REVIEW

Protein Tyrosine Phosphatase 1B (PTP1B) in the immune system

Le Sommer Samantha[1,±], Pesaresi Martina[1,±], Martin-Granados Cristina[2], Delibegovic Mirela[1]

[1]*Institute of Medical Sciences, University of Aberdeen, College of Life Sciences and Medicine, Aberdeen, UK, AB25 2QE*
[2]*Department of Pharmacology, University of Cambridge, Cambridge, UK, CB2 1PD*
[±]*SLS and MP contributed equally to this work*

Correspondence: Samantha Le Sommer or Mirela Delibegovic
E-mail: r02sl13@abdn.ac.uk or m.delibegovic@abdn.ac.uk
Received: August 14, 2015
Published online: September 02, 2015

Protein Tyrosine Phosphatase 1B (PTP1B) is best known for its role in insulin and leptin signalling. Its ability to directly dephosphorylate the insulin receptor (IR) has made it a prime target for the development of anti-diabetic drugs. In recent times the role of PTP1B has been substantially expanded from a simple regulator of insulin signalling to a complex and dynamic regulator of multiple signalling pathways including the Janus kinase and signal transducer and activator of transcription (JAK-STAT) signalling, thus providing a link between metabolism and inflammation. Here, we review the inflammation associated with obesity and diabetes and the role that PTP1B may play in the development and regulation of this inflammation. We will discuss the role of PTP1B in both the innate and adaptive immune system and how the development of tissue specific knock out models have allowed us to delineate this complex system. Finally, we discuss how this new knowledge may allow us to develop safe and effective treatments for a multitude of conditions, including type 2 diabetes mellitus (T2DM), autoimmunity, and chronic inflammation.

Keywords: PTP1B; inflammation; obesity, diabetes; innate immunity; adaptive immunity

To cite this article: Le Sommer Samantha, *et al*. Protein Tyrosine Phosphatase 1B (PTP1B) in the immune system. Inflamm Cell Signal 2015; 2: e965. doi: 10.14800/ics.965.

Obesity induced inflammation is the main risk factor for development of Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder characterised by hyperglycaemia and hyperinsulinaemia, most commonly associated with being overweight or obese. Incidences of T2DM have increased greatly over the last twenty years. In 1996 within the United Kingdom alone the number of people diagnosed with T2DM was 1.4 million. This has risen steadily, reaching 3.2 million in 2013 (6% of the population) and it is estimated that, by 2025, 5 million people within the UK will have been diagnosed with T2DM [1], this rise in prevalence is not specific to the UK and is mirrored across all developed countries. Recent evidence has now demonstrated systemic obesity-induced inflammation to be the main risk factor for development of T2DM [2], alongside a number of other serious health problems such as, cardiovascular disease and stroke. This increase in incidences is thought to be due to a combination of a rapidly aging population and an increase in prevalence of obesity. Therefore, understanding of how T2DM is promoted and exacerbated is fundamental in the fight against the world's most prevalent metabolic disorder.

The highest risk factor for the development of T2DM is obesity, a complex disorder which can be defined as an expansion of the white adipose tissue due to increased adipocyte number and size [3]. By 2050, obesity is predicted
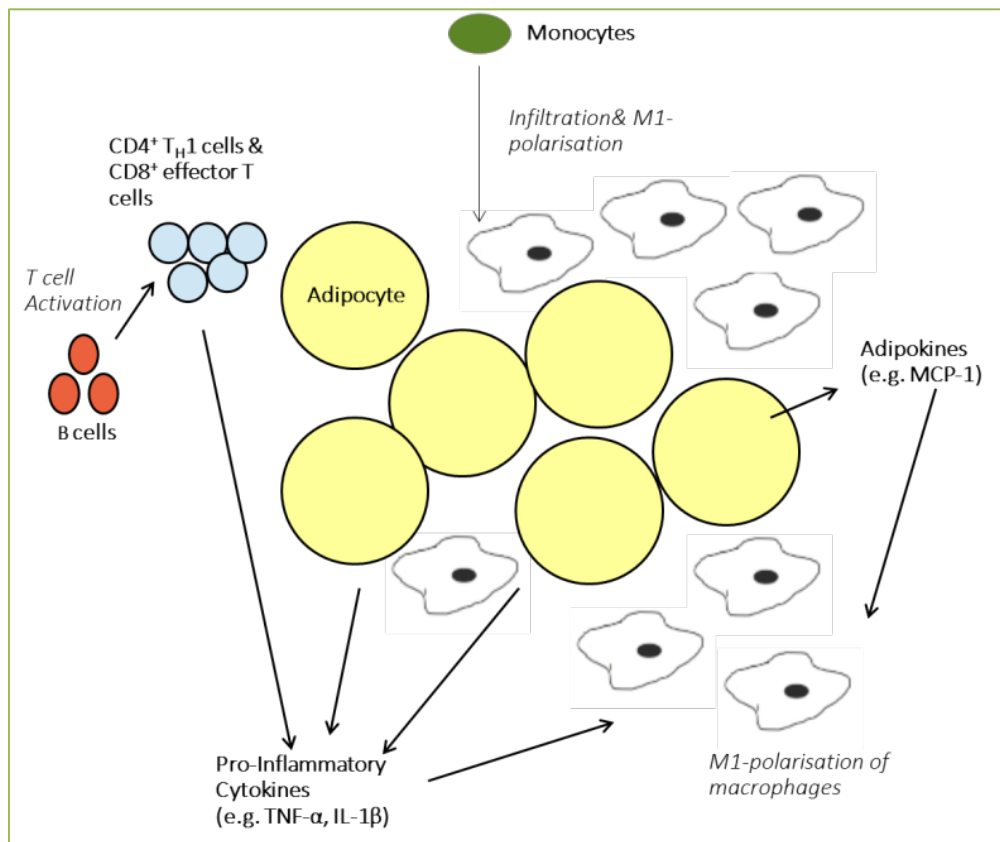
Figure 1. Immune cells mediate inflammation in adipose tissue. In obesity-induced inflammation, monocytes are recruited to the adipose tissue via chemoattractants initially secreted by the adipocytes themselves. The pro-inflammatory response is further reinforced by recruited CD4+ T_H1 and CD8+ effector T cells and B cells, resulting in a shift of the tissue environment away from the homeostatic M2 end of the scale to pro inflammatory M1.

to affect 60% of the adult population [4] and now represents a pandemic health problem. Recent research has extensively shown obesity to correlate with a chronic state of sub-acute, low-grade inflammation, possibly owing to the intersection that exists between the metabolic and immune pathways [2].

