Concurrent administration of COVID-19 and influenza vaccines enhances Spike-specific antibody responses

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Background: The bivalent COVID-19 mRNA boosters became available in fall 2022 and were recommended alongside the seasonal influenza vaccine. However, the immunogenicity of concurrent versus separate administration of these vaccines remains unclear.

Methods: Here, we analyzed antibody responses in healthcare workers who received the bivalent COVID-19 booster and the influenza vaccine on the same day or on different days through systems serology. Antibody binding and functional responses were characterized at peak responses, and after 6 months following vaccination.

Results: IgG1 and neutralization responses to SARS-CoV-2 XBB.1.5 were higher at peak and after 6 months following concurrent administration compared with separate administration of the COVID-19 and influenza vaccines. While similar results were not observed for influenza responses, no interference was noted with concurrent administration.

Conclusions: These data suggest that concurrent administration of these vaccines may yield higher and more durable SARS-CoV-2 neutralizing antibody responses while maintaining responses against influenza.

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INTRODUCTION

The bivalent COVID-19 mRNA vaccines encoded ancestral and BA.5 Spike \(^1\), and subsequent Omicron lineages emerged that further escaped antibody recognition \(^2\) including XBB strains \(^3,4\). The rollout of the bivalent COVID-19 mRNA vaccines in fall 2022 coincided with the seasonal influenza vaccines. Previous work studying concurrent administration of seasonal influenza and ancestral COVID-19 vaccines such as BNT162b2 and ChAdOx1 showed no interference in immune responses to either vaccine. Additionally, rates of adverse events were similar in this placebo-controlled study \(^5\). However, it has remained unclear how concurrent administration of the updated COVID-19 mRNA and influenza vaccines may impact antibody profiles generated. Additionally, how the antibody profiles are sustained beyond peak immunogenicity \(^6-8\) is unclear when the two vaccines are co-administered.

Here we profiled antibody responses of healthcare workers who received the bivalent COVID-19 mRNA booster and the seasonal influenza vaccine on the same day or different days. We analyzed responses to the predominant variant at the time of the study, XBB.1.5 Spike. We observed significantly higher IgG1 responses and neutralization to XBB.1.5 at peak and after 6 months. While IgG1 responses to influenza antigens did not display a phenotype as XBB.1.5 Spike, no immune interference was noted when the influenza vaccine was concurrently administered with the bivalent COVID-19 booster. Our study suggests an immunological benefit to concurrent vaccination with these two vaccines for Spike-specific antibody responses.

METHODS

Experimental Outline and Study Participants

Participants were enrolled as a part of the Massachusetts Consortium on Pathogen Readiness (MassCPR) with informed consent. Individuals were divided into participants who received an influenza vaccine on the same day as the bivalent COVID-19 mRNA vaccine or those who received the two vaccines on different days within 4 weeks. Vaccines were administered September-December 2022. Serum samples were obtained 3-4 weeks and 6 months after the COVID-19 booster. The median ages were 36 (range 26-62) for those who received the vaccines concurrently and 39 (range 23-72) for those who received the vaccines on different days. Both groups were predominantly female (86% and 80% for the two groups, respectively) and had similar baseline medical conditions. Of the group who received a bivalent mRNA boost, 15 received the Pfizer-BioNTech and 29 received the Moderna booster. Individuals who received the influenza vaccine before the COVID-19 booster received it a median of 8.4 days (range 1-28)
before, and those who received the influenza vaccine after the COVID-19 booster received it a median of 13.2 days (range 2-29) after.

The flu vaccines received during this study period were Fluarix and Fluzone. The antigenic composition of the 2022-2023 influenza vaccine was used to perform antigen binding profiling, along with other influenza antigens. Neither of these vaccines contains a characterized adjuvant.

