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Sex-specific differences in immune response to SARS-CoV-2 vaccination vanish with age

To the Editor,

Early in the coronavirus disease 2019 (COVID-19) pandemic, age has been recognized as one of the major risk factors for poor clinical outcome(1). Based on hospitalization rates, it has also rapidly become evident that fewer women than men were affected by severe disease manifestation(2). With the primary goal to protect the most vulnerable populations, those older than 65, scientists around the world have successfully developed different vaccines with unprecedented speed(3). Although it is well established that immune responses against infections decline with age(4), it is less clear how vaccine-elicited immunity varies between different sex and age groups(5). Given the importance of understanding these biological parameters, which may directly affect translatability of research findings into the clinic, we sought to investigate the immune response against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a protein-based and vesicular stomatitis virus (VSV)-vectored vaccination approach in young and aged mice of both sexes.

First, we used the recombinant receptor binding domain (RBD) of the SARS-CoV-2 spike protein from the original reference strain emulsified in an aluminum hydroxide containing wet gel suspension (i.e. Alum) to subcutaneously immunize C57BL/6 mice (Figure S1). Seven days later, they received a second booster injection and the vaccine response was assessed on day 28 (Figure 1A,B). To test the induction of humoral immunity as a function of age, we measured antigen-specific IgG in young (2 months-old) and aged (18-19 months-old) mice by ELISA. Consistent with other studies, the systemic RBD-specific IgG response was significantly diminished in aged mice (Figure 1C,D). This age-related decline in total RBD-specific IgG is primarily due to a loss of IgG1 production since the other subclasses remained barely detectable (Figure S2). To further characterize humoral immunity, we measured the total number of plasma B-cells in spleen by flow cytometry and quantified RBD-specific plasma B-cells in the spleen of immunized mice by ELISpot. While the total number of splenic plasma B-cells were increased in aged mice the RBD-specific IgG positive B-cells were significantly diminished (Figure 1E and F) and correlated with serum IgG levels (Figure 1G), suggesting that the age-related reduction of RBD-specific plasma cell formation might contribute to the concomitant decrease in antibody titers. Previous studies have reported significant alterations in T follicular helper (Tfh) and regulatory (Tfr) cell numbers in lymphoid organs in aged mice contributing to impaired plasma B-cell generation and defective antibody production(6). Indeed, we measured an age-related increase in both Tfr and Tfh populations in the spleen as quantified by flow cytometry, while the number of classical T regulatory cells (Tregs) remained unchanged (Figure S3A). Most importantly, the live SARS-CoV-2 neutralization potency of serum from aged mice was significantly reduced for the original reference strain and different other variants of concern (i.e. alpha, gamma and delta), which is in line with the age-related decrease in RBD-specific serum antibody titers and plasma B-cells (Figure 1H).

Additionally, we evaluated sex-specific differences in vaccination response across age in the same cohorts of immunized C57BL/6 mice (Figure 2A). While RBD-specific IgG and IgG1 responses in serum were higher in young females as compared to young male controls (Figure 2B,C), these differences were no longer apparent in the aged mice, and there were no detectable sex-specific differences in the number of splenic plasma B-cells (Figure 2D). In line with higher RBD-specific antibody titers, young female C57BL/6 mice also showed more potent virus neutralization of the SARS-CoV-2 reference strain when subcutaneously immunized with a protein-based vaccine or intramuscularly injected with two VSV-vectored COVID-19 vaccine candidates (i.e. VSV-SD21 and VSV-Mq-SD21) as compared to male controls (Figure 2E and S4A,B). However, neutralization of the other tested variants of concern was diminished and equally weak in both sexes (Figure 2E), indicating that mutations in the RBD domain of these variants were sufficient to escape the vaccine-induced antibody response. To test whether these findings were conserved across different mouse strains, we repeated the same immunization regimen in young BALB/c mice. The observed outcome was essentially the same with females showing a better vaccination response than male mice (Figure S5A-E). The sex-specific differences in humoral immune response of young C57BL/6 mice persisted even after an additional injection with a protein-based vaccine 21 days after the first boost as assessed on day 42 (Figure 2 F-J). Strikingly, we found increased numbers of RBD-specific plasma B-cells in the bone marrow of young female mice in this context.

