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DATA NOTE

REVISED A dataset for the analysis of antibody response to glycan alpha-Gal in individuals with immune-mediated disorders [version 2; peer review: 2 approved, 1 not approved]

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Abstract

Humans evolved by losing the capacity to synthesize the glycan Galα1-3Galβ1-(3)4GlcNAc-R (α-Gal), which resulted in the development of a protective response mediated by anti-α-Gal IgM/IgG/IgA 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 mediated by anti-α-Gal IgE antibodies and associated with allergic reactions to mammalian meat consumption and tick bites. However, the anti-α-Gal antibody response may be associated with other immune-mediated disorders such as those occurring in patients with COVID-19 and Guillain-Barré syndrome (GBS). Here, we provide a dataset (209 entries) on the IgE/IgM/IgG/IgA anti-α-Gal antibody response in healthy individuals and patients diagnosed with AGS, tick-borne

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	Invited Reviewers		
	1	**2**	**3**
version 2 (revision) 25 May 2021	✓ report	✗ report	✓ report
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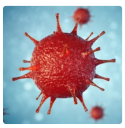
allergies, GBS and COVID-19. The data allows correlative analyses of the anti-α-Gal antibody response with factors such as patient and clinical characteristics, record of tick bites, blood group, age and sex. These analyses could provide insights into the role of anti-α-Gal antibody response in disease symptomatology and possible protective mechanisms.

Keywords
alpha Gal, immune response, antibody, allergy, tick, coronavirus, COVID-19, Guillain-Barré syndrome, alpha-Gal syndrome



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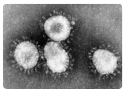

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Any reports and responses or comments on the article can be found at the end of the article.

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REVISED **Amendments from Version 1**

The paper was revised in response to reviewer comments by (a) adding information on BSA coated with α-Gal, (b) updating references, (c) adding new information to dataset validation, and (d) including the statement "For the validation of the ELISA with 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 please refer to Pacheco *et al.* (2021)"

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Introduction

The gene coding for α-1,3-galactosyltransferase (*α1,3GT*) was inactivated in old-world monkeys, an evolutionary adaptation that resulted in the production of high antibody titers against glycan Galα1-3Galβ1-(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 & Galili, 2008). Bacteria in the human gut microbiome express *α1,3GT* genes to produce α-Gal epitopes (Montassier *et al.*, 2020), suggesting that natural anti-α-Gal antibodies are produced in response to gut microbiota (Bello-Gil *et al.*, 2019; Galili *et al.*, 1988; Mañez *et al.*, 2001; Yilmaz *et al.*, 2014). 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, 2019; Hodžić *et al.*, 2020a). In contrast, the presence of α-Gal in tick salivary glycoproteins and glycolipids (Araujo *et al.*, 2016; Cabezas-Cruz *et al.*, 2018; Chinuki *et al.*, 2016; Crispell *et al.*, 2019) and 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 (Cabezas-Cruz *et al.*, 2019; Commins *et al.*, 2009; Contreras *et al.*, 2020; de la Fuente *et al.*, 2019a; de la Fuente *et al.*, 2020; Fischer *et al.*, 2016; Levin *et al.*, 2019; Mateos-Hernández *et al.*, 2017; Platts-Mills *et al.*, 2020; Steinke *et al.*, 2015; van Nunen *et al.*, 2007).

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, ABO blood group, co-occurring disorders and exposure to cats and other pets (Cabezas-Cruz *et al.*, 2017; Cabezas-Cruz *et al.*, 2019; Commins, 2016; Commins *et al.*, 2014; de la Fuente *et al.*, 2020a; Fischer *et al.*, 2014; Fischer *et al.*, 2016; Morisset *et al.*, 2012; Platts-Mills *et al.*, 2020; Wölbing *et al.*, 2013). 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; Wilson *et al.*, 2019). Furthermore, α-Gal-mediated innate and adaptive immune response mechanisms have been associated with protection against pathogen infection in various animal models (Hodžić *et al.*, 2020a). However, little is known about the influence of anti-α-Gal immune response on immune-mediated disorders such as those occurring in patients with COVID-19 and Guillain-Barré syndrome (GBS).

