Microbiota Control of Malaria Transmission

Miguel P. Soares^{1,*} and Bahtiyar Yilmaz^{1,2,*}

¹Instituto Gulbenkian de Ciência, Rua da Quinta Grande, 6, 2780-156, Oeiras, Portugal

²Current address: Maurice Müller Laboratories (DKF), Universitätsklinik für Viszerale Chirurgie und Medizin Inselspital, Murtenstrasse 35, University of Bern, 3010 Bern, Switzerland

*Correspondence: mpsosares@igc.gulbenkian.pt (M.P. Soares) and bahtiyar_yilmaz@outlook.com (B. Yilmaz).

Stable mutualistic interactions between multicellular organisms and microbes are an evolutionarily conserved process with a major impact on host physiology and fitness. Humans establish such interactions with a consortium of microorganisms known as the microbiota. Despite the mutualistic nature of these interactions, some bacterial components of the human microbiota express immunogenic glycans that elicit glycan-specific antibody (Ab) responses. The ensuing circulating Abs are protective against infections by pathogens that express those glycans, as demonstrated for *Plasmodium*, the causative agent of malaria. Presumably, a similar protective Ab response acts against other vector-borne diseases.

Trends

Immunogenic bacteria in the human gut microbiota express α -gal glycans. Humans produce α -gal-specific antibodies in response to immunogenic bacteria in the microbiota.

Plasmodium spp. express α -gal glycans.

Abs directed against α -gal glycans confer protection against malaria transmission.

Microbiota and Natural Antibodies

Humans establish structured and often **mutualistic** interactions with their **microbiota** (see Glossary), which are vertically transmitted and, to some extent, maintained throughout life via horizontal transmission [1]. This occurs mainly at epithelial interfaces such as the intestinal and urogenital tracts, as well as at the lung and skin, where tightly juxtaposed epithelial cells limit systemic access to potentially damaging microbes and/or their component parts [2]. Resident immune cells at these epithelial barriers sense components of the microbiota via pattern recognition receptors (PRRs), eliciting a host response that maintains the functional integrity of epithelial barriers [3]. This involves the production of mucus and antimicrobial peptides, as well as IgA and IgM **antibodies** (**Abs**) [4,5] that transverse epithelial barriers and bind to immunogenic components of the microbiota [6], modulating its composition and impact on host physiology [4,5]. Here, we explore how Ab responses directed against the Gal α 1–3Gal (α -gal) glycan expressed by bacteria in the gut microbiota confer protection against malaria [7] and presumably other vector-borne diseases.

Glycan-Specific Natural Abs

Humans have relatively high levels of circulating anti-glycan Abs [8,9], including α -gal-specific Abs that account for up to approximately 1–5% of circulating IgM and IgG, and are produced by 1% of the B cell repertoire of healthy adult individuals [10–12]. Anti-glycan Abs, including α -gal-specific Abs, are often referred to as natural Abs (NAbs) because they are present in the circulation of healthy individuals in the ‘absence’ of a traceable immunization [13]. While animals maintained under **germ-free** conditions can produce relatively low levels of NAbs, production of physiologic levels of circulating glycan-specific Abs require the establishment of host microbiota interactions [7,14]. In

keeping with this notion, a significant proportion of circulating NAbs recognize glycans expressed by components of the gut microbiota [15], as illustrated for anti-blood group NAbs [16], which include α -gal-specific NAbs [17].

Based on the immediate glycan recognition at the outer surface of microorganisms, NAbs act as a first-line of defense against virus and bacteria [18,19] and possibly protozoan parasite [20] infections (reviewed in [21,22]). When present above a certain threshold level at the time of infection, circulating NAbs can target pathogens as soon as these breach epithelial barriers [21]. Activation of the classical complement pathway and Ab-dependent cell-mediated cytotoxicity limit pathogen expansion and dissemination into vital organs [19] (reviewed in [21]).

Anti-glycan NAbs, including α -gal-specific Abs, are generated in mice by long-lived B cells known as B1 cells [8,23,24], as well as by marginal zone B cells in the spleen [25] (reviewed in [21]). The production of these Abs is triggered upon engagement of PRRs and/or the B cell receptor by microbial-associated molecular patterns, including glycans such as those in bacterial lipopolysaccharide (LPS). This results in the generation of low affinity ($K_d = 10^{-4} - 10^{-7}$ M) glycan-specific IgM Abs, via a mechanism that does not require T cell help and does not involve Ig class-switch recombination or affinity maturation. However, some glycan-specific Ab responses are associated with the production of high affinity ($K_d > 10^{-7}$ M) T cell-dependent IgG Abs [13,26].

Expression of identical or similar glycans by pathogens and their mammalian hosts raises the question as to how Ab responses targeting these glycans are generated. Presumably, glycan-specific Ab responses should only target xeno-glycans that are not expressed as part of self [27], thus avoiding autoimmunity and disease [13]. This constraint was circumvented for some self-glycans such as α -gal through an evolutionarily based process whereby loss-of-function mutations in genes responsible for the expression of such glycans were selected for and fixed in populations [28] (Box 1, Box 2, and Figure 1).

In contrast to humans, most mammals including mice carry a functional *GGTA1* gene, which encodes a UDP-galactose: β -D-galactosyl-1,4-*N*-acetyl-D-glucosaminide α -1,3-galactosyltransferase (α 1,3GT) that generates the Gal α 1-3Gal β 1-4GlcNAc-R (α -gal)

glycan (Box 1, Box 2, and Figure 1). Mammals also express a functional isoglobotriaosylceramide synthase (*iGb3S*) gene, which encodes a UDP-gal:β-D-galactosyl-1,4-glucosyl-ceramide α1,3GT that generates the Galα1-3Galβ1-4Glc-ceramide glycan (Box 1, Box 2, and Figure 1). As a result, α-gal-specific B cells are purged from the B cell repertoire of adult mice, which fail to generate anti-α-gal Ab responses. Deletion of the *Ggtal* gene in mice eliminates the expression of Galα1-3Galβ1-4GlcNAc-R glycan and allows for the production of anti-α-gal Abs [29]. This is possible despite the expression of Galα1-3Galβ1-4Glc-ceramide [30], presumably because α-gal-specific Abs can discriminate Galα1-3Galβ1-4GlcNAc from Galα1-3Galβ1-4Glc-ceramide, based on GlcNAc or Glc-ceramide recognition, respectively [31,32]. A similar mechanism is likely to explain how α-gal-specific Ab responses are possible in individuals expressing the Galα1-3Gal(Fuca1-2)β1-3GlcNAc B blood group antigen, suggesting again that anti-α-gal Abs can discriminate Galα1-3Galβ1-4GlcNAc or Galα1-3Galβ1-4Glc-ceramide from Galα1-3Gal(Fuca1-2)β1-3GlcNAc [12] (Box 1).

