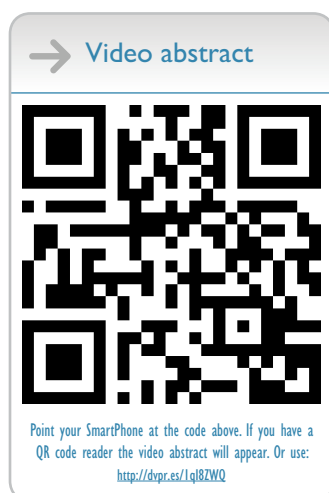Open Access Full Text Article

REVIEW

TGR5 ligands as potential therapeutics in inflammatory diseases

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Abstract: Takeda G protein-coupled receptor 5 (TGR5), also known as Gpbar1, membrane-type bile acid receptor (M-BAR), or GPR131 is a G protein-coupled receptor that is best known for its activation by bile acids. TGR5 has been found to regulate a number of specific processes, including energy expenditure and glucagon-like peptide-1 release. Other actions in which TGR5 is implied range from regulating bile acid homeostasis to playing a role in the nervous system. The receptor is increasingly associated with the regulation of inflammatory responses in a number of cells that are relevant to the immune response. TGR5 exerts antiinflammatory actions by decreasing adhesion molecule expression in endothelial cells and inhibiting proinflammatory cytokine production in macrophages. A number of animal models also hint toward the antiinflammatory actions of TGR5. These include models of atherosclerosis, colitis, and inflammation-driven liver diseases. In the current review, we provide a comprehensive overview of TGR5 with a focus on its role in inflammation. We furthermore describe the currently known agonists of TGR5 and discuss some of the recent findings on TGR5 signaling. The potential drawbacks, as well as the encouraging prospects, of TGR5 will be discussed in view of TGR5 as a therapeutic target in diseases with inflammatory facets.

Keywords: metabolism, inflammation, macrophages, G protein-coupled receptor, bile acids, Takeda G protein-coupled receptor 5

Physiological expression and function of TGR5

Takeda G protein-coupled receptor 5 (TGR5), also known as Gpbar1, membrane-type bile acid receptor (M-BAR), or GPR131, is one of ~800 human G protein-coupled receptors (GPCRs) that impact all levels of biology by transducing signals from a diverse spectra of events.[1] TGR5 belongs to the family A group of GPCRs, also known as the rhodopsin-like subfamily of GPCRs. The receptor is conserved among mammals, and is also found in lower vertebrates, such as fish.[1] TGR5 was discovered as a membrane receptor responsive to bile acids (BAs).[2–4] Since then, TGR5 has been investigated in several cell types, such as muscle cells, adipocytes, endothelial cells (ECs), and macrophages.[1,5] The functions of TGR5, which are dependent on cell type, range from glucagon-like peptide-1 (GLP-1) release and mitochondrial energy homeostasis to the control of inflammatory responses (see Figure 1).[6–9]

In the current review, we provide an extensive introduction to TGR5 and its function in different tissues, with a focus on the effects of TGR5 in inflammation. We describe the possible problems and promising outlook of TGR5 as a therapeutic target in diseases with inflammatory aspects.

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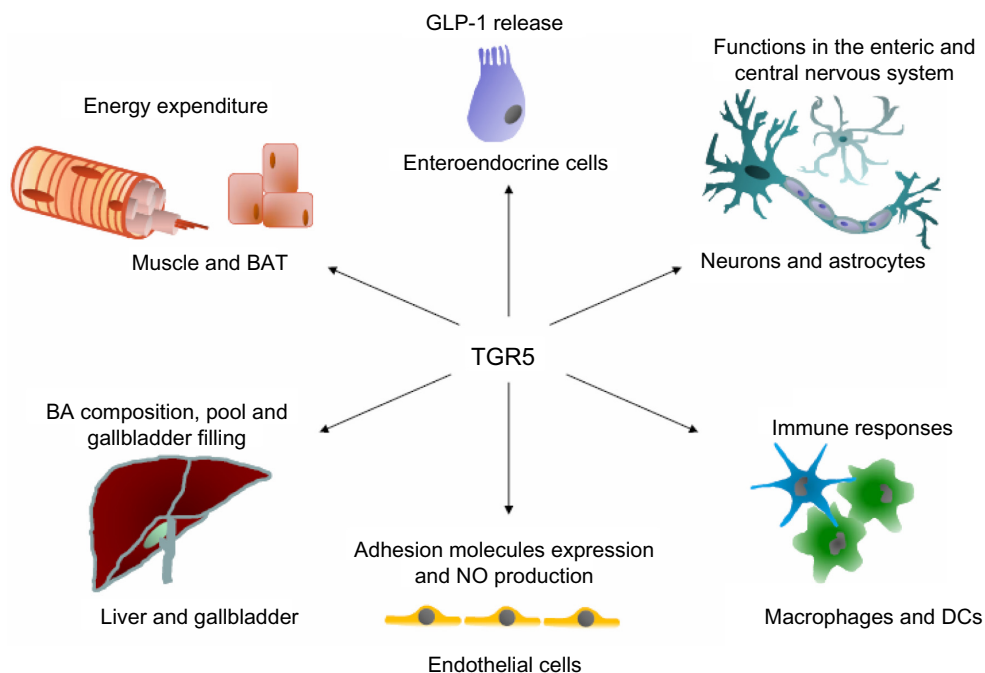Fax +31 020 691 5519
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Figure 1 Schematic overview of the action of TGR5 in different cells and tissues.
Notes: TGR5 is present and functional in different cell types and tissues throughout the body. It is involved in BA metabolism, energy homeostasis and immune response.
Abbreviations: GLP-1, glucagon-like protein 1; BAT, brown adipose tissue; TGR-5, Takeda G protein-coupled receptor 5; BA, bile acid; NO, nitric oxide; DCs, dendritic cells.