These results raise questions and hypothesis regarding the role of α-Gal-mediated immune responses in disease symptomatology and possible protective mechanisms (de la Fuente *et al.*, 2019b; de la Fuente *et al.*, 2020b; Pacheco *et al.*, 2021; Urra *et al.*, 2021). Consequently, to advance in addressing these questions and hypothesis, here we provide data on the IgE/IgM/IgG/IgA anti-α-Gal antibody response in healthy individuals and patients diagnosed with AGS, tick-borne allergies, GBS and COVID-19. These data contribute to correlative analyses of the anti-α-Gal antibody response with factors such as patient and clinical characteristics, record of tick bites, blood group, age and sex. These analyses could provide insights into the role of anti-α-Gal antibody response in disease symptomatology and protection against immune-mediated disorders.

Materials and methods

Essential methods used for the generation of the dataset (de la Fuente *et al.*, 2020) were described in Urra *et al.* (2021) with additional information in Pacheco *et al.* (2021) and Doncel-Pérez *et al.* (2020).

Patients and healthy individuals

A retrospective case-control study was conducted in patients suffering from COVID-19 admitted to the University General Hospital of Ciudad Real (HGUCR), Spain from March 1 to April 15, 2020. The infection by SARS-CoV-2 was confirmed in all patients included in the study by the real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay from Abbott Laboratories (Abbott RealTime SARS-COV-2 assay, Abbott Park, Illinois, USA) from upper respiratory tract samples after hospital admission. Clinical features, as well as laboratory determinations were obtained from patient's medical records. The patients were grouped as hospital discharge, hospitalized and intensive care unit (Urra *et al.*, 2021). Patients were hospitalized for developing a moderate-severe clinical condition with radiologically demonstrated pneumonia and failure in blood oxygen saturation. Patients with acute respiratory failure who needed mechanical ventilation support were admitted to a hospital ICU. The patients were discharged from the hospital due to the clinical and radiological improvement of pneumonia caused by the SARS-CoV-2, along with the normalization of analytical parameters indicative of inflammation, such as C-reactive protein (CRP), D-Dimer and blood cell count (Urra *et al.*, 2021). Samples from asymptomatic COVID-19 cases with positive anti-SARS-CoV-2 IgG antibody titers but negative by RT-PCR were collected in May 22–29, 2020 and included in the dataset (Urra *et al.*, 2021). Samples from healthy individuals (individuals without record of tick bites and allergic reactions) and patients diagnosed with tick-borne allergic reactions (AGS, anaphylaxis or urticaria) were collected prior to COVID-19 pandemic in April 2019 (Pacheco *et al.*, 2021). The use of human peripheral blood serum samples from healthy individuals and patients diagnosed with tick-borne allergic reactions 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 dataset were provided by the BioB-HVS, integrated into the Spanish National Biobanks Network. All samples were processed following standard operating procedures with the

appropriate approval of the Ethical and Scientific Committees (Toledo Hospitable Complex 29012014-No17, University Hospital of Ciudad Real C-352 and SESCAM C-73).

Preparation of serum samples

For the preparation of serum samples, a sterile tube without anticoagulant 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.

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 (Galα1-3Gal-BSA, 3 atom spacer, product code NGP0203, 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 µl/well PBS with 0.05% Tween 20 (PBST) (Sigma-Aldrich), blocked with 100 µl/well of 1% human serum albumin (HAS) in PBST (Sigma-Aldrich) for 1 h at RT and then washed four times with 100 µl/well of PBST. Human serum samples were diluted 1:100 in PBST with 1% HAS and 100 µl/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 µl/well of goat anti-human immunoglobulins-peroxidase IgG (FC specific) (Cat. No. I2136), IgM (µ-chain specific) (Cat. No. I1636), and IgE (ε-chain specific) (Cat. No. I6284) 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 µl/well of PBST and 100 µl/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 µl/well of 2 N H_2SO_4 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.

