

## REVIEW

# Diabetes and susceptibility to infections: Implication for COVID-19

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## Abstract

A number of mechanisms have been proposed to explain the well-established link between diabetic status and an increased susceptibility to infection. Notably, diabetes has been shown to be one of the strongest factors influencing healthcare outcome in COVID-19 infections. Though it has long been noted that lymphocytes upregulate insulin receptors following immune activation, until recently, this observation has received little attention. Here, we point out key findings implicating dysregulated insulin signalling in immune cells as a possible contributing factor in the immune pathology associated with diabetes. Mechanistically, insulin, by activating the PI3K/Akt/mTOR pathway, regulates various aspects of both myeloid cells and lymphocytes, such as cell survival, metabolic reprogramming and the polarization and differentiation of immune cells. PI3K signalling is also suppressed by immune checkpoint proteins, suggesting that insulin signalling may antagonize peripheral tolerance. Remarkably, it has also recently been shown that, following insulin binding, the insulin receptor translocates to the nucleus where it plays a key role in regulating the transcription of various immune-related genes, including pathways involved in viral infections. Taken together, these observations suggest that dysregulated insulin signalling may directly contribute to a defective immune response during COVID-19 infections.

## KEYWORDS

COVID-19, diabetes, immune checkpoint blocker, inflammation, insulin, insulin resistance

## INTRODUCTION

While the 1918 influenza pandemic was associated with an increased mortality in young adults [1], COVID-19 is remarkably less lethal in the younger population, with most adverse effects observed in the older individuals. Reports indicate that ‘children were as likely to be infected as adults’ [2], suggesting that the lower mortality rate does not seem to be driven by lower rates of infection in children. A number of reasons have been proposed for the lower

disease severity in children, including lower expression of angiotensin-converting enzyme 2 in the respiratory tract of children [3]. Children who were hospitalized often exhibited underlying comorbidities. Of particular interest is the observation that amongst children who were hospitalized ‘obesity was notable as a comorbidity’ [4]. In fact, it is evident that T2D strongly predisposes to a more unfavourable disease trajectory with a number of proposed mechanisms underlying this observation in adults [5–8]. Indeed, it has long been argued that diabetes associated with immune

**Abbreviations:** CTLA-4, cytotoxic T-lymphocyte antigen 4; FOXO4, forkhead box O; HIF-1 $\alpha$ 4, hypoxia-inducible factor 1-alpha; ICPs4, immune checkpoint proteins; INSR, insulin receptor; mTOR4, mammalian target of rapamycin; PD-14, programmed cell death protein 1; PI3K4, phosphoinositide 3-kinases; T2D4, type 2 diabetes.

dysfunction in the context of increased oxidative stress, mitochondrial dysfunction, hyperglycaemia and the formation of advanced glycation end products. Another level of complexity related to the fact that T2D may be associated with other risk factors, such as old age and obesity. As an example, although the CORONADO study clearly established a key role for T2D in impacting on a more severe disease course, it should be noted that most patients were also old (mean age  $69.8 \pm 13$  years). Furthermore, BMI was also reported as an independent prognostic factor for predicting disease severity [7].

Interestingly, a number of recent observations have implicated insulin in regulating critical aspects of various immune cells. Here, we review various aspects of immune cell function that may be dysregulated as a result of defective insulin signalling in T2D. Collectively, these observations suggest that altered insulin homeostasis may play an important role in directing the course of COVID-19 infections.

## INSULIN: NOVEL IMMUNOLOGICAL IMPLICATIONS

Though insulin is well known for its rapid signal transduction via the PI3K/Akt pathway, it was recently shown that insulin also regulates transcriptional events [9]. Following binding of insulin, the insulin receptor complex translocates to the nucleus where it associates with RNA polymerase II. Importantly, nuclear translocated insulin receptor complex regulates the transcription of various genes involved in adaptive immunity such as *MHC-I*. Furthermore, other disease pathways that are also regulated include the hosts' response to viral infections, such as the influenza life cycle and HIV infections [9]. It was also demonstrated that diabetes has an impact on insulin's transcriptional activity, as *ob/ob* mice exhibited altered chromatin remodelling [9]. Given that insulin regulates gene networks involved with adaptive immunity and viral responses, and in the light of the fact that insulin resistance alters the transcriptional function of insulin, it is clear that more detailed studies on insulin's transcriptional role in immune genes are warranted.