Signaling of TGR5

TGR5 binds and responds to several BA species with different affinities,[3,4] which results in the upregulation of cyclic adenosine monophosphate (cAMP) levels. Other compounds that have been described to activate TGR5 include triterpenoids, bile alcohols, and certain steroid hormones, including neurosteroids.[10–13] A large number of semisynthetic and synthetic agonists have also been developed to target TGR5.[14] These are more elaborately described in the section titled, "TGR5 agonists".

The receptor has no known sorting motifs and is synthesized in the endoplasmic reticulum, after which it translocates to the plasma membrane to function. This translocation process was addressed by Spomer et al,[15] who demonstrated that this trafficking depends on the membrane proximal C-terminal alpha helix of TGR5. In contrast to most GPCRs, TGR5 does not traffic to endosomes, but relocates to plasma microdomains where it elevates cAMP levels for a prolonged period.[16] This is in line with the weak interaction of TGR5 with GPCR kinases or β-arrestin-1/2.[16]

It is well described that TGR5 is able to interact with G protein α_S (Gα_S).[3,4] Upon the binding of agonists to the ligand-binding pocket of TGR5, Gα_S is activated and regulates adenylyl cyclase activity. This results in the elevation of cAMP levels (among others) and further downstream

signaling.[3,4] TGR5 is also able to bind Gα_{i3} and Gα_Q in the FLO cancer cell line, as well as in the human AGS gastrinoma cancer cells.[17,18] These G proteins respectively dampen cAMP activity and increase phospholipase activity.[19] Additionally, TGR5 couples to Gα_S in H69 nonciliated cholangiocytes, but couples to Gα_i in ciliated H69 cholangiocytes,[20] which underlines the binding of TGR5 to different G proteins. TGR5 also activates ERK1/2 (extracellular signal-regulated kinases-1/2) in an epidermal growth factor receptor (EGFR)-dependent manner.[16,21] Finally, it has been demonstrated that TGR5 induces JNK (c-Jun-N terminal kinase) activation, and it has been proposed to play a role in hepatocyte apoptosis.[22] Of interest, a human study has revealed a number of genetic variants of TGR5 that show abrogated cAMP signaling.[23]

Taken together, TGR5 is able to bind to multiple G proteins and subsequently induces distinct downstream signaling cascades in several cell types. This could suggest that the downstream signaling of TGR5 is dependent on cell type and its differentiation state.

Physiological role of TGR5 in BA homeostasis

TGR5 is expressed in many cells that are relevant to the homeostasis of BA metabolism. These cells include gallbladder epithelial cells, gallbladder smooth muscle

cells, as well as cholangiocytes.[20,23–27] A role of TGR5 in BA metabolism is underlined by findings that the BA pool size is decreased in *Tgr5*^{−/−} mice.[28,29] In gallbladder epithelial cells, TGR5 is demonstrated to increase chloride secretion via activation and translocation of the cystic fibrosis transmembrane conductance regulator, and as such to modulate bile composition.[24] In cholangiocytes, TGR5 is proposed to function as a sensor to couple biliary BA concentration to ductular bile formation and bile flow.[20,23] TGR5 is also demonstrated to relax gallbladder smooth muscle cells, leading to gallbladder filling.[26,27] In short, evidence suggests that TGR5 regulates bile composition and bile flow.

Physiological role of TGR5 in metabolic tissues

Accumulating evidence suggests that TGR5 influences glucose homeostasis and energy expenditure.[7,8] Activation of TGR5 by BAs triggers the release of GLP-1 in murine intestinal L-cells.[6,7] GLP-1 is an incretin with beneficial effects on glucose homeostasis, such as increased insulin secretion and thus improved glucose tolerance.[7] Upon treatment of obese mice with BA sequestrants that increase the BA pool size, GLP-1 secretion increases in a TGR5-dependent manner, with improved glucose tolerance when compared to controls.[7,30,31]

TGR5 increases energy expenditure in thermogenic tissues such as mouse brown adipose tissue and human skeletal muscle.[8] Stimulation of the TGR5 pathway in these tissues triggers the thyroid hormone-activating enzyme type 2 iodothyronine deiodinase (D2), converting inactive thyroxine (T4) into the active iodothyronine (T3) form, thereby increasing energy expenditure.[8] Mice on a high-fat diet were protected from obesity and insulin resistance when fed a BA-supplemented diet, whereas this effect was not observed in *D2*^{−/−} mice, which underlined the role of D2 in this response.[8] Plasma BA levels have also been associated with thyroid hormone control and energy expenditure in humans.[32] Thus, TGR5 plays an important metabolic role by which it positively influences glycemic control via GLP-1 release and increases energy expenditure by activating D2.

