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Review Article

Structure, metabolism and biological functions of steryl glycosides in mammals

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Steryl glycosides (SGs) are sterols glycosylated at their 3β-hydroxy group. They are widely distributed in plants, algae, and fungi, but are relatively rare in bacteria and animals. Glycosylation of sterols, resulting in important components of the cell membrane SGs, alters their biophysical properties and confers resistance against stress by freezing or heat shock to cells. Besides, many biological functions in animals have been suggested from the observations of SG administration. Recently, cholesteryl glucosides synthesized via the transglycosidation by glucocerebrosidases (GBAs) were found in the central nervous system of animals. Identification of patients with congenital mutations in GBA genes or availability of respective animal models will enable investigation of the function of such endogenously synthesized cholesteryl glycosides by genetic approaches. In addition, mechanisms of the host immune responses against pathogenic bacterial SGs have partially been resolved. This review is focused on the biological functions of SGs in mammals taking into consideration their therapeutic applications in the future.

Introduction

Steryl glycosides (SGs) are derivatives of sterols (steroids with a hydroxy group at the C3 carbon in ring A of four condensed aliphatic rings) glycosylated at the hydroxy group.

SG was first isolated from olives more than a century ago [1] and this compound was later identified as sitosteryl D-glucoside [2,3]. An acylated form of SG, phytosteryl 6′-*O*-acyl-glucoside, was then isolated from soybeans and potatoes [4]. SGs and acylated SGs are ubiquitously found in vascular plants, fungi, and algae, whereas their presence in bacteria and animals is relatively restricted. Comprehensive reviews on the structure, distribution, and biosynthesis of SGs were published in 1999 and 2010 [5,6]. As sterol biosynthesis in prokaryotes is relatively rare, SGs and acyl SGs are found only in limited bacteria species. Regarding the presence of SGs in animals, cholesteryl β-glucoside and its variants in the sugar moiety have been reported in sea cucumber [7], soft coral [8], snakes [9], birds [10,11], mice [12], and humans [13–17]. Recently, biosynthesis of cholesteryl glucosides in mammals was reported in which transglycosylation to sterols was catalyzed by glucocerebrosidases (GBA) using glucosylceramide (GlcCer)as a source of glucose ([12,18–20], reviewed in [21]). Genetic approaches became applicable to the functional characterization of these SGs owing to the accomplishment of the cloning of the responsible enzyme genes. On the other hand, sterol glycosyltransferases that are responsible for the biosynthesis of SGs in plants, fungi, and bacteria have not been identified in mammals.

Following the summary of the structure and metabolism of SGs, the present review focuses on the recent research progress on the biological functions of them in mammals. The results are classified as follows: (1) those obtained when subjects were exposed to exogenous SGs, (2) those obtained from the observation of subjects with GBA gene mutations, and (3) those when the subjects interacted with pathogenic bacteria producing SGs.

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Structural variations and distribution of steryl glycosides (SGs)

Identified SGs contain structural variations both in the sterol and sugar moiety. Typical structures are illustrated in Figure 1.

Sterol heterogeneity

The composition of sterols in SGs reflects the amount of free sterols in each organism, which are sitosterol, ergosterol and cholesterol in plants, fungi and animals, respectively (Figure 1A). SGs found in certain auxotrophic bacteria, such as *Helicobacter pylori* [22] and *Borrelia burgdorferi* [23], contain cholesterol. The cholesterol present in such pathogenic bacteria is considered to originate from the host cells [24,25].

The configuration of the 3-hydroxy group in sterols is usually β (extruding from the planar condensed rings), thus the configuration of their glycosides is considered to be β. The presence of exceptionally 3α-hydroxy stigmasterol and its glycosides in *Mimusops elengi* is proposed based on NMR spectroscopy [26].

Variations in the sugar moiety

The sugar species most commonly found in SGs is D-glucopyranose (Figure 1A,B). Most steryl glucosides are of β-anomeric configuration [5,6]. In addition to β-glucosides, steryl glucopyranosides with α-anomeric configuration are found in *H. pylori* [22] and *Acholeplasma axanthum* [27]. SGs with a sugar moiety other than glucopyranose are less abundant in many organisms [5,6]. Several examples of SGs other than glucopyranosides are as follows: β-D-galactopyranoside (*B. burgdorferi* [23], *Codium decorticatum* [28], and vertebrates [20]), β-D-glucuronopypanoside (*Homo sapiens* [15–17]), β-D-xylopyranoside (sea cucumber [7,29]), α-L-fucopyranoside (soft coral [8]), and *Candida albicans* α-mannopyranoside [30]. Some SGs consist of a diglycosyl or an oligoglycosyl sugar moiety (reviewed in [5]) such as α-Glc(1–3) α-Glc (*A. axanthum* [27]) and β-gentiotrioside (*Vigna angularis* [31]).

The C6′-primary hydroxy group of the pyranose constituting SGs is frequently acylated with a fatty acid, as initially found in soy beans and potatoes [4] (reviewed in [5]) (Figure 1C). Acylation of SGs affects the biophysical properties of cell membranes such as hydrophobicity. Furthermore, it alters the immunogenicity of cells in some cases, as will be described in the sections below.

Biosynthesis and degradation of SGs

Sterol glycosyltransferase and glucocerebrosidases

Sterol glycosyltransferase, the key enzyme responsible for the biosynthesis of SGs, transfers sugars from sugar nucleotides to the 3-hydroxy group of sterols. Cloning of the genes encoding sterol glycosyltransferase has been accomplished from plants, fungi, molds, and bacteria [6,32], but not from animal cells. Most cloned enzymes are sterol β-glucosyltransferase that transfers a glucose from UDP-glucose to sterols, whereas sterol β-galactosyltransferase and α-glucosyltransferase have been cloned from *B. burgdorferi* [6] and *H. pylori* [33], respectively. A β-glucosyltransferase cloned from *Candida bumbicola* exhibits a broad substrate specificity [32]. This enzyme catalyzes the transfer of a glucose from UDP-glucose to both sterols and hydroxy fatty acids. A sterol β-glucosyltransferase found in eggplant (*Solanum melongena*) was demonstrated to have the potential to transfer a galactose or xylose as well as a glucose from appropriate sugar nucleotides to sterols [34].

Recently, the generation of ChβG was reported in a heat-shocked human cell line [13]. Of note, this activity was due to the transglycosylation activity of mammalian GBA 1 and 2, which usually catalyze the hydrolysis of β-glucosylceramide (GlcCer) [12,18–20]. These enzymes reversibly catalyze the transfer of a glucose between cholesterol and ceramide depending on the concentration of accessible substrates. More recently, it was demonstrated that cholesteryl β-galactoside (ChβGal), as well as ChβG [11], was present in the vertebrate brain and that GBA 1 and 2 were responsible for the degradation and the synthesis of this cholesteryl glycoside, respectively [20]. These demonstrations are interesting because β-galactosylceramide (GalCer) and its derivative, sulfatide, are abundant in the vertebrate brain. The transglycosylation activity of galactosylceramidase, an enzyme responsible for the hydrolysis of galactosylceramide, has not been reported yet.

