α-Gal-Based Vaccines: Advances, Opportunities, and Perspectives

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ABSTRACT

Humans and crown catarrhines evolved with the inability to synthesize the oligosaccharide galactose-α-1,3-galactose (α-Gal). In turn, they naturally produce high quantities of the glycan-specific antibodies, which can be protective against infectious agents exhibiting the same carbohydrate modification on their surface coat. The protective immunity induced by α-Gal is ensured through an antibody-mediated adaptive and cell-mediated innate immune response. Therefore, the α-Gal antigen represents an attractive and feasible target for developing glycan-based vaccines against multiple diseases. In this review article, we provide an insight into our current understanding of the mechanisms involved in the protective immunity to α-Gal and discuss the possibilities and challenges in developing a single-antigen pan-vaccine for prevention and control of parasitic diseases of medical and veterinary concern.

The rationale for developing an α-Gal-based vaccine against parasites

Carbohydrates are abundantly expressed on the surface of nearly all cells in both prokaryotic and eukaryotic organisms, where they exist in the form of complex glycans, polysaccharides, and oligosaccharides linked to proteins and lipids [1,2]. Indeed, many pathogens causing diseases in humans and animals contain unique carbohydrate structures on their surface coat that serve as receptors by which the pathogenic organisms attach to and invade host cells [3-11]. The pathogen carbohydrate molecules are often structurally different from those displayed on mammalian host cells and are thereby recognized by the host immune system [2]. Natural infections and exposure to the carbohydrate antigens on infectious agents can elicit an innate immune response and activate B cells to produce protective glycan-binding antibodies (Abs) in the host. The potential of the surface **glycotopes** (see **Glossary**) to induce such a potent immune response makes carbohydrates attractive and feasible targets for vaccine development [1,2,12]. Accordingly, the scientific interest in exploiting the immunogenic glycan antigens as vaccine constituents has considerably increased in the last years and it resulted in the development of efficient glycoconjugate vaccines targeting encapsulated bacteria strains such as *Haemophilus influenzae* type B, *Streptococcus pneumonia*, and *Neisseria meningitidis* [1]. Significant progress has also been made with conjugate vaccine formulations for control of other viral, parasitic, and fungal diseases [2,13,14].

The oligosaccharide galactose-α-1,3-galactose (α-Gal) is currently one of the most interesting carbohydrates that attracts growing attention from the scientific community after it was recognized as a major allergen responsible for the production of specific immunoglobulin (Ig)E that mediates delayed and severe anaphylactic reaction to mammalian meat consumption in humans previously exposed to tick bites [15-20]. This novel type of tick-

induced food allergy is better known as red meat allergy or the α-Gal syndrome [21-23]. The terminal α-Gal residues have recently been found in the saliva of several tick species, but the mechanism by which ticks promote an anti-α-Gal response and high-level sensitization in humans is not known [24-28]. It has been, however, hypothesized that tick salivary prostaglandin E2 (PGE2) may induce class switch recombination on B cells leading to IgE production [29]. Another possible explanation is that α-Gal from tick salivary glycoconjugates is presented to **antigen-presenting cells (APCs)** and B lymphocytes in the context of T helper (Th) 2 cell-mediated immunity, which leads to the differentiation of α-Gal-specific B cells into IgE secreting plasma cells [29]. The way how α-Gal is captured, processed and presented to CD4+ T cells seems to determine whether the antigen is recognized by the host immune system as harmless, like possibly in case of helminths and fungi, or hazardous [9,23]. By contrast to the detrimental effect of the IgE immune responses to α-Gal, the production of IgG and IgM isotypes induced by gut microbiota seems to be beneficial as these Abs can be protective against different pathogens exhibiting carbohydrates with the similar α-Gal modification on their surface [4-6,8,10,11,30]. In this review, we provide a summary of the recent advances in our understanding of the mechanisms involved in the protective immunity to α-Gal and discuss the possibilities in developing an α-Gal-based vaccine for prevention and control of multiple parasitic diseases of medical and veterinary importance.