Statistical analysis

Anti-α-Gal IgE, IgM and IgG antibody titers (O.D. at 450 nm values) were compared for each Ig by one-way ANOVA test ($p < 0.05$) (https://www.socscistatistics.com/tests/anova/default2.aspx) (Figure 1A and 1C). A Spearman Rho correlation analysis

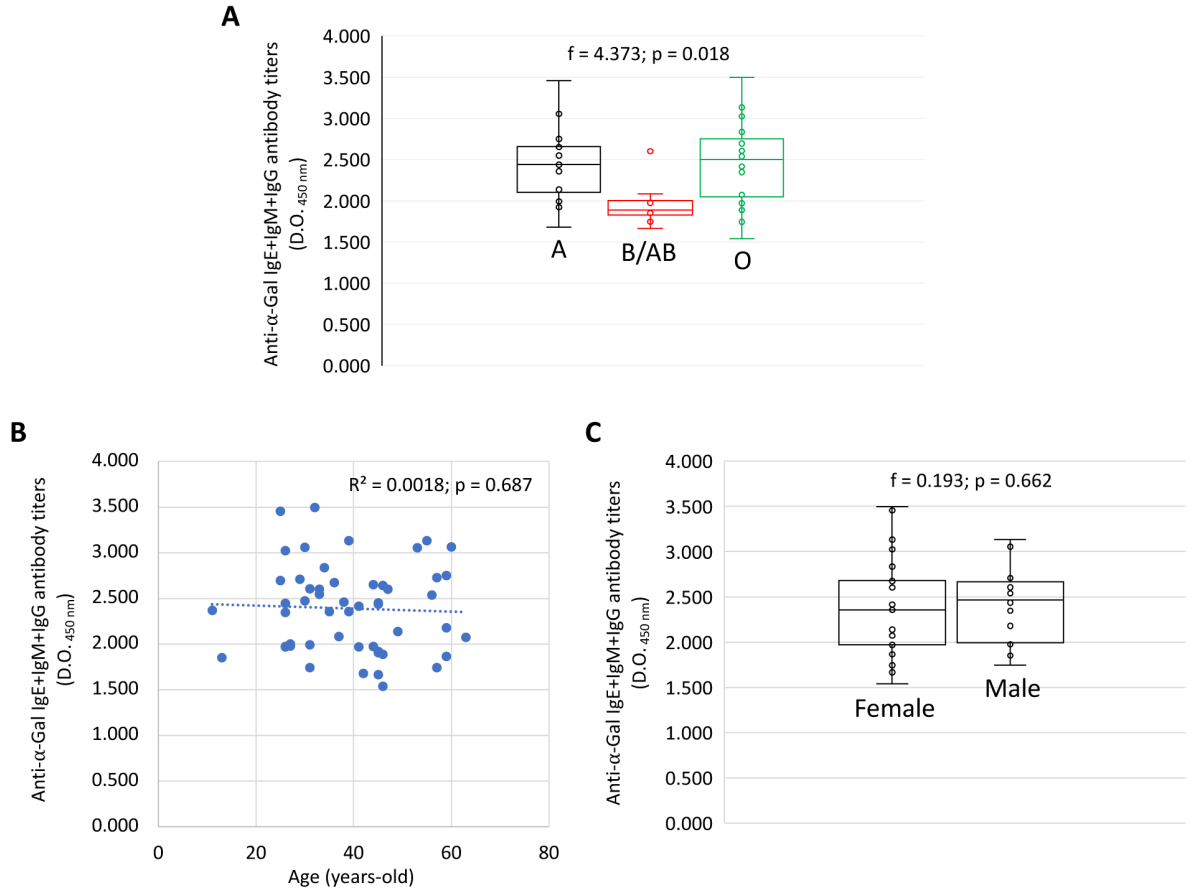

Figure 1. An example of the effect of certain factors such as (**A**) blood group, (**B**) age and (**C**) sex on the antibody response to α-Gal in healthy individuals. Anti-α-Gal IgE, IgM and IgG antibody titers were determined by ELISA. (**A, C**) The ELISA O.D. at 450 nm values were compared for each Ig by one-way ANOVA test ($p < 0.05$). (**B**) A Spearman Rho correlation analysis ($p < 0.01$) was conducted between anti-α-Gal IgE, IgM and IgG antibody titers and age. Correlation coefficient (R^2) is shown. Please refer to Pacheco *et al.* (2021) for validation of the ELISA with 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.

