The immuno-oncological implications of insulin

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# Abstract

Emerging evidence has implicated insulin in regulating the phenotypes of various immune cells through canonical downstream signalling effectors of insulin, namely, the PI3K/Akt/mTOR pathway. Notably, these signalling components also exhibit crosstalk with other immune signalling pathways, such as the JAK/STAT pathway (activated by cytokines and growth factors), and, importantly, are also negatively regulated by the immune checkpoint blockers (ICBs), PD-1 and CTLA-4. Here, we point out recent findings, suggesting that insulin may promote a pro-inflammatory phenotype with potential implications on ICB therapy. As an example, the contemporary paradigm holds that, while T cell receptor recognition of distinct MHC-expressed epitopes ensures specificity, co-activation of CD28 along with signal inputs form various cytokines and insulin operates to ‘fine-tune’ the immune response via PI3K and other downstream signalling molecules. These considerations highlight the urgent need for focused investigations into the role of insulin in regulating immune cell function in the context of ICB therapies.

# Introduction

Immune checkpoint blockade (ICB) and cell-based therapies (e.g. adoptive T cell therapy) represent exciting new developments in the field of cancer treatment. The explicit mobilisation of the immune system also forms a core strategy behind oncolytic viral therapy and cancer vaccines. Yet, an evolving understanding of the mechanisms by which certain conventional agents function suggests that the immune response likely contributes to the antineoplastic efficacy of various agents besides those used in ICB-based therapies. It is now understood that various chemotherapeutic agents, as well as ionising radiation, are able to promote immunogenic cell death (ICD) – a form of cell death ‘which involve increased antigenicity and adjuvanticity’ [1]. For example, the observation that ionising radiation of the primary tumour occasionally results in decreased sizes of metastatic tumours (i.e. tumours that are not directly irradiated) – the so-called abscopal effect – has been hypothesised to be mediated by the immune system following immunogenic cell death that promots antigen priming [2]. Anthracyclines are known to intercalate with DNA, thereby inhibiting DNA repair and polymerisation. However, these agents (e.g. doxorubicin) have also been found to promote the translocation of calreticulin (an ‘eat me’ signal) in pre-apoptotic cancer cells, thereby stimulating the engulfment of these cancer cells by dendritic cells and promoting the subsequent expression of cancer-derived neo-epitopes [3]. The immune system thus likely plays a notable role in antineoplastic therapies besides ICB-based therapies. Given the potential importance of the immune system on therapeutic efficacy, it is important to optimise therapeutic strategies in order to leverage the immune system for maximum effect.

Strikingly, emerging evidence implicates insulin to regulate various immunological functions in a range of immune cells [4]. As an example, in macrophages, insulin signalling has been implicated in antagonising FOXO1-mediated apoptosis via increased Akt activity [5]. Similarly, it has long been known that stimulated T cells rapidly increase the expression of insulin receptors (INSRs) [6]. Furthermore, recent reports suggest that insulin exerts functions beyond its canonical metabolic effects. For example, using a euglycaemic clamp, insulin was shown to regulate the transcription of several metabolic genes, but also of some genes that are less well-understood, such as those involved in the Notch signalling pathway [7]. Although this analysis was conducted on metabolic tissue (liver and muscle), it is interesting to note that Notch signalling also plays a well-established role in regulating both innate and immune cells [8]. For example, Notch 1 signalling plays an indispensable role in the activation of FOXP3, a signature transcription factor in regulatory T (Treg) cells, whereas in CD8+ T cells, Notch 2 expression is critical in the development of the cytotoxic T cell response [8]. Whether insulin similarly affects Notch signalling in immune cells warrants further investigation, but given the fact that many immune cells, including T cells, express INSRs, it seems very likely. Collectively, these observations strongly implicate insulin as a potential regulator of immune cells. Indeed, we have recently argued that inflammation-driven insulin resistance manifests as an adaptive strategy that permits the uncomplicated recruitment of insulin signalling mechanisms during infection [4].

However, this observation raises a question: what is the effect of insulin on cancer immunotherapies? As an example, type I diabetes (T1D) is associated with low insulin levels. In contrast, type II diabetes (T2D) may exhibit hyperinsulinemia, particularly in the context of intensive insulin therapy. If insulin can have an impact on immune function, what is the possible relevance of insulin on the therapeutic efficacy of ICB and the manifestation of adverse immunological events (AIE)? Similarly, the role of fasting prior to receiving chemotherapy are being investigated in several clinical trials, which raises the next question: could postprandial release of insulin have an impact on cancer therapy – particularly on agents that invoke ICD? For example, could suppressed insulin levels resulting from fasting compromise or enhance the efficacy of cancer therapies? Here, we review the effect of insulin on various immune cells and highlight the immune-oncological implications of insulin’s ability to modulate immune cell function. These observations strongly motivate the need to develop a model system to evaluate the unique role of insulin in various immune cells to optimise therapeutic settings such as fasting, chemotherapeutic agents that induce ICD, and the use of ICBs.