**Antibody binding profiling**

Antibody subclasses, isotypes, and Fc-receptor binding antibodies were assayed for binding to antigens listed in Supplementary Table 1 and described elsewhere. Assays for SARS-CoV-2 Spike and influenza antigens were done separately. The primary immunologic endpoint for SARS-CoV-2 responses were antibody responses to the predominant circulating SARS-CoV-2 variant at the time of this study, XBB.1.5. Exploratory endpoints were antibody responses to other SARS-CoV-2 variants. The breadth of antibody subclass and isotype binding was quantified by standardizing each subclass and isotype to Wu-1 Spike binding for receiving the vaccinations on different days (Supplementary Figure 1). Antibody binding responses to influenza antigens were to the HA components of the quadrivalent vaccine administered during the 2022-2023 season. Other influenza antigens and components of previous seasonal vaccinations were also used for exploratory analyses.

**Antibody functionality characterization**

Pseudovirus neutralization using serum from the cohort was performed as previously described. Antibody effector-mediated functions such as antibody-dependent cellular phagocytosis by monocytes (ADCP) and antibody-dependent neutrophil phagocytosis (ADNP) were done as previously described. For ADCP and ADNP, results were quantified using a previously validated flow cytometry-based assay, and readouts were quantified as a “Phagoscore” (see 12).

**Quantification and Statistical Analysis**

All figures and statistics were done using R Studio V 6.0 or GraphPad Prism V. 10. For correlation plots, a Spearman’s Rank correlation was calculated against individual pairings and plotted as a heatmap. For comparisons of responses, an initial Wilcoxon Rank Sum test was performed followed by a Bonferroni correction for multiple comparisons when appropriate. For SARS-CoV-2 responses, the primary endpoint was the antibody response to the predominant circulating SARS-CoV-2 variant at the time of the study XBB.1.5. Antibody responses to the two components of the bivalent mRNA vaccine booster, Wu-1 and BA.5 Spike, were assessed as exploratory endpoint. For influenza responses, antigens belonging to components of the seasonal influenza vaccine, both Influenza A and Influenza B, were assessed.
RESULTS

Concurrent bivalent COVID-19 and influenza vaccination led to higher XBB.1.5 Spike IgG1 responses

A cohort of 42 healthcare workers was followed longitudinally after bivalent COVID-19 mRNA boosting in fall 2022. Sera were evaluated at weeks 3-4 after boosting (peak immunogenicity) and at month 6 after boosting. The cohort was divided into individuals who received the COVID-19 booster and the influenza vaccine on the same day (n = 12) or different days (n = 30) (Figure 1A). The primary objective was to assess antibody responses to the predominantly circulating SARS-CoV-2 variant at the time of the study XBB.1.5.

IgG1 responses to XBB.1.5 Spike in individuals who received the bivalent mRNA COVID-19 vaccine concurrently with the influenza vaccine were 6.75-fold higher at peak and 4.69-fold higher at 6 months compared to those who received the two vaccines on different days (Figure 1B, purple box and whiskers, p = 0.033 and p = 0.016 at peak and 6 months, respectively). Antibody responses to Wu-1 and BA.5 Spike followed similar trends when the bivalent mRNA COVID-19 vaccine was administered concurrently with the influenza vaccine (6.47X and 4.29X for Wu-1 and BA.5 Spike at peak post, respectively, and 5.25 and 2.42X for Wu-1 and BA.5 Spike at 6 months, respectively) (Figure 1B – gray bars). In individuals who received the vaccines on different days, no differences were observed based on vaccination order (Supplementary Figure 2A-B). No IgG1 responses were observed to Ebolavirus glycoprotein (negative control, Supplementary Figure 2C).

No differences were observed in IgM responses (Figure 1C), suggesting that concurrent COVID-19 and influenza vaccination drove enhanced recall responses rather than de novo responses 12. Other IgG subclasses were also quantified for binding to these antigens including IgG2, IgG3, IgG4, and IgA. While trends were noticed, these comparisons were not statistically significant (Supplementary Figure 3).

