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T Cell Cross-reactivity in Autoimmune-like Hepatitis Triggered by COVID-19

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Running head: TCR Cross-reactivity in Hepatitis Triggered by COVID-19

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1 # T cell cross-reactivity in autoimmune-like hepatitis triggered
2 by COVID-19

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20 Over 1,000 cases of pediatric hepatitis of unknown etiology have been reported
21 worldwide since the first case was reported in the UK. To date, the etiology of pediatric
22 hepatitis remains unknown and controversial. Adenovirus was first suspected to be the
23 cause as it was present in the blood samples of the majority of cases. Partial cases have
24 also been tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-
25 CoV-2) [1]. However, it is still unclear how these viruses contribute to pediatric
26 hepatitis. In the case of a pediatric patient with SARS-CoV-2 infection, the liver biopsy
27 showed acute submassive hepatocyte necrosis, accompanied by a significant increase
28 in T cell infiltration [2]. Furthermore, CD8 T-cell dominant hepatitis induced by
29 coronavirus disease 2019 (COVID-19) vaccination has also been recently reported [3].
30 Although it is known that T cell receptors (TCRs) can discriminate between self- and
31 non-self-antigens, it is now well-accepted that TCRs exhibit cross-reactivity toward
32 similar and even distinct antigen peptides [4]. Thus, we hypothesized that following
33 SARS-CoV-2 infection or vaccination, T cells carrying TCRs that recognize self-
34 antigens undergo clonal expansion, which could eventually result in the onset of
35 autoimmune-like hepatitis (Figure 1A).

36 To test our hypothesis, we aimed to identify clonally expanded TCRs that recognize
37 self-antigens following SARS-CoV-2 infection. We cross-referenced two publicly
38 available TCR binding datasets, namely VDJdb and ImmuneCODE, as TCR with
39 similar complementarity determining region 3 of β chain (CDR3β) sequences may
40 recognize the same antigen [5, 6]. Recent studies have suggested cross-reactivity
41 between SARS-CoV-2 and a wide spectrum of viruses, such as cytomegalovirus (CMV)
42 and seasonal coronavirus [7]. As expected, we observed a substantial number of TCRs
43 displaying cross-reactivity with T cell epitopes derived from SARS-CoV-2, Epstein-
44 Barr virus (EBV), and/or the human proteome (Figure 1B). Ten CDR3β sequences were
45 identified, which demonstrated cross-reactivity with SARS-CoV-2- and human
46 proteome-derived T cell epitopes. Five of them were also present in an independent
47 dataset of TCR repertoires from COVID-19 patients (Figure 1C). Of note, two of them
48 (CASSLGQAYEQYF and CASSLGYEQYF) were found to exhibit specificity towards
49 T cell epitopes derived from EBV. We next compared the incidence of different CDR3

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50 sequences in the healthy control individuals and COVID-19 cohorts [6, 8] and found a
51 significant increase in the frequency of multiple CDR3β sequences, including the
52 CDR3β sequence CASSLGQAYEQYF ($p<2.2e-16$, Wilcox. test) identified above,
53 suggesting the clonal expansion of T cells with these CDR3β sequences in COVID-19
54 patients (Figure 1C). From a paired TCRαβ dataset that was identified by single-cell
55 TCR sequencing using DNA-barcoded TCR-dextramers in individuals with SARS-
56 CoV-2 antigen exposures [9], we identified a TCR containing the same COVID-19-
57 enriched CDR3β sequence CASSLGQAYEQYF (Figure 1D), namely CoV-TCR.
58 Coincidentally, CoV-TCR shares an identical TCRβ sequence with the LC13-TCR,
59 which has been previously reported to recognize an immunodominant epitope
60 (FLRGRAFGL) of EBV presented by human leukocyte antigen (HLA)-B*08:01 and
61 also cross-reacts with multiple other peptides, including a self-peptide (EEYLQAFTY)
62 from the ATP Binding Cassette Subfamily D Member 3 (ABCD3) protein. In addition,
63 their TCRα sequences were found to be highly similar, except for a single amino acid
64 substitution in the CDR3α region (CILPLAGGTSYGKLTF versus
65 CILPLLGGTSYGKTF). ABCD3 is a peroxisomal membrane protein that can transport
66 various fatty acids. Notably, its expression is much higher in liver tissues than in other
67 normal tissues, according to the Human Protein Atlas (Figure 1E). Additionally,
68 hepatocytes exhibit the highest level of ABCD3 gene expression compared to other
69 types of cells. Furthermore, both binding and cytotoxic assays demonstrated that the
70 CoV-TCR recognizes HLA-B*44:05-restricted self-peptide derived from ABCD3
71 (EEYLQAFTY) (Figure 1F-K). Unexpectedly, we failed to detect the interaction
72 between the HLA-A*01:01-restricted SARS-CoV-2 peptide (TTDPSFLGRY) derived
73 from Nonstructural protein 3 (NSP3) and the CoV-TCR, which had previously been
74 identified in a large-scale detection of SARS-CoV-2-specific T cells using DNA-
75 barcoded MHC-dextramers [9].

