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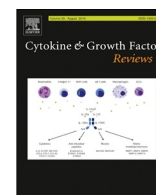
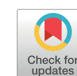


Insulin as an immunomodulatory hormone



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ABSTRACT

Insulin plays an indispensable role in the management of hyperglycaemia that arises in a variety of settings, including Type I and II diabetes, gestational diabetes, as well as is in hyperglycaemia following a severe inflammatory insult. However, insulin receptors are also expressed on a range of cells that are not canonically implicated in glucose homeostasis. This includes immune cells, where the anti-inflammatory effects of insulin have been repeatedly reported. However, recent findings have also implicated a more involved role for insulin in shaping the immune response during an infection. This includes the ability of insulin to modulate immune cell differentiation and polarisation as well as the modulation of effector functions such as biocidal ROS production. Finally, inflammatory mediators can through both direct and indirect mechanisms also regulate serum insulin levels, suggesting that insulin may be co-opted by the immune system during an infection to direct immunological operations. Collectively, these observations implicate insulin as a bona fide immune-modulating hormone and suggest that a better understanding of insulin's immunological function may aid in optimising insulin therapy in a range of clinical settings.

1. Introduction

The ability of insulin to lower blood glucose renders it one of the most widely administered hormones, with hundreds of millions of people globally receiving insulin annually. There is also good reason to suspect an increase in the application of insulin. Globally, the incidence of type I diabetes (T1D) is on the rise, with an annual increase between 2 % and 3 % [1]. Similarly, for patients suffering from Type II diabetes (T2D), insulin usage is projected to increase by 20 % from 2018 to 2030 [2]. Insulin is also central in the treatment of hyperglycaemia that develops after an inflammatory insult –as is typically observed in critical care patients. Although fewer statistics are available on the epidemiology of critical care patients, it is generally also believed to be on the rise [3]. Insulin also features an indispensable role in the treatment of gestational diabetes (GD). Here, due to the diverse criteria and other complications, the exact epidemiology is unknown, but the incidence of GD is similarly forecast to increase [4]. Given the substantial economic burden of treating hyperglycaemia and the attendant medical complications [5], there is a clear need to optimise interventions aimed at achieving effective glycaemic control.

Although the use of insulin is primarily administered with the goal to manage hyperglycaemia, insulin has a range of other physiological effects on diverse cell types. It has long been noted that immune cells express insulin receptors following activation [6], and that insulin mediates an anti-inflammatory effect in a range of clinical settings [7]. However, emerging evidence suggests a more complex view for insulin in tailoring the immune response. Here we review evidence that implicates insulin as a critical immune-regulatory hormone. We point out that insulin may both exert an anti-inflammatory response, while also augmenting certain aspects of immune cell function and likely play a central role in immune cell differentiation and development. In fact, reviewing emerging evidence, we argue that insulin plays a cardinal role in shifting the immune response from an innate to an adaptive response during prolonged immune activation. Given that insulin influences the immune system, we argue that insulin likely impact on the disease trajectory in a range of clinically relevant settings.

2. Insulin: an anti-inflammatory hormone

There is a large body of evidence which implicates insulin in mediating anti-inflammatory effects. In patients with T2D, insulin has been shown to suppress the transcription of various Toll-like receptors (TLRs), including 1, 2, 4, 7 and -9, on circulating mononuclear cells [8]. The same seems to hold true for patients with T1D, where insulin treatment was shown to moderate the immune system on multiple levels. As an example, an insulin infusion suppressed the circulating levels of both CRP and HMGB1, decreased the transcription of TLRs 1, 2 & 4, and CD14 in macrophages, and also attenuated ROS generation by

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neutrophils [9]. Human macrophages challenged with LPS not only exhibited dramatically lowered rates of apoptosis following insulin treatment (at 25 IU/mL insulin or higher), but also exhibited suppressed expression of IL-1β and TNF released at a dose of 15 IU/mL or higher [10]. The anti-inflammatory effects of insulin are likely evolutionary conserved, since animal models have revealed similar anti-inflammatory effects of insulin. In a rat model of severe inflammatory insult (third-degree burns over 30 % of body area), insulin therapy resulted in a significant suppression of circulating proinflammatory agents such as TNF-α, IL-6, and HMGB1 [11]. Additionally, in a swine model of sepsis, a hyperinsulinemic-euglycemic clamp suppressed the release of inflammatory mediators such as TNF and IL-6 in response to an LPS challenge [12].

An anti-inflammatory effect of insulin is also suggested by the fact that insulin may attenuate inflammation associated with autoreactive immune pathologies such as asthma [13]. Similarly, hyperinsulinemia was an independent predictor of long term remission in patients suffering from either Crohn's disease or ulcerative colitis [14]. Here it is possible that elevated insulin may promote a protective effect by antagonising the inflammatory response usually observed in these pathologies. However, it is also known that inflammatory mediators such as TNF can suppress insulin release in β-cells [15]. Thus, it is also possible that elevated insulin levels reflect a lower disease severity with a concomitant lower inflammatory response, which in turn would result in higher insulin levels. None the less, in severe inflammatory conditions such as in a critical care setting, it is believed that at least part of the beneficial effects of intensive insulin therapy relates to the anti-inflammatory effect of insulin [16]. Finally, severe inflammatory responses are also known to promote a hypercoagulable state. In this regard, induced hyperinsulinemia antagonised the clotting cascade by increasing the serum levels of plasminogen activator-inhibitor-1 (PAI-1) [17], indicating that insulin may attenuate various overlapping aspects of pro-inflammatory cascades.

2.1. Glucose lowering as an anti-inflammatory mechanism

A key putative mechanism through which insulin may mediate an anti-inflammatory effect is by its canonical function as a glucose lowering agent (Fig. 1). Although hyperglycaemia is nearly universally viewed as a key contributor towards the manifestation of pathologies, “the mechanisms behind glucose toxicity remain elusive” [18]. Presumably, the glucose-lowering effect of insulin may exert an anti-inflammatory effect reducing “glucose toxicity” and preventing cell stress, thereby avoiding the secretion of inflammatory mediators. None the less, there are other well-described mechanisms by which hyperglycaemia may induce inflammation. As an example, hyperglycaemia may facilitate the formation of advanced glycation end products (AGE), which in turn activate receptors of AGE (RAGE) expressed on immune cells and stimulate the release of various pro-inflammatory mediators.

