

Characterization of the anti- α -Gal antibody profile in association with Guillain-Barré syndrome, implications for tick-related allergic reactions

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ABSTRACT

Humans evolved by losing the capacity to synthesize the glycan Gal α 1-3Gal β 1-(3)4GlcNAc-R (α -Gal), which resulted in the capacity to develop a protective response mediated by anti- α -Gal IgM/IgG antibodies against pathogens containing this modification on membrane proteins. As an evolutionary trade-off, humans can develop the alpha-Gal syndrome (AGS), a recently diagnosed disease mainly associated with allergic reactions to mammalian meat consumption. The etiology of the AGS is the exposure to tick bites and the IgE antibody response against α -Gal-containing glycoproteins and glycolipids. The objective of this study was to characterize the anti- α -Gal antibody response in association with the immune-mediated peripheral neuropathy, Guillain-Barré syndrome (GBS), and compare it with different factors known to modulate the antibody response to α -Gal such as exposure to tick bites and development of allergic reactions in response to tick bites. The results showed a significant decrease in the IgM/IgG response to α -Gal in GBS patients when compared to healthy individuals. In contrast, the IgM/IgG levels to α -Gal did not change in patients with allergic reactions to tick bites. The IgE response was not affected in GBS patients, but as expected, the IgE levels significantly increased in individuals exposed to tick bites and patients with tick-associated allergies. These results suggest that the immune pathways of anti- α -Gal IgM/IgG and IgE production are independent. Further studies should consider the susceptibility to allergic reactions to tick bites in GBS patients.

1. Introduction

Inactivation of the α -1,3-galactosyltransferase (α 1,3 G T) gene in old world monkeys, apes and humans resulted in an almost unique ability of this group of primates to produce high antibody titres against Gal α 1-3Gal β 1-(3)4GlcNAc-R (α -Gal) (Galili, 2015). Previous results showed that up to 1 %–5 % of the circulating IgM/IgG found in healthy individuals are directed against α -Gal (Macher and Galili, 2008). Bacteria in the human gut microbiome produce α 1,3 G T genes (Montassier et al.,

2020) and experimental enrichment of gut milieu with *Escherichia coli* O86:B7 with high levels of α -Gal on its surface, elicited an anti- α -Gal IgM/IgG response that protected α 1,3 G T-deficient mice against malaria transmission (Yilmaz et al., 2014). These results strongly suggest that natural anti- α -Gal antibodies are produced in response to gut microbiota (Galili et al., 1988; Mañez et al., 2001; Bello-Gil et al., 2019). This evolutionary adaptation has been associated with the protective response of anti- α -Gal IgM/IgG antibodies against pathogens containing this modification on membrane proteins (Galili, 2018; Hodzić et al.,

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2020a). In contrast, the presence of α -Gal in tick salivary glycoproteins and glycolipids (Araujo et al., 2016; Chinuki et al., 2016; Cabezas-Cruz et al., 2018; Crispell et al., 2019) and possibly tick cement (Villar et al., 2020), induces anti- α -Gal IgE antibodies that mediate delayed anaphylaxis to mammalian meat consumption and immediate anaphylaxis to tick bites, xenotransplantation and certain drugs such as cetuximab (van Nunen et al., 2007; Commins et al., 2009; Steinke et al., 2015; Fischer et al., 2016; Mateos-Hernández et al., 2017; Cabezas-Cruz et al., 2019; Levin et al., 2019; Platts-Mills et al., 2020; de la Fuente et al., 2019a, 2020; Contreras et al., 2020).