As argued above for other glycan-specific NAb responses, steady-state production of α-gal-specific Abs in humans is sustained most probably by continuous exposure to bacterial components of the microbiota that express α-gal [14,33]. These are likely to include members of the *Enterobacteriaceae* family of Gram-negative bacteria, such as *Klebsiella*, *Serratia*, and *Escherichia coli* spp. as well as Gram-positive bacteria, that is, *Lactobacillus casei* [14]. In *Enterobacteriaceae*, α-gal is conjugated to LPS at the outer surface of the cell wall, as illustrated for *Salmonella minnesota* and *Klebsiella* spp. [14]. In some cases, α-gal is conjugated to the O-antigen of LPS, as illustrated for *E. coli* O86:B7 [34].

E. coli O86:B7 is a **pathobiont** of the human microbiota that is likely to contribute to the production of circulating anti-α-gal Abs. *E. coli* O86:B7 expresses the Galα1-3Gal(Fuca1-2)β1-3(or4)GlcNAc glycan conjugated to the to the O-antigen of LPS [34] and feeding live or dead *E. coli* O86:B7 to humans triggers the production of circulating anti-α-gal Abs directed against the Galα1-3Gal(Fuca1-2)β1-3(or4)GlcNAc human B blood group [17]. Moreover, when colonized by *E. coli* O86:B7, GGTA1-deficient

(*Ggtal*^{-/-}) mice produce anti- α -gal IgM Abs, in the range of adult individuals from a malaria-endemic region [7]. Expression of Gal α 1-3Gal by *E. coli* O86:B7 is under the control of an α 1,3s(GT) encoded by the *wbnI* gene, which shares high homology with other genes expressed in related *Enterobacteriaceae* such as *E. coli* O55:H7 [34]. To what extent orthologs of the *wbnI* gene control the expression of α -gal glycan in other bacterial species that express α -gal conjugated to LPS, for example, *Bacteroides ovatus*, *Helicobacter mustelae*, *Shigella dysenteriae*, *Klebsiella pneumoniae*, *Campylobacter coli*, *Serratia marcescens*, or *Salmonella typhimurium* remains to be established [35].

Levels of circulating anti- α -gal IgM Abs in humans are low to undetectable during the first years of postnatal life [7,10], which is also the case for NAbs directed against other glycans [8], including ABO blood group glycans [36]. Levels of circulating anti- α -gal Abs increase over time to reach a steady state at 3–5 years of age [36,37]. Maternal transfer of anti- α -gal IgG Abs accounts for the relatively high levels of these Abs in the circulation of newborns, decreasing during the first few months of postnatal life and increasing thereafter to reach steady state levels within the first 3–5 years [7,36]. Anti- α -gal IgA NAbs are also detected in secretory fluids, such as saliva and colostrum, and as such can be vertically transferred by the mother to newborns [38].

One possible explanation for the relative low levels of circulating α -gal-specific IgM NAbs in newborns is that the early B cell repertoire lacks glycan-specific B cells, although they arise thereafter either spontaneously or in response to microbiota colonization [8]. Consistent with this notion, gut colonization by *E. coli* and *Bifidobacteria* promotes B cell maturation in newborns and infants [39]. Moreover, newborns harbor a simplified microbiota [40], which may be linked to the impact of newborn diet on microbiota composition [41]. In addition, some components of the microbiota of newborns exert a negative impact over potentially virulent *Enterobacteriaceae* that express α -gal, that is, colonization resistance [42]. This protective mechanism that prevents enteric infections during early postnatal life might also avoid exposure to immunogenic bacteria expressing α -gal during a developmental time frame, likely inducing a state of immunological tolerance [43].

146

147 **Expression of α -Gal by *Plasmodium* and Other Related Protozoan Parasites**

148 Malaria is transmitted to humans through inoculation of a relatively small number of
149 *Plasmodium* sporozoites, upon the bite of an infected female *Anopheles* mosquito [44].
150 Despite having a reportedly poor **glycosylation** profile [45], *Plasmodium* spp. express α -
151 gal glycans detected at the surface of sporozoites [7], as well during the blood stage of
152 infection [7,46–48]. In *Plasmodium* sporozoites, α -gal glycans are conjugated to
153 glycosylphosphatidylinositol (GPI)-anchored proteins [7,49], other than
154 circumsporozoite protein (CSP) [7], a GPI-anchored protein that covers the surface of
155 *Plasmodium* sporozoites [50]. Whether these α -gal glycans are conjugated directly to
156 proteins or lipids and their exact structure remains to be established.

157 An α L,3GT orthologous gene has been characterized in vector-borne protozoan parasites
158 from *Trypanosoma* spp. [51], which include *Trypanosoma brucei*, the causative agent of
159 African human trypanosomiasis, that is, sleeping sickness, and *Trypanosoma cruzi*, the
160 causative agent of American trypanosomiasis, that is, Chagas disease. Both diseases are
161 transmitted to humans via the inoculation of *Trypanosoma* metacyclic trypomastigotes in
162 the skin, either through the bite of a tsetse fly from the *Glossina* spp. or upon deposition
163 of infected feces by blood-sucking *Triatomine* bugs, respectively. *T. brucei* [52] and *T.*
164 *cruzi* [53,54] metacyclic trypomastigotes express α -gal glycans, conjugated to GPI-
165 anchored proteins as well to other glycoproteins and glycolipids. Presumably, this
166 explains why individuals infected by *T. cruzi* produce high levels of circulating anti- α -
167 gal Abs [54–56]. Whether this is also the case for *T. brucei* infection is not clear.

168 *Leishmania* is another genus of trypanosomatid protozoan parasites that expresses α -gal
169 [55,57], as illustrated for *Leishmania chagasi*, the causative agent of human visceral
170 leishmaniasis, and *Leishmania mexicana* or *Leishmania braziliensis*, the causative agent
171 of localized cutaneous and mucocutaneous leishmaniasis. Infection is transmitted to
172 humans via inoculation of promastigotes upon the bite of sand flies from the
173 *Phlebotomine* spp. [58]. Leishmaniasis is associated with the production of high levels of
174 circulating anti- α -gal Abs, as illustrated in humans [56] and *Ggtal*^{-/-} mice [59]. Abs

directed against α -gal bind to the lipid fraction and associated GPI anchors of *Leishmania* promastigotes [60,61] and opposite to the flagellar pocket of *Leishmania* amastigotes [55,62]. This suggests that the α -gal glycans expressed by *Leishmania* spp. are immunogenic and that α -gal-specific Abs might confer to leishmaniasis, which remains to be tested experimentally.

Targeting of *Plasmodium* and Possibly Other Vector-Borne Protozoan Parasites by α -Gal-Specific NAbs

Circulating anti- α -gal IgM and IgG Abs target *Plasmodium* sporozoites immediately after inoculation in the skin, conferring sterile protection against malaria transmission [7] (Figure 2). Consistent with their well-established cytolytic effect [63], anti- α -gal Abs kill *Plasmodium* sporozoites via an Fc-dependent mechanism involving the activation of the classical complement pathway [7] (Figure 2). Presumably, this explains why in malaria-endemic areas, individuals with higher levels of circulating anti- α -gal IgM Abs have a decreased risk of *Plasmodium falciparum* infection, as compared with infected individuals [7].