($p < 0.01$; https://www.socscistatistics.com/tests/spearman/default2.aspx) was conducted between anti-α-Gal IgE, IgM and IgG antibody titers and age (Figure 1B).

Dataset validation

The dataset (de la Fuente *et al.*, 2020) was validated in studies reported by Urra *et al.* (2021), Pacheco *et al.* (2020) and Doncel-Pérez *et al.* (2020). A recent study correlated blood group with anti-α-Gal antibody response and SARS-CoV-2 infection (Hodžić *et al.*, 2020b). Despite the presence of relatively high anti-α-Gal IgE levels in healthy individuals, factors such as tick bites or allergy correlate with higher IgE antibody titers against α-Gal (Pacheco *et al.*, 2021). Additionally, a comparative analysis was conducted between the IgE+IgM+IgG antibody response to α-Gal and blood groups (Figure 1A), age (Figure 1B) and sex (Figure 1C) in healthy individuals (n = 75) to illustrate lower antibody titers in blood group B/AB individuals as previously reported (Cabezas-Cruz *et al.*, 2017) but no differences regarding age and sex, which have been reported before as factors affecting the antibody response to α-Gal, infection and vaccination (Buonomano *et al.*, 1999; Giefing-Kröll *et al.*, 2015; Wang *et al.*, 1995).

The main limitation of the dataset is sample size for some factors (i.e. age, sex or blood group), which were not disclosed by all individuals, and anti-α-Gal IgA antibody titers that could be considered in the analysis (Mateos-Hernández *et al.*, 2020; Urra *et al.*, 2021).

Data availability

Underlying data

Harvard Dataverse: A dataset for the analysis of antibody response to glycan alpha-Gal in individuals with immune-mediated disorders. https://doi.org/10.7910/DVN/RBU2VR (de la Fuente *et al.*, 2020).

This dataset contains characteristics and serum antibody levels of the individuals included in the study and was used in analyses reported in publications by Urra *et al.* (2021), Pacheco *et al.* (2021) and Doncel-Pérez *et al.* (2020).

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

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PubMed Abstract | Publisher Full Text | Free Full Text

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Version 2

Reviewer Report 12 August 2021

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✔ **Mona Mahmoud**
Parasitology and Animal Diseases Department, National Research Center, Giza, Egypt

The article is scientifically sound. The data includes a dataset of anti-alphaGal IgM, IgG, and IgE levels in the serum of healthy people and patients, including COVID-19 patients, tick bite patients, and patients with Guillain-Barré syndrome. The rationale and methods are stated clearly, and the dataset is provided in a way that is both accessible and convenient.

Is the rationale for creating the dataset(s) clearly described?
Yes

Are the protocols appropriate and is the work technically sound?
Yes

Are sufficient details of methods and materials provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: immuoparasitology, Molecular biology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 20 July 2021

https://doi.org/10.5256/f1000research.57019.r86098

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Tilo Biedermann
[1] Department of Dermatology and Allergology, Technical University of Munich, Munich, Germany
[2] Clinical Unit Allergology, Hemholtz Zentrum Munchen, German Research Centre for Environmental Health, Neuherberg, Germany

Response to 1
The presentation of the data set in Figure 1 is still not clear to me. What is the rational to sum up OD_{450} values of α-Gal IgM, IgG and IgE ELISAs? Since Figure 1 is an example to demonstrate the possible usage of the data set, it should in my opinion consist of analyzes with a scientific value. Thus, I would recommend to exemplary show one of the isotypes instead of the summation of all measured isotypes.

