JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
EDITORIAL

Targeting inflammation in type 2 diabetes by antibody-mediated Tyro-3, Axl, Mer receptor activation

Multiple mechanisms can cause type 2 diabetes, and the relative contribution of each remains equivocal. Notably, all of these mechanisms are associated with inflammatory response. Some evidence from basic research included islet-derived interleukin-1β (IL-1β) production under hyperglycemia or hyperlipidemia, abundant secretion of tumor necrosis factor (TNF) and monocyte chemoattractant protein 1 (MCP1) by hypertrophy adipocytes, and macrophage infiltration closely linked with insulin resistance[1]. Furthermore, epidemiological studies have shown elevated circulating levels of C-reactive protein, IL-1β and interleukin-6 (IL-6) in patients with type 2 diabetes. Current medications for type 2 diabetes are insulin, metformin, sulphonylureas, thiazolidinedione, α-glucosidase inhibitors, incretin hormone-based therapy and sodium-dependent glucose cotransporter inhibitors. Those therapeutic targets were mainly focused on hyperglycemia improvement, and adverse effects were still unresolved. Therefore, researchers should not only consider glycemia palliation, but also modulate hyperactivation of the immune system by metabolic stress. The proof-of-concept studies can be briefly categorized into TNF antagonism, inhibitor of nuclear factor-kappa B kinase β/nuclear factor-kappa B inhibition, IL-1 receptor blockade and IL-1β antagonism. Each item will be further discussed respectively.

The initial two trials of TNF antagonism failed to significantly promote insulin sensitivity in diabetes patients, and this consequence might be owed to short duration and small sample sizes (10 and seven patients for 4 weeks and 2 days, respectively)[1]. In contrast, prolonged (6 months) TNF inhibition did show a decrease in fasting glucose, and probably improved insulin sensitivity, despite the participants being obese individuals without diabetes[1]. Large cohort studies have established that TNF inhibition reduces the risk of developing diabetes. According to the aforementioned convincing studies, TNF-related therapy should be translated into a more comprehensive clinical application.

Salsalate is a prodrug form of salicylic acid, and has been suggested to ameliorate diabetes progression through suppression of nuclear factor-kappa B. From the evaluation of several independent clinical studies, treatment improved glycemia, reduced levels of C-reactive protein and increased adiponectin levels in blood plasma. The authors recognized these results as powerful support that inflammation has a substantial impact on type 2 diabetes disease progression[1].

The pioneering investigation of ankinra (a recombinant human IL-1 receptor antagonist) was published in the *New England Journal of Medicine* in 2007. After 13 weeks of injection, it was noted that ankinara reduced fasting glucose level, restored β-cell function and palliated markers of systemic inflammation. Those therapeutic effects were preserved during 39-week follow up after anakinra withdrawal. The long-lasting effect was contributed to the interruption of IL-1β autostimulation. As a result of injection-site reactions and daily injection protocol by anakinra, several humanized antibodies against IL-1β were also developed in the treatment of type 2 diabetes. Each IL-1β-specific antibody (gevokizumab, canakinumab and LY2189102) has shown benefits in patients with type 2 diabetes. In comparison with blockade of IL-1 receptor, neutralizing anti-IL-1β antibodies spared the action of IL-1α, and thus provided safety advantages.

The currently marketed antibody-based drugs are mainly focused on diseases affecting large numbers of patients (such as cancer and inflammatory diseases). Based on that, type 2 diabetes could be a promising candidate to explore beyond the present IL-1β studies. Antigen target selections play fundamental roles in this development.