The α-Gal epitope and anti-α-Gal antibodies

The α-Gal epitope is a unique oligosaccharide naturally produced on glycoproteins and glycolipids of non-primate mammals, prosimians, and New World monkeys [31]. The glycosylation enzyme α-1,3-galactosyltransferase (α1,3GT), encoded by the *ggta1* gene in mammals, catalyzes the synthesis of the epitope by transferring galactose molecule from

uridine diphosphate (UDP)-Gal to N-acetyllactosaminide [32]. Conversely, humans and Old World primates lack the synthetic machinery to produce the α-Gal due to the functional inactivation of the *ggta1* gene, caused by several deletions in the DNA sequence that encodes premature stop codons [33-35]. Non-mammalian vertebrates (e.g. birds, fish) do not have the *ggta1* gene and also lack the α-Gal [10,11,36]. In humans, the α-Gal epitope is expressed only in the blood group B glycoconjugates (Galα1-3[Fucα1-2]Galβ1-4Glc-ceramide) [37]. Therefore, humans and other vertebrates with this *ggta1* gene modification lost immune tolerance to non-cryptic α-Gal moieties and are consequently able to produce large quantities of Abs that specifically bind to the non-self-antigen [38,39]. However, due to the structural similarity between antigen B and α-Gal, individuals with blood groups AB and B produce lower Ab levels against the related antigens α-Gal (Galα1-3Galβ1-4GlcNAc) and gal2 (Galα-1,3-Gal) [40]. The disruption of the *ggta1* gene in our primate ancestors, estimated to have occurred almost 28 million years ago, was likely exerted by a strong selective pressure of an infectious agent and the subsequent acquisition of immune-resistance to pathogens expressing α-Gal [31,39]. This evolutionary scenario can be further supported by the following facts: (*i*) enveloped viruses express α-Gal when propagated in cells with active *ggta1* gene; (*ii*) helminths, protozoa, bacteria, and fungi also express carbohydrates with terminal α-Gal moieties; and (*iii*) anti-α-Gal Abs can neutralize pathogenic organisms exhibiting α-Gal epitopes [37].

Anti-α-Gal Abs, in particular IgG, IgM, and IgA isotypes are naturally generated in healthy human individuals as an immunological response to continuous antigenic stimulation by Gram-negative bacteria of gut flora, which express very diverse terminal and non-reducing α-Gal-linked glycans, predominately in Galα1,2-, Galα1,4-, and Galα1,6-R forms [41,42]. A high proportion of individuals (>70%) in healthy populations exhibit α-1,3-

galactosyltransferase sequences in the bacteria of their gut microbiome [43]. Notably, α-1,3-galactosyltransferase genes in bacteria and mammals were not evolutionarily related. However, other immune-mediated mechanisms may also be activated in response to α-Gal [44]. For instance, high levels of anti-α-Gal Abs have also been reported in human patients infected with *Trypanosoma* spp. (Kinetoplastida), *Leishmania* spp. (Kinetoplastida), and *Plasmodium* spp. (Apicomplexa), indicating the polyreactive nature of the Abs [4,45-48]. However, pathogen-specific anti-α-Gal seems to have different specificities than Abs produced towards the α-Gal from enterobacteria [3-5,45,49]. Nevertheless, IgG2 and IgM Abs induced by α-Gal expressed on pig cells are a major immunological barrier preventing the transplantation of pig organs into humans [50].

Mechanisms of the protective α-Gal immunity against parasites

In general, the α-Gal epitope has significant clinical potential in the possibility of developing an α-Gal glycovaccine or other interventions such as pro- and postbiotics to induce a protective α-Gal immune response against multiple diseases (**Figure 1, Key Figure**) [4,5,8,10,11,13,14,30]. The immunity induced by α-Gal influences pathogen infection and its multiplication by various mechanisms including B cell maturation, antibody-mediated **opsonization** of α-Gal-containing pathogens, activation of the **complement system**, Fc-receptor (FcR)-mediated phagocytosis, activation of macrophage response, antibody-mediated interference with the α-Gal antagonistic effect to promote **toll-like receptor 2 (TLR2)/nuclear factor kappa-light-chain-enhancer (NF-κB)**-mediated immune response and upregulation of proinflammatory **cytokines** (**Table 1**).

Plasmodium spp.