Response to 2
I agree that different factors such as tick bites correlate with higher α-Gal IgE titers. However, a rational explanation for the high α-Gal IgE titers in a large proportion of healthy controls is still missing (atopy? History of repetitive tick bites?). The article by Pacheco *et al.* mentioned by the authors (DOI: 10.1016/j.ttbdis.2021.101651) shows a quantitative analysis of α-Gal IgE (ImmunoCAP) in addition to the ELISA assay results. However, while, as expected and also in accordance with previous publications by others (DOI: 10.1111/all.13400), only a small proportion of healthy, asymptomatic individuals (15%) were positive for α-Gal IgE in the ImmunoCAP analysis, almost all sera analyzed by ELISA showed α-Gal IgE levels above the cut-off for positivity (OD_{450} = 0.3) defined by the authors in another publication (DOI: 10.1038/emm.2016.164), regardless of the tick bite or allergy history of the respective individual. Additionally, the correlation analysis in Supplementary Figure 1 (Pacheco et al.) is not clear to me. What is the x axis corresponding to (number of individuals)? Is it not the case that each individual corresponding to one of the different allergy-type reaction groups is defined by one individual symbol in the graph? If I understood correctly, the formula to convert antibody titers into kU/l is based on a correlation of OD_{450} values generated by ELISA and kU/l values of the corresponding samples determined by ImmunoCAP. Could you please share the corresponding correlation with me in order to reproduce the results shown in Supplementary Figure 1C? Using the formula to determine α-Gal IgE titers as stated in Pacheco et al., only OD_{450} values higher than 2.7 result in kU/l values higher than the cut-off of 0.35 kU/l (which is not fitting to the cut-off of OD_{450} of 0.3 defined for positivity of the ELISA). Although I agree that the relation between α-Gal IgE levels of healthy individuals and AGS patients fits the literature (higher OD_{450} in AGS and anaphylactic patients compared to healthy), I am still missing an explanation for the surprisingly high OD_{450} values of α-Gal IgE in a large proportion of healthy individuals, especially if the cut-off for positivity of the α-Gal IgE ELISA is set to OD_{450} 0.3.

References
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3. Cabezas-Cruz A, Mateos-Hernández L, Alberdi P, Villar M, et al.: Effect of blood type on anti-α-Gal immunity and the incidence of infectious diseases. *Experimental & Molecular Medicine*. 2017; **49** (3). Publisher Full Text

Is the rationale for creating the dataset(s) clearly described?
Yes

Are the protocols appropriate and is the work technically sound?
Yes

Are sufficient details of methods and materials provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 21 Jul 2021

Jose de la Fuente, Instituto de Investigación en Recursos Cinegéticos IREC, Ciudad Real, Spain

We appreciate reviewer comments, but consider that the information addressing these comments was provided in the previous response and revised manuscript. Exposure to ticks is always a variable that is difficult to fully address although this question was included in data collection as reflected in the database associated to the paper. Therefore, we have no further revisions in response to these comments.

Competing Interests: No competing interests were disclosed.

Reviewer Report 26 May 2021

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Jacques Le Pendu

CRCINA, Université de Nantes, Inserm, Nantes, France

No further comments to make.

Is the rationale for creating the dataset(s) clearly described?
Yes

Are the protocols appropriate and is the work technically sound?
Yes

Are sufficient details of methods and materials provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Glycobiology, Host-pathogens interactions, histo-blood group antigens

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 18 May 2021

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Tilo Biedermann
[1] Department of Dermatology and Allergology, Technical University of Munich, Munich, Germany
[2] Clinical Unit Allergology, Hemholtz Zentrum Munchen, German Research Centre for Environmental Health, Neuherberg, Germany

The data note presented by José de la Fuente and colleagues contains α-Gal specific antibody titers of IgE, IgM, IgG isotypes in the serum and, for some individuals, IgA isotype in saliva of healthy individuals as well as patients with tick bite-associated symptoms, Guillain-Barré syndrome and COVID-19 infection, respectively. The dataset was already used in a published study by the authors correlating α-Gal specific antibodies and COVID-19 disease symptoms (Urra *et al*. 2020,[1]). Although the dataset is given in an easily accessible format (Excel sheet) and methods are adequately described, I have some major concerns in regard to the benefit of the data for other research

applications which are listed below.

1. The authors validate their dataset using comparative analysis of antibody titers of healthy individuals with blood group, age and sex. What is the rational to use a summation of α-Gal IgG, IgM and IgE antibody levels for these analyses? Since different antibody isotypes are associated with certain immunological functions (effector functions mediated by Fc part of the antibody) and certain diseases are associated with certain antibody isotypes (e.g. AGS and α-Gal IgE), a summation of different antibody isotype levels is in my opinion not correct and does not provide meaningful scientific insights. Additionally, since the entries for the key characteristics are incomplete for a large proportion of the individuals included, the used comparative analysis is not generally applicable for the presented dataset and thus not appropriate for its validation.

