

## Diabetic Animal Models with Infectious Diseases: Focus on the Dysfunction of Immune System

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### Abstract

Diabetes Mellitus (DM) is a metabolic disease that can lead to a variety of complications, such as neuropathy, retinopathy, nephropathy, and cardiovascular disease. Furthermore, pathogen infection accompanied by considerable morbidity and mortality is common among diabetic patients. Increased susceptibility to pathogen infection results from impaired immune responses, such as lower cytokine production and reduced function or migration of immune cells. However, existing clinical data remains controversial because multiple diabetes-related factors such as obesity, hyperglycemia, hyperinsulinemia, and other comorbidities also increase the risk of infection. In recent decades, several animal models have been used to investigate the role played by immune dysfunction in increasing susceptibility to pathogens and related diseases in diabetes. This review focuses on studies that used diabetic animal models to study infectious diseases and summarizes potential mechanisms underlying dysfunction of the immune system in diabetes patients.

### Introduction

Worldwide, the number of people suffering from diabetes mellitus increased from 153 million to approximately 347 million between 1980 and 2008 [1]. Estimates predict that the global prevalence of diabetes will reach 366-440 million by 2030 [2]. Diabetic patients are more susceptible to infection by certain microorganisms, such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Mycobacterium tuberculosis (Mtb)* [3], and these infections often require hospitalization. One recent study calculated the cause-specific risk of death in 820,900 people according to diabetes status or fasting glucose level [4]. Those authors found that the hazard ratios of mortality from infectious diseases (excluding pneumonia) ranked second only to renal disease as a cause of noncancerous and nonvascular death in diabetes patients.

Previous clinical investigations and experimental studies using diabetic rodent models have shown that the dysfunctional immune system of diabetes patients leads to increased susceptibility to pathogens and infections of greater severity. Those research identified various impairments to innate immunity in DM patients, including reduced production of inflammatory cytokines and loss of function (chemotaxis, phagocytosis, superoxide production, or killing activity) in neutrophils, macrophages or natural killer cells [5-7]. Defects in adaptive immunity have also been reported in diabetic patients, including: abnormal delayed-type hypersensitivity, attenuation of lymphocyte proliferation, and decreased serum antibody levels [8-11]. However, diabetes is a metabolic disease that is often accompanied by abnormalities, which impair the resistance to pathogenic infections, such as chronic inflammation and associated alterations to the lipid profile, neuropathy, and chronic vascular or renal diseases [12]. Therefore, the mechanism underlying the role played by the immune system in high susceptibility to pathogen infection among diabetes patients are still far from established.

For decades, animal models have been used to investigate the pathophysiology of Type 1 and Type 2 diabetes (T1D and T2D) for the development of therapeutic treatment strategies. More recently, diabetic animal models have been applied to investigate how immune dysfunction contributes to infection among diabetes patients. Most

previous studies have focused on Tuberculosis (TB) and sepsis; however, reports of diabetes-related infections such as pneumonia, Urinary Tract Infections (UTI), Surgical Site Infections (SSI), or infections on the feet have provided intriguing data, which have indicated various mechanisms that could explain the defective immune responses. In this review, we classify diabetic animal models according to various infectious diseases and describe the microorganisms and diabetic models used in diabetes studies. We also discuss various mechanisms associated with immune dysfunction, in order to identify cellular and/or molecular properties that could be promising therapeutic targets for future research and treatment of diabetes.

### Diabetic Animal Models of Various Infectious Diseases

#### Diabetic animal models of respiratory infections

**Tuberculosis pneumonia:** *Mtb*, a bacterial species in the Mycobacteriaceae family, is the causative agent in most cases of TB. Approximately 10% of *Mtb*-infected patients develop an active disease as a result of inherited and acquired risk factors, including human immunodeficiency virus, malnutrition, alcohol use, smoking, and indoor air pollution. Recently, diabetes has been deemed a risk for the growing number of TB worldwide, and the World Health Organization estimates that the current number of diabetes patients (347 million) will double by 2030 [13]. Therefore, the combination of TB and diabetes represents a worldwide health threat, and identifying potential mechanisms by which DM increases TB incidence is crucial to deal with this.

