We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter

Neutrophil Function Impairment Is a Host Susceptibility Factor to Bacterial Infection in Diabetes

Daniella Insuela, Diego Coutinho, Marco Martins, Maximiliano Ferrero and Vinicius Carvalho

Abstract

Diabetes mellitus is a highly prevalent noncommunicable disease globally. One of the main complications of diabetes is the increased susceptibility to bacterial infection. Neutrophils play a crucial role in inflammatory response against bacterial infections, once they are the first cells recruited to the sites of injury. In diabetes, there is a failure in the neutrophil functions, including migration, ROS production, phagocytosis, and bacterial killing, which are associated with the high incidence of bacterial infections. Herein, we point out pieces of evidence revealing the primary molecular mechanisms involved with impairment of neutrophil functions in diabetes, with relationship with high susceptibility to bacterial infections.

Keywords: diabetes, bacterial infection, neutrophils, inflammation, chemotaxis

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by a hyperglycemic condition that results in several complications, such as neuropathy, nephropathy, retinopathy, and increased risk of cardiovascular disease [1]. DM can be classified into type 1 (T1DM) and type 2 (T2DM). T1DM is common in childhood or young adulthood and is a result of autoimmune destruction of beta-cells in pancreatic islets mediated by T cells, leading to defect in insulin synthesis [2, 3]. The T2DM appears mainly in adulthood, affecting people with the most productive age. This type of diabetes is associated with insulin resistance and inadequate compensation by beta-cells, leading to a relative insulin deficiency [1, 4]. Currently, it is known that there are over 425 million people with DM globally. Worryingly, it is estimated that in 2045, this number will grow to over 600 million [5].

Hyperglycemia, a hallmark of DM, is associated with patient vulnerability to bacterial infections, such as tuberculosis and pneumonia, besides more severe sepsis of bacterial origin [5]. In fact, diabetic patients generally present microbial persistence, greater susceptibility to new infections, recurrences, and an increase in the risk of mortality if compared to nondiabetic individuals [5, 6]. This is due to the compromised immune response presented by diabetic patients, which leads to failure in leukocytes protective effects. Cyclically, infection profile in these patients can worsen glycemic control [5]. Neutrophils present an important role in host immune response to bacterial infection, once they are one of the first leukocytes

that arrive in the infected area [7]. In normal conditions, these cells act by different manners against microorganism, leading to infection control and resolution of the inflammatory process. However, the immune response in diabetic patients is characterized by impairment in neutrophil function [7, 8]. Here, we revised the mechanisms involved with the failure of neutrophil functions noted in DM and its relationship with the high susceptibility to bacterial infections.

2. Role of neutrophils in bacterial infections

2.1 Migration of neutrophils to infected sites

Neutrophils are polymorphonuclear (PMN) versatile innate effector cells essential for immune defense, which arise from hematopoietic stem cells (HSCs) in bone marrow [9]. Under normal conditions, about 5×10^{10}–10×10^{10} new neutrophils are produced in the bone marrow daily [10, 11]. Chemokine gradients and adhesion molecules are central players that regulate neutrophil release from the bone marrow [11]. Neutrophils express CXC receptors (CXCR)-1 and CXCR2 that interact with CXC chemokines (CXCL1/KC, CXCL2/MIP-2, and CXCL8/IL-8) and result in neutrophil migration from bone marrow into the bloodstream. Neutrophils also express CXCR4, which interacts with CXCL12/SDF-1 produced by osteoblasts and other stromal cells to mediate neutrophil maintenance in the bone marrow [10, 12]. Thereby, only a small fraction of mature neutrophils is released into the blood. However, after a bacterial invasion, the host defense activates strong neutrophil release from bone marrow and migration toward infected sites [11, 12].

Under bacterial infection, sentinel cells detect the microorganisms via pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs). These receptors identify highly conserved pathogen-associated molecular patterns (PAMPs), including peptidoglycan (PGN) and lipopolysaccharide (LPS) expressed in the cell membrane surface of bacteria. They can also recognize danger-associated molecular patterns (DAMPs), such as high mobility group protein B1 (HMGB1), ATP, and uric acid, released from damaged and necrotic cells after tissue injury. Then, sentinel cells release mediators such as granulocyte colony-stimulating factor (G-CSF), which leads to neutrophil production and release from bone marrow via upregulation of CXCR2 and its ligands, and reduces expression of CXCL12/SDF-1 and CXCR4. After this event, neutrophils can be mobilized to sites of infection and combat microorganism [13–15].

Correct leukocyte recruitment requires the adhesive interactions between P-selectin glycoprotein ligand-1 (PSGL-1), E-selectin ligand-1 (ESL-1), and CD44 expressed on the neutrophil membrane surface to the P- and E-selectin which are upregulated in endothelial cells of inflamed tissue. These processes will lead to neutrophil capture and fast rolling [16, 17]. Rolling event exposes neutrophils to chemokines that are arrested on the glycocalyx of endothelial cells, such as CXCL8/IL-8. Then occurs the activation of integrin molecules such as VLA-4 (CD49D/CD29), macrophage-1 antigen (MAC-1 or CD11b/CD18), and lymphocyte function-associated antigen-1 (LFA-1 or CD11a/CD18) on neutrophils [10, 18]. The integrin binds to their ligands such as intercellular adhesion molecule (ICAM)-1, ICAM-2, and platelet endothelial cell adhesion molecule-1 (PECAM-1) on endothelial cells, resulting in slow rolling and firm adhesion of the neutrophil to endothelial cells [17]. Thence, the neutrophils perform diapedesis toward the tissue and migrate along a chemokine gradient until they arrive in the infected site. The long-distance recruitment is mediated by chemoattractants, including leukotriene B4 and CXCL8/IL-8, while near chemoattractants are peptides and C5a [17].

Despite the canonical neutrophil migration during infections, in sepsis occurs an inadequate migration of neutrophils even with high levels of chemokines at the infection site [12]. The decrease of CXCR2 expression on the cell surface of neutrophils is among the mechanisms leading to this failure. The prolonged exposure to CXCR2 agonists, which leads to phosphorylation of G protein-coupled receptors (GPCRs) by GPCR kinases (GRKs) and induces the desensitization and internalization of CXCR2, can explain the down-regulation of this receptor [16]. In addition, the activation of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) by inflammatory products, such as C-reactive protein (CRP), bacterial products, apoptotic cells, or activated platelets, can also account for CXCR2 neutrophil endocytosis [12, 19].

2.2 Actions of neutrophils in infected sites

At the sites of infection, neutrophils can combat pathogenic microorganisms and clear infections by different ways including phagocytosis, degranulation of microbicidal molecules, production and secretion of reactive oxygen species (ROS), and release of neutrophil extracellular traps (NETs) [20]. For efficient bacterial phagocytosis, the microorganism needs to be covered with opsonins, such as immunoglobulins (Igs) and components of the complement system, which are recognized by neutrophil specific surface receptors. After phagocytosis of an opsonized pathogen by neutrophils, there is a mobilization of intracellular granules or lysosomes, leading to the killing of the ingested bacteria [21].

Neutrophil activation can induce the production of ROS to combat infection. It happens mainly due to the action of NADPH oxidase complex (NOX), but can also be generated by mitochondria. After neutrophil activation, NOX acts converting molecular oxygen (O_2) into superoxide anion ($O_2^{\cdot -}$) which suffers dismutation, spontaneously or catalyzed by myeloperoxidase (MPO), generating hydrogen peroxide (H_2O_2) [22, 23]. In addition to ROS, neutrophils can also enhance the inducible nitric oxide synthase (NOS2) expression, which will convert O_2 to nitric oxide (NO), resulting in reactive nitrogen species (RNS). Both ROS and RNS contribute to microbicide activity and are crucial for the defense against intracellular microorganisms [22]. Curiously, NO is supposed to be involved in the failure of neutrophil migration during sepsis, once it stimulates the internalization of CXCR2 on the neutrophil surface and reduces expression of adhesion molecules, leading to diminished leucocyte rolling and adhesion to the endothelium [12]. After neutrophil migration, degranulation occurs, which is the process mediated by microbial or inflammatory stimuli in which neutrophils release the granule contents, such as MPO, defensins, cathepsin G, neutrophil elastase, and collagenase. These granule contents are released by exocytosis or into the phagosome to kill microorganisms [24].

Neutrophils may also perform the antimicrobial activity directly attacking and restraining microorganisms by releasing NETs (NETosis) [25, 26]. NETs are extracellular fibrous structures composed by a network of extracellular chromatin fibers, histones, antimicrobial peptides, and enzymes, including MPO, α-defensins, cathepsin G, elastase, and lactoferrin, to capture and kill microorganisms [12, 20, 26]. NETosis occurs after neutrophil exposure to bacteria or stimulation with mediators such as interleukin CXCL8/IL-8. This neutrophil stimulation will result in activation of intracellular pro-inflammatory kinases, such as Akt, p38 MAPK, or MEK/ERK, a release of neutrophil elastase, oxidative burst, and actin polymerization [20, 26, 27]. This mechanism will result in microorganism destruction and neutrophil death [25]. NETs also limit the microorganism growth and dissemination; however, excessive formation of NETs in association with the

uncontrolled inflammatory response that occurs in sepsis can result in multiple organ damage to the host [12, 20].

