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Anti α 1-3Gal antibodies and Gal content in gut microbiota in immune disorders and multiple sclerosis

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Abstract

Recent observations suggest that Gal antigen content in gut microbiota and anti-Gal antibody response may influence inflammation in immune related disorders. In this review we summarized the current knowledge on antibody response to the Gal epitope in various immune disorders. We discuss the origin of Gal antigen associated to gut microbiota. In multiple sclerosis, the possible mechanisms by which the altered microbiota and/or circulating anti-Gal level could affect the immune response in this disease are presented.

I. Introduction

Inflammation is a complex mechanism involved in immune responses that could be deleterious when becoming chronic. To decrease inflammation state in various immune related disorders appears crucial. Until now, immunosuppressive drugs are efficient but side-effects remain important and can cause serious events to patients. More targeted therapies are sought. Multiple sclerosis is a chronic inflammatory disease of the central nervous system. Despite immunosuppressive drugs are efficient to reduce lesions associated with the remitting relapsing form of the demyelination disease and possible to reduce a subsequent evolution toward chronic and debilitating phases of the disease, strategies based on immunoregulation would therefore represent a major progress.

Recently alterations of the gut microbiome associated with MS have been described [1] and evidence that some microorganisms contributing to the specific microbiota profiles of MS patients can curve the outcome of the disease in animal models have brought hope that interfering with gut microbiota may open therapeutic opportunities in MS clinical management.

Recent observations of lower anti-Gal antibody levels in blood and CSF of MS patients have spurred the analysis of microbiota microorganisms able to synthesize $\alpha 1,3\text{Gal}$ the antigen lacking in human following a loss mutation of GGTA1 gene and suggested that the Gal positive species are less represented in MS. There is thus a possibility that either low anti-Gal levels directly affect immunity in MS or are only a marker of a specific microbiota profile lacking Gal antigen and able to interfere in the mechanisms of the disease.

We review here the current knowledge on a possible role of anti-Gal antibodies in MS and of alteration of microbiota components able to synthesize Gal in the gut of MS patients and healthy subjects. Origins of anti-Gal antibodies in Humans are discussed

as well as the recent progresses regarding the species synthesizing the Gal antigen in gut microbiota. We also review anti-Gal antibody responses assessed in various infections and immune related disorders exhibiting mostly increase of anti-Gal antibody (Ab) levels and particularly in multiple sclerosis where, in contrast, the anti-Gal antibody levels were found decreased and associated with a lower content of bacteria able to synthesize the Gal antigen in the gut microbiota.

II. Gal antigen and anti-Gal antibody response in mammals

The α -galactosyl carbohydrate epitope Gal α 1-3Gal β 1-4GlcNAc-R (Gal) is highly expressed on the glycoproteins and glycolipids of mammals except from Old World monkeys, Apes and Humans [2,3]. As a result, human develop in the first month of life anti-Gal antibodies able to bind to Gal epitopes [4]. This absence of Gal epitope in Old World monkeys, apes and Humans results from a mutation event in the glycoprotein galactosyltransferase alpha1,3 (GGTA1) gene, which led to the inactivation of the enzyme α 1-3-galactosyltransferase (α 1,3GT) corresponding to the glycotransferase family EC 2.4.1.87. This mutation is estimated to have occurred around 28 millions of years ago in Old World monkeys [5] and was spread among individuals through natural selection. Indeed, it is highly probable that an infectious agent carrying Gal epitope submitted Old World primates to a strong selective pressure and that α 1,3GT mutants were then able to recognize the infectious Gal epitope as part of the non-self and subsequently overcame the infection by generating anti-Gal antibodies [5-13]. Anti-Gal antibodies are also found in human secretions, at the interface with pathogens [14]. Distributions of anti-Gal antibodies and Gal epitope expression are summarized in **table 1**.

Mammals	α 1,3GT activity	Gal epitope	Anti-Gal antibodies
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Non-primate mammals	+	+	-
Prosimians (lemurs, lorisoids...)	+	+	-
New world monkeys (marmosets ...)	+	+	-
Old World monkeys (baboons, macaques...)	-	-	+
Apes (orangutans, gorillas, chimpanzees...)	-	-	+
Humans	-	-	+

Table 1: Distribution of Gal epitope, α 1,3GT activity and anti-Gal antibody responses in Mammals. Common ancestor of Old world monkeys, Humans and Apes lost α 1,3GT activity and Gal expression due to GGTA1 gene inactivation and developed anti-Gal antibodies.

α 1,3GT-KO mice are able to produce anti-Gal antibodies due to the lack of Gal antigen contrary to wild-type mice lacking anti-Gal antibodies [15-18]. Anti-gal antibodies are the most abundant natural antibodies in humans, apes and Old World monkeys constituting up to 1% of the circulating antibodies [19]. Although some anti-Gal IgE are observed and associated to red meat allergy after tick bites [20], these antibodies are mainly IgM and IgG class. By their abundance and beside their highly deleterious role in vascularized xenotransplantation, anti-Gal antibodies have raised several challenging working hypotheses regarding their possible involvement in a number of clinical entities and particularly in the innate defense against Gal positive microorganisms.