Using a hyperinsulinaemic–euglycaemic clamp, insulin was shown to regulate the transcription of various unexpected pathways in muscle and liver, including Notch signalling [10]. Of note, Notch signalling is involved in various immune-related functions, including antigen presentation by mast cells [11] (which also express insulin receptors [12]), activation and differentiation of T cells [13], and lineage commitment in monocytes [14]. These observations raise the possibility that insulin may also impact on immune function via Notch signalling. Collectively, these observations implicate insulin in mediating immune regulatory functions not

directly associated with its canonical metabolic regulatory effect.

## INSULIN'S EFFECT ON MYELOID CELLS

Both macrophages and neutrophils express insulin receptors and are responsive to insulin [15]. However, in healthy human subjects, neutrophils were more responsive and exerted greater chemotaxis towards formyl-Met-Leu-Phe, phagocytosis and bactericidal activity. Another differential effect of insulin on macrophages vs. neutrophils reported in this study includes the ability of insulin to stimulate ROS production in neutrophils, while suppressing ROS production in macrophages [15]. Notably, the effect of insulin was blunted in aged individuals. In mice, insulin enhanced the LPS-mediated release of TNF and IL-6 in macrophages, while wortmannin, a PI3K inhibitor, counteracted this effect [16]. However, in tissue-resident macrophages, insulin suppressed LPS-mediated TNF and IL-6 in alveolar macrophages, while suppressing IL-6 and IL-1 $\beta$  in peritoneal macrophages, indicating that the tissue compartment might implicate the specific response of macrophages to insulin.

Mechanistically, insulin may solicit a pro-inflammatory phenotype in innate immune cells by upregulating the activity of hypoxia-inducible factor 1- $\alpha$  (HIF-1 $\alpha$ ). Earlier studies have reported that a pro-inflammatory phenotype in myeloid cells requires HIF-1 $\alpha$  [17], a transcription factor that regulates the expression of various innate immune genes [18]. In particular, mammalian target of rapamycin (mTOR) complex 1, downstream of the phosphoinositide 3-kinases (PI3K) and Akt, has been shown to be critical in promoting HIF-1 $\alpha$  activity, through the expression of various glycolytic enzymes [19]. This observation suggests that insulin, via PI3K/Akt/mTOR pathway, may also mediate a pro-inflammatory response by prompting HIF-1 $\alpha$  activity in response to enhanced mTOR activity. However, there are conflicting reports on the effect of insulin on macrophages. As an example, in the context of wound healing, insulin has been reported to down-regulate pro-inflammatory responses in macrophages [20]. In contrast, U937 macrophages exposed to insulin solicited an increased release of pro-inflammatory cytokines, including IL-1 $\beta$ , IL-8 and CCL2 [21]. This might suggest a modulating role of the microenvironment in the response of macrophages to insulin. Another consideration is that various isoforms of the signalling molecules relaying insulin signalling are expressed in immune cells. As an example, Akt2 deletion in macrophages is associated with M2 polarization, whereas Akt1 ablation prompted M1 polarization [22]. Thus, the differential expression of Akt isoforms may pivot the effect of insulin in these immune cells.

A state of insulin resistance has been reported in macrophages and is associated with an anti-inflammatory M2 polarization [23]. Here, chronically elevated insulin (either *in vivo* or in diet-induced glucose-intolerant mice) resulted in decreased insulin signalling, manifesting with decreased Akt2 phosphorylation and a more M2-like phenotype. However, in contrast to this study, it was reported that macrophages derived from insulin-resistant obese *db/db* mice exhibited a pro-inflammatory phenotype characterized by enhanced IL-1 $\beta$  transcription [24]. There are thus conflicting results on the effect of insulin resistance in macrophages. Elucidating the consequence of insulin resistance in immune cells has direct clinical implications. As an example, do elevated levels of insulin seen during the early phase of T2D result in increased insulin signalling in immune cells, or does insulin resistance in immune cells impede insulin signalling? Do tissue-resident macrophages exhibit disparities in their inflammatory phenotype as a result of insulin resistance?

