O-GlcNAcylation in immunity and inflammation: An intricate system (Review)

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Abstract. Chronic, low-grade inflammation associated with obesity and diabetes result from the infiltration of adipose and vascular tissue by immune cells and contributes to cardiovascular complications. Despite an incomplete understanding of the mechanistic underpinnings of immune cell differentiation and inflammation, O-GlcNAcylation, the addition of O-linked N-acetylglucosamine (O-GlcNAc) to cytoplasmic, nuclear and mitochondrial proteins by the two cycling enzymes, the O-linked N-acetylglucosamine transferase (OGT) and the O-GlcNAcase (OGA), may contribute to fine-tune immunity and inflammation in both physiological and pathological conditions. Early studies have indicated that O-GlcNAcylation of proteins play a pro-inflammatory role in diabetes and insulin resistance, whereas subsequent studies have demonstrated that this post-translational modification could also be protective against acute injuries. These studies suggest that diverse types of insults result in dynamic changes to O-GlcNAcylation patterns, which fluctuate with cellular metabolism to promote or inhibit inflammation. In this review, the current understanding of O-GlcNAcylation and its adaptive modulation in immune and inflammatory responses is summarized.

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Abbreviations: O-GlcNAc, O-linked N-acetylglucosamine; OGT, O-linked N-acetylglucosamine transferase; OGA, O-GlcNAcase; GlcNAc, β-D-N-acetylglucosamine; UDP-GlcNAc, uridine diphosphate N-acetylglucosamine; HBP, hexosamine biosynthetic pathway; TPR, tetratricopeptide repeat; EGF, epidermal growth factor; AMPK, AMP-activated protein kinase; HAT, histone acetyltransferase; TCR, T cell receptor; Th, T helper; iTregs, inducible regulatory T cells; IL, interleukin; NF-κB, nuclear factor-κB; IκBα, inhibitor of NF-κB α; LPS, lipopolysaccharide; IKK, IκB kinase; CBP, CREB-binding protein; NFAT, nuclear factor of activated T cells; TNF-α, tumor necrosis factor-α; TNFAIP3, TNF-α induced protein 3; BCR, B cell receptor; TAK1, transforming growth factor-β activated kinase 1; VCAM-1, vascular cell adhesion molecule-1; NO, nitic oxide; iNOS, inducible nitric oxide synthase; STAT3, signal transducer and activator of transcription 3; CUL3, cullin 3; TAB1, TAK1-binding protein 1; GlcN, glucosamine; PUGNAc, O-(2-acetamido-2-deoxy-D-glucopyranosylidene) amino-N-phenylcarbamate; MCAO, middle cerebral artery occlusion; ICAM-1, intercellular adhesion molecule; MPO, myeloperoxidase; GPX1, glutathione peroxidase 1; Glc, Glucose; Fruc, fructose; Nod2, Nucleotide-binding oligomerization domain 2; Sp1, specificity protein 1

Key words: O-GlcNAcylation, O-linked N-acetylglucosamine transferase, O-GlcNAcase, immunity, inflammation

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1. Introduction

Over 450 types of protein post-translational modifications have been discovered, including phosphorylation, glycosylation, ubiquitination, acetylation, nitrosylation, methylation and ADP-ribosylation (1). Such modifications dramatically expand the repertoire of potential protein functions and contribute to the high diversity of cellular activities due to their reversible nature, relatively small metabolic cost, and their ability to extensively modulate the functions of target proteins (2,3). Among all post-translational modifications, phosphorylation and glycosylation are considered the most common and the best investigated.

Glycosylation was, until recently, considered a stable and conserved post-translational modification, while phosphorylation was generally considered as a dynamic and reversible modification. A specialized form of O-glycosylation refers to the presence of β-D-N-acetylglucosamine (GlcNAc) monosaccharide units linked O-glycosidically to intracellular proteins (O-GlcNAcylation). This type of modification was not discovered until 1984 and was found to be directly involved

in the modulation of a number of cellular processes (4). O-GlcNAcylation differs from N-glycosylation and other O-glycosylation like O-GalNAcylation by occurring mainly on nuclear, cytoplasmic and mitochondrial proteins, and by being temporally dynamic. Unlike N-glycosylation, O-GlcNAcylation is more analogous to phosphorylation as it occurs rapidly and reversibly. Interestingly, O-GlcNAcylation can either compete or co-operate with phosphorylation since it targets either the same or the proximal serine/threonine (Ser/Thr) residue sites required for phosphorylation (5). However, unlike phosphorylation that is regulated by a diverse array of kinases and phosphatases, O-GlcNAcylation is controlled by the action of a single pair of enzymes, OGT and OGA (6). OGT is the specific enzyme responsible for catalyzing the reaction between a single GlcNAc moiety and the hydroxyl group of a ser or thr residue on proteins, while OGA is the specific glycoside hydrolase that removes it (6,7). The substrate of the transferase reaction is uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), which is synthesized by the hexosamine biosynthetic pathway (HBP). HBP co-ordinates with almost every other metabolic cellular pathway since O-GlcNAcylation is sensitive to the levels of insulin, glucose, amino acids (glutamine), fatty acid (acetyl-CoA), nucleotide (UDP) and cellular stress. Therefore, O-GlcNAcylation has mainly been considered to serve in the regulation of cellular signaling, transcription and translation in response to nutrients and stresses (8) (Fig. 1). Signaling pathways convert environmental cues into intracellular events, such as immune cell activation and inflammation. Combined aberrant O-GlcNAcylation may be the cause of excessive nutrient intake, diabetes and/or autoimmune diseases progressions, including those involved in inflammatory and immune responses in individual cell types and tissues (Table I).