Steryl glucoside acyl transferase

6′-*O*-acylated steryl glycopyranosides are widely distributed in many organisms. The 6′-*O*-acylation activity *in vitro* was detected in the extracts of plants [35–38]. The supposed acyltransferases included in the extracts

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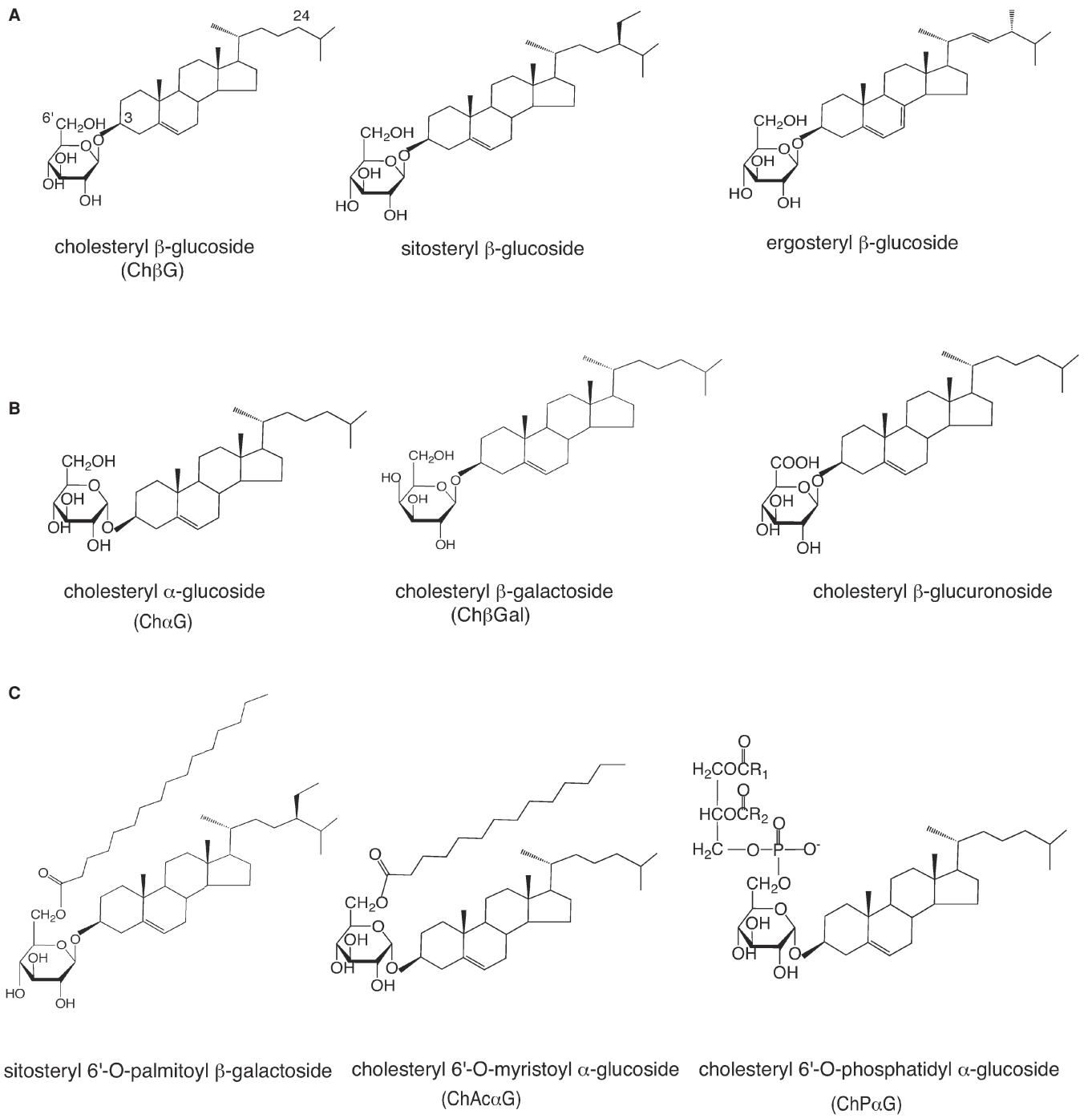

Figure 1. Structure of typical steryl glycosides.

(**A**) Polymorphism of the sterol moiety in steryl glycosides. Cholesteryl β-glucoside (found in humans, snakes, and chicken), sitosteryl β-glucoside (plants and algae), and ergosteryl β-glucoside (fungi) are shown. (**B**) Polymorphism of glycosides in steryl glycosides. Cholesteryl α-glucoside found in *Helicobacter pylori*, cholesteryl β-galactoside in *Borrelia burgdorferi* and vertebrate brain, and cholesteryl β-glucuronoside in human liver are shown. (**C**) Modification of the 6' hydroxyl group of the pyranosyl residue in steryl glycosides. Sitosteryl 6′-*O*-palmitoyl β-glucoside (plants), cholesteryl 6′-*O*-myristoyl α-glucoside (*Helicobacter pylori*), and cholesteryl 6′-*O*-phosphatidyl α-glucoside *(Helicobacter pylori)* are shown.

require exogenous acyl lipids such as glycerophospholipids as fatty acid sources for acylation and transfer acyl group from them to SGs. Regarding acyltransferases in bacteria, activation of the gene *Hp0499* in *H. pylori* was demonstrated to increase the production of cholesteryl 6′-*O*-acyl α-glucoside (ChAcαG) [39]. Suggesting the

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gene products to be involved in the acylation. Recently, *hp0499* was identified to be the gene for acyltransferase that catalyzes the acyl transfer from phosphatidylethanolamine to ChαG to form ChAcαG [40].

Steryl glycoside hydrolases

SGs hydrolases for the catabolism of SGs have been found in several organisms. A specific SGs hydrolase was first found in mustard (*Sinapis alba*) seedlings [41], followed by in Golden butterwax bean (*Phaseolus vulgaris*) hypocotyls [42]. The latter enzyme simultaneously catalyzes the transfer of a glucose residue from cholesteryl glucoside to ceramide to produce glucosylceramide similar to GBA1 and GBA2 described above.

Recently, SGs hydrolases were identified from other kingdoms such as fungi [43] and *Saccharomyces cerevisiae* [44].

Biological functions of steryl glycosides in mammals

Glycosylation of sterols alters their physicochemical properties such as hydrophobicity and mobility. The introduction of SGs to a cell membrane lipid bilayer is expected to alter its characteristics. Thus, several attempts have been made to examine the effects of SG introduction to reconstituted lipid bilayers on their thermostrophic phase transition or fluidity that will confer resistance against stress by freezing, as reported in plants (reviewed in [6]). SGs with amphipathic property distribute differently from the constituents of them and may play their own roles *in vivo*.

This review is focused on the biological functions of SGs in mammals which were classified into the following three for convenience as suggested in the introduction, (1) effects of SG administration to subjects, (2) functions of endogenous SGs synthesized by transglycosylation reaction of GBAs, and (3) SGs produced by microbial pathogens that elicit host immune responses during infection.

(1) Effects of SG administration to subjects

Before describing the effects of SG administration in detail, it may be important to discuss the efficiency of absorption of orally administered SGs through the intestines. Weber reported that following oral administration of ^{14}C-labeled sitosteryl β-glucoside to rats, the radioactivity of the tracer was hardly detectable outside the alimentary canal [45]. In another report, orally administered ^{3}H-labeled sitosteryl β-glucoside was absorbed from the intestinal tract, incorporated into lipoproteins, such as chylomicrons, and only ∼0.6% of the tracer was recovered from the thoracic duct in rats [46]. Thus, when evaluating the experimental results, it should be taken into consideration that the incorporation of SGs *in vivo* is limited following their oral administration. As for steryl *O*-acyl glycosides, quantitative analyses of their absorption are lacking.