76 Thus, our study identified a CoV-TCR that recognizes self-peptide derived from
77 ABCD3, which is one of the most abundant peroxisomal membrane proteins in
78 hepatocytes, from COVID-19 patients. Although this TCR does not recognize the
79 previously reported SARS-CoV-2 antigenic peptide, it is possible that it could

80 recognize other antigenic peptides derived from SARS-CoV-2 that have not yet been
81 identified or reported. Alternatively, the expansion of T cell clones with the same
82 CDR3β sequence CASSLGQAYEQYF could also be attributed to the reactivation of
83 EBV in COVID-19 patients. In conclusion, our findings support the notion that cross-
84 reactivity of clonally expanded T cells could be one of the causes of COVID-19-related
85 autoimmune-like hepatitis, including pediatric hepatitis of unknown etiology. Further
86 comprehensive research and investigation are warranted to explore this possibility in
87 greater detail.

88 **MATERIALS AND METHODS**

89 **T cell receptor repertoire sequencing data collection**

90 The T cell receptor (TCR) repertoire data were obtained from the immuneACCESS®
91 database. TCR sequencing data for coronavirus disease 2019 patients were accessed
92 from http://adaptivebiotech.com/pub/covid-2020 [6], while the healthy control data
93 were derived from http://adaptivebiotech.com/pub/Dean-2015-GenomeMed [8].

94 **TCR repertoire data analysis**

95 Initially, Immunarch was utilized to convert and load the ImmunoSEQ format files.
96 Following this, data from 1,318 coronavirus disease 2019 (COVID-19) patients and
97 587 healthy controls were imported into the application for analysis. The V(D)J
98 distribution features of the COVID-19 group as well as healthy controls were extracted,
99 and the clonotype differences between the two groups were assessed. Subsequently,
100 TCR data were annotated using VDJdb, and the further annotated TCRs were
101 transformed into frequencies for inter-group statistical evaluation [10]. Finally,
102 unpaired Wilcoxon tests and data visualization were performed using relevant R
103 packages.

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104 **Cell lines and primary cells**

105 HEK-293T (ATCC, Manzas, Virginia, USA), Jurkat E6-1 (ATCC, Manzas, Virginia,
106 USA), and HepG2 (ATCC, Manzas, Virginia, USA) cells were obtained from the
107 American Type Culture Collection (ATCC, Manzas, Virginia, USA). Primary human
108 peripheral blood mononuclear cells (PBMC) from healthy donors were purchased from
109 Sailybio (Sailybio, Shanghai, China). HEK-293T and HepG2 cells were cultured in
110 DMEM medium (Gibco, Grand Island, NYC, USA) supplemented with 10% (*v*/*v*) fetal
111 bovine serum (FBS) (Gibco, Grand Island, NYC, USA) and 1% (*v*/*v*) penicillin-
112 streptomycin (Gibco, Grand Island, NYC, USA). Jurkat cells were cultured in RPMI
113 1640 medium (Hyclone, Logan, Utah, USA) supplemented with 10% (*v*/*v*) FBS, 1%
114 (*v*/*v*) penicillin-streptomycin, 0.1M HEPES (Gibco, Grand Island, NYC, USA), 1mM
115 sodium pyruvate (Gibco, Grand Island, NYC, USA), 1% (*v*/*v*) non-essential amino
116 acids (Gibco, Grand Island, NYC, USA) and 50 μM β-mercaptoethanol (Sigma-Aldrich,
117 St.Louis, Missouri, USA).

