Neutralisation activity of mucosal IgA against XBB sublineages and BA.2.86

Recent publications in *The Lancet Infectious Diseases* have reported a marked decrease in neutralisation activity against the current SARS-CoV-2 omicron subvariants, such as XBB.2.3 or BA.2.86, when using serum samples or plasma from vaccinated individuals or those who have had a breakthrough infection.[1–4] More specifically, Keiya Uriu and colleagues[3] suggested that the increased transmission potential of BA.2.86 is related to its higher immune escape capacity. In our previous study, we have shown that high concentrations of salivary receptor-binding domain (RBD)-specific secretory IgA are associated with protection against breakthrough infection in individuals who have received mRNA-based vaccines.[5] Subsequent investigations showed that breakthrough infections caused by omicron might elicit a more potent, long-lasting, and cross-reactive mucosal immune response than vaccination alone, thus potentially conferring increased protection against emerging variants.[5–7] However, it remains unclear whether the mucosal IgA response elicited by vaccination or infection is protective against new omicron subvariants such as BA.2.86.

In this Correspondence, we evaluated the level and breadth of mucosal antibody responses against the currently circulating variants in a study cohort comprising 15 vaccinated individuals, 13 of whom had confirmed breakthrough infection (appendix p 2). The infections occurred mainly during the waves of BA.1, XBB, and BQ.1 (appendix p 6). Matched saliva, nasal fluid, and tear samples from 15 participants (n=45), together with matched serum samples from 11 participants, were collected 18–19 months after their last vaccine dose or 3–20 months after having had breakthrough infection (appendix p 6). The concentrations of binding and neutralising antibodies in mucosal fluids and serum samples were quantified using ELISA and a lentivirus-based pseudovirus neutralisation assay, respectively (appendix pp 2–4).[5,7,8]

To compensate for the different mucosal flow rates between individuals and potential variations in amount of sampling material, the concentrations of RBD-specific antibodies were first normalised according to the total immunoglobulin concentrations for the respective antibody class (ie, IgA, IgG, IgM, and secretory immunoglobulin) in each sample (figure A; appendix p 7). G614 variant (B.1 lineage, spike Asp614Gly mutation) RBD-specific IgA antibodies were present in most of the mucosal samples, with the highest amount being identified in nasal fluid (geometric mean 0·025% of total IgA). However, these specific IgA antibodies were not detected in the majority of serum samples (figure A). Furthermore, the IgA antibodies measured were probably produced locally at mucosal sites as secretory IgA, as evidenced by the strong correlations observed between the concentrations of G614 RBD-specific IgA antibodies in tears (r_s=0·80, p=0·0008), nasal fluid (r_s=0·90, p<0·0001), and saliva (r_s=0·91, p<0·0001) and the concentrations of G614 RBD-specific secretory immunoglobulin in the corresponding fluids (appendix p 8). By contrast, the specific IgA concentrations in the mucosal samples did not correlate with those in the serum samples (appendix p 8).

The concentrations of G614 RBD-specific IgG antibodies were similar in nasal fluid (0·114% of total IgG), saliva (0·101%), and serum (0·272%), but they were much lower in tears (0·006%; figure A). Concentrations of salivary (r_s=0·97, p<0·0001) and nasal fluid (r_s=0·84, p=0·0022) G614 RBD-specific IgG antibodies strongly correlated with serum IgG antibodies, suggesting a serum origin (appendix p 8).

We further assessed the level of neutralising antibodies against pseudovirus bearing the spike proteins of G614, XBB.1.5, XBB.1.16, XBB.2.3, and BA.2.86 in tears, nasal fluid, and serum. The neutralisation activity of tears and nasal fluid against all pseudovirus variants showed little or no change (0·8–1·3 fold) compared with their activity against the G614 pseudovirus. Conversely, a marked decrease in neutralisation activity against these variants was observed when testing the matched serum samples, especially for XBB.1.16 (19·1-times decrease) and BA.2.86 (27·5-times decrease; figure B). The neutralisation activity in serum was exclusively correlated with serum anti-RBD binding IgG, whereas the neutralisation activity in tears and nasal fluid was correlated with anti-RBD binding IgG and IgA in the respective mucosal fluids, with a stronger correlation with IgA (r_s=0·82, p=0·0004) than with IgG (r_s=0·47, p=0·082) in tears (appendix p 9). However, neutralisation activity in mucosal fluids did not significantly correlate with the concentration of serum anti-RBD IgG antibodies (appendix p 9).