Suppression of cholesterol absorption

In 1950, it was reported that the administration of plant sterols (phytosterols), natural food ingredients, lowered serum levels of low-density lipoprotein cholesterol. Since then, a lot of studies have been published. The administration of phytosterols was suggested to reduce cholesterol absorption via the intestines by competing with intestinal cholesterol and also to induce the secretion of cholesterol from the circulation into the intestinal lumen [47–49]. Then, cholesterol-lowering effects of SGs were examined because SGs were usually included in the plant sterol preparations to a certain degree. Lin et al. measured the excretion of orally administered deuterium-labeled cholesterol into the feces of mice as a tracer and found that the simultaneous administration of phytosteryl 6′-*O*-acyl β-glucoside reduced cholesterol absorption (increased tracer excretion) as efficiently as phytosterol ester administration. They also demonstrated that the levels of the deuterium-labeled tracer in the serum and liver of the mice administered phytosteryl 6′-*O*-acyl β-glucoside were much lower than those administered phytosterol ester [50]. Thus, the cholesterol-lowering effects of SGs were suggested to be stronger than those of phytosterol ester. Tateo et al. [51] examined lipid profiles in the plasma, liver, and feces of rats following soybean SG administration. They found that SG administration significantly promoted the excretion of cholesterol into feces when compared with the administration of soybean oil or other lipid components. Thus, it is suggested by these two reports that SGs reduce cholesterol absorption by promoting the excretion of cholesterol into the intestinal lumen.

Th1 shift following phytosteryl β-glucoside administration

When human helper T cells, previously stimulated with a sub-optimal concentration of phytohemagglutinin *in vitro*, were subsequently treated with sterolin formulation (the 100 : 1 ratio of sitosterol and sitosteryl

β-glucoside), cell proliferation and the production of type I helper T cell (Th1)-mediated cellular immunity-promoting cytokines, such as IFN-γ and IL-2, increased [52]. Similarly, when T cells were collected from volunteers who previously ingested sterolin (20 mg 3 times a day for 4 weeks) and stimulated with PHA in culture, their proliferation was greater than that of T cells treated with placebo. As the average intake of plant sterols is ∼300 mg per day [53], the amount of sitosterol administered to the subjects was much more than that included in the sterolin formulation. Thus, it is likely that the sitosteryl β-glucoside included in sterolin was responsible for the induction of Th1-skewed immune responses. Based on these findings, the same group assessed the effects of sterolin on diseases that may be improved by increased Th1 immunity such as type I allergy, systemic autoimmunity, and infection (reviewed in [54]). For example, the daily administration of sterolin accompanied by regular drug treatments promoted general recovery from pulmonary tuberculosis with increased proliferation of lymphocytes [55]. However, Nair and Kanfer raised concerns [56] about the effective concentrations of sitosterol and sitosteryl glucoside contained in sterolin that was prepared from the extract of *Hypoxis hemerocallidea* and used in the series of the studies described above [52–55].

The effects of β-sitosteryl glucoside administration were examined by another group using SGs prepared from an origin other than *H. hemerocallidea*. The mice injected with sitosteryl β-glucoside survived longer than the untreated mice following infection with *C. albicans* [57]. As the protective effects were dependent on the presence of $CD4^+$ T cells, depletion of these cells from mice abrogated the effects of SG treatment to prolong their survival. Moreover, when spleen lymphocytes prepared from mice previously treated with sitosteryl β-glucoside were stimulated with an anti-CD3 monoclonal antibody ex vivo, they produced five-times more IFN-γ and four-times more IL-2 than splenocytes from untreated mice. This suggests that the resistance against *C. albicans* infection was due to increased Th1 immunity in mice treated with sitosteryl β-glucoside.

Collectively, these studies suggested that sitosteryl β-glucoside is a potent immune regulator that can shift the Th1/Th2 balance towards Th1-dominant, although the mechanism underlying these findings remains unclear.

Analgesic activity

It was previously demonstrated that oral administration of SGs induced analgesic effects in recipients [58]. For example, when mice were subjected to the acetic acid-induced writhing test with or without the oral administration of sitosteryl β-glucoside isolated from the leaves of *Mentha cordifolia* Opiz (100 mg/kg) prior to the test, the number of writhes induced by acetic acid decreased by 70%. In another test by the hot plate method, the administration of SG to recipients increased in pain tolerance to a degree comparable to the known analgesic mefenamic acid. Thus, the physiology of the central nervous system may be affected by oral SG administration.

Anti-complementarity

One of the SG species isolated from *Orostachys japonicus*, 6′-*O*-palmitoyl β-sitosteryl glucoside, exhibited anti-complementary activity on the classical pathway of the complement of human serum [59] (IC50 of the SG was 1.0 μM, whereas that of tiliroside (a typical anti-complementary agent) was 76.5 μM). Of note, β-sitosteryl glucoside (lacking 6′-*O*-acyl group) purified from the same plant had no anti-complementary activity in the same assay, suggesting the importance of the presence of an acyl group in the 6′-*O*-palmitoyl β-sitosteryl glucoside to exert anti-complementary activity.

Macrophage activation

Sitosteryl 6′-*O*-acyl β-glucosides (a mixture of palmitoyl and oleoyl, etc.) isolated from the leaves of *Phaleria cumingii*, a Palauan medical herb, was found to stimulate macrophages [60]. The mouse macrophage line Raw 264.7 cells exhibited augmented phagocytosis activity toward latex particles in the presence of this SG *in vitro*. Similarly, the addition of cholesteryl β-glucoside (ChβG) to the culture of *H. pylori* increased the phagocytosis of the microbes by macrophages [24]. In contrast, cholesteryl α-glucoside found in *H. pylori* prevented phagocytosis [24]. Moreover, this particular SG consisting of α-glucosyl residue is recognized by immune cells, including macrophages [61] and iNKT cells [62], and induces immune responses of the host *in vivo* and *in vitro*. The details of this SG found in *H. pylori* will be discussed in a new chapter below.

Insulin release and antihyperglycemic effects

Effects of sitosterol β-glucoside administration on insulin release and antihyperglycemia were previously examined in rats [63]. The secretion of insulin was induced in the culture of islets of Langerhans isolated from rats

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by stimulation with SGs purified from *Centaurea seridis* even at a non-stimulatory concentration of glucose. In addition, oral administration of the SGs to rats increased the fasting plasma insulin level [64]. However, this treatment did not change serum insulin and glucose levels in rats with severe diabetes induced by streptozotocin injection [63]. This suggests that intact, but not lesioned, pancreatic β-cells are able to respond to stimulation with SGs purified from *C. seridis* to secrete insulin.

Heat shock

Induction of SG biosynthesis in cells of several organisms [6], including humans [13], in response to heat shock was previously reported. Reversibly, human fibroblast cells up-regulated the expression of the transcription factor HSF1 responsible for the transcription of the heat shock protein 70 gene after stimulation with cholesteryl β-glucoside (ChβG) *in vitro* [14]. As cholesterol glycosyltransferases have not been identified in animals to date, it is likely that ChβG is biosynthesized in human cells via transglycosilation by GBAs, as will be discussed in a chapter below.

Parkinson's disease

The high prevalence of amyotrophic lateral sclerosis (ALS) and its clinical variant Parkinsonism-dementia complex (PDC) has long been recognized among the indigenous population of Guam, and the environmental neurotoxins included in a local traditional food, seeds of cycad, was suspected to be one of the causative substances of the diseases [65]. Shaw et al. reported that mice fed cycad flour developed many neurological deficits that resemble major features of ALS-PDC in humans [66], and that SGs isolated from cycad seeds were neurotoxic and the causative substances of the diseases [67]. Sitosteryl β-glucoside was the major component among more than three forms of steryl β-glucosides isolated from cycad seeds. The neurotoxicity of the isolated sitosteryl β-glucoside, synthetic sitosteryl β-glucoside and cholesteryl β-glucoside, was compared in these studies. These compounds induced lactose dehydrogenase release from a slice of rat cortex in a similar degree, whereas synthetic cholesteryl β-glucoside induced greater caspase-3 expression from human astrocytes in culture [67]. The neurotoxic effects of SGs were demonstrated not only *in vitro* but also *in vivo*. Chronic exposure to dietary sitosteryl β-glucoside (for 10–15 weeks) was neurotoxic to motor neurons of mice and induced an ALS-PDC phenotype in the recipients [68]. Recently Van Kampen et al. [69] presented a rat model of Parkinsonism in which rats after chronic exposure to dietary sitosteryl β-glucoside (for 10 months) exhibited phenotypes recapitulating multiple key features of the human disease.

