Neutrophils in type 1 diabetes

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ABSTRACT

Type 1 diabetes is an autoimmune disease that afflicts millions of people worldwide. It occurs as the consequence of destruction of insulin-producing pancreatic β-cells triggered by genetic and environmental factors. The initiation and progression of the disease involves a complicated interaction between β-cells and immune cells of both innate and adaptive systems. Immune cells, such as T cells, macrophages and dendritic cells, have been well documented to play crucial roles in type 1 diabetes pathogenesis. However, the particular actions of neutrophils, which are the most plentiful immune cell type and the first immune cells responding to inflammation, in the etiology of this disease might indeed be unfairly ignored. Progress over the past decades shows that neutrophils might have essential effects on the onset and perpetuation of type 1 diabetes. Neutrophil-derived cytotoxic substances, including degranulation products, cytokines, reactive oxygen species and extracellular traps that are released during the process of neutrophil maturation or activation, could cause destruction to islet cells. In addition, these cells can initiate diabetogenic T cell response and promote type 1 diabetes development through cell–cell interactions with other immune and non-immune cells. Furthermore, relevant antineutrophil therapies have been shown to delay and dampen the progression of insulitis and autoimmune diabetes. Here, we discuss the relationship between neutrophils and autoimmune type 1 diabetes from the aforementioned aspects to better understand the roles of these cells in the initiation and development of type 1 diabetes.

INTRODUCTION

Neutrophils, produced in the bone marrow from myeloblasts, were first discovered by Paul Ehrlich in 1879[1,2]. They were also called polymorphonuclear leukocytes by Elie Metchnikoff in 1893[3]. After a 14-day maturation in the bone marrow, neutrophils can be provisionally stored in a pond before being released into the blood[4], where they circulate as dormant cells[2]. When activated, neutrophils are the first cells to be recruited to the locations of inflammation, and provide the first line of defense. They have traditionally been considered as short-lived effector cells (just 8–12 h in the circulation and 1–2 days in tissues), possessing limited capacity for biosynthetic activity and releasing granules and reactive oxygen species (ROS)[5,6]. However, this classical view was challenged by the development of more sensitive approaches and research tools. It has recently been shown that neutrophils have a longer circulatory life span (up to 5 days) than first suggested (Figure 1)[5,6]. As an important element of the inflammatory response, neutrophils direct

and guide the innate immune response by engaging in complex interactions with macrophages, natural killer cells, dendritic cells and through cross-talk with most of the cellular effector mediators[6,7]. Stimulated by type I interferons (IFNs), neutrophils can contribute to the host response towards intracellular pathogens by involving gene expression[8]. After activation, neutrophils can promote an innate immune response through releasing soluble pattern recognition molecules (PRMs), which have the capacity to augment phagocytosis, stimulate complement and modulate inflammation. They can also secrete a diversity of cytokines, neutrophil extracellular traps (NETs), and microorganism- and tissue-damaging molecules to participate in innate resistance[6]. Through a combination of these cytotoxic substances, neutrophils not only eliminate inflammation, but also damage cells and tissues of the host. Additionally, neutrophils are implicated in the activation, recruitment, programming, and modulation of both innate and adaptive immune cells, and can help mediate the initiation of specific T and B cell immunity through soluble mediators or by direct cell–cell contact[3,7,9]. Accordingly, an aberrant neutrophil
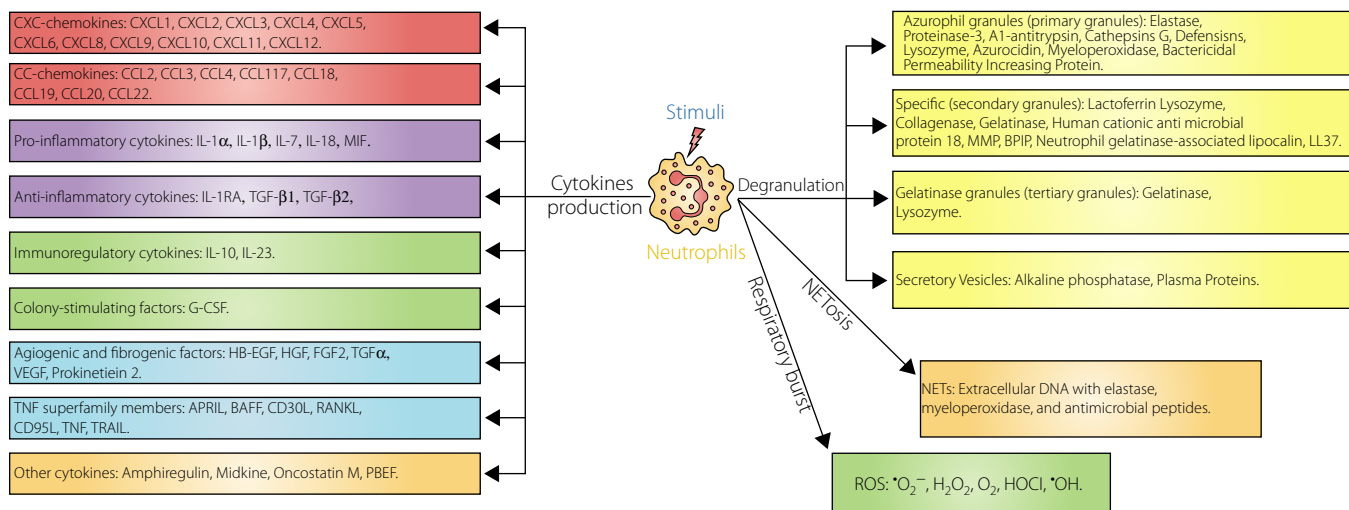
Figure 1 | Neutrophil-derived multiple effector molecules. During the process of maturation or on stimuli activation, neutrophils can express and/or release numerous cytotoxic substances. Three categories of granules, including azurophil granules, specific granules and gelatinase granules, are discharged during the degranulation process and are recognized as the basis content of their enzyme. Apart from these classical granules, neutrophils contain highly mobilizable secretory vesicles that serve as a reservoir primarily for plasma membrane receptors. Simultaneously with their degranulation, the initiation of nicotinamide adenine dinucleotide phosphate oxidase activity (a part of the cellular respiratory burst) in neutrophils occurs, and then various reactive oxygen species (ROS) are generated. Furthermore, neutrophils can produce numerous cytokines, which are considered to be the most critical effectors because of their vast and diverse of biological activities. In addition, neutrophils can be activated to undergo NETosis (a novel form of cell death) and extrude extracellular fibrillary networks termed neutrophil extracellular traps (NETs). APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor; BPIP, bactericidal permeability increasing protein; DNA, deoxyribonucleic acid; EGF, epidermal growth factor; G-CSF, granulocyte colony stimulating factor; HB-EGF, heparin binding epidermal growth factor; HGF, hepatocyte growth factor; IL-1RA, interleukin-1 receptor antagonist; MIF, macrophage migration inhibitory factor; PBEF, pre-B cell colony enhancing factor; RANKL, receptor activator for nuclear factor-κ B ligand; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; TRAIL, tumor necrosis factor-related apoptosis inducing factor.