Several epidemiological studies uncovered a strong correlation between α-Gal-specific IgM Abs and protection from *Plasmodium* infections in humans [47,48,51,52,53], but the first experimentally proved evidence for the protective role of α-Gal-induced immunity was provided by Yilmaz et al. [4]. In their seminal study, C57BL/6 *ggta1*-knockout (*ggta1*-KO) mice, which like humans do not express α-Gal on their cells, were used as an animal model to assess the protective effect of anti-α-Gal Abs against *Plasmodium berghei* and *Plasmodium yoelii* infections. *Plasmodium* parasites express the α-Gal carbohydrate possibly bound to glycosylphosphatidylinositol (GPI)-anchored surface proteins [4,54]. The results of the experimental study showed that gut colonization by *Escherichia coli* O86:B7 that expresses high levels of α-Gal inhibits the parasite transmission by *Anopheles* mosquitoes. In particular, anti-α-Gal IgM Abs produced in response to *E. coli* O86:B7 blocked infection with *Plasmodium* spp. in 60% of the mice, but this was not the case when the mice were or were not exposed to *E. coli* K12, a serotype which does not produce α-Gal. Immunization of *ggta1*-KO mice with rabbit red blood cell membranes (rRBCM) or synthetic α-Gal linked to bovine serum albumin (α-Gal-BSA) elicited the production of serum IgM and IgG (IgG1, IgG2b, and IgG3 subclasses) Abs, but the levels of circulating IgA and IgE Abs were not detectable. A significant increase in anti-α-Gal Ab production was also observed when mice were immunized with rRBCM supplemented with TLR9 agonist adjuvant and this was associated with an 88% reduction in the relative risk of *Plasmodium* infection compared to 61% risk reduction without adjuvant in combination. Further experiments revealed that the protective effect of α-Gal immunization is mediated via B cell-dependent mechanisms, as well as that both IgM and IgG Ab classes confer protection against malaria transmission, thereby providing **sterile immunity**. The cytotoxic effect of Abs produced towards α-Gal appears to be mediated by the classical complement activation. Furthermore, the protective activity of anti-α-Gal Abs was not observed when *Plasmodium* sporozoites were introduced

intravenously, suggesting that the protection induced by α-Gal immunization is only exerted in the dermis, where the pathogen is inoculated by mosquitoes. Once the parasite reaches the blood, the protective effect of the α-Gal immunity is no longer productive, and the parasitaemia, disease severity, as well as mortality rate were similar among infected *ggta1*-KO mice regardless of the source of the α-Gal residues, i.e. oral administration of *E. coli* O86:B7 or α-Gal immunization. Based on these findings, it has been proposed that α-Gal-induced immunity protects against *Plasmodium* transmission, but not against the blood stages of this parasite. These results suggest that anti-α-Gal immunity can influence malaria incidence, but not disease severity or protection once the disease is established. In agreement with the hypothesis, individuals from Mali and Senegal exposed to mosquito bites were not infected by *Plasmodium falciparum* when having high anti-α-Gal Ab levels [4,48]. In addition, the frequency of blood type B in African countries was positively correlated with the incidence of malaria, possibly due to weak anti-α-Gal immunity in populations with a high frequency of blood type B [48]. By contrast, a negative correlation was observed between the frequency of blood type A and the incidence of malaria [48]. A 4-year prospective cohort study in childhood malaria in Mali showed that children having blood types B and AB had higher incidence rate (blood type B: 1.63 and blood type AB: 1.65) compared to those children with blood types A and O (blood type A: 1.57 and blood type O: 1.45). This supports the role of blood type B in reducing anti-α-Gal Abs, which in turn increases the incidence of malaria [55].

Leishmania spp.

Leishmania spp. is another group of important and widespread protozoan parasites containing terminal α-Gal epitopes and thus they represent potential targets for vaccine development against human visceral and cutaneous **leishmaniasis** [5,6,46]. Variable levels of the α-Gal

