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Psychoneuroimmune Implications of Type 2 Diabetes: Redux

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Introduction

The psychoneuroimmune (PNI) response can be broadly described as the “brain-based” component of innate immune activation. Upon innate immune system triggering, cytokines are elicited that orchestrate the physiological, metabolic, behavioral and psychological changes that occur in an individual as a result of infection. Then, like a counter-weight, negative regulation of these pathways restores homeostatic balance. A sizable body of knowledge has arisen demonstrating that type 2 diabetes (T2D) is associated with alterations in the innate immune system. In addition, this proinflammatory leaning imbalance is implicated in the development of secondary disease complications and comorbidities in T2D, such as delayed wound healing, accelerated atherosclerosis and retinopathy. The consequences of T2D-associated proinflammation on the PNI response has only of late become recognized. New experimental data and recently published health related quality of life surveys indicate that individuals afflicted with T2D experience diminished happiness, feelings of well-being and satisfaction with life. These emotional/psychological consequences of T2D point to altered neuroimmunity as a previously unappreciated complication of T2D. This review article will discuss recent data detailing the impact of T2D on the host PNI response.

Type 2 diabetes

Diabetes is a widespread disease of impaired glucose metabolism that can be divided into two major categories. Type 1 diabetes (T1D) occurs when insulin, the primary hormone that increases tissue uptake of circulating blood glucose, is absent. Sometimes, T1D is referred to as insulin-dependent or juvenile onset diabetes, and its cause is typically an autoimmune-based destruction of insulin secreting, pancreatic beta cells. T2D, also known as non-insulin dependent or adult onset diabetes, is the more prevalent form of diabetes affecting over 23.6

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million individuals in the United States [1]. T2D accounts for 85-90% of all cases of diabetes [2]. In fact, the prevalence of T2D is predicted to grow to affect upwards of 300 million people globally over the course of the next decade [3]. Typically, the onset of T2D occurs after age 30, and there is a significant genetic component. The concordance rate among identical twins is nearly 100% [4], while first-degree relatives of those with T2D have a 20 to 40% chance of developing T2D [5].

T2D is a multifaceted disease that is associated with a number of maladies, and effective treatment of T2D and its associated complications is made difficult due to a complex and poorly understood disease progression. Data indicate that diabetes develops over the course of decades [2], and the elevated risk of complications begins many years before the clinical diagnosis of diabetes [6]. Overt T2D is preceded by a number of underlying conditions, such as insulin resistance, impaired glucose tolerance (IGT) [2,7], subclinical proinflammation [8], and the metabolic syndrome [2]. Developing diabetes, indicated by the presence of insulin resistance and IGT, often goes undetected because of the body's ability to compensate. Euglycemia is usually maintained for many years even though a state of insulin resistance exists. When the ability of insulin sensitive tissues to uptake glucose starts to decline, the pancreatic beta cells can increase their output of insulin to compensate for diminished tissue sensitivity and rising blood glucose. With increasing insulin resistance, beta cells are unable to compensate. Even at this extreme, normal fasting glucose levels are often maintained, but individuals begin to experience bouts of postprandial hyperglycemia, hence, IGT [2].

T2D is strongly associated with a sedentary lifestyle and obesity [9,10]. In addition, a recent downward shift in onset age [2] and a rise in the incidence in Asian populations, with traditionally lower rates of obesity [2,11], suggest that other precipitating factors may be involved. Early detection of diabetes is paramount to reducing associated morbidity and mortality, because progression of the T2D often goes undetected due to lack of routine screening during the "pre-diabetic" IGT period.

T2D is associated with a long list of complications, including increased risk of cerebrovascular and cardiovascular disease, impaired wound healing, increased susceptibility to infection and increased incidence of Alzheimer's disease [1,12-19]. The mechanism by which T2D accelerates and exacerbates the development of these conditions is not fully understood; however, the close relationship between these disease states and T2D suggests a shared common pathogenic precursor [20]. One likely suspect is chronic inflammation, as many diabetic complications, including altered psychoneuroimmunity, have an inflammatory component to their pathology. Overall, T2D is now considered to be a proinflammatory disease involving chronic activation of the innate immune system [3,21] likely impinging on quality of life. In those with T2D, health related quality of life assessments show lower satisfaction than control subjects [22] especially in regard to negative impact on the emotional attribute, which measures perceived happiness [23]. In sum, T2D not only debilitates physically, but also depresses emotional health and well-being.