## INSULIN REGULATES T- AND B-CELL FUNCTION

Recent findings have implicated insulin in regulating diverse functions in T cells. In Treg cells, insulin-mediated activation of the Akt/mTOR signalling pathway resulted in suppressed IL-10 release; however, insulin did not impact on other anti-inflammatory regulators such as cytotoxic T-lymphocyte antigen 4 (CTLA-4), CD39 and TGF- $\beta$  [25]. This observation may also underlie the observation that Treg-specific deletion of the insulin receptor (INSR) protects against diet and age-associated metabolic syndrome [26]. In this setting, deletion of insulin receptors on Treg cells may prevent insulin-mediated suppression of Treg's anti-inflammatory function. In turn, this Treg-specific deletion of insulin receptors resulted in enhanced anti-inflammatory function, thus preventing the diet and age-induced inflammation that precipitate in inflammation-mediated metabolic derangement. Taken together, these observations implicate a potential pro-inflammatory effect of insulin. One likely mechanism by which insulin signalling antagonizes Treg function might relate to an increased activity of Akt. It has long been known that Akt can inhibit forkhead box O (FOXO) protein transcriptional activity by phosphorylating these proteins, resulting in their nuclear exclusion [27]. In this regard, FOXO proteins play a critical role in both Treg development and function [28]. As an example, FOXO1 deletion impedes Foxp3<sup>+</sup> regulatory T (Treg)-cell development and also results in a dramatic decline in CTLA-4 receptor expression [28]. Thus, enhanced insulin signalling may exert a pro-inflammatory effect by enhancing Akt activity, which subsequently results in the phosphorylation of FOXO proteins and a decline in Treg function.

Insulin signalling has also been shown to play an indispensable role in mediating effector function in CD8<sup>+</sup> and CD4<sup>+</sup> T cells. In mice, the T-cell-specific deletion of INSRs resulted in defective influenza viral clearance [29]. Mechanistically, insulin signalling plays an indispensable role in activating the PI3K/AKT/mTOR pathway in T cells necessary for engaging effector functions [29]. Similar results have been reported in a rat model of autoimmune disease [30]. In this setting, the T-cell-specific deletion of INSRs was protective against experimental autoimmune encephalomyelitis. The observation that the insulin mediated the activity of T cells also correlated with the fact that CD28 also activated PI3K [31]. Because CD28 plays an indispensable role as a costimulatory signal that promotes T-cell activation, and given that CD28 also activates PI3K, it strongly implicates that insulin may augment T-cell function via a synergistic activation of PI3K. These observations clearly implicate downstream insulin signalling as an important role player in T-cell function.

Mechanistically, insulin seems to play a key role in mediating the metabolic reprogramming that is necessary for T-cell function. Firstly, insulin-activated PI3K regulates a range of downstream cascades that are involved in glucose and amino acid uptake [32]. However, evidence suggests that insulin not only promotes the uptake of nutrients but also regulates the aspects of glycolysis and aerobic metabolism necessary for T-cell proliferation and cytokine production. As an example, in CD4<sup>+</sup> T cells, mTOR activation is necessary for regulating the steady-state levels of HIF-1 $\alpha$  or HIF-2 $\alpha$ , which in turn is necessary for cytokine production involved in mediating effective humoral immunity [33]. In CD8<sup>+</sup> T cells, a very similar role in mTOR-mediated activation of HIF-1  $\alpha$  has been reported [34]. HIF-1 is an established transcription factor for enzymes involved in glycolysis in T cells [35], thus exemplifying the link between T-cell function and metabolism. Taken together, these observations mechanistically implicate a crucial role for insulin signalling in activated T cells by driving the metabolic phenotype necessary for a competent effector response.

Interestingly, it was noted early on during the COVID-19 pandemic that lymphopenia correlated with disease severity [36] and often associated with T-cell dysfunctions, including lymphopenia and T-cell exhaustion [37,38]. This observation was also confirmed in the CORONADO study that similarly reported a number of biological parameters that reflected disease severity, including lymphopenia [7]. These observations suggest that defective T-cell function may play a key role in mediating an unfavourable prognosis for patients with COVID-19 infections. Given the link between T2D and a more severe disease trajectory, it is tempting to speculate that dysregulated insulin signalling may play a major role in the manifestation of dysfunctional T-cell activity. As an example, could hyperinsulinaemia in T2D promote a form of insulin resistance in T cells?