Another major mechanism by which glucose may increase inflammatory tone is via the production of reactive oxygen species (ROS). As an example, exposure of Jurkat T-cells to hyperglycaemia (11 −20 mM), induced a 3–4 fold increase in intracellular ROS production, stimulated the transcriptional activity of NF-κB and AP-1, which in turn manifested in increased expression of immunological proteins such as cytokines and adhesion molecules [19]. The observation that antioxidants such as N-acetylcysteine (NAC) could prevent hyperglycaemia-induced transcription of Il-6 and Il-17, coupled with the fact that NF-κB is a ROS-sensitive transcription factor, collectively support the hypothesis that hyperglycaemia may in itself promote immune function via increased ROS signalling [19]. Mechanistically, increased pyruvate oxidation may result in enhanced mitochondrial ROS production, particularly from complexes I and III [20]. Indeed, inflammatory mediators have been shown to induce mitochondrial ‘retooling’, resulting in increased mitochondrial ROS production (reviewed [21]). Another key mechanism by which glucose can promote ROS formation is via the production of NADPH which in turn is necessary for NADPH oxidase (Nox). As an example, neutrophils exhibited increased flux of glycolytic intermediates through the pentose phosphate pathway (PPP), where NADPH generated during the oxidative phase of the PPP is used to produce superoxide [22]. Thus, controlling hyperglycaemia may act to reduce glycolytic flux, thereby limiting pyruvate and avoiding mitochondrial- and Nox-derived ROS production.

Notably, inflammatory intermediates have a well-established role in promoting insulin resistance in peripheral tissue, while also promoting gluconeogenesis and the breakdown of muscle to liberate amino acids as carbon sources for glucose production in the liver, collectively

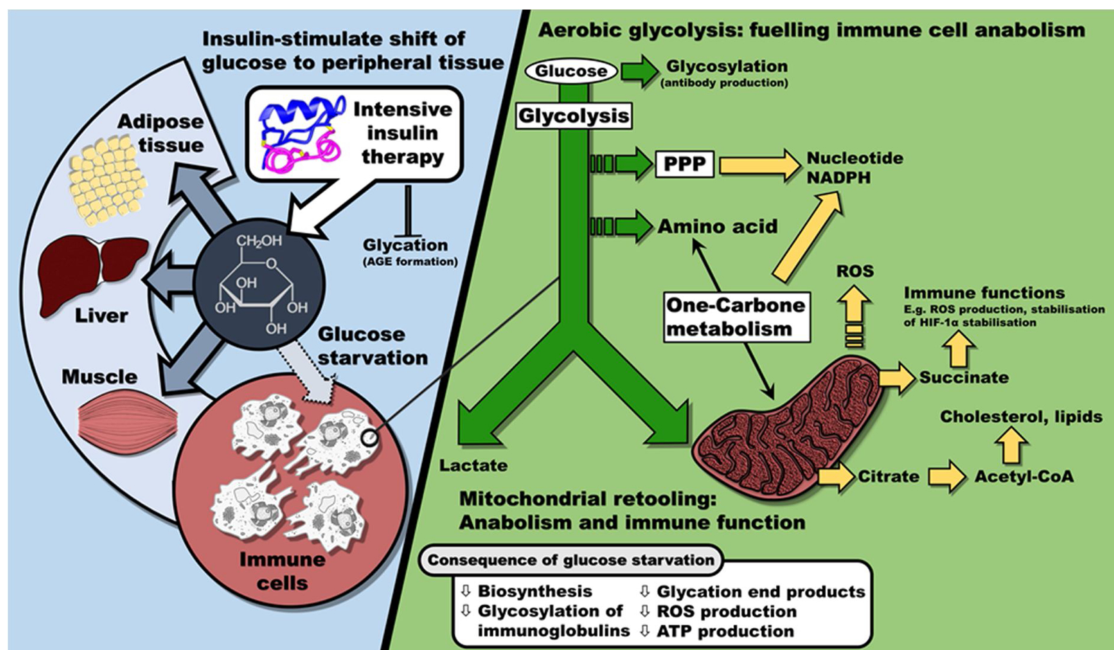

Fig. 1. Intensive insulin therapy may promote a decrease in serum glucose levels, thereby avoiding the formation of AGE as well as mitochondrial ROS production, thereby impeding immune cell metabolism.

culminating in hyperglycaemia. Based on recent developments in the field of immunometabolism, we have recently argued that these physiological events represent an evolutionary conserved strategy to sustain increased anabolism in immune cells [23]. Briefly, it is now clear that various immune cells with a pro-inflammatory or effector phenotype exhibit an increased demand for glucose. As an example, M1 polarised macrophages (i.e., 'pro-inflammatory') exhibit increased rates of glycolysis, whereas M2 macrophages (i.e. 'tolerogenic') are more reliant of mitochondrial respiration [24]. Neutrophils are also highly dependent on glucose: Indeed, a key feature of circulating neutrophils is that they are sparsely populated with mitochondria and are generating virtually all ATP via glycolysis [25]. Similarly, although anti-inflammatory, Th1 and Th17 cells exhibit increased aerobic glycolysis following exposure to inflammatory solicitors, while Tregs are dependent on OXPHOS [26]. The same theme is repeated in the activation of dendritic cells (DC) across the spectrum of immunogenic versus tolerogenic phenotypes, with increase OXPHOS in DCs with an immature or tolerogenic phenotype [27].

Why is it then the case that immune cells with pro-inflammatory phenotypes exhibit such a strong dependency on glucose? Pfeiffer and colleagues have used theoretical modelling to argue that although the yield of ATP per glucose may be low, the rate at which ATP are produced may be higher than in OXPHOS which is a slower process [28]. However, glucose is not merely used as energy substrate, but also contributes carbon to various biosynthetic pathways. This is well exemplified by serine, a non-essential amino acid which can be synthesised from the glycolytic intermediate, 3-phosphoglycerate (along with cysteine and glycine), and used in the *de novo* synthesis of purines and glutathione [29]. Similarly, serine plays an important role in immune cells: following activation, demand outstrips supply and serine becomes conditionally essential for rapidly proliferating effector T cells [30]. As mentioned, glycolytic intermediates also flux into the PPP where NADPH is formed during the oxidative phase of the pathway in order to support ROS production via NADPH oxidase system [22]. Yet, NADPH, as a universal electron donor, is also used in various anabolic processes [31]. Indeed, glycolytic intermediates fluxed into the PPP are also consumed in the production of nucleotides during the non-oxidative phase of the PPP. Glucose can also enter the TCA as pyruvate where various TCA intermediates may be syphoned off to be utilised in other immunological activities. As an example, accumulation of succinate promotes the release of Il-1β by stabilising HIF-1α [32]. In fact, evidence suggests that succinate can contribute towards pro-inflammatory signalling and ROS production (reviewed [33]). Citrate, another TCA intermediate also promotes immune cell anabolism: Citrate exported from the mitochondria are converted to Acetyl-CoA which fuels lipid synthesis and histone acetylation [34]. Finally, lymphoid cells, such as antibody producing plasma cells, require glucose for energy, biosynthesis of effector components, and the glycation of IgG proteins [35]. These observations establish glucose as a key substrate in energy production, biosynthetic activities and the generation of metabolic intermediates that participate in the regulation of immune function (e.g. HIF-1 stabilisation by succinate). Accordingly, we argue that strict glycaemic control via intensive insulin therapy in critical care patients, who invariably exhibit robust inflammatory states, may result from 'starving' immune cells of insulin [36].