Type 2 diabetes as a proinflammatory disease

The relationship between T2D and the innate immune system is bi-directional. Dysregulated innate immunity characterized by persistent subclinical chronic inflammation is intimately associated with the insulin resistant state, obesity and T2D [24-26]. Most evidence indicates that chronic inflammation directly contributes to the pathogenesis of T2D [8,24,26-32]. Historically, the concept that inflammation is associated with insulin resistance has been explored for over 20 years [33,34]. More recently, Pickup and Crook advanced the hypothesis that T2D is a disease of the innate immune system [3,21]. This hypothesis was based on findings in a large number of individuals with T2D that showed subtle elevations in proinflammatory

biomarkers and acute phase reactants including IL-1 β , TNF α , IL-6, sialic acid, serum amyloid, C-reactive protein, cortisol [3,21,29,35-37]. In addition, prospective studies have shown that alterations in C-reactive protein (CRP) and IL-6 predict the development of T2D in human subjects [37] as do elevations in the acute phase reactants fibrinogen and plasminogen activator inhibitor (PAI)-1 [38].

Recent T2D work has solidified the mechanism by which chronic inflammation can cause insulin resistance. Cai et. al. reported that NF- κ B and inflammatory transcriptional targets are activated in the liver by both diet-induced obesity and genetic onset obesity [32]. Moreover, they were able to reproduce similar changes in the hepatic inflammatory profile by selectively expressing a constitutively active IKK- β in hepatocytes, which, in turn, caused mice to exhibit a T2D phenotype. The activation of the NF- κ B pathway resulted in increased hepatic production of TNF- α , IL-1 β , and IL-6. Finally, Cai et. al. neutralized IL-6 systemically reversing the T2D phenotype [32]. In support of Cai et. al., Arkan et. al. selectively knocked out IKK- β in either hepatocytes or myeloid cells [31]. Hepatocyte specific deletion of IKK- β preserved hepatic insulin sensitivity after high fat diet, genetic obesity or aging. However, deletion of IKK- β in myeloid cells resulted in global protection of insulin sensitivity after high fat diet, genetic obesity or aging [31]. These results underscore the potential importance of myeloid cells (monocytes/macrophages) to chronic inflammation-induced insulin resistance.

On the other hand, there is still some uncertainty as to whether chronic inflammation leads to insulin resistance or whether insulin insensitivity brings about a condition of chronic inflammation. Symptoms inherent to T2D, such as hyperglycemia, hyperinsulinemia and insulin resistance can independently induce proinflammation via dysregulation of macrophage activation. In diet induced obesity, over-nutrition and increased fat mass results in infiltration of macrophages into adipose tissue, where adipokines, low oxygen and/or adipocyte apoptosis may stimulate macrophages to secrete proinflammatory cytokines [25,26,39]. Proinflammatory cytokines such as TNF- α [36,40] and IL-6 [41-44] induce insulin resistance in a number of tissues, and insulin resistance leads to IGT. In turn,, hyperglycemia [45,46] and hyperinsulinemia [47] independently induce proinflammation as measured by IL-6 secretion [48] and heightened acute inflammatory responses to stimuli, like LPS [49]. In healthy subjects, insulin inhibits acute phase protein synthesis [21], and, in animal models of T2D, the acute phase response is increased by the relative insulin deficiency that results from insulin resistance [21,50]. Hyperglycemia has been postulated to play a role in the generation of acute phase proteins and the inflammatory response [51,52] because treatments that both increase insulin sensitivity and lower blood glucose reduce the levels of serum acute-phase proteins [52,53]. Additionally, macrophage function, which plays a crucial role in the inflammatory response as an initiating event of the neuroimmune response, is altered by hyperinsulinemia and hyperglycemia [49,54-59]. Diabetes-associated alterations in the innate immune response have primarily been described within the context of the peripheral complications of T2D. Not only does the proinflammation observed during with insulin resistance likely contribute to accelerated progression of T2D and its complications it also likely exacerbates PNI-based sequela negatively impacting the diabetic patient's emotional well-being and quality of life.

The Psychoneuroimmune Response

The PNI response or “brain-based” innate immune reaction is initiated by infection via pathogenic microorganisms that trigger, in the host, a set of immunological, physiological, microbiological, and behavioral response mediated by innate immune cells which recognize pathogen-associated molecular patterns via Toll-like receptors (TLR)s. LPS is an archetypal pathogen-associated molecular pattern and a component of Gram negative bacterial cell walls. LPS binds to TLR-4 and CD-14 on the surface membrane of monocytes and macrophages. The proinflammatory cytokines that are produced as a result of subsequent TLR-4-dependent NF-

κ B pathway activation are part of a cytokine network that includes both proinflammatory and antiinflammatory cytokines. These antiinflammatory cytokines can specifically inhibit a single proinflammatory cytokine, for instance interleukin-1 receptor antagonist (IL-1ra) binds to the IL-1 receptor 1 to competitively block IL-1-mediated signaling, or these antiinflammatory cytokines can non-specifically downregulate production and/or action of proinflammatory cytokines, for instance IL-4 and IL-10 reduce the secretion of IL-1 β , IL-6 and TNF- α by macrophages [60]. Importantly, dysregulated and/or excessive inflammation can exacerbate pre-existent disease states [61,62] and profoundly depress mood and the feeling of well-being [63,64]. The possible association between heightened peripheral inflammation and central inflammation has only recently been systematically tested in animal models of T2D, despite the obvious clinical implications.