Considering that circulating α -gal-specific IgM Abs can be produced in response to α -gal expressing bacteria in the microbiota, gut colonization by those bacteria should confer protection against malaria transmission. In support of this hypothesis, *GgtaI*^{-/-} mice are protected from malaria transmission when (mono)colonized by the *E. coli* O86:B7 strain that expresses high levels of α -gal glycans [7] (Figure 2). This protective effect is mediated by the production of circulating α -gal-specific IgM Abs, as demonstrated by loss of protection in *E. coli* O86:B7 colonized *GgtaI*^{-/-} μ S^{-/-} mice, which lack circulating IgM [7]. Whether a similar protective mechanism occurs in humans when colonized by this or other bacterial strains expressing α -gal remains to be established. The recent observation that enteric colonization by *Enterobacteriaceae*, including *E. coli* and *Shigella*, is associated with reduced risk of *P. falciparum* infection in individuals from malaria-endemic areas supports this hypothesis [64]. Whether these *Enterobacteriaceae* include α -gal expressing bacteria that trigger the production of

circulating α -gal-specific IgM Abs [7,65] conferring protection against malaria transmission [7], remains to be established (Figure 2).

The protective effect exerted by α -gal-specific IgM Abs against malaria appears to be restricted to the initial prehepatic stage of infection, targeting *Plasmodium* sporozoites in the skin but not in the blood [7]. Moreover, neither α -gal-specific IgM nor IgG Abs appear to target the later, liver, or blood stages of *Plasmodium* infection in mice, and as such do not influence parasitemia or the pathogenesis of severe forms of malaria in mice, for example, experimental cerebral malaria [7]. The reasons for this are not clear but might relate to specific biologic aspects of IgM Abs, as discussed elsewhere [22].

Given the expression of α -gal by trypanosomatid protozoa parasites from *Trypanosoma* and *Leishmania* spp., it is reasonable to hypothesize that anti- α -gal NAbs might exert a similar protective effect against transmission of these vector-borne pathogens. While this has not been formally tested, there is experimental evidence to support this hypothesis. Anti- α -gal Abs can target *T. cruzi* for complement and cell-mediated cytotoxicity *in vitro*, thereby reducing parasite infectivity in mice [66,67]. Whether this is also the case for *T. brucei* was, to the best of our knowledge, not established. Even though α -gal-specific Abs can target *Leishmania* spp. promastigotes and amastigotes, it is not clear whether they confer protection against leishmaniasis. Possibly, these parasites evolved to escape the cytotoxic effect of circulating anti-glycan NAbs including α -gal-specific IgM NAbs [68,69]. Supporting this notion, *Trypanosoma* spp. can evade Ab cytotoxicity via different strategies, including antigenic variation of α -gal-conjugated GPI-anchored variant surface glycoproteins [51] or shedding of α -gal glycolipids, as demonstrated for *T. brucei* [69]. Moreover, rapid establishment of intracellular infection by *T. cruzi* or *Leishmania* might also contribute to escape α -gal-specific IgM cytotoxicity [70].

Evolutionary Constraints Imposed by α -Gal-Specific Abs on Vector-Borne Pathogens

Cytolytic targeting of *Plasmodium* and eventually other protozoan parasites by α -gal-specific IgM Abs would be expected to select loss-of-function mutations in putative

parasite $\alpha 1,3GT$ orthologous genes involved in α -gal expression, a host pathogen antagonistic coevolution process known as the ‘**Red Queen Hypothesis**’ [71]. There is indeed evidence that some families of glycosyltransferase genes were purged out of the *Plasmodium* genome [72]. However, this is less likely to occur when the glycosyltransferase is required to support the life cycle of these parasites in their arthropod vectors, which could be the case for $\alpha 1,3GT$. Yet another possibility is that expression of α -gal glycans is controlled by putative $\alpha 1,3GT$ orthologous genes from the parasites as well as their arthropod vectors.

Plasmodium spp. might not express O-glycans, that is, glycans covalently attached to proteins at serine/threonine (Ser/Thr) residues, but can express short N-glycans, that is, glycans covalently attached to proteins at asparagine (Asn), as illustrated on the surface of *P. falciparum* trophozoites and schizonts [73]. Moreover, *Plasmodium* spp. can synthesize uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) and guanosine diphosphate mannose (GDP-Man), the two α -gal precursors used by $\alpha 1,3GT$ [74]. It is possible therefore that N-linked and perhaps O-linked oligosaccharide synthesis occurs in *Plasmodium* spp. via an unconventional and yet noncharacterized pathway. However, a *Plasmodium* $\alpha 1,3GT$ orthologous gene has so far not been identified [49], which raises the possibility that α -gal detected at the surface of *Plasmodium* sporozoites is produced, at least in part, by the *Anopheles* mosquito vector. That arthropods express α -gal is supported by the finding that the bite of a star tick, *Amblyomma americanum*, can trigger the production of α -gal-specific IgE Abs, eventually associated with the development of red meat allergy [75]. However, there is no clear putative arthropod $\alpha 1,3GT$ orthologous gene, and as such the origin of the α -gal glycans detected in *Plasmodium* sporozoites remains to be established.

If the expression of α -gal at the surface of *Plasmodium* sporozoites is controlled, to at least some extent, by the *Anopheles* mosquito, how would these glycans transfer from the vector into the parasite? The observation that the α -gal is conjugated to GPI-anchored proteins expressed at the surface of *Plasmodium* sporozoites [7], *Trypanosoma* [53], and possibly *Leishmania* spp. [60,61,76] opens the possibility that arthropod GPI anchors may transfer from arthropod vectors to these parasites, via a process known as

intermembrane transfer of GPI-anchored proteins [77,78]. In support of this notion, *Trypanosoma* GPI-anchored variable surface glycoproteins can transfer to the membranes of mammalian red blood cells [79], and as such it is possible that arthropod GPI-anchored proteins carrying α -gal might also transfer into the membranes of protozoan parasites. This remains, however, to be tested experimentally.

Using α -Gal Glycans as Target Antigens in Vaccines against Vector-Borne Diseases

Vector-borne diseases account for 17% of all infectious diseases worldwide, corresponding to an estimated 1 billion infected individuals and 1 million associated deaths per yearⁱ. While the death toll of malaria decreased by 20–30% over the past decade, presumably attributable to the implementation of global malaria control programs, there are still 219 million infected individuals yearly, of which 660 000 succumb to severe diseaseⁱⁱ. The incidence of African human trypanosomiasis has also been steadily decreasing over the past decade, likely resulting from vector control strategiesⁱⁱⁱ. The situation is somehow different for American trypanosomiasis with an estimated 6–7 million individuals infected by *T. cruzi* and a fraction of those developing Chagas disease^{iv}. There is an estimated 1.3 million individuals infected by *Leishmania* spp. with an associated 20 000 deaths per year^v.