2.3 Resolution of neutrophilic inflammation

Resolution phase is an essential process to interrupt the inflammatory response after the danger signal or when microorganism has been eliminated, preventing the development of chronic inflammation and fibrosis [28]. Resolution of inflammation was previously considered a passive response, associated with clearance of inflammatory stimulus, reduction of pro-inflammatory mediators, and prevention of leukocyte recruitment. Currently, it is known that resolution is an active and tightly controlled process, carried out by specialized pro-resolving mediators (SPM) such as resolvins, lipoxins, maresins, and protectins, which are produced locally from polyunsaturated fatty acids and act orchestrating the end of inflammation, but do not evoke unwanted immunosuppression [29, 30]. For a correct resolution of inflammation, the neutrophil reverse migration, lymphatic drainage, exudation to the external environment, apoptosis of activated neutrophils followed by efferocytosis, and autophagic clearance of intracellular inflammatory signals are necessary [31, 32].

The reduction in neutrophil recruitment is regulated by a class-switch from the production of pro-inflammatory to pro-resolving mediators, resulting in down-regulation of CXCR2 on neutrophils [28]. Pro-resolving lipid mediators also resolve inflammation by promoting neutrophil apoptosis [28, 32]. Apoptotic neutrophils or cell bodies are phagocytosed by professional phagocytes, mainly macrophages, in a process known as efferocytosis. This event is mediated by an interaction between phosphatidylserine expressed on the neutrophil surface and macrophage receptors, such as TIM1 and TIM4 [32, 33]. During resolution, macrophages change their profile decreasing the pro-inflammatory feature and acquiring anti-inflammatory and pro-resolving functions, acting in apoptotic cell clearance, and producing immune regulatory intracellular messengers, including cyclic adenosine monophosphate (cAMP) [28, 34]. Macrophage phagocytosis allows the complete elimination of dead neutrophils and tissue debris of the infected and inflamed area. Generally, this process is followed by macrophage autophagy [35, 36]. Together, all these processes contribute to the resolution of neutrophil inflammation and tissue homeostasis [31].

3. Impaired neutrophil migration in diabetes

The causes of increase in susceptibility to infections in DM are not yet fully known, but one of the possible and well-established explanations is that diabetics present an impairment in defense mechanisms of innate immunity, including neutrophil migration to the site of inflammation, phagocytosis, ROS production, and bactericidal activity [37].

The number of neutrophils in the circulation is also altered in DM. Older studies have shown that in T1DM patients, there is an increase in neutrophil counts compared to healthy individuals [38, 39]. Recent researches described a decrease in circulating neutrophil numbers in T1DM patients in comparison with nondiabetics [40, 41]. Impairment in neutrophil yield and maturation in bone marrow, increase in peripheral neutrophil consumption, and/or tissue sequestration could explain this reduction in blood neutrophil counts observed in T1DM [42]. This divergence between studies can be attributed to differences between ethnic groups and the discovery of the existence of various stages of DM [43]. While in T1DM, the data about circulating neutrophil counts are still controversial, most of the studies

described that in T2DM patients, there is an increase in the number of neutrophils in circulation in comparison to healthy individuals [40, 44]. This neutrophilia was related to elevation in the circulation levels of inflammatory cytokines, including TNF-α, IL-1β, and IL-6, and CRP, a known marker of inflammation [45].

Regarding migration, *in vitro* studies described a reduction in CXCL8/IL-8, platelet-activating factor (PAF), or N-formyl-methionyl-leucyl-phenylalanine (fMLP)-induced chemotaxis of neutrophils from T1DM or T2DM patients compared to cells from healthy subjects [40, 46]. *In situ* evaluation of chemotaxis toward fMLP using T1DM rat neutrophils also revealed a deficiency in migration. This impairment in neutrophil chemotaxis was positively related to DM severity which was characterized by glycaemia values greater than 400 mg/dL [47]. In addition, blood neutrophils of diabetic animals presented a decreased migratory response to CXCL2/MIP-2 *in vitro* and *in vivo* compared to nondiabetic animals. Despite the deficiency in CXCL2/MIP-2 induced-neutrophil migration, there was no difference between the expression of CXCR-2, a CXCL2/MIP-2 receptor, on neutrophils from diabetic animals [48, 49]. Similarity in CXCR-2 mRNA levels was also found among bone marrow neutrophils obtained from NOD mice (a strain that spontaneously develops T1DM), NOR mice (a strain that is resistant to diabetes), and control mice strain. However, CXCR-1 mRNA levels were reduced in neutrophils isolated from NOD mice in comparison to neutrophils from NOR and control mice [50]. Then, it is possible to consider that alterations in CXCR-1 expression and activity may also contribute to the impairment of neutrophil migratory activity in diabetics.

A feature well described in DM is the increase in oxidative stress which may also be related with impairment of neutrophil migration. Oxidative stress can induce glutathionylation (S-thiolation) of several proteins, including L-plastin (LPL) [51] that is expressed exclusively in leucocytes and controls polarization and migration of neutrophils through bundling of β-actin filaments [52]. Neutrophils from diabetic patients and from T2DM mice showed enhanced S-thiolation of LPL in comparison to neutrophils from nondiabetic subjects, which culminate with impaired fMLP-chemotaxis of neutrophils from diabetics. S-thiolation of LPL reduces its interaction with β-actin and this may be another mechanism involved in defective migration of neutrophils in DM [51].

In addition, T1DM rats administered with LPS by intra-tracheal route exhibited a reduction in neutrophil accumulation in the bronchoalveolar fluid (BAL), which occurred in association with a decrease in TNF-α and IL-1β levels, when compared with nondiabetic rats provoked with LPS. However, no difference was observed in relation to the expression of ICAM-1 and E-selectin in lung vascular endothelium and cytokine-induced neutrophil chemoattractant-1 (CINC-1) amount in BAL [53]. A deficiency in neutrophil migration to airways after LPS intra-tracheal injection was also observed in a spontaneous rat model of T2DM, using Goto-Kakizaki (GK) rats. This reduction in neutrophil migration to the airways in GK rats stimulated with LPS occurred despite an increase in the number of neutrophils in the blood. These data showed that there was no failure in the production of these cells by the bone marrow, but impairment in the recruitment mechanisms of these leukocytes to the lungs. Indeed, GK rats exhibited a decrease in IL1-β, IL-6, and TNF-α concentration in BAL and also a reduction in the expression of adhesion molecules, such as LFA-1 and ICAM-2, on neutrophils. All these alterations were associated with a reduction in the TLR4 expression and activation in neutrophils [54].

3.1 Failure in neutrophil migration associated with hyperglycemia

Hyperglycemia can influence various components of the immune response, including activities of inflammatory cells [55]. Incubation of human neutrophils

with supraphysiological levels of glucose decreased both chemotaxis in response to zymosan and phagocytosis/killing of the intracellular bacteria *Staphylococci in vitro*. In addition, high glucose concentrations increased neutrophil adherence *in vitro*, and this also can limit neutrophil locomotion from blood vessels toward infected tissues *in vivo* [56].

It is debated which mechanisms are involved in the benefit of insulin treatment on the immune response of diabetics. While some authors argue that the beneficial effects are dependent on the correction of hyperglycemia by insulin, others believe that the insulin may have direct actions on immune system independently of glycemic control [55]. Indeed, it has been shown that insulin *in vitro* increases human neutrophil chemotaxis induced by fMLP, calcium ionophore, or phorbol-myristyl acetate (PMA) [57, 58]. Besides, insulin presents a chemokinesis effect which required activation of tyrosine kinase and phosphatidylinositol 3-kinase (PI3K), but did not depend on protein kinase C (PKC) stimulation [59, 60]. Interestingly, in a hyperglycemic medium, the chemokinetic action of insulin in neutrophils is blocked through a mechanism that involved activation of PKC [60]. These data suggest that insulin is able to exert direct effects on neutrophils, but the maintenance of glucose levels is also important for actions of this hormone on these leukocytes. In addition to acting on neutrophils, insulin can increase expression of the PECAM-1 in endothelial HUVEC cells and thus enhance transmigration of neutrophils across these cells in response to fMLP *in vitro* [61]. Finally, *in vivo* studies showed that insulin restored neutrophil migration to the lungs in T1DM rats subjected to LPS provocation. This effect of insulin occurred in parallel to a reduction of 50% glycemia; however, the glycemic levels continued to be high in these animals compared to nondiabetic rats [53]. These data suggest that the action of insulin on LPS-induced inflammatory response was not totally dependent on its effect on blood glucose.