III. Gal antigen sources

Anti-Gal antibodies are produced in responses to various stimuli and represent an important part of the humoral immune repertoire in Human. Gal antigen can be provided to human body by several ways: infectious agents, food and microbiota.

Gal antigen from pathogens

Several pathogens can increase anti-Gal antibody secretion in Human (Table 2). Parasites as *Trypanosoma cruzi* or *Leishmania* [21-24], *Plasmodium falciparum* [7]

express Gal antigen. Following infestation, parasite load (*Trypanosoma cruzi*) is decreased in α 1,3GT-KO mice compared to wild-type mice [9,25].

Pathogenic bacteria are also providers of Gal antigen (Table 2): *Escherichia coli* [26-28], *Salmonella typhimurium* [26,29,30], *Klebsiella pneumoniae* [26], *Neisseria meningitides* [31], *Serratia marcescens* [12], *Streptococcus pneumoniae* [32]. *E. coli* O86:B7 expresses high levels of Gal antigen [33], which is not the case for the *E. coli* K12 strain [7]. Urinary tract infection by *Escherichia coli* [34] or enteric exposure to *E. coli* O86:B7 [8] were also found associated with an increase of anti-Gal antibody titers.

A pathogenic fungus: *Paracoccidioides brasiliensis* has also been described expressing Gal antigen on its fungal cell wall and in intracellular vacuoles [35]. α 1,3GT genes are absent in viral genomes. However, viruses exhibit Gal antigen when they propagated in membrane vesicles from mammal cells expressing α 1,3GT enzyme but are Gal negative when they infect human or Old World monkey cells [36]. Anti-Gal antibodies from human sera inactivate retroviruses produced from animal cells [10,11].

However, to which magnitude common pathogens can modulate the basal levels, affinity and the isotype profile of anti-Gal antibodies in adults is not precisely known.

Gal antigen from food and allergy

Food can also contain Gal antigens, particularly in red meat from cattle, sheep, goat and pig. Anti-Gal IgE responses against meat occur in some patients, inducing urticarial and anaphylaxis, but not asthma [37]. This allergic response to red meat is mostly restricted to tick bites inducing the production of IgE antibodies against Gal antigen [38]. *Ixodes ricinus* ticks indeed exhibit Gal antigen on their glycoproteins [39]. Allergy to cat also induces IgE anti-Gal recognizing IgA and IgM from cats [40]. However, these anti-Gal IgE allergic symptoms are rare events. Concerning IgG directed against Gal antigen, a vegetarian diet in healthy individuals seems to be associated with a

decrease of anti-Gal antibody levels [41], however, vegetarian diet modifies as well gut microbiota [42], the potent Gal antigen provider. Finally, the respective rank of the role of Gal positive diet and of Gal provided by the gut microbiota (not exclusive of cooperative effects) in inducing anti-Gal “natural antibodies”, particularly in the first months of life contemporary to the maturation of the microbiota and of the introduction of Gal positive diet, is not yet well known.

Gal antigen in gut microbiota

Anti-Gal antibody titers increase with age, are low in babies [43,44] and higher in elderly individuals [45,46], suggesting that colonization of intestine by microbiota provides the main Gal antigen immunization in Human. The formation of anti-Gal antibody repertoire is concomitant with gut flora colonization in $\alpha 1,3$ GT-KO mice [47]. Moreover, depletion of gram-gut bacteria by antibiotics in baboons resulted in an important decrease of circulating anti-Gal antibodies, even more potent than immunosuppressive treatment [48]. Anti-Gal antibodies are able to interact with bacteria from gut microbiota [26]. In mice, colonization of gut flora by *E. coli* results in anti-Gal IgM and IgG antibody production in response to Gal Immunization and confers protection against Malaria transmission [7]. So, Gal antigen from gut microbiota can lead to anti-Gal antibody increase and protect against infections from Gal-positive pathogens.

However, the abundance of bacteria expressing $\alpha 1,3$ GT enzymes were only poorly characterized in human gut microbiome. Recently, we characterized the distribution of the Gal producing bacteria in healthy individual microbiota [49] from whole sequencing microbiome sequences collected from the Human microbiome project [50], from LifeLines-DEEP [51] cohort and from a multi-generational Asian American cohort [52]. The phylogenic tree from 193 bacterial gene sequences of $\alpha 1,3$ GT compared to

mammal sequences showed an important distance with large sequence disparities in microbiota bacteria (Figure 1). All mammal sequences are clustered and differed from bacterial sequences. As an attempt to explore the possibility that Gal positive food could also provide a substantial fraction of gut Gal antigen, we also assessed the presence of sequences specific of α 1,3GT gene (GGTA1) from red meat of cattle, sheep and pigs in 110 healthy human gut whole metagenome from the Human microbiome project. There were no homologous sequences of GGTA1 gene from cattle, sheep and pig detected in our screening using the same method of sequence alignment used to detect bacterial α 1,3GT gene. Altogether, our data suggest that Gal antigen immunization from diet red meat may be minimal, likely due to a rapid degradation by digestion, in contrast to bacterial derived Gal antigen, continuously produced.