Shaw et al. then addressed the mechanism of neurotoxicity in motor neuron-derived cells after exposure to cholesteryl β-glucoside (ChβG) *in vitro* [70]. When cells were transiently treated with ChβG (1 h), the phosphorylation of the so-called 'survival' protein kinase B (PKB) or Akt increased. Akt signaling is known to lead to cytoprotection against stress from serum deprivation. This result is consistent with the findings of Kunimoto et al. [13] that ChβG treatment of human fibroblast cells induces the expression of the stress response protein HSP 70. On the other hand, prolonged exposure of motor neuron-derived cells to ChβG was cytotoxic rather than cytoprotective [70]. This reflects the disease profiles found in the recipients exposed to dietary SGs for months as described above [66–68]. To address the mechanism of cytotoxicity of SGs at a subcellular level, Panov et al. [71] investigated the effects of SGs on respiration and reactive oxygen species (ROS) generation in brain mitochondria. They reported that ChβG treatment of non-synaptic brain mitochondria enhanced succinate dehydrogenase activity together with ROS generation. They suggested that ChβG influenced mitochondrial function by altering the packing of the bulk lipids of membranes and that the resulting changes in the integrity of the membrane led to the increased oxidative damage of neurons and eventual development of PD. In this study, neurotoxicity was not demonstrated by the sterols, the aglycones of steryl glucosides, whereas the role of cholesterol and its metabolites (oxidized forms of cholesterol) in the pathophysiology of PD has been reported by another group [72]. Accordingly, it is necessary to identify the inducers of neurodegenerative disease development, SGs, their aglycones, or both.

Recently, the presence of phytosteryl glycosides (sitosteryl β-glucoside, etc.) and cholesteryl β-glucoside in the embryonic chicken brain was demonstrated [11,20]. Although the possibility that phytosterols are synthesized *de novo* in the chicken brain is not formally excluded, the phytosteryl glycosides or at least their aglycones, phytosterols are likely to be transported from the egg yolk sac consisting of maternal components. The report on the transport of maternal cholesterol to the fetus in pregnant mice [73] supports this hypothesis. The finding that sitisteryl β-glucoside is susceptible to digestion by a recombinant human GBA1 formulation [11], Cerezyme [74], implys that phytosteryl β-glucosides found in the chicken embryo brain are recognized by

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GBA1 and that they are synthesized by the transglycosylation activity of the enzyme from phytosterol and β-GlcCer.

The presence of phytosterols in the murine adult brain has been reported [75,76]. Although the concentration of phytosterols in circulation are maintained at a low level in animals by an efflux pump, ATP-binding cassette transporter G5/G8 [77], a substantial portion of dietary phytosterol can be transported to the brain and accumulate there when its level exceeds the limit [76]. Analysis of the permeability of SGs across the blood-brain barrier, the permissible concentration of them in the brain, and the sterol specificity in the transglycosylation reaction by GBAs may be important to determine whether dietary SGs become beneficial or cause neurodegenerative diseases in recipients.

(2) Functions of endogenous ChβGs synthesized by the transglycosylation reaction of GBAs in mammals

Generation of endogenous ChβGs by GBAs

Taking into account the reports on the effects of dietary phytosteryl glycosides, it is possible that the accumulation of endogenous ChβGs in the central nervous system becomes neurotoxic and raises the incidence of neuropathy. As mentioned in the previous section, cholesterol glycosyltransferases have not been identified in animals. Instead, ChβGs are biosynthesized by the transglycosylation activity of GBAs using glucosyl ceramide (GlcCer) as a glucose donor (reviewed in [21,78]) (Figure 2). Thus far, four GBAs have been reported in humans or mice, and transglycosylation activity was demonstrated for GBA1 and GBA2 [21]. The presence of patients with genetic defects in the GBA1 or GBA2 gene, and their model mice established by gene manipulation makes it possible to investigate the functions of the products of these enzymes, including ChβGs, by genetic approaches.

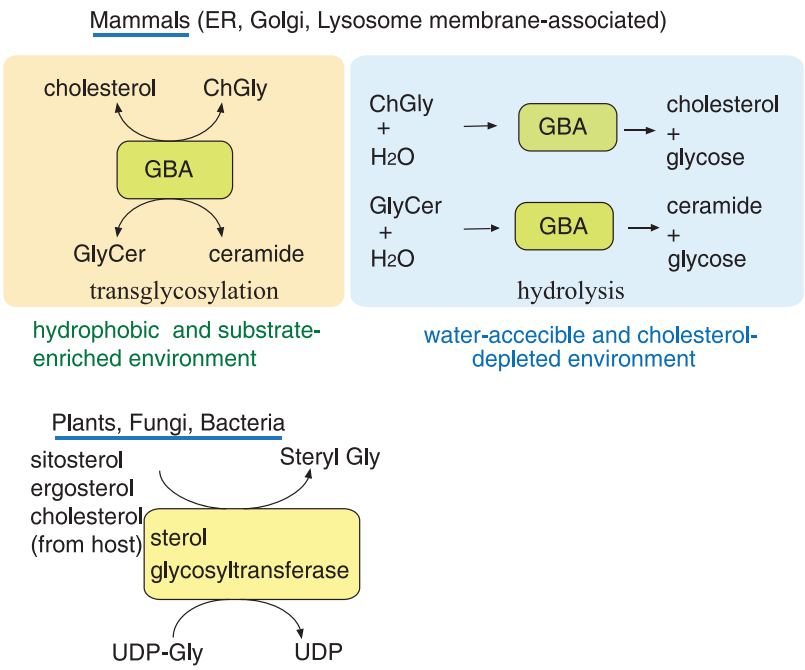

Figure 2. Biosynthesis of SGs.

In mammals, glucocerebrosidases (GBAs) function as enzymes for the biosynthesis of SGs. GBAs catalyze transglycosylation from β-glycosylceramide (GlyCer) to cholesterol to generate cholesteryl β-glycoside (ChGly) and ceramide, and vice versa. As ChGlys, cholesteryl galactoside, as well as cholesteryl glucoside, was recently found [20]. GBAs promote transglycosylation when they are associated with hydrophobic surface of membranes abundant in cholesterol and GlyCer, such as lipid rafts, otherwise they catalyze hydrolysis of GlyCer or ChGly. Sterol glycosyltransferases play roles in biosynthesis of SGs in plants, fungi, and bacteria utilizing UDP-Gly or an appropriate sugar nucleotide as a glycosyl donor. Sterol glycosyltransferases remain to be identified in animals. Gly, glycoside or glycosyl.

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GBA1 and GBA2 are non-integral membrane-associated glucosidases. GBA1 localizes at the lumenal surface of lysosome membranes, whereas GBA2 is associated with the cytoplasmic side of membranes of the endoplasmic reticulum (ER) and Golgi apparatus [79,80]. These enzymes function as glycosidases for GlcCer under usual physiological conditions. In addition, they catalyze the transglycosylation reaction between a glucose donor and an acceptor under appropriate conditions. The transglycosylation activity of GBAs was identified in GBA1 between artificial substrates in the previous studies [81,82]. Then, Akiyama et al. confirmed transglycosylation from β-GlcCer to cholesterol by GBA1 to generate ChβG utilizing a cell line lacking β-GlcCer synthase (ceramide glucosyltransferase) activity in which transglycosylation did not take place due to the lack of the glucose donor β-GlcCer [19,21]. Transglycosylation is assumed to become dominant at hydrophobic sites of membranes according to the study on the mechanism of transglycosylation reactions of rosewood β-glucosidases [83]. The glucosyl-enzyme intermediates react with a proximal glucose acceptor which has been fixed by the specific hydrophobic amino acid residues properly located at the active site of the enzyme. If the glycosyl-enzyme intermediates are exposed to water, they will be hydrolyzed. It is suggested that the membrane domains abundant in β-GlcCer and cholesterol tend to organize hydrophobic lipid rafts [84], thus lipid rafts are one of the possible milieus suitable for transglycosylation from β-GlcCer to cholesterol. Indeed, ChβGs are detectable in the membrane raft-enriched fraction of heat-shocked animal cells [85], although the association of GBAs with lipid raft domains have not been formally indicated.