response can exacerbate and even initiate a variety of diseases, including autoimmune diseases, such as antineutrophil cytoplasmic antibodies-mediated vasculitis, systemic lupus erythematosus and multiple sclerosis[10–12].

Over the past decades, a number of studies have implied that neutrophils are involved in the initiation and perpetuation of autoimmune diabetes as follows: (i) circulating neutrophil counts and functions were found to change in diabetes patients; (ii) they can be activated and recruited to pancreatic islets, and were found to initiate autoimmune diabetes in non-obese diabetic (NOD) mice. Furthermore, neutrophils were observed to infiltrate the pancreas in type 1 diabetes patients; (iii) neutrophil toxic tools, such as neutrophil elastase, were proved to increase in type 1 diabetes patients and associate with the pathogenesis of β-cell autoimmunity as well as diabetic complications; and (iv) related antineutrophil treatments were shown to dampen and reduce the development of insulitis and autoimmune diabetes. Therefore, it is logical to hypothesize that neutrophils are involved in the pathogenesis of type 1 diabetes.

NEUTROPHIL COUNTS AND FUNCTIONS IN TYPE 1 DIABETES

Early studies showed that type 1 diabetes patients had higher neutrophil numbers than the controls, and increased neutrophil counts were reported to correlate with an augmented risk of vascular disease (Table 1 lists the changes of neutrophil numbers)[13,14]. It has been concluded that increased levels of circulating neutrophils could be caused by immoderate enlistment from the bone marrow and/or the return of marginated cells to the circulatory pond[15]. However, more recent studies showed that circulating neutrophil numbers decreased in patients with type 1 diabetes and healthy autoantibody-positive subjects, which might be associated with β-cell specific autoimmunity[15–17]. Reduced numbers of blood neutrophils in type 1 diabetes could be a result of abnormal neutrophil yield and maturation, peripheral consumption or damage, and tissue detainment. It was suggested that neutrophils confined in the pancreas should account for the decreased blood neutrophils[16,18]. The discrepancy in alterations of neutrophil counts from different studies might result from the discoveries from different stages of diabetes or different ethnic groups. Further investigations are required to explain the variance in the changes of neutrophil counts, and a more comprehensive and longitudinal study might clarify the difference. Neutrophil functions were also reported to change at different steps in type 1 diabetes. Clinical studies and experimental data in animal models clearly showed that consistent defects in neutrophil chemotaxis[19,20], adhesion[21] and microbicidal activities (Table 1)[22–24].

