

The SARS-CoV-2 Omicron BA.1 spike G446S mutation potentiates antiviral T cell recognition



Open Access This file is licensed under a Creative Commons Attribution 4.0

International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to

the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. In the cases where the authors are anonymous, such as is the case for the reports of anonymous peer reviewers, author attribution should be to 'Anonymous Referee' followed by a clear attribution to the source work. The images or other third party material in this file are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

This paper describes a comprehensive analysis of T-cell responses to the SARS-CoV-2 Spike protein in recipients of mRNA Covid vaccines with the common Asian HLA class I allele, A*2402. The authors first demonstrate the immunodominance hierarchy of 3 A24-restricted epitopes in a large group of vaccinees, then look in depth at responses to the 2 immunodominant epitopes (NF9 and QI9) in different SARS-CoV-2 strains.

The main finding is that NF9 specific responses are enhanced towards spike proteins expressing the G446S substitution, found in Omicron BA.1, which lies outside the NF9 epitope, and this is reflected in the ability of some donor cell-lines to suppress viral replication of BA.1 better than other strains. Further characterisation demonstrates that the TCRs of NF9 specific cells are better able to respond to processed antigen, suggesting that this mutation affects antigen processing. Using a series of protease inhibitors, the authors conclude that the mutation enhances processing by the TPP11 protease (however, I note that whilst the use of the TPP11 inhibitor in figure 3f just reaches significance, the graph suggests that Bestatin also diminishes T-cell recognition).

Overall, this paper is well-written and clear, and the experiments have been carefully performed. I only have one comment:

In previous studies of viral (HIV) escape from antigen processing, it was shown that the mutations affected proteasomal processing. I do not understand why the authors did not look at proteasomal processing as well as enzymes involved in e.r. peptide trimming.

Reviewer #2 (Remarks to the Author):

In this manuscript Motozono and colleagues report a fascinating observation on a mutation within SARS-CoV-2 spike protein (G446S) which significantly enhances the endogenous presentation of a HLA A24-restricted epitope. Enhanced endogenous presentation is not due alteration the binding of the peptide epitope to HLA molecule or TCR recognition of MHC-peptide complex. Authors show that the enhanced recognition of NF9 epitope is due to improved endogenous processing through an ER resident tripeptidyl peptidase II. Interestingly, authors found that G446S mutation which is specifically seen in Omicron BA.1 variant and leads to enhanced immune recognition, loss of this mutation in BA.2 and delta variants does not enhance immune recognition. Overall this an elegantly designed study with very impressive data. Authors have conducted all experimental studies diligently and have provided strong supporting evidence to argue their case. I have few minor comments which authors may like to consider while revising their manuscript.

Data presented in Fig. 1d & e shows expression of CD25 and CD137 on T cell following stimulation with NF9 and QI9 peptide epitopes. I was bit surprised why authors did not use HLA-peptide tetramers for these epitope which they already used in the data presented Fig. 1C. Expression of these markers are highly unreliable as a marker for antigen specificity. If authors want to include this data, they should provide a proper controls. No peptide is not an appropriate control. I would suggest they use another viral peptide (e.g. HIV or influenza).

I was wondering if authors can provide pairwise analysis of T cell responses to NF9 and QI9 peptides in same donors. In addition, it would be nice if they can also include some data from individuals who have been infected with BA.1 and BA.2 variants to show the dynamics of T cell response to NF9 and QI9 peptides. Do authors have any clinical data from infected (symptomatic and asymptomatic) HLA A24+ individuals and how their T cell responses to NF9 and QI9 differ and evolve over the course of primary infection.

Please correct figure number in Line 215. This should read Fig. 3f not Fig. 3d.

Reviewer #3 (Remarks to the Author):

The authors provide a report of mutational changes in SARS-CoV-2 variants that have differential impact on T cell recognition. They demonstrate that while single amino acid changes in delta ablate recognition of an HLA-A24 restricted epitope the response is augmented in Omicron by an amino acid change that is adjacent to the epitope. They provide convincing evidence to support their hypothesis using cell lines over-expressing the spike variants and using infection with different viral variants.