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In the 1980s, Saiki et al. [14] demonstrated that streptozotocin (STZ)-induced diabetic ICR mice suffered an increase in mortality when injected intravenously with live *Mtb* (Schacht strain) (90% mortality among diabetic mice compared with 10% among normal mice). Their study revealed that T-cell function and phagocytic activity of macrophages were depressed in STZ-induced diabetic mice; however, B-cell function and intracellular killing of macrophages remained normal. Neither ketoacidosis nor ketone bodies were detected in diabetic mice, suggesting that the defective immune function was associated with hyperglycemia.

In 2004, Sugawara et al. [15] infected GK/Jcl rats, a T2D animal model, with *Mtb* (Kuronno strain) via the airborne route to mimic the natural pathway of TB infection. They found that GK/Jcl diabetic rats had more colony-forming unit cells in lung and spleen tissues than did non-diabetic Wistar rats [15]. Furthermore, the production of TNF- $\alpha$ , IL-12, and Nitric Oxide (NO) was lower in the alveolar macrophages obtained from diabetic rats, compared with control rats [15]. Those findings suggest that low expression of inflammatory cytokines and NO coupled with incomplete macrophage activation allows granulomas to grow larger than normal. Similar results were observed in STZ-induced diabetic ICR mice intravenously infected with *Mtb* (H37Rv strain). The authors of that study suggested that reduced expression of IL-12, a critical inducer of IFN- $\gamma$ , was the primary reason for the inhibition of iNOS expression, and thus also explained the increased susceptibility of diabetic mice to *Mtb* infection. In another study, insulin therapy was shown to lower the blood glucose level of diabetic mice and appeared to improve bacterial clearance, implying that hyperglycemia (but not STZ itself) was involved in impairing host immune responses against *Mtb* [16]. Collectively, the aforementioned studies demonstrated that defects of innate immune responses, such as lower production of Th1-related cytokines and NO as well as reduced macrophage function, limit the ability of diabetic hosts to defend against *Mtb* infection.

In 2007, Martens et al. [17] demonstrated the participation of adaptive immune response to *Mtb* infection (Erdman strain) in STZ-induced diabetic C57BL/6 mice. They observed that chronically diabetic mice are more susceptible to *Mtb* infection than are acute diabetic mice. Although the levels of IFN- $\gamma$ , IL-1 $\beta$  and TNF- $\alpha$  were higher in lung lysates of chronically diabetic mice with *Mtb* infection, the production of IFN- $\gamma$  in lung leukocytes re-stimulated with Con A, anti-CD3, or *Mtb* antigens decreased *ex vivo*. Those results suggest that impairments to adaptive immune function in chronic hyperglycemia

patients are associated with increased susceptibility to *Mtb* infection in the lung. Strengthening that hypothesis, *Ins2<sup>Akita</sup>* (Akita) mice were found to spontaneously become hypoinsulinemic and hyperglycemic at 3 to 4 weeks of age. The same research group recently demonstrated that increased TB susceptibility in chronic STZ-induced diabetic mice is the result of a delay in the innate immune response to *Mtb*-infected alveolar macrophages [18]. This in turn drains the lymph nodes, delays the delivery of antigen-bearing antigen-presenting cells to the lung, and delays priming of the adaptive immune response. These immune-related activities are all necessary for the restriction of *Mtb* replication.

Multiple studies have shown that susceptibility to *Mtb* infection increases in diabetes patients and that dysfunction in both innate and adaptive immune responses may be the cause (Table 1). Nonetheless, the underlying mechanism behind immune dysfunction in diabetes remains unclear. Recently, Podell et al. [19] demonstrated that non-diabetic hyperglycemia exacerbates the severity of *Mtb* infection in guinea pigs. They also suggested that Advanced Glycation End Products (AGEs) are the link between hyperglycemia and immune dysfunction in diabetes patients. However, additional evidence is required to confirm this hypothesis.