Eradication of vector-borne diseases will require the development of highly efficient vaccines conferring sterile protection against disease transmission. The finding that α -gal in bacterial components of the gut microbiota can trigger a systemic Ab response that confers sterile protection against malaria transmission argues that vaccination against α -gal might contribute to achieve this goal, at least in the context of malaria. Likely, the protective effect conferred by low-affinity α -gal-specific IgM NAbS produced against enteric bacteria should be enhanced via vaccination approaches eliciting high-affinity α -gal-specific IgG Ab responses [7]. Of note, newborns have only residual levels of circulating anti- α -gal IgM Abs and would benefit particularly from such an approach. Different strategies may be considered, namely glycan-based vaccines [80] or mucosal immunization by bacteria expressing glycans [81].

When conjugated to protein antigen, immunogenic glycans such as α -gal can trigger

high-affinity T cell-dependent IgG Ab responses directed against peptide and glycan epitopes [82]. Briefly, glycoconjugates containing α -gal are rapidly captured by circulating anti- α -gal Abs and shuttled, via an Fc receptor-mediated mechanism, to dendritic cells that present peptide epitopes to naïve CD4⁺ T helper (T_H) cells and cross-present to naïve CD8⁺ T cytotoxic (T_C) cells [25,83]. These glycoconjugates are also captured by peripheral α -gal-specific B cells, which account for 1% of the peripheral B cell repertoire and can present peptide epitopes to T_H cells [83]. Using such glycoconjugates in a vaccination strategy is particularly well suited when targeting *Plasmodium*, *Trypanosomes*, or *Leishmania*, which have an initial extracellular stage of infection in the skin that can be targeted by high-affinity glycan-specific IgG Abs. The intracellular stages of infection can be targeted by antigen-specific CD4⁺ T_H and CD8⁺ T_C cells, recognizing peptides derived from the glycoconjugate-based vaccine.

Oral vaccination by live bacteria expressing specific antigens is a widely used approach to obtain protective immunity against pathogens. In one approach, antigens are expressed in attenuated nonpathogenic bacterial strains such as *Salmonella typhi* [84] or *Vibrio cholerae* [85]. Alternatively, antigens can be expressed in live food grade bacteria such as *Lactococcus* [86], probiotic bacteria such as *Bifidobacteria*, or gut commensal *Bacteroidetes*, *Alphaproteobacteria*, *Actinobacteria*, *Firmicutes* or *Fusobacteria*. The finding that when expressed in *Lactococcus lactis*, antigenic peptides derived from *Plasmodium* proteins can elicit a protective Ab response against *Plasmodium* infection [86,87], suggests that this approach might be used in bacteria expressing α -gal to confer immune protection against malaria and possibly other vector-borne diseases.

Concluding Remarks

The finding that when expressed by bacterial components of the gut microbiota glycans, such as α -gal, can elicit a systemic Ab response that confers sterile protection against malaria transmission could have several implications, not only to our current understanding of host–microbial interactions but possibly to the eradication of malaria and likely other vector-borne diseases. The realization that mutualistic host–microbiota interactions can exert such protective effects suggests that these might be manipulated to reach therapeutic benefit (see Outstanding Questions). This may be achieved either by

diet manipulation in combination with gut colonization by natural or genetically engineered probiotic bacterial strains expressing α -gal. To translate these approaches into clinical practice, it would be important to compare the relative efficiency of anti- α -gal Abs, as compared with other Ab responses targeting *Plasmodium* sporozoites such as those directed against the CSP antigen. Glycoconjugate-based vaccines can be generated to combine the immunogenic effect of CSP and achieve robust and long-lasting sterile protection against malaria transmission. The uniqueness of the α -gal immunization approach is that it can be used, in combination or not with other antigens, to prevent the transmission of malaria as well other major vector-borne diseases.

Outstanding Questions

What are the immunogenic bacteria in the human gut microbiota that drive the production of α -gal-specific Abs?

What are the cellular and molecular mechanisms via which immunogenic bacteria in the gut microbiota trigger the production of α -gal-specific Abs?

What is the molecular mechanism driving the expression of α -gal glycan in *Plasmodium* spp.?

Do α -gal-specific Abs confer protection against vector-borne pathogens, other than *Plasmodium* spp.?

Should vaccination against α -gal glycans be considered in the development of malaria vaccines?

Should vaccination against α -gal glycans be considered in the development of vaccines against vector-borne pathogens, other than *Plasmodium* spp.?

Acknowledgments

The authors thank Jessica Thompson, Susana Ramos (Instituto Gulbenkian de Ciência, IGC), Silvia Portugal (National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA), and João Rodrigues (Instituto de Medicina Molecular,

Lisboa, Portugal) for reviewing the manuscript, as well as members of the Inflammation Group (IGC) for insightful discussions. Support from the Bill & Melinda Gates Foundation (OPP1024563), Fundação para a Ciência e Tecnologia (FCT), Portugal, RECI-IMI-IMU-0038-2012, and European Research Council ERC-2011-AdG 294709-DAMAGECONTROL to M.P.S., as well as FCT SFRH/BD/51176/ 2010 to B.Y. are acknowledged.

Disclaimer Statement

An article by Cabezas-Cruz *et al.* was published in this Journal, *Trends in Parasitology* (<http://doi.org/10.1016/j.pt.2015.06.016>) during submission of our manuscript. Despite the time overlap we had no access to this article and as such the hypothesis put forward in our manuscript should be considered as original and emanating from its authors.

Resources

ⁱwww.who.int/mediacentre/factsheets/fs387/en/

ⁱⁱwww.who.int/mediacentre/factsheets/fs094/en/

ⁱⁱⁱwww.who.int/mediacentre/factsheets/fs259/en/

^{iv}www.who.int/mediacentre/factsheets/fs340/en/

^vwww.who.int/mediacentre/factsheets/fs375/en/

References

1. Lozupone, C.A. *et al.* (2012) Diversity, stability and resilience of the human gut microbiota. *Nature* 489, 220–230
2. Marchiando, A.M. *et al.* (2010) Epithelial barriers in homeostasis and disease. *Annu. Rev. Pathol.* 5, 119–144
3. Rakoff-Nahoum, S. *et al.* (2004) Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 118, 229–241