It is well known that chronic hyperglycemia upregulates the generation of advanced glycation end-products (AGE). AGEs are produced by a nonenzymatic reaction between reducing sugars, such as glucose, and amino acids of proteins. AGEs can induce cross-link between proteins and also can bind cellular receptors; among them, the best described is the receptor for AGE (RAGE) [62]. AGE accumulation has been associated with the development of several diabetic complications, including retinopathy, nephropathy, and neuropathy [63]. RAGE is expressed on neutrophils and its activation by AGEs, like glycated albumin, induces a transient rise in intracellular free-calcium levels and actin polymerization. Nevertheless, the dimension of increase in calcium levels induced by glycated-albumin is smaller than that induced by fMLP. In addition, glycated-albumin pre-treatment in neutrophils inhibited elevation of intracellular calcium levels promoted by fMLP, causing a defective signal processing and, consequently, a reduction in fMLP-induced-transendothelial migration *in vitro* [64]. Furthermore, glycated collagen also inhibited chemotaxis in response to fMLP, and this effect was associated with the capacity of glycated collagen to increase adhesion strength of neutrophils *in vitro* [62]. In addition, the blockade of AGE formation in diabetic animals restored leucocyte rolling, adhesion, and migration in response to zymosan *in vitro* [65], and also restored neutrophil accumulation toward traumatic skin tissue induced by hot water [66]. Therefore, it is possible that in DM, AGEs promote sustained stimulation of neutrophils which decreases the responses of these cells to chemotactic stimulus.

A positive relation between hyperglycemia and serum NO levels was also described in rats [67], and some studies have reported an increase in serum or plasma NO concentrations in T1DM and T2DM patients [67, 68]. Human neutrophils treated with L-Arginine, a NO precursor, have decreased chemotaxis toward CXCL8/IL-8 *in vitro*, while treatment with NOS inhibitor increased CXCL8/

IL-8-induced-chemotaxis of neutrophils *in vitro* [69]. In addition, a NO donor inhibited human chemotaxis promoted by fMLP *in vitro* and incubation with a guanylate cyclase inhibitor did not interfere with the effect of NO donor. These data suggested that the inhibitory action of NO on neutrophil chemotaxis is independent of cGMP [51]. The NO-induced impairment of neutrophil migration was confirmed using bone marrow neutrophils isolated from $NOS2^{-/-}$ mice stimulated with fMLP *in vitro,* which showed increased chemotaxis in comparison to that isolated from $NOS2^{+/+}$ mice [51]. Furthermore, the pre-treatment with NOS inhibitor prevented impairment of neutrophil recruitment toward peritoneal cavity observed in severe sepsis [70]. Therefore, it is possible to hypothesize that deficiency on migration activity of neutrophils may be associated with increased serum levels of NO in diabetics.

3.2 Failure in neutrophil migration independent of hyperglycemia

DM has altered levels of several molecules in serum that are not directly related to hyperglycemia, some of which can interfere with components of immune response, including neutrophils. Alpha-1-acid glycoprotein (AGP) is one of the main acute-phase proteins in organisms; its synthesis depends mainly on liver, and during an inflammatory response, the concentration in serum increases. [71]. AGP can bind to hormones and interfere with functions of endothelial cells, platelets, and leukocytes, and in fact, it inhibits human neutrophil chemotaxis in response to fMLP *in vitro* [72]. In addition, intravenous administration of AGP in rats prevented migration of neutrophils to peritoneal cavity, reducing rolling and adhesion of these leukocytes on endothelium of mesenteric microcirculation induced by carrageenan [73]. DM patients present high serum levels of AGP [48], so it is feasible that AGP can mediate the impairment of neutrophil locomotion described in DM.

Furthermore, AGP-mediated neutrophil dysfunction was also demonstrated in diabetic animals upon sepsis induction by cecal ligation and perforation (CLP). Neutrophils from septic T1DM mice showed impaired rolling, adhesion, and migration from mesenteric tissue toward the peritoneal cavity, while accumulated in lung tissue. These observations were associated with an altered expression of adhesion molecules (CD62L-CD11b) and a clear reduction in CXCR2 in neutrophils from diabetic animals compared to nondiabetic, after CLP. Accordingly, neutrophils from diabetic mice presented an increased expression of GRK2, a key modulator of CXCR2 receptor desensitization, upon sepsis induction compared to control septic mice. AGP administration in septic nondiabetic mice impaired neutrophil migration to peritoneal cavity, augmenting GRK2 expression, and reducing CXCR2, which reproduced the diabetic condition. On the other hand, insulin treatment reduced GKR2 and augmented CXCR2 on neutrophils obtained from diabetic mice, while decreased AGP serum concentrations. Thus, AGP increased production is involved in neutrophil impaired migration to infection during diabetes, possibly by enhancing GRK2 expression and/or augmenting NO production in these cells [48]. Notably, CXCR2 downregulation in diabetic animals seems to depend on the presence of comorbidity since several studies showed no difference in CXCR2 expression between normal and diabetic mice.

Histamine for a long time was considered as a pro-inflammatory mediator whose main role is played in allergic inflammation. However, some evidence has shown that histamine can modulate other immunological events. Neutrophils express both histamine receptors, H1 and H2 [74] and activation of H2 inhibited human neutrophil chemotaxis *in vitro* [75]. Furthermore, blood neutrophils obtained after systemic or inhalatory administration of histamine in normal volunteers showed a reduction in chemotactic response to zymosan *in vitro* [75].

After septic stimuli, T1DM mice exhibited mast cell accumulation in the peritoneal cavity and higher plasma levels of histamine than nondiabetic mice. In addition, the augmented activation of H2 receptor promoted an increase in intracellular expression of GRK2 and cAMP levels in diabetic septic mice neutrophils, favoring CXCR2 desensitization [74].

Resistin is a cysteine-rich protein that belongs to the resistin-like molecule (RELM) family that, in humans, is released mainly by macrophages but can be also produced by adipose tissue [76]. Resistin impairs glucose tolerance and insulin action and therefore has been related to obesity-induced insulin resistance and T2DM [77]. Beyond metabolic effects, resistin can act directly in immune cells, including neutrophils. Resistin decreased fMLP-induced neutrophil chemotaxis *in vitro* through inhibition of PI3K pathway activation. Resistin also decreased oxidative burst in neutrophils after stimulation with PMA and *Escherichia coli* [78]. Since resistin directly affects neutrophil function and T2DM patients present higher serum levels of this hormone [79], it can be suggested that resistin is also involved with the deficiency of neutrophil responses in DM independently of hyperglycemia.

4. Neutrophil response to bacterial infections in diabetes

It is now generally accepted that high glucose concentrations impaired several functions of neutrophils beyond their migratory capacity, including phagocytosis and bacterial killing. Hyperglycemia hinders neutrophil activity by inducing higher concentrations of intracellular calcium and thereby reducing ATP levels, which in turn leads to reduced phagocytic ability of PMN cells. Nevertheless, under glycemic control, diabetic patients restored intracellular calcium levels and increased cellular ATP content in neutrophils, which consequently improved phagocytosis. In addition, hyperglycemia was shown to affect other immune and hemostatic responses during experimental human endotoxemia. Healthy patients submitted to high blood glucose levels presented a reduction of *E. coli* endotoxin-induced neutrophil degranulation and exaggerated coagulation. A reversal of these effects was observed when glucose was controlled with insulin therapy [55].

Neutrophils from diabetic patients showed increased production of inflammatory cytokines [80] and ROS without any stimulation, although neutrophil oxidative responses to certain pathogens appear to be predominantly suppressed in diabetes [64, 81, 82]. Furthermore, hyperglycemia led to decreased mRNA synthesis of different pro-inflammatory cytokines in neutrophils after LPS stimulation, compared with the euglycemic state [55]. In addition, T1DM mice showed a hyperglycemia-induced pre-activation of NOX, resulting in a significantly higher release of superoxide. Sustained hyperglycemic condition may, therefore, induce oxidative damage and the onset of diabetic complications, particularly at sites with neutrophilia [83, 84].

In DM, neutrophils increased basal ROS generation in a close-relationship to sustained hyperglycemia and the generation of AGEs [64]. On the other hand, decreased pathogen-stimulated ROS production is thought to be related to impaired glucose metabolism by the pentose-phosphate pathway, which produces NADPH that is a requirement for optimal superoxide generation by NOX [6]. Off noted, phagocytosis and NETosis were shown to depend on oxidative burst in neutrophils. Nevertheless, the relevance of the ROS production misbalance noted in neutrophils obtained from diabetics is not clear, since not all the diabetic patients with diminished ROS production presented recurrent bacterial infections [82].