Growth arrest-specific 6 (Gas6) was cloned in 1988 and characterized in 1993. It belongs to the family of vitamin K-dependent coagulation proteins and is recognized as a growth factor-like molecule, as it interacts with receptor tyrosine kinases of the Tyro-3, Axl, Mer (TAM) family. Gas6 expression is widespread in many tissues, including immune cells, endothelial cells, vascular smooth muscle cells and adipocytes. The Gas6/TAM system regulates an intriguing mix of processes, including cell survival and proliferation, cell adhesion and migration, blood clot stabilization, and inflammatory cytokine release. Over the years, the role of the Gas6/TAM system has been found to be important in injury, repair, inflammation, hemostasis, autoimmune disease, vascular diseases and cancer[2]. Several previous reports showed that the Gas6/TAM system was involved in the pathogenesis of diabetic renal and vascular disease[2]. Expression of Gas6/TAM was increased in the glomerulus of diabetic rats, which led to mesangial and glomerular

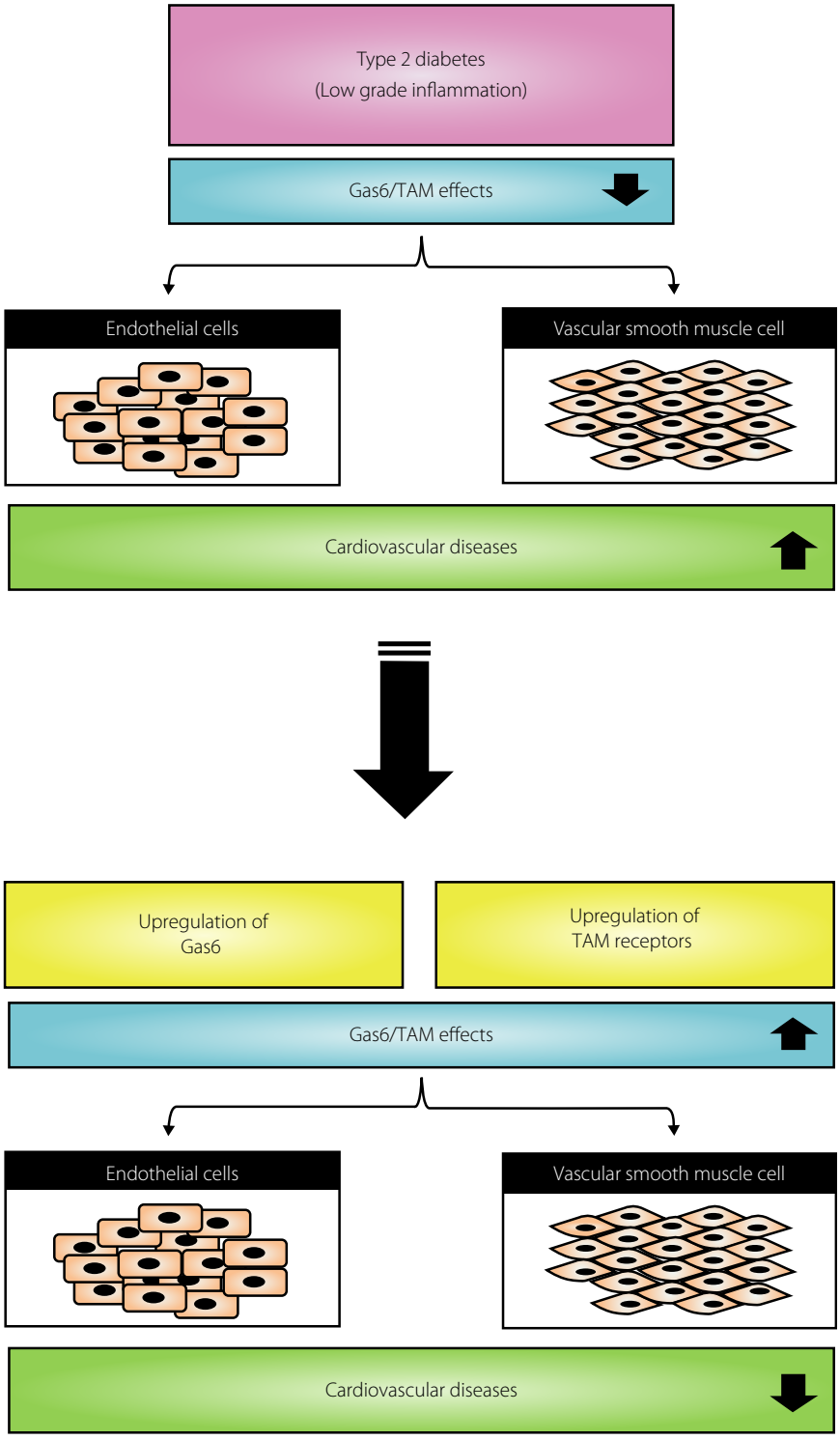

Figure 1 | Possible new anti-inflammation therapeutic strategy to type 2 diabetes and vascular complications through increased growth arrest-specific 6/Tyro-3, Axl, Mer (Gas6/TAM) effects. Current medications for type 2 diabetes were mainly focused on hyperglycemia improvement. Multiple mechanisms could cause type 2 diabetes, and all of these mechanisms are associated with inflammatory response. Type 2 diabetes with low-grade inflammation might cause and be associated with decreased Gas6/TAM effects. There are several strategies to increase Gas6/TAM effects including direct injection of Gas6 and anti-Mer, anti-Axl antibody-based treatment. Previous animal studies pointed out the potential of activating antibodies to anti-Mer/anti-Axl as TAM-specific immunosuppressive modalities. Once the TAM antibody-based diabetes treatment can be extended to humans, it could offer a promising new approach to treating diabetes-related cardiovascular diseases.

hypertrophy. In vascular smooth muscle cells, Gas6/Axl signaling increased cell survival in the presence of low glucose (LG), and increased cell migration in the presence of high glucose (HG). Recently, our report also showed that plasma Gas6 levels were associated with altered glucose tolerance, inflammation and endothelial dysfunction[3]. We found the concentration of plasma Gas6 was significantly lower among patients with type 2 diabetes compared with normal glucose tolerance subjects ($P < 0.001$), and was inversely correlated with fasting glucose, TNF-α, IL-6, high-sensitive