Factors that may affect the antibody response to α -Gal include but are not limited to age, repeat consumption of certain food and meats of different origin or innards with higher α -Gal content, exposure to tick bites, alcohol consumption, exercise, use of non-steroidal anti-inflammatory medications, ABO blood group, co-occurring disorders and exposure to cats and other pets (Commins, 2016; Wölbing et al., 2013; Commins et al., 2014; Fischer et al., 2014; Morisset et al., 2012; Fischer et al., 2016; Cabezas-Cruz et al., 2017, 2019; Platts-Mills et al., 2020; de la Fuente et al., 2020). Additionally, the anti- α -Gal-specific IgE response has been associated with other diseases such as atopy, coronary artery disease and atherosclerosis (Gonzalez-Quintela et al., 2014; Wilson et al., 2017, 2019). However, little is known about the influence of immune-mediated peripheral neuropathies on the antibody response to α -Gal. In this regard, a particularly interesting question is whether immune-mediated disorders such as Guillain-Barré syndrome (GBS) that are associated with deregulation of the response to self-antigens also produce deregulation of the immune response to α -Gal. GBS is an autoimmune disease associated with recent bacterial or viral infection and with the involvement of both cellular and humoral immune responses (Van den Berg et al., 2014; Doncel-Pérez et al., 2016). The objective of this study was to evaluate the anti- α -Gal response in GBS patients. A cohort of patients from the same geographical origin and suffering tick-related allergies was used for comparative purposes. This study is of clinical relevance because factors affecting the levels of anti- α -Gal may influence the risk of patients to develop tick-induced allergies or their susceptibility to infectious diseases.

2. Materials and methods

2.1. Experimental design

The study focused on the characterization of the effect of GBS on the IgE, IgM and IgG antibody response to α -Gal. Tick bites and allergic reactions to tick bites in healthy individuals and cases of AGS, anaphylaxis and urticaria were included for comparative purposes (Table 1). A correlation analysis between the anti- α -Gal-specific IgE antibody response, which has been associated with the AGS, was conducted with respect to the different cases of GBS and allergy-type reactions to tick bites to provide new insights into the immune-mediated mechanisms associated with GBS.

2.2. Patients and healthy individuals

The use of human peripheral blood serum samples from patients and healthy individuals was done with their written informed consent in compliance with the Helsinki Declaration. Nursing personnel at the General University Hospital of Ciudad Real, Spain, extracted blood samples. Samples and data from patients with GBS included in this study were provided by the BioB-HVS, integrated into the Spanish National Biobanks Network. Both groups of samples were processed following standard operating procedures with the appropriate approval of the Ethical and Scientific Committees (Toledo Hospitable Complex 29012014-No17 and SESCAM C-73).

Table 1
Patients and healthy individuals.

Group	Description	Data of individuals*
Factor: GBS (Fig. 1)	Patients diagnosed with GBS and hospitalized	n = 22 51 $\pm$ 21 (12–83) years-old F/M = 0.6 Spain
	No record of tick bites and no allergic reaction	n = 37 40 $\pm$ 11 (21–60) years-old F/M = 1.5 Spain
Healthy (Figs. 1, 2A and B, A1B, A1C)	No allergic reactions to tick bites or no record of tick bites	n = 75 49 $\pm$ 9 (21–60) years-old F/M = 1.2 Spain
	Record of tick bites	n = 52 42 $\pm$ 14 (13–74) years-old F/M = 0.6 <i>A. americanum</i> <i>Ixodes scapularis</i> <i>Ixodes ricinus</i> <i>Rhipicephalus bursa</i> <i>Rhipicephalus sanguineus</i> <i>Hyalomma lusitanicum</i> <i>Hyalomma marginatum</i> <i>Dermacentor marginatus</i> <i>Rhipicephalus</i> spp. Unknown Spain, USA, Brazil, Portugal, France, Morocco
Factor: Tick bites (Fig. 2A)	No record of tick bites	n = 37 40 $\pm$ 11 (21–60) years-old F/M = 1.5 Spain
	Record of tick bites and allergic reaction	n = 12 45 $\pm$ 16 (26–74) years-old F/M = 0.7 <i>A. americanum</i> <i>Rhipicephalus bursa</i> , <i>Rhipicephalus sanguineus</i> <i>Rhipicephalus</i> spp. <i>Hyalomma marginatum</i> Unknown Spain, Brazil, France, USA, Morocco
Factor: Tick bites and allergy (Fig. 2B)	Record or not of tick bites and no allergic reaction	n = 78 40 $\pm$ 12 (13–60) years-old F/M = 0.9 <i>A. americanum</i> <i>Ixodes scapularis</i> <i>Ixodes ricinus</i> <i>Rhipicephalus bursa</i> <i>Hyalomma lusitanicum</i> <i>Dermacentor marginatus</i> <i>Rhipicephalus</i> spp. Unknown Spain, USA, Brazil, Portugal
	Record of tick bites and no allergic reaction	n = 41 40 $\pm$ 12 (13–57) years-old F/M = 0.6 <i>A. americanum</i> <i>Ixodes scapularis</i>