In summary, our data demonstrate significant age- and sex-related differences in the humoral immune response to different vaccine candidates administered via different application routes in different mouse strains, which is coherent with results previously reported in human clinical trials(4). Moreover, we identified important cellular changes in the T- and B-cell compartment of lymphoid organs potentially representing a mechanistic basis for the observed age- and sex-specific differences in humoral immunity. Further studies are required to investigate these mechanisms in more detail. Finally, our study clearly highlights that age and sex are important biological variables that should be considered in pre-clinical development and evaluation of novel vaccine candidates.

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Author Contributions
DB and AE have conceptualized the study and wrote the manuscript. DB, PG and HRJ have performed experiments and analyzed data. LFP, TSJ, AT, NGFL, GZ, CB and BW have produced reagents and provided scientific input.

Keywords
SARS-CoV-2, vaccine, sex, age, immune response

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Conflict of interest
The authors declare that there are no conflicts of interest in relation to this work.

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Figure Legends
Figure 1.

Decreased humoral immune response in aged mice. A) Vaccination approach with recombinant SARS-CoV-2 RBD emulsified in alum. B) Vaccination scheme. C) RBD-specific IgG response in serum on day 28 from young and aged mice was assessed by ELISA. D) Quantification of half maximal serum concentration for RBD-specific IgG and IgG1 (EC50) by ELISA. E) Representative dot plots and numbers of splenic plasma CD138$^+$ Sca-1$^+$ plasma B-cells as measured by flow cytometry. F) Number of RBD-specific IgG plasma cells in splenocytes from young and aged mice was measured by ELISPOT. G) Correlation between half maximal serum concentration for RBD-specific IgG and number of RBD-specific plasma B-cells. H) Live SARS CoV-2 neutralization with sera from young and aged mice was determined by the inhibition of virus-induced cytopathic effect (CPE). Data are representative for one experiment with 10 mice per group. Statistical significance was calculated by unpaired two-tailed Student's t-test (D,E,F,H) and linear regression analysis (G). Results are displayed as individual data points with mean ± s.e.m. for young mice (2 months of age) in white and aged mice (18-19 months of age) in black. *P < 0.05, **P < 0.01, ***P < 0.001. ns, not significant.

Figure 2.

Sex-specific humoral immune response vanishes with age. A) Vaccination scheme. B) RBD-specific IgG response in serum of young and aged mice from both sexes was assessed by ELISA. C) Quantification of half maximal serum concentration for RBD-specific IgG and IgG1 (EC50) by ELISA. D) Number of RBD-specific IgG plasma cells in splenocytes from indicated groups was measured by ELISPOT. E) Live SARS CoV-2 neutralization response in young and aged mice from both sexes were determined by the inhibition of virus-induced cytopathic effect (CPE). Data is representative for one experiment with 5-7 mice per group. F) Vaccination scheme. G) RBD-specific IgG response in serum on day 42 from young female and male mice was assessed by ELISA. H) RBD-specific IgG and IgG1 was determined by ELISA. I) Number of RBD-specific IgG plasma cells in the bone marrow from young female and male mice was measured by ELISPOT. J) Live SARS CoV-2 neutralization with sera from young female and male mice was determined by the inhibition of virus-induced cytopathic effect (CPE). Data is representative for one experiment with 9-10 mice per group. Statistical significance was calculated by unpaired two-tailed Student's t-test (B,C,D, H, I, J). Results are shown as individual data points with mean ± s.e.m. for young female (blue) and male (pink) mice as well as aged female (grey) and male (black) mice. *P < 0.05, **P < 0.01, ***P < 0.001. ns, not significant.