2. Enzymes controlling O-GlcNAc cycling

O-linked N-acetylglucosamine transferase (OGT). OGT is encoded by a single gene in mammals, however, its transcript produces multiple variants after selective splicing which are as follows: Nucleocytoplasmic, (ncOGT; 116 kDa); mitochondrial, (mOGT; 103 kDa) and short, (sOGT; 78 kDa) (9). They share a common catalytic and phosphorylase-derived C-terminal domain but differ in length owing to variable numbers of the N-terminal tetratricopeptide repeat (TPR) domain (10), separated by a spacer region. It had long been believed that OGT isoforms are present in the cytoplasm and nucleus, such as ncOGT and sOGT; and in mitochondria, such as mOGT (7). However, an atypical OGT termed epidermal growth factor domain-specific O-GlcNAc transferase (EOGT) was identified, which transfers GlcNAc to Ser or Thr in secreted and membrane proteins containing the epidermal growth factor (EGF) repeat with a specific consensus sequence. Interestingly, OGT and EOGT exist in separate cellular compartments and have mostly distinct substrates (11,12).

OGT modulates a variety of cellular processes like transcription, protein synthesis (9), protein degradation (13,14), protein-protein interaction or localization (7,15,16) and stress response (17) using several substrates. However, it has been difficult to elucidate the mechanisms by which OGT recognizes its substrates since it targets only a fraction of Ser/Thr

in different biological situations for glycosylation. There have been several suggested potential mechanisms. OGT primarily recognizes most of its substrates through asparagine ladder binding in the TPR domain (18,19). The interaction between OGT and its substrates also contributes to the OGT catalytic mechanism. The N-acyl group in UDP-GlcNAc is crucial for its affinity to sOGT. In this process, the backbone carbonyl oxygen of L653 and the hydroxyl group of T560 in sOGT are important for the recognition of the UDP-GlcNAc (20). Additionally, OGT can also non-specifically modify proteins which contain regions of intrinsic disorder (e.g. tau and nuclear pore proteins) without recognizing any specific sequences or structures (7). Different UDP-GlcNAc concentrations or varying UDP-GlcNAc gradients may influence OGT binding specificity (21). Finally, OGT is also regulated by phosphorylation. The phosphorylation of OGT at T444 by AMP-activated protein kinase (AMPK) is closely associated with OGT nuclear localization in myotubes and phospho-mimetic T444E-OGT exhibits altered substrate selectivity. Acute treatment (2 h) with a highly specific activator of AMPK (A-769662) induced significant global changes in O-GlcNAcylated proteins bound to wild-type OGT compared with T444E-ncOGT. It demonstrated the placement of a large highly charged moiety on residue T444 is sufficient to dramatically alter the substrate selectivity of OGT (22).

Genetic and chemical approaches are used to inhibit both OGT expression and activity to comprehensively study the biological role of O-GlcNAc and assess the catalytic vs. non-catalytic roles of OGT. Complete OGT knockout is embryo-lethal in a range of animal models. As such, tissue-specific OGT knockout mice have been employed (23). Consequently, chemical inhibitors are a worthwhile means to explore the biological role of O-GlcNAcylation without affecting OGT protein levels. UDP, alloxan, compound 4 (ST045849), compound 5 and (αR)-α-[[(1,2-Dihydro-2-oxo-6-quinolinyl)sulfonyl] amino]-N-(2-furanylmethyl)-2-methoxy-N-(2-hienylmethyl)-benzeneacetamide are small molecules that compete with the donor substrate (24-26) (Fig. 1). In addition, a new series of competitive inhibitors, goblin1 (OGT bisubstrate-linked inhibitor 1) and goblin 2, derived from both OGT and the substrate, were designed based on the understanding of the OGT catalytic mechanism (27). However, off-target effects, lack of specificity and low-grade cell permeability are limitations that need to be resolved (28).

O-GlcNAcase (OGA). The human OGA (hOGA) gene MGEA5 is alternatively spliced to generate nucleocytoplasmic (nc; 130 kDa) and short (s; 75 kDa) isoforms. The ncOGA contains both an N-terminal O-GlcNAc hydrolase and a C-terminal histone acetyltransferase-like (HAT-like) domain; while sOGA lacks the HAT-like domain and is localized to the endoplasmic reticulum and lipid droplets (29,30).

hOGA belongs to the carbohydrate-active enzymes database glycoside hydrolase 84 family of enzymes (31). The substrate recognition mechanism of hOGA was not considered to be sequence specific until the crystal structure for hOGA was reported (32). The crystallographic data for hOGA suggested that the full-length and nuclear spliced isoforms could be structurally distinguished by
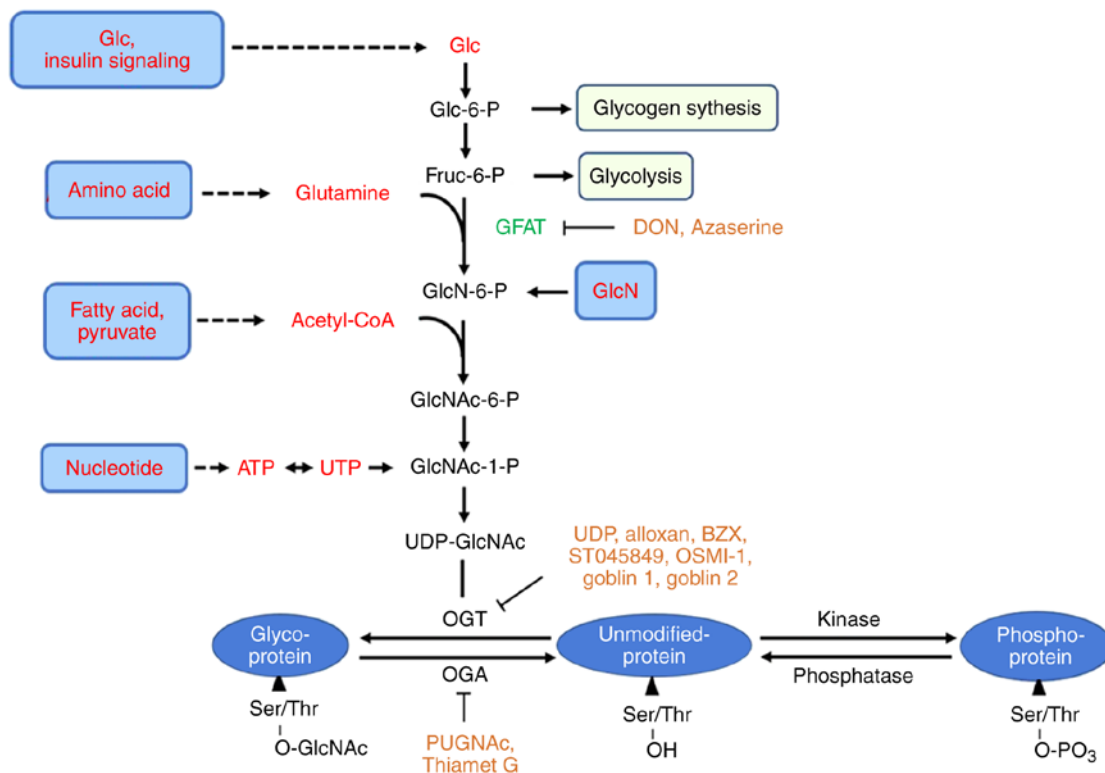
Figure 1. Nutrient flux through the HBP modulates protein O-GlcNAcylation. The majority of glucose is taken for glycolysis and glycogen synthesis, but a small percentage (~2-5%) is funneled directly into the HBP for UDP-GlcNAc synthesis. OGT catalyzes the addition of GlcNAc from UDP-GlcNAc to serine and threonine residues, while OGA catalyzes their removal. Together these two enzymes tune the dynamic addition and removal of O-GlcNAc for thousands of diverse proteins. O-GlcNAcylation sites are usually directly at, or close to, the same serine or threonine residues used by kinases. HBP, hexosamine biosynthetic pathway; OGT, O-linked N-acetylglucosamine transferase; OGA, O-GlcNAcase; GlcNAc, N-acetylglucosamine; UDP, uridine diphosphate; GFAT, glutamine-fructose-6-phosphate aminotransferase; OSMI-1, (αR)-α-[[(1,2-Dihydro-2-oxo-6-quinolinyl)sulfonyl]amino]-N-(2-furanylmethyl)-2-methoxy-N-(2-thienylmethyl)-benzeneacetamide.