Specific comments.

1. The manuscript would be enhanced if it was possible to provide real-world data on what happens to the magnitude of peptide restricted responses after exposure to Omicron or Delta.
2. Figure 1b: Tetramer staining isn't completely convincing given some background staining shown in A24- volunteers. Could be enhanced by including non-vaccinated, non-infected A24+ controls.
3. Figure 1c: How was the cut-off of 0.1% defined as a positive response

1 REVIEWER COMMENTS

2
3 **Reviewer #1 (Remarks to the Author):**

4 This paper describes a comprehensive analysis of T-cell responses to the SARS-
5 CoV-2 Spike protein in recipients of mRNA Covid vaccines with the common Asian
6 HLA class I allele, A*2402. The authors first demonstrate the immunodominance
7 hierarchy of 3 A24-restricted epitopes in a large group of vaccinees, then look in
8 depth at responses to the 2 immunodominant epitopes (NF9 and QI9) in different
9 SARS-CoV-2 strains.

10
11 The main finding is that NF9 specific responses are enhanced towards spike proteins
12 expressing the G446S substitution, found in Omicron BA.1, which lies outside the
13 NF9 epitope, and this is reflected in the ability of some donor cell-lines to suppress
14 viral replication of BA.1 better than other strains. Further characterisation
15 demonstrates that the TCRs of NF9 specific cells are better able to respond to
16 processed antigen, suggesting that this mutation affects antigen processing. Using
17 a series of protease inhibitors, the authors conclude that the mutation enhances
18 processing by the TPPII protease (however, I note that whilst the use of the TPPII
19 inhibitor in figure 3f just reaches significance, the graph suggests that Bestatin also
20 diminishes T-cell recognition).

21
22 Overall, this paper is well-written and clear, and the experiments have been carefully
23 performed. I only have one comment:

24
25 **Our reply:**

26 We appreciate Reviewer 1's positive comments and are happy to hear that
27 "*Overall, this paper is well-written and clear, and the experiments have been*
28 *carefully performed*".

29
30 As suggested by the reviewer, it could be interpreted as bestatin modestly reduced
31 the average of T-cell recognition (**Fig. 3f**), although the difference was not
32 statistically significant ($p = 0.2765$ by ANOVA, with multiple comparisons by
33 Bonferroni correction; versus DMSO alone). Accordingly, we have added the
34 sentence to mention this in the revised manuscript (**page 7, line 224-226**).

35
36 Additionally, we noticed that the statistical analysis of DMSO alone in **Fig. 3f** was
37 determined by ANOVA, with multiple comparisons by Bonferroni correction, but not
38 Mann-Whitney test in the original manuscript (**page 7, line 215 and page 15, line**

39 457-458). We sincerely apologize for the mistake. We have corrected the sentence
40 in the revised manuscript (page 7, line 223-224 and page 15, line 470-471).

41

42 In previous studies of viral (HIV) escape from antigen processing, it was shown that
43 the mutations affected proteasomal processing. I do not understand why the authors
44 did not look at proteasomal processing as well as enzymes involved in e.r. peptide
45 trimming.

46

47 **Our reply:**

48 Thank you very much for this important suggestion. We agree that this is a
49 possible scenario. To confirm whether G446S involves proteasomal processing,
50 we performed a TCR sensitivity assay in the presence of MG-132 (a proteasome
51 inhibitor). There was no statistical difference between the sensitivity of NF9/A24
52 and QI9/A24-specific TCRs in the presence of MG-132 ($p = 0.8850$ and >0.9999 by
53 unpaired two-tailed Student's t-test; versus DMSO alone, respectively). We
54 included this observation in the revised manuscript with the data (page 7, line 213-
55 218 and page 17, line 502-508; **Extended Data Fig. 2c**).

