DOI: 10.1002/jmv.27022

LETTER TO THE EDITOR

JOURNAL OF MEDICAL VIROLOGY WILEY

Reply to: The antibody response to the glycan α-Gal correlates with COVID-19 symptoms

To the Editor:

We read with interest two recent papers which discussed possible connections between the immune response to galactose-α-1,3-galactose (α-Gal) and coronavirus disease 2019 (COVID-19).[1,2] First described nearly a hundred years ago by Landsteiner and Miller as a "B-like" blood group substance of non-primate mammals (but not higher primates), work over the last 40 years has made it clear that α-Gal is also expressed on some species of bacteria and multi-cellular parasites, and that all immunocompetent humans produce large quantities of immunoglobulin M (IgM), IgG and IgA antibodies (Ab) specific for the oligosaccharide.[3,4] More recent research indicates that a subset of the population can also produce IgE specific for α-Gal. IgE sensitization to α-Gal has largely been attributed to tick bites and is an important cause of allergic reactions to mammalian meat and dairy.[5,6]

On this backdrop, Urra et al. reported that patients with COVID-19 had altered levels of anti-α-Gal IgG, IgM, IgA, and IgE Ab as compared to a control cohort.[1] Specifically, they found that levels of α-Gal-specific IgG, IgM, and IgE (but not IgA), were lower in patients hospitalized in an intensive care unit (ICU) with severe COVID-19 as compared to healthy uninfected controls. They also reported that relative amounts of different anti-α-Gal antibody isotypes varied in relation to disease severity. Interestingly, they noted that IgE represented 14%–45% of the overall repertoire of anti-α-Gal antibody levels, with the highest amount of specific IgE observed in asymptomatic COVID-19 patients. The authors speculate that dysbacteriosis could have caused the reduced antibody response to α-Gal, which in turn translated to greater severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral loads and systemic inflammation. This hypothesis leads to the idea that restoring anti-α-Gal antibodies could be protective against COVID-19. This paper raises some intriguing points, but we think additional commentary is merited.

In our recent investigation of COVID-19, which utilized a quantitative ImmunoCAP-based approach, we did not observe differences in levels of anti-α-Gal IgG when comparing patients with severe COVID-19 to a reference cohort of healthy uninfected controls.[7] The explanation for the discrepancy between our findings and Urra et al. is not clear. One possibility is that inflammatory mediators which are present during acute severe COVID-19 could be interfering with one or both of our assays. However, we would highlight a recent report that used a glycan array and did not find lower anti-α-Gal IgG levels in COVID-19 patients compared to controls.[8] To look at this question in a different light, here we have extended our previous analysis by monitoring anti-α-Gal IgG levels among five patients with severe COVID-19 in which longitudinal data were available. The data indicate that levels were relatively stable across time, including at a follow-up timepoint where the patients had convalesced and recovered from their infection (Figure 1A). We also measured IgG to tetanus toxoid as a reference control antigen (to which most individuals are vaccinated) and found little fluctuation in antibody levels across the time points (Figure 1B). It is also important to note that the anti-α-Gal antibody levels reported by Urra and

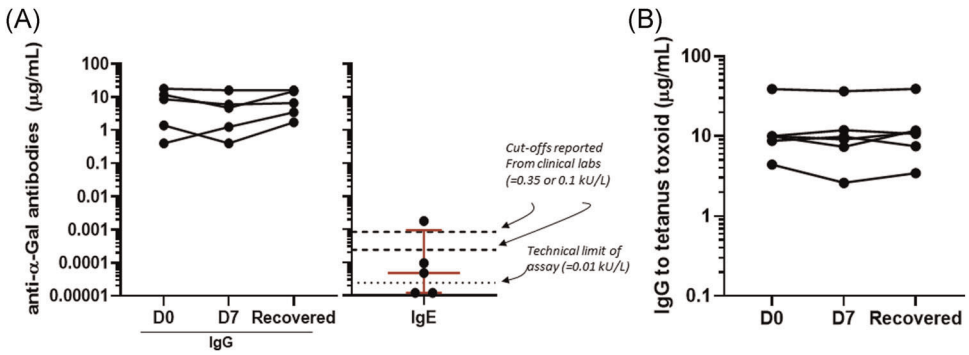

FIGURE 1 Quantitative assessment of antibodies in five patients admitted to the intensive care unit (ICU) with severe COVID-19 using ImmunoCAP. (A) Immunoglobulin G (IgG) levels to galactose-α-1,3-galactose (α-Gal) assessed at day of admission (D0; median 10 days post-symptom onset), Day 7 of admission (D7; median 17 days post-symptom onset) and at a recovery follow-up clinic (median 74 days post-symptom onset). IgE levels to α-Gal were measured at the recovery timepoint, expressed in µg/ml using the same units/axis as for IgG. Two samples in which no IgE was detected were plotted as ×0.5 the technical limit of the assay. Both the IgG and IgE assays used α-Gal-HSA as the assay solid-phase, as previously described.[7] (B) IgG to tetanus toxoid was measured by ImmunoCAP using the commercial assay (Thermo-Fisher/Phadia)

colleagues were based on semi-quantitative enzyme-linked immunosorbent assays, where the read-out is in OD_{450} units. As there is not an internal calibrator curve, the antibody levels cannot be expressed in a quantitative fashion. As a consequence, there are major limitations in comparing isotype-specific antibody levels with each other. This is particularly true when considering IgE, which usually represents only a minor fraction of the antibody repertoire. Using the quantitative ImmunoCAP assay, here we show that levels of anti-α-Gal IgE were log orders of magnitude lower than specific IgG in the five severe COVID-19 patients (Figure 1A).