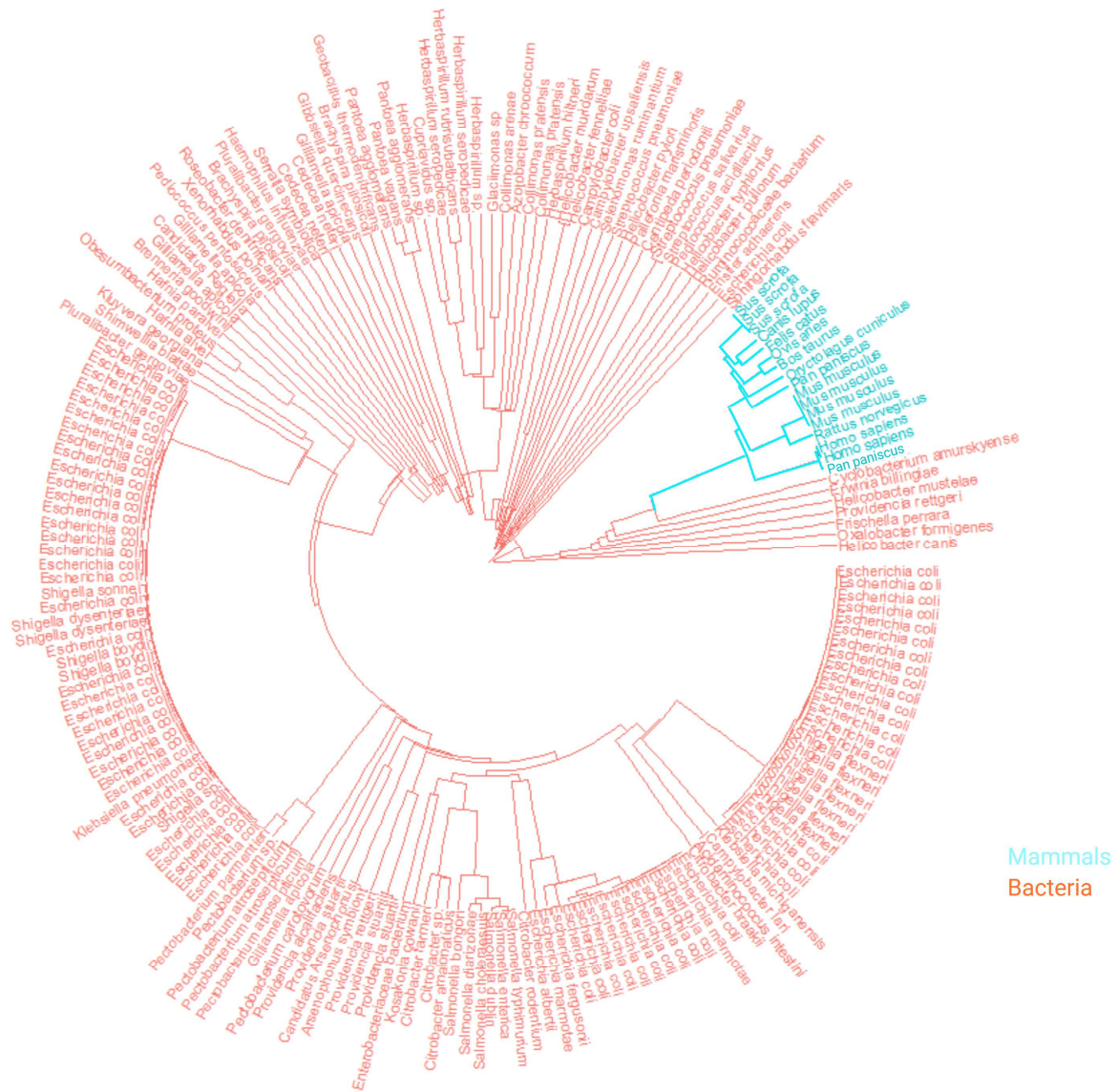


Figure 1: Phylogenetic tree of $\alpha 1,3GT$ genes. All GGTA1 gene sequences from mammals are clustered. The genetic distance between bacterial (red) and mammal (blue) $\alpha 1,3GT$ gene sequences is important.