their ability to form an interlinked dimer. Based on the crystal structure, novel inhibitors targeting hOGA other than O-(2-acetamido-2-deoxy-D-glucopyranosylidene) amino-N-phenylcarbamate (PUGNAc) and thiamet G could be designed (32,33) (Fig. 1). Notably, OGA can be O-GlcNAcylated by OGT at S405; however, the implications of O-GlcNAcylation of OGA have remained elusive (1,34).

3. Immunoregulatory role of O-GlcNAcylation

O-GlcNAcylation activates T cells. The T cell receptor (TCR) is a molecule present on the surface of T cells that is responsible for recognizing antigens as peptides which are bound to major histocompatibility complex II molecules on the surface of antigen presenting cells. This triggers the initial activation of cluster of differentiation (CD)4+ T cells (35) and directs CD4+ T cell differentiation into four effectors: T helper 1 (Th1), which target intracellular bacteria and viruses; Th2, which target extracellular parasites; Th17, which target fungi and extracellular pathogens and inducible regulatory T cells (iTregs), which have the opposing function of decreasing an inflammatory immune response. T cell activation induces expression of GLUT1 and amino acid transporters on the cell surface which transport adequate glucose and glutamine for the differentiation of Th1, Th2 and Th17 cells. Moreover, the conversion of glutamine to acetyl CoA boosts fatty acid synthesis during T cell activation (36,37). Taken together,

glucose, glutamine and acetyl CoA are all substrates that participate in the HBP, driving an increase in UDP-GlcNAc synthesis and thus protein O-GlcNAcylation by OGT (37,38) (Fig. 2).

T cell activation enhances both OGT expression and overall protein O-GlcNAylation levels. More than 2,000 intact O-linked glycopeptides have been identified on activated primary human T cells via a technique termed as isotope targeted glycoproteomics. A large portion (>45%) of identified O-GlcNAc sites lie in proximity to or coincide with a known phosphorylation site, indicating a possibility of post-translational modification crosstalk (39). It has been reported that deficiency in protein O-GlcNAcylation results in the blocking of T cell proliferation, development, transformation and differentiation (40,41). Importantly, Notch, TCR and c-Myc (T58) have been identified as key controllers of T cell protein O-GlcNAcylation by regulation of glucose and glutamine transport (41,42).

The nuclear factor (NF)-κB transcription factor family consists of five proteins, including p65 (RelA), RelB, c-Rel, p105/p50 (NF-κB1) and p100/p52 (NF-κB2). NF-κB p65/p50 is the most commonly occurring heterodimer complex among NF-κB homodimers and heterodimers and is the functional component involved in nuclear translocation and activation of NF-κB (43). Under quiescent conditions, the inactive NF-κB p65/p50 heterodimer is mainly sequestered in the cytoplasm and is associated with inhibitor of NF-κB α (IκBα). However,

Table I. Functions of O-GlcNAcylation in immune and inflammatory responses.