2.2. Anti-inflammatory effects of insulin: cell signalling

The observation that insulin exerts anti-inflammatory effects are also supported in a mechanistic manner. This is well demonstrated by insulin's ability to antagonise the transcriptional activity of FOXO transcription factors. As an example, the role of lineage-specific FOXO1 deletion in transgenic mice has demonstrated that FOXO1 plays a critical role in promoting phagocytosis, chemotaxis and bactericidal functions of neutrophils [37]. Similarly, FOXO1 also promotes the transcription of various pro-inflammatory genes in macrophages [38]. In fact, FOXO1 activity has been shown to play a key role in promoting the polarisation of macrophages towards a pro-inflammatory M1 phenotype [39]. Furthermore, FOXO1 plays an important role in the activation and mobilisation of neutrophils following a pathogen challenge [37]. FOXO1 also play diverse roles in various immune cells involved in the adaptive immune system (reviewed [40]). In this regard, the PI3K-Akt pathway has a well-documented ability to antagonise FOXO1 : Specifically, Akt suppresses the transcriptional activity of FOXO1 by phosphorylation, promoting its association with 14-3-3 proteins which subsequently result in its exclusion from the nucleus [40]. Similarly, insulin treatment promoted mTORC2-mediated phosphorylation of Akt (S473), which in turn phosphorylates FoxO1 (T24) and FoxO3 (T32), culminating in supressed TLR4 signalling in response to LPS in leukocytes [41]. This suggests that insulin may mediate an anti-inflammatory effect via the activation of PI3K/Akt pathway which in turn supresses the transcriptional activity of FOXO proteins.

Another potential mechanism by which insulin may exert its anti-inflammatory effects includes the indirect regulation of insulin on Nrf2 (NF-E2-related factor-2). Nrf2 is a transcription factor that plays a major role in regulating antioxidant defence mechanisms. Under basal conditions, Nrf2 activity is supressed by its association with Keap1, which also facilitates Nrf2 ubiquitination and subsequent degradation. However, under certain stress conditions, such as ROS exposure, thiol groups of Keap1's cysteine becomes oxidised, resulting in the Nrf2 disassociating from Keap1; alternatively, phosphorylated p62 (S349) induces Keap1 to preferentially associate with p62, which is subsequently targeted for autophagic degradation, resulting in the accumulation of Nrf2 which subsequently translocates to the nucleus [42]. In turn, Nrf2 may promote anti-inflammatory effects via a range of distinct mechanisms (recently reviewed [43]). Because NF-κB can be activated by ROS [44], and since Nrf2 is a classic inducer of antioxidant defence mechanisms, it is natural to suspect that Nrf2 may mediate its anti-inflammatory effect by promoting the expression of ROS-scavenging mechanisms. Indeed, macrophages from mice lacking Nrf2 exhibited enhanced NADPH oxidase-dependent ROS generation and elevated TLR4 activation following an LPS challenge [45]. However, it has subsequently been shown that Nrf2 can also directly bind to cis-regulatory elements of key pro-inflammatory genes such as Il-6 and Il-1β, suppressing their transcription by abolishing RNA polymerase II recruitment [46]. Notably, the ability of Nrf2 to antagonise inflammatory cytokine expression was not abolished by antioxidant treatment, thus implicating a ROS-independent mechanism for Nrf2 in regulating inflammation. In this regard, indirect evidence suggests the potential role of insulin signalling in mediating an anti-inflammatory effect through enhanced insulin signalling. Briefly, it was recently demonstrated that phosphorylation of p62 at S351 (a phosphorylation site conspicuously conserved across vertebrates) increased binding affinity of Keap1 for p62 (which targets proteins for selective autophagic degradation), resulting in enhanced degradation of Keap1 and the subsequent liberation of Nrf2 [47]. Ensuing experiments indicated mTORC1 as the putative kinase responsible for p62 phosphorylation [47]. Thus, because mTORC1 is activated by various growth factors, including insulin [48], it is tempting to speculate that mTORC1 activation following insulin binding to its receptor may exert enhanced p62 phosphorylation, thereby enhancing the p62-mediated degradation of Keap1, allowing for the enhanced Nrf2-mediated antagonism of pro-inflammatory processes.

It is also often claimed that insulin may exert anti-inflammatory effects by antagonising the canonical pro-inflammatory transcriptional activity of NF-κB. As an example, earlier studies reported a 45 % decline in NF-κB nuclear translocation in aortic endothelium cells following treatment with 100 μU/mL insulin [49]. Previous studies have similarly demonstrated an anti-inflammatory effect of insulin on macrophages derived from obese individuals, mediated through the ability of insulin to attenuate NF-κB transcriptional activity [50]. In both peritoneal macrophages and RAW 264.7 cells, insulin promoted the expression of

IL-10, suppressed the translocation of NF-κB to the nucleus, and antagonised the expression of pro-inflammatory mediators following an LPS challenge [51].

Notwithstanding these observations, the mechanism by which insulin may antagonise NF-κB remains to be fully elucidated. In streptozotocin induced diabetes, intensive insulin therapy increased inhibitor κB (IκBα) protein (the canonical inhibitor of NF-κB) which is associated with a corresponding decline in NF-κB DNA binding activity [52]. However, it should be noted that these effects may reflect lower serum glucose levels, rather than the direct effect of elevated insulin. This suggest that a decrease in NF-κB signalling may be secondary to insulin's other immune-suppressive mechanisms rather than a direct effect of insulin. That is, insulin may supress other pro-inflammatory inputs, thereby removing impetus for NF-κB activation.