Transglycosylation by GBA2 from GlcCer to cholesterol was demonstrated by Marques et al. [12] in cells with overexpressing GBA2 genes. They also suggested the involvement of GBA2 in the biosynthesis of ChβGs *in vivo* by the reduced ChβG levels in the plasma, liver, and thymus of GBA2-deficient mice. On the other hand, LIMP2 deficiency (with markedly reduced GBA1 activity due to its impaired transport from the ER to lysosome) increased the ChβG levels, thus it is assumed that GBA2 rather than GBA1 primarily synthesizes ChβGs *in vivo* under physiological conditions. This notion is supported by their findings that the markedly increased ChβG synthesis in the glucosylceramide synthase gene-transfected cells (due to the increase in the glucose donor GlcCer for transglycosylation) was inhibited in the presence of a GBA2 inhibitor (AMP-DNV), but not in the presence of a GBA1 inhibitor (CBE). However, Marques et al. suggest the involvement of GBA1 in transglycosylation *in vivo* under pathological conditions. They noted markedly increased ChβG concentrations in the liver of Niemann pick C1 mutant mice in which cholesterol is accumulated in lysosomes due to the defect in cholesterol export. They also found the elevation of ChβG levels in the liver of Gaucher disease (GD) model mice with GBA1 deficiency in the white blood cell lineage in which β-GlcCer accumulation in lysosomes is induced. Thus, it is suggested that lysosomal glucocerebrosidase GBA1 generates ChβG when the transglycosylation donor or acceptor is accumulated in lysosomes to a certain extent.

The transglycosylation by GBAs is reversible, thus these enzymes also catalyze transglycosylation from ChβG to ceramide to produce GlcCer. In addition, GBAs are able to hydrolyze not only β-GlcCer but also ChβG when when water, instead of glucose-acceptors, is accessible to the enzyme-substrate intermediate [21,83].

Degradation of GlcCer takes place mainly in lysosomes by GBA1, and this enzyme is accessible to membrane-bound GlcCer via the aid of the lysosome lipid-binding protein saposine C [86]. It remains unclear whether saposine C or similar activator proteins function in ChβG formation or hydrolysis by GBAs.

Gaucher disease (GD) and Parkinson's disease (PD)

GD is a lysosomal storage disorder caused by homozygous mutations in the GBA1 gene. The accumulation of GlcCer in lysosomes, especially phagolysosomes, is one of the prominent features of GD patients [87]. The manifested symptoms of GD are diverse among individual patients and this disease is classified into several types based on the criteria, for example, whether neurological disorders are present [88]. In GD patients and their model mice (deletion of GBA1 gene in white blood cell lineage), GBA2 activity is enhanced [89,90]. GBA2 activity may compensate for the defects in lipid metabolism caused by GBA1 deficiency to maintain homeostasis. Plasma levels of ChβG in GD patients are higher than those in healthy controls, and treatment of the patients with GlcCer synthase inhibitor (Eliglustat, Miglustat) reduced the levels [12]. Plasma levels of ChβG in Niemann-Pick C1 patients accompanying lysosomal accumulation of cholesterol, another substrate for transglycosylation of GBAs, are also higher [12]. These results are consistent with the findings in the model mice for GD and Niemann-Pick C1 patients described above. Thus, accumulation of the substrates for transglycosylation of GBAs (GlcCer, cholesterol) in lysosomes may induce the increase in ChβG levels. Mistry et al. [91] reported that GBA2 gene deletion in GD model mice (with GBA1 gene deficiency in white blood cell lineage) rescued the symptoms of GD. The introduction of GBA2 gene deficiency into Niemann-Pick C1

model mice also ameliorated the symptoms of neuropathology [92]. These reports suggest that the increased levels of ChβG caused by the unbalance between GBA1 and GBA2 activity are closely associated with the development of the disorders. However, congenital loss of function mutations in GBA2 alone in subjects, where lower ChβG levels are expected, also cause abnormalities in many tissues including male infertility in mice [93,94] and humans [95–97], presumably due to the accumulation of GlcCer, thus suggesting that the levels of lipid metabolites other than ChβG are also important factors for the development of GD. Examination of GBA1 activity, and the balance between ChβG and GlcCer levels in the tissues of GBA2-deficient mice are of interest.

PD, as well as GD, is a common lipid metabolism disorder characterized by the accumulation of fibers of misfolded α-synuclein proteins included in Lewy bodies in neurons, resulting in the marked loss of dopaminergic neurons of the substantia nigra [98,99]. Investigations of several thousand patients suspected of having PD revealed that GBA1 gene mutations are a common risk factor for this disease. As excessive feeding of SGs for months causes ALS-PDC and endogenous ChβG can accumulate in neurons under pathological conditions due to abnormal lipid metabolism, it is possible that the accumulation of ChβG and GlcCer is closely associated with the development of PD. Garcia-Sanz et al. [100] demonstrated that a GBA1 gene point mutation (asparagine 370 to serine) caused lysosomal accumulation of cholesterol and GlcCer due to the loss of function of GBA1 in PD patients. They also described that fibroblasts isolated from the patients exhibited phenotypes similar to those of the cells in the subjects fed excessive amounts of SGs such as impaired autophagy leading to incomplete removal of damaged mitochondria by ROS and increased cell death [100]. Gegg et al. reported that the GBA1 activity is down-regulated in PD patients carrying heterozygous GBA1 gene mutations, especially in the substantia nigra [101]. Although the role of ChβG in PD development was not directly described in these two reports, the accumulation of the substrates for transglycosylation (cholesterol, GlcCer) by the GBA1 loss of function and the up-regulated GBA2 activity to compensate for the loss is likely to induce the elevation of ChβG. The balance of GBA1 and GBA2 activity may be important to maintain the homeostasis of the metabolism of glycosphingolipids and cholesteryl glycosides, and influence the development of PD. The relationship among GBA activity, dysfunction of lysosomes and PD pathogenesis is discussed in a previous review [102]. To understand the mechanism underlying the development of neurodegenerative disorders, including PD in which accumulated ChβG is suspected to be involved, regional GBA1 and GBA2 activity, and ChβG levels in the brain of patients should be investigated and will be useful for the diagnosis and treatment [21,78].

(3) Steryl glycosides produced by microbial pathogens that elicit the immune responses of the hosts during infection

Cholesteryl 6′-*O*-acyl β-galactoside produced by *B. burgdorferi* as an immunogen in patients with Lyme disease

Spirochete *B. burgdorferi*, the etiological agent of Lyme disease, is usually transmitted to humans and animals by bites of ticks of the genus *Ixodes*.

B. burgdorferi requires cholesterol for survival but does not have ability to synthesize this lipid. Thus, this pathogenic bacterium acquires cholesterol from the cell membrane of the host [103]. The acquired cholesterol is glycosylated to make cholesteryl glycosides. Most *B. burgdorferi* genospecies produce cholesteryl β-galactoside (ChβGal) [6]. The biological significance of this glycolipid is not clearly understood, but its glycosylation is likely to reduce the antigenicity of cholesterol toward phagocytosis by host macrophages, which was corroborated as in *H. pylori* (see the section of *H. pylori* below).