2. The OD_{450} values and thus the titers of α-Gal specific IgE are unexpectedly high in almost half of the healthy individuals. Is there an explanation for α-Gal IgE titers in serum of healthy individuals which are as high as in AGS patients or individuals with tick-bite associated allergic reactions? Can these individuals for certain be classified as “healthy” or how can sensitization to α-Gal be explained (atopy)? In my opinion, the ELISA data presented in this manuscript requires validation due to the unexpected and controversial high titers of α-Gal specific IgE antibodies in “healthy” individuals e.g. by using α-Gal ImmunoCAP or the commercially available α-Gal IgE ELISA Kit.

3. As also stated by the authors as main limitation of their dataset, key characteristics such as age, sex and blood group are missing for a large proportion of the entries. This incompleteness significantly limits the benefit of the dataset for other research questions.

References

1. Urra JM, Ferreras-Colino E, Contreras M, Cabrera CM, et al.: The antibody response to the glycan α-Gal correlates with COVID-19 disease symptoms.*J Med Virol*. **93** (4): 2065-2075 PubMed Abstract | Publisher Full Text

Is the rationale for creating the dataset(s) clearly described?

Yes

Are the protocols appropriate and is the work technically sound?

No

Are sufficient details of methods and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Immunology, Allergy, Dermatology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 18 May 2021

Jose de la Fuente, Instituto de Investigación en Recursos Cinegéticos IREC, Ciudad Real, Spain

Thanks for your comments to our paper. In response to your comments:

The data note presented by José de la Fuente and colleagues contains α-Gal specific antibody titers of IgE, IgM, IgG isotypes in the serum and, for some individuals, IgA isotype in saliva of healthy individuals as well as patients with tick bite-associated symptoms, Guillain-Barré syndrome and COVID-19 infection, respectively. The dataset was already used in a published study by the authors correlating α-Gal specific antibodies and COVID-19 disease symptoms (Urra *et al*. 2020,[1]). Although the dataset is given in an easily accessible format (Excel sheet) and methods are adequately described, I have some major concerns in regard to the benefit of the data for other research applications which are listed below.

1. The authors validate their dataset using comparative analysis of antibody titers of healthy individuals with blood group, age and sex. What is the rational to use a summation of α-Gal IgG, IgM and IgE antibody levels for these analyses? Since different antibody isotypes are associated with certain immunological functions (effector functions mediated by Fc part of the antibody) and certain diseases are associated with certain antibody isotypes (e.g. AGS and α-Gal IgE), a summation of different antibody isotype levels is in my opinion not correct and does not provide meaningful scientific insights. Additionally, since the entries for the key characteristics are incomplete for a large proportion of the individuals included, the used comparative analysis is not generally applicable for the presented dataset and thus not appropriate for its validation.

Response: We agree with reviewer on the differences between antibody isotypes as disclosed in the Introduction of the paper. However, Figure 1 is only an example to illustrate the effect of some factors such as blood group, age and sex on the total antibody response to α-Gal. This is why total antibody titers for subtypes IgE, IgM and IgG were added to illustrate these correlations or the absence of it. In no case we are using these data for validation or immunologically related analyses. For this purpose, please refer to these papers published using this dataset and with analyses using different antibody isotypes:
Urra JM, Ferreras-Colino E, Contreras M, Cabrera CM, Fernández de Mera IG, Villar M, Cabezas-Cruz A, Gortázar C, de la Fuente J. The antibody response to the glycan α-Gal correlates with COVID-19 disease symptoms. J Med Virol. 2021 Apr;93(4):2065-2075. doi: 10.1002/jmv.26575.
Hodžić A, de la Fuente J, Cabezas-Cruz A. COVID-19 in the Developing World: Is the Immune Response to α-Gal an Overlooked Factor Mitigating the Severity of Infection? ACS Infect Dis. 2020 Dec 11;6(12):3104-3108. doi: 10.1021/acsinfecdis.0c00747.
Doncel-Pérez, E., Contreras, M., Gómez Hernando, C., Vargas Baquero, E., Blanco García, J., Rodríguez Gómez, J., Velayos Galán, A., Cabezas-Cruz, A., Gortázar, C., de la Fuente, J. 2020.