2.3. Insulin and autophagy

Another plausible mechanism by which insulin could alter immune cell function is through its modulating effects on autophagy. Autophagy, being a major catabolic apparatus, is potently inhibited by branch chain amino acids and growth factors, such as insulin, in major insulin-sensitive tissue. Additionally, the autophagic apparatus is also utilised in degrading pathogens, a process term xenophagy [53], as well as the processing and presentation of epitopes on antigen presenting cells such as dendritic cells [54]. More recently, inhibition of mTOR, a canonical inhibitor of autophagy, resulted in an enhancement of neutrophil extracellular trap (NET) formation [55]. These findings suggested that mTOR activators, such as insulin, may compromise NET formation in neutrophils. There is thus strong evidence that induction of autophagy may promote immune function, a notion also supported by the observation that various pathogens have evolved a barrage of subversive strategies to manipulate autophagy [56]. This raises the question: given that autophagy plays a central role in pathogen degradation and processing of antigens for MHC presentation, could insulin, a canonical inhibitor of autophagy, alter these processes? In this regard, it is intriguing to note that culturing macrophages in media devoid of amino acids or serum (including insulin) has been shown to augment phagocytosis and lysosomal degradation of *Mycobacterium tuberculosis* through the manipulation of the autophagic pathway [57]. Similarly, mice treated with rapamycin, a potent inhibitor of mTOR and thus also an effective inducer of autophagy, exhibited an enhanced vaccine response to *Mycobacterium tuberculosis* [58]. There are thus a number of observations implicating the anti-inflammatory functions of insulin, although the underlying mechanisms remains to be fully elucidated (Fig. 2).

3. Insulin promotes immune function

Notwithstanding the preceding discussion, there are also considerable evidence implicating insulin in the enhancement of at least certain immunological functions (Fig. 3). Tsai and co-workers [59], employing a T cell-specific conditional *Insr*-knockdown mouse model, revealed the critical role of insulin in promoting the effector function of T cells. In this setting, insulin was shown to play an indispensable role in driving the metabolic adaptation of T cells towards an effector phenotype that was characterised by elevated levels of aerobic glycolysis and a corresponding increase in glucose uptake. Though insulin-signalling does not appear to be critical under normal conditions, following an immunogenic challenge, T cells lacking *Insr* exhibited compromised epitope expression and an attenuation of pro-inflammatory cytokine production. These results are consistent with earlier reports demonstrating upregulation of insulin receptors following an immunogenic challenge [6]. Mechanistically, it was shown that insulin promotes glucose uptake and also induces the activation of the anabolic PI3K-AKT-mTOR signalling pathway in T cells [59]. Remarkably, there is also evidence to suggest that insulin may enhance a pro-inflammatory state as a result of insulin's effect on regulatory T (Treg) cells. Hyperinsulinemia (10 ng/ml) compromised the ability of Treg cells to secret Il-10 as a result of AKT/mTOR activation [60]. In addition, Tregs isolated from hyperinsulinemic obese mice, exhibited elevated expression of IFN-γ and supressed IL-10 release [60]. This suggests that insulin may be pro-inflammatory by antagonising the function of anti-inflammatory Treg cells. Collectively, these observations raise the prospect that insulin may promote a pro-inflammatory tone in T cells.

There is also evidence that insulin may promote natural killer (NK) cell function. As an example, treatment of NK cells (20 IU/mL) with insulin promoted the expression of INF-γ and also increased cytotoxicity towards LX2 cells (a human hepatic stellate cell) [61]. Conversely, either hypoinsulinemia, or suppression of insulin receptors promoted NK cell death [61]. Notably, the mechanism by which insulin enhanced NK cell function seems to be mTOR-dependent as rapamycin (mTOR inhibitor) not only antagonised cytotoxicity, but also downregulated insulin receptor expression on NK cells. Thus, NK cells, similar to T cells (also derived from lymphoid progenitor cells), seem to be dependent on insulin signalling via the mTOR pathway [61], suggesting that insulin may be particularly relevant to these cells. However, though B-1 cells (also lymphoid derived) do transcribe insulin receptors, insulin did not seem to have an effect on the production of natural antibodies [62]. Here it is worth noting that this particular study made use of db/db mice (i.e. lacking leptin receptor) and, because leptin influences various aspects of the immune system [63], the extent to which these findings may be generalised remains to be established. Collectively, these observations suggest that insulin may play a pro-inflammatory effect on at least certain lymphoid-derived immune cells.

Insulin may also augment the immune response of the innate immune system. Although insulin treatment had no effect of bone marrow derived macrophages (BMDM) of diabetic (alloxan treated) mice, insulin did potentiate LPS-mediated release of cytokines (Il-6 and TNF) [64]. This same insulin-potentiating release of cytokines was also observed in alveolar and peritoneal macrophages derived from these diabetic mice [64].

Insulin has also been implicated in promoting ROS production. As an example, pre-incubation of neutrophils with 1 nM insulin did not induce a respiratory burst, but instead primed neutrophils for more robust ROS production following a N-formylmethionyl-leucyl-phenylalanine (fMLP) challenge [65]. In human subjects receiving a hyperinsulinemic-euglycemic clamp, insulin supressed ROS production by macrophages, while increasing ROS production in neutrophils [66]. It is tempting to speculate that the 'priming effect' of insulin on neutrophil ROS production might be mediated by insulin-induced glucose uptake [67]. In this scenario, glucose loading, which occurs because insulin-mediate glucose uptake, promotes the formation of NADPH: Glycolytic intermediates fluxed into the oxidative phase of the pentose phosphate pathway (PPP) are used to convert NADP + to NADPH [68], which in turn may be used to produce ROS via the NADPH-oxidase (Nox) system [69]. However, it remains unclear why macrophages are not similarly affected, since LPS-stimulation also upregulated the PPP in macrophages [70]. In fact, it has been shown that hyperglycaemia can also activate the NADPH-oxidase system in macrophages [71].

In addition to promoting NADPH production (necessary for Nox function) recent findings also suggested two additional pathways for insulin-mediated activation of Nox2 [72]. Firstly, hyperinsulinemia (1 nmol/L) resulted in 3-fold increase in Nox2 protein levels. Secondly, insulin promoted $P47^{phox}$ phosphorylation which subsequently result in Nox2 binding to the P47 regulatory element, thus promoting Nox2 activation. Similar results have been reported for human fibroblasts where insulin stimulated PI3K-dependent ROS production [73]. However, the mechanism by which insulin promotes both the expression and activity of Nox2 remains to be fully elucidated. Additionally, mTOR have been show to promote mitochondrial ROS production, thereby promoting NLRP3 inflammasome activation in an NF-κB-independent manner [74]. Significantly, because ROS have been implicated in