- 380 4. Macpherson, A.J. *et al.* (2015) The bilateral responsiveness between intestinal
381 microbes and IgA. *Trends Immunol.* 36, 460–470
- 382 5. Hooper, L.V. and Macpherson, A.J. (2010) Immune adaptations that maintain
383 homeostasis with the intestinal microbiota. *Nat. Rev. Immunol.* 10, 159–169
- 384 6. Palm, N.W. *et al.* (2014) Immunoglobulin A coating identifies colitogenic
385 bacteria in inflammatory bowel disease. *Cell* 158, 1000–1010
- 386 7. Yilmaz, B. *et al.* (2014) Gut microbiota elicits a protective immune response
387 against malaria transmission. *Cell* 159, 1277–1289
- 388 8. Kearney, J.F. *et al.* (2015) Natural antibody repertoires: development and
389 functional role in inhibiting allergic airway disease. *Annu. Rev. Immunol.* 33,
390 475–504
- 391 9. Schneider, C. *et al.* (2015) The human IgG anti-carbohydrate repertoire exhibits a
392 universal architecture and contains specificity for microbial attachment sites. *Sci.*
393 *Transl. Med.* 7, 269ra261
- 394 10. Galili, U. *et al.* (1984) A unique natural human IgG antibody with anti-alpha-
395 galactosyl specificity. *J. Exp. Med.* 160, 1519–1531
- 396 11. Galili, U. *et al.* (1985) Human natural anti-alpha-galactosyl IgG. II. The specific
397 recognition of alpha (1-3)-linked galactose residues. *J. Exp. Med.* 162, 573–582
- 398 12. Galili, U. *et al.* (1987) The human natural anti-Gal IgG. III. The subtlety of
399 immune tolerance in man as demonstrated by crossreactivity between natural
400 anti-Gal and anti-B antibodies. *J. Exp. Med.* 165, 693–704
- 401 13. Avrameas, S. (1991) Natural autoantibodies: from ‘horror autotoxicus’ to ‘gnothi
402 seauton’. *Immunol. Today* 12, 154–159
- 403 14. Galili, U. *et al.* (1988) Interaction between human natural anti-alpha-galactosyl
404 immunoglobulin G and bacteria of the human flora. *Infect. Immun.* 56, 1730–
405 1737
- 406 15. Bovin, N.V. (2013) Natural antibodies to glycans. *Biochemistry (Moscow)* 78,
407 786–797
- 408 16. Landsteiner, K. (1900) *The Specificity of Serological Reactions*, Courier Dover
409 Publications

- 410 17. Springer, G.F. and Horton, R.E. (1969) Blood group isoantibody stimulation in
411 man by feeding blood group-active bacteria. *J. Clin. Invest.* 48, 1280–1291
- 412 18. Briles, D.E. *et al.* (1981) Antiphosphocholine antibodies found in normal mouse
413 serum are protective against intravenous infection with type 3 streptococcus
414 pneumoniae. *J. Exp. Med.* 153, 694–705
- 415 19. Ochsenbein, A.F. *et al.* (1999) Control of early viral and bacterial distribution and
416 disease by natural antibodies. *Science* 286, 2156–2159
- 417 20. Navin, T.R. *et al.* (1989) Effect of immunoglobulin M from normal human serum
418 on *Leishmania donovani* promastigote agglutination, complement-mediated
419 killing, and phagocytosis by human monocytes. *Infect. Immun.* 57, 1343–1346
- 420 21. Ochsenbein, A.F. and Zinkernagel, R.M. (2000) Natural antibodies and
421 complement link innate and acquired immunity. *Immunol. Today* 21, 624–630
- 422 22. Pleass, R.J. *et al.* (2015) Immunoglobulin M: restrainer of inflammation and
423 mediator of evasion by *Plasmodium falciparum* malaria. *Trends Parasitol.*
424 <http://dx.doi.org/10.1016/j.pt.2015.09.007>
- 425 23. Thurnheer, M.C. *et al.* (2003) B1 cells contribute to serum IgM, but not to
426 intestinal IgA, production in gnotobiotic Ig allotype chimeric mice. *J. Immunol.*
427 170, 4564–4571
- 428 24. Kawahara, T. *et al.* (2003) Peritoneal cavity B cells are precursors of splenic IgM
429 natural antibody-producing cells. *J. Immunol.* 171, 5406–5414
- 430 25. Benatuil, L. *et al.* (2008) Ig knock-in mice producing anti-carbohydrate
431 antibodies: breakthrough of B cells producing low affinity anti-self antibodies. *J.*
432 *Immunol.* 180, 3839–3848
- 433 26. Wang, L. *et al.* (1995) Human anti-Gal heavy chain genes. Preferential use of
434 VH3 and the presence of somatic mutations. *J. Immunol.* 155, 1276–1285
- 435 27. Oyelaran, O. *et al.* (2009) Profiling human serum antibodies with a carbohydrate
436 antigen microarray. *J. Proteome Res.* 8, 4301–4310
- 437 28. Bishop, J.R. and Gagneux, P. (2007) Evolution of carbohydrate antigens –
438 microbial forces shaping host glycomes? *Glycobiology* 17, 23R–34R
- 439 29. Thall, A.D. *et al.* (1996) alpha 1,3-Galactosyltransferase-deficient mice produce
440 naturally occurring cytotoxic anti-Gal antibodies. *Transplant Proc.* 28, 556–557

- 441 30. Diswall, M. *et al.* (2011) Antigen-binding specificity of anti-alphaGal reagents
442 determined by solid-phase glycolipid-binding assays. A complete lack of
443 alphaGal glycolipid reactivity in alpha1,3GalT-KO pig small intestine.
444 *Xenotransplantation* 18, 28–39
- 445 31. Milland, J. *et al.* (2007) Carbohydrate residues downstream of the terminal
446 Galalpha(1,3)Gal epitope modulate the specificity of xenoreactive antibodies.
447 *Immunol. Cell Biol.* 85, 623–632
- 448 32. Milland, J. *et al.* (2006) The molecular basis for galalpha(1,3)gal expression in
449 animals with a deletion of the alpha1,3galactosyltransferase gene. *J. Immunol.*
450 176, 2448–2454
- 451 33. Macher, B.A. and Galili, U. (2008) The Galalpha1,3Galbeta1,4GlcNAc-R (alpha-
452 Gal) epitope: a carbohydrate of unique evolution and clinical relevance. *Biochim.*
453 *Biophys. Acta* 1780, 75–88
- 454 34. Guo, H. *et al.* (2005) Molecular analysis of the O-antigen gene cluster of
455 *Escherichia coli* O86:B7 and characterization of the chain length determinant
456 gene (wzz). *Appl. Environ. Microbiol.* 71, 7995–8001
- 457 35. Hennet, T. (2002) The galactosyltransferase family. *Cell Mol. Life Sci.* 59, 1081–
458 1095
- 459 36. Doenz, U. *et al.* (2000) Anti-Galalpha1-3Gal IgM/IgG antibody levels in infants:
460 do they have a clinical relevance in pediatric xenotransplantation? *J. Heart Lung*
461 *Transplant* 19, 1108–1113
- 462 37. LaTemple, D.C. and Galili, U. (1998) Adult and neonatal anti-Gal response in
463 knock-out mice for alpha1,3galactosyltransferase. *Xenotransplantation* 5, 191–
464 196
- 465 38. Hamadeh, R.M. *et al.* (1995) Anti-alpha-galactosyl immunoglobulin A (IgA),
466 IgG, and IgM in human secretions. *Clin. Diagn. Lab. Immunol.* 2, 125–131
- 467 39. Lundell, A.C. *et al.* (2012) Infant B cell memory differentiation and early gut
468 bacterial colonization. *J. Immunol.* 188, 4315–4322
- 469 40. Yatsunenko, T. *et al.* (2012) Human gut microbiome viewed across age and
470 geography. *Nature* 486, 222–227