Fever

Fever is one of the host defense mechanisms to infection, injury and inflammation. Fever is an adaptive homeostatic state that is characterized by an increased set point in body temperature [65]. Elevated body temperature stimulates the proliferation of immune cells and is unfavorable for the growth of many bacteria and viruses [66]. When exogenous pyrogens (experimentally represented by LPS) invade the organism, fever is caused through the activation of myeloid cells that subsequently release endogenous pyrogens, proinflammatory cytokines, prostaglandins and free radicals. This is of clinical importance because cytokine immunotherapy, which can activate cytokine cascades, is also associated with fever [67]. Importantly, evidence indicates that both peripheral and brain-based IL-1 β and TNF- α are involved directly in the pyrogenic response to inflammation [68-70]. In the periphery, IL-1 β and TNF- α cause increased production of IL-6, the principal endogenous pyrogen [69]. The liver is the main clearance organ for circulating LPS [71-73]. Kupffer cells account for 80–90% of the total population of fixed tissue macrophages in the body [74,75] and are believed to be responsible for the liver's clearance of LPS. Additionally, Kupffer cells may be a principle producer of cytokines and, hence, the fever induced by LPS [76]. Therefore, altered peripheral macrophage function caused by T2D likely has a pronounced effect on the febrile response.

The effect of insulin resistance has been studied in the Zucker (fa/fa) rat model of obesity/T2D [77]. In this study, various cytokines were infused directly into the lateral ventricle of the brains via a surgically implanted cannula. The obese rats exhibited a differential febrile response to the pyrogenic cytokine IL-1. They reached a peak febrile temperature that was about 0.5°C higher than their lean counterparts, and interestingly, the febrile response of these obese rats persisted over a longer duration than control lean rats. A second independent study using both fa/fa rats and Otsuka Long-Evans Tokushima Fatty (OLETF) rats (which are obese due to the absence of the cholecystokinin-A receptor), also, demonstrated a febrile responses to intravenous *E. coli*-derived LPS which trended higher in obese animals when compared to lean counterparts [78]. It is important to point out that some conflicting data have been reported regarding the effect of obesity and T2D on febrile responsiveness as changes in ambient temperature alter the fever response in fa/fa rats [79] and IL-6 administered directly into the brain does not induce a higher fever in obese rats [77]. These findings underscore the importance of the experimental conditions, specific exogenous pyrogen and method of administration of the exogenous pyrogen in making an interpretation of data regarding an effect of T2D on febrile responsiveness.

In our laboratory, we have utilized the *db/db* (C57BL/6J-lepr^{db/db}) mouse model of T2D. The *db/db* mouse displays characteristic, hyperphagia, metabolic dysfunction, morbid obesity and neuroendocrine abnormalities that parallel uncontrolled human T2 D (Table 1). Using this model, we have observed exacerbated febrile responsiveness to intraperitoneal LPS in diabetic mice compared to non-diabetic heterozygote control (*db/+*) mice (Fig 1). When 8-wk old *db/*

db mice and *db/+* mice were injected intraperitoneally with LPS, LPS-induced fever was significantly increased in *db/db* mice when compared to *db/+* mice. Peak change in colonic temperature as measured by a rectally inserted thermocouple was 1.5 °C for *db/db* mice but only 0.6 °C for *db/+* mice. In addition, *db/db* mice had a marked extension in the duration of fever when compared to *db/+* mice. Together, these results indicate that the febrile responsiveness, an important aspect of brain-based innate immunity is exacerbated in an experimental model of T2D.

Sickness behavior

LPS is a potent activator of the neuroimmune system [80], and LPS-induced sickness has been a key tool in PNI research. Sickness behavior refers to a coordinated set of nonspecific behavioral modifications that occur in individuals during an infection [66,81-84]. Sickness behavior is typically accompanied by fever and a variety of behavioral responses, including decreased appetite, fatigue, sleep disturbances, retardation of motor activity, reduced interest in the physical and social environment, loss of libido, impaired cognitive abilities, anhedonia and depressed mood [66,85,86]. While these behavioral changes are often interpreted to be an unavoidable consequence of a degraded state, increasing evidence suggests that these cytokine-mediated symptoms are part of an organized and evolutionarily conserved adaptive defense response to infection. Sickness behavior reflects motivational reorganization; whereby, the individual's priorities are restructured to maximize the immune efficiency in fighting infection. The main proinflammatory cytokines responsible for the initiation of the hosts behavioral response to infection are IL-1 β , IL-6, and TNF- α [81,87]. These cytokines are secreted by activated monocytes and macrophages. However, as in models of experimental stroke, brain IL-1 β appears to be the predominant cytokine that mediates sickness behavior [87,88], as intracerebroventricular administration of IL-1RA blocks some sickness behaviors caused by peripheral and/or central innate immune system activation [89,90].