moieties have been so far observed in *Leishmania major*, *Leishmania infantum*, and *Leishmania amazonensis* [5,6,56]. *Leishmania major*, and perhaps other *Leishmania* species, synthesize Type-II glycoinositolphospholipids (GIPL)-2 and GIPL-3, which are capped with terminal, non-reducing and highly immunogenic α-galactopyranosyl residues with different structural configurations [5,57]. These glycolipids are expressed in abundance in the amastigote stages, residing in macrophages of mammalian hosts [57]. In a recent study, three different neoglycoproteins (NGPs) containing synthetic α-Gal in different configurations, namely Galα(1,6)Galβ-BSA (NGP5B), Galα(1,4)Galβ-BSA (NGP12B), and Galα(1,3)Galα-BSA (NGP17B), were evaluated as potential prophylactic vaccine candidates against *L. major* [5]. The *ggta1*-KO mice immunized with the three NGPs tested produced high levels of specific anti-α-Gal IgG Abs. However, only NGP5B administered alone or in combination with CpG adjuvant displayed a significant complement-independent lytic activity against infective metacyclic *L. major* promastigotes. Incubation of NGP5B and NGP5B + CpG immunized mice sera with *L. major* promastigotes caused lysis of 44% and 60% promastigotes, respectively. In contrast to *Plasmodium* parasites [4], the lytic effect was documented only when the complement was heat inactivated. Furthermore, mice immunized with NGP5B had significantly reduced parasite load and the size of footpad lesions by 96% compared to control groups. Similarly, a significant reduction in *L. infantum* and *L. amazonensis* parasite load in liver and spleen along with increased levels of anti-α-Gal IgG were observed in another study in which *ggta1*-KO mice were immunized with Qβ virus-like particle bearing α-Gal trisaccharide [6]. In NGP5B immunized mice, a strong protective Th1 cellular immune response with increased levels of proinflammatory cytokines such as interleukin (IL)-12p40, IL-2, and interferon (IFN)-γ were recorded. NGP5B and NGP5B + CpG in mice induced a robust $CD4^+$ and a $CD8^+$ T cell response, which is essential for the protection against *L. major*. Taken together, immunization with this NGP5B alone or with

CpG in combination induce partial, but significant protective α-Gal immunity against the *L. major* infection in mice.

Trypanosoma cruzi

Trypanosoma cruzi has a complex carbohydrate-rich surface coat that contains GPI-anchored mucins (tGPI-mucins) with the linear and immunodominant α-Gal glycotope and several as yet uncharacterized branched terminal α-Gal *O*-glycans [3]. Previous studies showed that patients with acute and chronic **Chagas disease** produce high levels of anti-α-Gal Abs that specifically recognize the tGPI-mucins on trypomastigotes and are considerably different in terms of specificity and biological activity than natural anti-α-Gal Abs produced in response to gut microbiota [3,45,49]. The latter also exhibits substantially lower lytic activity against *Trypanosoma* trypomastigotes [3,45]. Therefore, Portillo et al. [8] recently investigated the efficacy of the *T. cruzi* immunodominant α-Gal epitope as a prophylactic glycovaccine candidate in the acute model of Chagas disease. They immunized *ggta1*-KO mice by intraperitoneal injection of synthetic α-Gal linked to human serum albumin (Galα3LN-HSA or Galα1-3Galβ1-4GlcNAc-HSA), which is almost structurally identical to that found on tGPI-mucins. This comprehensive study clearly demonstrated that mice immunized with Galα3LN-HSA alone or in combination with liposomal-monophosphoryl lipid A (LMPLA) adjuvant had significantly reduced parasite load (91.7-99.9%) in all tissues tested, reduced cardiac inflammation, myocyte necrosis, and T cell infiltration in comparison to control groups. The trypanolytic effect of anti-α-Gal Abs occurred in a complement-independent manner, similar to that in *Leishmania* parasites [5]. These Abs specifically bind to the parasites and destabilize the plasma membrane leading to the surface coat disruption, agglutination, and death. The authors further demonstrated that purified murine IgG Abs to Galα3LN-HSA effectively block the host cell infection and intracellular proliferation of the

parasite. Immunization with Galα3LN-HSA + LMLP increased the production of IgG1 and IgG2b subclasses and significantly increased levels of serum cytokines, chemokines, and growth factors, in particular IL-2, IL-4, IL-9, IL-15, C-C chemokine motif ligand 3 (CCL3), and vascular endothelial growth factor (VEGF). Furthermore, a significant increase of antigen-induced $CD4^+$ and $CD8^+$ T cells was observed along with a considerable expansion of memory $CD4^+CD44^+$ T cells, but not memory $CD8^+CD44^+$ T cells, which are considered to be critical for protective and long-lasting α-Gal immunity against *T. cruzi* infection. Based on the overall results, the authors propose that the production of protective anti-α-Gal Abs to Galα3LN-HSA is mediated through a T cell-dependent B cell memory mechanism.