- 471 41. De Filippo, C. *et al.* (2010) Impact of diet in shaping gut microbiota revealed by a
472 comparative study in children from Europe and rural Africa. *Proc. Natl. Acad.*
473 *Sci. U.S.A.* 107, 14691–14696
- 474 42. Buffie, C.G. and Pamer, E.G. (2013) Microbiota-mediated colonization resistance
475 against intestinal pathogens. *Nat. Rev. Immunol.* 13, 790–801
- 476 43. Fan, X. *et al.* (2004) Donor-specific B-cell tolerance after ABO-incompatible
477 infant heart transplantation. *Nat. Med.* 10, 1227–1233
- 478 44. Cox, F.E. (2010) History of the discovery of the malaria parasites and their
479 vectors. *Parasites Vectors* 3, 5
- 480 45. Macedo, C.S. *et al.* (2010) Overlooked post-translational modifications of
481 proteins in *Plasmodium falciparum*: N- and O-glycosylation – a review. *Mem.*
482 *Inst. Oswaldo Cruz* 105, 949–956
- 483 46. Ramasamy, R. and Reese, R.T. (1985) A role for carbohydrate moieties in the
484 immune response to malaria. *J. Immunol.* 134, 1952–1955
- 485 47. Ramasamy, R. and Reese, R.T. (1986) Terminal galactose residues and the
486 antigenicity of *Plasmodium falciparum* glycoproteins. *Mol. Biochem. Parasitol.*
487 19, 91–101
- 488 48. Ramasamy, R. *et al.* (2001) Antibodies and *Plasmodium falciparum* merozoites.
489 *Trends Parasitol.* 17, 194–197
- 490 49. Ramasamy, R. and Field, M.C. (2012) Terminal galactosylation of
491 glycoconjugates in *Plasmodium falciparum* asexual blood stages and
492 *Trypanosoma brucei* bloodstream trypomastigotes. *Exp. Parasitol.* 130, 314–320
- 493 50. Nardin, E.H. *et al.* (1982) Circumsporozoite proteins of human malaria parasites
494 *Plasmodium falciparum* and *Plasmodium vivax*. *J. Exp. Med.* 156, 20–30
- 495 51. Pingel, S. *et al.* (1999) Purification and characterization of an alpha-
496 galactosyltransferase from *Trypanosoma brucei*. *Biochem. J.* 338, 545–551
- 497 52. Zamze, S.E. *et al.* (1991) Structural characterization of the asparagine-linked
498 oligosaccharides from *Trypanosoma brucei* type II and type III variant surface
499 glycoproteins. *J. Biol. Chem.* 266, 20244–20261
- 500 53. Almeida, I.C. *et al.* (1994) Lytic anti-alpha-galactosyl antibodies from patients
501 with chronic Chagas disease recognize novel O-linked oligosaccharides on

502 mucin-like glycosyl-phosphatidylinositol-anchored glycoproteins of
503 *Trypanosoma cruzi*. *Biochem. J.* 304, 793–802

504 54. Couto, A.S. *et al.* (1990) The N-linked carbohydrate chain of the 85-kilodalton
505 glycoprotein from *Trypanosoma cruzi* trypomastigotes contains sialyl, fucosyl
506 and galactosyl (alpha 1-3)galactose units. *Mol. Biochem. Parasitol.* 39, 101–107

507 55. Avila, J.L. *et al.* (1989) Immunogenic Gal alpha 1-3Gal carbohydrate epitopes
508 are present on pathogenic American *Trypanosoma* and *Leishmania*. *J. Immunol.*
509 142, 2828–2834

510 56. Towbin, H. *et al.* (1987) Circulating antibodies to mouse laminin in Chagas
511 disease, American cutaneous leishmaniasis, and normal individuals recognize
512 terminal galactosyl(alpha 1-3)-galactose epitopes. *J. Exp. Med.* 166, 419–432

513 57. Avila, J.L. and Rojas, M. (1990) A galactosyl(alpha 1-3)mannose epitope on
514 phospholipids of *Leishmania mexicana* and *L. braziliensis* is recognized by
515 trypanosomatid-infected human sera. *J. Clin. Microbiol.* 28, 1530–1537

516 58. Bates, P.A. (2007) Transmission of *Leishmania* metacyclic promastigotes by
517 phlebotomine sand flies. *Int. J. Parasitol.* 37, 1097–1106

518 59. Avila, J.L. (1999) alpha-Galactosyl-bearing epitopes as potent immunogens in
519 Chagas disease and leishmaniasis. *Subcell. Biochem.* 32, 173–213

520 60. McConville, M.J. *et al.* (1993) The glycoinositol phospholipids of *Leishmania*
521 *mexicana* promastigotes. Evidence for the presence of three distinct pathways of
522 glycolipid biosynthesis. *J. Biol. Chem.* 268, 15595–15604

523 61. McConville, M.J. *et al.* (1990) Structure of the lipophosphoglycan from
524 *Leishmania major*. *J. Biol. Chem.* 265, 19611–19623

525 62. Bretana, A. *et al.* (1992) American *Leishmania* spp. and *Trypanosoma cruzi*:
526 galactosyl alpha(1-3) galactose epitope localization by colloidal gold
527 immunocytochemistry and lectin cytochemistry. *Exp. Parasitol.* 74, 27–37

528 63. Ding, J.W. *et al.* (2008) Hyperacute rejection by anti-Gal IgG1, IgG2a, and
529 IgG2b is dependent on complement and Fc-gamma receptors. *J. Immunol.* 180,
530 261–268

531 64. Yooseph, S. *et al.* (2015) Stool microbiota composition is associated with the
532 prospective risk of *Plasmodium falciparum* infection. *BMC Genomics* 16, 631

- 533 65. Posekany, K.J. *et al.* (2002) Induction of cytolytic anti-Gal antibodies in alpha-
534 1,3-galactosyltransferase gene knockout mice by oral inoculation with
535 *Escherichia coli* O86:B7 bacteria. *Infect. Immun.* 70, 6215–6222
- 536 66. Abrahamsohn, I.A. and Silva, W.D. (1977) Antibody dependent cell-mediated
537 cytotoxicity against *Trypanosoma cruzi*. *Parasitology* 75, 317–323
- 538 67. Almeida, I.C. *et al.* (1991) Complement-mediated lysis of *Trypanosoma cruzi*
539 trypomastigotes by human anti-alpha-galactosyl antibodies. *J. Immunol.* 146,
540 2394–2400
- 541 68. Gazzinelli, R.T. *et al.* (1991) Direct lysis of *Trypanosoma cruzi*: a novel effector
542 mechanism of protection mediated by human anti-gal antibodies. *Parasite*
543 *Immunol.* 13, 345–356
- 544 69. Souto-Padron, T. *et al.* (1994) Distribution of alpha-galactosyl-containing
545 epitopes on *Trypanosoma cruzi* trypomastigote and amastigote forms from
546 infected Vero cells detected by Chagasic antibodies. *J. Eukaryot. Microbiol.* 41,
547 47–54
- 548 70. Pereira-Chiocola, V.L. *et al.* (2000) Mucin-like molecules form a negatively
549 charged coat that protects *Trypanosoma cruzi* trypomastigotes from killing by
550 human anti-alpha-galactosyl antibodies. *J. Cell Sci.* 113, 1299–1307
- 551 71. Van Valen, F. (1973) A new evolutionary law. *Evol. Theor.* 1, 1–30
- 552 72. Samuelson, J. *et al.* (2005) The diversity of dolichol-linked precursors to Asn-
553 linked glycans likely results from secondary loss of sets of glycosyltransferases.
554 *Proc. Natl. Acad. Sci. U.S.A.* 102, 1548–1553
- 555 73. Bushkin, G.G. *et al.* (2010) Suggestive evidence for Darwinian selection against
556 asparagine-linked glycans of *Plasmodium falciparum* and *Toxoplasma gondii*.
557 *Eukaryot. Cell* 9, 228–241
- 558 74. Ramasamy, R. (1987) Studies on glycoproteins in the human malaria parasite
559 *Plasmodium falciparum*—lectin binding properties and the possible carbohydrate-
560 protein linkage. *Immunol. Cell Biol.* 65, 147–152
- 561 75. Cabezas-Cruz, A. *et al.* (2015) Regulation of the immune response to alpha-Gal
562 and vector-borne disease. *Trends Parasitol.* 31, 470–476