Importantly, T cells do not express insulin receptors during rest and only upregulate insulin receptors following CD3/CD28 stimulation [29], suggesting that these cells would be insensitive to the prevailing hyperinsulinaemia during the early phases of T2D. However, as in patients with more advanced T2D, a decline in insulin production might result in defective PI3K/Akt activation in T cells. Supporting this view, the CORONADO study also reported lower mortality in patients receiving metformin, though this was argued to reflect a less advanced stage of diabetes rather than metformin *per se* [7].

Finally, insulin is also implicated in B-cell function, suggesting that dysregulated insulin signalling may adversely affect a competent antibody response. It has long been noted that both B and T lymphocytes upregulate insulin receptors following cell activation [39]. Insulin promotes the growth and proliferation of myeloma cells, implicating insulin as a potent trophic factor in B cells. Specifically, it was reported that insulin signalling through insulin/IGF-1 hybrid receptor activation promoted myeloma proliferation [40]. This observation is consistent with the already well-established role of PI3K in B-cell function (reviewed in ref. [41]). Notably, the PI3K pathway is activated following B-cell receptor engagement, which is also enhanced by CD19, a key coreceptor that, as expected, also activates other canonical insulin signalling molecules, such as Akt and mTOR [41]. Very similar to T cells, PI3K/Akt/mTOR components also seem to play critical roles in B-cell survival and metabolic reprogramming associated with glucose utilization [42]. In addition to fuelling the metabolism of antibody synthesis, glucose is also consumed during the glycosylation of antibodies [43].

In summary, there is clear evidence implicating insulin signalling in T cells and most likely also in B cells. However, how T2D impacts on these signalling cascades is currently unknown. A compensatory increase in insulin early during T2D may suggest enhanced insulin signalling in T cells, whereas more advanced states of T2D may be associated with pancreatic  $\beta$ -cell depletion and a corresponding decline in insulin. Another possibility is that T and B cells of T2D patients may exhibit a form of insulin resistance. Because both B and T lymphocytes upregulate insulin receptors only after cell activation [39], it may be expected that these cells are less likely to be insulin-resistant. However, there is evidence that suggests a state of insulin resistance in T cells of obese diabetic patients [44]: Here, supra-physiological doses of insulin resulted in increased Akt phosphorylation and a decreased Th1/Th2 ratio in healthy controls, but not in obese patients with diabetes, suggesting insulin resistance may attenuate Akt activation. Elucidating the role of altered insulin sensitivity in immune cells may thus cast light on the immune pathology in T2D and explain the unfavourable disease trajectory associated with COVID-19 in patients with diabetes.

## INSULIN SIGNALLING AND IMMUNE CHECKPOINT PROTEINS

Central tolerance is not completely effective with some residual self-reactivity being unavoidable [45]. Here, peripheral tolerance plays an important role in limiting the immune response to self-antigens. Accordingly, whereas antigen presentation on MHC provides specificity, immune checkpoint proteins (ICPs) play an indispensable role in directing the magnitude of the immune response. This is well exemplified by the observation that ICP inhibitors utilized in cancer therapy often result in the manifestation of autoimmune diseases [46]. Mechanistically, ICP attenuates T-cell effector function, thereby exerting an intrinsic anti-inflammatory effect and promoting tolerance. In this regard, emerging evidence implicates insulin in regulating the same signalling cascades antagonized by ICP.

As mentioned, insulin-mediated activation of PI3K/Akt plays a critical role in mediating the metabolic reprogramming necessary for T-cell effector function. Remarkably, ICPs such as programmed cell death protein 1 (PD-1) and CTLA-4 both antagonize PI3K activity. PD-1 antagonizes PI3K activity by increasing the phosphatase activity of PTEN [47], the canonical negative regulator of PI3K. CTLA-4 may negatively regulate PI3K via indirect mechanisms. As mentioned, CD28, an indispensable costimulator necessary for effective T-cell function, acts as a PI3K activator [31]. CD28 is activated by binding to B7 expressed by activated antigen-presenting cells. In this regard, CTLA-4 antagonizes CD28 activity through a number of mechanisms, including competitive binding to B7 (for which CTLA-4 exhibited a higher affinity) [48]. Thus, ICPs such as CTLA-4 and PD-1 both antagonize PI3K signalling, suggesting an antagonistic effect towards insulin.