Although the gene sequences are present in most of the gut microbiome of healthy individuals, we reported that the $\alpha 1,3GT$ gene positive fraction of the gut microorganisms represented only a small percentage (estimation 0.5% to 1%) of the total microbiota bacteria [49]. Enterobacterales (including *Escherichia coli*) was the predominant order of bacteria bearing $\alpha 1,3GT$ gene sequences. The family distribution of gut bacteria bearing the $\alpha 1,3GT$ enzyme needed to produce Gal epitope obtained in this study [49] is summarized in figure 2. Seventy-five to 95% of healthy individual

samples had at least one α 1,3GT identified sequence depending on the considered cohort. The Gal antigen itself, in addition to the α 1,3GT enzyme activity were detected in the stools from all of a small group of tested healthy donors (n=6). Moreover, in this group the Gal antigen amount in stools correlated to the number of bacterium species bearing α 1,3GT gene sequences [49]. Altogether, these data suggest that α 1,3GT gene presence in bacteria from gut microbiota result in Gal expression in a quantitative manner [49]. Importantly, the direct pondered assessment of the gut Gal antigen amounts will offer the opportunity to investigate the working hypothesis that the level of a given sugar antigen in the gut governs the level of production of antibodies against this antigen and the corresponding blood levels.

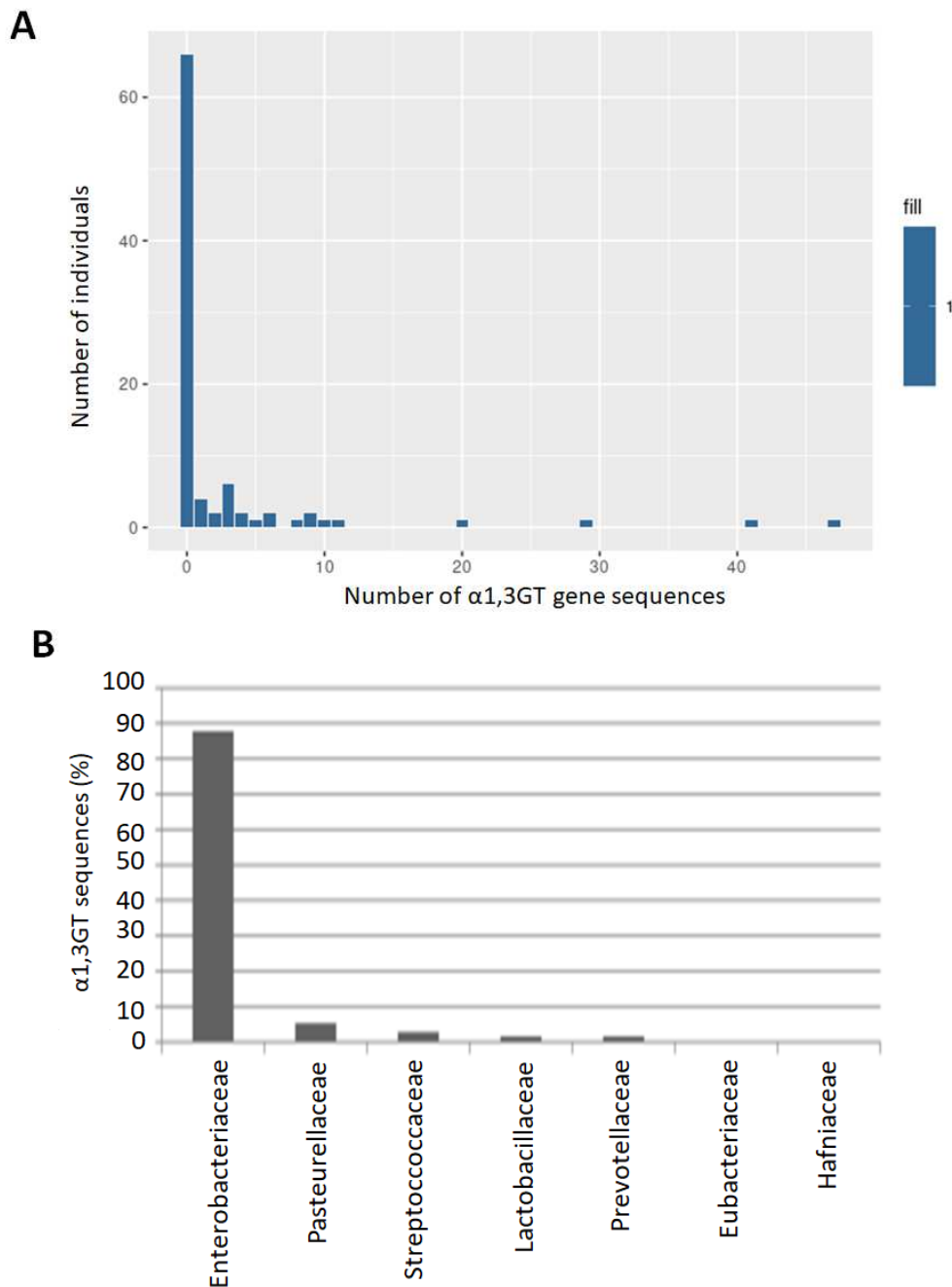


Figure 2: α1,3GT gene sequences and distribution in gut bacteria. A. Distribution of α1,3GT gene sequences in gut microbiota from healthy individuals **B.** Family distribution of Gal-bearing gut bacteria, from the whole metagenomic sequencing (results from stools of 110 healthy individuals of Human microbiome project are compiled). More than 80% of the total α1,3GT gene count are originated from the Enterobacteriaceae family (mainly of *E. coli*). Other families were represented below 10% (Pasteurellaceae, Streptococcaceae and Lactobacillaceae).

Some yeasts from gut microbiota also express Gal antigen via α1,3GT activity such as *Schizosaccharomyces pombe* [53]. However, the gut fungal and protozoal microbiomes are currently not enough characterized to assess α1,3GT gene presence.

IV. Anti-gal antibodies in immune related disorders