TGR5 in the nervous system

TGR5 is present in the enteric nervous system, as well as in the central nervous system.[3,12,28,33] In the murine enteric nervous system, TGR5 was found in inhibitory motor neurons and descending interneurons, but not in glial cells.[33] Stimulation of TGR5 with the BA deoxycholic acid (DCA) inhibited intestinal motility and secretion, suggesting a neural role for TGR5 in digestion.[33] In humans, a link has been made between a TGR5 single-nucleotide polymorphism and bowel transit, although this relationship was not very strong.[34] In addition, the receptor has been proposed to mediate itch and analgesia.[35]

In the central nervous system, *TGR5* messenger ribonucleic acid (mRNA) expression has been detected in mice, rats, and humans.[3,12,28] The TGR5 protein is expressed throughout the rat brain in both neurons and astrocytes.[12] However, the function of TGR5 in the brain remains enigmatic. It is possible that TGR5 plays a role as a neurosteroid receptor because TGR5 can be activated in vitro by several neurosteroids, predominantly by 5β-pregnan-3α-ol-20-one.[12] Stimulation of astrocytes with 5β-pregnan-3α-ol-20-one leads to an increase in cAMP and calcium, and to the downregulation of *Tgr5* mRNA.[12] Interestingly, BAs with moderate TGR5 affinity are also present in the human and rat brain.[36,37] In the rat brain, only the unconjugated primary BAs – chenodeoxy cholic acid (CDCA) and cholic acid (CA) – and secondary BA (DCA) have been detected, while their tauro-conjugated forms and other BAs were also found in peripheral tissue.[37] In rats, as well as in humans, BAs are able to pass the blood–brain barrier.[38,39] So, although BAs seem to be common ligands for TGR5 in the periphery, this might not be the case in the central nervous system.

TGR5 in the immune system

TGR5 is also expressed in several cells of the immune system, and it has been demonstrated to regulate immune responses. These cells include monocytes, macrophages, ECs, and dendritic cells (DCs). We discuss the function of TGR5 in these cells in detail in the section titled, "TGR5 in the immune response".

TGR5 agonists
Development of TGR5 agonists

Endogenous BAs are physiological ligands that activate TGR5, albeit with different affinities.[3,4] There has been a rush to design and validate new compounds that are able to modulate the activity of the TGR5 receptor and, until now, this research has focused on TGR5 ligands that improve glucose homeostasis and limit body weight gain. The design and synthesis of TGR5 agonists has especially been deemed necessary because endogenous BAs generally have a lower affinity and selectivity for TGR5, and they have specific biological properties with regard to localization and cycling. The hierarchy of BA affinity for TGR5 is as follows: lithocholic acid (LCA) > DCA > CDCA > CA.[40] In line with computational methods that have been used to

investigate BA binding to TGR5, a recent TGR5 mutation study confirmed key residues within the TGR5 binding pocket that are involved in BA binding to TGR5.[41,42]

Different research groups and companies have used step-by-step strategies to design selective agonists via the extensive screening of BA derivatives and the subsequent modification of their individual structures. Medicinal chemistry strategies yielded potent BA (steroidal) derivatives via modification of the BA scaffold and screening of chemical libraries. Subsequent hit-to-lead optimization produced potent nonsteroidal heterocyclic compounds, as reviewed earlier.[14] Evidently, the steroidal agonists contain a steroidal core that includes BAs, steroid hormones, and semisynthetic derivatives, whereas this is not the case for the nonsteroidal agonists. Besides the selective TGR5 agonists, compounds have been developed with dual affinity for both TGR5 and the nuclear BA receptor, farnesoid X receptor (FXR).[43] Although many compounds have been identified and tested for affinity, efficacy, and selectivity, only a limited number have made it to preclinical testing in the fields of atherosclerosis, colitis, and liver diseases. Most compounds were investigated with respect to specific aspects of obesity and type 2 diabetes mellitus.[14]

Semisynthetic bile acids as TGR5 agonists

One of the best known semisynthetic BA TGR5 agonists might be 6α-ethyl-23(S)-methylcholic acid (S-EMCA, INT-777), which was developed via the incorporation of crucial 6α-ethyl and 23(S)-methyl moieties in the scaffold of CA.[44] Likewise, other semisynthetic BA receptor ligands have been developed solely with FXR (INT-747) or with combined FXR/TGR5 (INT-767) activity.[43,45,46] The TGR5 agonist, INT-777, stimulates receptor activation and GLP-1 release from enteroendocrine L-cells, increases energy expenditure, reduces hepatic steatosis, improves insulin sensitivity, and stimulates insulin release in cultured pancreatic MIN6 cells and human pancreatic islets.[6–8,47] Furthermore, INT-777 stimulates bile flow, gallbladder filling, and gallbladder relaxation.[26,27] In 2011, it became clear that INT-777 was also able to inhibit macrophage inflammation and atherosclerosis, which is discussed in the sections titled, "TGR5 function in monocytes and macrophages" and "Atherosclerosis".[5] In addition, INT-777 decreases basal secretory tone and inhibits cholinergic-induced secretory responses in the colon via TGR5 activation.[48] Other semisynthetic BAs include multiple ursodeoxycholic acid analogs studied by Iguchi et al,[49] among which 7α-methylated ursodeoxycholic acid (3α,7β-dihydroxy-7α-methyl-5β-cholanoic acid) had a remarkably high affinity to bind to and activate TGR5.