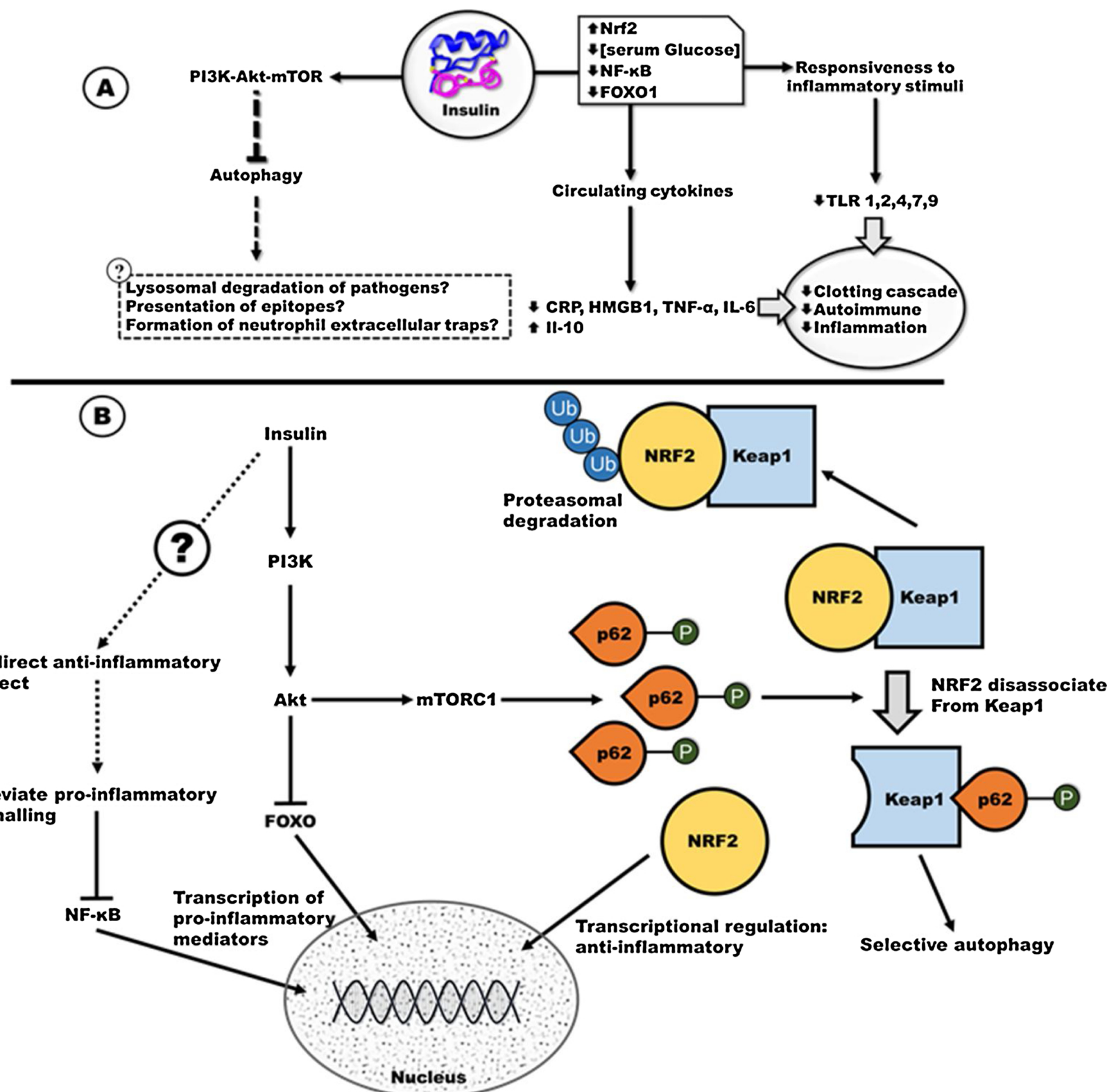

Fig. 2. Although insulin has a well-established role in attenuating inflammation (**A**), the exact mechanisms by which insulin signalling mediates these remain to fully elucidated (**B**).

canonical inflammatory pathways such as NF-κB [44] and inflammasome [75], insulin stand poised to potentiate the activation of these cardinal pro-inflammatory pathways.

Insulin has also been implicated in immune cell differentiation (Fig. 3A) as insulin signalling has been shown to direct the differentiation of multipotent progenitor cells in bone marrow towards a lymphoid cell lineage [76]. Specifically, mice lacking *Insr* exhibited increased differentiation of pluripotent cells towards myeloid lineage at the expense of lower levels of lymphoid cells [76], suggesting that insulin polarised the immune cell differentiation towards a lymphoid-dominated immune response. Very similar results have also been reported in monkeys, where streptozotocin treatment resulted in lymphopenia [77]. Since streptozotocin results in the depletion of insulin-producing β-cells, it is tempting to speculate that the supressed neutrophil counts may relate to the streptozotocin-induced compromised insulin signalling. These observations implicate insulin in promoting the differentiation of myeloid-derived immune cells such as neutrophils and macrophages.

However, the preceding observations stand in contrast to findings in patients suffering from T1D, where a decline in circulating neutrophils (which are myeloid-derived cells) already manifest in the pre-clinical phase and “persists for some years before long-term resolution” [78]. The decrease in neutrophil counts in patients with T1D did not occur as a result of increased attrition (e.g. resulting from apoptosis) or neutrophil-specific antibodies, but rather “seems to mirror the continuing destruction of β-cell mass” [78]. The observation that a decline in neutrophils mirrored a decrease in β-cell mass suggests that insulin may act as a trophic factor for neutrophils. In this scenario, the decrease in β-cell mass led to a decline in insulin, precipitating in suppressed neutrophil development. Supporting this view, earlier studies in healthy human subjects have found that administering insulin caused an abrupt increase in circulating levels of leukocytes which seemed to be driven by a specific increase in the number of circulating neutrophils (and not as a result of decreased neutrophil apoptosis) [79]. Significantly, however, these authors also noted that insulin can suppress the expression of various adhesion molecules of neutrophils [79]. This raises the possibility that insulin does not act as a trophic factor, but instead increases neutrophil counts by down regulating adhesion molecules which results in the release of neutrophils from the bone marrow niche. Thus, it is not clear if insulin promotes neutrophil counts by either increasing the number of neutrophils produced in bone marrow, or if insulin causes a rise in neutrophil count as a result of more neutrophils entering circulation.