Table 1 | Neutrophil counts and functions in autoimmune type 1 diabetes

Neutrophil counts and functions	Alteration	Reference
Counts		
Neutrophil counts	Upregulated	13,14
	Downregulated	15–17
Functions		
Chemotaxis	No change	19,20
Adhesion	Downregulated	21
Microbicidal activities	Downregulated	22–24
Oxidative burst activity	Downregulated	24,25
	Upregulated	26
Migration	Upregulated	19
	Downregulated	22,27
Phagocytosis	No change	23
	Downregulated	22,24,28
Apoptosis	Upregulated	27,29,30

However, literature to date is contradictory in regard to other host defense functions of neutrophil including oxidative burst activity[24–26], migration[19,22,27], phagocytosis[22–24,28] and apoptosis[27,29,30]. The changed neutrophil functions can be caused by upregulated expression of adhesion molecules[28], downregulated receptors[31], impaired calcium signaling[32] and anomalous activities of adenosine triphosphate synthases on neutrophils[33]. Although the neutrophil apoptosis rate was significantly correlated with glycated hemoglobin (HbA1c) levels in patients with type 2 diabetes[30], research has shown that HbA1c level and history of infection did not seem to affect neutrophil functions in type 1 diabetes patients[28]. It was reported that neutrophil functions might be closely related to β-cell autoimmunity, as a significant decrease in neutrophil numbers can be detected in type 1 diabetes patients diagnosed within 1 year and in prediabetes, but not in type 1 diabetes patients with long duration[17,18]. There is abundant evidence showing the effects of environmental factors, such as hyperglycemia and advanced glycation end-products (AGEs) on neutrophil functions, but literature regarding the association of neutrophil dysfunction and genes is scarce. It was suggested that human leukocyte antigen-D related-associated genes were not related to impaired neutrophil functions, though they were closely correlated with type 1 diabetes pathogenesis.

NEUTROPHIL-DERIVED EFFECTOR MOLECULES AND TYPE 1 DIABETES

Adhesion Molecules

Adhesion molecules, existing as soluble and membrane forms, not only play an essential role during neutrophil migration from the bloodstream into target tissue[34], but are also involved in the regulation of the immune system[35]. In autoimmune diabetes, the migration of immune cells from blood vessels into the pancreatic islet is important to exert their potential[36]. Vari-

ous studies have shown an involvement of two families of adhesion molecules including selectins and integrins in the development of type 1 diabetes. The selectin family consists of P-selectin (CD62P, GMP-140), L-selectin (CD62L) and E-selectin (CD62E; Table 2 summarizes the neutrophil-related adhesion molecules). L-selectin is predominantly expressed on the surface of various immune cells[37]. It is rapidly effluxed after the activation of neutrophil[38]. Soluble L-selectin (sL-selectin) was found to alter in patients with type 1 diabetes, as well as subjects in the preclinical stage of the disease[39]. Early studies suggested that L-selectin could be one of the new risk markers for type 1 diabetes development in humans and animal models[40]. Subsequent studies further suggested that elevated levels of sL-selectin are associated with high titers of insulinoma-associated protein 2 antibody in children with type 1 diabetes[41] and siblings of diabetic children[34]. Therefore, augmented sL-selectin expression in patients can manifest an active destructive insulitis procedure[34,41]. Furthermore, increased levels of sL-selectin were also reported to correlate with seroconversion to autoantibody positivity, proposing that the activation of leukocytes coincides with the occurrence of β-cell autoimmunity[42]. However, from the prospective aspect (10 and 2 years, respectively), it was shown that early-onset of β-cell autoimmunity cannot be displayed entirely by elevated concentrations of circulating adhesion molecules[42,43]. No differences were discovered in the integrated concentrations of sL-selectin associating with distinct autoantibody specificities and titers.

Integrins, heterodimers with an α chain and a common β chain, are composed of β1-integrins and β2-integrins. The three β2-integrin complexes comprise CD11a (leucocyte function antigen-1 [LFA]), CD11b (macrophage-1 [MAC-1]) and CD11c (p150,95). LFA is expressed on neutrophil membranes, whereas MAC-1 glycoprotein complex is stored in secondary granules[4]. LFA-1 was shown to be involved in the initiation of insulitis in humans and type 1 diabetes animal models[44,45]. Treatment with anti-LFA-1 monoclonal antibodies can delay the spontaneous onset of the disease in NOD mice[46,47], and

Table 2 | Neutrophil-related adhesion molecules

Type	Expressed cells	Ligand
Slectins		
P-selectin (CD62P)	Endotheliocyte	PGSL
L-selectin (CD62L)	Neutrophils	Unknown
E-selectin (CD62E)	Endotheliocyte	Unknown
Integrins		
VLA-4 (CD49d)	Neutrophils	VCAM-1 (CD106)
LFA-1 (CD11a)	Neutrophils	ICAM-1 (CD54)
Mac-1 (CD11b)	Neutrophils	ICAM-1 (CD54)
p150 (CD11c)	Neutrophils	Unknown
CD29	Unknown	Unknown

ICAM-1, intercellular adhesion molecule 1; LFA-1, leucocyte function antigen-1; Mac-1, macrophage-1; PGSL, P-selectin glycoprotein ligand; VCAM-1, vascular adhesion molecule-1; VLA-4, very late antigen 4.