**Other bacterial pneumonia:** Diabetic patients also face increased risk of mortality due to pneumonia (hazard ratio, 1.67) [4]. In the 1960s, Drachman et al. [20] demonstrated that the death rate attributable to type 25 pneumococci infections is higher in alloxan-induced diabetic rats than in non-diabetic rats, due to an overgrowth of bacteria in pulmonary lesions. The inability of diabetic rats to control bacterial growth is due primarily to a reduction in the phagocytic capabilities of leukocytes. Data from *in vitro* experiments indicates that hyperglycemia-induced hyperosmolarity is the depressive factor associated with inferior phagocytic defense [20]. To mimic the natural path of pneumococcus infection, Hebert et al. [21] exposed alloxan-induced diabetic mice to an aerosol containing 10<sup>8</sup> type III *Streptococcus pneumoniae*/ml, and monitored the ability of the pulmonary barrier to defend against infection. In that study, the survival rate of diabetic mice was lower than that of non-diabetic mice. However, insulin treatment significantly increased the survival rate of diabetic mice, suggesting that insulin therapy could help to control pulmonary infection in diabetes patients. Other earlier work also showed that phagocytic functions including chemotaxis, phagocytosis, and the adhesion of leukocytes to the endothelium, are impaired in diabetes patients [22-24]. Authors of one study therefore suggested that impaired macrophage function may account for the high susceptibility to pulmonary infection. Another

Animal models	Characterization of infections in diabetic mice	Infection route	Cellular or molecular observation in diabetic mice with TB	Reference
STZ (200 mg/kg)-induced diabetes in ICR mice (T1D) with <i>Mtb</i> (Schacht strain) infection	Reduced survival time and decreased survival incidence after <i>Mtb</i> infection	Intravenous injection	Depression of T cell function and macrophage phagocytosis	[14]
GK/ Jcl rat (T2D) with <i>Mtb</i> (Kuronno strain) infection	Larger granulomas, higher colony-forming units count in lung and spleen tissues after infection for 3 weeks	Airborne route infection	Less TNF- $\alpha$ , IL-12 secretion and NO production in alveolar macrophages stimulated with <i>Mtb</i> .	[15]
STZ (150 mg/kg)-induced diabetes in ICR mice (T1D) with <i>Mtb</i> (H37Rv strain) infection	Increased bacterial loads in lung, liver and spleen	Intravenous injection	Less expression level of Th1-related cytokines and iNOS in lung, liver and spleen	[16]
STZ (150 mg/kg)-induced diabetes in C57BL/6 mice (T1D) with <i>Mtb</i> (Erdman strain) infection Akita mice (T1D) with <i>Mtb</i> (Erdman strain) infection	Higher bacterial lung burden and increased extent of lung inflammation	Airborne route infection	Increase in IFN- $\gamma$ and inflammatory cytokines in pooled lung lysates. Reduced antigen-specific and -nonspecific IFN- $\gamma$ production in lung T cells.	[17, 18]
Diet-induced hyperglycemia in non-diabetic guinea pig (non-DM) with <i>Mtb</i> (H37Rv strain) infection	Higher lung and extrapulmonary <i>Mtb</i> lesion burden in sucrose-induced hyperglycemia guinea pig	Airborne route infection	Hyperglycemia-mediated AGE accumulation in lung	[19]

**Table 1:** Comparison of the characteristics and immune dysfunction in diabetic mouse models of TB pneumonia.

recent study demonstrated that diabetes increases the risk of *Chlamydia pneumoniae* spreading from the lung to the peripheral blood in NOD mice [25]. In last two decades, several studies demonstrated that *C. pneumoniae* is associated with the development atherosclerosis [26,27], suggesting that increased dissemination of *C. pneumoniae* may accelerate the formation of atherosclerotic plaques in diabetes. A relationship between diabetes and pneumonia-related death has been established; however, current animal models are inadequate to identify underlying mechanisms. Thus, the development of new animal models is urgently required to elucidate these effects and facilitate the development of effective clinical treatments.