Earlier studies have implicated insulin signalling as playing an

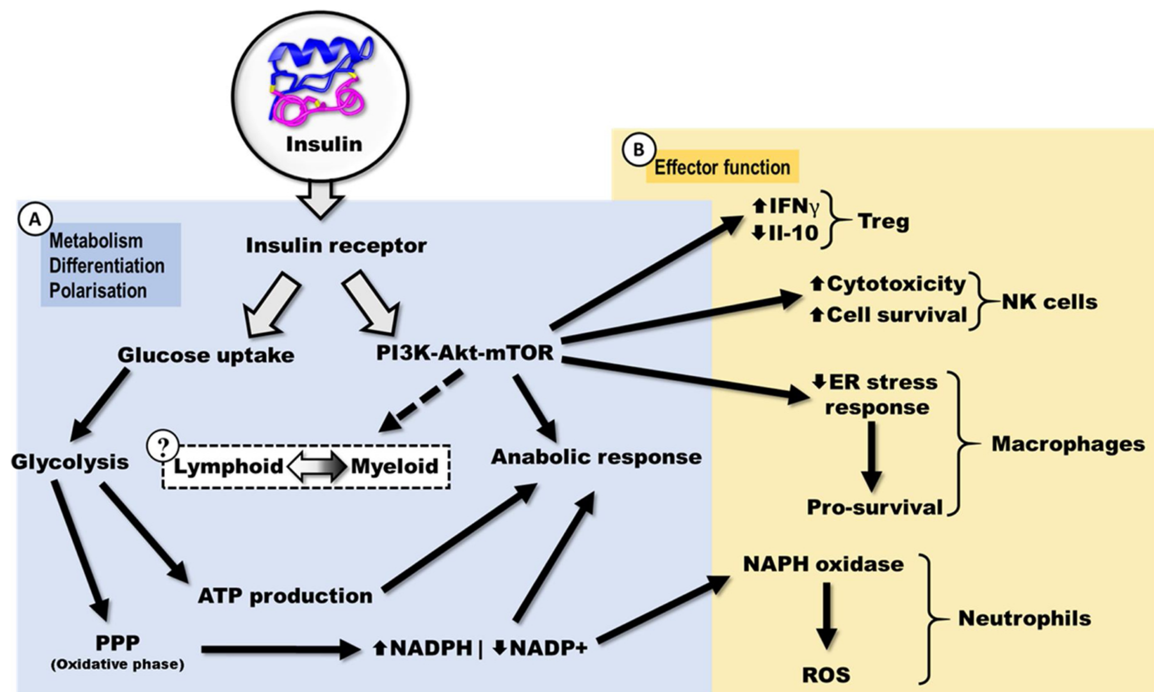

Fig. 3. Insulin regulates a range of immunological functions in various immune cells.

important pro-survival function in macrophages. In transgenic mice prone to form atherosclerotic lesions, *Insr*$^{-/-}$ bone marrow transplants resulted in the arteriosclerotic plaques populated by *Insr*$^{-/-}$ macrophages that exhibited decreased Akt signalling with increased apoptosis as a result of an enhanced ER stress response [80]. Since *Insr*$^{-/-}$ bone marrow gives rise to functional macrophages, insulin signalling must be dispensable for differentiation, but necessary for long term survival, suggesting an immune-enhancing function. However, it is important to note that increased macrophage survival does not necessarily indicate that insulin promotes macrophage function. That is, the fact that insulin exerts a pro-survival function in macrophages does not necessarily translate to enhanced immune cell function, as un-activated immune cells may also exhibit enhanced survival. It is also not obvious to which extent the unique biology of plaque-forming macrophages translate to macrophages in general.

4. How can insulin be both pro and anti-inflammatory?

4.1. Isoform and signalling crosstalk

A major contributing factor to the diverging effect solicited by insulin likely relate to the fact that insulin signalling may crosstalk with other immunological pathways active in a cell-type specific manner (Fig. 4). This is well exemplified by the observed crosstalk between the JAK/STAT pathway (activated by cytokines and growth factors) and the insulin signalling pathway. As an example, Il-6 induces classical activation (non-inflammatory) via Il-6 recognition of glycoprotein 130 (gp130) or trans-activation (soliciting a pro-inflammatory response) by first binding to a soluble IL-6 receptor (IL-6R) before binding to gp130. Notably, while Il-6 activated JAK/STAT3 through the classical pathway in human vascular endothelial cells, trans activation also activated Akt (S473 phosphorylation) via PI3K [81]. This observation suggests that insulin may promote the pro-inflammatory signalling function of Il-6 by Akt phosphorylation, thereby promoting a 'trans-activated' phenotype to IL-6 signalling. Co-operative interaction between insulin signalling and STAT is also evident in immune cells. The profound increase in glycolysis observed in naïve T cells following T cell receptor (TCR) binding is mediated by Akt, but also requires STAT5, which promotes glutaminolysis (i.e. the consumption of glutamine to replenish carbon loss from the TCA from syphoning of TCA-intermediates used to sustain anabolism) in an mTORC1-dependent manner [82]. Similar crosstalk between downstream signalling effectors of insulin and STAT is also observed in innate immune cells. As an example, M1 polarisation of intestinal macrophages have been shown to be dependent on hyper-activation of STAT1 and 3 downstream of PI3K/Akt/mTORC1 signalling cascade [83]. Taken together, the emerging view is that crosstalk between insulin signalling pathway (PI3K/Akt/mTOR) and canonical cytokine signalling pathways (JAK/STAT) allow for the detailed tailoring of immune response and likely also contribute towards the observed pleiotropic effects of certain immune effectors such as Il-6.

Another factor contributing to the diverging roles of insulin on immune cell function involves the fact that the majority of proteins in the insulin signalling pathway are expressed as different isoforms, suggesting a putative mechanism by which cell-specific immunological processes may be solicited. As an example, insulin receptor substrate (IRS) are expressed as either IRS1 or IRS2. Here, evidence suggest that the relative expression of IRS1 versus IRS2 may relay immune-cell specific effects, as is illustrated by the observation that Il-4 selectively activates IRS2 but not IRS1 in macrophages [84]. Earlier studies have also suggested that IRS2 activation by Il-4 can potentiate the release of Th2 cytokines (i.e. promote humoral immunity) [85], suggesting that both lymphoid and myeloid cell function are impacted on by IRS isoform expression. Similar, the class I PI3K forms heterodimers between catalytic subunits (p110α, p110β, p110δ and p110γ) and different isoforms of the regulatory subunits (p85α, p55α, p50α, p85β and p55γ) [86]. While the α and β regulatory subunits are ubiquitously expressed, the γ or δ are predominantly expressed by immune cells. Isoform-specific knock-out studies have established that these isoforms may exert distinct functions. As an example, in LDLR$^{-/-}$ mice, loss of p110γ resulted in stunted macrophage proliferation (but did not influence polarisation) while in contrast, T cell proliferation was unaffected by p110γ loss [87], suggesting perhaps isoform-specific functions in myeloid versus lymphoid cells. There is also strong evidence to suggest that Akt isoforms may play a formative role in directing immune cell phenotype. In mice, ablation of Akt1 resulted in increased M1 macrophage polarisation, while deletion of Akt2 promoted the M2 phenotype