Concurrent bivalent COVID-19 and influenza vaccinations increased Spike-specific IgG1 breadth to multiple variants 6 months post-vaccination

We next performed an exploratory analysis to assess if concurrent vaccination increased IgG1 binding breadth to SARS-CoV-2 Spikes to multiple variants. These included Alpha, Beta, Delta, Gamma, and BQ.1.1, in addition to the previously tested Spikes. Comparisons between concurrent and different vaccination days showed consistently increased IgG1 responses and sustained FcγRIIIA responses at 6 months to all these Spike variants in individuals who received the vaccines concurrently (Figure 2). We also observed a more robust correlation between IgG1 and IgG3 with FcγRs at both peak immunogenicity and 6 months in individuals who received the vaccines concurrently compared with those who received the vaccines on different days (Supplementary Figure 4).

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Antibody functions including pseudovirus neutralization and effector functions were then quantified. Both at peak and 6 month following vaccination, pseudovirus neutralization to BA.5 and XBB.1.5 was higher in those who received the two vaccines concurrently (Supplementary Figure 5A-B). This increase was not observed for effector functions such as antibody dependent cellular phagocytosis by monocytes (ADCP) and antibody-dependent neutrophil phagocytosis (ADNP) to the bivalent vaccine components or to XBB.1.5 Spike (Supplementary Figure 5C-D). Interestingly, subclass quantitation showed a consistently lower IgG4 as a fraction of the whole IgG repertoire for individuals who received the two vaccines concurrently (Supplementary Figure 6).

No significant differences were observed for antibody responses to SARS-CoV-2 nucleocapsid, arguing against differential infection impacting humoral profiles (Supplementary Figure 7).

**Concurrent bivalent COVID-19 and influenza boosting did not impact influenza HA antibody responses**

As we defined peak responses in relation to when participants received the bivalent mRNA COVID-19 vaccine, we did not capture peak responses to the influenza vaccine in this study. However, we assayed antibody responses to influenza HA at ~ 6 months following immunization.

Unlike SARS-CoV-2 Spike responses, we did not detect differences in influenza HA responses as a consequence of vaccination on the same day or different days (Supplementary Figure 8). We tested for components of the 2022-2023 seasonal influenza vaccine, including Influenza A Darwin 9, Influenza A Victoria 2570, Influenza A Wisconsin 588 pdm09, Influenza B Austria 1359, and Influenza B Phuket 3073 (for the quadrivalent vaccine) ⁹.

**DISCUSSION**

This study shows that concurrent administration of the bivalent COVID-19 booster and the inactivated influenza vaccine on the same day resulted in higher XBB.1.5 Spike-specific binding IgG1 responses at peak and 6 months as compared with administration of these vaccines on separate days. Moreover, neutralizing antibodies towards BA.5 and XBB.1.5 were higher at both peak and 6-month timepoints when the bivalent booster was administered concurrently compared with different days.

Safety profiles of concurrent COVID-19 and influenza vaccination have been reported ¹⁷, but limited data exist on the durability of antibody responses following different vaccination schedules. Lazarus *et al.* reported no interference in antibody generation to either influenza HA or SARS-CoV-2 Spike when the ancestral COVID-19 vaccines were co-administered with seasonal influenza vaccines ⁵. Another previous report analyzing quadrivalent influenza and mRNA-1273 vaccines showed no antigen interference or safety concerns ¹⁸. Our data extend
these prior studies by evaluating the bivalent mRNA COVID-19 vaccine with the seasonal influenza vaccine in the context of widespread population immunity in 2022-2023. It has been estimated that over half of the population in the United States was infected during the BA.1 wave 19. Moreover, we show that the enhanced antibody responses observed at peak were also durable for at least 6 months.