### Diabetic animal models of urinary tract infections

The risk of UTIs, including those of the bladder (cystitis) and kidney (pyelonephritis), is increased in diabetes patients, and many infected diabetic patients commonly suffer from UTI-related complications [28]. Although immune system defects have been suggested to contribute to diabetic UTIs, few studies have established this link directly. Rosen et al. [29] used a STZ-induced diabetic mouse model to investigate the susceptibility of diabetic animals to uropathogenic *Escherichia coli*, the most common etiological agent of UTIs. Those researchers found that the burden of infections by *E. coli* (UT189), *K. pneumoniae* (TOP52), and *Enterococcus faecalis* (0852) in the bladder and kidney are more severe in diabetic mice than in non-diabetic mice. Moreover, compared with *E. coli*, the prevalence of *K. pneumoniae* was higher in the bladder. Those results were consistent with the epidemiological findings in diabetic patients. In addition, the *E. coli* titers in bladders of Toll-like receptor 4 (TLR-4) mutant mice (C3H/HeJ strain) were less than that of normal mice (C3H/HeN strain), indicating that TLR-4-regulated cells are associated with increased susceptibility to UTIs [29]. Nevertheless, increased susceptibility to UTI infection was still observed in diabetic C3H/HeJ mice, suggesting that other TLR-4-independent factors are also involved in diabetes-related UTI.

### Diabetic animal models of foot infection

Foot infections following skin ulceration commonly require hospitalization and are the most common cause of lower-extremity amputations among diabetic patients [30]. Although diabetic foot infections are polymicrobial, *Staphylococcus aureus* (*S. aureus*) is frequently implicated [31]. To investigate the pathogenesis of foot infections in diabetes, one research group developed diabetic foot infection animal models by inoculating the hind paw of NOD mice (T1D) [32] and *db/db* mice (T2D) with *S. aureus* [33]. In those studies, diabetic mice exhibited more severe foot infection than did non-diabetic mice. The control of glycemia was helpful to improve *S. aureus* clearance and leukocytes bacterial activity, suggesting that hyperglycemia is a risk factor to increase the susceptibility to foot infections in diabetes. Moreover, in the early stages of bacterial infection, the expression of chemokines such as KC and MIP-2 was shown to decrease, and fewer polymorphonuclear leukocytes reached the infected hind paw of diabetic NOD mice. The authors hypothesized that delayed innate immune responses allowed invading bacteria to gain a foothold in the tissue of diabetic mice. However, delayed immune responses were not observed in diabetic *db/db* mice, suggesting that the mechanism underlying immune dysfunction in the two types of diabetes are not necessarily the same.

### Diabetic animal models of surgical site infection

The frequency of surgical site infection is higher in diabetes

patients than in healthy individuals [34-36]. SSI can lead to the failure of medical implants such as prosthetic joints, implantable cardioverter defibrillators, urinary catheters, orthopedic implants, breast implants, and glucose sensors [37]. To investigate the effect of insulin treatment on SSI and neutrophil function, one research group recently developed T2D models of *S. aureus* SSI in *db/db* mice and mice that were fed a high-fat-diet (HFD-fed mice) [38]. Those authors demonstrated that SSIs in diabetic *db/db* mice and hyperglycemic HFD-fed mice were more severe than in non-diabetic and euglycemic mice, respectively. However, insulin treatment decreased the severity of SSI substantially and also improved the ability of neutrophils to kill *S. aureus*. Moreover, *ex vivo* insulin treatment largely restored neutrophil function and ameliorated SSI, suggesting that insulin may activate neutrophil function directly [38]. More recently, Lovati et al. [39] used a NOD mice model to investigate the susceptibility of T1D animals to orthopedic implant-related *S. aureus* infection. Those authors observed more severe osteomyelitic change surrounding the implant in diabetic NOD mice than in control mice. Although the immune cells responsible for increased susceptibility to implant-related infection were not identified, this study still provided a useful animal model for related studies.