Challenges in developing an α-Gal-based vaccine

Carrier protein

The evolutionary adaptation in crown catarrhines and their ability to induce a protective α-Gal immune response against infection and multiplication of pathogens suggests the possibility of developing α-Gal-based interventions for control of multiple diseases affecting humans, birds, and fish [4-6,8,10,11,13,14,30]. The prospective vaccine antigen should be capable to induce antigen-specific plasma cells secreting protective Abs and development of memory T and B cells in order to provide efficient and long-lasting protection [2,4,5,8]. Nevertheless, the target carbohydrate antigen must not induce autoimmune- or allergy-related anti-α-Gal IgE Abs following immunization [2,4,5,8]. In contrast to protein antigens, carbohydrates are classified as thymus-independent antigens with poor immunogenic reactivity [58]. Due to the lack of T cell activation, B cells predominantly produce IgM and IgG Abs of low affinity and the immune response induced by carbohydrates is often short-lived [59-62]. Therefore, covalent conjugation of carbohydrate antigens to carrier protein has been proposed to overcome the immunoreactive issue [2]. Synthetic α-Gal-containing NGPs

252 coupled to BSA or HSA have been often used for classical immunization by injection with
253 great success in mice [4,5,8,11,30]. It is important, however, to note that the protein linker
254 can sometimes induce the production of anti-linker Abs and influence the immune response
255 against desired glycan antigen [5]. To minimize the cross-reactions and to achieve a strong
256 and more specific immune response towards specific glycotope, smaller and more flexible
257 protein linkers should be used for glycan conjugation [63,64]. It has also been shown that
258 pre-existing natural anti-α-Gal Abs can enhance the immunogenicity of antigens exhibiting α-
259 gal epitopes by increasing a T cell-dependent immune response following immunization [65-
260 68]. In this sense, combining the α-Gal carbohydrates with other protein vaccine candidates
261 could improve the immunogenicity of the α-Gal vaccine formulations [4,69].
262
263 ***Structure of the α-Gal epitopes and immunization route***
264 The structural configuration of the epitope and the immunization route are other important
265 considerations for the successful α-Gal vaccine development. Immunization with linear
266 Galα3LN (Galα1-3Galβ1-4GlcNAc trisaccharide), which is structurally nearly identical to
267 native *T. cruzi* surface glycotope, provided the full protection against lethal *T. cruzi* challenge
268 in *ggta1*-KO mice [3,8]. The naturally occurring human anti-α-Gal Abs induced by gut
269 microbiota have a considerably weaker binding activity to the linear Galα3LN on tGPI-
270 mucins of *T. cruzi* and lower trypanolytic effect on metacyclic trypomastigotes than parasite-
271 specific anti-α-Gal Abs [3,45,49]. Indeed, the immunodominant Galα3LN glycan on tGPI-
272 mucins is a major target for the protective anti-α-Gal Abs and it has not been yet reported in
273 any enterobacteria [41], suggesting that gut enrichment with α-Gal-containing bacteria may
274 not be as efficient as immunization with synthetic NGPs for *T. cruzi* infection [8]. On the
275 other hand, the experimental study by Mateos-Hernández et al. [10] showed that oral
276 administration of *E. coli* O86:B7 protects turkeys (*Meleagris gallopavo*) from developing

277 respiratory clinical aspergillosis, while subcutaneous immunization with the synthetic Galα1-
278 3Gal-BSA failed to protect against an infectious challenge with *A. fumigatus*. *Escherichia*
279 *coli* O86:B7-treated turkeys had substantially lower granulomatous lesion score,
280 inflammatory response, and lower hyphae score than immunized birds or those from control
281 groups. Interestingly, the protective effect of *E. coli* O86:B7 was not due to the increased
282 production of serum anti-α-Gal IgY, but it is likely associated with a considerable reduction
283 of anti-α-Gal IgA Abs in the lungs of infected birds. The mechanism by which *E. coli*
284 O86:B7 abrogates anti-α-Gal IgA response in the lungs of immunized turkeys remains to be
285 elucidated, but it may be associated with the production of α-Gal-specific **regulatory T cells**
286 **(Tregs)** in the guts, which can then migrate to the lungs and induce tolerance to the infection.
287 Based on these findings, it can be suggested that the efficacy of immunization with α-Gal and
288 the activation of the specific protective immunity may be dependent on the source, i.e.
289 configuration of the α-Gal epitopes and the route of its administration (immunization through
290 injection *vs* oral administration).
291
292 ***Infection route***
293 Nevertheless, the protective effect of the α-Gal-induced immunity may also be depending on
294 the route of pathogen infection. In a recently published study, zebrafish (*Danio rerio*) was
295 introduced as a new animal model to investigate the possibility of using α-Gal-based vaccine
296 formulation for the control of tuberculosis [11]. In two separate experiments, zebrafish were
297 first immunized by intraperitoneal injection of synthetic Galα1-3Gal-BSA either in
298 combination with ISA 71 VG adjuvant or without it and then challenged with *Mycobacterium*
299 *marinum*, a bacterium causing chronic tuberculosis-like diseases in fish [70-72]. The
300 protection against the mycobacterial infection, characterized by increased production of
301 protective anti-α-Gal IgM Abs, opsonization of *M. marinum,* promotion of FcR-mediated