4.1 Neutrophil dysfunction and sepsis

According to The Third International Consensus Definitions for Sepsis and Septic Shock, "sepsis is a life-threatening organ dysfunction secondary to a deregulated host response to an infection" [85]. During septic processes, serum inflammatory marker concentration increases in patients although innate immune response appears to be impaired. Particularly, defective neutrophil recruitment to the sites of infection was reported in animal models of sepsis [86, 87]. Clinical studies reported that the incidence of sepsis is increased in diabetic patients [5]. Accordingly, DM is associated with high severity of sepsis, likely due to compromised immune responses, such as adhesion, chemotaxis, phagocytosis, and bacterial killing by immune cells [88]. Few studies reproducing septic inflammations in the context of diabetes had been performed in animals. T1DM or T2DM animals have worse prognosis upon CLP-induced sepsis even though plasma levels of systemic pro-inflammatory cytokines, like TNF-α, CXCL2/MIP-2, and IL-6, are increased in diabetic animals compared with control animals upon sepsis induction. This situation is normally attributed to neutrophil dampened activity [48, 74, 89, 90].

On the other hand, results obtained upon CLP-induced sepsis in a mouse model of T2DM showed an increased neutrophil infiltration in the peritoneal cavity in diabetic animals compared to nondiabetic upon sepsis induction. Nevertheless, neutrophils from diabetic animals presented reduced phagocytic activity and ROS generation after sepsis induction compared to control animals in the same condition. This impairment in neutrophil functions was related to a downregulation of TAM family of receptor tyrosine kinases. The lack of an appropriated innate immune response results in deficient bacterial elimination and augmented death rate in diabetic septic animals compared to control septic animals [90].

Similar results were observed in T1DM NOD mice intraperitoneally challenged with *Staphylococcus aureus*. The augmented neutrophil presence in the peritoneum of diabetic mice was associated with a sustained TNF-α production which prevents apoptosis in these leukocytes. Despite it, diabetic mice were more susceptible to *S. aureus* infection possibly associated to neutrophil decreased oxidative burst [91]. In addition, administration of GM-CSF, a cytokine known to activate PMNs, in diabetic animals submitted to CLP was able to restore neutrophilic activity and prevent the increased mortality of the animals. These effects of GM-CSF were associated with an increased neutrophil phagocytic activity and ROS generation, which controlled bacterial proliferation in the peritoneal cavity [90].

4.2 Neutrophil counts and function in tuberculosis

Several clinical and epidemiological studies have identified DM as a risk factor for the development of pulmonary tuberculosis (TB). T2DM and TB are two of the most common co-morbid conditions in many parts of the world. In addition, DM has been associated with a greater severity of TB disease among the infected population and worse outcome in response to treatment [92]. TB-DM co-morbidity is characterized by heightened levels of bacterial loads in sputum accompanied by increased neutrophil counts in peripheral blood [93]. Neutrophilic inflammation is a central feature of TB-DM, accompanied by elevated levels of biomarkers associated with macrovascular complications.

Whole blood gene expression and plasma analyses showed that several inflammatory markers, including IL-1β, CXCL8/IL-8, IL-17A, CCL3/MIP-1, TNF-α, and VEGF, associated with neutrophilic activity and absolute neutrophil counts were highly increased in TB-DM patients compared to TB or DM patients. A higher

frequency of participants with high molecular degree of perturbation (MDP) was also noted in the TB-DM subgroup. MDP is a parameter that reflects the "distance to health," based on molecular expression scores in comparison with a healthy population. Consequently, they suggest that epigenetic reprogramming and neutrophilic inflammation determine the pattern of plasma cytokines and growth factors in TB-DM co-morbidity, highlighting neutrophilic inflammation as the main cause of susceptibility to develop TB by DM patients. Thereby, neutrophilic inflammation may be a useful target to improve TB treatment outcomes in this growing TB-DM patient population [94]. In addition, increased levels of three of the most prominent antimicrobial peptides, cathelicidin (LL37), human β-defensin 2 (HBD2), and human neutrophil peptide 1–3 (HNP1–3), principally secreted by neutrophils were found in individuals with TB-DM and TB compared with individuals with latent TB or non-TB-infected [7]. However, neutrophils isolated from T2DM patients showed a decreased capacity to phagocyte *Mycobacterium tuberculosis* or other *M. tuberculosis*-related molecules compared to control donors [95].

There are few studies using animal models of TB-DM co-morbidity focusing on neutrophil activity. Even though it is frequently observed that diabetic animals have an increased accumulation of neutrophils within lung tissue upon infection [96, 97], T2DM animals were more vulnerable to *M. tuberculosis* showing a decreased survival rate compared to control infected animals. Also, diabetic animals recruited more neutrophils and express higher levels of CXCL8/IL-8 in lung tissue than control infected animals [96]. In T1DM mice, infection with *M. tuberculosis* led to a decreased survival rate associated with an impaired bacterial control compared to nondiabetic infected mice. This high mortality of T1DM mice was accompanied by a lung neutrophilia and IL-6 overexpression. The treatment of TB-DM animals with neutralizing anti-IL-6 antibodies reduced neutrophil numbers and controlled bacterial burden in lung tissue, improving the survival rate [97].

4.3 Neutrophil counts and function in pneumonia

DM increases the risk of patients acquiring a pneumococcal disease, and besides, adversely affects the severity and outcome of this infectious illness [98]. In fact, DM has been shown to be a significant predictor of hospitalization in patients with community-acquired pneumonia (CAP) and also, a risk factor for the development of bacteremia in patients with pneumococcal pneumonia. T2DM is frequently associated with increased mortality rate from pneumonia, which appeared to be highest in the early phase of infection where neutrophilic inflammation is more important [99]. *Streptococcus pneumoniae* is the most frequent cause of CAP irrespective of age and comorbidity. The phagocytosis of *S. pneumonia* was reduced in neutrophils recovered from eight patients with poorly controlled DM, but this defect improved with insulin treatment. Notably, control neutrophils incubated with serum taken from patients with diabetes also demonstrated a defective phagocytosis, suggesting that the inefficient bacterial opsonization might be occurring in the diabetic patient's serum [100].

Once phagocytosed, bacterial killing by neutrophils depends on the generation of ROS. *Ex-vivo* studies using neutrophils from T2DM patients have demonstrated a defect in the intracellular killing of *S. pneumoniae* together with a reduced O_2 production, reduced MPO activity, and H_2O_2 generation. In addition, chronic hyperglycemia induces inactivation of the source of leukocyte ROS, which results in high prevalence of oral abscesses, progressive interstitial inflammation, and fibrosis in the lung of mice in the absence of an inflammatory stimulus, leading to cachexia and death. These data suggested that ROS generated by NOX is not only

beneficial but also essential to oral and respiratory health in DM, particularly when the glycemia is uncontrolled [84].

Klebsiella pneumoniae is emerging as an agent which induces severe CAP. DM is associated with increased susceptibility to *K. pneumoniae* and poor prognosis of infection. Streptozotocin-induced diabetic mice are more susceptible to oropharyngeal infection with *K. pneumoniae*, presenting increased mortality rate and less bacterial control. There was no difference in the antibacterial activity of neutrophils recovered from nondiabetic and diabetic mice, indicating that the higher bacterial burden in hyperglycemia is probably related to a defective inflammatory signaling and late neutrophil recruitment. In fact, *K. pneumoniae* LPS induced a fewer recruitment of neutrophils to the alveolar airspace in diabetic mice compared to nondiabetic mice. Also, diabetic mice reduced neutrophil accumulation and early production of CXCL1/KC, CXCL2/MIP-2, IL-1β, and TNF-α in lung. Additionally, TLR2 and TIRAP, a Toll receptor and adaptor protein, were under-expressed in lungs of diabetic mice following *K. pneumoniae*-LPS provocation compared to nondiabetic infected mice, while no differences were observed for TLR-4 expression. These observations suggested that the failure in neutrophil recruitment and activation during the first hours of infection with *K. pneumoniae* is a most probable mechanism for high susceptibility to pneumonia in diabetics [101].

Commonly, *K. pneumoniae* infections cause pneumonia or urinary tract infections; however, during the past two decades, a distinct invasive syndrome that causes liver abscesses (KLA) has been increasingly reported in Asia, and this syndrome is emerging as a global disease [102]. DM is the most common comorbidity in KLA patients. It was shown that DNA and MPO levels were elevated in the plasma of KLA patients compared to uninfected individuals, indicating neutrophil activation independently of diabetic status. In addition, clinical *K. pneumoniae* isolates induced phagocytosis, bacterial killing, and NETosis comparable by neutrophils from diabetic and nondiabetic patients. Notably, the IL-12-IFNγ axis and its downstream chemokines CXCL9/MIG, CXCL10/IP-10, and CCL5/RANTES were produced at lower levels by peripheral blood mononuclear cells (PBMCs) from T2DM compared to PBMCs from healthy individuals in response to *K. pneumoniae* strains. These observations indicated that although T2DM does not overtly impact on neutrophil intra- and extra-cellular killing of *K. pneumoniae*, it may influence cytokine/chemokine production and intracellular killing by PBMCs.