Of clinical importance, these behavioral responses have been observed during the course of infection and also during systemic or central administration of cytokines. Cancer therapies, involving treatment with proinflammatory or antiviral cytokines (mainly IL-2, TNF- α and IFN- α), have been associated with flu-like and depressive symptoms, as well as, signs of cognitive impairment [91]. Administration of IFN- α , which has been used in the treatment of chronic hepatitis C, is associated with symptoms of cognitive impairment, behavioral despair, fatigue and depressed mood [91]. The symptoms of sickness behavior almost immediately disappear after termination of cytokine administration, supporting a causal role for cytokines in the mediation of these behaviorally-based sequela. Therefore, patients with T2D, or even some pre-diabetic risk factors, may not only experience more debilitating conditions during an illness or infection, but may not tolerate immunotherapy as well. The heightened PNI responses may also interfere with a patient's diabetes treatment regimen because depressed mood or loss of the feeling of well-being is also a strong predictor of patient non-compliance with a prescribed course of therapy [92,93].

LPS-induced social withdrawal is a classic behavioral feature of the innate immune response that is routinely used as a quantitative measure in rodents because of a large and repeatable change in interaction patterns that are easy to measure [66]. When tested in the *db/db* mice, LPS-induced sickness behavior was significantly augmented as compared to non-diabetic control mice. Consistent with the experimental results showing the impact of T2D on the febrile response, *db/db* mice are more sensitive to the behavioral effects of intraperitoneal LPS. Using a weight-adjusted dose of LPS, both the magnitude and duration of sickness behavior were exacerbated in *db/db* mice. However, while a dose of 100 μ g/kg LPS is commonly used in the study of sickness in rodents, under these conditions *db/db* mice would receive a significantly larger dose of LPS because of their obesity-associated increase in body weight [49]. Clearance

of LPS from the peritoneal cavity occurs primarily by the peritoneal lymphatic system and hematogenously via the portal vein [94]. Since abdominally based inflammation utilizes the vagus nerve to communicate with the brain, locally elevated LPS levels in the peritoneum (due to a weight based strategy of LPS administration) would likely artificially heighten sickness behavior in obese mice versus thin mice. Therefore, LPS-dependent behavioral changes utilizing a fixed dose of LPS were examined. Interestingly, these data also showed an exacerbated sickness behavior response in *db/db* mice where recovery from sickness was significantly delayed [49]. *Db/db* mice, also, had a significant increase in peritoneal levels of IL-1 β and diminished up-regulation of the important IL-1 negative regulatory molecules, IL-1ra and IL-1R2 (the IL-1 decoy receptor) in both the periphery and the brain [49]. The importance of these studies is that they were the first to show in a mouse model of T2D decreased ability to recover from brain-based innate immune activation.

Heightened brain-based immune responsiveness has been identified in the non-obese diabetic (NOD) mouse (a model of T1D) [95] indicating that both T1D and T2D (ie diabetes in general) are associated with dysregulated neuroimmunity. Hence, altered central innate immunity may be a common feature or possibly a complication of diabetes. The mechanism responsible for neuroimmune dysregulation in diabetes is likely hyperglycemia. We have shown that in a mouse model of T1D, LPS-dependent social withdrawal was augmented and reliant on elevated blood glucose [96]. T1D was induced in mice with streptozotocin. Streptozotocin is a potent pancreatic beta cell toxin that in 4 days causes blood glucose levels to exceed 400 mg/dl [96]. When T1D mice were challenged with LPS, LPS-induced social withdrawal was more than double that of non-diabetic mice. Examination of peritoneal proinflammatory cytokine levels 2 h after LPS administration showed that diabetic mice had 4-, 2.5- and 3.6-fold greater concentrations of IL-1 β , IL-6 and TNF- α , respectively, when compared to non-diabetic mice. Control of blood glucose levels moderated LPS-induced social withdrawal. Finally, administration of STZ to hyperglycemic/hyperinsulinemic *db/db* mice did not alter LPS-induced social withdrawal [96]. Together these findings indicate that mice with T1D have augmented sickness in response to innate immune challenge that is due to hyperglycemia and not to hyperinsulinemia.