# Onco-immunological implications of insulin signalling in T cells

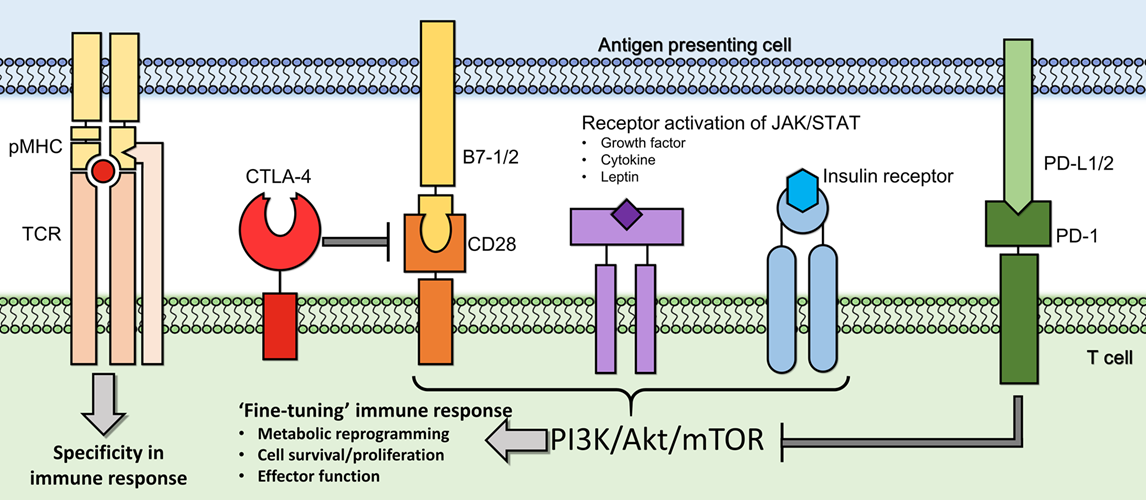
Previous studies on rat thymocytes revealed that INSRs are exclusively expressed on activated T cells [6]. Subsequent studies have pointed out that the increase in INSR expression co-occurs with increases in protein and nucleotide syntheses [9]. These early observations suggested that insulin plays a key role in driving T cell anabolism. This notion is supported by the recent findings that although naïve CD4+ T cells of rats exhibited very low INSR levels, INSRs are rapidly upregulated following immune activation, with consequent enhanced expression of GLUT1, -3 and -4 and a corresponding increase in glucose consumption [10]. Specifically, insulin signalling is implicated in promoting aerobic glycolysis, as conditional knockdown of INSRs stunted aerobic glycolysis, which was reflected in lower GLUT3 and -4 expression as well as attenuated lactate production [10]. Since canonical anabolic pathways such as PI3K/Akt/mTOR are also downregulated following INSR knockdown, it is suggested that INSRs play a key role in mobilising T cells by promoting cell proliferation. Indeed, INSR knockdown did not lead to a shift in Th1 versus Th2 cytokine responses and was found to be similarly dispensable for the function of Tregs [10], suggesting that insulin signalling plays a role in the expansion of T cell numbers, but not necessarily in the differentiation or polarisation of T immune cells. These findings in rats [10] are comparable to what was observed in mice [11]. Although insulin signalling is not necessary for T cell homeostasis under steady-state conditions, following an immunogenic stimulus, INSR-deficient T cells exhibited a marked decline in the expression of *Myc* (a transcription factor downstream of mTOR involved in orchestrating glycolytic metabolism) and the glucose transporters, GLUT1, -3, -4 and -6, with a corresponding decline in glucose uptake [11]. This defective glucose metabolism translated to lower rates of cell proliferation as well as compromised effector function (e.g. IFNγ production) and functionally which precipitated in a compromised immune response against H1N1 influenza infection [11].