4.4 Neutrophil function in bacterial infection-induced deficiency in wound healing

Delayed wound healing is one of the main diabetes-related morbidities. Neutrophil inefficient activity has been pointed as one of the major responsible factors for the impaired wound healing in diabetes, since neutrophil depletion accelerates wound resolution independently of the presence of an infection [103]. Furthermore, increased serum elastase levels, a marker of neutrophilic inflammation, predicted delayed wound healing in diabetic patients. In addition, proteomic analyses of the diabetic patient's foot ulcers (DFUs) showed elevated expression of NET components, including elastase, histones, neutrophil gelatinase-associated lipocalin, and proteinase-3, in nonhealing wounds as also in circulating blood. Consistently, neutrophils isolated from blood of DFU patients showed an increase of spontaneous NETosis but an impaired inducible NETosis [104]. Isolated neutrophils from T2DM patients presented higher NETosis rate than neutrophils from healthy patients in the absence of stimulation, which was associated with elevated intracellular calcium levels. Hyperglycemia is strongly related to these effects since

neutrophils derived from healthy patients produced more NETosis after pre-incubation with high glucose medium *in vitro*. In addition, large amounts of NETs were found in excisional skin sterile-wounds of streptozotocin-induced diabetic mice. Although the role of NETosis in wounds remains elusive, it has been confirmed that the inhibition of NETosis or degrading NETs improved sterile-wound healing and reduced NET-driven chronic inflammation in diabetic mice [105].

Gram-positive bacteria cause more than half of cases of diabetes-related wound infections. Especially, *Staphylococcus aureus* is a major pathogen in these infections, and its presence correlates with significant delays in wound healing [106]. Wounds induced by *S. aureus* in T2DM mice showed delayed resolution compared to non-diabetic mice. Seven days after infection, the lesions of diabetic mice presented exacerbated NETosis, while nondiabetic mice had their inflammatory process already resolved and healing was nearly completed. Although neutrophils derived from both T1DM and T2DM patients produced greater amounts of NETs compared to healthy volunteer's neutrophils, the induction of NETosis cannot be explained just by hyperglycemia. In fact, some works showed that high glucose exposure reduced LPS- or IL-6-induced NETosis *in vitro* [105, 107, 108].

Some mechanisms that could also explain the increased neutrophil NETosis in diabetic patients are the elevated levels of zonulin and the overexpression of PAD4. Zonulin is a protein that modulates the permeability of tight junctions between cells of the digestive tract. Interestingly, the increased zonulin levels in diabetic patients revealed a strong correlation with neutrophil elastase concentration and NET formation in a glucose-independent way [109]. PAD4 is a calcium-dependent enzyme that mediates NETosis. In diabetes, PAD4 was upregulated in neutrophils from individuals with diabetes and was responsible for the unbalanced NET production by these leukocytes.

In T2DM mice, although neutrophil infiltration toward the lesion was augmented, the impaired wound healing upon surgical site infection with *S. aureus* was related to a significant reduction in phagocytic activity and bacterial killing by neutrophils. Consistently, *S. aureus*-induced phagolysosome maturation was abolished and PMA-stimulated superoxide production was decreased in neutrophils recovered from diabetic mice. In addition, treatment of neutrophils with insulin significantly restored neutrophil killing activities and increased phagocytosis. Interestingly, phagosome maturation and superoxide production restoring were dependent on glycemic control and not on a direct effect of insulin. These abnormalities in neutrophil functions were closely related with impaired wound healing in DM, once treatment with insulin restored normal wound healing in diabetic mice [110].

5. Conclusion

The increased susceptibility to bacterial infections is one of the hallmarks of diabetic complications. Under comorbidity with diabetes, the high prevalence and severity of bacterial infections, as observed in tuberculosis, pneumonia, and sepsis, is closely associated to impairment in neutrophil functions, such as migration, phagocytosis, ROS production, and NET formation. The alterations in neutrophil functions noted in diabetics occur both dependently and independently of the glycemic control. Among the mechanisms that lead to neutrophil dysfunction in diabetic conditions not related to glycemic control, some targets have been highlighted, such as AGP, H2 receptor, IL-6, PAD4, resistin, and zonulin. These potential targets should be better explored in clinical studies concerning their putative benefits to diabetic patients.

Acknowledgements

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Instituto Nacional de Ciência e Tecnologia-NIM, and Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) for financial support.

Conflict of interest

The authors declare no conflict of interest, including with the financial support agencies.

Author details

Daniella Insuela[1], Diego Coutinho[1], Marco Martins[1], Maximiliano Ferrero[1] and Vinicius Carvalho[1,2*]

1 Laboratory of Inflammation, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil

2 National Institute of Science and Technology on Neuroimmunomodulation (INCT-NIM), Brazil

*Address all correspondence to: viniciusfrias@hotmail.com

References

[1] Al-Awar A, Kupai K, Veszelka M, Szűcs G, Attieh Z, Murlasits Z, et al. Experimental diabetes mellitus in different animal models. Journal Diabetes Research. 2016;**2016**:1-12. DOI: 10.1155/2016/9051426

[2] Saberzadeh-Ardestani B, Karamzadeh R, Basiri M, Hajizadeh-Saffar E, Farhadi A, Shapiro AMJ, et al. Type 1 diabetes mellitus: Cellular and molecular pathophysiology at a glance. Cell Journal. 2018;**20**(3):294-301. DOI: 10.22074/cellj.2018.5513

[3] Yi B, Huang G, Zhou Z. Different role of zinc transporter 8 between type 1 diabetes mellitus and type 2 diabetes mellitus. Journal of Diabetes Investigation. 2016;**7**(4):459-465. DOI: 10.1111/jdi.12441

[4] Pedron S, Emmert-Fees K, Laxy M, Schwettmann L. The impact of diabetes on labour market participation: A systematic review of results and methods. BMC Public Health. 2019;**19**(1):19-25. DOI: 10.1186/s12889-018-6324-6

[5] Toniolo A, Cassani G, Puggioni A, Rossi A, Colombo A, Onodera T, et al. The diabetes pandemic and associated infections: Suggestions for clinical microbiology. Reviews in Medical Microbiology: A Journal of the Pathological Society of Great Britain and Ireland. 2019;**30**(1):1-17. DOI: 10.1097/mrm.0000000000000155

[6] Hodgson K, Morris J, Bridson T, Govan B, Rush C, Ketheesan N. Immunological mechanisms contributing to the double burden of diabetes and intracellular bacterial infections. Immunology. 2015;**144**(2):171-185. DOI: 10.1111/imm.12394

[7] Kumar Nathella P, Babu S. Influence of diabetes mellitus on immunity to human tuberculosis. Immunology. 2017;**152**(1):13-24. DOI: 10.1111/imm.12762

[8] Chiang CY, Bai KJ, Lin HH, Chien ST, Lee JJ, Enarson DA, et al. The influence of diabetes, glycemic control, and diabetes-related comorbidities on pulmonary tuberculosis. PLoS One. 2015;**10**(3):1-15. DOI: 10.1371/journal.pone.0121698

[9] Grieshaber-Bouyer R, Nigrovic PA. Neutrophil heterogeneity as therapeutic opportunity in immune-mediated disease. Frontiers in Immunology. 2019;**10**:1-13. DOI: 10.3389/fimmu.2019.00346

[10] Alvarenga DM, Mattos MS, Araujo AM, Antunes MM, Menezes GB. Neutrophil biology within hepatic environment. Cell and Tissue Research. 2018;**371**(3):589-598. DOI: 10.1007/s00441-017-2722-9

[11] Lerman YV, Kim M. Neutrophil migration under normal and sepsis conditions. Cardiovascular & Hematological Disorders Drug Targets. 2015;**15**(1):19-28. DOI: 10.2174/1871529X15666150108113236

[12] Shen XF, Cao K, Jiang JP, Guan WX, Du JF. Neutrophil dysregulation during sepsis: An overview and update. Journal of Cellular and Molecular Medicine. 2017;**21**(9):1687-1697. DOI: 10.1111/jcmm.13112

[13] Liu L, Sun B. Neutrophil pyroptosis: New perspectives on sepsis. Cellular and Molecular Life Sciences. 2019;**76**:1-10. DOI: 10.1007/s00018-019-03060-1

[14] de Oliveira S, Rosowski EE, Huttenlocher A. Neutrophil migration in infection and wound repair: Going forward in reverse. Nature Reviews. Immunology. 2016;**16**(6):378-391. DOI: 10.1038/nri.2016.49

[15] Rosales C. Neutrophil: A cell with many roles in inflammation or several cell types? Frontiers in Physiology. 2018;**9**:1-17. DOI: 10.3389/fphys.2018.00113

[16] Maas SL, Soehnlein O, Viola JR. Organ-specific mechanisms of transendothelial neutrophil migration in the lung, liver, kidney, and aorta. Frontiers in Immunology. 2018;**9**(2739):1-24. DOI: 10.3389/fimmu.2018.02739

[17] Caster DJ, Powell DW, Miralda I, Ward RA, McLeish KR. Re-examining neutrophil participation in GN. Journal of the American Society of Nephrology. 2017;**28**(8):2275-2289. DOI: 10.1681/ASN.2016121271

