11c\(^-\) and CD4\(^+\) Th1 cells play an important role in T1DM since they are infiltrated around the islets of Langerhans in the pancreas, provoking the destruction of insulin-producing \(\beta\) cells in the islets with a consequence of deficiency in insulin production [40]. This phenomenon can be reproduced by transferring the T cell autoimmunity involved from one affected animal to another (healthy animal) [41]. Cytolytic T lymphocytes (CTLs), reacting with antigen of islet cells, mediate the lysis along with the generation of inflammatory cytokines that damage the islet cells, and autoantibody which contribute to their injury [42].

It has been reported that MCs are also determinant for the maturation of Th17 cells, which are recognized as key cells in autoimmune disorders including T1DM [43]. In this disease, which is a T-cell-dependent process, MCs participate in the activation of T cells by producing inflammatory TNF [44]. It has been reported that diabetic mice had decreased numbers of T regulatory (T reg) cells and a reduction in IL-10, TGF-\(\beta\), and IL-6 expression in the pancreatic tissue [45]. These deficiencies make the mice more susceptible to contracting T1DM. Many genes are involved in T1DM, including HLA-DR3 and DR4 genes, which are present in the white population in about 93% [46]. In an experimental animal model, it is interesting that IL-2 (a T cell growth factor) knockout mice readily develop autoimmune diseases, including T1DM, through an unknown mechanism [47].

Experiments are ongoing in our laboratory to verify whether W/W\(^v\) mast cell deficient mice are insulin resistant compared with healthy mice, and this could explain some of the clinical findings in patients affected by T1DM.

Type 2 diabetes mellitus and mast cells

Type 2 diabetes is the most common type of diabetes and is a major health problem in the 21st century [49]. Mast cells are inflammatory cells which perform pleiotropic roles in autoimmunity and atherosclerosis...
as well as other inflammatory diseases and metabolic disorders including diabetes [50]. A limited number of mast cells are located in healthy human organs, but in patients with metabolic diseases the number is high, with increased activity.

Mast cell mediators, such as chemokines, cytokines, growth factors, heparin, histamine, proteases, chymase and tryptase, are biomarkers of metabolic diseases including T2DM and contribute to their pathogenesis [50]. Tryptase is the major mast cell enzyme which promotes fibroblast proliferation and collagen synthesis involved in vascular disorders associated with diabetes, while chymase is a serine protease which converts TGF-β from inactive to active form [51]. The chymase is stored in mast cells and, when released, degrades HDL components (apolipoprotein E, apoA-I and apoA-IV) in atherosclerotic lesions present in diabetic disease [52].

Histamine is a classical inflammatory mediator generated by activated receptors of mast cells from the histamine-forming enzyme histidine decarboxylase (HDC), the sole enzyme responsible for the production of histamine from histidine, and is induced in a variety of tissues in response to various stimuli including bacterial components, cytokines/chemokines, and other biochemical compounds [53]. Data are accumulating to support the idea that diabetes is under the control of numerous cytokines and hormones, and mast cell products [54]. Histamine is a biogenic amine inflammatory mediator stored in the granules of mast cells which binds to specific receptors in various tissues and causes an increase of vascular permeability and contraction of smooth muscle, and is involved in the regulation of diabetes mellitus [55]. Histamine participates in the pathophysiological actions of mast cells, immune responses, inflammation and a variety of diseases, including diabetes and its complications [55]. Therefore, HDC may mediate the inflammatory processes in T2DM [56]. Moreover, histamine and VEGF induce microvascular hyperpermeability with an angiogenic effect and chemotactic recruitment of lymphocytes, macrophages and mast cells that are able to contribute to the angiogenesis-modulating molecules and inflammation [57]. Recently, the anti-histamine anti-H3R antagonists have been studied in early-phase clinical trials for possible application in T2DM and obesity, a major risk factor for insulin resistance.

An in vitro experimental model using diabetes mellitus leukocytes activated with lipopolysaccharide (LPS) presented low superoxide generation, reduction of prostaglandin E2, in macrophages, TNF, IL-1β, and inhibition of granulocytes in the generation of inflammatory arachidonic acid products [58]. Therefore, in diabetes mellitus there is compromised immunity with consequent risk of infections and cardio-circulatory diseases.