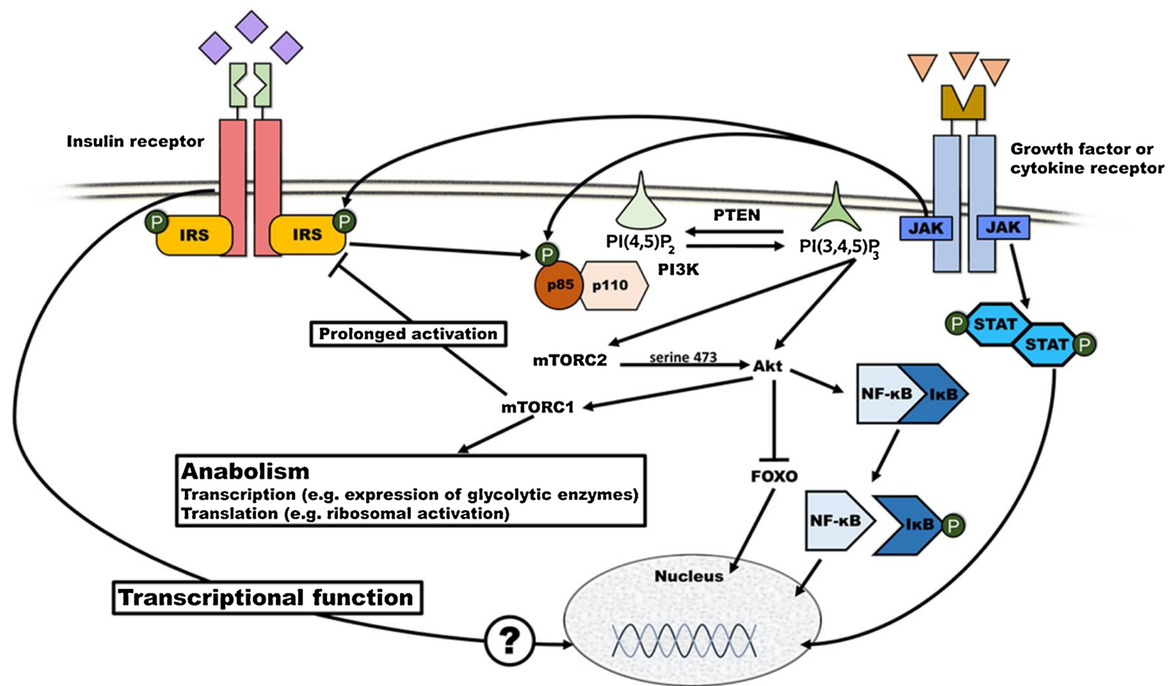

Fig. 4. Insulin signalling pathway intersect and converge on other signalling molecules and also influences transcription both directly and indirectly.

in a cell-autonomous manner [88]. Similar findings have been reported for neutrophils [89]: neutrophils derived from Akt2-knockout mice exhibited a decrease in superoxide production, cell migration, and compromised granule enzyme release, suggesting that the Akt2-isoform may play a key role in promoting a pro-inflammatory phenotype in myeloid cells. It is thus evident that there are differential expression of specific isoforms in immune cells with functional consequences. Since immune cells co-express different isoforms, it likely that the ratio between specific isoforms may contribute to immune cell heterogeneity and contribute to the immunological response solicited by insulin.

Finally, it has recently been shown that, in mouse liver, insulin stimulation resulted in the nuclear translocation of insulin receptor (IR) [90]. IR was found to associate with RNA polymerase II and HCF-1 (Host cell factor-1) on genomic regions that mostly corresponded to promoter regions. Notably, IR-targeted genes were enriched for proteins usually associated with various metabolic functions, but intriguingly, also immune responses. As an example, IR was found to bound to promoter regions of genes involved in the adaptive immune system, MHC I antigen processing and presentation, B cell receptor signalling and genes associated with viral infections such as influenza and HIV (see Fig. 3 A and B in [90]). The transcriptional activity of IR induced by insulin thus suggest another level of complexity that needs to be addressed in order to explain the divergent roles of insulin on the immune system.

4.2. Dose and time

As pointed out, one key reason for these diverging effects related to the effect of insulin on glucose: while intensive insulin therapy may lower glucose levels, thereby starving immune cells of glucose, milder dosages of insulin may actually enhance immune function by promoting the uptake of glucose by immune cells. This strongly implicates a dose-dependent effect of insulin and suggests that part of the differential effects on insulin may relate to varying dosages of insulin exposure. Additionally, there is reason to suspect that insulins' effect may also depend on duration of exposure. As an example, it is know that insulin release normally follows an oscillating pattern [91], and that high-dose insulin can increasing the period between cycles (i.e. cause a delay in insulin release) [92]. Moreover, chronic exposure to insulin actually stimulated decreased insulin signalling by promoting the inactivation of IRS1 by mTORC1 and mTORC2 [93]. As a result, both the duration of exposure, as well as the duration between insulin exposure and measurement of a response, likely impact on immune cell phenotype/response.

4.3. Model systems and their limitations

The interaction between the immune system and insulin have, until very recently, predominantly focused on the pathology of chronic immune activation on insulin sensitivity (i.e. inflammation-mediated insulin resistance) and glucose homeostasis. A major workhorse in these studies include obesogenic mice, specifically ob/ob (leptin deficient) and db/db (leptin receptor-deficient) mice. But, as mentioned, leptin exert profound effects on both innate and adaptive immune cells [63]. As an example, earlier studies have shown that in macrophages, leptin promotes diverse cellular functions, including phagocytosis of *C. parasilopsis* and the release of TNF and Il-6 [94]. In human B cell, leptin promotes the release of pro-inflammatory cytokines IL-6 and TNF-α, but also Il-10 [95]. Similarly, leptin promotes the proliferation of human T cells and also potentiates the expression of IL-2 and IFN-γ [96]. Furthermore, JAK2 activation by leptin have been shown to enhance signalling through the PI3K/Akt/mTOR pathway in various cancers, including colorectal carcinoma [97], indicating that leptin and insulin signalling may converge/synergise. Given the wide range of immune cell function impacted on by leptin, these mice probably do not represent optimum model systems for studying insulin's immunological functionality.