### Diabetic animal models of sepsis

A variety of diabetic animal model has been developed to investigate the pathophysiology of sepsis, due to the fact that sepsis accounts for the highest risk ratio among all infectious diseases requiring that diabetic patients undergo hospitalization [40]. In the early 1980s, Kitahara et al. [41] demonstrated the association between diabetes and sepsis by inoculating STZ-induced diabetic mice with *Pseudomonas aeruginosa* (*P. aeruginosa*) [41]. No difference in mortality was observed between diabetic and control mice; however, reduced resistance to bacterial growth was observed in the liver, spleen, kidney and peripheral blood of diabetic mice. The protective activities of blood serum from vaccinated diabetic mice were significantly lower than that from vaccinated non-diabetic mice, suggesting a link between resistance to *P. aeruginosa* infection and antibody- or cytokine-mediated immunity. The diabetic mice that received serum from normal vaccinated mice also showed a decrease in resistance to bacterial growth, suggesting the existence of abnormalities in immune cells against *P. aeruginosa* infection. In addition, reduced resistance to sepsis induced by Group B streptococci (GBS) was consistently observed in diabetic mice. The higher mortality rate associated with GBS-induced sepsis in diabetic mice was likely due to dysregulation of the cytokine network and prolonged local inflammatory responses [42].

In a study of Akita mice using a spontaneous T1D model, the mortality rate associated with Cecal Ligation and Puncture (CLP) induced-sepsis was exacerbated [43]. Authors of those results demonstrate that increased mortality among diabetic mice is not dependent on the pre-lethal activation of cytokines but rather coincides with a widespread reduction in the inflammatory response [43]. In addition, Jacob et al. [44] used a Goto-Kakizaki (GK) rat T2D animal model, to investigate sepsis-induced inflammation in animals that underwent CLP [44]. Those authors observed higher levels of plasma IL-6 and IL-10 in diabetic GK rats than in non-diabetic Wistar-Kyoto rats at 20 hours post-CLP, suggesting a relationship between T2D and sepsis-induced inflammation.

In addition to the dysregulation of cytokine production, defective neutrophil function has also been associated with the severity of sepsis in both diabetic patients and diabetic animal models. Spiller et al. [45] demonstrated that alloxan-induced diabetic mice are highly susceptible to polymicrobial-induced sepsis due to reductions in rolling, adhesion,

and migration of neutrophils. They also observed G-protein-coupled receptor kinase-2 (GRK2)-mediated downregulation of CXCR-2 in blood neutrophils and higher expression of  $\alpha$ 1-acid glycoprotein (AGP) in the serum of diabetic mice, compared with control mice [45]. Moreover, administration of AGP eliminated the protective effects of insulin in diabetic mice, suggesting that a diabetes–insulin–sepsis–AGP axis is involved in regulating the migration of neutrophils to infection sites [45]. The same research team recently demonstrated that mast cells also participate in the increased susceptibility of diabetic mice to septic peritonitis. Specifically, the histamine released by mast cells appears to impair neutrophil migration through histamine H<sub>2</sub> receptor signaling [46].

Collectively, studies involving various diabetic animal models support the idea that diabetic hosts are more susceptible to microbial-induced bacteremia or sepsis (Table 2). Insulin treatment and appropriate glycemic control can increase the resistance to sepsis in diabetic individuals and animals. Additionally, impaired neutrophil migration resulting from mast cell degranulation may partially responsible for the increase in the severity of sepsis among diabetes patients. Other impaired neutrophil functions, such as chemotaxis and reduced phagocytic capacity, have also been described in diabetic hosts. In the future, a more comprehensive suite of immune cell functions should be investigated in order to extend our knowledge of how immune dysfunction affects microorganism-induced sepsis in diabetes patients.