As expected, in B cell disorders inducing antibody defects, anti-Gal antibody production is impacted and titers of anti-Gal antibodies are decreased [54]. However, in auto-immune processes, anti-Gal antibody titers are differently modified. The group of Uri Galili has been the first to suggest that the couple Gal antigen/anti-Gal antibodies may be also involved in some autoimmune processes. In Graves' disease, an autoimmune disorder that causes hyperthyroidism, patients display elevated titers of anti-Gal antibodies [55]. Cultured Graves' disease thyrocytes bind anti-Gal and are stimulated by these antibodies to proliferate [56] whereas anti-gal antibodies do not bind to normal thyrocytes and do not affect them. These observations suggest that Gal-mimicking epitopes are aberrantly expressed on patient thyrocytes through an unknown mechanism or that a cross-reaction of anti-Gal antibodies occurs. Indeed, some studies described peptides mimetic to the Gal epitope [57,58] whereas others favor polyreactivity of anti-Gal antibodies against auto-antigens [59,60]. Increase in anti-Gal antibodies has also been reported in other inflammatory diseases such as Crohn's disease [61,62], rheumatoid arthritis [63-65], spondyloarthropathies [64], Sjogren's syndrome [63], glomerulonephritides such as IgA nephropathy and IgA vasculitis [34]. Anti-Gal antibodies were shown to bind to glomerular cells through laminin in mouse without inducing lesions [66]. Table 2 summarized the reported findings of abnormal levels of anti-Gal antibodies in human diseases including auto-immune diseases as well when available the source of Gal antigen.

It is however currently not clear whether anti-Gal antibodies contribute to the pathology of these diseases or are inflammation biomarkers resulting from pathological processes. Another possibility may be that the Gal antigen is provided by pathogens such as *Klebsiella pneumoniae* which has been shown to be able to add a galactose in α 1-3 position, generating Gal antigen [67]. Moreover, anti-Gal antibodies bind to

senescent [4] or pathological red blood cells (sickle cell anemia [68] and thalassemia [69]).

Several works have also shown a Janus like effect of anti-Gal antibodies in several infectious diseases with either a possible protective or a deleterious effect. Chagas disease is caused by the intra-cellular parasite *Trypanosoma cruzi* that continuously elicits Gal antigen production by releasing glycoinositolphospholipids and lipophosphoglycans carrying Gal epitopes [70-72]. In response, anti-Gal antibodies are highly produced, participating to the lysis of the parasite [23,73-76]. However, this phenomenon in Chagas patients could contribute to chronic inflammation [77-79]. In virus infection, anti-Gal antibodies were associated with local inflammation. Indeed, the increase of anti-Gal antibodies in blood [80] is also observed in cervical mucus which is associated with cervical lesions during human papillomavirus infection [81]. A direct binding of anti-Gal antibodies to infected cervical squamous cells was observed [82].