Insulin also antagonises Treg cell differentiation and function, where activation of the PI3K/Akt/mTOR signalling pathway antagonises FOXP3 transcriptional activity, which prevents the induction of a characteristic Treg-like transcriptional profile [12]. In rats, the inducible silencing of INSRs of T cells resulted in compromised cytotoxic function of CD8+ T cells, but did not alter the ability of Tregs to suppress inflammation [10], suggesting that perhaps insulin signalling is not necessary in promoting the anti-inflammatory capacities of Tregs. In fact, evidence from a mouse study suggests that insulin may in fact impair the anti-inflammatory function of Tregs. In C57BL/6 mice, where Tregs were subjected to hyperinsulinemia, the ability of these cells to modulate TNFΑΑ by macrophages were suppressed [13]. Mechanistically, the upregulation of insulin signalling was responsible for enhanced Akt and downstream mTOR signalling, which in turn compromised IL-10 production by Tregs [13]. The fact that both *in vitro* and *in vivo* settings produce the same response suggests that insulin (and not an obesogenic diet), are indeed the key mediator of this IL-10 suppression in Tregs. These results are also in line with previous *in vitro* and *in vivo* studies that showed that activation of the Akt/mTOR cell signalling pathways negatively regulates the differentiation of CD4+ T cells into Tregs in mice [14]. It should be noted, however, recent evidence suggests that the intensity of Akt signalling may be critical in delineating Treg cell phenotype. Using differential ‘dosages’ of antigens, variable levels of T cell receptor (TCR) activation was achieved, which in turn regulated the phosphorylation status of Akt [15]; critically, low activation of Akt promoted Treg differentiation whereas high Akt activity promoted T helper (Th) cell differentiation. Since insulin can promote Akt activity, it is tempting to speculate that insulin may skew T cell differentiation from a Treg towards a Th response.

# Insulin-mediated antagonism of immune checkpoint blockers

There is intriguing evidence implicating insulin signalling in modulating the functional output of immune checkpoint blockers. CD28, an indispensable co-stimulatory activator of T cells, is activated following binding to B7-1 or B7-2 (also known as CD80 and CD86, respectively). Following ligand activation, CD28 mediates its co-stimulatory effect by upregulation of PI3K activity in T cells [16]. However, CTLA-4 exhibits higher affinity for B7 and is believed to prevent CD28 activation by either outcompeting CD28 for B7 or by the ‘capturing’ of B7 by CTLA-4-expressing cells (e.g. Tregs), followed by the trans-endocytosis of these B-7 proteins [17]. Regardless of the exact mechanism, it is evident that CTLA-4, by preventing B7-mediated activation of CD28, also potentially antagonises PI3K signalling. Similarly, the binding of PD-1 to PD-L1 is associated with an increase in PTEN activity, thereby antagonising PI3K signalling [18]. Because ICB-based therapies modulate the same signalling effectors involved in insulin signalling, it is exceedingly likely that insulin may impact the therapeutic outcomes of these therapies.

These findings suggest that insulin signalling may have an important function in driving the metabolic reprogramming of T cells that takes shape as these cells mature and develop an effector function through two complimenting mechanisms (**Figure 1**). Firstly, insulin may promote the import of metabolites such as glucose, amino acids and phosphate, necessary for sustaining immune cell anabolism. Secondly, the activation of anabolic signalling pathways such as PI3K/Akt/mTOR downstream of canonical insulin signalling mobilises transcriptional and translational machinery to promote immune cell anabolism and direct T cell differentiation. Thus, while TCR recognition of distinct MHC-expressed epitopes ensures specificity, co-activation of CD28, along with signal integration form cytokines and insulin, operate to ‘fine-tune’ the immune response via PI3K and other downstream signalling molecules [19]. Insulin thus stands poised to regulate T cell differentiation and effector function, suggesting that serum insulin levels may likely impact on the efficacy of immune checkpoint therapies.



**Figure 1**. Various cytokines and growth factors, as well as co-activator CD28, converge on components of insulin signalling pathways. CD28 is a critical co-activator necessary for T cell effector function following recognition of MHC-presented peptides. Here, CD28 activation promotes PI3K/Akt signalling. Similarly, various growth factors and cytokines often activate the JAK/STAT pathway which in turn have been shown to promote PI3K/Akt activation. PI3K/Akt and downstream mTOR activation is also associated with pro-survival and anabolic signalling that promotes effector immune cell function. Conversely, PD-1 activation antagonises this signalling pathway directly, while CTLA-4 indirectly attenuates PI3K/Akt activation by competitive binding to B7-1 or B7-2, thereby preventing CD28 activation. Because canonical insulin signalling promotes the PI3K/Akt/mTOR pathway, hyperinsulinemia may represent an increase in the inflammatory tone of T cells by upregulating the same pathway antagonised by ICBs.