[18] Mortaz E, Alipoor SD, Adcock IM, Mumby S, Koenderman L. Update on neutrophil function in severe inflammation. Frontiers in Immunology. 2018;**9**:1-14. DOI: 10.3389/fimmu.2018.02171

[19] Rios-Santos F, Alves-Filho JC, Souto FO, Spiller F, Freitas A, Lotufo CM, et al. Down-regulation of CXCR2 on neutrophils in severe sepsis is mediated by inducible nitric oxide synthase-derived nitric oxide. American Journal of Respiratory and Critical Care Medicine. 2007;**175**(5):490-497. DOI: 10.1164/rccm.200601-103OC

[20] Li RHL, Tablin F. A comparative review of neutrophil extracellular traps in sepsis. Frontiers in Veterinary Science. 2018;**5**:1-11. DOI: 10.3389/fvets.2018.00291

[21] van Kessel KPM, Bestebroer J, van Strijp JAG. Neutrophil-mediated phagocytosis of *Staphylococcus aureus*. Frontiers in Immunology. 2014;**5**:1-12. DOI: 10.3389/fimmu.2014.00467

[22] Phan QT, Sipka T, Gonzalez C, Levraud J-P, Lutfalla G, Nguyen-Chi M. Neutrophils use superoxide to control bacterial infection at a distance. PLoS Pathogens. 2018;**14**(7):1-29. DOI: 10.1371/journal.ppat.1007157

[23] Witter AR, Okunnu BM, Berg RE. The essential role of neutrophils during infection with the intracellular bacterial pathogen *Listeria monocytogenes*. Journal of Immunology. 2016;**197**(5):1557-1565. DOI: 10.4049/jimmunol.1600599

[24] Taheri N, Fahlgren A, Fällman M. Yersinia pseudotuberculosis blocks neutrophil degranulation. Infection and Immunity. 2016;**84**(12):3369-3378. DOI: 10.1128/iai.00760-16

[25] van Dam LS, Rabelink TJ, van Kooten C, Teng YKO. Clinical implications of excessive neutrophil extracellular trap formation in renal autoimmune diseases. Kidney International Reports. 2018;**4**(2):196-211. DOI: 10.1016/j.ekir.2018.11.005

[26] Gray RD, Hardisty G, Regan KH, Smith M, Robb CT, Duffin R, et al. Delayed neutrophil apoptosis enhances NET formation in cystic fibrosis. Thorax. 2018;**73**(2):134-144. DOI: 10.1136/thoraxjnl-2017-210134

[27] Behnen M, Möller S, Brozek A, Klinger M, Laskay T. Extracellular acidification inhibits the ROS-dependent formation of neutrophil extracellular traps. Frontiers in Immunology. 2017;**8**:1-22. DOI: 10.3389/fimmu.2017.00184

[28] Schett G, Neurath MF. Resolution of chronic inflammatory disease: Universal and tissue-specific concepts. Nature Communications. 2018;**9**(1):1-8. DOI: 10.1038/s41467-018-05800-6

[29] Philippe R, Urbach V. Specialized pro-resolving lipid mediators in cystic fibrosis. International Journal of Molecular Sciences. 2018;**19**(10):1-11. DOI: 10.3390/ijms19102865

[30] Galvão I, Queiroz-Junior CM, de Oliveira VLS, Pinho V, Hirsch E, Teixeira MM. The inhibition of phosphoinositide-3 kinases induce resolution of inflammation in a gout model. Frontiers in Pharmacology. 2019;**9**:1-10. DOI: 10.3389/fphar.2018.01505

[31] Rahtes A, Geng S, Lee C, Li L. Cellular and molecular mechanisms involved in the resolution of innate leukocyte inflammation. Journal of Leukocyte Biology. 2018;**104**(3):535-541. DOI: 10.1002/jlb.3ma0218-070r

[32] Cartwright JA, Lucas CD, Rossi AG. Inflammation resolution and the induction of granulocyte apoptosis by cyclin-dependent kinase inhibitor drugs. Frontiers in Pharmacology. 2019;**10**:1-18. DOI: 10.3389/fphar.2019.00055

[33] Rossaint J, Margraf A, Zarbock A. Role of platelets in leukocyte recruitment and resolution of inflammation. Frontiers in Immunology. 2018;**9**:1-13. DOI: 10.3389/fimmu.2018.02712

[34] Bang S, Xie Y-K, Zhang Z-J, Wang Z, Xu Z-Z, Ji R-R. GPR37 regulates macrophage phagocytosis and resolution of inflammatory pain. Journal of Clinical Investigation. 2018;**128**(8):3568-3582. DOI: 10.1172/jci99888

[35] Skendros P, Mitroulis I, Ritis K. Autophagy in neutrophils: From granulopoiesis to neutrophil extracellular traps. Frontiers in Cell and Development Biology. 2018;**6**:1-12. DOI: 10.3389/fcell.2018.00109

[36] Prieto P, Rosales-Mendoza CE, Terrón V, Toledano V, Cuadrado A, López-Collazo E, et al. Activation of autophagy in macrophages by pro-resolving lipid mediators. Autophagy. 2015;**11**(10):1729-1744. DOI: 10.1080/15548627.2015.1078958

[37] Knapp S. Diabetes and infection: Is there a link?—A mini-review. Gerontology. 2013;**59**(2):99-104. DOI: 10.1159/000345107

[38] Collier A, Jackson M, Bell D, Patrick AW, Matthews DM, Young RJ, et al. Neutrophil activation detected by increased neutrophil elastase activity in type 1 (insulin-dependent) diabetes mellitus. Diabetes Research. 1989;**10**(3):135-138

[39] Jackson MH, Collier A, Nicoll JJ, Muir AL, Dawes J, Clarke BF, et al. Neutrophil count and activation in vascular disease. Scottish Medical Journal. 1992;**37**(2):41-43. DOI: 10.1177/003693309203700205

[40] Huang J, Xiao Y, Zheng P, Zhou W, Wang Y, Huang G, et al. Distinct neutrophil counts and functions in newly diagnosed type 1 diabetes, latent autoimmune diabetes in adults, and type 2 diabetes. Diabetes/Metabolism Research and Reviews. 2019;**35**(1):1-10. DOI: 10.1002/dmrr.3064

[41] Valle A, Giamporcaro GM, Scavini M, Stabilini A, Grogan P, Bianconi E, et al. Reduction of circulating neutrophils precedes and accompanies type 1 diabetes. Diabetes. 2013;**62**(6):2072-2077. DOI: 10.2337/db12-1345

[42] Battaglia M. Neutrophils and type 1 autoimmune diabetes. Current Opinion in Hematology. 2014;**21**(1):8-15. DOI: 10.1097/MOH.0000000000000008

[43] Huang J, Xiao Y, Xu A, Zhou Z. Neutrophils in type 1 diabetes. Journal of Diabetes Investigation. 2016;7(5):652-663. DOI: 10.1111/jdi.12469

[44] Zhang H, Yang Z, Zhang W, Niu Y, Li X, Qin L, et al. White blood cell subtypes and risk of type 2 diabetes. Journal of Diabetes and its

Complications. 2017;**31**(1):31-37. DOI: 10.1016/j.jdiacomp.2016.10.029

[45] Yang M, Liu J, Xu J, Sun T, Sheng L, Chen Z, et al. Elevated systemic neutrophil count is associated with diabetic macroalbuminuria among elderly Chinese. International Journal of Endocrinology. 2015;**2015**:1-6. DOI: 10.1155/2015/348757

[46] Trevelin SC, Carlos D, Beretta M, da Silva JS, Cunha FQ. Diabetes mellitus and sepsis: A challenging association. Shock. 2017;**47**(3):276-287. DOI: 10.1097/SHK.0000000000000778

[47] Ozsoy N, Bostanci H, Ayvali C. The investigation of the ultrastructural neutrophil changes in alloxan-induced diabetes in rats: Response to a chemotactic challenge. Cell Biochemistry and Function. 2004;**22**(2):81-87. DOI: 10.1002/cbf.1059

[48] Spiller F, Carlos D, Souto FO, de Freitas A, Soares FS, Vieira SM, et al. alpha1-Acid glycoprotein decreases neutrophil migration and increases susceptibility to sepsis in diabetic mice. Diabetes. 2012;**61**(6):1584-1591. DOI: 10.2337/db11-0825

[49] Veenstra M, Ransohoff RM. Chemokine receptor CXCR2: Physiology regulator and neuroinflammation controller? Journal of Neuroimmunology. 2012;**246**(1-2):1-9. DOI: 10.1016/j.jneuroim.2012.02.016

[50] Haurogne K, Pavlovic M, Rogniaux H, Bach JM, Lieubeau B. Type 1 diabetes prone NOD mice have diminished Cxcr1 mRNA expression in polymorphonuclear neutrophils and CD4+ T lymphocytes. PLoS One. 2015;**10**(7):1-16. DOI: 10.1371/journal.pone.0134365