The hypothesis of a link between inflammation and metabolic disease was first proposed over a century ago due to the observation that the anti-inflammatory drug salicylate (aspirin) exerted beneficial effects on glucose homeostasis in diabetic patients [5]. However, with the discovery of insulin this hypothesis become less popular, only to re-emerge in recent years, due to a flagship paper published by Pickup and Crooks which correlated inflammatory cytokines with insulin resistance [6], and inflammation once again became thought of as a root cause of T2DM.

Homeostatic environment of the adipose tissue is maintained by M2 macrophages

Obesity is associated with a significant number of differentially regulated genes, including adipokines,

cytokines and enzymes involved in lipid metabolism. Adipose tissue is comprised of a number of cell types, from pre-adipocytes, adipocytes, vascular epithelial cells to a number of immune cells such as macrophages, which infiltrate into the adipose tissue and are estimated to reach over 20-30 million per kilogram of excess fat [7]. Adipocyte tissue macrophages (ATM) are the most abundant immune cell type, these ATM are highly plastic cells, capable of adapting to different microenvironments within the tissue [7].

During normal homeostatic conditions in the adipose tissue the immune population is dominated by T_H2 cells - T regulatory cells (T_{regs}) and eosinophils - which act in concert to induce and maintain an anti-inflammatory environment. Eosinophils produce IL-4 and IL-13 [8], whereas resident macrophages and Tregs primarily release IL-10 [9]. The displayed phenotypes of macrophages can exist anywhere upon a sliding scale between the M2 anti-inflammatory side, under normal homeostatic conditions to highly polarised M1 pro-inflammatory ATM.

Homeostatic ATM are typically IL-10^{high} and IL-12^{low},

and are involved in immunoregulatory roles and thus lay at the M2 end of the macrophage polarisation scale. Indeed, not only do they secrete anti-inflammatory cytokines, but also a number of growth factors, including transforming growth factor beta (TGF-β), which can act as an immunosuppressant and regulates adipogenisis [10], along with insulin-like growth factor (IGF-1) [11], a hormone similar in structure to insulin that regulates cell growth. In particular, M2a and M2b macrophages (respectively induced by IL-4/IL-13 and combined exposure to immune complexes and Toll Like Receptor or Interleukin 1-Receptor agonists) orchestrate T_H2 responses. IL-10-induced M2c macrophages, instead, primarily function in immune suppression and tissue remodelling. However, it is important to stress that this schematic classification does not fully reflect the complexity of macrophage activation, and indeed macrophages can be seen as lying on a continuum spectrum characterised by 3 fundamental functions, namely: host defence, wound healing and immune regulation [12].

Obesity disrupts adipose tissue homeostasis, inducing inflammation and insulin resistance.

Excessive weight gain leads to the pathological expansion of adipocytes, the stress of lipid uptake and cell expansion in order for adequate lipid storage causes cellular stress. Eventually, cells can no longer compensate for this stress and a portion of adipocytes enter necrotic cell death; thus, the presence of these necrotic cells is considered to be one of the pathological hallmarks of obesity. The death of these cells results in a release of a plethora of cytokines and chemokines that induce the infiltration of immune cells, mostly macrophages, from the circulation [13]. These infiltrating macrophages form crown like structures (CLS) around the necrotic cells which can be easily identified by perilipin stain [14]. In addition, this infiltration of macrophages is associated with a phenotypic switch from the homeostatic M2 end of the scale to the pro-inflammatory M1 side [15] (Fig.1).

This increase in ATM recruitment and the phenotypic switch subsequently leads to an increase in inflammatory cytokines which, are derived mostly from the presence of these M1 macrophages rather than the adipocyte cells [13]. Interestingly, weight loss is also associated with infiltration of macrophages into the tissue [16], although it is thought that this population is transitory in nature and display a different phenotype which serves to promote and regulate lipolysis and transcriptionally display increased levels of lipid metabolism genes such as *apoE* and fatty acid handling protein 4 [17].

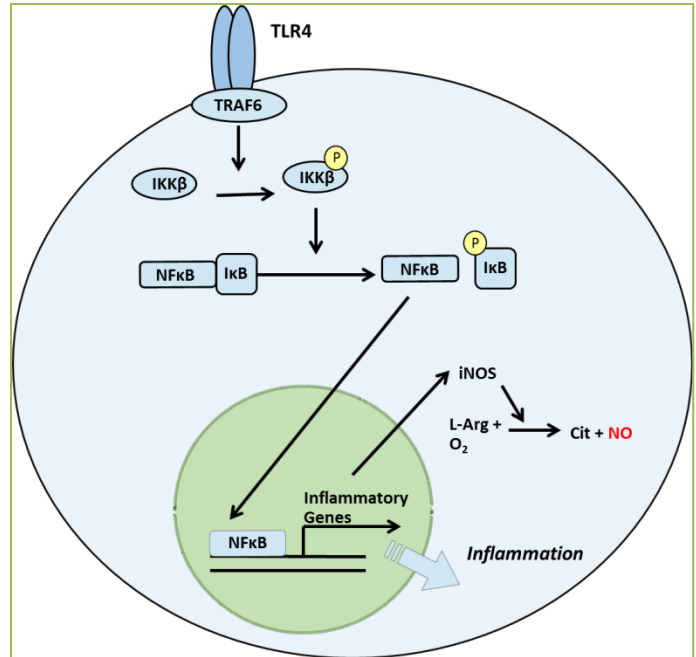This phenotypic switch results in potent activation of the JNK and IKKβ/NFkB pathways, which directly impairs

