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SARS-CoV-2-neutralizing humoral IgA response occurs earlier but modest and diminishes faster compared to IgG response.

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Review Timeline:

Submission Date:	July 15, 2022
Editorial Decision:	August 1, 2022
Revision Received:	September 8, 2022
Accepted:	September 13, 2022

Editor: Takamasa Ueno

Reviewer(s): The reviewers have opted to remain anonymous.

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

DOI: https://doi.org/10.1128/spectrum.02716-22

August 1, 2022

Dr. Hiroaki Mitsuya
National Center for Global Health and Medicine
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Re: Spectrum02716-22 (SARS-CoV-2-neutralizing humoral IgA response occurs earlier but modest and diminishes faster compared to IgG response.)

Dear Dr. Hiroaki Mitsuya:

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Takamasa Ueno

Editor, Microbiology Spectrum

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Reviewer comments:

Reviewer #1 (Comments for the Author):

The authors examined the role of IgA antibodies against COVID-19 and determined the neutralizing activity of serum/plasma from infected and vaccined individuals. They found that IgA possesses modest neutrazing activity compared to IgG, which showed great potential in ab testing. They also provided vaccination data for individuals infected with the virus but no detectable ab activity. I found the data are nicely presented and informative. Could the author provide more ab responses data from other studies especially with the different vaccines. Also, could the author give more explanation for the second dose seems having no protection?

Reviewer #2 (Comments for the Author):

IgA is known to be important for the mucosal immunity to prevent invasion of pathogens including SARS-CoV-2, but its role in blood hasn't been well-understood. In this paper, the authors analyzed the neutralizing activity of serum/plasma anti-Spike IgG and IgA in patients with COVID-19 over time. It was found that the neutralizing activity of serum IgA increased faster than that of IgG after the disease onset, and the former quickly attenuated whereas the latter lasted for longer time. Furthermore, IgA neutralizing activity increased rapidly after vaccination in the subjects with a history of SARS-CoV-2 infection. Although IgG is thought to play a central role in prevention against SARS-CoV-2, this study suggests that serum IgA also functions in the early stages of infection and immediately after vaccination. Thus, these findings provide useful information for control of SARS-CoV-2 infection. However, several corrections should be made in this manuscript.

1. Line 248. The subheading "Neutralizing activity is greater in patients with severe COVID-19 than with moderate disease" isn't correct. Figure 2 shows that there are no significant differences in the activities of IgG and IgA between moderate and severe diseases.

2. Line 244. How could the authors conclude that "The comparative data showed that S1-binding IgA production significantly predominated over S1-binding IgG production (p=0.009, Wilcoxon signed-rank test)" by Fig 1e and 1f? The authors should explain in more detail about this description.

3. Lines 282-285. Citation of the figures in the text seems to be wrong. It is supposed that Fig 3a (line 282) is Fig S3a, and Fig. S2c, S2d, and S2e (line 285) are from Fig. S3 not S2.

4. Figure 1. There are no explanations about the markers in the graphs. What do red and blue markers mean?

Staff Comments:

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Reviewer #1's comments:

1. *Could the author provide more ab responses data from other studies especially with the different vaccines.*

Our response:

The kinetics of SARS-CoV-2-S-binding (S-binding) IgG, IgA, and IgM upon natural SARS-CoV-2 infection has been well described over two years from COVID-19 pandemic (*Ma H, Cell Mol Immunol.* 2020.; *Iyer AS, Sci Immunol.* 2020, *Sterlin D, Sci Transl Med.* 2021, *Marot S, Nat Commun.* 2021). There have also been a good body of literature regarding SARS-CoV-2-neutralizing IgG antibody responses; yet, there are only a few reports on neutralizing IgA antibody. However, such a few reports describe the neutralizing activity of monoclonal IgA antibodies produced by peripheral (*Pisil Y, Pathogens.* 2021) or mucosal (*Planchais C, J Exp Med.* 2022) memory B cells. There are also a number of reports describing immune responses elicited by various types of vaccines, such as mRNA (BNT162b2 or mRNA1273), non-replicating adenoviral vector (AZD1222, Sputnik V, or Ad26.COV2.S) and inactivated (BBIBP-CorV) vaccines (*Lafon E., J Allergy Clin Immunol.* 2022, *Adjobimey T, Front Immunol.* 2022); however, such articles have also only described S-binding IgA antibodies, and no neutralizing activity of such IgA antibodies have been evaluated. Thus, our present report, which describes the neutralizing IgA activity in detail together with those of neutralizing IgG activity in individuals with COVID-19, should shed light in the understanding of the immune response upon SARS-CoV-2 infection.

As per suggestion by Reviewer#1, we have added the points above toward the end of the Discussion section in the revised version of the manuscript.