Cholesteryl galactosides constitute a significant portion of the total lipid content of *B. burgdorferi* (45%), and the acylated form of ChβGal (ChAcβGal) is one of the most abundant components [23,104,105].

Glycolipids of *B. burgdorferi* become antigens in Lyme disease patients and two glycolipid components, ChAcβGal [23,105] and α-galactosyl diacylglycerol [106], have been identified to be most reactive with the isolated antisera. ChAcβGal was reported to frequently induce antibody production, especially in the late stage of Lyme disease [104]. The minimum essential structural requirements for this glycolipid to be antigenic are the galactose, cholesterol, and fatty acid residues with a minimal chain length of four carbon atoms [107], thus suggesting the importance of the acylated form of the cholesteryl galactoside. As ChAcβGal is ubiquitously synthesized by the genus *Borrelia* regardless of genospecies (except for *Borrelia hermsii*: this species expresses cholesteryl glucosides), this glycolipid is useful as a vaccinogen [108].

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Another important function of cholesteryl galactosides in *B. burgdorferi* is their role in the formation of lipid raft domains in the cell membrane [109]. ChAcβGal is thought to be especially important to form eukaryotic cell-like well-ordered raft structures in *B. burgdorferi* where sphingolipid biosynthesis is absent [110]. The interaction and lipid exchange between the raft domains of the pathogenic bacteria and the host cells was exhibited in the co-culture of *B. burgdorferi* and human epithelial cells [25]. This interaction is beneficial for the pathogenic bacteria to acquire cholesterol and other nutritional resources, and this mechanism may also facilitate their adherence and transmission of virulent factors to host cells. However, incorporation of the components of the pathogens into host cells may induce host immune responses.

Ergosteryl β-glucoside produced by *Cryptococcus neoformans* as a target of vaccination

Cryptococcus neoformans is an encapsulated fungal pathogen. The fungal cells initially infect the lungs, and disease progression results in a highly lethal form of meningoencephalitis. However, there are few effective prophylactic tools for cryptococcosis. Del Poeta et al. previously demonstrated that the introduction of mutations to the gene encoding sterylglucosidase (Δ*sgl)* in *C. neoformans* rescued the host in murine models of *C. neoformans* infection [111]. Thus, the accumulation of ergosteryl β-glucoside may function in protection. Antibodies against the main polysaccharide that composes the fungal capsule, glucuronoxylomannan (GXM), are ubiquitously detected in human serum, although this antibody-mediated protection is insufficient for survival. Colombo et al.[112] tested a vaccination strategy for mice using GXM-containing extracellular vesicles (EVs) prepared from the Δ*sgl1* line of *C. neoformans* (ergosteryl β-glucoside enriched in the EVs), and found that the host was protected from *C. neoformans* infection. The authors interpreted that the accumulating SGs in the Δ*sg11* EVs altered the properties of GXM and that vaccination with Δ*sgl1* EVs elicited effective immunity from recipients. Sterylglycosidase-deleted EVs may be a potent tool for vaccination strategies against fungal infection. Recent studies on the vaccination strategies against fungus infection using fungal SGs as an adjuvant are reviewed [113].

Involvement of cholesteryl 3-α-D-glucoside (ChαG) and its derivatives produced by *H. pylori* in the interaction between the host and pathogen during infection

(a) Glycosylation of cholesterol facilitates *H. pylori* to evade immune surveillance of the host

H. pylori is a Gram-negative helix-formed microaerophile bacterial pathogen. This bacterium has colonized the stomach of humans since their early stage of evolution [114]. Following the first demonstration of the pathogenic potential of *H. pylori* in 1984 by Marshall and Warren [115], there is accumulating evidence that infection by this bacterium is a causative factor of gastric ulcers, carcinoma, and mucosal-associated lymphoid tissue lymphoma [116–118]. *H. pylori* is auxotrophic for cholesterol, one of the important components of the cell membrane [119]. *H. pylori* follows a cholesterol gradient and extracts it from plasma membranes of epithelial cells of the host stomach [24]. Incorporation of cholesterol promotes phagocytosis of *H. pylori* by antigen-presenting cells (APCs) and enhances the following antigen-specific immune response of the host [24], in which Th1 $CD4^+$ T cells play essential roles [120–123].

H. pylori converts cholesterol to cholesteryl α-glucoside (ChαG) using cholesterol α-glucosyltransferase (encoded by the *hp0421* gene) [24,33,124] just a few hours after incorporating cholesterol to evade immune surveillance. Most ChαG is further converted to cholesteryl 6′-*O*-acyl (mainly $C_{14:0}$ myristoyl), α-glucoside (ChAcαG), or cholesteryl 6′-*O*-phosphatidyl α-glucoside (ChPαG) [22,24]. Cholesteol α-glucosyltransferase, responsible for the conversion from cholesterol to ChαG, is expressed as a precursor form in the cytoplasm, transmitted to the cell membrane of the bacterium, and activated [125]. Consistent with these results, localization of ChαGs at the outer side of cell membrane has been reported [126]. Expression of ChαGs is important for *H. pylori* to maintain morphology, cell wall integrity, and resistance to antibiotics [127]. When human macrophage cells were cocultured with either *H. pylori* of cholesterol α-glucosyltransferase-deficient or wild-type strains, they incorporated and phagocytosed the microbes of cholesterol α-glucosyltransferase-deficient mutant more efficiently than the microbes of wild type [24]. In addition, the proliferation of human $CD4^+$ T cells was inhibited by the presence of microbes of wild-type *H. pylori* in culture, whereas the presence of α-glucosyltransferase-deficient *H. pylori* in the culture did not affect the growth of the host T cells [128]. The importance of glycosylation of cholesterol for the survival of invading *H. pylori* was also demonstrated in *in*

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vivo studies. When *H. pylori* or related *H. hepaticus* of cholesterol α-glucosyl transferase-deficient mutant or wild-type strains were orogastrically inoculated to mice, the microbes of the mutant strains were more effectively cleared from the digestive organs of the recipients than those of the wild type [129,130]. Morey and Meyer [131] recently reported that depletion of cholesterol in the epithelial cells in host gastric glands by *H. pylori* infection destroys raft structure of their plasma membrane and prevents the signaling of IFN-γ and other pro-inflammatory cytokines thereby promoting the persistence of the infection. They also demonstrated that the escape from host immune surveillance is dependent on the activity of cholesterol α-glucosyltransferase in the pathogens although the roles of the resultant cholesteryl α-glucosides in the escape were not directly described [131].

Taken together, these reports suggest that conversion of the incorporated cholesterol to its glycosides helps *H. pylori* escape from the immune surveillance of the host. Wang et al. compared the infection of human adenocarcinoma cells by *H. pylori* of cholesterol α-glucosyl transferase-deficient mutant with the infection by wild-type *H. pylori* and proposed that ChαGs expression on the outer membrane of *H. pylori* facilitates the coalescence between *H. pylori* and host cell membrane raft domains, and the subsequent introduction of virulent factors into host cells via type IV secretion system pili [132]. Wang et al. then compared the phagocytosis of *H. pylori* of cholesterol α-glucosyl transferase-deficient with wild-type strains by murine macrophage cells. They found that ChαGs transmitted from *H. pylori* to macrophage membranes retarded phagosome formation, maturation, and fusion with lysosomes thereby facilitating the survival of incorporated *H. pylori* in macrophages [133].

It was recently reported that acylation of the 6' hydroxy group of ChαG by cholesteryl α-D-glucopyranoside acyltransferase enhanced the adhesion of *H. pylori* to host gastric epithelium [40], thus suggesting that the acylation as well as the glycosylation of cholesterol facilitates the infection of the bacterium to host.