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Table 1 (continued)

Group	Description	Data of individuals*
		<i>Ixodes ricinus</i> <i>Rhipicephalus bursa</i> <i>Hyalomma lusitanicum</i> <i>Dermacentor marginatus</i> <i>Rhipicephalus</i> spp. Unknown Spain, USA, Brazil, Portugal n = 2 70 ± 6 (65–74) years-old F/M = 1.0 <i>Amblyomma americanum</i> USA n = 6 54 ± 16 (26–74) years-old F/M = 1.0
AGS (Fig. A1B and A1C)	Patients with diagnosed AGS with history of tick bites, delayed anaphylaxis to mammalian meat consumption and positive anti- α -Gal IgE response (> 6 kU/l)	
Anaphylaxis (Fig. A1B and A1C)	Patients with immediate anaphylaxis to tick bites	<i>A. americanum</i> <i>Rhipicephalus bursa</i> <i>Rhipicephalus</i> spp. <i>Hyalomma marginatum</i> Unknown USA, Spain, Morocco n = 6 35 ± 9 (27–52) years-old F/M = 0.5
Skin local reactions (urticaria) (Fig. A1A–A1C)	Individuals with reported skin local reactions to tick bite	<i>Rhipicephalus sanguineus</i> Unknown Spain, Brazil, France

* Data for each group includes number of individuals (n), age (years-old), female to male ratio (FM), tick species and countries where tick bites were recorded.

2.3. Preparation of serum samples

For the preparation of serum samples, a sterile tube without anti-coagulant was used to collect blood samples. The blood from each patient and the healthy individual was maintained in standing position at room temperature (RT) for clotting (20–30 min), and centrifuged at 1,500×g for 20 min at RT. Serum was collected and conserved at -20 °C until used for analysis.

2.4. Determination of antibody titers against α -Gal

For ELISA, high absorption capacity polystyrene microtiter plates were coated with 50 ng of BSA coated with α -Gal (BSA- α -Gal, thereafter named α -Gal; Dextra, Shinfield, UK) per well in carbonate-bicarbonate buffer (Sigma-Aldrich, St. Louis, MO, USA). After an overnight incubation at 4 °C, coated plates were washed one time with 100 μ L/well PBS with 0.05 % Tween 20 (PBST) (Sigma-Aldrich), blocked with 100 μ L/well of 1% human serum albumin (HAS) in PBST (Sigma-Aldrich) for 1 h at RT and then washed 4 times with 100 μ L/well of PBST. Human serum samples were diluted 1:50 in PBST with 1% HAS and 100 μ L/well were added into the wells of the antigen-coated plates and incubated for 1 h at 37 °C. Plates were washed four times with PBST and 100 μ L/well of goat anti-human immunoglobulins-peroxidase IgG (FC specific), IgM (μ -chain specific), and IgE (ϵ -chain specific) secondary antibodies (Sigma-Aldrich) diluted 1:1000, v/v in blocking solution were added and incubated for 1 h at RT. Plates were washed four times with 100 μ L/well of PBST and 100 μ L/well of 3,3',5,5'-tetramethylbenzidine TMB (Promega, Madison, WI, USA) were added and incubated for 20 min at RT. Finally, the reaction was stopped with 50 μ L/well of 2 N H₂SO₄ and the O.D. was measured in a spectrophotometer at 450 nm. The average of two technical replicates per sample was used for analysis after