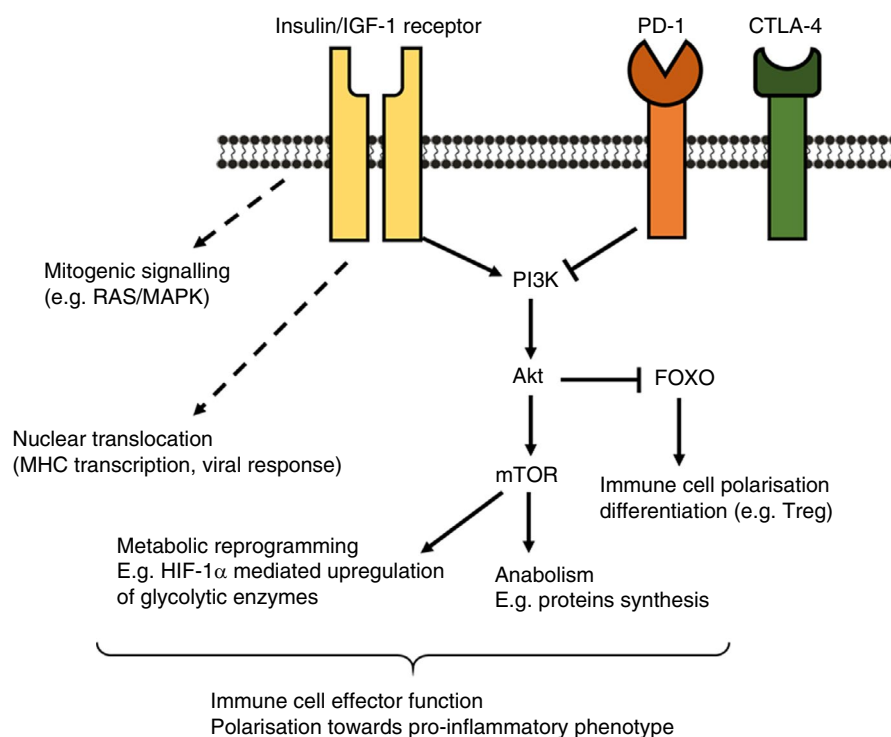
The so-called cytokine storm (or cytokine release syndrome) represents a comorbidity often observed in COVID-19 infections [49]. In this regard, insulin-mediated activation of the PI3K pathway may contribute towards a state of hypercytokinaemia by overriding the PI3K inhibition usually exerted by ICPs. As an example, a phase I trial reported a severe cytokine storm solicited by TGN1412, a superagonist anti-CD28 monoclonal antibody [50]: in this regard, CD28 activation is, as mentioned, associated with upregulation of PI3K. There is thus evidence to suggest that insulin signalling may augment PI3K activation, thus curbing the anti-inflammatory effect of ICPs mediated by their antagonism of PI3K signalling. However, a link between the ICP effect and T2D has not been investigated and it is currently not known how diabetes impacts on ICP signalling in context of COVID-19 infections. On the one hand, elevated insulin levels may promote pathological inflammation such as a cytokine storm by overriding the ICP-mediated inhibition of PI3K activation.

On the other hand, it is also possible that immune cell insulin resistance may result in reduced PI3K activation resulting in enhanced immunosuppression in patients with T2D. Supporting this narrative, defective T-cell function is often observed in COVID-19 patients with increased risk of morbidity [37,38]. Here, attenuated T-cell function may result in reduced viral clearance, which in turn results in immune hyperactivation by host response to elevated viral antigens. Clearly, how insulin signalling is affected in T cells of patients with T2D needs to be investigated to resolve these questions and to develop more effective treatment strategies for COVID-19 infection in diabetic patients.

## INSULIN SIGNALLING IN THE IMMUNE SYSTEM: AN INTEGRATIVE VIEW

Here, we have outlined a range of observations that implicate insulin signalling in regulating immune cell activity (Figure 1). An intriguing observation is the upregulation of viral response genes, including MHC proteins, following insulin receptor translocation. As discussed, the PI3K/Akt/mTOR pathway plays a key role in mediating metabolic reprogramming associated with effector function and in promoting immune cell anabolism. These pathways are also antagonized by ICPs such as PD-1 and CTLA-4, highlighting the importance of this pathway in immune cell activation.