[51] Dubey M, Singh AK, Awasthi D, Nagarkoti S, Kumar S, Ali W, et al. L-Plastin S-glutathionylation promotes reduced binding to beta-actin and affects neutrophil functions. Free Radical Biology & Medicine. 2015;**86**:1-15. DOI: 10.1016/j.freeradbiomed.2015.04.008

[52] Arpin M, Friederich E, Algrain M, Vernel F, Louvard D. Functional differences between L- and T-plastin isoforms. The Journal of Cell Biology. 1994;**127**(6 Pt 2):1995-2008

[53] de Oliveira Martins J, Meyer-Pflug AR, Alba-Loureiro TC, Melbostad H, Costa da Cruz JW, Coimbra R, et al. Modulation of lipopolysaccharide-induced acute lung inflammation: Role of insulin. Shock. 2006;**25**(3):260-266. DOI: 10.1097/01.shk.0000194042.18699.b4

[54] Kuwabara WMT, Yokota CNF, Curi R, Alba-Loureiro TC. Obesity and type 2 diabetes mellitus induce lipopolysaccharide tolerance in rat neutrophils. Scientific Reports. 2018;**8**(1):1-13. DOI: 10.1038/s41598-018-35809-2

[55] Schuetz P, Castro P, Shapiro NI. Diabetes and sepsis: Preclinical findings and clinical relevance. Diabetes Care. 2011;**34**(3):771-778. DOI: 10.2337/dc10-1185

[56] Wierusz-Wysocka B, Wysocki H, Wykretowicz A, Klimas R. The influence of increasing glucose concentrations on selected functions of polymorphonuclear neutrophils. Acta Diabetologica Latina. 1988;**25**(4):283-288

[57] Walrand S, Guillet C, Boirie Y, Vasson MP. Insulin differentially regulates monocyte and polymorphonuclear neutrophil functions in healthy young and elderly humans. The Journal of Clinical Endocrinology and Metabolism. 2006;**91**(7):2738-2748. DOI: 10.1210/jc.2005-1619

[58] Cavalot F, Anfossi G, Russo I, Mularoni E, Massucco P, Burzacca S, et al. Insulin, at physiological concentrations, enhances the polymorphonuclear leukocyte chemotactic properties. Hormone and Metabolic Research. 1992;**24**(5):225-228. DOI: 10.1055/s-2007-1003298

[59] Oldenborg PA, Sehlin J. Insulin-stimulated chemokinesis in normal human neutrophils is dependent on D-glucose concentration and sensitive to inhibitors of tyrosine kinase and phosphatidylinositol 3-kinase. Journal of Leukocyte Biology. 1998;**63**(2):203-208

[60] Oldenborg PA, Sehlin J. Hyperglycemia in vitro attenuates insulin-stimulated chemokinesis in normal human neutrophils. Role of protein kinase C activation. Journal of Leukocyte Biology. 1999;**65**(5):635-640

[61] Okouchi M, Okayama N, Imai S, Omi H, Shimizu M, Fukutomi T, et al. High insulin enhances neutrophil transendothelial migration through increasing surface expression of platelet endothelial cell adhesion molecule-1 via activation of mitogen activated protein kinase. Diabetologia. 2002;**45**(10):1449-1456. DOI: 10.1007/s00125-002-0902-x

[62] Toure F, Zahm JM, Garnotel R, Lambert E, Bonnet N, Schmidt AM, et al. Receptor for advanced glycation end-products (RAGE) modulates neutrophil adhesion and migration on glycoxidated extracellular matrix. The Biochemical Journal. 2008;**416**(2):255-261. DOI: 10.1042/BJ20080054

[63] Singh VP, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. The Korean Journal of Physiology & Pharmacology. 2014;**18**(1):1-14. DOI: 10.4196/kjpp.2014.18.1.1

[64] Collison KS, Parhar RS, Saleh SS, Meyer BF, Kwaasi AA, Hammami MM, et al. RAGE-mediated neutrophil dysfunction is evoked by advanced glycation end products (AGEs). Journal of Leukocyte Biology. 2002;**71**(3):433-444. DOI: 10.1189/jlb.71.3.433

[65] Sannomiya P, Oliveira MA, Fortes ZB. Aminoguanidine and the prevention of leukocyte dysfunction in diabetes mellitus: A direct vital microscopic study. British Journal of Pharmacology. 1997;**122**(5):894-898. DOI: 10.1038/sj.bjp.0701448

[66] Tian M, Qing C, Niu Y, Dong J, Cao X, Song F, et al. Effect of aminoguanidine intervention on neutrophils in diabetes inflammatory cells wound healing. Experimental and Clinical Endocrinology & Diabetes. 2013;**121**(10):635-642. DOI: 10.1055/s-0033-1351331

[67] Adela R, Nethi SK, Bagul PK, Barui AK, Mattapally S, Kuncha M, et al. Hyperglycaemia enhances nitric oxide production in diabetes: A study from south Indian patients. PLoS One. 2015;**10**(4):1-17. DOI: 10.1371/journal.pone.0125270

[68] Assmann TS, Brondani LA, Boucas AP, Rheinheimer J, de Souza BM, Canani LH, et al. Nitric oxide levels in patients with diabetes mellitus: A systematic review and meta-analysis. Nitric Oxide. 2016;**61**:1-9. DOI: 10.1016/j.niox.2016.09.009

[69] Nolan S, Dixon R, Norman K, Hellewell P, Ridger V. Nitric oxide regulates neutrophil migration through microparticle formation. The American Journal of Pathology. 2008;**172**(1):265-273. DOI: 10.2353/ajpath.2008.070069

[70] Benjamim CF, Ferreira SH, Cunha FQ. Role of nitric oxide in the failure of neutrophil migration in sepsis. The Journal of Infectious Diseases. 2000;**182**(1):214-223. DOI: 10.1086/315682

[71] Hochepied T, Berger FG, Baumann H, Libert C. Alpha(1)-acid glycoprotein: An acute phase protein with inflammatory and immunomodulating properties. Cytokine & Growth Factor Reviews. 2003;**14**(1):25-34. DOI: 10.1016/S1359-6101(02)00054-0

[72] Laine E, Couderc R, Roch-Arveiller M, Vasson MP, Giroud JP, Raichvarg D. Modulation of human polymorphonuclear neutrophil functions by alpha 1-acid glycoprotein. Inflammation. 1990;**14**(1):1-9

[73] Mestriner FL, Spiller F, Laure HJ, Souto FO, Tavares-Murta BM, Rosa JC, et al. Acute-phase protein alpha-1-acid glycoprotein mediates neutrophil migration failure in sepsis by a nitric oxide-dependent mechanism. Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**(49):19595-19600. DOI: 10.1073/pnas.0709681104

[74] Carlos D, Spiller F, Souto FO, Trevelin SC, Borges VF, de Freitas A, et al. Histamine h2 receptor signaling in the pathogenesis of sepsis: Studies in a murine diabetes model. Journal of Immunology. 2013;**191**(3):1373-1382. DOI: 10.4049/jimmunol.1202907

[75] Bury TB, Corhay JL, Radermecker MF. Histamine-induced inhibition of neutrophil chemotaxis and T-lymphocyte proliferation in man. Allergy. 1992;**47**(6):624-629

[76] Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochemical and Biophysical Research Communications. 2003;**300**(2):472-476. DOI: 10.1016/S0006-291X(02)02841-3

[77] Kusminski CM, McTernan PG, Kumar S. Role of resistin in obesity, insulin resistance and type II diabetes. Clinical Science. 2005;**109**(3):243-256. DOI: 10.1042/CS20050078

[78] Cohen G, Ilic D, Raupachova J, Horl WH. Resistin inhibits essential functions of polymorphonuclear leukocytes. Journal of Immunology. 2008;**181**(6):3761-3768. DOI: 10.4049/jimmunol.181.6.3761

[79] Hodges SK, Teague AM, Dasari PS, Short KR. Effect of obesity and type 2 diabetes, and glucose ingestion on circulating spexin concentration in adolescents. Pediatric Diabetes. 2018;**19**(2):212-216. DOI: 10.1111/pedi.12549

[80] Hatanaka E, Monteagudo PT, Marrocos MS, Campa A. Neutrophils and monocytes as potentially important sources of proinflammatory cytokines in diabetes. Clinical and Experimental Immunology. 2006;**146**(3):443-447. DOI: 10.1111/j.1365-2249.2006.03229.x

[81] Chao WC, Yen CL, Wu YH, Chen SY, Hsieh CY, Chang TC, et al. Increased resistin may suppress reactive oxygen species production and inflammasome activation in type 2 diabetic patients with pulmonary tuberculosis infection. Microbes and Infection. 2015;**17**(3):195-204. DOI: 10.1016/j.micinf.2014.11.009

[82] Wykretowicz A, Wierusz-Wysocka B, Wysocki J, Szczepanik A, Wysocki H. Impairment of the oxygen-dependent microbicidal mechanisms of polymorphonuclear neutrophils in patients with type 2 diabetes is not associated with increased susceptibility to infection. Diabetes Research and Clinical Practice. 1993;**19**(3):195-201. DOI: 10.1016/0168-8227(93)90114-K

[83] Gyurko R, Siqueira CC, Caldon N, Gao L, Kantarci A, Van Dyke TE. Chronic hyperglycemia predisposes to exaggerated inflammatory response and leukocyte dysfunction in Akita mice. Journal of Immunology.