has a strong preventive effect on the progression of the disease[48,49]. The mechanism of prevention appears to result from suppressing effector cells to home to the pancreas. It is well known that LFA-1 can be expressed on neutrophil membranes as well as lymphocytes, monocytes and natural killer cells. To our knowledge, the expression of LFA-1 on neutrophils in type 1 diabetes has not been reported. However, a variety of studies have reported the expression of LFA-1 on monocytes. An early study found that LFA-1 alpha chain expression decreased and the percentage of LFA-1 β chain-positive monocytes was normal in newly diagnosed patients with type 1 diabetes[50]. While another study showed that a higher level of LFA-1 on mononuclear cells was observed in overt diabetics in comparison with the controls[51], there was a positive interrelation between LFA-1 expression and islet cell autoantibodies (ICA) titer, indicating that LFA-1 plays an important role in type 1 diabetes pathogenesis[51]. MAC-1, another member of the β2-integrins, is considered to be associated with vascular event rates, because it is prothrombotic and can mediate leukocytes vascular infiltration[52]. In type 1 diabetes patients, neutrophils showed higher expression of MAC-1 receptors that are independent of duration of diabetes and HbA1c[53]. Increased expression of MAC-1 can be caused by acute hyperglycemia[52], and might result from impaired neutrophil actin polymerization[54]. It was observed that elevated expression of MAC-1 could contribute to the pathogenesis of diabetic complications by enhancing adhesion between neutrophils and endothelial cells[54,55].

Degranulation Products

Neutrophils are sophisticated cells that communicate with their environment by dislodging and releasing various kinds of granules and vesicles[1], such as azurophil granules, specific granules, gelatinase granules and secretory vesicles (Figure 1)[1,56,57]. Normally, a variety of proteases, formed during the process of neutrophil maturation, are stored intracellularly in granules, and might be liberated into the extracellular space after neutrophil activation and degranulation[58]. It has been suggested that neutrophil proteases show activity in membrane-bound form and soluble form, the latter of which acts extracellularly in plasma and tissues[59]. Among these proteases, neutrophil elastase and myeloperoxidase, found in the azurophil granules, are considered as relatively neutrophil-specific. Plasma lactoferrin, a member of the specific granules, is thought to derive principally from neutrophils, and is markedly associated with circulating neutrophil numbers, although it can be excreted by other diversified cells[60].

Neutrophil elastase is capable of degrading most of the extracellular matrix proteins and plasma proteins[61]. In addition, neutrophil elastase can regulate inflammation by splitting different agents, such as cytokines, chemokines and cell surface receptors[62,63]. Plasma and total neutrophil elastase were reported to rise substantially in type 1 diabetes patients when compared with the control[13,14]. High concentrations of plasma neutrophil elastase can also be considered as a marker of the development of complications, such as diabetic angiopathy and coronary artery disease, because it might contribute to the progression of vascular disease[64–66]. Furthermore, the previous work in our laboratory discovered that levels of circulating neutrophil elastase released from activated neutrophils was positively associated with the counts and titers of the autoantibodies against β-cell-specific antigens, which suggested that neutrophil activation and elevated proteases activities might play an anetiogenic role in the process of β-cell autoimmunity. Therefore, serum neutrophil elastase could act as a susceptible biomarker for the prediction and early diagnosis of type 1 diabetes[17]. Although some studies showed that poor short-term glycemic and metabolic control in type 2 diabetes patients were correlated with higher elastase concentration in plasma and neutrophils[59,64], it has been shown in several studies that increased plasma neutrophil elastase level in type 1 diabetes patients was not related to age, leucocyte count, HbA1c, plasma glucose or duration of diabetes[13,14]. Myeloperoxidase (MPO), a powerful oxidative medium, is located mainly in neutrophil primary granules and constitutes approximately 5% of the total neutrophil protein[56]. MPO is relevant to oxidative stress, because it catalyzes ROS formation that can facilitate atherogenesis and alter lipid as well as proteins[60]. Activity of MPO can be inhibited in patients with diabetes, resulting in diminished phagocytic activity of neutrophils and thus increasing susceptibility to infections[67]. It has been shown that no significant differences were found in plasma MPO values when children and adolescents with type 1 diabetes were compared with controls. However, another study found that diabetic children had significantly higher serum concentrations of MPO than the healthy control group, which can reflect increased risk of cardiovascular diseases in type 1 diabetes patients[68]. Regardless of whether the MPO level is increased or decreased, there are no significant interrelations between MPO serum concentration and diabetes duration, HbA1c value, and the level of actual blood glucose[68].

Cytokines

Neutrophils, either constitutively or after appropriate motivation, not only synthesize a variety of polypeptides that are directly involved in their effector functions, but can also produce numerous inflammatory modulation proteins (Figure 1)[69,70]. Among these molecules, cytokines are considered to be the most critical effectors because of their vast and diverse biological activities[69,71]. Cytokines are not liberated immediately after synthesis, but are deposited in temporarily intracellular ponds and are released quickly when neutrophils are activated[72]. Human and mouse neutrophils were reported to have a different capacity to express cytokines, especially interleukin (IL)-6, IL-17A, IL-17F and IFN-γ[6]. Whether activated neutrophils secrete cytokines or not depends on the state of the cells and the type of triggering stimulus. Although it is evident that neutrophils make substantially less quantities of cytokines than other immune cells, it must be stressed that neutrophils constitute most of the infiltrating cells in inflamed tissues, and might be considered as an important origin of cytokines in

those tissues[69]. In view of the broad spectrum of biological activities exerted by cytokines, it is reasonable to infer that neutrophils have crucial effects not only on inflammatory response, but also on innate and adaptive immune responses[6,70].