Author, year	Cell type and tissue	Stimuli and treatment	Target protein and O-GlcNAcylation site	Function	(Refs.)
Golks *et al*, 2007;	T cells	Anti-CD3/CD28 Ab	NFAT, p65,	T cell activation,	(52)
Lund *et al*, 2016;			c-Rel (S350)	IL-2, IFNG, and CSF2 ↑	(40)
Bunting *et al*, 2007;			c-Myc (T58)	T cell clonal expansion	(53)
Swamy *et al*, 2016					(41)
Golks *et al*, 2007;	B cells	Anti-IgM Ab	NFAT, p65 Lyn (S19)	B cell activation	(52)
Wu *et al*, 2017		-		B-cell activation and expansion	(56)
Kneass and Marchase, 2005;	Neutrophils	fMLF/PMA	-	Cellular migration	(66)
Kneass and Marchase, 2004					(67)
James *et al*, 2002	Mesangial cells	High glucose, GlcN	p65	VCAM-1 ↑	(73)
Dela *et al*, 2017	Placentas cells	High glucose	p65	TNF-α and IL-6 ↑	(74)
Krick *et al*, 2018	Epithelial cells	FGF23	-	NFAT activation ↑ IL-6 ↑	(71)
Donovan *et al*, 2014		high glucose	Sp1	VEGF-A ↑	(81)
Zhang *et al*, 2015	Pancreatic acinar cells	caerulein	p65, IKKα	TNF-α and NO ↑	(75)
Li *et al*, 2014;	Endothelial cells	LPS	p65	Inflammatory mediators ↑	(76)
Donovan *et al*, 2014;		High glucose	Sp1	VEGF-A, ICAM-1 ↑	(81,82)
Zhang *et al*, 2017					
Allison *et al*, 2012;	293T	TNF-α	p65 (T305, S319)	IL-6 and TNF-α ↑	(78,79)
Ma *et al*, 2017;					(85,88)
Pathak *et al*, 2012		IL-1/osmotic stress	TAB1 (S395)		
Hou *et al*, 2016		MDP	Nod2	NF-κB activity ↑	
Hwang *et al*, 2017;	Macrophages	LPS IL-1/osmotic stress	STAT3(T717)	NO/iNOS ↑;	(62,63,84 85,87)
Ryu and Do, 2011;			TAK1(S427)	IL-12, CXCL1 and	
Li *et al*, 2017;				CXCL2 ↑; IL-10 ↓;	
Pathak *et al*, 2012;				polarization	
Zhang *et al*, 2016					
Hwang *et al*, 2010;	Macrophages smooth Muscle cells	GlcN with LPS	p65 and c-Rel, RNAPII	COX-2, iNOS, IL-1β, IL-6, TNF-α ↓	(64,65)
Hwang *et al*, 2014					
He *et al*, 2017		Thiamet G with MCAO or LPS	-	Iba+ cells ↓ iNOS and COX2 ↓ p65 translocation ↓ M1 polarization ↓	(103)
Yang *et al*, 2008		High glucose	p65 (T352)	VCAM-1 ↑	(77)
Yang *et al*, 2010;	Smooth muscle cells	High glucose	PGX1 p65 (S536),	anti-oxidant activity ↑	(112)
Xing *et al*, 2011;	Colon epithelial cell	GlcN/OGA inhibitor with TNF-α	A20	MCP-1, P-selectin, VCAM-1 ↓	(98,111)
Yao *et al*, 2018					
Hirata *et al*, 2018		dextran sodium sulfate	-	phosphor-p65 ↓ IL-1β ↓	(99)
Zou *et al*, 2007	Heart	GlcN with trauma-hemorrhage	-	cardiac output and organ perfusion recovered	(91)
Guo *et al*, 2015	Heart	Intermittent hypoxia	-	MAPK activity ↑ p65 levels ↑ inflammatory mediators ↑	(72)

IL, interleukin; LPS, lipopolysaccharide; IKK, IκB kinase; NFAT, nuclear factor of activated T cells; TNF-α, tumor necrosis factor-α; TAK1, transforming growth factor-β activated kinase 1; VCAM-1, vascular cell adhesion molecule-1; NO, nitic oxide; iNOS, inducible nitric oxide synthase; STAT3, signal transducer and activator of transcription 3; TAB1, TAK1-binding protein 1; GlcN, glucosamine; MCAO, middle cerebral artery occlusion; ICAM-1, intercellular adhesion molecule; MPO, myeloperoxidase; FGF23, fibroblast growth factor 23; Nod2, Neucleotide-binding oligomerization domain 2; Sp1, specificity protein 1.
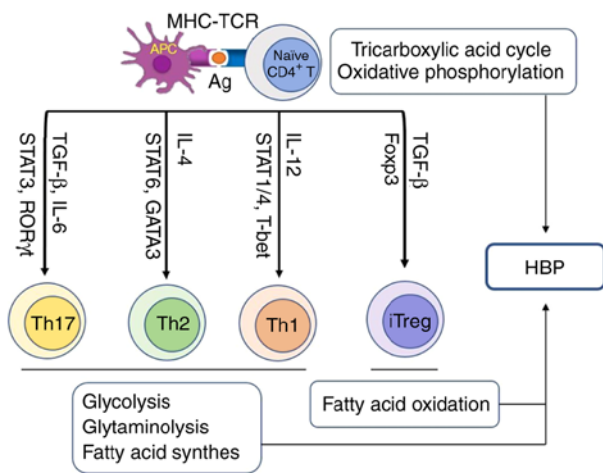
Figure 2. Connection between helper T cell differentiation and HBP. After T cell antigen receptor engagement, naïve CD4+ T cells differentiate into effector T cells including Th1, Th2 and Th17 cells, as well as iTreg. Effector T cells utilize glucose through glycolysis and amino acids through glutaminolysis to meet their energy need for differentiation, whereas regulatory T cells use energy from fatty acid oxidation. Finally, HBP integrates glucose, amino acid and fatty acid metabolism to generate UDP-GlcNAc for O-GlcNAc modification. CD, cluster of differentiation; HBP, hexosamine biosynthetic pathway; iTregs, inducible regulatory T cells; MHC, major histocompatibility complex; TCR, T cell receptor; IL, interleukin; UDP-GlcNAc, uridine diphosphate N-acetylglucosamine; APC, antigen presenting cell.