Overall, most conditions with an inflammatory component comorbid with diabetes are made more ominous by T2D. For example, delayed wound healing has long been recognized as a complication of T2D. In rodents, prolonged secretion of proinflammatory cytokines are identified at the wound site in T2D mice [97]. Likewise, in experimental animal models of cerebral ischemia, diabetic mice suffer more severe damage and have poorer outcomes when compared to non-diabetic mice. We have shown that acute hypoxia, triggers neuroimmune system activation causing loss of interest in the social environment, and that recovery from hypoxia-induced neuroimmune system activation was impaired in the *db/db* mouse model of T2D [101]. Importantly, recovery from the behavioral consequences of acute hypoxia was nearly ablated in mice that lack IL-1R1 signaling and in mice intracerebroventricularly administered a caspase-1 inhibitor that blocks conversion of pro-IL-1 β to IL-1 β . Diabetic mice had prolonged recovery from neuroimmune system activation due to loss of brain-based up-regulation of IL-1ra and IL-1R2, but speed of behavioral recovery could be doubled by administration of subcutaneous IL-1ra to these mice [101]. Such results demonstrate that acute hypoxia activates the IL-1-arm of the neuroimmune system, which diabetes exacerbates due to brain-based loss of IL-1 counter-regulation and that treatment with IL-1RA ameliorates.

On the flip side, antiinflammatory cytokines have effectively been used to attenuate the behavioral changes that occur during immune activation. Central administration of recombinant IL-1ra [89], IL-10 [95], IL-4 [98] and IGF-1 [99,100] dampen the sickness behavior response induced by activation of the innate immune system. Importantly, we found that IGF-1 attenuates sickness behavior in response to peripheral LPS challenge in *db/db* mice

[100]. While *db/db* mouse peritoneal macrophages elaborate more proinflammatory cytokines they also, as noted above, fail to up-regulate two key counter-regulators of IL-1 in response to LPS: IL-1ra and IL-1R2 [49]. In addition, this failure to up-regulate IL-1ra and IL-1R2 occurs not only in microbial-based innate immune activation (LPS) but also during non-microbial-based innate immune activation (acute hypoxia) [101]. Thus, an imbalance in IL-1-based proinflammation and antiinflammation appears key to T2D-associated dysregulated neuroimmunity. However, the story is not quite so simple due to an important difference between human T2D and the *db/db* mouse model of T2D and that is the absence of functional leptin signaling in *db/db* mice. In very recent work, we reported that leptin is key acute hypoxia recovery because it dramatically augments IL-1ra production in mice [102]. In fact, leptin appears to be a more potent inducer of IL-1ra than hypoxia. In leptin receptor defective (*db/db*) and leptin deficient (*ob/ob*) mice, sickness recovery from hypoxia was delayed and in *ob/ob* mice, leptin administration completely reversed this delayed recovery. In addition, leptin administration to normal mice cuts hypoxia recovery time by 1/3 while, in turn, boosting serum IL-1ra. Finally, leptin fails to alter hypoxia recovery in IL-1ra knockout mice [102]. These results show that by enhancing IL-1ra production leptin promoted sickness recovery from hypoxia but they also suggest that human diabetes may be different because it is often associated with up-regulation of IL-1ra [103].

Humoral and neural routes communicate immune status to the brain

Activated macrophages are the primary cellular source of IL-1 β , TNF- α and IL-6 and these proinflammatory cytokines are key to the communication between the immune system and the brain [104]. There are a number of different routes by which the peripheral generated cytokines may communicate with the brain [87]. The first of these routes involves the circumventricular organs, those regions of the brain that lack a fully intact blood—brain barrier (BBB). In these organs, cytokines can freely diffuse from the blood into the brain parenchyma where they then may interact with macrophages [104]. Another route by which cytokines communicate with the brain is across the intact BBB. Blood borne cytokines may interact with endothelial cells, which, in turn, signal perivascular macrophages located on the brain side of the BBB. Cytokines can also be actively transported across the BBB via a saturable transporter mechanism [105]. Regardless of the signal, once activated, perivascular macrophages can communicate with microglia, the resident macrophages of the brain.

Another route facilitates communication between the periphery and brain. Locally, peripheral inflammation is initially communicated to the brain through afferent vagus nerve fibers which results in up-regulation of glial-cell derived proinflammatory cytokines in the brain [66]. Sensory vagal afferent terminals express receptors for IL-1, and following peripheral activation of the innate immune system, increased expression of c-fos occurs in the brain in the projection areas of the vagus. Subdiaphragmatic vagotomy in rodents prevents both behavioral depression and activation of the limbic system following intraperitoneal administration of LPS or recombinant IL-1 β [106]. Induction of central IL-1 β expression by peripheral LPS or IL-1 β is blocked in vagotomized animals, but when IL-1 β is injected centrally, vagotomy has no effect on the activation of the brain-based innate immune response [106]. Recently, the relative contribution of the humoral versus neural communication pathways in mediating PNI has been debated, as subdiaphragmatic vagotomy does not block all aspects of the PNI response [107]. Therefore, cytokines mediate immune to brain communication in a complex, and often redundant, network that involves both neural and humoral components.

