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Peer Review File

Evolving antibody response to SARS-CoV-2 antigenic shift from XBB to JN.1

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Referee #2

(Remarks to the Author)
Jian et al explore the immune response to SARS-CoV-2 variants, focusing on the shift from XBB to JN.1 lineage. The study provides an extensive analysis of the humoral immune response in seven different categories of SARS-CoV-2-naive or previously exposed and vaccinated individuals. The authors also used a mouse model to analyze the shift in humoral responses against previous Omicron and JN.1 lineages. The work highlights the antigenic singularity of JN.1-derived viruses, which elicit superior plasma neutralization titers against its sub-variants compared to the XBB lineage. The authors characterized ~2000 RBD-specific monoclonal antibodies (mAbs) with their targeting epitopes characterized by deep mutational scanning (DMS) and underscore the “superiority” of JN.1-elicited memory B cells. The authors also report a strong immune evasion and ACE2 binding of the currently dominant KP.3 sub-variant, confirming the need for JN.1 or KP.3-based vaccine boosters. The authors advocate for updating vaccine compositions. This is an important work. I don’t have major concerns. The manuscript is dense and huge, sometimes uneasy to read for non-specialists.

Main concerns

1. The authors could provide more detailed descriptions of the cohorts. Analyzing individuals that were COVID-naïve and infected with JN.1 strains is of great interest, even if only a few individuals were studied. How can the authors be sure that they have not been previously infected? Similarly, they describe individuals successively infected with different virus strains. Were the viruses sequenced in all individuals?
2. Fig. 2 were all individuals sampled at the same time after their last viral infection? This is not clear from the cartoon in Fig. 2A.

Referee #3

(Remarks to the Author)
In this detailed manuscript the authors defined the molecular transition from JN.1 to KP variants of SARS-CoV-2 and interrogate humoral immune responses. This has been an important viral evolution over the last few months.

The work is of technically high-quality

Points:

The high affinity of KP3, achieved through epistasis, is very interesting. The work of Li et al (PMID 39013463) showing the decreased ACE2 affinity of JN.1 is of note and could be quoted.

mRNA vaccines have formed a cornerstone of vaccine protection in many populations and as such this work is perhaps of less relevance to this population.

KP.3 is stated as the strongest variant for immune escape although the 1.9 to 2.4 fold reduction in IC50 compared to JN.1 is

relatively modest.The clinical significance of this reduction could be considered.

Are Omicron-specific antibodies completely selective for Omicron or cross-reactive with Wuhan/ancestral?

It is noted that variant subtype was inferred based on sampling timepoint and as such was not definitively assigned at infection.

The work would benefit from a demographics table of the donors with their age and gender, or at least mention which inactivated vaccine the donors have received.

It appears that that some vaccine-naïve cohorts generate WT-reactive antibodies and that the BA.5 + JN.1 infection cohort induced higher percentage of WT-reactive mAbs compared to vaccinated donors that had BA.5 + JN.1 infection, which is the opposite of what one might expect. It is suggested that those cross-reactive mAbs target non-neutralizing epitopes but it may be interesting to see why the authors think this percentage difference happened.

Of course this field moves very quickly. If a major aim of the paper is to prompt the consideration of changing vaccine composition from XBB to JN.1 then this has already happened in many countries. Recent European guidance has suggested continuing with JN.1 vaccine booster and not to shift to KP.2 variant. Information to guide this decision has been based on post-vaccine samples delivered by manufacturers and as such there is already a considerable evidence base. The need to shift to KP.3 has been deferred. As such, the work is slightly retrospective in nature for policy decision, although of strong technical ability.

Response to Referees

Referee #2 (Expertise: SARS-CoV-2, serology)

Jian et al explore the immune response to SARS-CoV-2 variants, focusing on the shift from XBB to JN.1 lineage. The study provides an extensive analysis of the humoral immune response in seven different categories of SARS-CoV-2-naive or previously exposed and vaccinated individuals. The authors also used a mouse model to analyze the shift in humoral responses against previous Omicron and JN.1 lineages. The work highlights the antigenic singularity of JN.1-derived viruses, which elicit superior plasma neutralization titers against its sub-variants compared to the XBB lineage. The authors characterized ~2000 RBD-specific monoclonal antibodies (mAbs) with their targeting epitopes characterized by deep mutational scanning (DMS) and underscore the "superiority" of JN.1-elicited memory B cells. The authors also report a strong immune evasion and ACE2 binding of the currently dominant KP.3 sub-variant, confirming the need for JN.1 or KP.3-based vaccine boosters. The authors advocate for updating vaccine compositions. This is an important work. I don't have major concerns. The manuscript is dense and huge, sometimes uneasy to read for non-specialists.

Response: Thanks for your careful review and positive comments for this study. We have improved the clarity and made the manuscript more friendly to the broad readership in the revised version.

Main concerns

1. The authors could provide more detailed descriptions of the cohorts. Analyzing individuals that were COVID-naïve and infected with JN.1 strains is of great interest, even if only a few individuals were studied. How can the authors be sure that they have not been previously infected? Similarly, they describe individuals successively infected with different virus strains. Were the viruses sequenced in all individuals?