# Insulin and dendritic cells

Dendritic cells (DCs) are the single most important type of immune cells capable of activating T cells. As an example, ICD-based DC vaccines (i.e. vaccines derived from cancer cells killed via a mechanism that solicits immunogenic cell death) have been shown to shift T cell phenotype from regulatory to a Th1/Th17-dominated anti-tumour response [20]. This interaction between T cells and DCs is likely to be reciprocal; in mice, selective ablation of Tregs resulted in an almost 10-fold increase in mature DC counts in lymph nodes [21], suggesting that T cells also regulate DC function and survival. Evidence also implicates DC cells in mediating antitumour effects via T cell activation. As an example, tumour cells engrafted intradermally (richly populated with DCs) exhibited increased DC recruitment to draining lymph nodes compared to subcutaneous (low DC density) engraftments [22]. Although DCs have not been reported to express INSRs, they do express IGF-1 and -2 which can be activated during elevated levels of insulin. This raises the question: how does insulin impact on the ability of DCs to either induce tolerance or to augment T cell function?

A recent study on BALB/c mice revealed that the treatment of bone marrow-derived DCs with insulin (100 nM) or IGF-1 (100 ng/ml) prior to an LPS challenge stimulated phagocytosis of FITC-conjugated dextran, but suppressed the release of TNFΑΑ in a PI3K/Akt-dependent manner [23]. This increase in phagocytosis of FITC-conjugated dextran is also supported by the observation that insulin treatment stimulates DC maturation and increased expression of scavenger receptors [24]. This may suggest that insulin-mediated activation of PI3K can enhance the expression of cancer-derived epitopes as PI3K-activity promotes DC phagocytosis.

However, as mentioned, PI3K/Akt signalling suppressed TNFΑΑ expression [23], whereas in earlier studies it was shown that PI3K-phosphorylation following DC stimulation also attenuated IL-12 [25], a cytokine that plays a critical role in mobilising a Th1-based response. These observations suggest that PI3K activation may suppress inflammatory escalation. Thus, insulin likely promotes phagocytosis (for instance, class A scavenger receptors are involved in the uptake of apoptotic bodies), while also attenuating inflammatory signalling (suppression of TNFΑΑ expression). This suggests that PI3K/Akt activation likely mediates an anti-inflammatory effect through DCs (a) by enhanced clearance of apoptotic bodies which may prevent pro-inflammatory secondary apoptosis and (b) by suppressing the release of pro-inflammatory cytokines. Resolving the effect of elevated insulin levels may be important in optimising cancer therapies which are dependent on epitope presentation and the development of an immunogenic tumour micro-environment (e.g. the Th1 cytokine response). As an example, cancer patients who are pre-diabetic or exhibit insulin resistance may benefit from scheduling immunotherapy sessions so that it does not coincide with insulin administration to avoid elevated insulin levels that may drive the tolerogenic effects of DCs.

# Insulin and macrophages

From tumour initiation to the promotion of metastasis, macrophages play a well-established role in cancer progression [26]. The expansion of macrophages in the tumour seems to be primarily mediated through enhanced recruitment of these cells from the bone marrow and the spleen and not by local expansion of resident macrophages [27]. Thus, disruption of chemokine signalling is currently explored as a strategy to prevent macrophage recruitment [28]. Besides the expansion of tumour-associated macrophages (TAMs), it is apparent that macrophage polarisation plays a pivotal role in cancer progression. For example, a high M1/M2 ratio is associated with a better clinical response in ovarian cancer [29]. Indeed, the ability of TAMs to present tumour antigens have recently been shown to play a key role in mediating effective immune surveillance and re-establishing the efficacy of ICB therapy in mouse models [30]. It is thus apparent that therapeutic interventions that impact either the expansion of TAMs or the polarisation of macrophages can direct therapeutic outcome. In this regard, evidence implicates insulin in potentially modulating both macrophage recruitment and polarisation.

Chemokines such as CCL2 have been shown to play a key role in promoting monocyte recruitment to the tumour site [31]. Interestingly, although CCL2 expression is usually driven by the NF-κB signalling pathway, recent findings have shown that insulin signalling, acting through mTORC1 activation may enhance the expression of CCL2 on macrophages, thereby promoting their recruitment to the tumour micro-environment [32]. These observations thus strongly suggest that insulin therapy may enhance macrophage recruitment to the tumour bed. Support for this mechanism is also provided by observations implicating insulin as a chemokinetic agent in macrophages during wound healing – in THP-1 macrophages, insulin stimulates cell migration in a dose-dependent manner. Specifically, the increase in cell migration was dependent on PI3K-Akt as well as SAPK/JNK signalling pathways that converge on Rac1 [33]. This suggests that elevated levels of insulin may promote the accumulation of TAMs. Although the accumulation of macrophages is usually a poor prognostic marker, the tumour micro-environment as well as the polarisation of these cells likely contribute to these diverging findings [34].