Our findings thus indicate that mucosal IgA, induced by breakthrough infection of previous omicron variants, exhibits no or very small decrease in viral neutralising capability, in contrast to systemically generated IgG antibodies, even when challenged by the newly emerging variant BA.2.86. Because neutralisation in nasal fluid correlates with the concentrations of both nasal fluid IgA and IgG anti-RBD antibodies, we cannot dismiss the potential for locally produced IgG to synergistically act with dimeric IgA anti-RBD antibodies in the neutralisation of the virus in mucosal fluid. Considering that 13 of 15 participants had breakthrough

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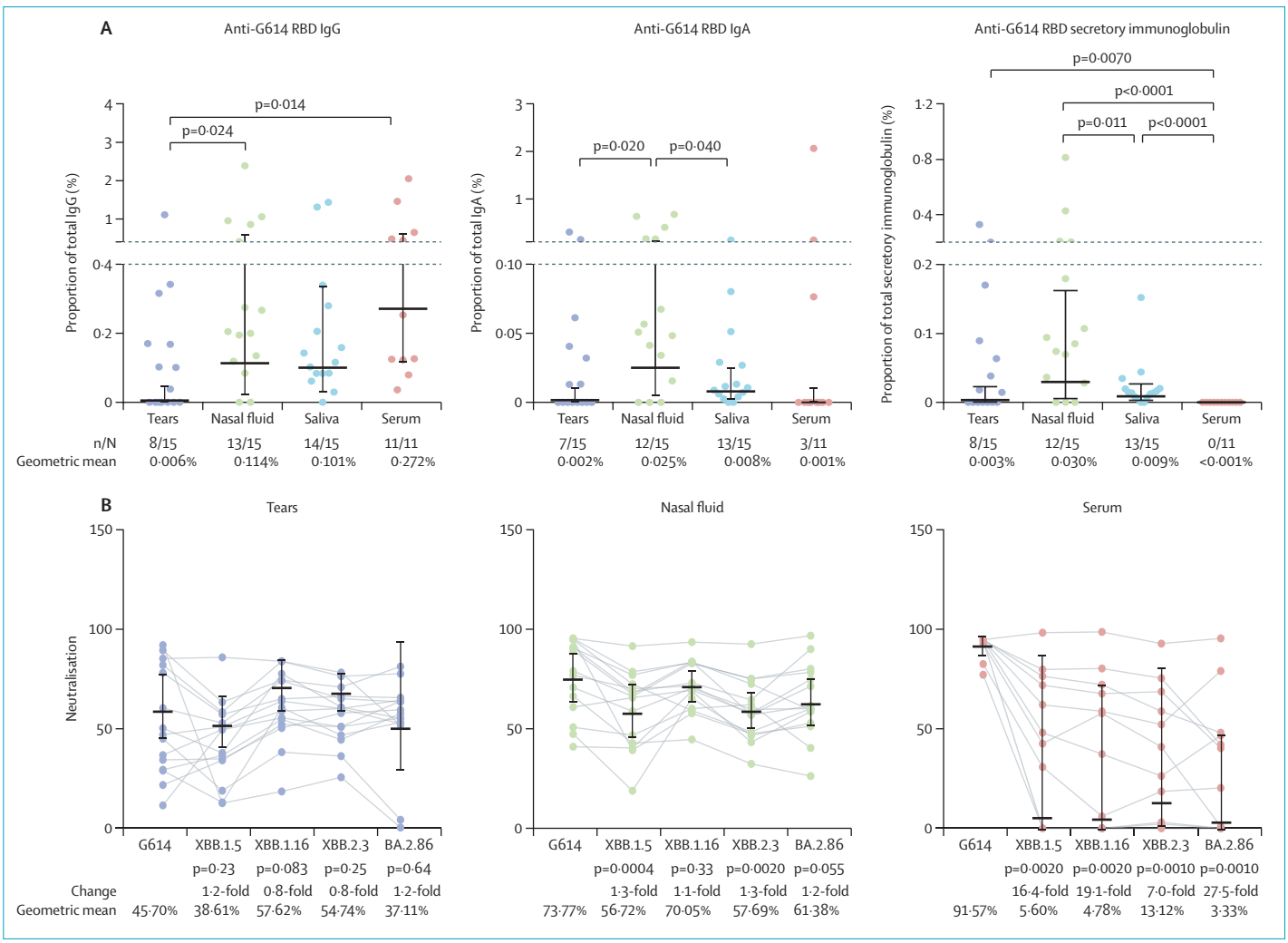