Furthermore, MCs are rich sources of proinflammatory cytokines which cause insulin resistance in adipocytes [59]. Cytokines, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-16, IL-33, IFN-α, and TNF, mediate the biological effects of mast cells, including the recruitment of other inflammatory cells, and are important in diabetes mellitus infections [60]. Mast cells may store the TNF which increases the expression of E-selectin in the endothelium during bacterial infection and enhances the recruitment of dendritic cells [61].

Tumor necrosis factor (like other cytokines) is generated with the transcript and translation protein method, and it is stored in the granules of MCs for rapid and immediate release after activation. Tumor necrosis factor is also expressed in adipocytes, constituting a link between inflammation, obesity, and diabetes. Tumor necrosis factor and/or TNF receptors impair insulin sensitivity, and mice lacking TNF had improved insulin sensitivity in healthy and genetic (ob/ob) models of obesity [62].

Insulin resistance is an important sign of T2DM and has been associated with a state of low-grade inflammation. Blocking or inhibiting TNF and its receptor p55, TNF leads to an improvement of glucose homeostasis and insulin sensitivity, with a significant reduction of blood insulin level and the ratio of insulin and glucose, suggesting that this inflammatory response is relevant for the regulation of insulin [62].

Mast cells and their secretion are important for the innate immune response to bacterial infections, which are very common in diabetes, due to damage of immunity [63, 64]. Mast cells participate in the high susceptibility to and severity of infections in diabetes mellitus [65]. They are abundant in mucosal tissues, which are portals of entry for common infectious agents, in addition to allergens, and are relevant cells for the clearance of several types of bacteria [66].

Mast cell deficiency or receptor inhibition restrains generation of chemical compound mediators or cytokines/chemokines, an effect that ameliorates glucose regulation, reduces body weight gain in obesity and improves survival in diabetic experimental animals [67].

During bacterial infections in diabetes, the most reactive compounds are PGD2 (an MC product from arachidonic acid cascade), histamine, IL-6, IL-1, IL-18, TNF and IL-33, which is an alarmin cytokine repeatedly identified as predisposing to inflammation risk and allergy [68, 69]. They are all mediators of MC activity. It is generally believed that MCs are detrimental to bacteria and with their mediators help macrophages, dendritic cells, and neutrophils to clear them from tissues and circulation [70]. Bacteria may bind CD48 on MCs and induce MC activation and degranulation. In addition, MCs can be found in bacterial inflammatory tissue in response to specific C-C chemokines, such as RANTES and MCP-1, since we
previously demonstrated that injection of these two chemokines into the skin tissue, in vivo in a rat animal model, has a significant effect on MC recruitment and histidine decarboxylase (HDC) mRNA production [20]. Mast cell-deficient C57BL/6j-W/Wv/+ mice are more vulnerable to bacterial infections, but they have fewer inflammatory diseases. When the inflammatory chemokine RANTES or MCP-1 is injected intramuscularly or intradermally in genetically mast-cell-deficient W/Wv mice, the inflammatory effect is almost absent [48], and they have higher insulin sensitivity and glucose tolerance [33].

It is likely that some bacteria and Toll like receptors (TLRs) can interfere with the cell surface of MCs, reducing the expression of the FccRI receptor, influencing the generation of chemical mediators and cytokine/chemokine production, including TNF [71]. Toll like receptors play an important role in innate immunity where bacterial infections in diabetes are involved and are present in immune cells, including MCs. Receptors TLR1, 2, 4, 5, 6, 10, and 11 are expressed in cell membrane, while TLR3, 7, 8, and 9 are expressed intracellularly [72]. Toll like receptor microbial ligands and inflammatory cytokines stimulate and induce VCAM-1 expression by stimulation of TLRs [73]. Mast cells express TLRs, including TLR-9 and TLR-3, which stimulate and have a positive influence in VCAM-1 expression in diabetic patients [73]. They can be activated by several compounds, including bacteria, an effect that leads to the release of several cytokines and chemokines. These studies suggest that infections that occur in diabetes may modify the course of inflammation orchestrated by MC activity.

According to the recently published international consensus, data are accumulating to support the view that MCs are involved in insulin resistance and type 2 diabetes and participate in diabetes and other metabolic diseases where immunity is compromised [33].

It has been reported in in vitro studies that activated MCs may contribute to the uptake of low-density lipoproteins (LDL) to cultured macrophages, increasing the generation of foam cells which exert a series of potentially detrimental effects on many cell types, as well as atherogenic actions and diabetogenic functions on the β cells of the endocrine pancreas [74]. Leptin is also involved in T2DM and obesity, which, in some cases, is linked to asthma in children [75], and it acts through histamine produced in the brain [76]. The treatment of diabetic patients with histamine-2 receptor blockers (H2RBs) could reduce the risk of cancer diseases [76].