There are, however, some conflicting findings regarding the role of insulin in macrophage polarisation. In U937-derived macrophages, insulin promotes the synthesis and release of pro-inflammatory cytokines such as IL-1β [35]. However, recent findings have demonstrated that insulin signalling through PI3K/Akt and PPAR-γ antagonises pro-inflammatory signalling (MAPK) and transcription activity (NF-κB) in THP-1 cells, promoting the transition from M1 towards an M2 (i.e. a more tolerogenic) phenotype [36]. These discrepancies may be the result of different model systems (e.g. THP-1 versus U937). Another explanation relates to the dosage of insulin and the duration for which cells are exposed. For example, at elevated levels, insulin activated the IGF-1 signalling pathway. In this regard, it has recently been demonstrated that IGF-1 exposure can promote an M2-like phenotype in macrophages[37], suggesting that very high insulin levels may exert an anti-inflammatory effect via the IGF-1 signalling pathway.

There also exists evidence that suggests that the activation of signalling factors downstream of insulin signalling cascades may invoke anti-inflammatory effects. Activation of alpha-7 nicotinic receptor (α7 nAChR) has been shown to attenuate inflammation in a range of pathological settings [38]. Interestingly, α7 nAChR activation on monocytes attenuated the production of pro-inflammatory cytokines such as TNFΑΑ-α, IL-1β and IL-6 in a PI3K-dependent manner [39]. Mechanistically, cotinine (an α7 nAChR agonist) pre-treatment of monocytes exerted this anti-inflammatory effect by enhanced PI3K-dependent Akt activation, which in turn inhibited GSK3-β (via phosphorylation) following a *P. gingivalis* challenge.

Another mechanism through which PI3K/Akt/mTOR signalling activation via insulin may regulate macrophage polarisation and cytokine production is through mTOR-mediated transcriptional regulation. In response to pathogenic infection, for example, macrophages downregulate mTOR, which results in a biased translation of pro-inflammatory cytokines [40]. Mechanistically, mTOR phosphorylate 4E-BP1 which results in the release of eIF4E, a translation initiation factor which forms the eIF4F complex which is necessary for cap-dependent translation. This mTOR-mediated transcriptional regulation predisposes macrophages towards the synthesis of anti-inflammatory cytokines such as IL-1and IL-10. Conversely, mTOR inhibition resulted in increased pro-inflammatory (e.g. IL-6) cytokine production. Importantly, mTOR was regulated by the ubiquitination of Akt, suggesting that enhanced Akt activation may promote anti-inflammatory cytokine production. Since insulin is a potent inducer of the PI3K/Akt/mTOR pathway, these results suggest the possibility that insulin may promote a tolerogenic phenotype in macrophages. Crucially, however, this observation was made in a pathogen model system; whereas in a metabolic mouse model, a novel form of macrophage insulin resistance was described where suppressed Akt activity was associated with an anti-inflammatory phenotype [41].

# Insulin signalling in other immunogenic roles

In addition to regulating cells of the immune system, there is also evidence that suggests that insulin may enhance the immunogenicity of cancer cells by upregulating MHC I proteins. It is now clear that insulin not only mediates its effect through the canonical cell signalling pathway, but that it also regulates transcription. Following insulin binding to the INSR, the INSR forms a complex with Pol II, and associates with the transcription initiation sites of various genes [42]. Although most of these regulated genes are typical of insulin’s function, some of the genes are not. As an example, the genes involved in pathways such as in Class I MHC-mediated antigen processing and presentation, viral infection, and the adaptive immune system, were all enriched [42]. This raises the possibility that insulin may augment the expression of neo-epitopes. However, this study utilised human hepatocellular carcinoma (HepG2) cells that may reflect the fact that liver cells are intrinsically more responsive to insulin. It therefore remains to be established whether these observations also extend to cancers derived from other tissue types.

Although we have focused primarily on the effects of insulin on immune cells, inflammatory pathways in non-immune cells also co-opt PI3K/Akt signalling. As an example, lung epithelial cells, which also express INSRs [43], crosstalk with other inflammatory pathways to induce the expression of chemokines such as IL-8 [44]. It is thus likely that insulin may have an impact on the inflammatory function of various tumour-associated cells, thereby altering the inflammatory micro-environment which might, in turn, reshape immune cell function.