upon stimulation by pro-inflammatory cytokines, lipopolysaccharide (LPS) or glucose, IκBα is phosphorylated by the IκB kinase (IKK) on S32 and S36. This phosphorylation results in IκB ubiquitination and subsequent degradation by the 26S proteasome. NF-κB p65/p50 then detaches from IκBα and translocates to the nucleus where it binds to NF-κB promoter/enhancer sites. Through its acidic transactivation domain, p65 has the capacity to interact with several different transcriptional regulatory proteins, such as CREB-binding protein (CBP)/p300, to initiate transcription of NF-κB target genes (44,45). NF-κB is primarily modulated by post-translational modifications, such as phosphorylation, acetylation and glycosylation. It regulates several cellular processes like innate immunity, adaptive immunity, inflammation, cell apoptosis, cell survival and differentiation. The activity of NF-κB is closely associated with the pathogenesis of metabolic syndrome (46). Metabolic syndrome comprises hyperglycemia, hyperlipidemia, insulin resistance, obesity and hepatic steatosis which result from nutrient excess (47). Mounting an immune response to infection resulting in the release of cytokines is energy intensive. NF-κB is activated by cytokines to promote re-localization, activation and differentiation of macrophages at the site of infection. These activated macrophages defend against invading microorganisms by producing antimicrobial molecules and recruiting leukocytes, subsequently removing pathogens and clearing dead cells (48,49). NF-κB-regulated expression of the inflammatory mediators drives the differentiation of monocytes into either M1 or M2 macrophages which are vital to the development of the inflammation-associated metabolic disease. M1 macrophages produce and release IL-1, IL-6, tumor necrosis factor (TNF)-α, and other pro-inflammatory cytokines, while M2 macrophages secrete the anti-inflammatory cytokine

IL-10 (49,50). Therefore, NF-κB controls the expression of the inflammatory mediators that recruit monocytes, drive differentiation to macrophages and direct macrophage cell fate. NF-κB therefore modulates inflammation in the liver, adipose tissue and central nervous system in the development of metabolic diseases (49,51). Furthermore, an increase in NF-κB p65 O-GlcNAcylation contributes to T cell activation. It has been reported that OGT is required for the O-GlcNAcylation and transcriptional activity of both p65 as well as nuclear factor of activated T cells (NFAT). This leads to IL-2 production which is consistent with T cell activation (52). Additionally, NF-κB subunit c-Rel is modified and activated by O-GlcNAcylation at S350. Conversely, mitigating the O-GlcNAcylation of this residue disturbs the c-Rel-mediated expression of IL-2, IFN-γ and colony stimulating factor (CSF)2 in response to TCR activation. However, mutating the O-GlcNAcylation site of c-Rel or treating cells with PUGNAc has no effect on TCR or TNF-α induced expression of *TNFAIP3* (encodes TNF-α induced protein 3) and *NFKBIA* (encodes NF-κB inhibitor α), genes that are considered to contain c-Rel binding sites in their promoters (53). Although TCR and TNF both elevate the nuclear translocation of c-Rel, only TCR activity resulted in the O-GlcNAcylation of c-Rel in the nucleus. These results suggest a stimulus-specific role or O-GlcNAcylation of c-Rel in promoting T cell-mediated autoimmunity. It is the generation of these autoimmune T helper cell cytokines which result in pancreatic β-cell injury leading to type 1 diabetes (54,55).

O-GlcNAcylation activates B-cells. B cell activation, like T cell activation, is triggered by the specific recognition of antigens by the B cell receptor (BCR). In activated B cells, NF-κB and NFAT are O-GlcNAcylated by OGT through direct interaction and this correlates strongly with their translocation to the nucleus (52). Intriguingly, O-GlcNAcylation of Lyn at S19 is critical for Lyn activation and Syk interaction in BCR-mediated B-cell activation and expansion (56). Taken together, O-GlcNAcylation mediated by OGT contributes to maintain homeostasis, transduce BCR-mediated activation signals and activate humoral immunity.

O-GlcNAcylation modulates the activation of monocytes and macrophages. Monocytes and macrophages play vital roles in acute and chronic inflammation. Obesity, diabetes and insulin resistance are concomitant with the aggregation of pro-inflammatory monocytes and macrophages in different organs, such as the pancreas, adipose tissue, liver and blood vessel walls (57-61). O-GlcNAcylation is implicated in the inflammatory reaction in monocytes and macrophages, such as c-Rel. At normal glucose concentrations, glucosamine (GlcN) dose-dependently increases LPS-stimulated c-Rel O-GlcNAcylation and production of NO/inducible nitric oxide synthase (6,62). Of interest, OGT de-nitrosylation is triggered by LPS in macrophage cells and results in p62 and p65 hyper-O-GlcNAcylation and NO/cytokine production. As mentioned previously, this study supports a role for de-nitrosylation of S-nitrosylated OGT in controlling the LPS-stimulated innate immune response due to upregulation of OGT activity (63).

Numerous previous studies (64,65), on the other hand, indicate that O-GlcNAcylation participates in anti-inflammatory

responses in monocytes and macrophages and have tried to uncover the mechanisms by which O-GlcNAcylation protects monocytes and macrophages from inflammatory stresses. GlcN may serve as a novel neuroprotective or anti-inflammatory agent, not only because it reduces infarct volume and affords a reduction in motor impairment and neurological deficits in a rat middle cerebral artery occlusion model, but also because it suppresses LPS-induced upregulation of pro-inflammatory mediators both in microglia (BV2) and macrophages (RAW264.7) (64). GlcN is a substrate for glutamine: Fructose-6-phosphate amidotransferase (GFAT), the rate-limiting enzyme in the HBP. Thus, when the glucose and glutamine influx rapidly increases, UDP-GlcNAc levels and subsequent O-GlcNAcylation can also rise. However, in this case, GlcN inhibits the O-GlcNAcylation of NF-κB, probably by disturbing the association between OGT and NF-κB (64).

O-GlcNAcylation activates neutrophils. Neutrophils (polymorphonuclear leukocytes) are a key member of the innate immune system and are activated when presented with a large and diverse group of stimuli. The process of activation initiates a cascade of events that lead to spreading and finally migration. It has been reported that O-GlcNAcylation mediates the activation of the small GTPase Rac and the downstream p38 and p44/42 mitogen-activated protein kinase (MAPK) signaling pathways in neutrophils. These MAPKs are known to modulate cellular chemotaxis leading to cell movement and inflammatory response (66-68).

4. Pro-inflammatory role of O-GlcNAcylation

Positive regulation of the transcription factors by O-GlcNAcylation. A total of ~4,000 proteins have been identified as OGT targets to-date and this will continue to increase as the technology for mapping and quantifying O-GlcNAc sites improves (69,70). O-GlcNAcylation affects NF-κB, NFAT and specificity protein 1 (Sp1) which are related to inflammation and immune reactions. For example, fibroblast growth factor 23 augments the global changes in the O-GlcNAc modification of proteins in human bronchial epithelial cells to regulate airway inflammation through NFAT activation and IL-6 upregulation (71). Intermittent hypoxia raises the O-GlcNAc in proteins, accompanied by an increase in the levels of myocardial NF-κB, inflammatory cytokines, caspase-3 and cardiomyocyte apoptosis (72).