What is the impact of the antibody response to glycan alpha-Gal in Guillain-Barré syndrome associated with SARS-CoV-2 infection? Merit Research Journal of Medicine and Medical Sciences (MRJMMS) 8: 730-737.
https://digital.csic.es/bitstream/10261/230769/1/whatinfect.pdf
Pacheco I, Fernández de Mera IG, Feo Brito F, Gómez Torrijos E, Villar M, Contreras M, Lima-Barbero JF, Doncel-Pérez E, Cabezas-Cruz A, Gortázar C, de la Fuente J. Characterization of the anti-α-Gal antibody profile in association with Guillain-Barré syndrome, implications for tick-related allergic reactions. Ticks Tick Borne Dis. 2021 May;12(3):101651. doi: 10.1016/j.ttbdis.2021.101651.

2. The OD_{450} values and thus the titers of α-Gal specific IgE are unexpectedly high in almost half of the healthy individuals. Is there an explanation for α-Gal IgE titers in serum of healthy individuals which are as high as in AGS patients or individuals with tick-bite associated allergic reactions? Can these individuals for certain be classified as “healthy” or how can sensitization to α-Gal be explained (atopy)? In my opinion, the ELISA data presented in this manuscript requires validation due to the unexpected and controversial high titers of α-Gal specific IgE antibodies in “healthy” individuals e.g. by using α-Gal ImmunoCAP or the commercially available α-Gal IgE ELISA Kit.

Response: This is an interesting observation that has been reported in other studies. However, factors such as tick bites or allergy do correlate with higher IgE antibody titers (see figure 2 in Pacheco et al. Ticks Tick Borne Dis. 2021 May;12(3):101651. doi: 10.1016/j.ttbdis.2021.101651). For the validation of the ELISA with ImmunoCAP please see Supplementary Figure A1 in this paper by Pacheco et al. with correlation analysis between different allergy-type reactions to tick bites and anti-α-Gal IgE antibody response using a Spearman Rho (rs) correlation analysis ($p < 0.01$) conducted between allergy-type reactions and anti-α-Gal IgE antibody titers determined by ELISA (O.D. at 450 nm) and converted to kU/l. Positive anti-α-Gal IgE levels were considered at cut-off value of 0.35 kU/l. Due to scope of Data note papers, we cannot disclose all this information in the paper, but can be referred to in the revised paper.

3. As also stated by the authors as main limitation of their dataset, key characteristics such as age, sex and blood group are missing for a large proportion of the entries. This incompleteness significantly limits the benefit of the dataset for other research questions.

Response: We acknowledge these limitations of the dataset that are due to ethic issues in some of the studies. Nevertheless, we consider that this information is useful for the scientific and medical community interested in the study of the antibody response to α-Gal.

Competing Interests: None

Reviewer Report 04 December 2020

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Jacques Le Pendu
CRCINA, Université de Nantes, Inserm, Nantes, France

The data note presents a dataset of levels of anti-alphaGal IgM, IgG and IgE in the serum of healthy individuals and of patients, including COVID-19 patients, patients with tick bites and Guillain Barré syndrome patients. The rationale and methods are clearly presented and the dataset is given in an easily accessible and convenient format.

I would only like to see a clarification concerning the exact alphaGal-BSA antigen that was used for coating. The structure of the oligosaccharide should be given.

Is the rationale for creating the dataset(s) clearly described?
Yes

Are the protocols appropriate and is the work technically sound?
Yes

Are sufficient details of methods and materials provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Glycobiology, Host-pathogens interactions, histo-blood group antigens

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 04 Dec 2020

Jose de la Fuente, Instituto de Investigación en Recursos Cinegéticos IREC, Ciudad Real, Spain

Thanks for your positive feedback to our paper. In response to your question, the alphaGal-BSA antigen used for coating the ELISA plates was Galα1-3Gal-BSA (3 atom spacer) (Product Code: NGP0203; https://www.dextrauk.com/products/neoglycoproteins/gala1-3gal-series-neoglycoproteins/product/288-gala1-3gal-bsa-3-atom-spacer).

Competing Interests: No competing interests were disclosed.

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