Triterpenoid TGR5 agonists

The screening of a library of plant extracts for TGR5 activity led to the discovery of oleanolic acid (OA) as a triterpenoid TGR5 agonist.[40] OA was known as an olive leaf-derived oleanane-type triterpenoid and has weak antiinflammatory and anticarcinogenic effects.[40,50] Multiple studies, primarily on glucose metabolism, have shown the biological action of OA as a TGR5 agonist. OA improved glucose tolerance in mice fed a high-fat diet.[40] Additionally, OA increases pancreatic beta cell insulin release via TGR5 and improves prokinetic action (motility) of the colon.[35,47]

Betulinic acid (BTA) was identified as a triterpenoid able to activate TGR5 by a biological screening of a collection of naturally occurring triterpenoids.[51] In this study, triterpenoid derivatives were generated, that were also potent TGR5 agonists.[51] Even the most potent BTA derivative, however, showed marginal effects on glucose metabolism, despite its beneficial toxicity profile; these effects may have been due to its low bioavailability.[51] Other findings indicate that BTA may play a role in intraluminal duodenal HCO_3^- secretion after luminal administration.[52] BTA also inhibits high glucose-induced vascular smooth muscle cell proliferation and migration.[53] Finally, BTA decreased body weight, as well as blood glucose and lipid levels, while increasing insulin and leptin in mice fed a high-fat diet.[54] However, none of these studies definitely proved a TGR5-mediated mechanism, leaving the relevance of TGR5 in these effects of BTA uncertain.

The same holds true for the triterpenoid, ursolic acid (UA).[51] Before the discovery of UA as a TGR5 agonist, UA was shown to improve glucose metabolism without affecting body weight.[55] Later on, these findings were more or less confirmed in other studies that showed ameliorated glucose metabolism, decreased abdominal obesity, and improved hepatic steatosis.[56–58] Of relevance, TGR5 dependence was not investigated in these studies.

Other TGR5 agonists

A number of other TGR5 agonists have been published that show therapeutic potential. One of those agonists is SB-756050 (1,4-bis[{3,4-bis(methyloxy)phenyl} sulfonyl] hexahydro-1H-1,4-diazepine). SB-756050 is a selective TGR5 agonist, and its use in humans has been researched published.[59] Chronic enteral administration of SB-756050 improved glucose metabolism in diabetic rats; however, these effects were not reproduced in humans.[59] This could indicate a difference between rat and human TGR5 targeting.

Zambad et al[60] screened ~400 compounds for TGR5 activation in vitro and identified TRC210258

(N-[4-chlorophenyl]-2-[4-fluorophenoxy]-N-methylimidazo [1, 2-a] pyrimidine-3-carboxamide) as a new TGR5 activator. TRC210258 efficiently activated TGR5 and improved many metabolic parameters, such as GLP-1 secretion, energy expenditure, cholesterol, and glucose metabolism in diet-induced obese mice and hamsters.

The 3-aryl-4-isoxazolecarboxamides have been shown to activate TGR5 as well.[61,62] These specific compounds demonstrated improved GLP-1 secretion after intraluminal administration in the colon of dogs together with a concomitant glucose challenge.[61] Another class of compounds is the class of specific 2-aryl-3-aminomethylquinones. These molecules are demonstrated to activate TGR5, increase GLP-1 secretion, and increase glucose clearance in diet-induced obese mice.[62]

Other synthetic TGR5 agonists include 6-methyl-2-oxo-4-thiophen-2-yl-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid benzyl ester and (S)-1-(6-fluoro-2-methyl-3,4-dihydroquin-olin-1[2H]-yl)-2-(isoquinolin-5-yloxy)ethanone, although, follow-up on their biological activity has never been published.[63] Another group of TGR5 agonists are the 5-phenoxy-1,3-dimethyl-1H-pyrazole-4-carboxamides.[64]

The fluoroquinolone, ciprofloxacin, has also been shown to activate TGR5.[65] Before its discovery as a TGR5 agonist, ciprofloxacin was already known to increase cAMP in monocytes and macrophages, and to counterregulate cytokine production.[66] Cipriani et al[65] demonstrated that ciprofloxa-cin functions as a TGR5 agonist, as determined by in silico screening, docking calculation, and in vitro experiments.

Finally, the TGR5 agonist, XL-475, has been reported to beneficially alter blood glucose, glucose tolerance, hepatic steatosis, and hepatic and plasma lipid levels in animal models.[14] XL-475 was designed to selectively target the intestines, which could limit potential adverse effects by preventing systemic exposure.[14]

In summary, the interest in TGR5 as a target receptor has led to the identification and synthesis of several compounds. Many of these compounds did not advance to clinical application.[14] However, much research is currently ongoing, and clinical trials have started to test XL-475 for its benefit in preventing diabetes in humans, as reviewed earlier.[14]