The most widely and intensively studied O-GlcNAcylated transcription factor is NF-κB (7). High glucose and pro-inflammatory factors, risks for metabolic syndrome and diabetes mellitus are deemed to activate the NF-κB pathway and initiate the transcription of relevant downstream target genes. Therefore, the role of O-GlcNAcylation in NF-κB activation has gained more attention.

It has been reported that glucosamine, high glucose and overexpression of GFAT are employed to prompt the binding between nuclear proteins and NF-κB consensus sequences. This enhances vascular cell adhesion molecule-1 (*Vcam*-1) promoter activity in glomerulus cells isolated from male Sprague-Dawley rats (73). This set of data suggest that NF-κB O-GlcNAcylation can participate in inflammation (73).

Furthermore, high glucose has been found to augment O-GlcNAcylation of NF-κB and production of cytokines TNF-α and IL-6 in rat placenta (74). However, little is known about the mechanisms through which O-GlcNAcylation upregulates NF-κB activity. In the caerulein-stimulated acute pancreatitis model, OGT-mediated O-GlcNAcylation of NF-κB p65 and IKKα promotes NF-κB signaling activation, TNF-α secretion and nitric oxide (NO) production in AR42 J rat pancreatic acinar cells which might exacerbate the progression of acute pancreatitis (75). In addition, the authors' previous study has confirmed that LPS triggers the OGT-dependent O-GlcNAcylation of NF-κB and thereby induces a vascular endothelial inflammatory response (76). However, the O-GlcNAc modification sites on NF-kB have not been confirmed and the mechanism by which O-GlcNAc modification promotes NF-kB activation has not been completely elucidated.

An increasing amount of research is focused today on demonstrating the pro-inflammatory role of O-GlcNAcylation in the NF-κB signaling pathway. In rat vascular smooth muscles, O-GlcNAcylation of NF-κB p65 on T352 inhibits the interaction between NF-κB p65 and IκB, thus elevating the translocation of NF-κB to the nucleus and increasing the transcription of VCAM-1 under hyperglycemic conditions (77). These results suggest that a specific O-GlcNAcylation site at T352 on NF-κB p65 may contribute to a prolonged increase in NF-κB activity during the progression of diabetes (77). Moreover, the transcriptional activity of NF-κB seems to increase its concomitant phosphorylation and acetylation, which may interact with O-GlcNAcylation in 293T cells (78,79). Under the stimulation of TNF-α, OGT enhances the CBP/p300-dependent acetylation of NF-κB p65 on K310 by combining with NF-κB regulated promoters and subsequently promotes full NF-κB transcription. Previous mapping studies reveal T305 to be an important residue required for attachment of the O-GlcNAc moiety on p65 (78,79). Similarly, Ma *et al* (79) have verified that O-GlcNAcylation of p65 on T305 and S319 increases CBP/p300-dependent acetylation of p65 on K310, facilitating NF-κB transcriptional activation. In addition to enhancing O-GlcNAcylation, OGT increases the expression of p300, IKKα and IKKβ, and elevates IKK-induced p65 phosphorylation on S536, leading to NF-κB activation. However, O-GlcNAcylation of p65 at T305 is likely impaired by the phosphorylation of p65 at T308 (79). In addition, O-GlcNAcylation of IKKβ occurring at S733 has been discovered to prompt the NF-κB activity by heightening IKK activity, IκB phosphorylation and IκB degradation in cultured mouse and human fibroblasts (80).

Sp1, another transcription factor, besides NF-κB, is also O-GlcNAcylated under high glucose concentration. Donovan *et al* (81) indicated that hyperglycemia significantly increases the binding of Sp1 to the vascular endothelial growth factor (VEGF)-A promoter, while the downregulation of OGT or Sp1 by shRNA significantly abro gates glucose-induced changes in proangiogenic protein VEGF-A in ARPE-19 (human retinal pigment epithelial cells) and TR-iBRB (rat retinal microendothelial cells). This suggests that hyperglycemia-driven elevation of O-GlcNAcyaltion of Sp1 mediates VEGF-A production in the retinal endothelium and pigment epithelium. Furthermore, Zhang *et al* (82) confirmed that

hyperglycemia also stimulated intercellular adhesion molecule (ICAM)-1 expression by O-GlcNAc modification of Sp1 in human umbilical vein endothelial cells and rat retinal capillary endothelial cells. Taken together, this group of studies helps to enhance understanding of how O-GlcNAcylation modulates inflammatory factors during diabetic retinopathy.

Signal transducer and activator of transcription 3 (STAT3) plays a key role in the cytokine-cytokine receptor signaling pathway in colitis (83). Nevertheless, the critical mechanism that regulates STAT3 phosphorylation and its target pro-inflammatory cytokine production remains unclear. Li *et al* (84) demonstrated that modification of STAT3 with O-GlcNAc at T717 suppresses its phosphorylation and the expression of the downstream anti-inflammatory gene, IL-10, in bone marrow-derived macrophages (BMMs) isolated from cullin (*Cul*) 3-deficient mice led to increased disease severity in azoxymethane (AOM) induced colitis and a colitis-associated cancer model. Meanwhile, the expression of pro-inflammatory cytokines, such as IL-12, CXCL1 and CXCL2 are upregulated by O-GlcNAcylated STAT3 in BMMs generated from *Cul* 3-deficient mice make them more susceptible to AOM-induced colitis and colitis-associated cancer. Furthermore, the researchers found that myeloid-derived CUL3, a cullin family E3 ubiquitin ligase, attenuated OGT expression and downregulated STAT3 O-GlcNAc modification by facilitating the interaction between the *Ogt* promoter region and nuclear factor-erythroid-2 related transcription factor-2. The depletion of STAT3 O-GlcNAcylation mediated by CUL3 is concomitant with the upregulation of STAT3 phosphorylation and IL-10 gene expression.