2006;**177**(10):7250-7256. DOI: 10.4049/jimmunol.177.10.7250

[84] Zamakhchari MF, Sima C, Sama K, Fine N, Glogauer M, Van Dyke TE, et al. Lack of p47(phox) in akita diabetic mice is associated with interstitial pneumonia, fibrosis, and oral inflammation. The American Journal of Pathology. 2016;**186**(3):659-670. DOI: 10.1016/j.ajpath.2015.10.026

[85] Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). Journal of the American Medical Association. 2016;**315**(8):801-810. DOI: 10.1001/jama.2016.0287

[86] Crosara-Alberto DP, Darini AL, Inoue RY, Silva JS, Ferreira SH, Cunha FQ. Involvement of NO in the failure of neutrophil migration in sepsis induced by *Staphylococcus aureus*. British Journal of Pharmacology. 2002;**136**(5):645-658. DOI: 10.1038/sj.bjp.0704734

[87] Arraes SM, Freitas MS, da Silva SV, de Paula Neto HA, Alves-Filho JC, Auxiliadora Martins M, et al. Impaired neutrophil chemotaxis in sepsis associates with GRK expression and inhibition of actin assembly and tyrosine phosphorylation. Blood. 2006;**108**(9):2906-2913. DOI: 10.1182/blood-2006-05-024638

[88] Shah BR, Hux JE. Quantifying the risk of infectious diseases for people with diabetes. Diabetes Care. 2003;**26**(2):510-513. DOI: 10.2337/diacare.26.2.510

[89] Filgueiras LR Jr, Martins JO, Serezani CH, Capelozzi VL, Montes MB, Jancar S. Sepsis-induced acute lung injury (ALI) is milder in diabetic rats and correlates with impaired NFkB activation. PLoS One. 2012;**7**(9):1-9. DOI: 10.1371/journal.pone.0044987

[90] Frydrych LM, Bian G, Fattahi F, Morris SB, O'Rourke RW, Lumeng CN, et al. GM-CSF Administration improves defects in innate immunity and sepsis survival in obese diabetic mice. Journal of Immunology. 2019;**202**(3):931-942. DOI: 10.4049/jimmunol.1800713

[91] Hanses F, Park S, Rich J, Lee JC. Reduced neutrophil apoptosis in diabetic mice during staphylococcal infection leads to prolonged Tnfalpha production and reduced neutrophil clearance. PLoS One. 2011;**6**(8):1-10. DOI: 10.1371/journal.pone.0023633

[92] Jeon CY, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: A systematic review of 13 observational studies. PLoS Medicine. 2008;**5**(7):1091-1101. DOI: 10.1371/journal.pmed.0050152

[93] Andrade BB, Kumar NP, Sridhar R, Banurekha VV, Jawahar MS, Nutman TB, et al. Heightened plasma levels of heme oxygenase-1 and tissue inhibitor of metalloproteinase-4 as well as elevated peripheral neutrophil counts are associated with TB-diabetes comorbidity. Chest. 2014;**145**(6):1244-1254. DOI: 10.1378/chest.13-1799

[94] Prada-Medina CA, Fukutani KF, Pavan Kumar N, Gil-Santana L, Babu S, Lichtenstein F, et al. Systems immunology of diabetes-tuberculosis comorbidity reveals signatures of disease complications. Scientific Reports. 2017;**7**(1):1-16. DOI: 10.1038/s41598-017-01767-4

[95] Raposo-Garcia S, Guerra-Laso JM, Garcia-Garcia S, Juan-Garcia J, Lopez-Fidalgo E, Diez-Tascon C, et al. Immunological response to mycobacterium tuberculosis infection in blood from type 2 diabetes patients. Immunology Letters. 2017;**186**:41-45. DOI: 10.1016/j.imlet.2017.03.017

[96] Podell BK, Ackart DF, Obregon-Henao A, Eck SP, Henao-Tamayo M,

Richardson M, et al. Increased severity of tuberculosis in Guinea pigs with type 2 diabetes: A model of diabetes-tuberculosis comorbidity. The American Journal of Pathology. 2014;**184**(4):1104-1118. DOI: 10.1016/j.ajpath.2013.12.015

[97] Cheekatla SS, Tripathi D, Venkatasubramanian S, Nathella PK, Paidipally P, Ishibashi M, et al. NK-CD11c+ cell crosstalk in diabetes enhances IL-6-mediated inflammation during mycobacterium tuberculosis infection. PLoS Pathogens. 2016;**12**(10):1-24. DOI: 10.1371/journal.ppat.1005972

[98] Joshi N, Caputo GM, Weitekamp MR, Karchmer AW. Infections in patients with diabetes mellitus. The New England Journal of Medicine. 1999;**341**(25):1906-1912. DOI: 10.1056/NEJM199912163412507

[99] Torres A, Blasi F, Dartois N, Akova M. Which individuals are at increased risk of pneumococcal disease and why? Impact of COPD, asthma, smoking, diabetes, and/or chronic heart disease on community-acquired pneumonia and invasive pneumococcal disease. Thorax. 2015;**70**(10):984-989. DOI: 10.1136/thoraxjnl-2015-206780

[100] Koh GC, Peacock SJ, van der Poll T, Wiersinga WJ. The impact of diabetes on the pathogenesis of sepsis. European Journal of Clinical Microbiology & Infectious Diseases. 2012;**31**(4):379-388. DOI: 10.1007/s10096-011-1337-4

[101] Martinez N, Ketheesan N, Martens GW, West K, Lien E, Kornfeld H. Defects in early cell recruitment contribute to the increased susceptibility to respiratory Klebsiella pneumoniae infection in diabetic mice. Microbes and Infection. 2016;**18**(10):649-655. DOI: 10.1016/j.micinf.2016.05.007

[102] Siu LK, Yeh KM, Lin JC, Fung CP, Chang FY. Klebsiella pneumoniae liver abscess: A new invasive syndrome. The Lancet Infectious Diseases. 2012;**12**(11):881-887. DOI: 10.1016/S1473-3099(12)70205-0

[103] Dovi JV, He LK, DiPietro LA. Accelerated wound closure in neutrophil-depleted mice. Journal of Leukocyte Biology. 2003;**73**(4):448-455. DOI: 10.1189/jlb.0802406

[104] Fadini GP, Menegazzo L, Rigato M, Scattolini V, Poncina N, Bruttocao A, et al. NETosis delays diabetic wound healing in mice and humans. Diabetes. 2016;**65**(4):1061-1071. DOI: 10.2337/db15-0863

[105] Wong SL, Demers M, Martinod K, Gallant M, Wang Y, Goldfine AB, et al. Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. Nature Medicine. 2015;**21**(7):815-819. DOI: 10.1038/nm.3887

[106] Yates C, May K, Hale T, Allard B, Rowlings N, Freeman A, et al. Wound chronicity, inpatient care, and chronic kidney disease predispose to MRSA infection in diabetic foot ulcers. Diabetes Care. 2009;**32**(10):1907-1909. DOI: 10.2337/dc09-0295

[107] Joshi MB, Lad A, Bharath Prasad AS, Balakrishnan A, Ramachandra L, Satyamoorthy K. High glucose modulates IL-6 mediated immune homeostasis through impeding neutrophil extracellular trap formation. FEBS Letters. 2013;**587**(14):2241-2246. DOI: 10.1016/j.febslet.2013.05.053

[108] Riyapa D, Buddhisa S, Korbsrisate S, Cuccui J, Wren BW, Stevens MP, et al. Neutrophil extracellular traps exhibit antibacterial activity against burkholderia pseudomallei and are influenced by bacterial and host factors. Infection and Immunity. 2012;**80**(11):3921-3929. DOI: 10.1128/IAI.00806-12

[109] You Q, He DM, Shu GF, Cao B, Xia YQ, Xing Y, et al. Increased formation of neutrophil extracellular traps is associated with gut leakage in patients with type 1 but not type 2 diabetes. Journal of Diabetes. 2018:1-9. DOI: 10.1111/1753-0407.12892

[110] Yano H, Kinoshita M, Fujino K, Nakashima M, Yamamoto Y, Miyazaki H, et al. Insulin treatment directly restores neutrophil phagocytosis and bactericidal activity in diabetic mice and thereby improves surgical site *Staphylococcus aureus* infection. Infection and Immunity. 2012;**80**(12):4409-4416. DOI: 10.1128/IAI.00787-12