IgG1 is the most abundant serum IgG subclass and is capable of both neutralizing and non-neutralizing functions. A correlate of protection against COVID-19 of neutralizing antibodies has been reported, but this was only studied for the ancestral Wu-1 virus 20. Other reports have suggested that Fc-effector functions may also be required for protection against Omicron variants Spike 12,21,22. While neutralization titers to XBB.1.5 in our study were higher in those who received the COVID-19 and influenza vaccines concurrently, we did not find a similar increase in effector-mediated functions. This IgG1 and neutralization response appeared to be a recall response 12 as no differences in IgM were noted.

Previous reports have shown that mRNA COVID-19 vaccine boosting can disproportionately expand IgG4 responses 23,24. We did not find evidence that concurrent administration of the mRNA COVID-19 boosters and seasonal influenza vaccines impacted IgG4 expansion. This is in agreement with previous literature that showed lack of interference when these two vaccines were co-administered 5.

In summary, our results suggest potential benefits of concurrent administration of the COVID-19 mRNA vaccines and the seasonal influenza vaccine for induction and durability of Spike-specific IgG1 and neutralizing antibody responses. Because of the expected seasonality of SARS-CoV-2 and influenza, both vaccines will likely continue to be recommended. Our results suggest that concurrent administration of these vaccines should be considered as a strategy to potentiate antibody responses to the COVID-19 vaccine and possibly improve vaccine effectiveness 20,30.

**Limitations**

A limitation of our study is the small size of this cohort, which primarily consisted of healthcare workers, which may not reflect the general population. Larger future studies are therefore needed. Future studies should also involve a broader age range than our cohort, including children and elderly adults 31-34. Another limitation was that the peak responses were defined relative to mRNA COVID-19 vaccination and we did not capture peak influenza responses. Moreover, how adenovirus and protein-based COVID-19 vaccine boosters 35-37 impact influenza responses generated by inactivated and live-attenuated vaccines 10 remains to be determined. Lastly, we were unable to assess durability beyond 6 months, although this timeframe covers a typical influenza season.
Author contributions


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Conflicts of interest statement

R.P.M. receives financial support from AbbVie, Pfizer, GSK, the Bill and Melinda Gates Foundation, the Wellcome Trust, the United States Department of Defense, and the National Institute of Health. The remaining authors declare no competing interests.

Patient consent statement

All participants were enrolled as a part of the Massachusetts Consortium on Pathogen Readiness (MassCPR) biorepository cohort with approval from the Institutional Review Board (IRB). All participants provided informed consent.

FIGURE LEGENDS

Figure 1. Concurrent bivalent COVID-19 mRNA and influenza boosters induce more durable IgG1 responses to Spike.
A. Cohort used in this study. Participants were divided into those who received a bivalent mRNA COVID-19 booster and flu vaccine on the same day (concurrently) or different days. Blood was drawn at peak immunogenicity (2-4 weeks) and 6 months after the bivalent COVID-19 mRNA booster.
B. IgG1 antibody responses to the predominantly circulating COVID-19 Spike variant at the time of this study XBB.1.5 (purple) as well as the ancestral (Wu-1) and Omicron BA.5 (grey) Spikes that represent the vaccine immunogens. White circles indicate means. Fold differences and p-values are shown. * = p<0.05, ns = not statistically significant, Mann–Whitney U test / Wilcoxon rank-sum test.

C. IgM antibody responses were quantified similarly to (B) and serves as a control to assess de novo antibody affinity maturation. Fold differences and p=values are shown. * = p<0.05, ns = not statistically significant, Mann–Whitney U test / Wilcoxon rank-sum test.

Figure 2. Concurrent COVID-19 mRNA and influenza vaccination selectively expand IgG1 binding breadth to multiple Spike variants.
received the vaccines on different days was used as a standard to compare across groups. The scale on the right represents fold MFI increase. All antibody isotypes, subclasses, and FcR-binding antibodies are shown in distinct colors, and a legend is shown at the bottom.

REFERENCES


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