118 **DNA constructs**

119 A murine stem cell virus-based retroviral vector encoding Cov-TCR, LC13-TCR, or
120 F5-TCR genes carrying murine TCR constant regions had the format LNGFR△-P2A-
121 TCRα-F2A-TCRβ. LNGFR△ is a transduction marker comprising low-affinity nerve
122 growth factor receptors with the truncated intracellular domain. A lentiviral vector
123 encoding eGFP and SCT composed of antigenic peptide (ABCD3, EEYLQAFTY;
124 NSP3, TTDPSFLGRY), β2-microglobulin, and HLA-B*44:05 or HLA-A*01:01
125 domains via flexible glycine-serine linkers were prepared with a disulfide trap

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126 modification. We also construct lentiviral vectors encoding β2-microglobulin, and
127 HLA-B*44:05 or HLA-A*01:01 domains, respectively. CD8 was subcloned into a
128 retroviral vector.

129 **Cell lines construction**

130 Retroviruses encoding Cov-TCR, LC13-TCR, F5-TCR or CD8 were produced in HEK-
131 293T cells by transient transfection of retroviral-based plasmids and their packaging
132 vectors (pRD114 and pHIT60) using TransIT-293 (Mirus Bio, USA) according to the
133 manufacturer's protocol. Lentiviruses encoding HLA molecule were produced in HEK-
134 293T cells by transient transfection of lentiviral-based vectors and their packaging
135 vectors (psPAX2 and pMD2.G). After 48 h transfection, the virus was collected and
136 filtered through a 0.45 μm syringe filter for infection. The Jurkat and HepG2 cells were
137 spin-infected with viral supernatant supplemented with 10 μg/mL polybrene (Sigma-
138 Aldrich, St.Louis, Missouri, USA) at 2500 rpm at 30 °C for 90 min respectively. After
139 48 h post-infection, $TCR^{hi}CD8^{hi}$ Jurkat cells and $\beta 2M^{hi}eGFP^{hi}$ HepG2 cells were sorted
140 by FACS to establish derivative cell lines.

141 **Primary T cell activation and retroviral transduction**

142 PBMC were cultured in RPMI 1640 medium supplemented with 5% (*v*/*v*) human serum
143 (Gemini), 1% (*v*/*v*) penicillin-streptomycin, 0.1M HEPES, 1mM sodium pyruvate, 1%
144 (*v*/*v*) non-essential amino acids and 50 μM β-mercaptoethanol in the presence of human
145 IL-2 (300U/mL, Peprotech). Anti-human CD3 (1μg/mL, Biolegend, California, USA)
146 and anti-human CD28 (1μg/mL, Biolegend, California, USA) monoclonal antibodies
147 were used to activate PBMCs. After 48 h activation, $1x10^6$ PBMCs per 24-well plate

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148 were used for viral transduction. The majority of the medium was replaced with viral
149 supernatant supplemented with 10 μg/mL polybrene. After 24 h, the cells were
150 centrifugation at 2500 rpm at 30 °C for 90 min, and further incubated with fresh
151 medium containing 300U/mL human IL-2 and 1μg/mL anti-human CD28 for 24 h.
152 Then the above transduction procedure was repeated, and all cells were cultured in a
153 fresh medium for further use. LNGFR was used to quantify the infection efficiency.
154 mTCRβ was used to quantify the expression of TCR on the cell membrane 24 h after
155 secondary infection. The transduced primary T cells were cultured for 48 h and then
156 used for cytotoxicity assay.