Pro-inflammatory effects of other O-GlcNAcylated proteins. In addition to transcription factors, other functional proteins are found to be O-GlcNAcylated in pro-inflammatory reaction. Transforming growth factor-β activated kinase (TAK) 1 is an important serine/threonine protein kinase that mediates signals transduced by pro-inflammatory cytokines such as TGF-β, TNF-α and IL-1. A number of experimental data suggest that O-GlcNAcylation of TAK1-binding protein 1 (TAB1) at S395 is required for full TAK1 activation upon stimulation with IL-1/osmotic stress, for the downstream activation of NF-κB and finally for the production of IL-6 and TNF-α in IL-1R cells 293 (cells stably expressing the IL-1 receptor) and immortalized *Tab1*-deficient MEFs (85). Another study indicated that TAK1 is O-GlcNAcylated as well. Upon stimulation with IL-1 and NaCl, O-GlcNAcylation of TAK1 at S427 is required for T187/S192 phosphorylation and full activation of TAK1 in RAW264.7 cells (86). Thus, TAK1 O-GlcNAcylation has been found to activate downstream JNK and NF-κB signaling pathways and increase pro-inflammatory cytokine production, and finally facilitate M1 polarization of macrophages which is closely associated with acute inflammatory responses in RAW264.7 cells (87).

Nucleotide-binding oligomerization domain 2 (Nod2) is a cytoplasmic human NOD-like receptor that recognizes bacterial components. Both wild-type Nod2 and a Nod2 Crohn's-associated variant are O-GlcNAcylated and this modification affects Nod2's ability to produce various inflammatory molecules such as cytokines and chemokines via NF-κB activation in 293T cells (88).

5. Anti-inflammatory role of O-GlcNAcylation

In terms of cardiac and vasculature diseases, augmenting O-GlcNAc modification of proteins in the vasculature may represent a novel anti-inflammatory and vasoprotective mechanism (89,90). On one hand, acute GlcN and PUGNAc treatment increases O-GlcNAc-modified protein levels and ameliorates acute inflammatory responses in balloon-injured rat carotid arteries; a 14-day GlcN treatment attenuates neointima formation (89). On the other hand, acute increases in protein O-GlcNAcylation relieve TNF-α triggered vascular dysfunction, at least partly, via mitigating the expression of iNOS (90). Furthermore, GlcN treatment and OGT overexpression attenuate the trauma-hemorrhage induced increase in cardiac levels of TNF-α and IL-6 mRNA, IκB α phosphorylation, NF-κB, NF-κB DNA binding activity, ICAM-1, and myeloperoxidase activity. The enhanced O-GlcNAcylation triggered by both GlcN and OGT overexpression mediates improvement in cardiac function after hemorrhagic shock occurred (91,92).

Negative regulation of the transcription factors by O-GlcNAcylation. Aberrant regulation of NF-κB activity has been associated with a variety of disorders such as autoimmune disease, cancer and diabetes (93). Chronic inflammation is the main culprit of the diseases mentioned above (94-96). Therefore, fully elucidating the molecular mechanisms that precisely tune NF-κB activity may have great biological and clinical significance. Although increasing O-GlcNAc levels are usually accompanied by NF-κB activation in diabetes and insulin resistance, O-GlcNAcylation inducing treatments appear to have anti-inflammatory and pro-survival effects during acute injuries like myocardial infarction, burns, trauma and sepsis (91,92,97). For instance, GlcN or PUGNAc administration after trauma-hemorrhage improve organ perfusion and function, and this is associated with increased protein O-GlcNAc levels (91). Moreover, GlcN and PUGNAc treatment protect against TNF-α induced inflammatory stress by enhancing O-GlcNAcylation at S536 of p65 and by inhibiting TNF-α induced phosphorylation of NF-κB p65, thus inhibiting NF-κB signaling in rat aortic smooth muscle cells (98). *In vivo*, dextran sodium sulfate-induced NF-κB p65 phosphorylation and IL-1β mRNA expression are significantly lower in Ogt-transgenic than in wild type mice. This suggests that O-GlcNAcylation could prevent acute colitis by reducing acute maximum inflammation (99).

Another study indicated that GlcN alleviates the O-GlcNAcylation of both nuclear and cytosolic forms of c-Rel and inhibits the binding of c-Rel to the NF-κB site in the iNOS promoter upon stimulation by LPS. The mechanism by which GlcN exerts these effects involves the suppression of the interaction between c-Rel and OGT (100). Moreover, OGT plays a key role in the inflammatory responses of macrophages. It has been observed that in N9 microglial cells, in response to LPS, the enhanced expression of iNOS, NO and ROS is mediated via the downregulation of OGT and protein O-GlcNAcylation, or via the upregulation of MAPKs phosphorylation and NF-κB translocation (101). In addition, overexpression of OGT exhibits inhibitory effects on the LPS-driven activation of NF-κB and iNOS through modulation of histone acetylation

either directly or indirectly (102). In addition to GlcN and OGT overexpression, thiamet G, an OGA inhibitor, has been found to dramatically reduce the infarct volume and ameliorate the neurological deficits either before or after middle cerebral artery occlusion (MCAO). Moreover, thiamet G administration reduces the number of Iba1^{+} cells in MCAO mice and decreases the expression of iNOS and cyclooxygenase 2 mainly by suppressing NF-κB p65 pathway (103).

Sp1 is a zinc finger transcription factor. As described above, elevated Sp1 activity upon O-GlcNAcylation could play a role in hyperglycemia-induced pro-inflammatory and pro-fibrotic factors involved in diabetic retinopathy (81,82). Alternatively, O-GlcNAc of Sp1 may also reduce its transcriptional activity, possibly by disturbing its interaction with its cooperative factors, such as Elf-1 (104), NF-Y (105), Ying-Yang 1 (106) and sterol regulatory element binding protein 2 (107). Thus, O-GlcNAc modification of Sp1 could be a participant in negative regulation of placental and embryonic expression of oncofetal protein gene (Pem) (104), hyaluronan synthesis (106) and lipid synthesis (107). Interestingly, Suh *et al* (108) and Lee *et al* (109) showed that O-GlcNAcylation of Sp1 protects against hypoxia-induced dysfunction of Na^{+}/glucose cotransporter (SGLT) in renal proximal tubule cells and hypoxia-induced apoptosis of mouse embryonic stem cells. A weakening association between Sp1 and its co-operative factors caused by O-GlcNAcylated Sp1 could be a mechanism postulated to explain these phenomena.