### Diabetic animal models of melioidosis

Melioidosis is an emerging tropical infectious disease with high

incidence and mortality rate in northern Australia and south-east Asia. Diabetic patients with preexisting or newly diagnosed T2D have a high incidence of melioidosis accompanied by pneumonia and septic shock [47-51]. Hodgson et al. [52] used *db/db* mice to investigate the dysfunction of immune responses underlying high susceptibility of melioidosis in T2D [52]. They observed that mice with T2D are more susceptible to *Burkholderia pseudomallei* (*B. pseudomallei*)-induced mortality accompanied by increased expression of inflammatory cytokines and hypoglycemia which is the response often observed in bacterial sepsis. The decrease of phagocytic and antimicrobial activities in macrophages from diabetic mice may contribute to the failure of controlling bacterial dissemination and disease progression. Recently, the same group generated a polygenic diet-induced diabetes model that more closely resembles the clinical criteria of T2D to investigate DM and melioidosis comorbidity [53]. Similarly, their results indicate that the impaired immune pathways contribute to the increased susceptibility to bacterial infection in diabetic mice. In addition to T2D, Williams et al. [54] used STZ-induced T1D model to demonstrate that uncontrolled hyperglycemia impairs Bone Marrow-Derived Dendritic Cells (BMDCs) and macrophage to internalize and kill *B. pseudomallei* and generate cytokine profiles not favor Th1-type immune response [54]. Collectively, these studies demonstrate how diabetes status and hyperglycemia affect the ability of BMDCs and macrophages to clear *B. pseudomallei*. Although the mechanisms underlying immune dysfunction are still unclear, these studies establish suitable animal models to investigate diabetes and melioidosis comorbidity.

Animal models	Characterization of symptoms in diabetic mice	Infection route or sepsis induction	Cellular or molecular observation in diabetic mice with sepsis	Reference
STZ (140 mg/kg)-induced diabetes in CF1 mice (T1D) with <i>P. aeruginosa</i> (NC-5 strain) infection	No increase in acute death rate. Increase in the number of bacteria in kidney, liver, spleen and peripheral blood.	Intravenous injection	(i) Less effect of pass protection in immune serum from diabetic vaccinated mice in normal recipients, implying the impairment of antibody- or cytokine-mediated immunity. (ii) Less effect of protection in immune serum from normal vaccinated mice into diabetic recipients, implying the impairment of immune cells-mediated immunity	[41]
STZ (50 mg/kg for 5 consecutive days)-induced diabetes in CD-1 mice (T1D) with type VI GBS (NCTC 1/82 strain) infection	Lower 50% lethal dose (>1 log <sub>10</sub> in CFU). Increase in bacterial growth in blood and kidney.	Intravenous injection	Increase in IL-6, IL-1 $\alpha$ , IL-10, TNF- $\gamma$ and decrease in IFN- $\gamma$ levels in the serum of diabetic mice with sepsis.	[42]
Goto Kakizaki (GK) rats (T2D) with CLP	Not validated	CLP-induced sepsis	Increase in plasma lactate, IL-6 and IL-10 20 hr after CLP in diabetic mice with sepsis	[44]
AKITA mice (T1D) with CLP	Increase in the sepsis-induced mortality	CLP-induced sepsis	(i) Greater number of circulating neutrophils at first 24hr after CLP in diabetic mice. (ii) Decrease in pro-inflammatory and anti-inflammatory cytokines in diabetic mice compared with died mice.	[43]
Alloxan (50 mg/kg)-induced diabetes in BALB/C mice or in C57BL/6 mice (T1D) with CLP	Increase in polymicrobial sepsis-induced mortality and the number of bacterial load in peritoneal cavity lavage or blood	CLP-induced sepsis	(i) Reduction in rolling, adhesion and migration of leukocytes to the sites of infection. (ii) Downregulation of CXCR2 and upregulation of GRK2 in the neutrophils. (iii) Impairment in intracellular adhesion molecule-1 expression on endothelium (vi) Increase in AGP serum protein levels.	[45]
Alloxan (50 mg/kg)-induced diabetes in BALB/c mice with CLP NOD mice (T1D) with CLP	(i) Acceleration of polymicrobial sepsis-induced mortality and increase of bacterial load in peritoneal cavity lavage or blood (ii) C48/80 or histamine receptor antagonist treatment or monocytes deficiency reversed the acceleration of sepsis-induced mortality and bacterial loading in peritoneal cavity lavage or blood	CLP-induced sepsis	(i) Reduce in neutrophil migration to the sites of infection. (ii) Downregulation of CXCR2 and upregulation of GRK2 in the neutrophils (iii) C48/80 or histamine receptor antagonist treatment or monocytes deficiency increased CXCR2 expression in the neutrophils (iii) C48/80 treatment prevented GRK2 induction in the neutrophils	[46]