phagocytosis and macrophage activation, was only observed in zebrafish vaccinated with Galα1-3Gal-BSA and infected by mucosal exposure to *M. marinum*, but not in those infected intraperitoneally.

Bacteria used for oral immunization

In most of the studies reported here, *E. coli* O86:B7 strain was proved to successfully elicit protective α-Gal immunity in *ggta1*-KO mouse and bird models [4,10]. No apparent morbidity or mortality occurred in any of the test or control *ggta1*-KO mice due to oral gavage with live *E. coli* O86:B7. Mice remained healthy and active, with no detectable weight loss, diarrhoea, or observable abnormalities [4,73]. Humans orally inoculated with the parental strain of *E. coli* O86:B7, *E. coli* O86, developed increased blood group B Abs [74]. However, *E. coli* O86:B7 was associated with a gastroenteritis outbreak [75]. Therefore, gut colonization with probiotic *E. coli* Nissle 1917 (ECN1917) strain offers a much safer and better alternative [47] also because this strain has higher α-Gal content compared to *E. coli* O86:B7 and it is possibly able to induce a stronger α-Gal immune response. Using Gram-positive bacteria as a probiotic-based vaccine would also be an option, but in contrast to Gram-negative enterobacteria, they do not have immunogenic lipopolysaccharide (LPS) components [76], which may have an impact on the immune response against α-Gal in the intestinal mucosa [47]. To overcome the possible low antigenicity, probiotic-based vaccines using Gram-positive bacteria could be combined with TLR4 agonist for LPS [13,47]. This can be implemented by transforming candidate Gram-positive bacteria with a plasmid containing LPS specific peptide mimotopes [77], as previously observed in *Lactobacillus casei* [78]. Alternatively, a plasmid containing bacterial *ggta1* reported in *E. coli* and other bacteria could be transferred to any bacteria and theoretically used as a probiotic-based vaccine [47].

Concluding remarks

Immunization by α-Gal either through injection of synthetic NGPs or oral administration of α-Gal-expressing bacteria represents a promising and innovative strategy for the prevention and control of parasitic diseases in humans and animals. A combination of protective protein antigens with α-Gal-containing compounds may improve vaccine efficacy by activating complementary immune protective mechanisms. Furthermore, these interventions have the potential to induce an efficient and long-lasting protective immune response targeting other pathogenic agents with the surface α-Gal modifications including *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, Newcastle disease virus, human immunodeficiency virus (HIV), and measles virus [7,14,23,79]. However, all currently available data are gathered in experimental studies employing the humanized mouse or turkey/chicken and fish models [4-6,8,10,11,30], which opens up the question of whether these promising findings in animals can be reliably translated to humans (see **Outstanding Questions**). Therefore, more clinical studies are still needed to optimize the vaccine antigen and standardize the immunization protocols and to better understand the mechanisms underlying the protective immunity induced by α-Gal. There is still much to be learned (see **Outstanding Questions**), but in the era of the -omics technologies, the development of efficient immunization interventions based on the α-Gal antigens against multiple pathogens appears to be closer to reach than ever.

Disclaimer Statement

The authors declare that they have no conflicts of interest.

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Glossary

Antigen-presenting cells (APCs): are a large group of various cells (i.e., macrophages, dendritic cells, B lymphocytes) that induce the cellular immune response by processing and exposing an antigen to T cells

Chagas disease: a severe tropical disease caused by the parasite *Trypanosoma cruzi*, which is transmitted to humans and animals mostly by triatomine bugs. It is also referred to as American trypanosomiasis.

Complement system: an integral part of the innate immune system that supports antibodies and phagocytes to clear foreign particles, microbes, and damaged body cells from an organism. The system also promotes inflammation and opsonization.