TGR5 in the immune response
TGR5 function in monocytes and macrophages
Upon its initial discovery as a BA responsive receptor, Kawamata et al[3] already linked TGR5 to the regulation of immune responses. In this study, high *TGR5* expression was demonstrated in human monocytes, as well as in rabbit alveolar macrophages.[3] Tumor necrosis factor (TNF)α secretion of human monocytic THP-1 cells over-expressing TGR5 was inhibited by several BA species and correlated to cAMP levels – an effect not observed in control THP-1 cells.[3] Concordantly, TGR5 is expressed in rat Kupffer cells (resident liver macrophages), which show a decrease in cytokine production in response to TGR5 agonist exposure.[67] To prove the physiological relevance and contribution of TGR5 in this response, primary mouse macrophages were isolated from *Tgr5*^{+/+} and *Tgr5*^{−/−} mice. It was demonstrated that, in contrast to *Tgr5*^{+/+} macrophages, *Tgr5*^{−/−} macrophages were deficient in inducing cAMP or a calcium flux upon treatment with the TGR5-specific agonist, INT-777 (see the section titled, "Semisynthetic bile acids as TGR5 agonists").[5] Furthermore, *Tgr5*^{−/−} macrophages showed an increased production of cytokines, thereby fur-ther establishing the role of TGR5 in regulating immune responses in macrophages (see Figure 2A), an effect con-firmed in the different mouse macrophage subsets by differ-ent research groups.[5,65,68] Additionally, human macrophages express TGR5 and show a similar inhibition of cytokine production upon treatment with compounds that are able to stimulate TGR5.[69–71]

A number of different downstream signaling routes have been proposed to contribute to the antiinflammatory properties of TGR5, which seem to converge on the inhibi-tion of nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB).[9] The precise contributions of these path-ways have not been investigated.

TGR5 in dendritic cells
A recent study addressed the role of TGR5 during the differentiation of monocytes into DCs in vitro.[72] In this study, it was confirmed that *TGR5* mRNA levels are high in monocytes, consistent with previous findings.[3] Fur-thermore, monocytes exposed to BAs showed activation of the CREB (cAMP response element-binding protein), indicating functional TGR5 downstream signaling.[72] Upon the differentiation of monocytes into DCs by granulocyte-macrophage colony-stimulating factor and interleukin (IL)-4, TGR5 gradually decreases but remains detectable.[72] Differentiation of DCs in the presence of a TGR5 compound results in DCs that are less reactive, as measured by low IL-12 and TNFα expression (see Figure 2B). DCs are able to regulate inflammation by interacting with T-cells and by secreting several cytokines.[72] These findings indicate that TGR5 affects the adaptive (specific) immune response by modulating DC function.
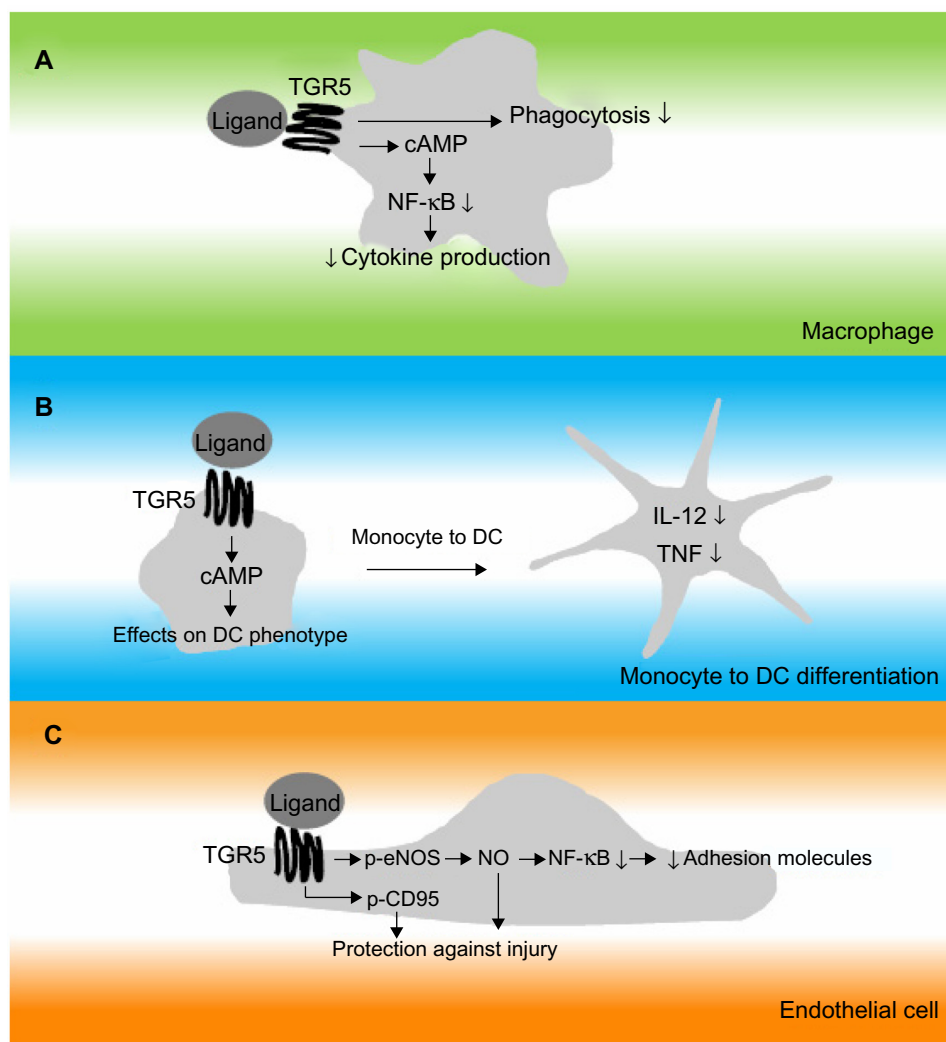
Figure 2 Simplified drawing of proposed action of TGR5 in cells relevant to the immune response.