Figure 2. Activation of the IKKβ and NFκB signalling results in induction of inflammatory genes. The canonical NFκB pathway is induced by a wide variety of signals such as Toll Like Receptors (TLR4 is shown as an example) and a number of cytokines. Induction of the pathway involves a number of adaptors (not shown) which cause phosphorylation of IKKβ subunit of IKK. In turn IKKβ phosphorylation of IκB proteins bound to NFκB leads to ubiquintation of the IκB proteins leaving NFκB free to translocate into the nucleus of the cell. In the nucleus NFκB binds the DNA at specific sequences called κB sites and controls the transcription of inflammatory genes.

insulin signalling, while also transcriptionally up-regulating pro-inflammatory genes, including TNF-α, IL-6 and MCP-1 [18] (Fig. 2). Furthermore, TNF was shown to be expressed at very high levels in the adipose tissue of obese humans [19] and rodents, and antagonises the action of insulin in cultured cells [20]. It has been proposed that TNFα antagonises the action of insulin via upregulating the expression of PTP1B which dephosphorylates the insulin receptor (IR) and thus terminates the signal transduced though the receptor. This hypothesis has been supported by a number of studies, which have demonstrated that *in vitro* PTP1B is upregulated in response to TNFα treatment in multiple tissues including hepatic, muscle and adipose tissue [21, 22]. This was mirrored *in vivo* in mouse models where an increase in PTP1B expression was observed in a number of tissues during inflammation; again including adipose and hepatic tissues [21].

Additionally, it has been demonstrated that *in vivo,* PTP1B can be upregulated via TNFα [23] and furthermore, that high fat diet (HFD) feeding of mice leads to a concomitant increase in both TNFα and PTP1B levels [2]. These findings were further supported by studies from clinical settings, demonstrating that T2DM patients exhibit high expression
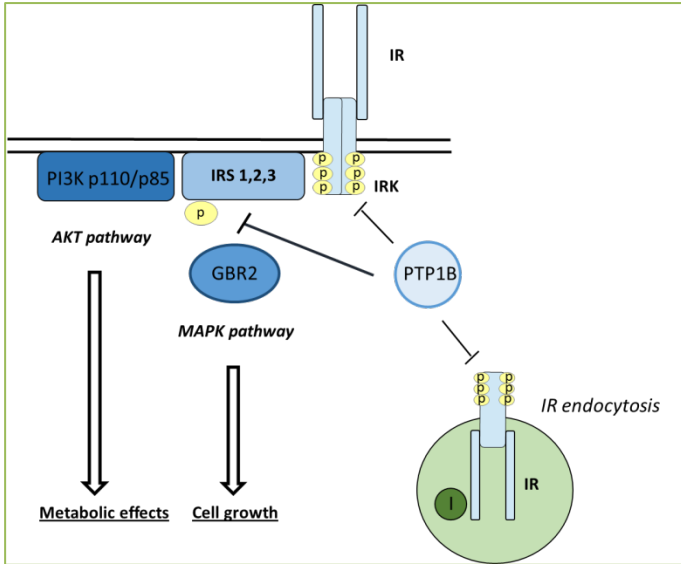
Figure 3. Protein Tyrosine Phosphatase 1B directly dephosphorylates the insulin receptor resulting in blockade of insulin signalling. In response to insulin ligation, the insulin receptor (IR) is phosphorylated and recruits multiple downstream targets; including insulin receptor substrates (IRS) and GBR2. In turn phosphorylation of IRS recruits phosphatidylinositol 3-kinase (PI3K) through binding of the p85 subunit which causes activation of the catalytic p110 subunit. PI3K causes activation of the downstream AKT pathway and leads to induction of metabolic effects such as glucose uptake into muscle and induction of lipid metabolism. GBR2 leads to activation of the mitogen-activated protein kinase (MAPK) pathway leading to downstream activation of genes which facilitate cell growth and proliferation.

levels of PTP1B [24]. It may be proposed that this upregulation of PTP1B, leading to disruption in insulin signalling in response to obesity-induced inflammation, may be an important mechanism by which insulin resistance and T2DM develops.

Generally, this inflammatory response is acute, and the associated hyperlipidemia and enhanced catabolism is only transient in nature. Indeed, the changes in insulin sensitivity and maintenance in glycaemia during acute inflammation may be of benefit, by diverting glucose away from tissues and into the blood stream, making it more available to the immune system during a response to neutralise challenges [25]. However, a continually expanding body of evidence supports the idea that disturbance in metabolic homeostasis can induce immune imbalance on a continuous spectrum that has immunosuppression and undernutrition at one end, and over-nutrition, obesity and sustained inflammation at the other. In this context, T2DM can be seen as an obesity-associated inflammatory disease.

Indeed, in all metazoans, the metabolic and immune pathways have evolved to be intimately connected and intrinsically overlapping, with a significant number of shared key molecules. In most cases, this integration is beneficial for the organism, as the normal inflammatory response is dependent on the mobilisation of stored energy [25].