Anti-inflammatory effects of other O-GlcNAcylated proteins. The zinc finger protein A20 (also known as TNFAIP3) has been identified as an inhibitory effector on NF-κB over-activation by using its deubiquitinase activity (110). However, the regulation of A20 activity remains poorly understood. GlcN and thiamet G significantly increase A20 O-GlcNAcylation and enhance binding to Tax1 binding protein 1, a key regulatory protein for A20 activity. These data suggest that O-GlcNAcylation is a critical regulatory mechanism for A20 activity, which in turn negatively regulates the NF-κB signaling cascade in TNF-α injured acute vascular smooth muscle cells (111).

Glutathione peroxidase 1 (GPX1), an anti-oxidant enzyme, is critical for cell survival during hyperglycemia and oxidative stress. Yang *et al* (112) discovered that hyperglycemia induces GPX1 activity by enhancing the O-GlcNAcylation of GPX1 and subsequently increases the association between non-receptor tyrosine kinase c-Abl and Arg in rat vascular smooth muscle cells. Also, pharmacological administration of the OGA inhibitor NTZ has been found to induce GPX1 activation in the mouse liver.

RNA polymerase II (RNAPII) catalyzes the transcription of all protein-coding genes and a number of non-coding RNAs. Hwang *et al* (65) discovered that GlcN relieves basal activities of transcription induced by LPS through the upregulation of RNAPII O-GlcNAcylation and DNA binding which are inhibited by LPS.

6. Conclusions and future perspectives

Since O-GlcNAcylation's involvement in various immune pathways appears complicated, the present review proposed several reasons why O-GlcNAcylation may exert different effects on inflammation and autoimmune diseases.

First, the O-GlcNAc modification as a dual-directional regulator of inflammation was discussed. On one hand, different stimuli lead to distinct outcomes, as exhibited by O-GlcNAcylation modifications in inflammatory responses. The key factor which determines the pro- or anti-inflammatory role of particular O-GlcNAcylation modifications is the type of insult (chronic hyperglycemia in diabetes vs. acute vascular injury). On the other hand, GlcN regulates inflammation by sensing energy states of normal and excess nutrients. At normal glucose concentrations, GlcN dose-dependently enhances LPS-triggered inflammation in macrophages. However, GlcN suppresses macrophage inflammation upon high glucose cell culture conditions. In addition, LPS-stimulates an increase in O-GlcNAcylation as well as an increase in DNA binding of c-Rel to the iNOS promoter by GlcN in normal glucose conditions, but a decrease in high glucose conditions (62). Moreover, hyperglycemia makes a pregnancy highly risky and may even have negative effects on the fetus. Since O-GlcNAcylation is believed to be a nutritionally responsive modification, it provides one means of explaining how excess nutrients in the intrauterine environment may affect metabolic deregulation of the offspring (113). Furthermore, GlcN-mediated O-GlcNAcylation participates in the inhibition of TNF-α and IL-8 gene expression in osteoarthritis (114). It therefore appears that, depending on the cellular nutrition state, the type of insult (chronic hyperglycemia in diabetes vs. acute vascular injury) and the cell state (inflammatory state vs. non-inflammatory state), HBP may quickly switch the management pattern to regulate inflammation, resulting in either pro- or anti-inflammatory outcomes (62). These results also suggest that O-GlcNAc may create a negative feedback loop between pro- and anti-inflammation. Thus, proper adjustment of O-GlcNAc presents a potential therapeutic strategy for combating metabolic dysregulation and inflammatory diseases such as diabetes, sepsis, and osteoarthritis.

Second, the present review considered O-GlcNAc as a target in relieving over-nutrition-related chronic inflammation and autoimmune diseases. The pathogenesis of obesity and type 2 diabetes are accompanied by long-term, low-grade, chronic inflammation. This inflammation has been implicated in much of the downstream pathology, including atherosclerosis, insulin resistance and a high risk for autoimmunity, which are associated with over-nutrition and adiposity (115,116). In addition, disruption in the metabolic pathways of effector T cells is integral to the progress of atherosclerosis and insulin resistance, which may result in enhanced shunting of metabolites into the HBP, fueling O-GlcNAc modification. Moreover, effector T cells such as Th1 and Th17 cells are critical for a number of autoimmune diseases, including multiple sclerosis (MS), rheumatoid arthritis, inflammatory bowel disease, and systemic lupus erythematosus (SLE) (117). SLE presents a reactivation of the silenced X-chromosome due to CD4^{+} T cell DNA demethylation and diet is thought to promote disease progression. The degree of overexpression of OGT in CD4^{+} T cells could potentially contribute to SLE in women (118). Additionally, Th17 cells are likely the most critical pathogenic factor of human MS. It was shown that miR-15b suppressed Th17 differentiation and the pathogenesis of MS by decreasing OGT expression in an NF-κB p65- and c-Rel-dependent manner (119). Thus, these observations logically led to the

investigation of the link between O-GlcNAcylation of proteins and T cell activation that are involved in metabolic and auto-immune diseases. Further characterization of the role of OGT and O-GlcNAc in autoimmunity may suggest new therapeutic targets for autoimmune diseases. Collectively, the recent explosion of research in this field indicates that the mechanisms by which O-GlcNAcylation regulates immune and inflammation responses will soon become clear.

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Authors' contributions

YL and JD contributed to the conception of the study, wrote the manuscript, performed the literature search and prepared the original draft preparation. YL wrote, reviewed and edited the manuscript. MX and LM performed the literature search and constructed the figures. All the authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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