Figure 1

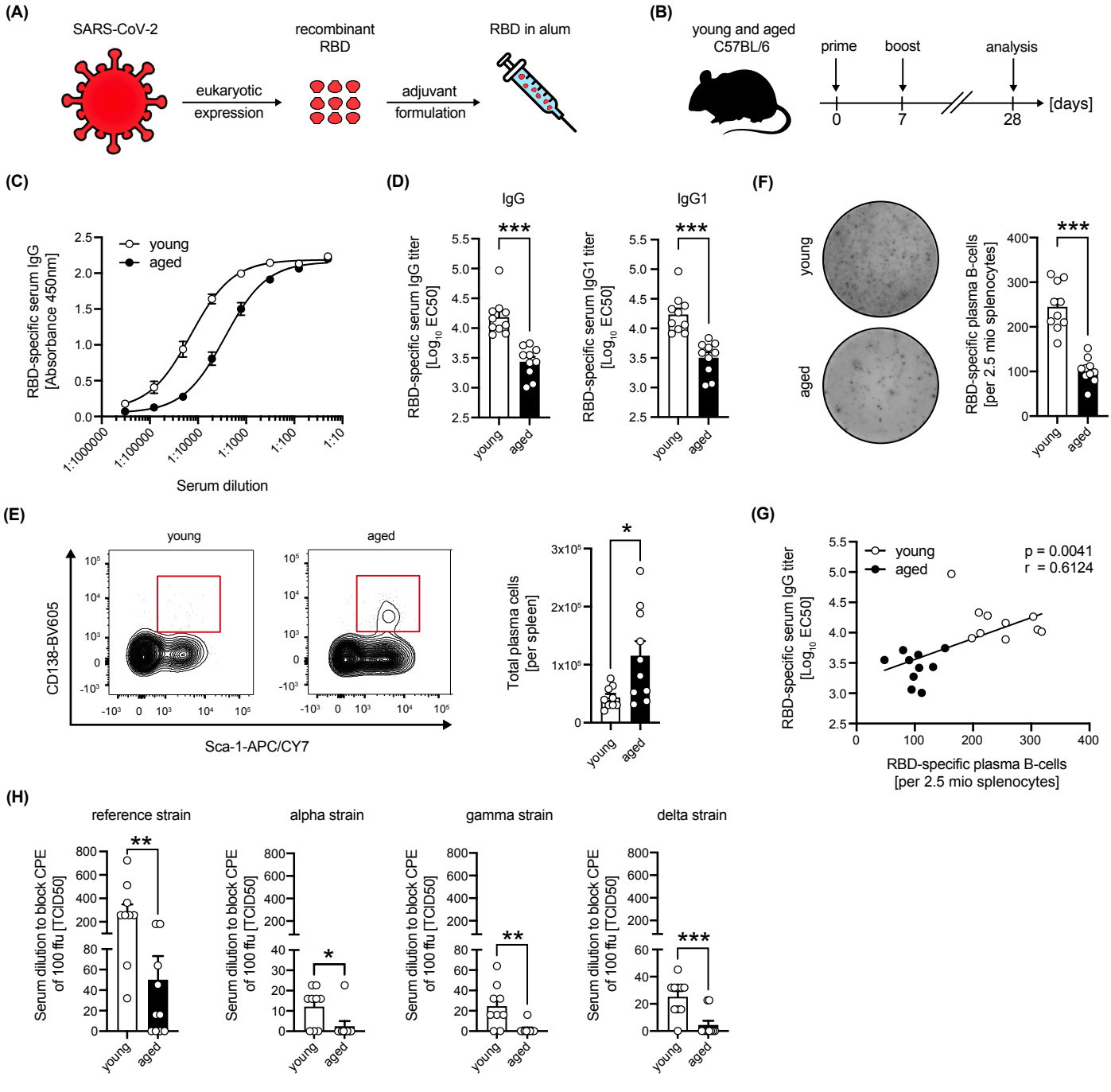

Figure 2