PI3K agonists such as insulin and IGF-1 may also exert immune regulatory effects via Akt-mediated phosphorylation of FOXO proteins, which in turn result in the nuclear exclusion (i.e. inactivation) of these transcription factors [51]. In turn, inhibition of FOXO proteins is known to impact on a number of immune functions. As an example, studies utilizing transgenic mouse models demonstrated a role of FoxO1 in promoting the polarization of macrophages towards an anti-inflammatory M2 phenotype [52], suggesting that Akt may promote a pro-inflammatory M1 phenotype by inactivating FoxO1. Similarly, in T cells, FOXO proteins are implicated in cell differentiation and polarization. As an example, the conversion of CD4 T cells towards Treg following TGF- $\beta$  stimulation is mediated by Foxo1 and Foxo3 [53]. Here, insulin-mediated Akt activation may antagonize Treg differentiation by phosphorylating FOXO proteins, resulting in their nuclear exclusion and preventing the transcriptional activation of genes necessary for Treg differentiation. Insulin/IGF-1 also results in activation of mitogenic signalling via RAS/RAF/MEK/MAPK/ERK pathway. Here, mitogen-activated protein kinases play diverse roles in innate immune cells and may not only provide negative feedback on the inflammatory response but also promote immune function [54]. In T cells, MAPK signalling and downstream ERK2 signalling have been shown to promote cell survival [55]. There is thus clear evidence implicating a mechanistic basis for insulin's immune regulatory effects. Specifically, insulin signalling seems to promote a pro-inflammatory state and assists in mobilizing the immune response.



**FIGURE 1** Insulin signalling plays a key role in activation and mobilization of immune responses in a range of immune cells

## FUTURE DIRECTIONS

There is clear evidence that dysregulated insulin signalling exerts a marked influence on COVID-19 disease trajectory. However, a persistent question remains [8]: ‘Why are people with diabetes at increased risk for disease severity and mortality due to COVID-19 infection?’ Here, we have reviewed emerging evidence implicating insulin in regulating various aspects of immune cell function, suggesting that dysregulated insulin signalling may promote immune dysfunction, thus enhancing susceptibility to COVID-19 infection. Indeed, both T1D and T2D are associated with an increased risk of infection-related mortality [56]. Emerging evidence implicates insulin as an underappreciated immune regulatory hormone. It is well appreciated that inflammatory mediators not only induce insulin resistance but also promote a state of hyperglycaemia by driving gluconeogenesis [57]. In addition, it is likely that insulin resistance may permit the repurposing of this metabolic hormone in directing the immune response during an infection. However, the functional significance of insulin signalling in the immune system is poorly understood. As an example, it is currently unknown whether and to which extent, insulin resistance manifests in immune cells such as T cells. Similarly, the immune-modulating effect of insulin on ICPs is also unexplored. Addressing these questions may open up avenues for more effective treatment strategies for patients with COVID-19 suffering from diabetes.

Another challenge related to the fact that T2D is a heterogeneous disease. Early on, compensatory hyperinsulinaemia may be the norm, followed by beta-cell depletion, resulting in suppressed insulin levels. In fact, a data-driven cluster analysis of patients with T2D resulted in the identification of five distinct clusters of risk factors and diabetic-associated complications [58], not only highlighting the heterogeneity within T2D but also raising the implication that patients with T2D may encounter differential disease trajectories as a result of different levels of insulin dysregulation.

As obesity often co-occurs with T2D, there might be important pathological crosstalk between diabetes and obesity-related immune dysfunction. Most notably, obesity is associated with chronic low-grade inflammation that is believed to contribute towards defective immune function [59]. Though a range of processes have been proposed (reviewed in ref. [60]), the trigger for adipose tissue inflammation is not fully understood. Adipose tissue is also a primary source of leptin, which in turn has been implicated in autoimmune diseases by virtue of its pro-inflammatory effect on cells of both the innate and adaptive immune system [61]. These processes represent a range of well-established mechanisms by which obesity may induce a pro-inflammatory state, which in turn may promote immune dysregulation. In turn, such chronic low-grade inflammation may promote immune dysregulation

that contributes to the unfavourable clinical outcome in both adults [62] and children [4] during COVID-19 infection.