- 563 76. McConville, M.J. and Bacic, A. (1989) A family of glycoinositol phospholipids
564 from *Leishmania major*. Isolation, characterization, and antigenicity. *J. Biol.*
565 *Chem.* 264, 757–766
- 566 77. Sloand, E.M. *et al.* (1998) Correction of the PNH defect by GPI-anchored protein
567 transfer. *Blood* 92, 4439–4445
- 568 78. Dunn, D.E. *et al.* (1996) A knock-out model of paroxysmal nocturnal
569 hemoglobinuria: Pig-a(–) hematopoiesis is reconstituted following intercellular
570 transfer of GPI-anchored proteins. *Proc. Natl. Acad. Sci. U.S.A.* 93, 7938–7943
- 571 79. Rifkin, M.R. and Landsberger, F.R. (1990) Trypanosome variant surface
572 glycoprotein transfer to target membranes: a model for the pathogenesis of
573 trypanosomiasis. *Proc. Natl. Acad. Sci. U.S.A.* 87, 801–805
- 574 80. Astronomo, R.D. and Burton, D.R. (2010) Carbohydrate vaccines: developing
575 sweet solutions to sticky situations? *Nat. Rev. Drug Discov.* 9, 308–324
- 576 81. Davitt, C.J. and Lavelle, E.C. (2015) Delivery strategies to enhance oral
577 vaccination against enteric infections. *Adv. Drug Deliv. Rev.* 91, 52–69
- 578 82. Cobb, B.A. *et al.* (2004) Polysaccharide processing and presentation by the
579 MHCII pathway. *Cell* 117, 677–687
- 580 83. Abdel-Motal, U.M. *et al.* (2009) Mechanism for increased immunogenicity of
581 vaccines that form in vivo immune complexes with the natural anti-Gal antibody.
582 *Vaccine* 27, 3072–3082
- 583 84. Hackett, J. (1993) Use of *Salmonella* for heterologous gene expression and
584 vaccine delivery systems. *Curr. Opin. Biotechnol.* 4, 611–615
- 585 85. Holmgren, J. and Czerkinsky, C. (1992) Cholera as a model for research on
586 mucosal immunity and development of oral vaccines. *Curr. Opin. Immunol.* 4,
587 387–391
- 588 86. Zhang, Z.H. *et al.* (2005) Oral vaccination of mice against rodent malaria with
589 recombinant *Lactococcus lactis* expressing MSP-1(19). *World J. Gastroenterol.*
590 11, 6975–6980
- 591 87. Eckburg, P.B. *et al.* (2005) Diversity of the human intestinal microbial flora.
592 *Science* 308, 1635–1638

- 593 88. Pradel, G. *et al.* (2002) Proteoglycans mediate malaria sporozoite targeting to the
594 liver. *Mol. Microbiol.* 45, 637–651
- 595 89. Frevert, U. *et al.* (1996) Cell surface glycosaminoglycans are not obligatory for
596 *Plasmodium berghei* sporozoite invasion in vitro. *Mol. Biochem. Parasitol.* 76,
597 257–266
- 598 90. Christiansen, D. *et al.* (2008) Humans lack iGb3 due to the absence of functional
599 iGb3-synthase: implications for NKT cell development and transplantation. *PLoS*
600 *Biol.* 6, e172
- 601 91. Galili, U. and Swanson, K. (1991) Gene sequences suggest inactivation of alpha-
602 1,3-galactosyltransferase in catarrhines after the divergence of apes from
603 monkeys. *Proc. Natl. Acad. Sci. U.S.A.* 88, 7401–7404
- 604 92. Hayakawa, T. *et al.* (2001) Alu-mediated inactivation of the human CMP-N-
605 acetylneuraminic acid hydroxylase gene. *Proc. Natl. Acad. Sci. U.S.A.* 98,
606 11399–11404
- 607 93. Tangvoranuntakul, P. *et al.* (2003) Human uptake and incorporation of an
608 immunogenic nonhuman dietary sialic acid. *Proc. Natl. Acad. Sci. U.S.A.* 100,
609 12045–12050
- 610 94. Varki, A. and Gagneux, P. (2009) Human-specific evolution of sialic acid targets:
611 explaining the malignant malaria mystery? *Proc. Natl. Acad. Sci. U.S.A.* 106,
612 14739–14740
- 613 95. Varki, A. *et al.* (2009) *Essentials of Glycobiology* (2nd edn), Cold Spring Harbor
614 Laboratory Press

615

616 [Box 1. Family 6 Glycosyltransferases](#)

617 Family 6 α 1,3GT are encoded by the *ABO*, *GGTA1*, and *iGB3* genes and catalyze the
618 formation of 1-3 glycosidic bonds between GalNAc or Gal and a Gal acceptor (EC
619 2.4.1.87). The human ABO α 1,3GT includes α 1,3GTA and α 1,3GTB, which generate
620 the A and B blood group glycan epitopes, that is, GalNAc α 1-3Gal(Fuc α 1-2) β 1-
621 3GlcNAc and Gal α 1-3Gal(Fuc α 1-2) β 1-3GlcNAc, respectively. The Fuc α 1-2 β 1-

3GlcNAc O blood glycan epitope is generated in the absence of α 1,3GTA and α 1,3GTB activity due to several loss-of-function mutations in these genes, maintained as balanced polymorphisms in human populations [88]. Functional deletions in genes encoding ABO α 1,3GT are not associated with overt **pathologic** outcomes and therefore the physiologic role of the ABO blood group system remains elusive. The glycoprotein α -galactosyltransferase 1 (GGTA1) α 1,3GT catalyzes the generation of Gal α 1-3Gal β 1-4GlcNAc-R glycan, bound essentially to proteins. The *GGTA1* gene is functional in nearly all mammals except in Old World monkeys, including humans, which carry a mutated *GGTA1* pseudogene and do not express the Gal α 1-3Gal β 1-4GlcNAc-R glycan [33]. The iGb3S α 1,3GT catalyzes the generation of Gal α 1-3Gal β 1-4Glc-ceramide glycan in a subset of isogloboside glycolipids [89]. In a similar manner to the *GGTA1* gene, humans carry a mutated *iGb3S* pseudogene [90] and do not express Gal α 1-3Gal β 1-4Glc-ceramide.