The various cytokines affecting the brain have two possible origins. First, cytokines originating from the peripheral immune organs can cross the BBB. Stimulation of the peripheral immune system could signal the brain in both a local and a systemic manner. Cytokines can reach the CNS directly by crossing at accessible areas in the BBB through the circumventricular organs

[108]. This was demonstrated by the appearance of significant quantities of human recombinant IL-1 in mouse cortex after its subcutaneous injection, without an elevation of mouse IL-1 levels [109]. In addition, there is convincing evidence for active, saturable, and specific transport of certain cytokines across the BBB [109,110]. Second, cytokines can be produced by cells within the CNS. Most of the cytokines and their receptors have been identified in various cell types of the CNS in both healthy and diseased states. It is believed that cytokines produced by neurons and glial cells within the brain participate in the complex autonomic, neuroendocrine, metabolic and behavioral responses to infection, inflammation, ischemia and other brain injuries [111-113].

Type 2 diabetes affects the blood brain barrier

As one of the primary interfaces between cytokines and the brain, the BBB has also been shown, in a few reports, to be compromised in T2D. Normally, the BBB refers to a specialized feature of the brain's capillary bed, where capillaries are connected via tight junctions. Brain endothelial cells have a significantly lower rate of endocytosis, and lack nearly all intracellular pores spanning the capillary walls. These features help prevent the uncontrolled entry of blood-borne molecules into the brain [114]. Magnetic resonance imaging has shown that patients with T2D have increased BBB permeability compared to healthy controls [115]. Experimentally, STZ-induced diabetes in rats was recently shown to increase BBB permeability, and treatment of these diabetic rats with statins (a class of cholesterol lowering pharmaceuticals) reduced BBB permeability [116]. In animal models with insulin deficiency and marked hyperglycemia, there is a regionally specific decrease in cerebral blood flow, which may be a compensatory/protective mechanism. Duckrow et. al. found that hindbrain blood flow was more reduced than forebrain blood flow. Moreover, decreased regional cerebral blood flow (CBF) was dependent acutely and chronically upon the degree of hyperglycemia [117]. In these studies, an osmotic effect was eliminated as a cause since control experiments with mannitol showed no CBF change. Human studies of CBF in hyperglycemia and hypoglycemia are more inconsistent than animal studies, which may be due to more extreme glucose ranges that are often seen in animal models. While the impact of diabetes-associated BBB permeability specifically on the PNI response has not been systematically studied, it seems reasonable to hypothesize that compromised BBB integrity may be a pathologic contributor to exacerbated brain-based responses to peripheral immune challenge.

Type 2 diabetes affects the source of proinflammatory cytokines

Macrophage activation, an initial step in cell-based innate immune activation, results in the elaboration of proinflammatory cytokines, and has repeatedly been shown in both human and animal studies of T2D to be perturbed. Since cytokines secreted by activated macrophages help direct the brain based response to infection, diabetes-associated alterations in cytokine secretion by macrophages would likely have significant impact on the concomitant metabolic and behavioral changes that ensue. However, in animal models of T2D, the specific model and the experimental conditions being used impact macrophage bioaction. In human patients with T2D, circulating monocytes were reported to have increased expression of CD14 [118], which is a co-receptor for LPS. The scavenger receptor, CD36, is also up-regulated in macrophages by T2D [119]. Moreover, data from our laboratory indicate that resident peritoneal macrophages isolated from *db/db* mice elaborate more IL-1 β in response to LPS than non-diabetic mice. We found that the peritoneal fluid of *db/db* mice exposed to a fixed dose of LPS had a peak increase in IL-1 β concentration more than double that of non-diabetic mice exposed to LPS, and resident peritoneal macrophages isolated from *db/db* mice produced more IL-1 β after LPS stimulation [49]. Naguib et. al. noted that the inflammatory response to bacteria is prolonged in *db/db* mice due to unresolved proinflammatory cytokine expression [97]. Studies conducted with macrophage cell lines [59,120] and primary macrophages [121,122] indicate

that the diabetic milieu heightens macrophage responsiveness to innate immune activators like LPS. Contrary reports also exist demonstrating that macrophage activity is diminished by T2D conditions. For example, Zykova *et al.* has shown that peritoneal macrophages elicited with thioglycollate from C57BL/KS-lepr-*db/db* mice had a diminished cytokine secretion response to LPS + IFN γ *ex vivo* [123].