(b) ChAcαG produced by *H. pylori* as a target of immune surveillance

H. pylori binds to the host cells using several kinds of adhesins, bacterial carbohydrate-binding proteins, to colonize gastric mucosa [134–136]. For example, BabA adheres to the host blood type-determining sugar chains. Kawakubo et al. [137] previously reported that gastric gland mucus-associated *O*-glycans carrying a terminal GlcNAcα1–4 unit inhibited the activity of bacterial cholesterol α-glucosyltransferase and that human carcinoma cells transfected with a GlcNAc transferase gene, responsible for the synthesis of the terminal GlcNAcα1–4 unit, became resistant to *H. pylori* infection. This is of interest because a specific sugar unit included in the targets for adhesins exhibited antibiotic activity.

H. pylori that breaks the barrier of the mucin layer in gastric organs becomes the target of host immune surveillance. Conversion of cholesterol to its glycoside affects the interaction between *H. pylori* and the host in a manner different from the manners described above (section 'Glycosylation of cholesterol facilitates H. pylori to evade immune surveillance of the host') that are beneficial for the pathogens to survive in the host. We recently demonstrated that ChAcαG produced by *H. pylori* is presented by the non-classical MHC class Ib molecules CD1d following phagocytosis and processing of the microbes by APCs, and recognized by invariant TCR-bearing NKT cells [62,138]. Murine invariant Vα14-Jα18 TCR^+ and their human counterpart invariant Vα24-Jα18 TCR^+ NKT (iNKT) cells are a subset of T lymphocytes [139] that are selected by non-classical MHC class I molecule CD1d in the thymus. These lymphocytes were reported to respond to marine sponge α-galactosylceramide (GalCer) presented by CD1d on APCs [140]. Then, antigenic glycolipids were discovered from bacteria, such as α-glucuronosyl and α-galacturonosyl ceramides, from α-proteobacteria [141,142], and α-galactosyl diacylglycerol from *B. burgdorferi* [143]. Thus, the involvement of iNKT cells in immune surveillance against bacterial infection was suggested. We paid attention to the structural similarities between ChAcαG and reported stimulants for iNKT cells, and examined whether ChAcαG was a potent antigen for this subset of T cells.

We demonstrated that glycolipid extracts from *H. pylori*-induced immune responses from murine and human iNKT cells in a CD1d-dependent manner, and that ChAcαG is responsible for the antigenic activity using synthesized ChAcαG [62,138]. To our knowledge, this is the first report on the recognition of cholesterol derivatives by TCR. ChAcαG has a unique molecular architecture (consisting of an α-glucoside, a 6′-*O*-acly group and a cholesterol residue) common to the glycolipid antigens for iNKT cells reported to date [144], i.e. they commonly possess two long hydrophobic moieties and an α-linked pyranose residue. It is presumed that each hydrophobic moiety anchors to either the A′ or the F′ pocket of CD1d and that the sugar moiety of the cholesteryl glycoside is correctly recognized by the invariant TCR of iNKT cells (Figure 3A). In addition to

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ChAcαG, ChPαG was shown to be antigenic recently [129], whereas ChαG, possessing only one long hydrophobic chain, demonstrated no detectable antigenic activity [138].

We have recently reported that splenocytes isolated from TCR Jα18-deficient mice (lacking iNKT cell development) produce only a trace amount of IFN-γ upon stimulation with *H. pylori* microbes in culture [145] (Figure 3B). Ito et al. [129] reported that the number of bacteria recovered from the stomach of TCR Jα18-deficient mice was larger than that from the stomach of wild-type mice after the orogastrical inoculation of *H. pylori* microbes. Collectively, these findings suggest that *H. pylori* that has converted cholesterol to its glycosides to prevent phagocytosis remains under the surveillance of the host immune system, where recognition of *H. pylori* ChAcαG by iNKT cells induces the subsequent immune responses. From this point of view, a clinical report on the accumulation of $CD161^{+}$ NKT-like cells at the sites of infection by *H. pylori* in the stomach is suggestive [146].

It is possible that localization of ChAcαG in the outer membrane of *H. pylori* enables the host to recognize the invading pathogen directly as a target of immune surveillance by using cell surface-expressed antigen

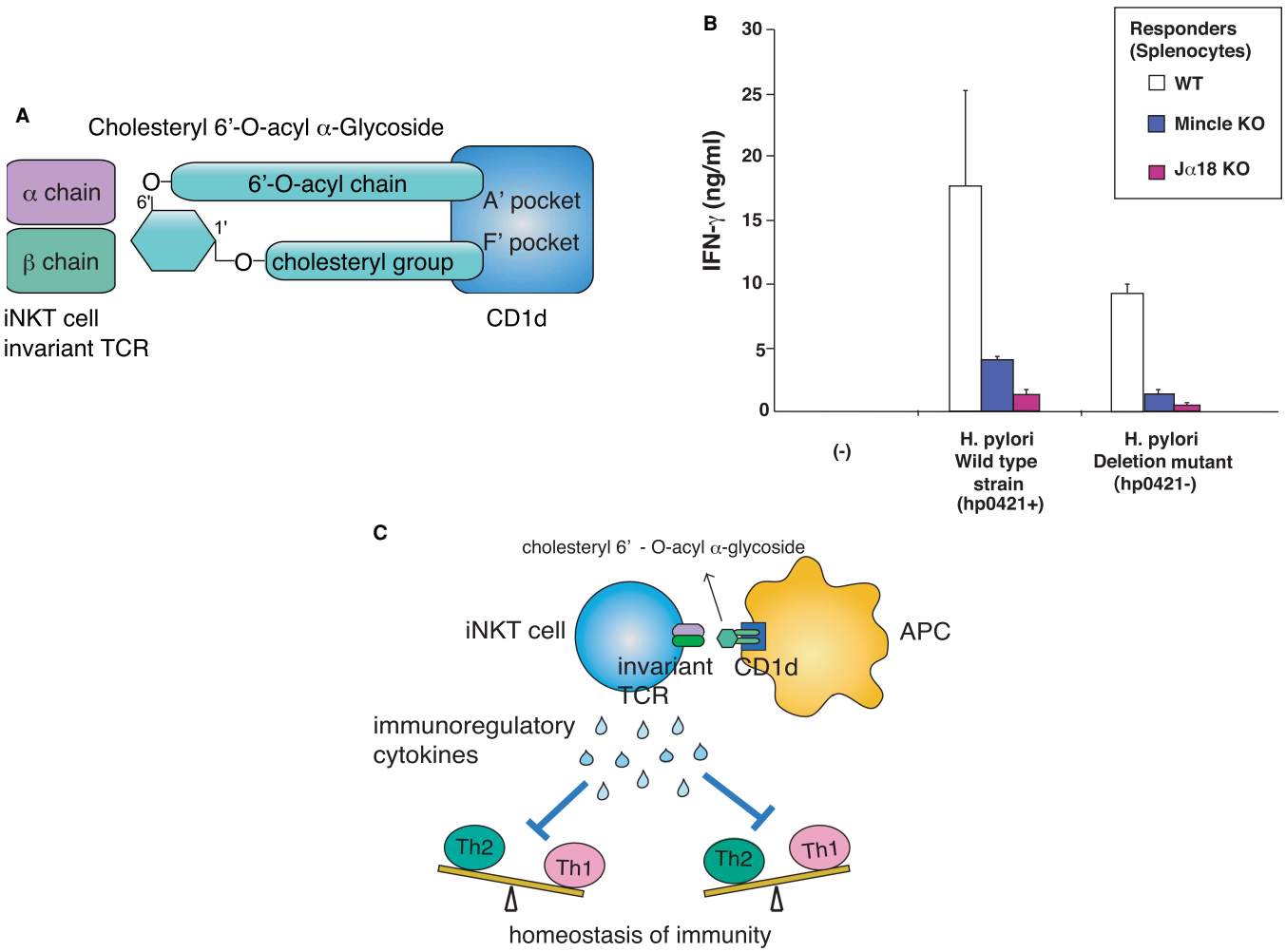

Figure 3. ChAcαG presented by antigen-presenting cells activates invariant NKT cells in a CD1-dependent manner.