The recent article by Chen discussed the possibility that α-Gal could be used as a means of enhancing immune responses to COVID-19 vaccinations.[2] The concept, which takes advantage of pre-existing humoral immunity to α-Gal, is interesting and biologically plausible. Data cited in that report from nonhuman studies was also encouraging that α-Gal can indeed boost vaccine-related immune responses. Nonetheless, we were struck that there was no mention of the fact that IgE to α-Gal could be an important confounder to this strategy. IgE is a critical mediator of allergic reactions and IgE specific for α-Gal has been linked with cases of anaphylaxis that were caused by gelatin-containing vaccines (in which the gelatin was a source of α-Gal).[9,10] As α-Gal occurs in some areas of the world with frequencies approaching or exceeding 20%, there are good reasons to think that inclusion of α-Gal could lead to issues with vaccine safety.[5,11] We don't doubt that α-Gal could have potential immune-enhancing benefits for certain personalized immunotherapies (e.g., cancer vaccines), but have reservations that it is a rational choice for designing vaccines that would be implemented widely on a population basis.[12]

Jeffrey M. Wilson
Thomas A. E. Platts-Mills
Behnam Keshavarz

Division of Allergy & Clinical Immunology, Department of Medicine, University of Virginia, Charlottesville, Virginia, USA

Correspondence
Jeffrey M. Wilson, PO Box 801355, University of Virginia, Charlottesville, VA 22908, USA.
Email: jmw2gc@virginia.edu

ORCID

Jeffrey M. Wilson http://orcid.org/0000-0002-5975-1760
Thomas A. E. Platts-Mills https://orcid.org/0000-0002-1263-329X
Behnam Keshavarz https://orcid.org/0000-0001-5121-6950

REFERENCES

1. Urra JM, Ferreras-Colino E, Contreras M, et al. The antibody response to the glycan alpha-Gal correlates with COVID-19 disease symptoms. *J Med Virol*. 2020:jmv.26575
2. Chen JM. SARS-CoV-2 replicating in nonprimate mammalian cells probably have critical advantages for COVID-19 vaccines due to anti-Gal antibodies: a minireview and proposals. *J Med Virol*. 2021;93(1):351-356.
3. Galili U. Anti-Gal: an abundant human natural antibody of multiple pathogeneses and clinical benefits. *Immunology*. 2013;140(1):1-11.
4. Cabezas-Cruz A, Hodzic A, Roman-Carrasco P, et al. Environmental and molecular drivers of the alpha-Gal syndrome. *Front Immunol*. 2019;10:1210.
5. Platts-Mills TAE, Commins SP, Biedermann T, et al. On the cause and consequences of IgE to galactose-alpha-1,3-galactose: a report from the National Institute of Allergy and Infectious Diseases Workshop on understanding IgE-mediated mammalian meat allergy. *J Allergy Clin Immunol*. 2020;145(4):1061-1071.
6. Wilson JM, Keshavarz B, Retterer M, et al. A dynamic relationship between two regional causes of IgE-mediated anaphylaxis: alpha-Gal syndrome and imported fire ant. *J Allergy Clin Immunol*. 2021;147(2):643-52 e7.
7. Keshavarz B, Wiencek JR, Workman LJ, et al. Quantitative measurement of IgG to severe acute respiratory syndrome coronavirus-2 proteins using immunoCAP. *Int Arch Allergy Immunol*. 2021:1-8.
8. Butler DL, Gildersleeve JC. Abnormal antibodies to self-carbohydrates in SARS-CoV-2 infected patients. *bioRxiv*. 2020.
9. Stone CA Jr., Hemler JA, Commins SP, et al. Anaphylaxis after zoster vaccine: implicating alpha-gal allergy as a possible mechanism. *J Allergy Clin Immunol*. 2017;139(5):1710-3 e2.
10. Stone CA Jr., Commins SP, Choudhary S, et al. Anaphylaxis after vaccination in a pediatric patient: further implicating alpha-gal allergy. *J Allergy Clin Immunol Pract*. 2019;7(1):322-324.e2.
11. Fischer J, Huynh HN, Hebsaker J, Forchhammer S, Yazdi AS. Prevalence and impact of type I sensitization to alpha-Gal in patients consulting an allergy unit. *Int Arch Allergy Immunol*. 2020;181(2):119-127.
12. Galili U. Amplifying immunogenicity of prospective COVID-19 vaccines by glycoengineering the coronavirus glycan-shield to present alpha-Gal epitopes. *Vaccine*. 2020;38(42):6487-6499.