Cytokines, produced locally in and around islets during islet insulitis by more than a single cell type, are shown to play considerable roles in the development of autoimmune diabetes[73]. It has been proposed that cytokines, such as tumor necrosis factor (TNF)-α, IL-1, IFN-γ and TNF-β, can stimulate toxic free radical production, which might initiate pancreatic β-cell destruction[74–76]. Furthermore, β-cell function can be impaired and β-cell mass can be reduced by IL-1 derived from macrophages[77]. In addition, cytokines like IFN-γ, IL-1 and TNF-α can exacerbate autoimmunity by intensifying adaptive immune responses[77]. Increased expression of cytokines (IFN-α, TNF-α and IL-1) is associated with β-cell destructive insulitis[73]. In patients with recent-onset type 1 diabetes, increased IL-18 is reported to be related to multiple autoantibody presence[78]. As a result of their important roles in type 1 diabetes, serum cytokines are promising candidates as additional markers and for monitoring interventions beyond autoantibodies to predict type 1 diabetes. However, many problems and conflicting observations exist – the major difficulty is the absence of a normal range for the majority of cytokines. Furthermore, the concept of physiological disparities, and technical alterations in assay methodologies and assay perturbations cannot be neglected. Another obstacle is the lack of specificity to diseases, even though certain cytokines have normal reference ranges and the levels in plasma can be measured by current technologies. Finally, cytokines concentrations are dynamic, especially under the condition of infections, allergies and medications[79]. Although cytokines have diverse biological activities and neutrophils can release a variety of cytokines, the role of cytokines derived from neutrophils in the pathogenesis and development of autoimmune diabetes is still unclear.

ROS

ROS, including superoxide anions, hydroxyl radicals and hydrogen peroxide, are produced as a consequence of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, which is a part of the cellular respiratory burst[56,80]. Neutrophils are rich sources of toxic oxygen species (Figure 1)[81], and the initiation of NADPH oxidase activity in neutrophils occurs almost simultaneously with their degranulation, with a delay of approximately 20 s[82]. Increased production of ROS from neutrophils has been reported in type 1 diabetes patients and rat models[26,83]. Elevated expression of these toxic substances not only initiate pancreatic β-cell destruction, but also predispose diabetic patients to infection by impairing anti-oxidant defense in neutrophils[84–86]. The enhancement of ROS production in type 1 diabetes is suggested to result from glycosylation and glyco-oxidation, lipid peroxidation, and activation of platelets and neutrophils[81]. Although AGEs possess no capacity to enhance respiratory burst alone, they appear to be capable of

increasing neutrophil ROS production by upregulating NADPH oxidase and by priming neutrophils[87]. However, other investigators found a reduction in respiratory burst responses, and it has also been documented that neutrophil NADPH oxidase activity *in vitro* was impaired and superoxide production was reduced in diabetic patients[24,88]. High-glucose levels rapidly decrease ROS from stimulated neutrophils, possibly by suppressing glucose-6-phosphate dehydrogenase[80]. The contradictory results of ROS levels in these studies might be derived from patients with different metabolic states and different disease durations[81]. Various studies show that ROS are generated from not only neutrophils, but also macrophages, mesangial cells and glomerular epithelial cells[89]. It has been shown that ROS mediated by macrophages can infiltrate islets and damage islet β-cells, directly resulting in autoimmune diabetes in NOD mice[90]. However, the role of ROS derived from neutrophils in the pathology of type 1 diabetes is not clear at this stage.

Neutrophil Extracellular Traps

Apart from producing classical effector molecules, such as proteases, cytokines and ROS, neutrophils might undergo NETosis (a novel kind of cell death procedure almost differentiated from both apoptosis and necrosis) and form fibrillary extracellular networks known as NETs in response to a number of stimuli[6]. NETs comprise nuclear constituents decorated by granular proteins and short peptidoglycan recognition protein, and are beneficial for antimicrobial processes. In addition, NETs are associated with autoimmunity, because they secrete self molecules extracellularly[3]. Dysregulated NET formation and NETosis were reported to be involved in a number of autoimmune diseases, such as type 1 diabetes, small vessel vasculitis and systemic lupus erythematosus[10–12]. Several studies have clearly proved that increased NETosis can induce autoimmunity and accelerate the occurrence of vascular disease in systemic lupus erythematosus patients[91]. However, literature regarding the role of NETs in autoimmune diabetes development is relatively scarce. In young NOD mice[92], NET formation was observed in pancreatic islets as early as 2 weeks after birth. In autoimmune type 1 diabetes patients, NET formation was found to be elevated and closely associated with increased circulating neutrophil elastase levels, suggesting that it might play a key role in the initiation of β-cell autoimmunity[17]. However, conflicting reports showed that neutrophils from diabetic patients (the diabetes type was not mentioned) released NETs at a lower level than that of healthy subjects, because a high-glucose condition might impair and delay neutrophil NET formation[93,94]. Therefore, expression of neutrophil NETs possessing antimicrobial property was reduced, providing a partial explanation for elevated susceptibility of diabetes mellitus patients to infections[93,94].