The expansion of PTP1B's role from insulin signalling to the immune system

PTP1B, in humans is encoded by the *ptpn1* gene, is a ubiquitously expressed, non-receptor, tyrosine phosphatase which is located on the cytoplasmic face of the endoplasmic reticulum (ER) [26]. It was the first tyrosine phosphatase discovered and was first isolated from human placenta [27] before being identified in all tissues [28]. More recently, the role of PTP1B has been expanded from being a simple regulator of insulin signalling to a multitude of substrates.

PTP1B has been well characterised as the master regulator of insulin signalling, where it dephosphorylates the insulin receptor (IR) resulting in a blockade of insulin signalling (Fig. 3), leading to insulin resistance and the development of hyperglycaemia [29]. PTP1B deficient mice display increased sensitivity to insulin and insulin dependent IR hyperphosphorylation; they also exhibit resistance to HFD induced obesity and have lower levels of adiposity [30]. Additionally, tissue specific knock out models of PTP1B (neuronal [31], hepatic [32] and muscle [33]) have all shown positive effects on metabolism indicating PTP1B could be a critical therapeutic target for T2DM treatment.

The dephosphoylation of pTyr by PTP1B is executed via a two-step mechanism; the first step involving neutrophilic attack of the phosphocenter by the reduced Cys215 residue, followed by subsequent protonation of Asp181 to yield the neutral tyrosine phenol. The thiophosphate intermediate is then hydrolysed and the enzyme is regenerated [34]. The development of substrate trapping mutants was a critical step in the identification of its targets. Specifically, mutation of the invariant Asp181 residue prevents the completion of the neutrophilic attack portion of the dephosphorylation process, effectively 'trapping' the substrate in a stable complex with the enzyme, thereby allowing the mutant PTP1B-D181A and its trapped substrate to be co-immnoprecipitated [35]. There are now several examples of substrate trapping mutants including PTP1B-Q262A, PTP1B-D181A-Q262A [36], and PTP1B-C215D [37]. It is through these approaches that molecules such as epidermal growth factor receptor (EGFR) [38], JAK 2, TYK2 [39], STAT3 [40], STAT6 [41], and many more have all been identified as PTP1B substrates and, in doing so, placing more focus on the importance of role of PTP1B in inflammation.

Role of PTP1B in development of inflammation in global knock out models

PTP1B has a complex role in the immune system which has not yet been fully characterised. Indeed, it is thought to be important in many areas ranging from haematopoiesis and macrophage polarization [42], regulation of dendritic cell (DC)[43] function and macrophage [44-46] function to the induction of B and T-Cell tolerance [47] in the periphery. During development of the myeloid lineages, PTP1B is involved in myelopoiesis since PTP1B deficiency altered signalling in response to colony stimulating factor 1 (CSF-1), via the JAK2/STAT3 pathway, subsequently resulting in a skewed ratio between monocytes and granulocytes *in vivo*. This bias is thought to be due to a combination of decreased threshold in response to MCSF and the hyper-phosphorylation of the activation loop tyrosine in PTP1B. Interestingly, it was demonstrated that this phenomenon was specific to the granulocyte-monocyte precursor stage, as this was not present at earlier stages of bone marrow differentiation. It was also reported that the resulting bone marrow derived macrophages (BMDM) exhibited an increase in sensitivity to endotoxin and upregulation of inflammatory markers, both *in vitro* and *in vivo*, providing evidence that PTP1B is a negative regulator of inflammation [42].

Global deletion of PTP1B has been shown to produce significant pro-inflammatory phenotype [48], splenic macrophages isolated from global PTP1B deletion models showed increased pro-inflammatory activity [49], additionally, *in vitro* knock down of PTP1B in RAW.264.7 cell line led to increased production of TNF-α, IL-6 and IFN-γ in response to LPS challenge [50]. This was corroborated in another study that reported similar results in peritoneal macrophages from global deletion model along with PTP1B knock downs in human macrophages from donors and cell lines [44]. Logically, it was hypothesised given macrophages importance in the development of T2DM and obesity, and the heightened inflammatory response, that macrophage specific PTP1B may be an important factor in the induction of insulin resistance and hence cell-specific targeting of PTP1B would be advantageous in the treatment of T2DM. However, myeloid specific deletion of PTP1B produced a pronounced anti-inflammatory phenotype, directly contradicting what was observed in global knock out, while maintaining the improvement in both, glucose homeostasis and resistance to the development of T2DM [46]. However, no changes in inflammation were detected in either muscle [33] or adipocyte [51] specific PTP1B deletion models, whereas modest improvement in liver inflammation was reported in the liver specific knock out models[32].

Myeloid cell PTP1B model reveals a role for PTP1B in the promotion of inflammation

When analysing the role of PTP1B, it is of critical importance to consider the methods by which its function has been studied. PTP1B appears to display cell and tissue specific roles, differing in its signalling roles between different cell and tissue types. Thus, creation of cell-type specific PTP1B knockout models has been invaluable in our understanding of the specific roles that PTP1B plays in different tissues. Using these collective results and comparing to the global knock out model combined with cell line studies, we can begin to untangle the complicated function(s) of PTP1B in inflammation and the immune system.

When compared to control littermates, myeloid specific deletion of PTP1B (LysM PTP1B) mice were resistant to LPS-induced endotoxaemia and HFD induced inflammation. Furthermore, elevated systemic IL-10 levels with a corresponding decrease in IL-6 was observed, which was in agreement with the *in vitro* data, indicating that bone marrow derived macrophages (BMDM) release lower levels of pro-inflammatory cytokines and increased IL-10 [46]. DC are also myeloid cells derived from the same cell lineage as macrophages, bone marrow derived DC from the LysM PTP1B model display defects in several key functions namely, LPS driven activation, presentation of peptide to T cells and their subsequent activation, along with a decrease in migratory capacity [43]; the ramifications of which leads to modulation of the adaptive immune system. More specifically, LysM PTP1B BMDC lack the ability to fully initiate T cell responses, and this, coupled with IL-10[46] increases seen in the macrophages, may act in an immunosuppressive manner to quash inflammatory responses and hence produce the heightened anti-inflammatory response seen in this model.