Notes: (**A**) TGR5 in macrophages. TGR5 activation is described to inhibit NF-κB activation through the elevation of cAMP, leading to the inhibition of proinflammatory cytokine production.[3,5,65,67–70] (**B**) TGR5 in monocytes/DCs. TGR5 activation in monocytes is described to result in a hypo-IL-12 and hypo-TNFα DC phenotype via the elevation of cAMP.[72] (**C**) TGR5 in endothelial cells. TGR5 activation in endothelial cells is found to enhance eNOS activation (p-eNOS) leading to NO production that decreases adhesion molecule expression via the inhibition of NF-κB. Additionally, NO and phosphorylated CD95, also proposed downstream of TGR5, protect endothelial cells from cell death.[73,74]

Abbreviations: TGR5, Takeda G protein-coupled receptor 5; cAMP, cyclic adenosine monophosphate; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B-cells; DC, dendritic cell; IL-12, interleukin-12; TNF, tumor necrosis factor; p-eNOS, phosphorylated endothelial nitric oxide synthase; NO, nitric oxide; p-CD95, phosphorylated cluster of differentiation 95.

TGR5 in endothelial cells

ECs form the lining between blood and tissue. These cells play a major role in inflammation, as they are involved in the trafficking of leukocytes to sites of inflammation via adhesion molecule expression. TGR5 expression has been investigated in ECs, and it was found that the receptor was present in several EC subsets, including rat sinusoidal ECs and bovine aortic ECs.[73,74] Also, human ECs were shown to express TGR5, including ECs derived from human umbilical cord, the pulmonary artery, and the dermal microvasculature.[73,74] In ECs, TGR5 was demonstrated to regulate phosphorylation of endothelial nitric oxide (NO) synthase (eNOS), resulting in the production of NO.[73,74] The NO produced has been proposed to subsequently inhibit adhesion molecule expression and monocyte adhesion by the inhibition of NF-κB (see Figure 2C).[74] NO also protects ECs from oxidative stress which, together with the demonstration that TGR5 induces cluster of differentiation (CD)95 phosphorylation in ECs, acts in an antiapoptotic manner and protects ECs from damage (see Figure 2C).[73,74] These observations are in line with the notion that TGR5 is suggested to protect against gastric and intestinal injury caused by acetylsalicylic acid and nonsteroidal antiinflammatory drugs via eNOS enzymes.[75] In addition, NO plays a role in vasodilation, which could

suggest that TGR5 plays a beneficial role in the regulation of blood pressure. This has been addressed by Fryer et al,[76] who found that activation of TGR5 using a novel synthetic compound induces vasodilatation in dogs without changes in NO production, but by activation of the large-conductance Ca^{2+}-activated potassium channel, $K_{Ca}1.1$. Lastly, TGR5 has been suggested to improve EC barrier function via cAMP–protein kinase A/Rac1-dependent cytoskeletal rearrangement.[77] Thus, TGR5 has several actions in ECs, including regulating eNOS activity, modulating adhesion molecule expression, and protecting them from cell death.

Clinical potential of TGR5 activation in inflammatory disease

The common denominator of diseases like atherosclerosis, colitis, hepatic disease, and the metabolic syndrome is inflammation.[78] As detailed in the section titled, "TGR5 in the immune response", activation of TGR5 has been shown to dampen the inflammatory response.[5,29,68]

Atherosclerosis

The expression of functional TGR5 in immune cells could suggest that TGR5 activation may play a protective role against atherosclerosis and coronary artery disease (CAD). Pols et al[5] showed that INT-777 lowered proinflammatory cytokine production via cAMP signaling and NF-κB inhibition and, as a consequence, decreased atherosclerosis in atherosclerosis-susceptible $Ldlr^{-/-}Tgr5^{+/+}$ mice, but not in $Ldlr^{-/-}Tgr5^{-/-}$ double-knockout mice. The authors found that INT-777 treatment resulted in less plaque formation, less inflammation, and less macrophage infiltration. Furthermore, INT-777 did not show effects on atherosclerosis in mice with leukocyte-specific deletion of $Tgr5$, whereas mice that normally express TGR5 in leukocytes did reveal an anti-atherogenic effect of INT-777. The latter finding emphasized the role of TGR5 in leukocytes in the antiatherogenic effects of INT-777. This concept has yet to be proven in humans. Of interest, Steiner et al[79] showed that elevated secondary BA levels were associated with protection against CAD in humans. In support of this, Duboc et al[80] demonstrated that subjects with CAD have lower levels of the secondary BA, LCA. LCA has a high affinity for the TGR5 receptor, possibly suggesting a protective role for TGR5 in human CAD.[40]

Colitis

TGR5 has been suggested to play a role in the integrity of the colon.[65] The receptor is expressed in enteric ganglia, the muscolaris externa, and the muscolaris mucosa, in enterocytes and in mononuclear cells migrated into the lamina propria.[65] Cipriani et al[65] showed that $Tgr5^{-/-}$ mice have altered gut architecture with lower colon cellularity and crypt distortion compared to wild-type mice. Moreover, $Tgr5^{-/-}$ animals had worse dextran sodium sulfate-induced colitis with higher colonic macroscopic injury scores, more severe intestinal permeability, and diarrhea. The fluoroquinolone, ciprofloxacin, may be of special interest in the case of inflammatory colitis because of both its antibiotic and TGR5 agonistic properties.[65] Additionally, Yoneno et al[70] showed that macrophage colony-stimulating factor and interferon-γ-stimulated macrophages expressed more TGR5 compared to other types of differentiated macrophages. Also, lamina propria mononuclear cells from Crohn's disease patients expressed more TGR5 compared to controls. In support of this, Fryer et al[76] found that rats showed faster recovery of 2,4-dinotrobenzene sulfonic acid-induced colitis when cotreated with a TGR5 agonist. This was demonstrated by lower colon weight and lower overall damage. Thus, TGR5 is protective in colitis-related diseases via the inhibition of inflammatory responses and by improving intestinal barrier function.