56

57

58

59

60

61

62

63

64

65

66

67

68

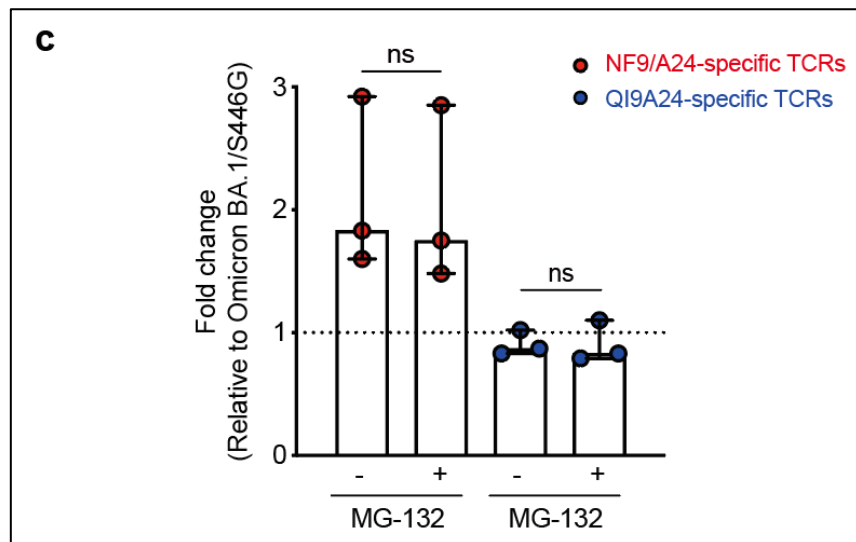
69

70

71

72

73



74 **Reviewer #2 (Remarks to the Author):**

75 In this manuscript Motozono and colleagues report a fascinating observation on a
76 mutation within SARS-CoV-2 spike protein (G446S) which significantly enhances the
77 endogenous presentation of a HLA A24-restricted epitope. Enhanced endogenous
78 presentation is not due alteration the binding of the peptide epitope to HLA molecule
79 or TCR recognition of MHC-peptide complex. Authors show that the enhanced
80 recognition of NF9 epitope is due to improved endogenous processing through an
81 ER resident tripeptidyl peptidase II. Interestingly, authors found that G446S mutation
82 which is specifically seen in Omicron BA.1 variant and leads to enhanced immune
83 recognition, loss of this mutation in BA.2 and delta variants does not enhance
84 immune recognition. Overall this an elegantly designed study with very impressive
85 data. Authors have conducted all experimental studies diligently and have provided
86 strong supporting evidence to argue their case. I have few minor comments which
87 authors may like to consider while revising their manuscript.

88
89 **Our reply:**

90 We are happy to hear that this reviewer feels that “*Overall this an elegantly designed*
91 *study with very impressive data*” and “*Authors have conducted all experimental*
92 *studies diligently and have provided strong supporting evidence to argue their case*”.

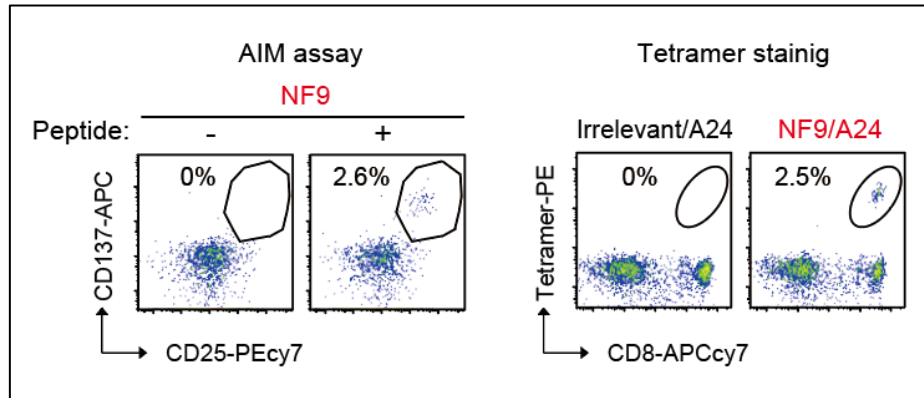
93
94 Data presented in Fig. 1d & e shows expression of CD25 and CD137 on T cell
95 following stimulation with NF9 and QI9 peptide epitopes. I was bit surprised why
96 authors did not use HLA-peptide tetramers for these epitope which they already used
97 in the data presented Fig. 1C. Expression of these markers are highly unreliable as
98 a marker for antigen specificity. If authors want to include this data, they should
99 provide a proper controls. No peptide is not an appropriate control. I would suggest
100 they use another viral peptide (e.g. HIV or influenza).