C-reactive protein and vascular cell adhesion molecule-1. Plasma Gas6 concentration could represent an independent risk factor of type 2 diabetes, and a potential surrogate marker of inflammation and endothelial dysfunction. Furthermore, we showed that Gas6 concentrations are significantly negatively correlated with abdominal obesity (body mass index, waist circumference, waist-to-hip ratio), insulin resistance (2-h insulin, homeostatic model of assessment of insulin resistance) and positively correlated with insulin sensitivity only in women[3]. Single-nucleotide polymorphisms are the most common genetic variations in genomes, and could affect factors that are involved in type 2 diabetes. Our study group also found that the intron 8 c.843 + 7G>A Gas6 polymorphism had a clearly significant lower frequency of the A allele and the homozygous AA genotype in type 2 diabetes compared with normal glucose tolerance subjects. In addition, the AA genotype group had significantly higher Gas6 concentrations and lower glucose, glycated hemoglobin and homeostatic model of assessment of insulin resistance than the GG genotype group. Hence, these findings showed that the Gas6 c.834 + 7G>A polymorphism could be an important pathogenic mechanism for type 2 diabetes. Our *in vitro* study results showed that HG caused downregulation of Gas6/Axl through Akt signaling to influence the expression of adhesion molecules and vascular endothelial growth factor/vascular endothelial growth factor receptor 2 in endothelial cells (ECs)[4]. These findings were supported by functional studies showing that a decrease of cell viability and angiogenesis and induction of monocyte-EC adhesion in ECs under HG. While we manipulated the Axl expression in HG cultured ECs, they could improve endothelial function with an increase of cell viability and angiogenesis and induction of monocyte-EC adhesion, and might be through Akt signaling then activating vascular endothelial growth factor/ vascular endothelial growth factor receptor 2 expression and suppressing adhesion molecules. These studies show that Gas6/Axl likely represents an important pathogenic mechanism for cardiovascular complications associated with diabetes. If Gas6/Axl/Akt signaling defects contribute to diabetic vascular complications, then therapeutic restoration of activity could have clinical significance. We suggest that upregulation of Gas6/Axl signaling pathways within the endothelium should be considered as a target for future therapeutic modalities in protecting diabetic patients from vascular complications (Figure 1).