Furthermore, various immune cells also express IGF-1 receptors, which also activate the PI3K/Akt pathway. In this regard, both the IGF-1 receptor and the insulin/IGF-1 hybrid receptors can be activated by insulin; however, at different affinities and at elevated insulin levels, insulin can also bind to and activate IGF-1 receptors [62]. As an example, dendritic cells treated with either 100 nM insulin or IGF-1 exhibited enhanced phagocytosis (as measured by FITC-dextran uptake) in a PI3K/Akt-dependent manner [63]. Also as mentioned, insulin seems to inhibit Treg function (e.g. IL-10 release [25]) suggesting a pro-inflammatory effect. In this regard, IGF-1 has similarly shown to antagonize Treg function. As an example, IGF-1 has recently been shown to skew T-cell differentiation from Treg towards Th17 [64]. This suggests that IGF-1, similar to insulin, may antagonize Treg cells. Further supporting this view, mice lacking IGF-1 receptors exhibited an attenuated inflammatory response following bleomycin-induced lung injury, suggesting a potential pro-inflammatory effect of IGF-1 [65]. These observations are also relevant to insulin as it suggests that hyperinsulinaemia, which can be very pronounced in critical care patients [66], may also engage IGF-1 signalling. Thus, insulin may exert an immune regulatory effect even in immune cells with a low abundance of insulin receptors. However, these observations are in conflict with others, which showed that IGF-1 treatment may actually stimulate Treg function [67]. This discrepancy may result from differences in IGF-1 concentration or from the fact that an osmotic pump was used, resulting in chronic IGF-1 exposure that may solicit a different response in Tregs. Furthermore, though IGF-1 and insulin do share common signalling effectors, the kinetics regarding the signalling effects are apparent. As an example, insulin is able to solicit greater glucose uptake in adipocytes than in IGF-1 [68]. From these observations, it is clear that a better understanding of the role of the IGF-1/insulin signalling system in immune cells is needed.

Finally, we have here reviewed the effect of insulin signalling on immune cells in a simplistic manner, neglecting some of the emerging complexities involved in PI3K signalling. As recently reviewed [69], the PI3K signalling pathways are highly branched, giving rise to a context-specific response and also exhibiting complex temporal properties (e.g. PI3K activation can solicit differential effects when chronically activated, vs. pulsatile stimulation). Indeed, these phenomena may explain the conflicting effects of IGF-1 stimulation on Tregs [67] vs. polarization towards Th17 reported by others [64]: chronic exposure via osmotic pumps may solicit different IGF-1-mediated PI3K signalling pathways. Resolving these dynamics will undoubtedly also have clinical implications. As an example, in diabetic patients, chronically elevated insulin levels may solicit defective PI3K signalling in

immune cells, thus contributing to the defective immune response to COVID-19.

## CONCLUSION

There is thus clear evidence implicating a direct immunomodulating function of insulin on various immune cells. Insulin plays an important role in metabolic reprogramming of immune cells' effector function and also impacting on immune cell differentiation. Furthermore, insulin signalling directly promotes the very same signalling cascades antagonized by ICPs, implicating insulin in regulating pro-inflammatory responses following MHC-I engagement. Given the importance of these mechanisms in controlling a viral infection, it is likely that dysregulated insulin signalling may explain why diabetes is closely associated with poor outcome in patients with COVID-19. However, how dysregulated insulin signalling in diabetes impacts on immune cell function, and how these immunological perturbations impact on COVID-19 infection, remains to be investigated. Of particular relevance is the manifestation of immune cell insulin resistance. Also, a better understanding of insulin's immune regulatory effects may also impact on other infections in diabetic patients, as well as guiding further studies on the role of insulin therapy in sepsis and other critical care settings. Insulin therapy or postprandial insulin spikes may also hold interesting implications for optimizing vaccine efficacy. Clearly, these considerations strongly urge for more research in exploring insulin signalling in immune cells.

## CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

## AUTHOR CONTRIBUTIONS

GVN conceived the initial idea for the manuscript. MVDM and AME contribute to the developed ideas in the manuscript. GVN, MVDM and AME drafted the manuscript.

## CONSENT FOR PUBLICATION

All authors have read and approved the final version of this manuscript.

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