**Table 2:** Comparison of the characteristics and immune dysfunction in diabetic mouse models of sepsis.

## Conclusions

The development of animal models for specific clinical diseases has been and will remain critical to our understanding of the pathophysiology of diseases and the development of novel therapeutic strategies to treat them. This review included a variety of diabetic animal infection models used to investigate immune dysfunction and diabetes-related infection. To summarize, STZ- and alloxan-induced T1D models have been widely used to investigate the susceptibility to and pathophysiology of various infectious diseases. The advantage of these models is that the development of hyperglycemia is uniform and controllable in different murine backgrounds. Moreover, STZ or alloxan-induced T1D can be used in knockout or mutant mice to identify mechanisms which may be responsible for increased susceptibility to infection. T2D animal models have also been used to investigate how immune dysfunction affects diabetes-related infections. The *db/db* mice with genetic mutation in leptin signaling are frequently used in different studies. However, the effect of leptin signaling deficiency on hormonal imbalance and immune system may complex the study of immune complications related to T2D. Hodgson et al. [53] therefore developed a polygenic model of diet-induced T2D that more closely reflect dietary intake in developed nations to investigate melioidosis [53]. In addition to the influence of leptin signaling mutation, researchers using T2D model observed the hyperproductivity of superoxide by neutrophils in HFD mice but not in *db/db* mice [38]. Patients suffering from T2D have elevated levels of proinflammatory cytokines, which can increase superoxide production in neutrophils [55]. This suggests that HFD mice may be the most appropriate animal model for the investigation of pathogen infection in T2D patients [38]. Finally, infections of greater severity have been observed in diabetic *db/db* mice as well as hyperglycemic HFD-fed mice, future investigators should take into account the differential superoxide production by neutrophils between these two models.

Many studies using diabetic animal models have focused on TB or microbial-induced sepsis on TB or microbial-induced sepsis. Those findings are in line with other animals models of diabetes-related infection, which show that diabetes and hyperglycemia can impair both innate and adaptive immune responses and thereby reduce resistance to pathogen infection and other associated diseases. Insulin treatment and proper glycemic control can help to reduce the amount of bacteria within lesions and ameliorate the severity of diseases. However, future researchers investigating immune response in diabetes subjects should consider the duration of diabetes because cytokine production differs between the acute and chronic stages of the disease. Although hyperglycemia has been shown to increase the dysfunction of phagocytes such as neutrophils or macrophages, and thus reduce resistance to different infections, the molecular mechanisms underlying these impairments remain largely unknown. Studies provided possible models of immune dysfunction in diabetes-related infection [45,46]. In addition, the immune system of diabetic subjects may be impaired by primary defects such as: (1) hyperglycemia-induced abnormalities in osmotic processes, (2) the accumulation of AGEs (3) high levels of AGP in serum, and (4) impairment of glucose metabolism in neutrophils. Interestingly, the effects of these defects may be in opposition to obesity-associated increase of adipose resident macrophages inflammation. In obese state, the increase accumulation of macrophage correlates with increased cytokines and chemokines. Leptin is a adipokine important in modulating the function of macrophages [56]. In obese *ob/ob* mice with *Mtb* infection, deficiency in leptin may attenuate the function of macrophage and IFN- $\gamma$ -driven Th1 responses [57]. However, the adipose resident macrophages phenotypically resemble M2 macrophage

and can produce anti-inflammatory cytokines, suggesting that the role of these cells in regulating immune responses in diabetes remain to be elucidated [58]. Future research should seek to further elucidate the effects of these abnormalities on immune system in patients suffering from diabetes.

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