Several receptor tyrosine kinase (RTK)-related drugs have been approved by the US Food and Drug Administration for treating cancers and other diseases. These drugs can be divided into two categories: small-molecule inhibitors that target intracellular kinase domain and monoclonal antibodies that target RTK-expressing cells. However, many small-molecule RTK inhibitors react with multiple tyrosine kinases in addition to their initially intended target. Notably, some therapeutic antibodies against RTK ectodomains have been shown to function as weak RTK agonists by driving receptor dimerization. In the recent work by Lemke *et al.*[5], they first applied affinity-purified, polyclonal anti-Axl (AF854; R&D Systems, Minneapolis, MN, USA) and anti-Mer (AF591; R&D Systems) to directly activate the TAM receptors *in vivo*. Based on their data, these activations were seen within 15 min after intravenous injection and declined to baseline by 24 h. They also found that the activations were dose-dependent, and the addition of anti-Axl or anti-Mer alone, in the absence of added Gas6, had no stimulatory effect on the phagocytosis of apoptotic cells. As the activation of Axl in dendritic cells suppressed the immune response *in vitro*, their group hypothesized that antibodies-mediated TAM receptor activation might be anti-inflammatory *in vivo*. In the mouse model of lipopolysaccharide-induced sepsis, they gave mice intraperitoneal injection of lipopolysaccharide (or saline as a control) together with either the activating antibody to Axl or control immunoglobulin G, then measured messenger ribonucleic acids encoding type I interferons in the spleen 2 h after injection. Pronounced suppression of *Ifn1* messenger ribonucleic acid and *Ifn4* messenger ribonucleic acid were detected in mice given injection of anti-Axl, but not in those given injection of control immunoglobulin G. Lemke *et al.*[5] pointed out the potential of activating antibodies to anti-Mer/anti-Axl as TAM-specific immunosuppressive modalities. Previous work in our laboratory has shown the importance of the Gas6/TAM system for diabetes progression in both cell-based models and epidemiological analysis from type 2 diabetes patients[3,4]. Those analyses have prompted us to search Gas6-associated therapeutics. Compared with direct injection of Gas6, anti-Mer and anti-Axl showed absolute receptor specificity, and thus enhanced their therapeutic potential. To test the possibility of *in vivo* application, we could initiate a high-fat diet-induced diabetes mouse model, which mimics human type 2 diabetes, and follow published antibodies injection protocol. Our data will not only clarify the role of each TAM receptor in diabetes, but provide a new direction for antibody-based diabetes treatment.

ACKNOWLEDGMENTS

This work was supported by research grants from the National Science Council (NSC-100-2314-B-016-026-MY3, NSC102-2314-B-016-044-MY2) and Tri-Service General Hospital (TSGH-C104-007-007-S03, TSGH-C104-007-007-S04), Taiwan.

DISCLOSURE

The authors declare no conflict of interest.

Chien-Hsing Lee[1], Chih-Ying Changchien[2], Yi-Jen Hung[1]*
[1]*Division of Endocrinology and Metabolism, Department of Internal Medicine, Tri-Service General Hospital, and* [2]*Department of Medicine, National Defense Medical Center, Taipei, Taiwan*
**E-mail: metahung@yahoo.com*

REFERENCES

1. Donath MY. Targeting inflammation in the treatment of type 2 diabetes: time to start. *Nat Rev Drug Discov* 2014; 13: 465–476.
2. van der Meer JH, van der Poll T, van't Veer C. TAM receptors, gas6, and protein S: roles in inflammation and hemostasis. *Blood* 2014; 123: 2460–2469.
3. Hung YJ, Lee CH, Chu NF, *et al.* Plasma protein growth arrest-specific 6 levels are associated with altered glucose tolerance, inflammation and endothelial dysfunction.. *Diabetes Care* 2010; 33: 1840–1844.
4. Lee CH, Shieh YS, Hsiao FC, *et al.* High glucose induces human endothelial dysfunction through an Axl-dependent mechanism. *Cardiovasc Diabetol* 2014; 13: 53.
5. Bhattacharyya S, Zagórska A, Lew ED, *et al.* Enveloped viruses disable innate immune responses in dendritic cells by direct activation of TAM receptors. *Cell Host Microbe* 2013; 14: 136–147.

Doi: 10.1111/jdi.12351