Hyperglycemia appears to be the predominant factor in making macrophages more responsive to immune stimulation. Several studies involving streptozotocin-induced diabetes in laboratory rodents have found hyperglycemia to cause heightened macrophage activation in response to stimuli, such as LPS. However, in terms of PNI, proinflammatory cytokine expression in the brain is critical for functional changes in the brain-based response. This has been demonstrated using cytokine receptor knockout mice. Specifically, IL-1 receptor knock out mice are resistant to the behavioral changes normally induced by direct central administration of IL-1 into the lateral ventricle. Studies have confirmed that the degree of hyperglycemia usually observed in T2D does, in fact, augment microglia-mediated inflammatory responses. In the Ins2 (Akita) mouse model of diabetes, hyperglycemic conditions resulted in morphologic changes in retinal microglia consistent with an activated state [124], and LPS in the presence of high glucose conditions synergistically increased cytotoxicity in primary rat microglia as a result of increased free radical production [122]. In an experimental model of ischemic stroke, microglia of *db/db* mice exhibited delayed expression of bfl-1, which is an endogenous bcl-2-related inhibitor of apoptosis [125]. The likely mechanism by which hyperglycemia enhances macrophage activation is due to an increase in oxidative stress or advanced glycation end products. During states of hyperglycemia, proteins can become non-enzymatically glycosylated, and glycosylated proteins are known to activate macrophages and render cells more susceptible to subsequent cytotoxic events [126]. However, in some models of T2D, like the Ins2 (Akita) or *db/db* mouse, hyperglycemia is associated with an induction of insulin resistance characterized by diminished signal transduction through the insulin receptor and insulin receptor substrate (IRS) proteins [124].

Antiinflammatory cytokine resistance

As discussed previously, experimental results from animal models of T2D suggest negative regulation of inflammatory processes is impaired, and chronic inflammation appears to induce a state of insulin resistance, likely mediated through impaired insulin receptor substrate (IRS) mediated signaling and increased expression of inhibitory proteins called suppressors of cytokine signaling (SOCS). Normal counter-regulation of proinflammation involves anti-inflammatory cytokines, such as IGF-1, IL-4, IL-10 and IL-13. These cytokines reduce secretion of proinflammatory cytokines by macrophages and stimulate the secretion of a number of antiinflammatory molecules, such as IL-1RA, IL-1R2, and soluble TNF receptors. IGF-1, IL-4, IL-10 and IL-13 utilize IRS proteins as a component of their own tyrosine phosphorylation signaling pathways. Importantly, up-regulation of SOCS proteins would inhibit certain actions of these antiinflammatory cytokines because SOCS proteins recognize and bind to tyrosine phosphorylated motifs on membrane receptors to inhibit downstream signal amplification. Therefore, it is likely that during insulin resistant states, there also exists a degree of antiinflammatory cytokine resistance, which compounds the inflammation inherent to diabetes-associated hyperglycemia.

In vivo data demonstrates diminished ability of antiinflammatory molecules to attenuate the PNI response [100]. The hypothesis of diabetes-induced resistance to antiinflammatory cytokines was described by Hartman *et al.* In this study, T2D conditions resulted in impaired IRS-2-mediated signal transduction in both a macrophage cell line and primary macrophages. Interestingly, the antiinflammatory molecules, IL-4 and IGF-1 had diminished ability to signal through this shared pathway [54]. In turn, others have shown that IRS-2 deficient lymphocytes

have a diminished capacity to secrete TH2 cytokines [127]. While the specific impact of SOCS up-regulation on PNI is not clear, SOCS proteins likely play an important role in diabetes-associated resistance to the immunological effects of IL-10, IL-4, and IGF-1. The ability of IGF-1 to attenuate LPS induced sickness behavior is impaired in *db/db* mice [100], and SOCS are up-regulated in number of models of T2D [128,129]. In addition, we were the first to report the relevance of the IL-4/IRS-2/phosphatidylinositol (PI3K) pathway in macrophages by showing that IL-4-dependent elaboration of IL-1RA requires IRS-2-mediated PI3K activity in primary macrophages [144]. We also demonstrated that macrophages isolated from *db/db* mice have impaired IRS-2-mediated PI3K activity and constitutively over-express SOCS-3. Examination of IL-4 signaling in *db/db* macrophages revealed that IL-4-dependent IRS-2/PI3K complex formation and IRS-2 tyrosine phosphorylation was reduced compared to *db/+* macrophages. SOCS-3/IL-4 receptor complexes, however, were increased in *db/db* mouse macrophages as compared to *db/+* mouse macrophages as was *db/db* mouse macrophage SOCS-3 expression. These results indicate that in the *db/db* mouse model of T2D macrophage expression of SOCS-3 is increased resulting in impaired IL-4-dependent IRS-2/PI3K formation that induces a state of IL-4 resistance disrupting IL-4-dependent production of IL-1RA. More studies, however, are needed to delineate the role of SOCS up-regulation in T2D-associated PNI because, as mentioned earlier, the *db/db* mouse model of T2D is significantly different than human diabetes.