157 **Peptide loading in antigen-presenting cells**

158 Lyophilized peptides (LifeTein, New Jersey, USA) were redissolved in
159 dimethylsulfoxide (Sigma-Aldrich, St.Louis, Missouri, USA) at 10mM and then diluted
160 in water to a final concentration of 10 μM. HepG2 cells (50,000 total, $0.5x10^6$ cells/mL)
161 were pulsed with 100 μL of peptide solution in a 96-well U-bottom plate and incubated
162 for 2 h at 37 °C. After incubation, 100 μL of the medium was added to each well and
163 then centrifuged for 5 min at 1500 r.p.m. The cells were washed once with 200 μL of
164 medium and then resuspended in 100 μL of medium for cytotoxicity assay.

165 **Co-culture of primary human T cells and cytotoxicity assay**

166 TCR-expressing primary T cells and peptide-pulsed APCs were washed and re-
167 suspended in a fresh RPMI 1640 medium and then plated on a 96-well U-bottom plate
168 in various effector-to-target ratios at 5:1 (target cells number is $2x10^4$) for co-incubated
169 for 6 h at 37 °C, 5% CO_2. After co-incubation, the plate was centrifuged for 5 min at

170 1500 rpm. The cells were resuspended with 50 μL of PBS and then frozen at -20°C for
171 1 h to lysis. The T cell-mediated cytotoxicity was determined by assessing the
172 intercellular luciferase activity of remaining living target cells. Briefly, the luciferase
173 substrate was dispensed into each cell-containing well at a 1:1 ratio. The plate was
174 gently agitated in the dark to stabilize the luminescence. The luminescent signal of each
175 plate was then read by a multifunctional microporous plate. Triplicate wells were
176 averaged and percent lysis was calculated from the data with the following equation: %
177 specific lysis=100× (Maximum fluorescence value – test fluorescence
178 value)/(Maximum fluorescence value).

179 **Flow cytometry and FACS**

180 Flow cytometric analysis of cell lines and primary T cells was performed according to
181 standard protocols. The following antibodies were purchased from BioLegend and used
182 at 1:200(*v/v*) in flow cytometry buffer (PBS With 2%FBS): PE-cy7-conjugated anti-
183 mouse TCRβ (clone H57-597, Biolegend, California, USA), APC-conjugated anti-
184 human NGFR (clone ME20.4, Biolegend, California, USA), Pacific Blue- conjugated
185 anti-human CD8 (clone HIT8a, Biolegend, California, USA). For surface marker
186 staining, cells were washed once in flow cytometers buffer before staining and stained
187 with fluorescently conjugated antibodies for 30 min on ice. Then, the stained cells were
188 washed twice in a flow cytometer buffer and 7-AAD viability staining solution
189 (Biolegend, California, USA) was added at 1:100 (*v/v*) before analysis using BD
190 LSRFortessa. FACS was performed using BD FACSAria III instruments.

191 **Lentiviral antigen-specific infections**

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192 Lentiviruses encoding SCT were produced in HEK-293T cells by transient transfection
193 of lentiviral-based vectors and their packaging vectors (psPAX2 and pMD2.G with
194 mutated VSVG) with linear 25 kDa polyethyleneimine (PEI) (Polysciences, USA) at a
195 3:1 mass ratio of PEI to DNA. After 48 h transfection, the virus was collected and
196 filtered through a 0.45 μm syringe filter for infection. The Jurkat cells expressing Cov-
197 TCR or LC13-TCR were spin-infected with viral supernatant supplemented with 10
198 μg/mL polybrene (Sigma-Aldrich, St.Louis, Missouri, USA) at 2500 rpm in 30 °C for
199 90 min respectively. After 24h postinfection, collected the cells and washed twice in a
200 flow cytometer buffer before analysis for infection via flow cytometry.