101
102 **Our reply:**

103 We thank the reviewer for this comment. The activation marker-induced (AIM) assay
104 has been extensively used to characterize antigen-specific T cell responses (Wolfl
105 et al. Blood, 2007. PMID: 17371945; Grifoni et al., Cell, 2020. PMID: 32473127;
106 Motozono et al. Cell Host Microbe, 2021. PMID: 34171266). We preliminarily
107 confirmed that there is no difference in the frequency of *in vitro*-expanded antigen-
108 specific T cells between AIM assay and tetramer staining, as shown below.

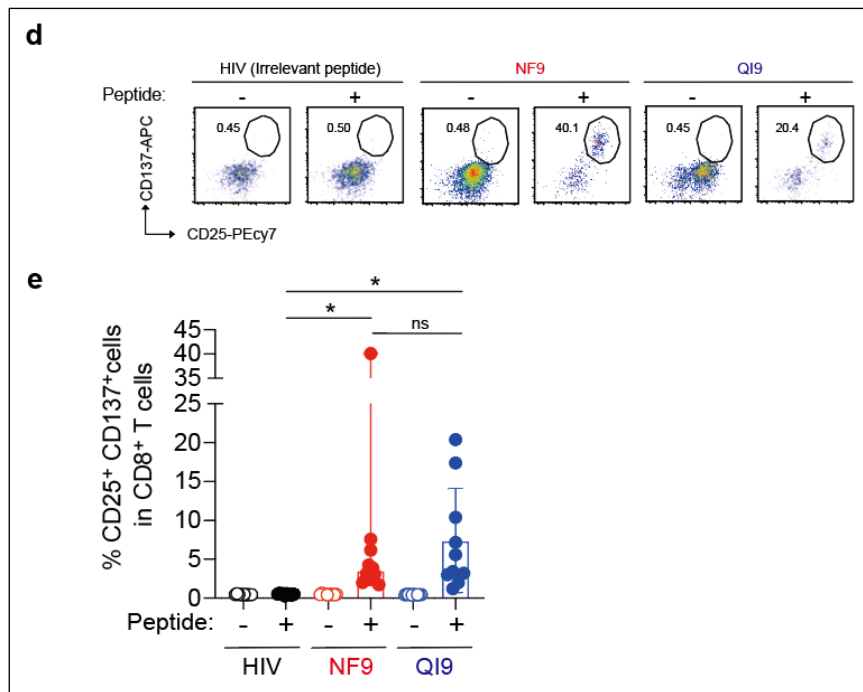
109
110

111
112
113
114
115
116
117
118
119



120 In addition to this assay (**Fig. 1d**), we confirmed the antigen-specificity of *in vitro*-
 121 expanded T cell lines used for functional assay by HLA tetramer in **Extended data**
 122 **Fig.1d**. However, we agree that the *in vitro* stimulation of PBMCs with the irrelevant
 123 peptide would be better as a negative control. According to the reviewer's suggestion,
 124 we stimulated PBMCs with representative HLA-A*24:02-restricted RF10 peptide
 125 (RYPLTFGWCF, residues 134-143 of the HIV Nef protein) and included it in **the**
 126 **revised Fig.1d and 1e** and manuscript (page 4, line 111, 112 and 114, page 14, line
 127 432 and page 19-20, line 594-595). Dr. Yoshihiko Goto has been added as an author
 128 to reflect the contribution of his experiments in the revised manuscript (page 1, line
 129 5, 6, 15-17, 19, 21, 23, 25, and page 11, line 308).