Bot,h BMDM and BMDC from the LysM PTP1B model have been shown to have hyperphosphorylation of STAT3, which is concurrent with PTP1B deletion after day 4 in culture in BMDC [43]. One of the few immune consistencies between the global and tissue specific PTP1B knock-out models is the hyper-phosphorylation of STAT3. Treatment of peritoneal macrophages from the global knock out with IL-10 leads to hyper-phosphorylation of STAT3, resulting in a qualitative change in the transcriptome downstream and induced genes associated with inflammation [45]. This suggests that PTP1B plays a role in the promotion of inflammation via STAT3, rather than negatively regulating it as previously thought, which wold be more consistent with the improved metabolic profile than a negative regulatory role.

Finally, it is important to consider the differences in PTP1B deletion between these models. In the LysM

Model	Deletion specificity	Observed phenotype	Ref
PTP1B-/-	Global PTP1B	Glucose sensitivity↑, insulin sensitivity ↑, inflammation ↑.	30
SA-PTP1B-/-	Hepatic PTP1B	Glucose sensitivity ↑, insulin sensitivity ↑, liver inflammation ↓.	32
Ptpn1loxP/loxP-nes Cre	Neuronal PTP1B	Glucose sensitivity ↑, weight ↓, activity ↑, no change in inflammation.	31
Adip-crePTP1B−/−	Adipocyte PTP1B	No changes in; glucose sensitivity, insulin sensitivity, inflammation.	51
MCK-PTP1B-/-	Muscle PTP1B	Glucose sensitivity ↑, insulin sensitivity ↑, no change in inflammation.	33
LysM-Cre-PTP1B-/-	Myeloid PTP1B	Glucose sensitivity ↑, insulin sensitivity ↑, inflammation ↓, IL-10 ↑, protected from LPS shock .	43, 46
Mb1-PTP1B-/-	B cell PTP1B	Inflammation ↑, autoimmunity ↑.	47
Lck-Cre-PTPN2-/-	T cell TCPTP	Inflammation ↑, autoimmunity ↑.	54

Table 1. Summary of PTP1B and TCPTP global and tissue specific knock out models in mice. Observed phenotypes in murine knock out models of PTP1B and TCPTP are not consistent, though this may not be surprising given PTP1B's differing roles in different tissues. Global, hepatic, neuronal, muscle and myeloid knockout models all exhibit increased glucose and insulin sensitivity with the myeloid and hepatic models offering protection against inflammation. Global and B cell PTP1B knock outs and the T cell TCPTP knock out all confer an increase in inflammation and increased disease. Therefore it would be logical to hypothesize that the increase in inflammation in the global model may be a consequence of PTP1B deficiency in B cells or possibly, T cells.

PTP1B model, PTP1B is excised via the LoxP-Cre system when Lysozyme-M gene is activated [52], whereas, in the global knock out, PTP1B is absent during the entire differentiation process, while it is known this alters the ratio of monocytes to granulocytes as previously discussed, it is currently unknown if this directly alters the immune phenotype. The possibility that the absence of PTP1B during the differentiation process has intrinsically altered the functionality of these cells cannot be discounted.

B cell PTP1B deficiency may account for discrepancies between myeloid and global knock out models

As mentioned previously, PTP1B is ubiquitously expressed and is also present in cells of the adaptive immune system. B cell PTP1B has been shown to play a critical role in the activation of B cells and the maintenance of immunological tolerance. PTP1B in B cells was demonstrated to negatively regulate TLR-4 signalling and B cell activating factor receptor (BAFF-R) signalling, which is important for B cell survival. Furthermore, PTP1B is involved in CD40 signalling, which is involved in the interaction with CD4+ T Cells prompting the switch to production of high affinity IgG antibodies [47].

The mb1-PTP1B model specifically deletes PTP1B in B cells. Deficiency of PTP1B in these cells results in an augmented response to LPS, which induces abnormal proliferation and differentiation into plasma cells. This is consistent with peripheral autoreactive B cells, which have a lower threshold for response and differentiate into self-reactive plasma cells [47]. Indeed, there is evidence to support this hypothesis as B cells from patients who suffer from rheumatoid arthritis appear to have lower levels of PTP1B. These cells behave consistently with those from the mb1-PTP1B model and display heightened responses to inflammatory stimuli [47] and therefore may account for the heightened inflammatory response seen in the global knock out models.

T cell - Protein Tyrosine Phosphatase (TC-PTP) is a close relative to PTP1B and is expressed mainly in T Lymphocytes. It is thought to play a similar role to PTP1B as it is able to directly dephosphorylate IR in T cells, this was confirmed through double PTP1B and TC-PTP knock out cell line studies which illustrated the non-redundant role of both proteins in insulin signaling [53]. It has also been demonstrated that TC-PTP lowers the threshold for T cell signalling, leading to autoimmunity and proliferation of cells. Thus, it is thought that TC-PTP mirrors the role of PTP1B

and negatively regulates T cell induced inflammation [54]. Given that PTP1B and TC-PTP are thought to have a similar role and also share substrates, delineating the PTP1B specific roles in T cells is a complex matter, further muddied by fact that PTP1B T cell specific knock outs have not yet been published. However, given the interest in PTP1B and the immune system it is a keen and exciting area of research that is fast developing.

While the phenotypes of the discussed models may appear contradictory (summarised in table 1) they give us valuable insight to the role PTP1B plays in individual tissues. The pro-inflammatory phenotype seen in the global PTP1B knock out is most likely a result of PTP1B deficiency in the B cells - or T cells if PTP1B plays a similar role as TCPTP as hypothesised - leading to a systemic increase in inflammation which modulates the tissue environment, causing a phenotypic switch of macrophages to a M1 profile, rather than an intrinsic PTP1B deficiency in the macrophages themselves as previously thought.