background (coated wells incubated with PBS and secondary antibodies) subtraction. The anti- α -Gal IgE antibody titers were determined in sera from selected individuals (n = 2 from each AGS, anaphylaxis, skin local reaction and no allergic reactions group) using the ImmunoCAP Phadia 250 automated platform (Thermo Fisher Scientific, Uppsala, Sweden) with the commercial ImmunoCap α -Gal bovine Thyroglobulin kit according to the manufacturer's instructions (Mateos-Hernández et al., 2017). These values were used to draw a trendline ($R^2 = 0.83$) to calculate the corresponding kU/l values for the ELISA O.D. at 450 nm values using the formula IgE titers (kU/l) = EXP ((O.D.450 nm – 2.9)/0.2). Positive anti- α -Gal IgE levels were considered at cut-off value of 0.35 kU/l (Platts-Mills et al., 2020). The ELISA O.D. at 450 nm values were compared between different groups by pairwise comparisons using the nonparametric Mann-Whitney U test (p = 0.05, <https://www.socscistatistics.com/tests/mannwhitney/default2.aspx>). A Spearman Rho (rs) correlation analysis (p = 0.01; <https://www.socscistatistics.com/tests/spearman/default2.aspx>) was conducted between anti- α -Gal IgE antibody titers and allergy-type reactions.

3. Results and discussion

3.1. Differential regulation of the antibody response to α -Gal in individuals with GBS or tick allergies associated with α -Gal

A new factor that may affect the antibody response to α -Gal was considered in this study. In patients with GBS we found that the anti- α -Gal IgM and IgG antibody levels were significantly lower (p ≤ 0.01) than in healthy control individuals (Fig. 1). These results suggested that the reduction in anti- α -Gal IgM and IgG antibody levels may increase the susceptibility to infectious diseases or contribute to the neuropathy in GBS patients. Considering the recognized pathogen-mediated etiology of GBS (Townson et al., 2007; Van den Berg et al., 2014), it would be interesting to test whether pre-existing low levels of anti-gal antibodies predispose to infection by pathogens triggering autoimmunity associated with anti-GM1 antibodies and GBS. It has been proposed that the GBS may be associated with infection by bacterial or viral pathogens such as *Campylobacter jejuni*, *Haemophilus* bacteria, cytomegalovirus (CMV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) Zika virus (Rosinska et al., 2012; Van den Berg et al., 2014; Nugent et al., 2017; de la Fuente et al., 2019b), and recently SARS-CoV-2 (Zhao et al., 2020; Toscano et al., 2020). Although still controversial (Koga et al., 2005), *Haemophilus influenzae*, a bacterium expressing α 1,3 G T-coding genes (Bello-Gil et al., 2019) and recognized by galactose-specific agglutinins from *Ricinus communis* (Kalograiki et al., 2018), is considered a causative agent of GBS (Mori et al., 2000; Ju et al., 2004). Furthermore, as recently suggested for SARS-CoV-2, enveloped viruses carrying blood type B antigen or virus-like particles (VLPs) with α -Gal modifications can be targeted by anti- α -Gal IgM and IgG (Breiman et al., 2020; Galili, 2020; Chen, 2020; Urra et al., 2020; Bogani et al., 2020). Based on the proposed protective mechanisms in response to α -Gal in the zebrafish animal model (Pacheco et al., 2020), it may be also considered that pathogen infection reduces anti- α -Gal antibody levels through interference with B-cell maturation while anti-GM1 antibodies mediate demyelination and nerve conduction failure. Additionally, it may be possible that targeting of the macrophage FcR by anti-GM1 antibodies may decrease FcR-mediated phagocytosis and macrophage response to facilitate pathogen infection. Another mechanism by which pathogen infection may decrease the levels of anti- α -Gal IgM and IgG is by inducing a dysbiosis in which the abundance of α -Gal-producing bacteria is reduced. Removal of aerobic gram-negative bacteria is more effective than immunosuppression to decrease natural alpha-galactosyl IgG antibodies (Mañez et al., 2001). Microbiota composition influences infection by *C. jejuni*, the major pathogen associated with GBS, and also gut colonization by the pathogen is associated with dysbiosis in different animal models and humans (Indikova et al., 2015; O'Loughlin et al., 2015; Kampmann et al., 2016). If proven right, these results