Hypothalamic-pituitary-adrenal axis

One of the methods of CNS regulation of innate immunity is through neuroendocrine control of immunocompetent cells via the hypothalamic-pituitary-adrenal (HPA) axis. In the HPA axis, the hypothalamus acts as a master gland, exhibiting control over the network. The bi-directional communication of the neuroendocrine and immune system is achieved through proinflammatory cytokines such as IL-1 β , TNF- α and IL-6 which are potent triggers to the HPA axis. Receptors for IL-1 have been identified in the hypothalamus and IL-1 β has been shown to induce ACTH release via CRF in a dose dependent manner [130]. It has been suggested that a negative feedback loop on macrophage IL-1 secretion is mediated by the HPA axis and sympathetic nervous system via central IL-1 β [131]. The primary route of HPA activation via peripheral IL-1 β appears to be accomplished through the stimulation of vagal afferents [132].

Glucocorticoids inhibit the production of IL-1 β via a negative feedback loop [133]. In fact, glucocorticoids serve as critical negative regulators of all myeloid cells [134]. Glucocorticoids which are often released in response to stress have anti-inflammatory and immunosuppressive effects. They not only negatively regulate macrophages, but also stimulate the secretion of IL-10 [135]. Hypophysectomy, surgical removal of the pituitary, has demonstrated the role of this gland in maintaining proper immune function. Hypophysectomy results in decreased lymphocytes, decreased antibody response and reduced thymus and spleen weights [136]. Adrenalectomy results in elevated pro-inflammatory cytokine expression in both the spleen and the brain in response to LPS [137] and increased mortality in response to LPS, IL-1 β , TNF- α and infection [138,139].

A number of studies have identified impaired HPA responsiveness associated with T2D as reviewed by Chan et. al. [140]. A recent study has shown that hyperinsulinemia, independent of glucose, increases the HPA response in rats and that diabetes significantly impaired the ability of the HPA response to appropriately match the potency of the stressor [141]. T2D is, also, associated with chronic activation of the HPA axis and hyper-secretion of glucocorticoids [142,143]. Chronic elevation of glucocorticoids results in a state of resistance and/or glucocorticoid insensitivity causing failure of glucocorticoid-dependent negative feedback

[142,143]. Hyperinsulinemia and insulin resistance likely alter the same pathways in the brain that are impacted in peripheral macrophages and tissues.

Summary/Conclusion

The idea that T2D is associated with augmented innate immune function characterized by increased circulating levels of acute phase reactants and altered macrophage biology is well established, even though the mechanisms involved in this complex interaction are still not entirely clear. Initially, the majority of studies investigating innate immune function in T2D were limited to the context of wound healing, atherosclerosis, stroke and other commonly identified comorbidities. Several important recurring themes, however, have come from these data. First, T2D is associated with a state of chronic, subclinical inflammation. Second, in macrophages, T2D conditions enhance proinflammatory reactions and impair antiinflammatory responses. Third, recovery from innate immune activation and resolution of inflammation in T2D is impaired. In sum, the impact of diminished emotional well-being on the quality-of-life for diabetes sufferers is significant, and, given the importance of inflammation to T2D, PNI-based T2D sequela should be considered a complication of diabetes that warrants serious clinical attention.

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Abbreviations

BBB, blood brain barrier
 CRP, C-reactive protein
 CNS, Central nervous system
 CVD, cerebro- or cardiovascular disease
 GC, glucocorticoid
 HPA, hypothalamic-pituitary-adrenal
 IGT, impaired glucose tolerance
 IGF, insulin-like growth factor
 IRS, insulin receptor substrate
 IFN, interferon
 IL, interleukin
 IL-1ra, interleukin-1 receptor antagonist
 IL-1R2, type 2 interleukin-1 receptor
 LPS, lipopolysaccharide
 PNI, psychoneuroimmune
 STZ, streptozocin
 SOCS, suppressors of cytokine signaling
 TLR, toll-like receptor
 TNF, tumor necrosis factor
 T1D, type 1 diabetes
 T2D, type 2 diabetes

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Fig. 1. Febrile response to LPS in *db/db* mice

8-week-old *db/db* and *db/+* mice were injected intraperitoneally with LPS (100 μ g/kg). Colonic temperature was measured at the times indicated. Data represent an average of 5 mice/group $\pm$ SEM. * $P < 0.05$ *db/db* vs. *db/+*

Table 1

Db/db mice have elevated body weight, blood glucose and serum insulin levels.

	<i>db/+</i>	<i>db/db</i>
Body Wt (g)	27.6 ± 0.55	36.7 ± 0.57[*]
FBG (mg/dl)	115.2 ± 19.08	400.4 ± 15.35[*]
RBG (mg/dl)	169.8 ± 12.23	423.0 ± 17.84[*]
FSI (ng/ml)	1.3 ± 0.05	2.4 ± 0.05[*]
RSI (ng/ml)	2.2 ± 0.08	5.4 ± 0.09[*]

Fasting blood glucose (FBG), random blood glucose (RBG), fasting serum insulin (FSI), and random serum insulin (RSI). Results represent the average of n=5 mice ± SEM.

Adapted from O'Connor et. al. J. Immunol. 2005, 174: 4991-7.

^{*}
P < 0.05