As an example, in mouse fibroblasts, treatment with IFNγ induced the expression of MHC II [45]. Here, PI3K plays a critical role in regulating the effect of IFNα and IFNγ signalling [46]. Since human fibroblasts also express INSRs [47], it is possible that insulin may enhance the expression of MHC II on these cells. This is of interest since MHC II usually express exogenously derived antigens and, since fibroblasts do phagocytose [48], it suggests that insulin may mobilise fibroblasts to present exogenously derived epitopes. Indeed, fibroblasts have been shown to affect antigen presentation in lymphoid organs [49] and have been induced to differentiate into DCs in both humans and mice [50]. Given the abundance of fibroblasts in the tumour micro-environment, there is a clear need to investigate the potential effect of insulin on fibroblasts and their possible contribution in presenting epitopes to T cells.

Insulin may also impact on the development of lymphoid structures. For example, PI3K plays a role in mediating lymphangiogenesis [51] and mice lacking the PI3K catalytic subunit *Pik3ca* exhibit defective lymphatic sprouting and maturation [52]. Given the importance of these structures in priming immune cells and their potential impact on immune-oncology (reviewed [53]), there is a clear need to evaluate the extent to which insulin may impact on the formation of lymphoid structures.

# Insulin as an immune modulator

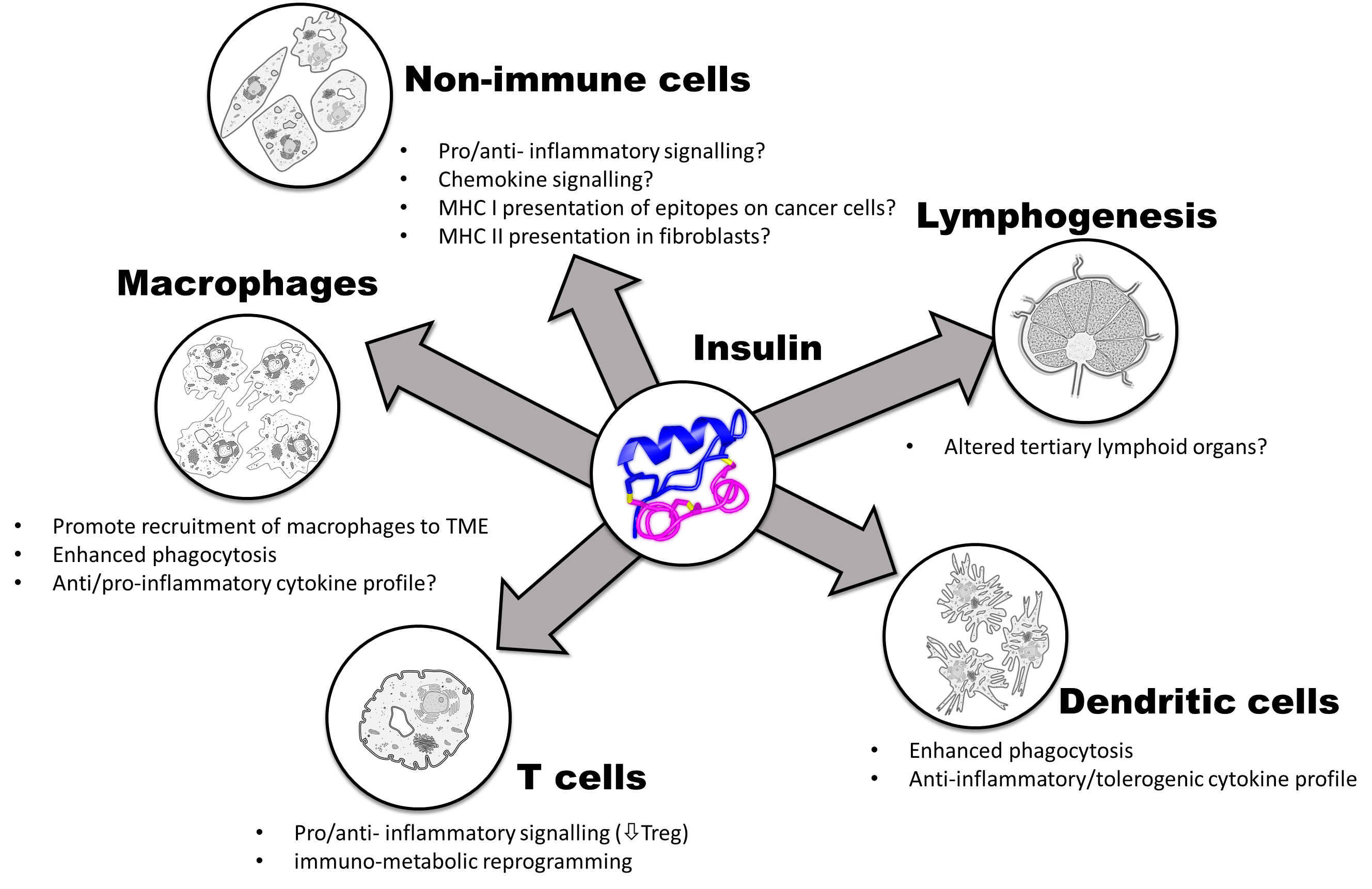
Exemplifying the integrative role of insulin in regulating the immune system is the observation that insulin levels are altered in response to pro-inflammatory solicitors. Both human and mice pancreatic beta cells express TLR4 [54] and, at least in mice, LPS (a classic TLR4 agonist) accentuate glucose-stimulated insulin release [55]. Conversely, low-dose TNFΑΑ appeared to lower serum insulin levels in healthy male volunteers, although this may have been through the enhanced clearance of insulin rather than decreased insulin production [56]. Regardless, the observation that insulin not only impacts on the immune system, but that insulin levels also respond to inflammatory stimuli suggests a reciprocal interaction between insulin and the immune system and thus consolidate the view that insulin is likely playing an integrated role in regulating the immune response.