2. *Also, could the author give more explanation for the second dose seems having no protection?*

Our response:

As Reviewer #1 noted, in the present study illustrated in Figure 4, while the 1st dose vaccination in the COVID-19-experineced individuals elicited a good response comparable to the response seen in COVID-19-unexperineced individuals following 2nd dose (*Walsh EE, N Engl J Med.* 2021; *Maeda K, Sci Rep.* 2021), the response following the 2nd dose was comparable to or even less than the response after the 1st dose in those COVID-19-experienced individuals. In this respect, it is noteworthy that the intervals following the 1st dose until the 2nd dose was administered were 3 or 4 weeks. These intervals were probably too short for eliciting the otherwise boosted immune response. In fact, there are several published articles that describe the antibody responses after the COVID-19 vaccination in previously COVID-19-expereineced individuals (*e.g. Mazzoni A, J Clin Invest.* 2021; *Ebinger JE, Nat Med. 2021*; *Anderson M, JAMA Netw Open. 2021*). In such articles, a single dose of COVID-19 mRNA vaccine (whether BNT162b2 or mRNA1273) or adenoviral vector-based vaccine (AZD1222) induced substantial neutralizing antibodies and T cells responses against the virus that are compatible with the responses seen after two doses of

vaccine in individuals without prior SARS-CoV-2 infection. While the mechanism of such robust immunogenicity seen with a single dose of vaccine in COVID-19-experienced individuals remains to be clarified, it is presumed that the prior COVID-19 served as the primary immunization.

In the revised version of the manuscript, these points have been described in the Discussion section.

Reviewer #2's comments:

1. *Line 248. The subheading "Neutralizing activity is greater in patients with severe COVID-19 than with moderate disease" isn't correct. Figure 2 shows that there are no significant differences in the activities of IgG and IgA between moderate and severe diseases.*

Our response:

As Reviewer #2 correctly pointed out, the paragraph in question describes a significant increase of serum/plasma or purified IgG neutralizing activity and the amount of S1-binding IgG in the convalescent phase than in the activity in the acute phase regardless disease severity, while there were no significant differences in the neutralizing activity between the moderate and severe symptom groups. Thus, the subheading was rephrased as follows:

> "Neutralizing IgG activity is greater in the COVID-19-convalescent phase than in the acute phase regardless disease severity"

2. *Line 244. How could the authors conclude that "The comparative data showed that S1-binding IgA production significantly predominated over S1-binding IgG production (p=0.009, Wilcoxon signed-rank test)" by Fig 1e and 1f? The authors should explain in more detail about this description.*

Our response:

According to Reviewer #2's suggestions, the sentences were rephrased as follows in the Results section of the revised manuscript.

> "Thus, we attempted to examine whether the amounts of S1-binding IgA produced predominated timewise over those of S1-binding IgG by using the slope indexes determined with the initial (first) value determined and the following (second) value determined for S1-binding IgA and IgG amounts in each individual. Then, the slope indexes of S1-binding IgA and IgG amounts were compared using Wilcoxon signed-rank test. We found that the slope indexes made with the first and second S1-binding IgA were significantly greater than those made with S1-binding IgG, suggesting that the amount of S1-binding IgA produced significantly predominated

over the amount of S1-binding IgG at the early phase of antibody response after the symptom onset (p=0.009) (Fig. 1e and 1f)."

Further, the sentences were rephrased as follows in the Materials and Methods section of the revised manuscript.

"To examine which of nIgG-EC_{50} and nIgA-EC_{50} values diminished faster in the convalescent-vaccine group, the values obtained by subtracting the lowest EC_{50} values from the highest EC_{50} values post-1st vaccine administration were compared. Then, the attenuation rates of nIgG-EC_{50} and nIgA-EC_{50}, and the differences after the vaccination were compared by Wilcoxon signed-rank test. To compare the amounts of S1-binding IgG and IgA timewise, the slope indexes were determined with the initial (first) value obtained and the following (second) value obtained for S1-binding IgA and IgG amounts in each individual. Then, the slope indexes of S1-binding IgA and IgG amounts were compared using Wilcoxon signed-rank test."

3. *Lines 282-285. Citation of the figures in the text seems to be wrong. It is supposed that Fig 3a (line 282) is Fig S3a, and Fig. S2c, S2d, and S2e (line 285) are from Fig. S3 not S2.*

Our response:

We thank Reviewer #2 for his/her careful review. The typos have now been corrected.

4. *Figure 1. There are no explanations about the markers in the graphs. What do red and blue markers mean?*

Our response:

The following description of colored markers in Figures 1, 2, and 3, and Supporting Figures 2 and 3 has been added.

"Blue symbols denote the samples collected from individuals with moderate symptom, while red symbols those from individuals with severe symptom."

September 13, 2022

Dr. Hiroaki Mitsuya
National Center for Global Health and Medicine
Shinjuku-ku
Japan

Re: Spectrum02716-22R1 (SARS-CoV-2-neutralizing humoral IgA response occurs earlier but modest and diminishes faster compared to IgG response.)

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Editor, Microbiology Spectrum

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Supplemental Material: Accept