Inhibition of PTP1B as a treatment for chronic inflammatory disorders

Given the importance of PTP1B both in the regulation of inflammation and insulin signalling, inhibitors of PTP1B have become a focused area of interest in drug discovery research. There are already several well-known small molecule inhibitors of PTP1B that are routinely used in the lab to study PTP1B both *in vitro* and *in vivo* which are made and distributed by multiple biotechnology companies for use in research. Many inhibitors (at least *in vitro*) have been discovered from a number of biological sources, such as polybromodiphenyl ether from an Indonesian marine sponge *Lamellodysidea herbacea* [55], multiple compounds in Angelica kesikei [56], a well-known anti-diabetic plant used in Japanese medicine as well as phylogenetic and computational methods and rational design approaches [37], all with the aim to develop small molecule inhibitors of PTP1B.

Designing PTP1B inhibitors is particularly difficult, due to the highly positively charged active site pocket and the fact PTP1B shares close homology with several other PTPs such as the aforementioned T-Cell PTP (TC-PTP). Thus creating a specific small molecular inhibitor which can be given via an oral route with desirable physiochemical properties, that is both efficacious *in vivo* and safe is a tremendous task. However, it is the nature of drug discovery that most compounds found to have the desired effect *in vitro* will fail during development and, to date, no compounds have successfully completed all phases of human clinical trials. As such, none have been licenced for use in the clinic due to

lack of *in vivo* efficacy; however there are small molecule inhibitors currently in phase II clinical trials.

While PTP1B inhibitors could be of great use in treatment of T2DM and chronic inflammatory disorders, care must be taken. As previously discussed, modulation of PTP1B in immune cells can greatly upset the homeostatic balance of the immune system. While clearly the over expression of PTP1B that creates insulin resistance is detrimental, equally, the absence of PTP1B in certain cell types such as B Cells can cause deleterious effects and exacerbate inflammation. Therefore an approach where excessive PTP1B is inhibited and returned to a baseline level in order to restore homeostasis would be the most beneficial in terms of treatment regimes. However this presents its own challenges, polymorphisms in genes encoding metabolising enzymes and drug transporters can affect the pharmokinetics and alter drug response. Dosages given to patients would have to be closely monitored in the clinic and tailored individually for the patient taking into account these factors, meaning a personalised approach would have to be taken bringing into question the cost-effectiveness of use of PTP1B inhibitor in the general population. Another approach may be the development of cell type specific delivery systems for a PTP1B inhibitor, inhibition of myeloid PTP1B would be highly advantageous and help minimise side effects associated with inhibition in B cells.

Concluding Remarks

In all metazoans, the mechanisms responsible for defence against pathogens have been evolutionarily coupled to metabolic control, with the aim of ensuring adequate host response to bacterial and viral pathogens, while subsequently preventing excessive inflammation that would lead to tissue damage. Metabolic homeostasis is the result of a delicate balance determined by the interplay of multiple factors. In particular, the functional cross-talk between adipocytes and resident M2-like macrophages ensures maintenance of insulin sensitivity; this is mostly mediated by signalling of anti-inflammatory cytokines like IL-4, IL-10 and IL-13.

The immune imbalance caused by nutritional overload can lead to chronic inflammatory status that is causal in the development of insulin resistance and hyperlipidemia. PTP1B, the master regulator of insulin signalling, forms a bridge between metabolism and inflammation and is a potent target for drug development. While its role is complex, multifactorial, cell type specific and often contradictory, however, by examining tissue specific knock outs we can delineate aspects of its complex role.

While global knock out models show improvement in

glucose homeostasis, this is accompanied by escalation of the LPS induced inflammatory response. In muscle, neuronal [31] and adipocyte specific [51] models this indicate no changes in inflammation, with hepatic specific [32] deletion offering some protection against liver inflammation. This led to the speculation that the pro-inflammatory phenotype observed with global PTP1B deficiency may be caused PTP1B absence from the immune cells.

LysM PTP1B model revealed enhanced anti-inflammatory properties, with a decrease in IL-6 and an increase in IL-10 both systemically and in vitro, coupled with decrease in BMDC migratory capacity and in capacity to activate T cells. A combination of inadequate migration and peptide presentation with a systemic anti-inflammatory environment may be working synergistically to supress the inflammatory responses. Consistently, across these models, STAT3 appears to be hyper-phosphorylated, indicating that the regulation of inflammation by macrophage and DC PTP1B is STAT3-dependent. The differences in these models point to speculation that PTP1B may have an effect on the polarisation of macrophages during development as it is also known to have a key role in haematopoiesis [42]. Evidence from the B cell specific deletion model mb1-PTP1B, suggests a highly pro-inflammatory phenotype due to PTP1B deficiency in B cells, along with development of autoimmunity, an observation echoed in human rheumatoid arthritis patient B cells [47]. This closely matches observations in the global knock out models and may account for the pro-inflammatory phenotype observed including some of the autoimmune aspects of the phenotype.

Given PTP1B's critical role in the regulation of inflammation and insulin signalling it is an important and critical target for the development of clinical treatments. If drugs are to be successful in this manner, they must be highly specific either in design or targeting to avoid disrupting the function of other closely related phosphatases and tailored for use in a manner that corrects the increased PTP1B expression associated with T2DM and obesity. While, to date, no inhibitors have made it through human clinical trials, work is ongoing apace to bring forward their use to the clinic and revolutionise the treatment of the world's most costly and prevalent metabolic disorder.

Conflict of Interest

The authors declare they have no competing interests.