The impact of insulin on the immune system is also exemplified by the observation that inflammatory mediators often activate signalling pathways that crosstalk with canonical insulin pathways. This is well illustrated by JAK/STAT pathways that are downstream effectors of various cytokines. Here, JAK2 activation has been shown to potentiate PI3K activity via insulin receptor substrate (IRS)-2 activation following leptin exposure [57]. Similarly, JAK1, -2 and -3 have also been shown to activate IRS-1 following, for example, IL-9 stimulation [58]. IL-13R activates both the JAK/STAT and the PI3K/AKT pathways [59], suggesting a synergistic effect between these pathways. It is thus apparent that various cytokines and growth factors also utilise insulin signalling pathways in mediating their effects, suggesting that immune regulation also recruits insulin signalling pathways. Similarly, the PI3K/Akt/mTOR pathway, downstream of canonical insulin signalling, plays diverse roles in promoting immune cell anabolism and metabolic adaptation in various immune cells (reviewed [4]).

Crosstalk between signalling cascades engaged by cytokines and insulin implicate insulin in potentiating these cytokine pathways. Due to the wide-ranging effect of cytokines, the exact effect of insulin is likely immune-cell specific. Indeed, insulin is known to exert tissue-specific effects: in a euglycaemic clamp setting, the transcription of muscle and liver following insulin exposure exhibited only a ~10% overlap in gene sets [7]. Such specificity is likely to also be partly mediated by the different isoforms expressed by various immune cells. For example, the stoichiometric ratios of different PI3K isoforms expressed by various immune cells [60], likely also mediate immune cell-specific responses.

# Insulin signalling and the immune system: unresolved questions

In summary, although in some cases direct evidence exists for the fact that insulin influences immune function (**Figure 2**), occasional conflicting findings also exist. There is thus a significant need for studies dedicated to specifically evaluate the uncomplicated effect of insulin on the immune system. To date, the majority of studies investigating the effect of insulin on the immune system have employed settings where insulin therapy was introduced in the context of hyperglycaemia manifesting with other co-morbidities (e.g. T1D, T2D, gestational diabetes or inflammation-driven hyperglycaemia in critical care patients). In addition to the unique nature of these systems, it is also not clear whether the effect of insulin on the immune system results from controlling hyperglycaemia (e.g. minimising the formation of pro-inflammatory advanced glycation end products) or a bona fide effect of insulin on immune cells. Complicating the problem, model systems for studying insulin is also problematic (reviewed [4]). For example, streptozotocin treatment, often used to induce T1D, results in lymphopenia with increasing Treg counts [61]. Such altered T cell function may render these models less appropriate for evaluating the effects of immune therapies that are dependent on T cell function (e.g. ICBs). There is thus a clear need to develop model systems for studying the immunogenic effect of insulin and its potential role in the context of onco-immunotherapies.



**Figure 2**. Insulin affects a wide range of cells types. Although evidence indicates that insulin promotes effector function of cytotoxic T cells as well as antagonising Treg cell function, its effect on other immune cells are more diverse and less defined.