Disorders	Gal epitope	α1,3GT enzyme	Anti-Gal antibodies
Chagas' disease (<i>Trypanosoma Cruzi</i> infection)	Gal Ag from <i>T. Cruzi</i> [70]	From <i>T. cruzi</i>	↗ anti-Gal IgG titers [23,73-76]
Malaria (<i>Plasmodium falciparum</i> infection)	Gal Ag from <i>P. falciparum</i>	From <i>P. falciparum</i>	↗ anti-Gal Ab titers (IgM and IgG) [7,83]
Leishmaniasis (<i>Leishmania</i> infection)	Gal Ag from <i>Leishmania</i> [21,22]	From <i>Leishmania</i>	↗ anti-Gal Ab titers (IgA, IgG, IgM) [22,24]
<i>Escherichia coli</i> infection	Gal Ag from <i>E. coli</i>	From <i>E. coli</i> [49]	↗ anti-Gal Ab titers (IgM and IgG) [8,34]
<i>Mycoplasma pneumoniae</i> infection	NFA	NFA	↗ anti-Gal IgG titers [34]
Human papillomavirus (HVP) infection	NFA	NFA	↗ anti-Gal Ab titers in blood [80] and in cervical mucus associated with cervical lesions [81] Binding of anti-Gal Ab on HPV infected cervical squamous cells [82]
Hepatitis C virus (HCV) infection	NFA	NFA	No difference (IgG) [63]
Hepatitis B virus (HBV) infection	NFA	NFA	No difference (IgG) [63]
Epstein-Barr virus (EBV) infection	NFA	NFA	↗ anti-Gal Ab titers (IgM and IgG) [84,85]
Red meat allergy	Gal Ag from red meat and ticks	From mammal meat and ticks	↗ anti-Gal IgE titers [20]

Cat allergy	Gal Ag from salivary cat IgA [40]	From cat	↗ anti-Gal IgE titers [40]
Breast cancer	NFA	NFA	Binding of anti-Gal IgG to human breast cancer cells [57,86]
Chronic lymphocytic leukemia			↘ anti-Gal Ab titers in blood (IgG, IgA, IgM) [54]
Common variable immunodeficiency			↘ anti-Gal Ab titers in blood (IgG, IgA, IgM) [54]
Graves' disease	NFA	NFA	↗ anti-Gal Ab titers [55] Binding of anti-Gal Ab to thyroid cells from patients [56] ↘ anti-Gal Ab titers in hyperthyroid patients but ↗ anti-Gal Ab titers in patients with ophthalmopathy [87]
Crohn's disease	NFA	NFA	↗ anti-Gal Ab titers (IgG, IgA, IgM) [61,62]
Ulcerative colitis	NFA	NFA	↗ anti-Gal Ab titers [61], or no difference (IgG, IgA, IgM) [62]
Rheumatoid arthritis	NFA	NFA	↗ anti-Gal Ab titers (IgG and IgM) [63-65]
Spondyloarthropathy (ankylosing spondylitis and psoriatic arthritis)	NFA	NFA	↗ anti-Gal IgG titers [64]
Osteoarthritis	NFA	NFA	No difference [65]
Sjogren's syndrome	NFA	NFA	↗ anti-Gal IgG titers [63]
Systemic lupus erythematosus	NFA	NFA	No difference (IgG) [63]
IgA nephropathy	NFA	NFA	↗ anti-Gal Ab titers (IgG and IgA, not IgM) [34] Binding of anti-Gal Ab to glomerular cells [66]
IgA vasculitis (Henoch-Schonlein purpura)	NFA	NFA	↗ anti-Gal Ab titers (IgA and IgG, not IgM) [34]
Atherosclerosis	NFA	NFA	↘ anti-Gal Ab titers in blood [88]
Multiple sclerosis	NFA	↘ α 1,3GT gene in gut microbiota [89]	↘ anti-Gal IgG titers in blood [84] and CSF [90]

Table 2: Gal antigen (Ag), α 1,3GT enzyme, anti-Gal antibody (Ab) titers in human diseases and infections. Inflammatory diseases and infections are associated (or not) with increase or decrease of anti-Gal Ab titers. NFA= no information available

Anecdotally, during viral infections, anti-Gal antibody structure can be modified. Indeed, during hepatitis viral infection, cirrhosis and fibrosis were found associated to glycosylation modifications of specific anti-Gal IgG supposed to modify their biological effects [91].

V. Multiple sclerosis and anti-Gal antibodies

Multiple sclerosis (MS) is an inflammatory disease affecting the central nervous system (CNS) and leading to myelin sheath destruction and degeneration of nerve fibers. Infiltrations of immune cells are observed in CNS lesions. Genetic as well as environmental factors are involved in the disease. Among the latter, smoking, low vitamin D levels and Epstein-Barr virus (EBV) are recognized risk factors for MS [92]. Interestingly, during primo-infection of infectious mononucleosis (IMN), an acute disease highly associated with an increase in subsequent MS occurrence, IMN patients exhibit higher titers of both anti-Gal and anti-Neu5Gc (an exogenic sialic-acid antigen also generating “natural” antibodies) antibodies than normal individuals [84,93]. **IgG and IgM classes were both increased.** The mechanism at the origin of this phenomenon is likely related to the extremely high frequency of EBV infected B cells during IMN [94] able to activate the large pool of precursor B cells dedicated to anti-Gal or Neu5Gc antibody production. In agreement with this interpretation, acute CMV disease did not elicit a similar profile [95]. However, MS patients (at distance of any past IMN episode) have a decreased serum anti-Gal **IgG** levels [84] despite they still exhibited an increase in anti-EBV (EBNA1) **IgG** titers compared to healthy individuals, a contrast which may be related to a chronic EBV antigen stimulation whereas the polyclonal stimulation contemporary to the IMN has disappeared. **Interestingly, Boligan et al. reported a decrease of anti-Gal antibody titers in cerebrospinal fluid samples from MS patients compared to inflammatory neurological disease patients [90]. By contrast anti-Neu5Gc and anti-Neu5Ac IgG responses were increased in blood and CSF of MS patients [90].** Altogether this anti-Gal **IgG** level decrease in MS suggested to investigate if it could be related to a concomitant abnormal low load of Gal antigen in the gut microbiota of MS patients and to speculate on how either low anti-Gal antibody level or abnormal gut Gal levels could contribute as an environment factor involved in MS occurrence.