Acknowledgements

References

1. Diabetes UK: Facts and stats. [https://www.diabetes.org.uk/Documents/About%20Us/Statistics/Diabetes-key-stats-guidelines-April2014.pdf]

2. Campa HS, Rena D, Leffb T. Adipogenesis and fat-cell function in obesity and diabetes. Trends Mol Med 2002; 8:442-447.

3. WHO. World health organisation-Obesity. [http://www.who.int/topics/obesity/en/]

4. Public Health England. UK and Ireland prevalence and trends in obesity [https://www.noo.org.uk/NOO_about_obesity/adult_obesity/UK_prevalence_and_trends]

5. Williamson RT. On the treatment of glycosuria and diabetes mellitus with aspirin. Br Med J 1901; 2:1946-1948.

6. Pickup JC, Crook MA. Is type II diabetes mellitus a disease of the innate immune system? Diabetologia 1998; 41:1241-1428.

7. Lumeng CN, DeYoung SM, Bodzin JL, Saltiel AR. Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. Diabetes 2007; 57:16-23.

8. Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, *et al*. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science 2011; 8:243-247.

9. Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, *et al*. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med 2009; 15:930-939.

10. Choy L, Derynck R. Transforming growth factor-beta inhibits adipocyte differentiation by Smad3 interacting with CCAAT/enhancer-binding protein (C/EBP) and repressing C/EBP transactivation function. J Biol Chem 2003; 278:9609-9619.

11. Lumeng CN, Del-Proposto JB, Westcott DJ, Saltiel AR. Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. Diabetes 2008; 57:3239-3246.

12. Mantovania A, Sicab A, Sozzanib S, Allavenab P, Vecchib A, Locatia M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 2004; 25:677-686.

13. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003; 112:1796-1808.

14. Murano I, Barbatelli G, Parisani V, Latini C, Muzzonigro G, Castellucci M, *et al*. Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. J Lipid Res 2008; 49:1562-1568.

15. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 2007; 117:175-184.

16. Red Eagle A, Chawla A. In obesity and weight loss, all roads lead to the mighty macrophage. J Clin Invest 2010; 120:3437-3440.

17. Kosteli A, Sugaru E, Haemmerle G, Martin JF, Lei J, Zechner R, *et al*. Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. J Clin Invest 2010; 120:3466-3479.

18. Gao Z, Hwang D, Bataille F, Lefevre M, York D, Quon MJ, *et al*. Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. J Biol Chem 2002; 277:48115-48121.

19. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 1995; 95: 2409-2415.

20. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance Science 1993; 259:87-91.

21. Zabolotny JM, Kim YB, Welsh LA, Kershaw EE, Neel BG, Kahn BB. Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. J Biol Chem 2008; 283:14230-14241.

22. Picardi PK, Caricilli AM, de Abreu LL, Carvalheira JB, Velloso LA, Saad MJ. Modulation of hypothalamic PTP1B in the TNF-alpha-induced insulin and leptin resistance. FEBS Lett 2010; 584:3179-3184.

23. Ahmad F, Goldstein BJ. Effect of tumor necrosis factor-a on the phosphorylation of tyrosine kinase receptors is associated with dynamic alterations in specific protein-tyrosine phosphatases. J Cell Biochem 1997; 61:117-127.

24. Ahmad F, Azevedo JL, Cortright R, Dohm GL, Goldstein BJ. Alterations in skeletal muscle protein-tyrosine phosphatase activity and expression in insulin-resistant human obesity and diabetes. J Clin Invest 1997; 100:449-458.

25. Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, *et al*. Effects of infection and inflammation on lipid and lipoprotein metabolism: Mechanisms and consequences to the host. J Lipid Res 2004; 45:1169-1196.

26. Frangioni JV, Beahm PH, Shifrin V, Jost CA, Neel BG. The nontransmembrane tyrosine phosphatase PTP-1B localizes to the endoplasmic reticulum via its 35 amino acid C-terminal sequence. Cell 1992; 68:545-560.

27. Tonks NK, Diltz CD, Fischer EH. Characterization of the major protein-tyrosine-phosphatases of human placenta. J Biol Chem 1988;263:6731-6739.

28. Chernoff J, Schievella AR, Jost CA, Erikson RL, Neel BG. Cloning of a cDNA for a major human protein-tyrosine-phosphatase. Proc Natl Acad Sci U S A 1990; 897:2735.

29. Seely BL, Staubs PA, Reichart DR, Berhanu P, Milarski KL, Saltiel AR, *et al*. Protein tyrosine phosphatase 1B interacts with the activated insulin receptor. Diabetes 1996; 45:1379-1385.

30. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, *et al*. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 1999; 283:1544-1548.

31. Bence KK, Delibegovic M, Xue B, Gorgun CZ, Hotamisligil GS, Neel BG, *et al*. Neuronal PTP1B regulates body weight, adiposity and leptin action Nat Med 2006; 12:917-924.

32. Delibegovic M, Zimmer D, Kauffman C, Rak K, Hong EG, Cho YR, *et al*. Liver-specific deletion of protein-tyrosine phosphatase 1B (PTP1B) improves metabolic syndrome and attenuates diet-induced endoplasmic reticulum stress Diabetes 2009; 58:590-599.

33. Delibegovic M, Bence KK, Mody N, Hong EG, Ko HJ, Kim JK, *et al*. Improved glucose homeostasis in mice with muscle-specific deletion of protein-tyrosine phosphatase 1B. Mol Cell Biol 2007; 27:7727-7734.

34. Tonks NK. PTP1B: From the sidelines to the front lines! FEBS Lett 2003; 546:140.

35. Flint AJ, Tiganis T, Barford D, Tonks NK. Development of "substrate-trapping" mutants to identify physiological substrates of protein tyrosine phosphatases. Proc Natl Acad Sci U S A 1997; 94:1680-1685.