Increasing numbers of drugs to lower and control blood glucose are used in patients affected by T2DM. These numerous glucose-lowering compounds act with different mechanisms, and one must take into account the relationship between the beneficial effect and their side effects [49].

Targeting and blocking TNF and/or ε-kit stem cell factor or their receptors on activated MCs, which regulates the recruitment and maturation of these cells, could be an effective therapeutic treatment for reducing the inflammatory phenomena which occur in T2DM [77].

Therapies targeting MCs may prove beneficial for treatment of inflammatory and autoimmune diseases (Table III). Therefore, anti-inflammatory therapy targeting MC degranulated mediators and cytokines/chemokines generated by these cells may improve insulin resistance and glycemic control.

However, several authors have reported that in diabetes, during the inflammatory response, there is re-

### Table III. Mast cell mediator release participates in innate acquired immunity, inflammation, autoimmunity and metabolic disorders, including diabetes

<table>
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<tr>
<th><strong>Mast Cells and Diabetes Cross-Talk</strong></th>
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<tr>
<td>Contribute to autoimmune diabetes by releasing interleukin-6</td>
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<tr>
<td>Mediate autoimmune diseases</td>
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<tr>
<td>Exert an anti-inflammatory action and protect mice from obesity and diabetes</td>
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<tr>
<td>Infiltrate pancreatic islets in human type 1 diabetes</td>
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<tr>
<td>Participate in cardio-metabolic diseases and diabetes</td>
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<td>Control insulinitis and increase Treg cells to confer protection</td>
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<tr>
<td>Have an impact on cardiovascular diseases in diabetes</td>
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<td>Have a role in wound healing in induced diabetic mice</td>
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<td>Their reduction improves vascular functions</td>
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<td>Are involved in adipose tissue fibrosis</td>
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<tr>
<td>Histamine H2 receptor signaling participates in the pathogenesis of sepsis in diabetes</td>
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<tr>
<td>Participate in myocardial remodeling in diabetes</td>
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<td>Proteases are potential risk factors of impaired fasting glucose and glucose tolerance</td>
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<td>Affect wound healing in a diabetic rat model</td>
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<td>Chymase and tryptase as targets for metabolic diseases including diabetes</td>
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<td>Inhibition of phospholipase A2 in mast cells modulates adipocyte signaling and diabetes</td>
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<td>Proteases are potential risk factors of human pre-diabetes and diabetes mellitus</td>
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<td>Anti-FcR antibody activates mast cells and delays T1DM</td>
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duced MC degranulation and production of reactive oxygen species, a decrease of microvascular response to MC mediators, including histamine, and reduced release of cytokines and arachidonic acid products [78]. All these effects can be ameliorated by insulin therapy. MCs have also emerged as modulators and down regulators of allergic inflammation. MCs may exert promotion or protection of wound healing, angiogenesis, cardiovascular diseases, obesity, diabetes and other disorders [67].

In addition, in some studies, it has been reported that mast cells may prevent obesity and diabetes in experimental animal models [79]. Moreover, diabetes mellitus may present inhibition of mast cell activation, a decrease of leukocyte-endothelial cell interactions, reduced numbers of leukocytes in inflammatory sites, a decrease of microvascular responses to inflammatory compounds, including histamine, a reduction in protein loss, and a decrease of acute inflammatory reactions [80].

We previously reported that MCs appear to be able to promote tumor development through the generation of certain inflammatory mediators and are therefore beneficial to the tumor, while producing other products may be detrimental to the tumor by participating in its rejection [81]. Regarding the involvement of the MCs in diabetes, it might be in the same situation. Indeed, MCs may be detrimental in diabetic patients by producing inflammatory mediators, PGD2 and cytokine/chemokine generation, but may also be protective, since their proteases may interfere with MC inflammatory mediators and cytokines/chemokines released may have inhibitory biological effects on diabetes. Moreover, manipulation of MCs could represent a new additional therapeutic strategy in diabetes and other metabolic disorders. However, the mechanism by which MCs and their products mediate metabolic diseases is still unclear.

More studies are needed to confirm the direct involvement of mast cell-derived inflammatory mediators in diabetic pathogenesis and other metabolic syndromes.

The authors declare no conflict of interest.

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