Liver diseases

Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis are part of the metabolic syndrome and are characterized by an inflammatory response.[78] Moreover, these conditions may progress to liver cirrhosis. Kupffer cells may play an important role in nonalcoholic fatty liver disease.[81] Wang et al[68] showed that $Tgr5^{-/-}$ mouse macrophages, Kupffer cells, and livers have more NF-κB activation in both a quiescent and activated state after lipopolysaccharide (LPS), as compared to control mice. Furthermore, the higher expression of NF-κB-regulated proinflammatory genes, such as monocyte chemo-attractant protein-1 and interferon-γ, was observed in the immune cells and livers of $Tgr5^{-/-}$ mice as compared to controls.[68] Immunohistochemistry staining also showed elevated Kupffer cell numbers in $Tgr5^{-/-}$ mice compared to $Tgr5^{+/+}$ mice, even in the absence of LPS. Moreover, treatment of animals with the TGR5 ligand, 23(S)-mCDCA, a specific semisynthetic TGR5 agonist, lowered these responses in $Tgr5^{+/+}$ mice, but not in the $Tgr5^{-/-}$ animals.[68,82] McMahan et al[83] treated obese db/db mice with a dual FXR/TGR5 agonist (INT-767) and found improved nonalcoholic steatohepatitis with increased intrahepatic antiinflammatory monocytes and a gene expression profile indicating alternative macrophage activation, thus pointing to an anti-inflammatory response.

Cholestasis is another area in which TGR5 targeting may be beneficial. Keitel et al[67] showed that TGR5 is present in isolated Kupffer cells, and that these cells are responsive to BAs. Subsequently, BAs lowered LPS-induced cytokines via the established TGR5–cAMP pathway. TGR5 immuno-reactivity in rat liver Kupffer cells increased after bile duct ligation, pointing to a possible protective role in obstructive cholestasis.[67]

After a partial hepatectomy, $Tgr5^{-/-}$ mice showed severe hepatocyte necrosis, cholestasis, a severe inflammatory response, and delayed regeneration in contrast to control mice.[84] Comparable severe liver injury was observed after bile duct ligation, which was worse in $Tgr5^{-/-}$ animals compared to controls.[84] It was proposed that TGR5 mediates these effects via the control of bile hydrophobicity and via inhibition of cytokine secretion of IL-1β, IL-6, and TNFα.[84]

Adverse effects of TGR5 ligands

As discussed in the section titled, "Physiological expression and function of TGR5", TGR5 is expressed in several organs such as the gut, liver, immune cells, kidney, spleen, brown adipose tissue, and the nervous system. This is important because targeting TGR5 for a specific diseased target site will result in TGR5 activation in other tissues as well.

Stimulation of TGR5 is demonstrated to result in modulated gastrointestinal motility.[33,85] Poole et al[33] showed that DCA lowered spontaneous phasic activity in segments of the ileum and colon, and it delayed gastric emptying and small intestinal transit. TGR5 slows down gastric motility, as shown by the treatment of gastric smooth muscle cells with OA, resulting in increased cAMP levels and relaxation of the carbachol-induced contraction of gastric muscle cells in wild-type, but not $Tgr5^{-/-}$, animals.[86] Interestingly, another study indicated that $Tgr5^{-/-}$ mice had slower colon and whole-gut transit, indicating that TGR5 agonism reduced peristalsis in the colon.[85] To what extent TGR5 agonism results in nausea or constipation in humans remains to be elucidated.

Limited data indicate that TGR5 agonism may affect the incidence of biliary stones (see the section titled, "Physiological role of TGR5 in BA homeostasis"). TGR5 is expressed in the gallbladder and may play a role in gallbladder emptying.[87] $Tgr5^{-/-}$ mice are protected from the development of gallstones when placed on a high-fat lithogenic diet.[27] This may be due to a reduced BA pool size or reduced CYP7a1 activity that reduces biliary cholesterol accumulation.[27] $TGR5$ ribonucleic acid (RNA) expression in human gallbladders has been shown to correlate with the incidence of gallstones, but this relationship was not found for the TGR5 protein.[24] Although the above data suggest a role for TGR5 in gallbladder physiology, the causal link to gallstones may be premature.