36. Boubekeur S, Boute N, Pagesy P, Zilberfarb V, Christeff N, Issad T. A new highly efficient substrate-trapping mutant of protein tyrosine phosphatase 1B (PTP1B) reveals full autoactivation of the insulin receptor precursor. J Biol Chem 2011; 286:19373-19380.

37. Romsicki Y, Scapin G, Beaulieu-Audy V, Patel S, Becker JW, Kennedy BP *et al*. Design and characterization of an improved protein tyrosine phosphatase substrate-trapping mutant. Biochemistry 2002; 41:4032-4039.

38. Sarmiento M, Puius YA, Vetter SW, Keng YF, Wu L, Zhao Y, *et al*. Structural basis of plasticity in protein tyrosine phosphatase 1B substrate recognition. Biochemistry 2000; 39:8171-8179.

39. Myers MP, Andersen JN, Cheng A, Tremblay ML, Horvath CM, Parisien JP, *et al*. TYK2 and JAK2 are substrates of protein-tyrosine phosphatase 1B. J Biol Chem 2001;276:47771-47774.

40. Zhang X, Guo A, Yu J, Possemato A, Chen Y, Zheng W, *et al*. Identification of STAT3 as a substrate of receptor protein tyrosine phosphatase. Proc Natl Acad Sci USA 2006; 104:4060-4064.

41. Lu X, Malumbres R, Shields B, Jiang X, Sarosiek KA, Natkunam Y, *et al*. Lossos. PTP1B is a negative regulator of interleukin 4-induced STAT6 signaling. Blood 2008; 112:4098-4108.

42. Heinonen KM, Bourdeau NDA, Lapp WS, Tremblay ML. Protein tyrosine phosphatase 1B negatively regulates macrophage development through CSF-1 signaling. Proc Natl Acad Sci U S A 2006; 103:277-2281.

43. Martin-Granados C, Prescott AR, Le Sommer S, Klaska IP, Yu T, Muckersie E, *et al*. A key role for PTP1B in dendritic cell maturation, migration, and T cell activation. J Mol Cell Biol 2015; 0:1-12.

44. Través PG, Pardo V, Pimentel-Santillana M, González-Rodríguez Á, Mojena M, Rico D, *et al*. Pivotal role of protein tyrosine phosphatase 1B (PTP1B) in the macrophage response to pro-inflammatory and anti-inflammatory challenge. Cell Death Dis 2014;e1125.

45. Pike KA, Hutchins AP, Vinette V, Théberge JF, Sabbagh L, Tremblay ML, *et al*. Protein tyrosine phosphatase 1B is a regulator of the interleukin-10-Induced transcriptional program in macrophages. Sci Signal 2014;7:ra43.

46. Grant L, Shearer KD, Czopek A, Lees EK, Owen C, Agouni A, *et al*. Myeloid-cell protein Tyrosine Phosphatase-1B deficiency in mice protects against high-fat diet and lipopolysaccharide-induced inflammation, hyperinsulinemia, and endotoxemia through an IL-10 STAT3-dependent mechanism. Diabetes 2014;63:456-470.

47. Medgyesi D, Hobeika E, Biesen R, Kollert F, Taddeo A, Voll RE, *et al*. The protein tyrosine phosphatase PTP1B is a negative regulator of CD40 and BAFF-R signaling and controls B cell autoimmunity. J. Exp Med 2014; 211:427-440.

48. Berdnikovs S, Pavlov VI, Valencia HA, McCary CA, Klumpp DJ, Tremblay ML, *et al*. PTP1B deficiency exacerbates inflammation and accelerates leukocyte trafficking in vivo. J Immunol 2012; 188:874-884.

49. Través PG, Pardo V, Pimentel-Santillana M, González-Rodríguez A, Mojena M, RicoD, *et al*. Pivotal role of protein tyrosine phosphatase 1B (PTP1B) in the macrophage response to pro-inflammatory and anti-inflammatory challenge. Cell Death Dis 2014.

50. Xu H, An H, Hou J, Han C, Wang P, Yu Y, *et al*. Phosphatase PTP1B negatively regulates MyD88- and TRIF-dependent proinflammatory cytokine and type I interferon production in TLR-triggered macrophages. Mol Immunol 2008;45:3545-3552.

51. Owen C, Czopek A, Agouni A, Grant G, Judson R, Lees EK, *et al*. Adipocyte-specific protein tyrosine phosphatase 1B deletion increases lipogenesis, adipocyte cell size and is a minor regulator of glucose homeostasis. PLOS one 2012.

52. Clausen BE, Burkhardt C, Reith W, Renkawitz R, Förster I. Conditional gene targeting in macrophages and granulocytes using LysMcre mice. Transgenic Res 1998; 8:819-829.

53. Galic S, Hauser C, Kahn BB,Haj FG, Neel BG, Tonks NK, *et al*. Coordinated regulation of insulin signaling by the protein tyrosine phosphatases PTP1B and TCPTP. Mol Cell Biol 2005;25:819.

54. Wiede F, Shields BJ, Chew SH, Kyparissoudis K, Vliet CV, Galic S, *et al*. T cell protein tyrosine phosphatase attenuates T cell signaling to maintain tolerance in mice. J Clin Invest 2011; 121:4758-4775.

55. Yamazaki H, Sumilat DA, Kanno S, Ukai K, Rotinsulu H, Wewengkang DS, *et al*. A polybromodiphenyl ether from an indonesian marine sponge lamellodysidea herbacea and its chemical derivatives inhibit protein tyrosine phosphatase 1B, an important target for diabetes treatment. J Nat Med 2013; 67:730-735.

56. Li JL, Gao LX, Meng FW, Tang CL, Zhang RJ, Li JY, *et al*. PTP1B inhibitors from stems of angelica keiskei (ashitaba). Bioorg Med Chem Lett 2015;25:2028-2032.