Cholesteryl 6′-*O*-acyl α-glycosides (α-glucoside, α-galactoside, and α-mannoside) are presented by CD1d and recognized by the invariant TCR of iNKT cells. (**B**) iNKT cell-dependent immune responses to ChAαG during *H. pylori* infection which is partially reduced by the absence of Mincle. Splenocytes isolated from wild type, Mincle-deficient, and Jα18-deficient mice were stimulated with ether wild type or *hp0421* (cholesterol α-glucosyl transferase gene)-deficient *H. pylori* microbes (MOI 0.01) *in vitro*, and IFN-γ secreted into the supernatants were determined by ELISA [145]. (**C**) Cholesteryl 6′-*O*-acyl α-glycosides presented by CD1d stimulate iNKT cells and induce the secretion of immunoregulatory cytokines. The activated iNKT cells are supposed to function in the homeostasis of immunity in disease models of either Th1 or Th2 excess.

receptors for pathogen-associated molecular patterns (PAMPs). Indeed, we recently found that one of the innate antigen receptors for PAMPs, the C-type lectin Mincle [147], expressed by APCs is involved in the recognition of ChAcαG [145]. Dendritic cells or macrophages are induced to secrete inflammatory cytokines, such as TNF-α, IL-6, and MIP2 [61,145] by stimulation with ChAcαG, and this secretion is depending on the expression of Mincle by dendritic cells or macrophages [145]. However, iNKT cells play indispensable roles in the subsequent activation of acquired immunity following ChAcαG stimulation because CD1d-deficient liver mononuclear cells, which include APCs normally expressing Mincle and T cells but not iNKT cells, produce only a negligible amount of IL-4 and IFN-γ upon stimulation with ChAcαG [61,138]. Here, it is suggested that iNKT cell activation is partially Mincle-dependent, because immune responses of splenocytes prepared from Mincle-deficient mice to *H. pylori* microbes in culture were diminished when compared with the immune responses of wild-type splenocytes (Figure 3B). Previously, an alternative mechanism of iNKT cell activation was proposed (cytokine-dominated activation mechanism) [148,149] in addition to TCR-dominated activation mechanism where iNKT cells are activated via direct stimulation to invariant TCR with exogenous glycolipid antigens on CD1d, as described above. In cytokine-dominated activation mechanism, iNKT cells are activated by concomitant stimulation with certain self-glycolipid antigens in the context of CD1d and cytokines, such as IL-12, produced by APCs stimulated with PAMPs. Mincle-dependent iNKT cell activation suggested here is presumably via a kind of cytokine-dominant activation mechanism in which ChAcαG plays the role for PAMPs. The precise mechanism is under investigation.

(c) Immunoregulation by the administration of ChAcαG and its sugar variants via the activation of iNKT cells

We previously demonstrated that the administration of ChAcαG to neonatal mice induces the activation of a subset of iNKT cells characterized by the CD4, CD8-double negative phenotype and the production of Th1-biased cytokines. We found that this induction resulted in a reduced incidence of allergic asthma after maturation [62] (Figure 3C). We also found that the production of anti-ovalbumin (OVA) antibodies of Th2-controlled isotypes, such as IgE and IgG1, but not Th1-controlled IgG2a, is suppressed in mice immunized with OVA when administered ChAcαG prior to immunization [138]. These observations strongly suggest that ChAcαG injection induces the activation of Th1 immunity from the host and functions in the homeostasis of the immune system to protect the host from allergic reactions or pathogenic infection.

We recently found that a glycoside isomer of *H. pylori* ChAcαG, cholesteryl 6′-*O*-acyl α-mannoside (ChAcαMan), previously reported to be present in *C. albicans* [30], induces the activation of iNKT cells in a CD1d-dependent manner [61]. It is assumed that this cholesteryl glycoside is stimulus to iNKT cells because it retains the molecular architecture common to the glycolipid antigens for iNKT cells as described above [144] in spite of having an unfavorable hydroxy group configuration at the 2′ position of the pyranose residue. The immunoregulatory potential of ChAcαMan was also suggested as found in ChAcαG. Suppression of infection by *Streptococcus pneumoniae* [61], delayed-type hypersensitivity, and type I allergy [150] by the administration of ChAcαMan were observed in mouse models by promoting immunoregulatory cytokine production by iNKT cells (Figure 3C).

Concluding remarks

The biological functions of SGs in mammals were summarized in this review. Dietary phytosteryl glucoside suppresses cholesterol absorption, whereas the accumulation of SGs, especially ChβG, in neurons is a risk factor for Parkinsonism or other lipid metabolic disorders. Glycosylation of cholesterol is a strategy for survival for cholesterol auxotrophic pathogenic bacteria during infection, whereas administration of cholesteryl glycosides stimulates macrophage phagocytosis and subsequent Th1-dominant immune responses. Therefore, it seems that SGs function as a double-edged sword *in vivo*.

Cholesterol glycosyltransferases in animals remains to be identified, while the biosynthesis of ChβG in animals via the transglycosylation reaction by GBAs was elucidated. GBAs play important roles in the control of lipid metabolism, and their abnormal activity induces the accumulation of endogenously synthesized ChβG in neurons and eventually neurodegenerative diseases. The induction of diseases by this mechanism is probably related to the high prevalence of ALS among the indigenous population of Guam. Examination of the regional concentration in the brain as well as the permeability through blood-brain barrier of phytosteryl glycosides, ChβG, ChAcβG and their metabolites may be useful to clarify the roles of SGs in the development of

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neurological disorders. It is also necessary to determine the acceptable dose of dietary SGs or ChβG for developing alimentary therapy with SGs.

The mechanisms for recognizing *H. pylori* ChAcαG by the host immune system have partially been revealed; ChAcαG can be recognized by both the C-type lectin receptor Mincle and the invariant TCR of iNKT cells following presentation by CD1d. On the other hand, the mechanisms of how the host immune system recognizes and interacts with SGs of various sources such as *B. burgdorferi* is still under investigation. Further investigations are necessary for developing vaccination strategies to pathogenic organisms.

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Competing Interests

The author declares that there are no competing interests associated with this manuscript.

Abbreviations

ALS, myotrophic lateral sclerosis; APC, antigen-presenting cell; ChAcG, cholesteryl 6′-*O*-acyl glycoside; ChAcαG, cholesteryl 6′-*O*-acyl α-glucoside; ChAcαMan, cholesteryl 6′-*O*-acyl α-mannoside; ChAcβG, cholesteryl 6′-*O*-acyl β-glucoside; ChG, cholesteryl glycoside; ChPαG, cholesteryl 6′-*O*-phosphatidyl glucoside; ChαG, cholesteryl α-glucoside; ChβG, cholesteryl β-glucoside; ER, endoplasmic reticulum; GalCer, galactosylceramide; GBA, glucocerebrosidase; GD, Gaucher disease; GlcCer, glucosylceramide; iNKT cell, invariant Vα14-Jα18 TCR α-bearing NKT cell; OVA, ovalbumin; PAMP, pathogen-associated molecular pattern; PD, Parkinson's disease; PDC, Parkinsonism-dementia complex; ROS, reactive oxygen species; SG, steryl glycoside; Th1, type I helper T cell.

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