130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145

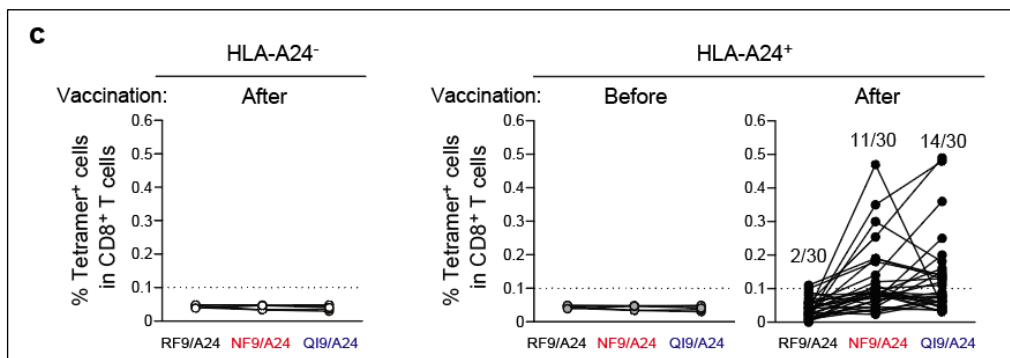


146 I was wondering if authors can provide pairwise analysis of T cell responses to NF9
 147 and QI9 peptides in same donors.

148

149 **Our reply:**

150 According to the reviewer's suggestion, we performed a pairwise analysis of T-cells
151 specific for the NF9 and the QI9 in the same donors in **the revised Fig.1c**. We have
152 added the data in non-vaccinated and seronegative HLA-A*24:02⁺ donors (n=5).
153 The new data are presented in **Fig. 1b and 1c** and (page 14, line 416-417, 419-
154 **422, and 430-431** of the revised manuscript).



165 In addition, it would be nice if they can also include some data from individuals who
166 have been infected with BA.1 and BA.2 variants to show the dynamics of T cell
167 response to NF9 and QI9 peptides. Do authors have any clinical data from infected
168 (symptomatic and asymptomatic) HLA A24⁺ individuals and how their T cell
169 responses to NF9 and QI9 differ and evolve over the course of primary infection.

170

171 **Our reply:**

172 We thank the reviewer for this interesting suggestion. In this study, we focused on
173 HLA-A*24:02-restricted vaccine-induced T cell responses against various SARS-
174 CoV-2 variants. However, we agree that it would be interesting to determine whether
175 an enhanced T cell response against Omicron BA.1 variant would be observed in
176 convalescents infected with Omicron BA.1 but not BA.2 variant, which is associated
177 with clinical outcome (severity, symptomatic or asymptomatic). We have started
178 collecting PBMC samples from convalescents with clinical data to address this
179 important question in a future study. Thank you again for the important suggestion.

180

181 Please correct figure number in Line 215. This should read Fig. 3f not Fig. 3d.

182

183 **Our reply:**

184 We sincerely apologize for the mistake. We have corrected it in the revised
185 manuscript (page 7, line 223).

186

187 **Reviewer #3 (Remarks to the Author):**

188 The authors provide a report of mutational changes in SARS-CoV-2 variants that
189 have differential impact on T cell recognition. They demonstrate that while single
190 amino acid changes in delta ablate recognition of an HLA-A24 restricted epitope the
191 response is augmented in Omicron by an amino acid change the is adjacent to the
192 epitope. They provide convincing evidence to support their hypothesis using cell
193 lines over-expressing the spike variants and using infection with different viral
194 variants.

195

196 **Our reply:**

197 We are happy to hear that this reviewer feels that “*They provide convincing evidence*
198 *to support their hypothesis using cell lines over-expressing the spike variants and*
199 *using infection with different viral variants*”.

200

201 Specific comments.

202

203 1.The manuscript would be enhanced if it was possible to provide real-world data on
204 what happens to the magnitude of peptide restricted responses after exposure to
205 Omicron or Delta.

206

207 **Our reply:**

208 We thank the reviewer for this important suggestion. We are now collecting SARS-
209 CoV-2-infected donors, rather than vaccine recipients in the current study, to
210 determine the magnitude of responses among peptides when exposed to Delta and
211 various sublineages of Omicron variants. We respectfully wish to address this
212 question in a future study.