VI. Microbiota, Gal antigen and multiple sclerosis

Recent studies suggest that gut microbiome could be an environmental key factor contributing to MS, as some gut microbiota might provide susceptibility or protection toward the disease [96,97]. In experimental autoimmune encephalomyelitis, transfer of gut microbiota from MS patients to mice induces proinflammatory responses and exacerbation of the disease whereas microbiota from healthy volunteers were less inflammatory [98,99]. Depletion of gut microbiota by antibiotics or germ-free breeding leads to improvement of neuroinflammation in mouse models [100,101]. MS patients tends to have altered microbiomes compared to healthy patients, with increases, at genus level, in *Methanobrevibacter*, *Akkermansia*, *Pseudomonas*, *Mycoplana*, *Haemophilus*, *Blautia* and *Dorea* and decreases in *Butyricimonas*, *Parabacteroides*, *Adlercreutzia* and *Prevotella* [102,103]. As microbiota is modified in MS patients, we hypothesized that the decrease of anti-Gal antibodies in MS could be linked to changes in bacterium abundances. To test this hypothesis, we performed bioinformatic analysis on publicly available 16S rRNA data using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) in order to compare α 1,3GT gene prediction levels in MS and in healthy individuals [89]. Briefly, PICRUSt uses 16S rRNA data to predict microbial community functional profiles (e.g. predicted metagenome). Here, we focused on the abundance of the enzyme EC 2.4.1.87 that corresponds to genes coding α 1,3GT in bacteria. We found that MS patients exhibit lower relative content in α 1,3GT genes suggesting that the decrease in anti-Gal antibodies is secondary to a drop of the number of bacteria expressing Gal antigen (Figure 3).

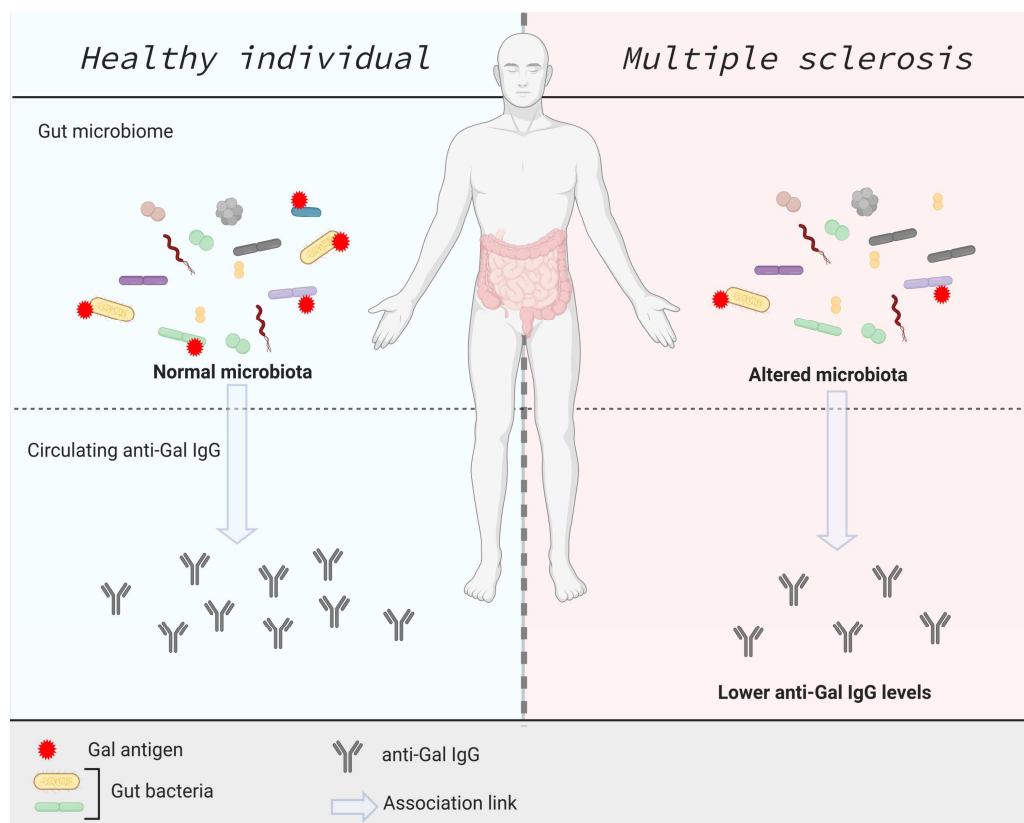


Figure 3: Gal antigen in gut microbiota and anti-Gal antibody responses in MS. Gal positive bacteria are found less abundant in gut microbiota from MS patients and anti-Gal antibody titers are decreased in blood and CSF.