Cytokines: a large group of small signalling proteins, peptides, or glycoproteins that are secreted by cells of the immune system. They are involved in the interactions and communications between cells, and the stimulation of the cell movement toward infection sites. Cytokines include interferons (IF), interleukins (IL), chemokines, and tumour necrosis factors (TNF).

Glycotope: a specific part of a carbohydrate antigen that is recognized by the immune system, in particular antibodies, B cells, or T cells.

Leishmaniasis: a vector-borne disease of medical and veterinary concern caused by the protozoan parasites from the genus *Leishmania*. The parasites are transmitted through the bites of infected phlebotomine sandflies. Over 20 *Leishmania* species infect humans worldwide and the diseases can be displayed as cutaneous, visceral, and mucocutaneous forms.

Nuclear factor kappa-light-chain-enhancer (NF-κB): a protein complex found in almost all animal cell types, which controls transcription of DNA, cytokine production, and cell survival. NF-κB is involved in rapid cellular responses to various stimuli and plays a key role in triggering the protective immune response to infections.

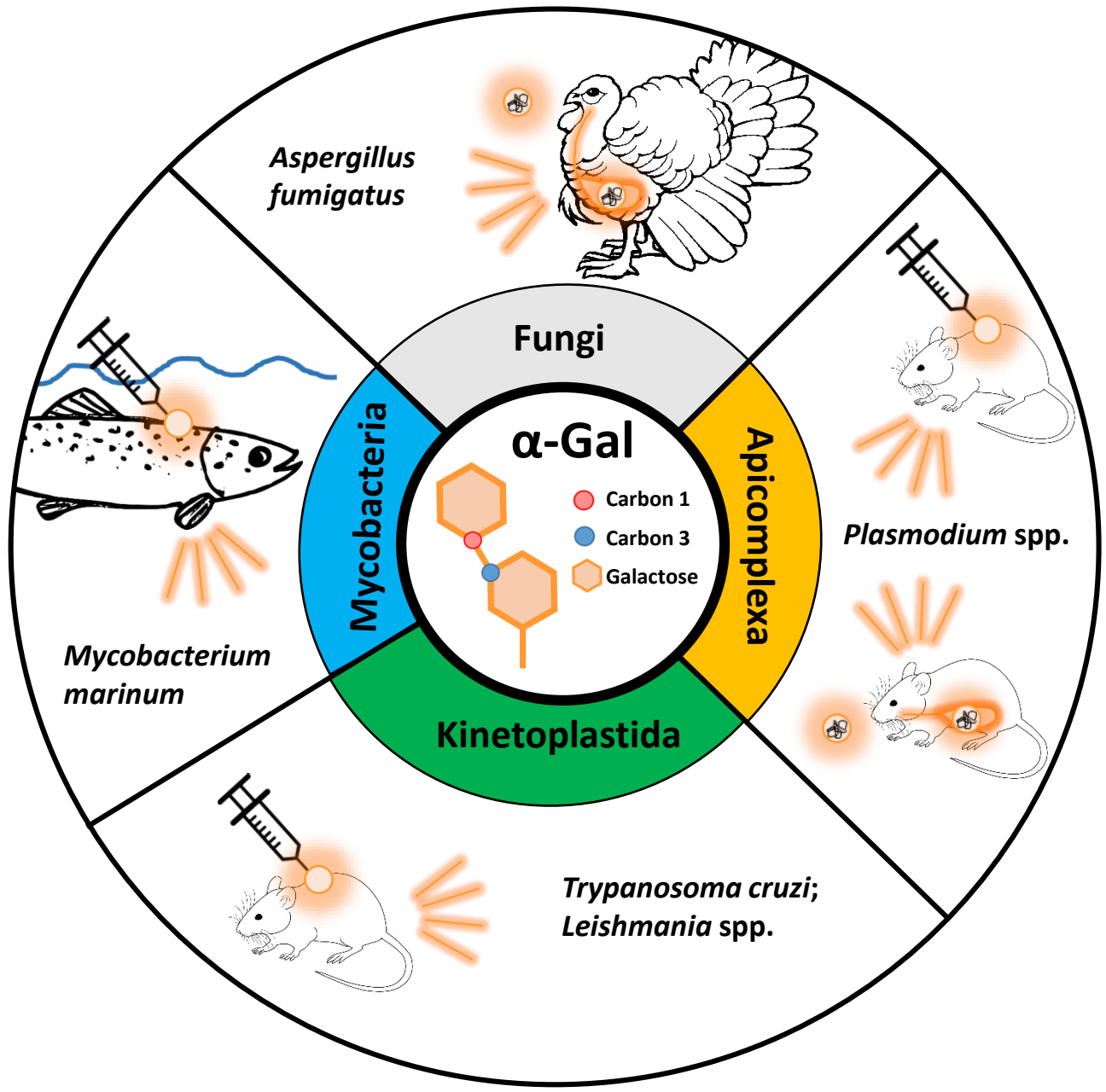
570 **Opsonization:** a process by which a microbial agent or a cell is marked for ingestion and
571 destruction by phagocytes.
572 **Regulatory T cells (Tregs):** subpopulation of T cells, which have a role in modulating other
573 cells of the immune system. Tregs control the immune response to self-antigens and prevent
574 autoimmune diseases. In general, they suppress or downregulate the induction and
575 proliferation of effector T cells.
576 **Sterile immunity:** a unique immune status, which prevents effective pathogen infection into
577 the host and is different from the immunity that allows infection but with subsequent
578 successful eradication of the pathogen.
579 **Toll-like receptors (TLRs):** a class of proteins that play a crucial role in the innate immune
580 system. They are usually expressed on macrophages and dendritic cells that pathogen-
581 associated molecular patterns derived from various infectious agents.
582
583 **Figure caption**
584 **Figure 1, Key Figure. α-Gal vaccination induces protection against several pathogens.**
585 Parental and/or oral immunization with preparations containing the carbohydrate α-Gal has
586 been shown to protect against kinetoplastid (*Trypanosoma cruzi* and *Leishmania* spp.),
587 apicomplexan (*Plasmodium* spp.), mycobacterial (*Mycobacterium marinum*), and fungal
588 (*Aspergillus fumigatus*) pathogens in different animal models.