Response: We have updated Supplementary Table 1 which include the information of the cohorts involved in this study. First, the COVID-naïve individuals involved in this study were actively under weekly or more frequent antigen or PCR tests before their first confirmed SARS-CoV-2 infection. The specific strains of infections were partially confirmed by sequencing, while others were inferred by the prevalent strain in the corresponding region (only when a dominant strain with over 90% prevalence exists). We discussed this approach in detail in the Methods section.

2. Fig. 2 were all individuals sampled at the same time after their last viral infection? This is not clear from the cartoon in Fig. 2A.

Response: This information is also included in the Supplementary Table 1. In brief, the interval between last exposure and sampling is 33±8.9 days (Mean±SD). We have added this information in the Methods.

Referee #3 (Expertise: Adaptive immunity, SARS-CoV-2):

In this detailed manuscript the authors defined the molecular transition from JN.1 to KP variants of

SARS-CoV-2 and interrogate humoral immune responses. This has been an important viral evolution over the last few months.

The work is of technically high-quality

Response: Thanks for your appreciation.

Points:

The high affinity of KP3, achieved through epistasis, is very interesting. The work of Li et al (PMID 39013463) showing the decreased ACE2 affinity of JN.1 is of note and could be quoted.

Response: Thanks for your suggestion. The newly published work of Li et al. provides important structural insights for the binding of JN.1 RBD to ACE2. We mentioned and cited it in the first section of results.

mRNA vaccines have formed a cornerstone of vaccine protection in many populations and as such this work is perhaps of less relevance to this population.

Response: We agree that this is an important drawback of this study, due to the limited accessibility of mRNA vaccinated cohorts in China. However, previous studies indicated that breakthrough infections and reinfections majorly induce WT-cross-reactive antibodies in mRNA vaccinated individuals (strong immune imprinting). Therefore, we separately analyzed the WT-reactive and Omicron specific humoral responses in the manuscript and the WT-reactive part is expected to imitate the responses in mRNA vaccinees. Indeed, recent studies also confirmed that Omicron BTI and reinfections in mRNA vaccinated individuals mainly elicit WT-reactive IGHV3-53/3-66-derived responses, which supports our approach and analysis (Paciello et al. bioRxiv 2024.03.03.583187).

KP.3 is stated as the strongest variant for immune escape although the 1.9 to 2.4 fold reduction in IC50 compared to JN.1 is relatively modest. The clinical significance of this reduction could be considered.

Response: The reduction in JN.1-infected cohorts is not so striking, which makes the KP.3 reinfection less likely occurred in patients who recovered from JN.1 infection. Therefore, JN.1-based vaccines are also likely to show acceptable protection. However, the stronger immune evasion and higher ACE2 binding will enable KP.3 and its subvariants to exhibit significant prevalence over JN.1 and other previous strains. If possible, KP.3 should be a better choice than JN.1 for vaccine boosters. We mentioned this in the Discussion part.

Are Omicron-specific antibodies completely selective for Omicron or cross-reactive with Wuhan/ancestral?

Response: The Omicron-specific antibodies are defined by negative ELISA to ancestral RBD, so they should be selective for Omicron.

It is noted that variant subtype was inferred based on sampling timepoint and as such was not definitively assigned at infection.

Response: Yes. We declared this in the Methods section. We believe this assignment of subtype is reasonable because the prevalent strain in China is not very diverse at the sampling timepoint

according to the epidemiological data. Generally, at the time of infection, the specific strain is over 90% in the corresponding region.

The work would benefit from a demographics table of the donors with their age and gender, or at least mention which inactivated vaccine the donors have received.

Response: We updated the Supplementary Table 1 which include more detailed information about the cohorts.

It appears that that some vaccine-naïve cohorts generate WT-reactive antibodies and that the BA.5 + JN.1 infection cohort induced higher percentage of WT-reactive mAbs compared to vaccinated donors that had BA.5 + JN.1 infection, which is the opposite of what one might expect. It is suggested that those cross-reactive mAbs target non-neutralizing epitopes but it may be interesting to see why the authors think this percentage difference happened.

Response: The phenomenon is not trivial but we believe it could be explained by the epitope masking of high amount of cross-reactive antibodies and B cells recalled by the first Omicron infection, which may enhance the response of Omicron-specific epitopes during the second Omicron exposure. This phenomenon is also observed in our previous study regarding BA.1/BA.2+BA.5 reinfection cohorts, which gave unexpected low ratio of WT-reactive mAbs (10.1038/s41586-023-06753-7).

Of course this field moves very quickly. If a major aim of the paper is to prompt the consideration of changing vaccine composition from XBB to JN.1 then this has already happened in many countries. Recent European guidance has suggested continuing with JN.1 vaccine booster and not to shift to KP.2 variant. Information to guide this decision has been based on post-vaccine samples delivered by manufacturers and as such there is already a considerable evidence base. The need to shift to KP.3 has been deferred. As such, the work is slightly retrospective in nature for policy decision, although of strong technical ability.

Response: Many preliminary data in this study have already provided valuable references supporting the changing of recommended vaccine composition in WHO and FDA meetings regarding SARS-CoV-2. KP.3 has already been dominant in multiple regions and it is foreseeable that KP.3-derived variants will become prevalent globally in the near future. The results could still provide important references for those that have not yet determined the vaccine update, and should also guide the determination of next seasonal SARS-CoV-2 vaccine updates.