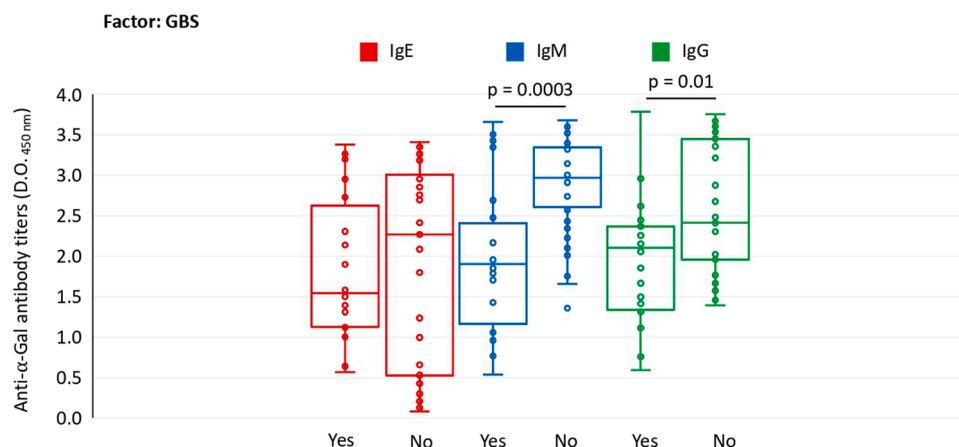


Fig. 1. Effect of GBS on the antibody response to α -Gal. Anti- α -Gal IgE, IgM and IgG antibody titers were determined by ELISA in patients with GBS and healthy individuals (Table 1). The ELISA O.D. at 450 nm values were compared between different groups by pairwise comparisons using the nonparametric Mann-Whitney *U* test ($p < 0.05$). Only significant differences ($p < 0.05$) are shown.

suggest the possibility of boosting the antibody response to α -Gal to prevent infections that may lead to GBS.

In contrast to GBS, the IgM and IgG antibody levels against α -Gal were not affected by tick bites or allergy factors (Fig. 2A and B). In contrast to humans, tick bites increase anti- α -Gal IgM levels but do not affect anti- α -Gal IgE levels in dogs, an animal that produces α -Gal as a self-antigen (Hodžić et al., 2019). These findings suggest that the production of anti- α -Gal IgE autoantibodies is tightly regulated, a hypothesis supported by growing evidence showing that IgE plays an important role in autoimmunity (Sanjuan et al., 2016).

The antibody response to α -Gal has been used to study the AGS but different factors may affect the anti- α -Gal IgE, IgM and IgG antibody levels (Commins, 2016; Wölbing et al., 2013; Commins et al., 2014; Fischer et al., 2014; Morisset et al., 2012; Fischer et al., 2016; Cabezas-Cruz et al., 2017, 2019; Platts-Mills et al., 2020; de la Fuente et al., 2020). To further expand the knowledge of the factors affecting the IgE response to α -Gal and potentially the AGS, we studied the IgE response in GBS. Patients exposed to factors unequivocally known to increase the IgE response (i.e. exposure to tick bites, development of allergic reactions in response to tick bites) were used as controls. As previously reported (recently reviewed by de la Fuente et al., 2020), the results showed a significant increase ($p < 0.05$) in anti- α -Gal IgE antibody levels in response to tick bites (1.3-fold) and allergic reactions to tick bites (1.4–1.5-fold) (Fig. 2A and B). These results support the use of the anti- α -Gal-specific IgE response for the diagnosis of the AGS in patients