CELL–CELL INTERACTIONS IN TYPE 1 DIABETES
Neutrophils and Other Immune Cells
Apart from using a set of membrane and intracellular molecules to respond to their local environment signals and to

modify their phenotype, neutrophils engage in complex bidirectional interactions with most other types of immune cells, and shape their activation, maturation and effector functions directly or indirectly, depending on the context[95]. They instruct other immune cells through secreting cytokines, granules and ROS. They can also participate in the communication networks through cell–cell contact[96]. By interplaying with other cells, neutrophils are representatively the predominant immune cells responding to inflammatory response and exacerbating inflammation[97]. In autoimmune type 1 diabetes, interactions between pancreatic β-cells and immune cells including neutrophils, as well as other immune cells, play significant roles in the progression of the disease. Recently, Diana et al.[92] found that physiological β-cell death can induce recruitment and activation of neutrophils, B-1a cells, and plasmacytoid dendritic cells in young NOD mice. The cross-talk between these innate immune cells was found to take place in the pancreas, and was required for the initiation of type 1 diabetes. They also found another novel cross-talk between macrophages and β-cells in the pancreas, which was responsible for neutrophil infiltration in the pancreas during the initiation phase of autoimmune diabetes (Figure 2)[98].

There is considerable evidence that T cells play an essential role in the development of type 1 diabetes in both animal models and humans. In the early stage of diabetes, CD8$^+$ T lymphocytes are considered as the most affluent immune cell type infiltrated in the pancreas during the occurrence of insulitis[99]. They can drive adaptive immune responses and damage pancreatic β-cells by major histocompatibility complex class I molecules-regulated pathogenic cytotoxicity, and both CD4$^+$ and CD8$^+$ T cells can secrete cytokines that could contribute to β-cell apoptosis directly or indirectly[100]. In NOD mice, neutrophil trafficking into the pancreatic islets is mainly regulated by CXCR2 ligands, and neutrophils are recruited by chemokines CXCL1 and CXCL2 produced by β-cells and macrophages[98]. CXCR2 blockade in early stages can lead to a dramatic reduction in specific CD8$^+$ T cells within the islets, and then can inhibit diabetogenic T-cell response, as well as the following development of diabetes[98]. Recently, decreased expressions of Cxcr1 messenger ribonucleic acid were found in both neutrophils and CD4$^+$ T lymphocytes isolated from NOD mice when compared with diabetes-resistant mice. Low Cxcr1 expression in NOD mice could contribute to the pathogenesis of autoimmune diabetes[101]. In addition, IL-17$^+$ β-cell-specific autoreactive CD4$^+$ T cells can be observed in the circulation of type 1 diabetes patients at diagnosis[102]. This pro-inflammatory IL-17 circumstance in the pancreas can attract neutrophils to infiltrate the pancreatic tissue[18]. It seems that both neutrophils and T cells might contribute to the pathogenesis of type 1 diabetes with a variable degree of synergism. Therefore, interactions between neutrophils and T cells in the development of type 1 diabetes deserve us to focus on.

Neutrophils and Endothelial Cells

Interaction between neutrophils and endothelial cells is one of the earliest events in the inflammatory process and normally occurs in post-capillary venules[38]. Adhesion of neutrophils to endothelial cells facilitates neutrophils to enter to the pancreatic islet and causes damage to it. However, most of the research about neutrophil–endothelial cell adhesion in diabetes have focused on the development of atherosclerosis-mediated diseases, such as diabetic microvascular and macrovascular complications[103,104], and studies about neutrophil–endothelial cell adhesion in type 1 diabetes pathogenesis are relatively scarce. Several endothelial adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), CD62E, CD62P and vascular adhesion molecule-1 (VCAM-1), can attract circulating neutrophils then bind with neutrophil adhesion molecules[103,105,106]. It has been shown that ICAM-1, which is the best characterized cell surface adhesion molecule, is implicated in the pathogenesis of type 1 diabetes by being involved in the extravasation of leukocytes from the circulation into the inflamed pancreas. ICAM-1 expression on vascular endothelial cells was reported to increase in the pancreatic islets of the NOD mouse[48,107], as well as in individuals with new-onset type 1 diabetes[108]. Serum levels of circulating ICAM-1 were also significantly increased in recent-onset type 1 diabetes patients and their first-degree relatives when compared with healthy controls[34,35]. Among these subjects, soluble ICAM-1 levels in prediabetics with positive autoantibodies were relatively higher than in patients with clinical diabetes, and were positively correlated with ICA and glutamicacid decarboxylase autoantibody (GADA) values[63]. These aforementioned advances, together with research reporting that neutrophils were found to infiltrate in the pancreatic islets, suggest that neutrophil–endothelial cell interactions are undoubtedly involved in the accumulation of inflammatory neutrophils into the pancreas and the destruction of pancreatic islets, but the mechanism that controls the process remains unclear.