Another major confounding factor when evaluating the effect of insulin on the immune system relates to the diabetic model implemented. Models emulating T1D often make use of animals treated with either Streptozotocin or Alloxan. In this regard, evidence indicated that both these agents exerted direct and indirect effects on the immune system. As an example, splenocytes treated *in vitro* with Streptozotocin undergo apoptosis and also abolish the proliferation of these cells following activation, whereas Treg cells are somewhat more resistant to this effect: as a result, Streptozotocin mice exhibited some level of

immunodeficiency [98]. In contrast, Alloxan have been argued to only transiently alter lymphocyte function and is therefore a more applicable system for immunological studies [99]. It should be stressed however that these findings are somewhat antiquated, warranting reinvestigation utilising more sensitive assays. Specifically, these earlier studies did not take the broad spectrum of immune cell heterogeneity into account and it remains possible that alloxan treatment may impose subtle changes in immune cell polarisation that was not taken into consideration by earlier studies.

Also, diseases associated with altered insulin/glucose homeostasis may also be accompanied by other co-morbidities, such as elevated chronic low-grade inflammation, which would necessarily also impact on immune cell function. Notably, these settings are also invariably associated with altered lipid profiles, which may also impact on immune cell phenotype. As an example, hypercholesterolemia resulted in lower mitochondrial mass with a corresponding decline in maximal respiration and affects the levels of metabolites such as itaconate in macrophages derived from mice fed a high fat diet [70]. Similarly, saturated fatty acids may activate TLR 2 and 4, thereby promoting inflammation [100]. Thus, other metabolic disturbances as well as the prevailing para-inflammatory context (e.g. inflammation of adipose deposits) may render it difficult to translate findings form one setting to another (e.g. T1D versus gestational diabetes).

5. Immunological regulation of insulin

Further implicating insulin as an immune-regulatory hormone is the observation that insulin not only impacts on the immune system, but that the immune system also influences serum insulin levels (Fig. 5). As an example, both a bolus injection of LPS as well as chronic administration via an osmotic pump, resulted in increased glucose-stimulated insulin secretion [101]. Indirectly, inflammatory mediators such as Il-1β and Il-6, in particular, have been shown to promote the expression of glucagon-like peptide-1 (GLP-1) [102], an incretin with a well-established role in promoting insulin release [103]. Another indirect mechanism by which inflammatory mediators may promote the release of insulin is via the increase of glucagon. In the presence of epinephrine, Il-6 enhances the release of glucagon [104]. In this regard, glucagon is also well known to stimulate the release of insulin [105]. Inflammation, via indirect signalling mediators such as glucagon and GLP-1, may thus enhance serum insulin levels.

Evidence suggests that the ability of inflammatory mediators to induce insulin secretion may be context dependent. As an example, under low glucose conditions, HMGB1 at a 200 − 500 ng/ml range promoted the release of insulin by INS-1 cells in a dose-dependent manner [106]. Though TNF does not acutely (30 min) diminish insulin secretion, prolonged (24 h) exposure did attenuate the release of insulin from cultured β-cells [15]. Mechanistically, JNK-p38MAPK-NF-κB inflammatory pathways have been implicated as downstream mediators of TNF activation, which prevents the glucose-mediated influx of Ca^{2+} which normally triggers insulin release [107]. Similarly, short term (2 h) exposure of Il-1β within the 5–500 pmol range, resulted in increased expression of insulin, whereas prolonged exposure (6 h) was associated with suppressed insulin release [108]. Here, recent studies implicated NF-κB transcriptional activity as a key mediator of the inhibitory effects of Il-1β on insulin release by β-cells [109]. Similarly, a sustained inflammatory insult may actually suppress insulin release by inducing apoptosis via the intrinsic pathways [110]. Thus, prolonged inflammation may result in suppressed insulin levels.

Another potential mechanism by which the immune system may enhance serum insulin levels is by supressing the clearance of insulin by insulin degrading enzyme (IDE). Nitric oxide (NO), often induced by inflammatory factors has been shown to inactivate IDE [111]. TNF initially induces a modest increase in IDE, followed by a more pronounced suppression of IDE protein levels in liver cells [112]. In mice liver cells (1c1c7 cells), glucagon is capable of inducing the expression of IDE [112]. As mentioned, inflammatory mediators may potentiate glucagon, suggesting another indirect mechanism by which inflammatory mediators may increase IDE activity. Interestingly, though IDE is ubiquitously expressed in various human tissue, it is not expressed in circulating lymphocytes but rather in other immune cells such as alveolar macrophages [113]. While IDE has diverse functions, it is not known if this expression pattern correlates with insulin removal. Also, more detailed studies are needed to provide a better understanding of the effect of inflammatory mediators on IDE activity and its potential effects on insulin.

6. Conclusion

Though it has been known for more than half a century that immune cells express insulin receptors, the functional role of insulin as an immunological mediator remains poorly understood. The emerging view is that insulin does not simply exert an anti-inflammatory effect, but rather that insulin differentially affects different immune cells, depending on their state of activation. A better understanding of how insulin impacts the immune system may be critical in the optimisation

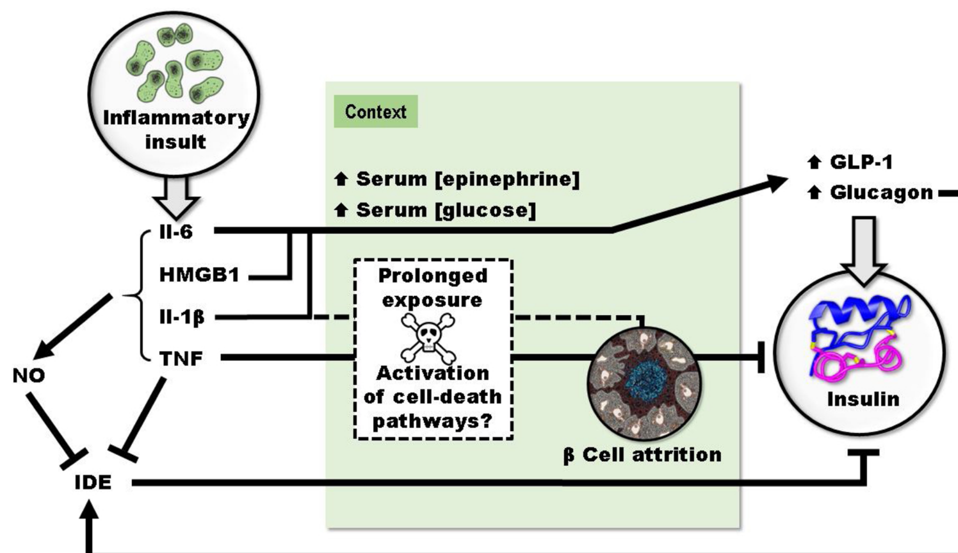

Fig. 5. Inflammatory mediators modulate insulin release both directly and indirectly in a context-dependent manner.

of therapeutic interventions in patients that also require glycaemic control.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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