Box 2. Evolutionarily Based Mechanisms of Self Versus Non-Self Glycan Discrimination

Several examples suggest that selection and fixation of loss-of-function in genes encoding glycosyltransferases that generate self-glycans acted as a major driving force in shaping the human anti-glycan Ab repertoire. These include loss-of-function mutations in ancestral anthropoid primates that deleted the *GGTA1* gene, which encodes an α 1,3GT generating the Gal α 1-3Gal β 1-4GlcNAc-R glycan [91]. Loss-of-function mutations were also selected and fixed in the human *iGb3S* gene, which encodes an α 1,3GT that generates the Gal α 1-3Gal β 1-4Glc-ceramide glycan [90]. Deletion of these α 1,3GT eliminated the expression of α -gal self-glycans and allowed for the emergence of immune reactivity against α -gal glycans, illustrated by the high levels of circulating anti- α -gal Abs detected in healthy humans [10]. Other examples of this evolutionarily based process include loss-of-function of the human cytidine monophosphate-N-acetylneuraminic acid hydroxylase-like (*CMAH*) gene, which suppressed the expression of N-glycolylneuraminic acid (Neu5Gc) [92] and allowed for immune reactivity against

this glycan [93]. Loss-of-function mutations in these glycosyltransferases also altered the ability of some pathogens to bind to host glycans in a manner that supports infection, as proposed for the impact of Neu5Gc elimination in *Plasmodium* infection [94]. It is likely that other loss-of-function mutations in glycosyltransferase genes shaped the human anti-glycan Ab repertoire and/or altered host–pathogen interactions [9].

Glossary

Antibodies (Abs): the product of *Immunoglobulin (Ig)* genes that specifically recognize molecular structures known as epitopes, that is, antigenic determinant. These are part of a larger molecule that can trigger an Ab response (i.e., antigen). Abs are composed of a fragment antigen-binding (Fab) region that binds the epitope and a fragment crystallizable (Fc) region, which endows the Ab with effector function through the engagement of immune-based mechanisms. Different Fc regions define IgM, IgA, and IgG isotypes and subclasses of IgA (IgA1 and IgA2) or IgG (IgG1, IgG2, IgG3, and IgG4). The main function of Abs is to recognize and neutralize pathogens as well as to avoid microorganism transition into a pathobiont.

Germ-free: absence of germs, that is, microorganisms. Animals or other multicellular organisms can be maintained under experimental germ-free conditions to establish a causal relationship between the microbiota and a given aspect of host physiology. When colonized by a specific microorganism or group thereof, germ-free animals or other multicellular organisms are referred to as gnotobiotic. This experimental approach is often used to determine the causal relationship between a given microorganism or group of microorganisms and a given aspect of host physiology.

Glycosylation: is an evolutionary conserved biologic process defined as an ‘enzyme-catalyzed covalent attachment of a carbohydrate to a polypeptide, lipid, polynucleotide, carbohydrate, or other organic compound’ [95]. Glycosylation is catalyzed, in most cases, by glycosyltransferases that use specific sugar nucleotide donors as substrates to generate glycans, that is, ‘any sugar or assembly of sugars, in free form or attached to another molecule’ [95].

Interspecies interactions: include **mutualistic** interactions (i.e., commensalism) when organisms from both species benefit from their interaction, **commensal** interactions (i.e., commensalism) when one of the species benefits from the interaction without detriment to the other, and **pathologic** interaction (i.e., parasitism) where one species (i.e., the pathogen) benefits from the interaction in detriment of the other (i.e., the host). In some cases mutualistic or commensal interactions change such that they become pathologic. In that case, the organism species that benefits from this transition, in detriment of its host, is referred to as a **pathobiont**.

Microbiota: multicellular organisms establish structured interactions with dynamic communities of microorganisms, including viruses, bacteria, and fungi, collectively known as the microbiota. This interspecies relationship can range from mutualistic to commensal or pathogenic, depending on the inherent composition of the microbiota or the immune status of the host. Deregulation of host microbiota interactions impacts on host homeostasis and can have pathogenic effects.

Red Queen Hypothesis: living organisms are under a continuous selective pressure of exerted by parasitic relationships so that both host and parasites coevolve to gain advantage over each other.

699

700 **Figure 1. Evolutionarily Based Mechanisms of Self Versus Non-Self Glycan**
701 **Discrimination.** Humans carry loss-of-function mutations that were inherited from
702 ancestral anthropoid primates and hominids, impairing the expression of
703 galactosyltransferase (GT)-encoding genes, such as *GGTA1* and *iGb3S* [90]. These
704 eliminated the expression of Gal α 1-3Gal (α -gal) glycans as self-antigens and allowed for
705 the emergence of anti- α -gal antibody (Ab) responses [10], which confer protection
706 against *Plasmodium* infection [7]. Presumably, this protective effect favored natural
707 selection and fixation of such mutations in modern humans. Similarly, loss-of-function
708 of the human *CMAH* gene (encoding a cytidine monophosphate-N-acetylneuraminic acid
709 hydroxylase-like protein) allowed for immune reactivity against N-glycolylneuraminic
710 acid (Neu5Gc) [93] and likely altered the ability of ancestral forms of *Plasmodium* to
711 infect ancestral hominids. This is thought to have driven *Plasmodium* spp. to coevolve,
712 which presumably gives rise to the modern human pathogen *Plasmodium falciparum*
713 [94]. It is likely that other loss-of-function mutations in glycosyltransferase genes shaped
714 the human anti-glycan Ab repertoire and/or altered host–pathogen interactions [9].

715

716 **Figure 2. Microbiota Driven Protection against Malaria Transmission.** Gut colonization
717 by the *Enterobacteriaceae Escherichia coli* O86:B7, which recapitulates the etiology of
718 anti-Gal α 1-3Gal (anti- α -gal) IgM antibody (Ab) production in mice [65] and humans
719 [17], confers protection against malaria transmission in mice [7]. This effect is mediated
720 via the production of circulating anti- α -gal IgM Abs that target α -gal glycans expressed
721 at the surface of *Plasmodium* sporozoites (Spz) [7]. Anti- α -gal IgM Abs trigger the
722 activation of the classical pathway of complement (C1q, C5b9), which kills *Plasmodium*
723 sporozoites in the skin and hence confers sterile protection against malaria [7].
724 Abbreviations: BCR, B cell receptor; PRR, pattern recognition receptor; α 3, Gal α 1-
725 3Gal.

726

727