Neutrophils and Platelets

Platelets and leukocytes, the latter of which consist of neutrophils, have been proven to regulate and influence each other's function by platelet–leukocyte contact and releasing soluble effector mediators[109]. Activated platelets promoted neutrophil activation and recruitment through expressing selectins, inflammatory cytokines, and chemokines[110]. Conversely, apoptotic and activated leukocytes can promote platelet recruitment and attachment[111,112]. Adhesion of activated platelets to leukocytes was reported to be implicated in the development of thrombotic occurrence, and apoptotic leukocytes can induce a prothrombotic course[113]. It was found that type 1 diabetes patients showed increased blood platelet–leukocyte aggregation and cross-talk in their blood[114]. Promotion of leukocyte–platelet interaction in the disease is probably caused by enhanced plasma elastase levels, which can induce platelet activation[115] and increase production of important soluble mediators, such
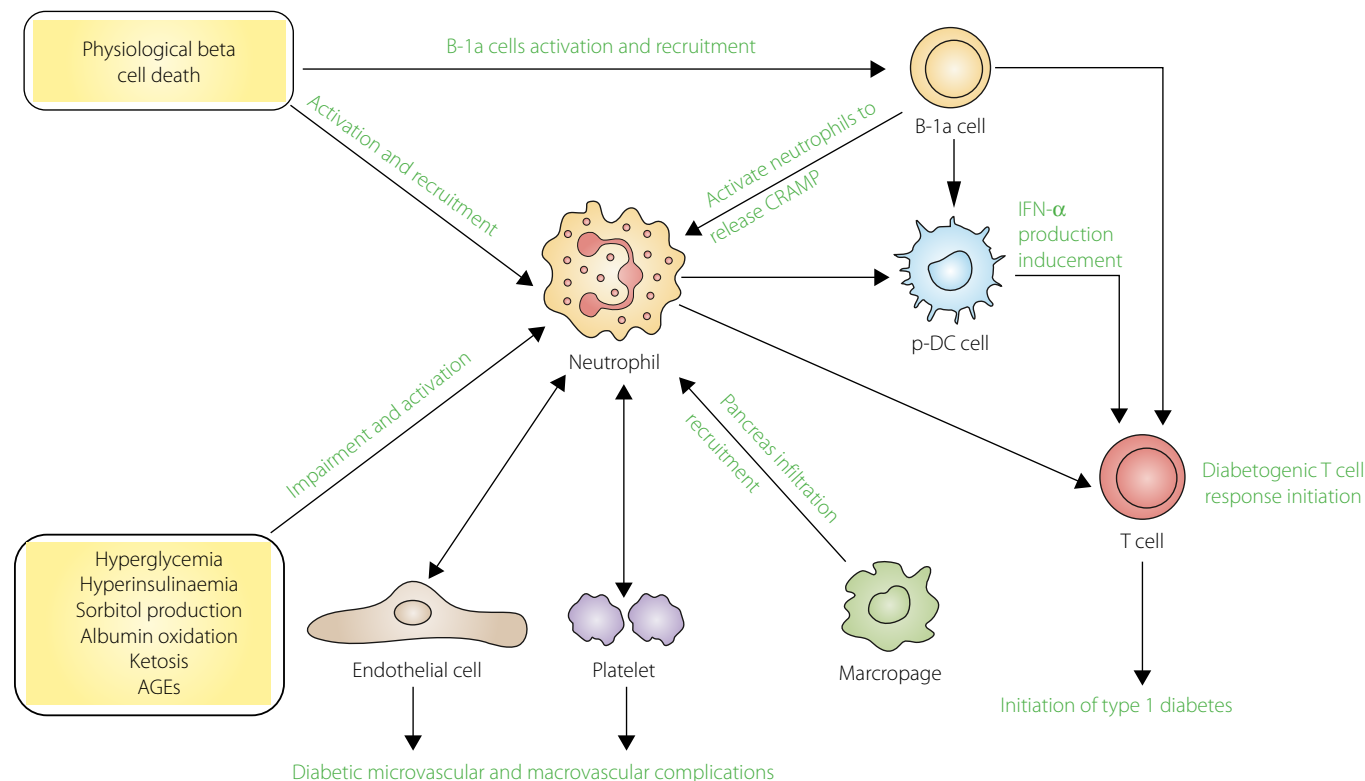
Figure 2 | Neutrophils and type 1 diabetes. The mechanism of the initiation and pathogenesis of type 1 diabetes still remains unclear. Physiological β-cell death was considered as an essential trigger in the development of the disease, which can recruit and activate immune cells, particularly neutrophils, to infiltrate in pancreatic islets. In the pancreas, neutrophils can release cathelicidin-related antimicrobial peptide (CRAMP), the process of which is activated by deoxyribonucleic acid (DNA)-specific immunoglobulin G secreted from B-1a cells. These immunoglobulin G and CRAMP peptide complex, together with β-cell debris like self-deoxyribonucleic acid, can induce plasmacytoid dendritic cells to produce interferon-α. The aforementioned cross-talk between these immune cells is required to induce diabetogenic T-cell response and then leads to the initiation of type 1 diabetes. Additionally, interaction between neutrophils and other non-immune cells, such as platelets in the blood or endothelial cells on the blood vessels, is supposed to play essential roles in diabetic microvascular and macrovascular complications. Conversely, neutrophils can be activated and impaired by metabolic changes in type 1 diabetes patients. AGEs, advanced glycation end-products; IFN, interferon.