with an appropriate clinical history (Platts-Mills et al., 2020). Symptomatic individuals included in the study showed different allergic reactions in response to tick bites (Table 1). Allergy-type reactions were rated from severe AGS and anaphylaxis to mild skin local reactions or urticaria to no reactions (Table 1). Patients with anaphylactic reactions to tick bite had different manifestations including generalized itching and erythema, difficult breathing, nausea and somnolence (Mateos-Hernández et al., 2017). Skin reactions to tick bite were manifested as urticaria (Case 1, Fig. A1A) or hives or disseminated rash (Case 2, Fig. A1A). The correlation analysis between anti- α -Gal IgE antibody levels and allergy-type reactions showed a significant positive correlation ($p < 0.0001$) with the severity of allergic manifestations (Fig. A1B). Using the quantitative analysis of anti- α -Gal IgE antibody titers at cut-off value of 0.35 kU/l (Platts-Mills et al., 2020), the results showed that 87.5 % (7/8) of the cases with AGS and anaphylaxis were positive while only 15 % (3/20) of the individuals with urticaria or asymptomatic showed antibody levels higher than 0.35 kU/l (Fig. A1C). In sharp contrast, GBS patients showed no significant changes in the IgE response (Fig. 1). These results are expected considering that switch to IgE is dependent on Th2 associated cytokines (e.g. interleukins IL-4, IL-13) (Aalberse et al., 2009) and that the anti- α -Gal IgE response has been associated to tick bites and parasite infection (Arkestål et al., 2011). In contrast, some parasites were found to inhibit the production of IgE antibodies to α -Gal in humans (Hodžić et al., 2020b). Several biomolecules in tick saliva such as prostaglandin E2 (PGE₂) were proposed to participate in

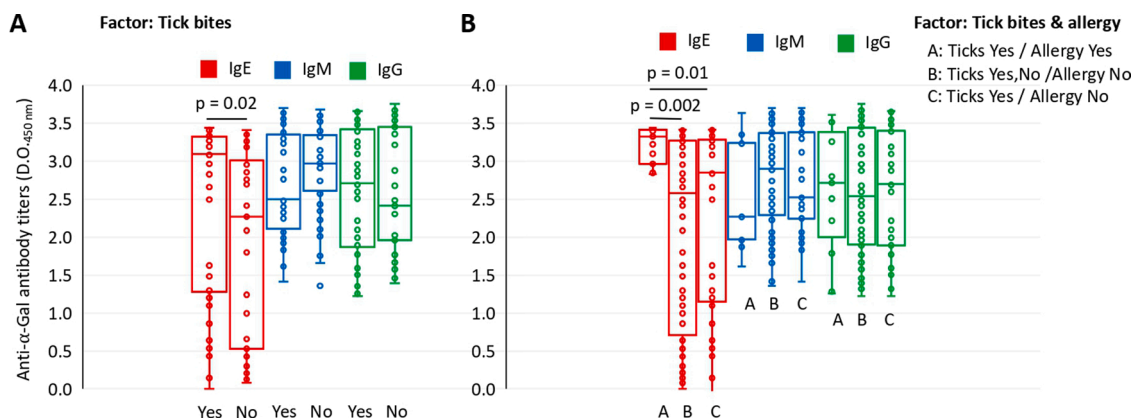


Fig. 2. Effect of different factors on the antibody response to α -Gal. The factors considered in this study included (A) exposure to tick bites (Tick bites) and (B) development of allergic reactions in response to tick bites (Tick bites & allergy) (Table 1). Anti- α -Gal IgE, IgM and IgG antibody titers were determined by ELISA. The ELISA O.D. at 450 nm values were compared between different groups by pairwise comparisons using the nonparametric Mann-Whitney *U* test ($p < 0.05$). Only significant differences ($p < 0.05$) are shown.

inhibition of the Th1 immune response and skew toward Th2 (Cabezas-Cruz et al., 2019).