213

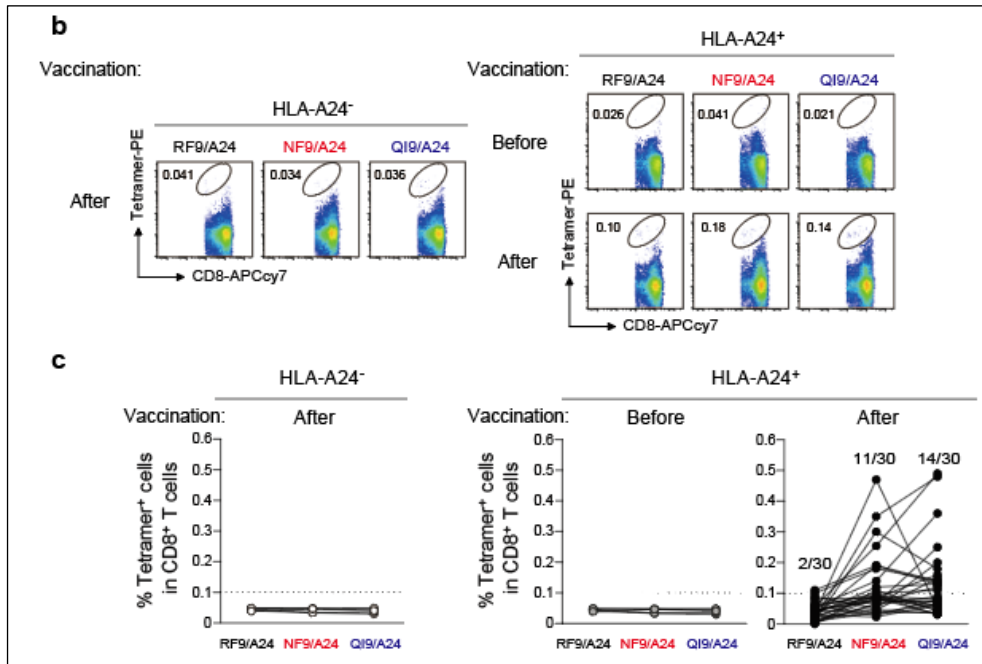
214 2. Figure 1b: Tetramer staining isn't completely convincing given some background
215 staining shown in A24- volunteers. Could be enhanced by included non-vaccinated,
216 non-infected A24+ controls.

217

218 **Our reply:**

219 In accordance with the reviewer's comment, we performed additional experiments
220 using HLA-A*24:02+ non-vaccinated and seronegative donors (n=5). The new data
221 are presented in **Fig. 1b and 1c** and (page 14, line 416-417, 419-422, and 430-431
222 of the revised manuscript).

223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243



3. Figure 1c: How was the cut-off of 0.1% defined as a positive response

Our reply:

We used a cut-off value determined by the median plus 4 x SD in the negative controls. To clarify this, we have added the sentence in the revised manuscript (page 14, line 430-431).

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have responded to all my concerns and I am very happy with the revised paper.

Reviewer #2 (Remarks to the Author):

Authors have addressed all issues raised by me and have provided detailed rebuttal for each comments. Text and figures have been appropriately revised to address all comments. I don't have any further comments or concerns.

Reviewer #3 (Remarks to the Author):

The authors have adequately addressed the requests from the reviewers

1 **REVIEWER COMMENTS**

2

3 Reviewer #1 (Remarks to the Author):

4 The authors have responded to all my concerns and I am very happy with the revised
5 paper.

6

7 **Our reply:**

8 We appreciate Reviewer 1's comments and are happy to hear that "I am very happy
9 with the revised paper".

10

11 Reviewer #2 (Remarks to the Author):

12 Authors have addressed all issues raised by me and have provided detailed rebuttal
13 for each comments. Text and figures have been appropriately revised to address all
14 comments. I don't have any further comments or concerns.

15

16 **Our reply:**

17 We thank the reviewer for this positive reply and are happy to hear that 'Text and
18 figures have been appropriately revised to address all comments'.

19

20 Reviewer #3 (Remarks to the Author):

21 The authors have adequately addressed the requests from the reviewers

22

23 **Our reply:**

24 We sincerely thank the reviewer for this positive reply.

25