In summary, it is clear that insulin likely exerts an under-appreciated role in regulating immune function. However, since most studies evaluating the effect of insulin on the immune system have been conducted in pathological settings (e.g. critical care or diabetes), the immune-regulatory role of insulin remains to be better resolved. Although the causal relationship is complex, it is well established that cancer is highly prevalent among patients that also exhibit altered glucose homeostasis (i.e. diabetic or obese individuals) [62], suggesting that many patients receiving onco-immune therapies will also receive insulin. Similarly, fasting prior to the induction of chemotherapy is currently being explored in a number of clinical trials [63]. Here, fasting-induced suppression of serum insulin may exert unappreciated effects that may benefit or counter therapies that induce ICD or make use of ICBs.

# Establishing the immune regulatory function of insulin: a call to action

Various lines of evidence reviewed here implicate insulin as an immune-regulatory hormone with direct implications for cancer immune-therapies. However, these observations also raise a key question: why, given the abundance of evidence implicating insulin signalling in modulating immune function, has insulin signalling scarcely received interest in an onco-immunological setting? Part of the reason may relate to a general neglect of insulin’s immune-regulatory function. Transgenic models were developed only recently to investigate specific ablation in immune cells. Similarly, we are unaware of any hyperinsulinemic-euglycemic clamp study evaluating the effect of insulin on immune cells. Though we focus on onco-immunological implications, it is worth noting that the effect of insulin-mediated immune regulation may also impact in other settings. As an example, in an evaluation of the Australian Diabetes register comprising of >1 million individuals, it was shown that diabetics are at an increased risk of mortality from various infections [64], suggesting that defective insulin signalling may negatively affect the immune system (though hyperglycaemia may also contribute to immune dysfunction).

In fact, dysregulated insulin signalling may also help explain the intriguing correlation between T2D and COVID mortality [65]. Though a host of theories have been proposed to explain this relationship, there is reason to suspect that dysregulated insulin signalling in T2D may impact on T cell function and contribute to defective clearance and tolerance of the viral infection. Remarkably, there is also evidence that defective insulin signalling may contribute towards coagulopathy often observed in COVID-19 infections [66]. Megakaryocyte-specific deletion of insulin receptors (platelets are derived from these cells) has been shown to modulate clotting cascades [67]. Here, the deletion of insulin receptors resulted in an increase in platelet count and volume, while platelet functions such as platelet aggregation, granule secretion, among others were negatively affected. This suggests that insulin signalling may promote a pro-thrombotic state. Indeed, diabetes has long been known to associate with a hypercoagulable state [68].

Both preeclampsia and gestational diabetes are well known to closely associate with each other [69]. Interestingly, immune dysfunction at the placenta is known to play a major role in this development of preeclampsia [70]. This raises the question of whether hyperinsulinemia during gestational diabetes [71] may contribute to the development of immune dysfunction that manifests as preeclampsia. Indeed, immune checkpoint proteins discussed here in the context of cancer are also role players in promoting maternal tolerance towards the placenta [72], suggesting that insulin-activated PI3K may antagonise the function of PD-1 and CTLA-4, resulting in compromised tolerance. It is also of interest to consider that insulin’s transcriptional upregulation of MHC I [42] also occurs in the placenta, where it may enhance the immunogenicity of placental tissue.

Taken together, these observations strongly implicate defective insulin signalling as a key mediator of immune dysfunction with a wide range of clinical implications. Insulin has extensively been studied for its metabolic functions in muscle, adipose and liver, as well as tissue directly impacted in diabetic pathologies (e.g. diabetic nephropathy). However, it is evident that insulin also influences the immune system directly, and numerous basic questions remain to be resolved. As an example, to which extend does an observation such as an increase in Notch signalling relate to other cells of the immune system? Similarly, is an upregulation of viral response genes seen in liver cells also relevant in other cells? Insulin resistance in immune cells is also poorly studied. There is thus a clear need to address the neglected immune regulatory functions of insulin to better optimise therapeutic interventions which include an immunological component.

# Conclusion

There is thus clear evidence indicating a potential role for insulin in modulating T cell function, as well as epitope presentation by immune cells, suggesting an under-appreciated role of this metabolic hormone in directing immune surveillance. In turn, these considerations suggest that insulin therapy may be exploited to maximise the efficacy of ICB-based therapies. However, a large part of insulin’s effect on the broader immune system remains poorly understood. There is also a significant need for the development of novel model systems to better understand the role of insulin on the immune system in order to maximise the therapeutic effect of fasting, ICBs or chemotherapies that induce ICD.

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# Declaration of Competing Interest

The authors declare no conflict of interest.

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