4. Conclusions

The main conclusions of our study could be summarized as (1) the regulation of anti- α -Gal IgE is not directly linked to that of anti- α -Gal IgM/IgG response, (2) the decrease in the IgM/IgG antibody response to α -Gal observed in GBS patients may reflect gut microbiota dysbiosis associated to infection by pathogens that trigger the neuropathy disorder, and (3) GBS should not be considered as a factor increasing the levels of anti- α -Gal IgE and thus the risk for tick bite-related allergies.

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CRediT authorship contribution statement

Iván Pacheco: Investigation, Data curation. **Isabel G. Fernández de Mera:** Investigation, Methodology, Data curation. **Francisco Feo Brito:** Investigation, Methodology. **Elisa Gómez Torrijos:** Investigation. **Margarita Villar:** Investigation, Methodology, Project administration, Funding acquisition. **Marinela Contreras:** Investigation. **José Francisco Lima-Barbero:** Investigation. **Ernesto Doncel-Pérez:** Investigation, Data curation. **Alejandro Cabezas-Cruz:** Investigation, Methodology, Writing - review & editing. **Christian Gortázar:** Investigation, Writing - review & editing. **José de la Fuente:** Conceptualization, Methodology, Investigation, Data curation, Supervision, Writing - review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

No competing interests declared.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ttbdis.2021.101651>.

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Appendices

Figure A1. Allergic reactions in response to tick bites. (A) Representative cases of patients with skin urticarial type reactions after tick bite. Case 1, local urticaria after *R. sanguineus* bite. Case 2, hives or disseminated rash after tick bite of unknown species. (B, C) Correlation analysis between different allergy-type reactions to tick bites and anti- α -Gal IgE antibody response (Table 1). A Spearman Rho (r_s) correlation analysis ($p < 0.01$) was conducted between allergy-type reactions and (B) anti- α -Gal IgE antibody titers determined by ELISA (O.D. at 450 nm) and (C) converted to kU/l. Positive anti- α -Gal IgE levels were considered at cut-off value of 0.35 kU/l (red dashed line).

A*Rhipicephalus sanguineus* male**Case 1**

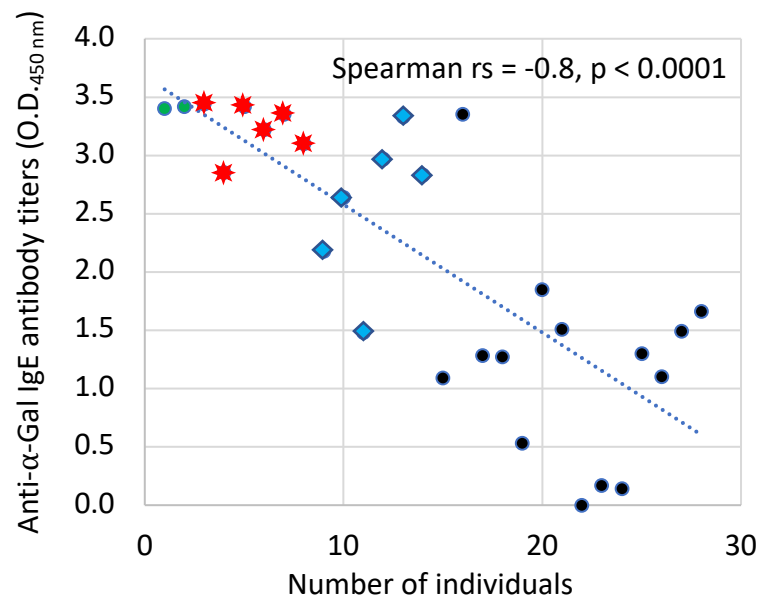
Urticaria at 2 hours post bite



Urticaria at 3 days post bite

**Case 2**

Urticaria at 3 hours post bite

**B****C**