Table 1. An overview of α-Gal antigens used as vaccine candidates against parasites and other infectious agents and mechanisms of protective α-Gal immunity.

Targeted pathogen	Source of α-Gal epitope	Animal model	Immunization route	Infection route	Mechanism of protective immunity	Reference
Plasmodium berghei, P. yoelii	*Escherichia coli* O86:B7, rRBCM, α-Gal-BSA	C57BL/6 α1,3GT-knockout mouse	PO, IP	ID, IV	Lytic activity of anti-α-Gal IgM and IgG antibodies (complement activation)	[4]
Leishmania major, L. infantum, L. amazonensis	Galα(1,6)Galβ-BSA, Qβ-α-Gal	C57BL/6 α1,3GT-knockout mouse	SC	FPI, IP	Lytic activity of anti-α-Gal IgG antibodies (complement independent), upregulation of Th1 cytokines, upregulation of memory $CD4^+CD44^+$, $CD8^+CD44^+$ and $CD4^+CD69^+$ T cells	[5,6]
Trypanosoma cruzi	Galα1-3Galβ1-4GlcNAc-HSA	C57BL/6 α1,3GT-knockout mouse	IP	IP	Trypanolytic activity of anti-α-Gal IgG antibodies (complement independent), upregulation of cytokines, chemokines and growth factors, expansion of antigen-specific memory $CD4^+CD44^+$ T cells	[8]
Mycobacterium marinum	Galα1-3Gal-BSA	Zebrafish (*Danio rerio*)	IP, ME	IP	B cell maturation, antibody-mediated opsonisation, FcR-mediated phagocytosis, macrophage response, interference with the α-Gal antagonistic effect of the TLR2/NF-kB-mediated immune response, upregulation of proinflammatory cytokines	[11]

Aspergillus fumigatus	*Escherichia coli* O86:B7, Galα1-3Gal-BSA	Turkey (*Meleagris gallopavo*)	PO, SC	IT	Decreased anti-α-Gal IgA levels in the lungs, production of α-Gal-specific Tregs (putative mechanism)	[10]

Abbreviations: FPI – footpad injection; ID – intradermally; IP – intraperitoneally; IT – intratracheally; IV – intravenously; ME – mucosal exposure (i.e., immersion in water containing *M. marinum*); PO – perorally; SC – subcutaneously.


Fungi
Mycobacteria
Apicomplexa
Kinetoplastida
α-Gal
Carbon 1
Carbon 3
Galactose
Aspergillus fumigatus
Mycobacterium marinum
Plasmodium spp.
Trypanosoma cruzi; Leishmania spp.