Figure: **Neutralisation activity of systemic IgG and mucosal IgA antibodies against omicron subvariants**
(A) G614 RBD-specific IgG, IgA, and secretory immunoglobulin were measured in matched saliva, nasal fluid, tears, and serum samples. Horizontal bars show geometric mean and 95% CIs. (B) Neutralising capacity of tears, nasal fluid, and serum against G614, XBB.1.5, XBB.1.16, XBB.2.3, and BA.2.86 pseudoviruses. Saliva could not be tested because of some components in saliva interfering with the neutralisation assay. The samples were run in duplicate, and each data point represents the mean value. Horizontal bars show geometric mean and 95% CIs. The fold-changes show the changes in geometric mean of neutralisation rate against each omicron subvariant compared with that of G614. n=samples positive for immunoglobulin antibodies. N=total number of samples. RBD=receptor-binding domain.

infection due to omicron subvariants, it is reasonable to propose that a portion of IgG antibodies in secretions is locally produced and might have higher affinity or greater neutralising capacity than their serum-derived counterparts. Nonetheless, mucosal IgA probably has a predominant role in neutralising these variants, as evidenced by our findings that monoclonal secretory IgA had more potent and broader neutralisation activity against omicron BA.1 and XBB variants than the parental IgG format.[7] The heightened efficacy of IgA might be attributed to an increased avidity resulting from a flexible hinge and antibody dimerisation.[7] In our previous study involving only vaccinated, non-infected individuals, we showed that a higher concentration of salivary IgA antibodies against the G614 RBD was associated with reduced breakthrough infections caused by the BA.1 variant during a 6-month follow-up.[5] Notably, the geometric means of RBD-specific salivary IgA in our current study cohort were four times higher than those for the previously studied individuals, who were vaccinated but not infected (appendix p 10). A strong mucosal IgA response induced by breakthrough infection due to earlier omicron variants might therefore be more potent and sustainable than systematic IgG response, potentially providing better protection against the circulating variant BA.2.86 and other subvariants. We propose that, together with the assessment of the infectivity of the virus and measurement of the IgG neutralising ability in serum or plasma, the mucosal antibody response, especially the IgA response, needs to be considered

when evaluating the immune escape capacity and transmission potential of new variants.

Our study is limited by the small cohort size and diverse vaccination and infection histories. Furthermore, it relied on self-reported COVID-19 infections, potentially underestimating the actual rate of breakthrough infections. The time of sampling after the final breakthrough infection might also have had an impact on the neutralising activity in various immune compartments because specific secretory IgA antibodies might have waned more slowly than specific serum IgG. Large-scale longitudinal studies are required to address these limitations.

YC and XSX have applied for a provisional patent for a series of antibodies, which includes BD55–5514 (SA55) used as a standard monoclonal IgA antibody in this study; they are also the founders of Singlomics Biopharmaceuticals. YC, XSX, LH, HM, and QP-H filed a US patent on the use of secretory IgA antibodies for therapy against SARS-CoV-2 (Secretory IgA Antibodies Against COVID Infection). All other authors declare no competing interests. This work was supported by the EU Horizon 2020 Research and Innovation Program (ATAC, 101003650; awarded to MH, DFR, LH, HM, and QP-H), the Swedish Research Council (2019-01302 and 2020-06116, awarded to QP-H), the Knut and Alice Wallenberg Foundation (KAW2020.0102, awarded to LH and QP-H), the Petrus och Augusta Hedlunds stiftelse (awarded to HM and FZ), the Stiftelsen Clas Groschinskys Minnesfond (awarded to HM and FZ), the Magnus Bergvalls Stiftelse (awarded to FZ), and the Eva & Oscar Ahréns Stiftelse (awarded to FZ). LH, HM, and QP-H are joint last authors.

*Fanglei Zuo, Yunlong Cao, Rui Sun, Ayijiang Yisimayi, Likun Du, Federico Bertoglio, Maren Schubert, Concetta Guerra, Andrea Cavalli, Michael Hust, Davide F Robbiani, Hassan Abolhassani, Xiaoliang Sunney Xie, Lennart Hammarström, Harold Marcotte, *Qiang Pan-Hammarström*
qiang.pan-hammarstrom@ki.se

Division of Immunology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm 17177, Sweden (FZ, RS, LD, HA, LH, HM, and QP-H); Biomedical Pioneering Innovation Center (BIOPIC), Peking University, Beijing, China (YC, AY, XSX); Changping Laboratory, Beijing, China (YC, AY, XSX); Department of Biotechnology, Institute of Biochemistry, Biotechnology and Bioinformatics, Technische Universität Braunschweig, Braunschweig, Germany (FB, MS, MH); Institute for Research in Biomedicine, Università della Svizzera italiana, Bellinzona, Switzerland (CG, AC, DFR)

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