Another side effect that has been proposed is itching (pruritus).[35] This was observed with the TGR5-selective agonist, OA, which induced hyperexcitability of the dorsal root ganglia neurons and stimulated the release of the itch-related transmitters, gastrin-releasing peptide and leucine-enkephalin.[35] Intradermal injection of the same agonist induced scratching behavior in $Tgr5^{+/+}$ mice, but not in $Tgr5^{-/-}$ mice.[35]

The infusion of TGR5 agonists reduces vascular tone and blood pressure in dogs, but not in rats, combined with reflex tachycardia and an inotropic response, resulting in enhanced cardiac output.[76] These effects were proposed to be mediated through the activation of the large-conductance Ca^{2+}-activated potassium channel, $K_{Ca}1.1$.[76]

Finally, some very limited in vitro evidence could suggest that TGR5 agonism induces (cancer) cell proliferation. Casaburi et al[88] suggested the involvement of TGR5 in endometrial cancer risk in obesity/type 2 diabetes. They used an in vitro approach in which low concentrations of CDCA stimulated the growth of an endometrial cancer cell line via a TGR5-dependent pathway. Perhaps more relevant is the finding of Yasuda et al,[21] who demonstrated that TGR5 signaling may be implicated in gastrointestinal tract cell carcinogenesis via the EGFR–extracellular signal-regulated kinase 1/2 pathway. This pathway was activated in human gastric carcinoma cells by BAs. Here, small interfering RNAs that target TGR5 mRNA suppressed phosphorylation of EGFR thereby inhibiting BA-induced activation. Additionally, TGR5 is proposed to increase in vitro cell growth of FLO cancer cells and AGS gastric adenocarcinoma cells.[17,18] It has also been observed that the methylation of $TGR5$ DNA is a hallmark of hepatitis B virus-associated hepatocellular carcinoma in humans.[89]

Although these possible side effects of TGR5 agonists in development should be taken into consideration, all data are based on animal and in vitro studies. While these preclinical models provide important clues, clear clinical evidence of the side effects of therapeutic TGR5 targeting is very limited at this point in time.

Future research required for therapeutic TGR5 ligands

As outlined in the previous sections, TGR5 may be a promising receptor in the adjunct treatment of a number of

diseases, including inflammatory disorders. With regard to future research, it should be underlined that many actions of TGR5 in different cells are not fully resolved or described. Studies elucidating the biological actions of TGR5 and its potential adverse effects are crucial, especially with the purpose of clinical application. Additionally, more translational and human studies investigating TGR5 will provide essential information regarding therapeutic ligands. An important step is to define the therapeutic window in which TGR5 activation is an effective treatment without resulting in adverse effects. So far, this has been done in one animal study that failed to show a therapeutic window between the inhibition of colitis and the TGR5 agonist's effects on vasodilation.[76]

Depending on the potential adverse effects of TGR5 signaling in humans, a number of approaches can be undertaken to minimize adverse effects. Three options are worth investigating, which include: 1) the biochemical properties of the ligand; 2) biased ligands; and 3) tissue-specific drug delivery, as will be discussed.

Firstly, a number of different synthetic agonists have been described that have different scaffolds.[14] These agonists differ in tissue distribution and metabolism, which will result in different properties. One example of such an agonist is XL-475 (discussed in the section titled, "Other TGR5 agonists"), which localizes to the intestine, thereby limiting systemic exposure.[14] This could potentially increase the therapeutic window. Other compounds that could have a beneficial tissue distribution are specific semisynthetic BA derivatives (see the section titled, "Semisynthetic bile acids as TGR5 agonists"), which cycle in the enterohepatic circulation and are consequently enriched in areas such as the colon and the liver.[90] If these latter tissues are desired target areas, these compounds could also have less adverse effects and consequently have a larger therapeutic window, as compared to TGR5 agonists that are also present throughout the body.

Secondly, a ligand of TGR5 can be designed in such a manner that downstream signaling is biased, which will result in a TGR5 agonist that has more specificity toward the desired signaling pathway.[91] This can potentially lead to a TGR5 ligand that discriminates between therapeutic and adverse effects, thereby enhancing the therapeutic window.

Finally, one could opt to target TGR5 in a tissue-specific manner, which could include a vehicle that directs TGR5 ligands to specific cells and tissues. Examples of these are liposomes, micelles, and biodegradable microparticles.[92] Via these vehicles, the TGR5 compound can be specifically routed to the desired sites and consequently reduce adverse effects in other tissues.

Conclusion

TGR5 is a G protein-coupled BA receptor present in a number of tissues and cell types throughout the body. The receptor functions in several processes, including metabolism and energy homeostasis. More recently, the role of TGR5 in suppressing immune responses has been highlighted, as outlined in this review. TGR5 dampens immune responses via different routes in immune-relevant cell types such as macrophages, DCs, as well as ECs. In line with these notions, TGR5 is involved in inflammatory diseases such as atherosclerosis, colitis, and liver diseases, and it could present an attractive target for therapeutic use. Next to its natural BA ligands, several semisynthetic agonists have been found that can activate TGR5 and subsequently exert positive effects on energy balance, glucose homeostasis, and inflammation. Further research is warranted to elucidate and map all actions of TGR5, especially in humans where information on TGR5 function is still scarce. Evidence indicates that TGR5 signaling may depend on the cell differentiation state and could differ between cell types. TGR5 downstream signaling should therefore be carefully dissected in immune cells, which could pave the way for biased signaling ligands to minimize potential adverse effects.

In our opinion, further studies, especially in humans, will be crucial to further validate TGR5. We are, however, optimistic that TGR5 is a promising target for adjunct intervention in inflammatory diseases. Innovative developments, such as biased signaling and tissue-specific drug targeting, could circumvent potential adverse effects of TGR5 targeting.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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