as platelet-activating factor and superoxide anion[109,116,117]. AGE-BSA, a model substance for AGEs, can augment platelet–neutrophil aggregation by inducing neutrophils apoptosis and enhancing Mac-1expression[113]. As the interplay between leukocytes and platelets connects inflammation with thrombosis and might facilitate vascular obstruction as well as tissue ischemia[118,119], elevated circulating platelet–leukocyte aggregation and cross-talk in type 1 diabetes individuals might contribute to platelet hyperactivity and the development of microvascular complications[117]. It was also found that an increase of platelet–neutrophil aggregation in patients with diabetes might be one of the factors leading to serious cardiovascular disease[120].

ANTINEUTROPHIL THERAPY
The previous studies showed that neutrophils play important roles in the progression of autoimmune type 1 diabetes, raising the possibility that neutrophils might be a candidate for therapeutic interventions for the disease. The processes of neutrophil activation, binding to the endothelium, transendothelial migration, emigration into the pancreatic islet and release of cytotoxic products, are all potential targets towards which pharmacological therapy can be achieved. Therapies directed against neutrophil-mediated injury in diabetes include direct inhibition of neutrophil recruitment and anti-adhesion therapy.

Recruitment of neutrophils from the circulation into the pancreas is necessary for these cells to infiltrate in the pancreatic islet and bring about their effects. It was reported that macrophages and β-cells recruit CXCR2-expressing neutrophils by producing chemokines CXCL1 and CXCL2[98]. Blockade of neutrophil recruitment by CXCR2 antagonist can depress diabetogenic T-cell response and dampen the subsequent progression of autoimmune diabetes. As aforementioned, neutrophil adhesion to endothelial venules within pancreatic sections is an important early step in the inflammatory response, and is considered as one of the essential steps in the initiation of autoimmune diabetes. Therefore, blocking different adhesion

molecules, such as selectins and integrins expressed on neutrophils by specific antagonists, might be an effective approach to reduce the migration of neutrophils to the inflamed pancreas, thus inhibiting the development of insulitis. Antibodies of anti-L-selectin and anti-VLA-4 were reported to delay the appearance of insulitis by inhibiting leukocyte adhesion to the inflamed blood vessels and interpreting the recruitment of leukocyte to the islets[47]. Administration of anti-α4-integrin and/or anti-LFA-1 antibodies has been found to lead to an inhibition of β-cells destruction, and protect against spontaneous and adoptively transferred diabetes in NOD mice[46,49,121,122]. Combination treatment with the two monoclonal antibodies might have a longer delayed influence in the initiation of diabetes[46]. Furthermore, long-term inhibition of both LFA-1 and α4-integrin not only prevents diabetes during treatment, but also has a persistent resistance to the disease long after the interference has ceased, suggesting that adhesion molecule inhibition can reduce islet insulitis by affecting effector cell functions through stimulation of suppressor cells involved in immunoregulation[46]. In addition, a short-term blockade of the LFA-1/ICAM-1 pathway at critical periods was shown to induce a unique peripheral tolerance against β-cell Ag(s) at an early age in NOD mice, resulting in complete protection from autoimmune diabetes[123]. Therefore, adhesion molecules, such as L-selectin, VLA-4 and LFA-1, play significant roles in the development of type 1 diabetes, and the disease can be prevented by blocking these adhesion pathways. However, it is known that adhesion molecules are expressed not only on neutrophils, but also on lymphocytes, monocytes and natural killer cells[124]. Studies have shown that anti-LFA-1 treatment can ameliorate neutrophil-mediated injury by reducing the adhesion, recruitment, accumulation and infiltration of neutrophils into tissues[125–128]. In addition, the activation of neutrophil respiratory burst can also be restricted by anti-LFA-1 interference[129]. Therefore, further exhaustive studies are required to better understand the preventive effects of neutrophil-specific anti-adhesion therapies in the progression of spontaneous diabetes.

CONCLUDING REMARKS

Progress over the past decades shows that neutrophils play essential roles in the onset and progression of autoimmune type 1 diabetes. Recently, exciting discoveries have provided new insights into the actions of neutrophils in the initiation of the disease, which found that neutrophils can induce type 1 diabetes through infiltrating into the pancreatic islets and interplaying with other immune cells. Unfortunately, only a few studies have specifically concentrated on the relationship between neutrophils and diabetes, though neutrophils are known to the most abundant cell type in the circulation and the first cells recruited to the site of inflammation. Detailed studies are required to elucidate how neutrophils are activated and recruited to cause damage to pancreatic islets through neutrophil-derived toxic substances or interactions with other cells. A better understanding of the role of neutrophils in type 1

diabetes pathogenesis will provide additional information for early diagnosis, therapy and even for the prevention of the disease. Although many challenges still remain in exploring the correlation between neutrophils and autoimmune type 1 diabetes, it is an encouraging and interesting research topic that deserves to be focused on.

DISCLOSURE

The authors declare no conflict of interest.

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