The mechanisms of a possible link between low levels of anti-Gal antibodies in MS patient blood and LCR and their disease may both involved the effect of some microbiota profiles on the immune response as already suggested in MS [98,99], or a direct role of anti-Gal antibodies on the immunity of patients with other MS risk factors. We recently reported that antibody responses against *Akkermansia muciniphila* are increased in cerebrospinal fluid from MS patients [104]. Interestingly, the increased abundance of this bacterium in MS gut microbiota was associated with an increased antibody titer against it in central nervous system of MS patients. This bacterium is devoid of $\alpha 1,3$ GT gene sequence in its genome. Altogether these results suggest that decreased abundance of Gal positive bacteria and increased abundance of Gal negative bacteria in MS gut microbiota may contribute to the decrease of anti-Gal antibody levels in this disease.

Experiments aiming at exploring the precise relationship between the level of gut Gal content and anti-Gal antibody response are not yet available. Nevertheless, besides being a marker of specific MS microbiota profile, the possibility of anti-Gal antibodies to bind cross reactive epitopes in human cells, and particularly on cells involved in autoimmunity, cannot be totally disregarded. Anti-Gal antibodies considered as “natural antibodies” [44,105,106] may contribute to normal immune system homeostasis [107,108]. The lack of anti-Gal antibodies in MS could affect inflammation regulation as natural antibodies exhibit anti-inflammatory activities [107,108]. Moreover, anti-Gal antibodies can inhibit the binding of some cytokines to their receptors [88,109]. The decrease of anti-Gal antibodies has also been observed in atherosclerosis [88] or in a subgroup of patients with Grave’s disease [87]. However, in contrast, the majority of auto-immune and inflammatory diseases are associated with an increase of anti-Gal antibodies titers in blood (Table 2). Monoclonal anti-Gal antibodies as well as the IB4 lectin have been shown to also recognize peptide mimotopes [110,111] and anti-Gal antibodies bind blood B group glycan as well as senescent human red blood cells [68]. Anti-Gal antibodies (from human or GGTA1 KO mice) also cross react with MUC1, a protein found in large amounts in breast cancer [112]. GGTA1 KO pig tissues were not stained by anti-Gal antibodies [113] which did not bind to total glycolipid fractions isolated from different human organs. In addition, although human anti-Gal antibodies bind several cytokine receptors, including TGF- β receptor in mice [109], no cross reactivity has been evidenced for receptors lacking Gal antigen (and Neu5Gc). Anti-Gal antibodies have also been shown to activate thyrocyte TSH receptor in pigs [114]. This last observation prompted studies in human thyroid Graves’ disease where high circulating anti-Gal are also found and where a TSH-like effect has been observed in vitro on thyrocytes [56]. However, this observation was restricted to Graves patient thyroid cells, no binding occurs on cells of patients without a concomitant presence of

anti-thyocyte auto-antibodies or on thyrocytes from normal individuals. Thus role of stimulation of thyrocytes by anti-Gal antibodies in Graves' disease physiopathology cannot be ascertained and at the best could likely be the result of a combined effect of anti-Gal and of these autoantibodies.

Therefore, the working hypothesis that a decrease of anti-Gal antibodies could be a marker of an abnormal content in Gal positive bacteria in MS gut microbiota, is more likely than that of a direct effect of anti-Gal antibodies on yet elusive cross reactive epitopes endowed with functional properties. However, there is currently no report regarding the possibility that microbiota lacking Gal positive species could curve the natural history of the disease in a mice MS model, as reported for other MS microbiota profiles.

Conclusion

Gal antigen provided by gut microbiota induces anti-Gal antibody responses in Humans. Modifications in MS gut microbiota may lead to the decrease of gut Gal antigen contents, possibly responsible of the observed decrease of circulating anti-Gal antibody titers in this disease. Whether the resulting modification in anti-Gal antibody amount interferes in the pathology is possible **but yet neither identified nor suggested** by observations made in the context other auto-immune disorders. Rather these new observations suggest that the Gal positive subset of gut microorganisms in MS could display an anti-inflammatory effect lacking in this disease, opening opportunities for a more targeted therapeutic.

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