201 **pMHC tetramer staining**

202 Cells were resuspended into a FACS buffer containing 1:20(*v*/*v*) pMHC tetramer and
203 incubated for 20 min at 4 °C. Cells were washed twice and then analyzed *via* flow
204 cytometry.

205 **Statistical analysis**

206 The cytotoxicity assay statistical analysis data was done in GraphPad Prism software
207 using an unpaired *t*-test. Data are reported as mean ± s.e.m. *$P < 0.05$; **$P < 0.01$;
208 ***$P < 0.001$; NS, not significant.

209

210

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221

222 **DECLARATION OF COMPETING INTERESTS**

223 The authors declare that they have no competing interests.

224

225 **ETHICS APPROVAL**

226 This study did not involve clinic studies or mouse experiments. Human peripheral blood
227 mononuclear cells (PBMCs) from healthy donors were purchased from Jun-X
228 biotechnology.

229

230 **DATA AVAILABILITY**

231 All data are available by requirements to the corresponding authors
232 wangjw28@163.com (J. Wang), and lgd@ism.cams.cn (G. Li).

233

234 **AUTHOR CONTRIBUTIONS**

235 Conceptualization, Y.L. and G.L.; Bioinformatics analysis, Y.L.; Experimental
236 validation, Y.W. and Z.P.; Data analysis, Y.W., Z.P., Y.L., J.W., and G.L.; Writing,
237 Y.W., Y.L., J.W., and G.L.; Supervision, J.W. and G.L. All authors had full access to
238 all study data and had final responsibility for the decision to submit for publication.

239

240 **REFERENCES**

241 [1] Brodin, P. & Arditi, M. Severe acute hepatitis in children: investigate SARS-CoV-2
242 superantigens, *Lancet Gastroenterol Hepatol* 2022; 7: 594-595.

243 [2] Osborn, J., Szabo, S. & Peters, A. L. Pediatric Acute Liver Failure Due to Type 2
244 Autoimmune Hepatitis Associated With SARS-CoV-2 Infection: A Case Report, *JPGN*
245 *Rep* 2022; 3:204.

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246 [3] Boettler, T., Csernalabics, B., Salié, H., Luxenburger, H., Wischer, L., Salimi Alizei, E,
247 et al. SARS-CoV-2 vaccination can elicit a CD8 T-cell dominant hepatitis, *J Hepatol*
248 2022; 77: 653-659.
249 [4] Joglekar, A. V. & Li, G. T cell antigen discovery, *Nat. Methods* 2021; 18: 873-880.
250 [5] Shugay, M., Bagaev, D. V., Zvyagin, I. V., Vroomans, R. M., Crawford, J. C., Dolton,
251 G, et al. VDJdb: a curated database of T-cell receptor sequences with known antigen
252 specificity, *Nucleic Acids Res* 2018; 46: 419-427.
253 [6] Nolan, S., Vignali, M., Klinger, M., Dines, J. N., Kaplan, I. M., Svejnoha, E, et al. A
254 large-scale database of T-cell receptor beta (TCRβ) sequences and binding associations
255 from natural and synthetic exposure to SARS-CoV-2, *Res Sq* 2020; 3.
256 [7] Pothast, C. R., Dijkland, R. C., Thaler, M., Hagedoorn, R. S., Kester, M. G. D., Wouters,
257 A. K., Hiemstra, P. S., van Hemert, M. J, et al. SARS-CoV-2-specific CD4(+) and CD8(+)
258 T cell responses can originate from cross-reactive CMV-specific T cells, *eLife* 2022; 11:
259 e82050.
260 [8] Dean, J., Emerson, R. O., Vignali, M., Sherwood, A. M., Rieder, M. J., Carlson, C. S, et
261 al. Annotation of pseudogenic gene segments by massively parallel sequencing of
262 rearranged lymphocyte receptor loci, *Genome Med* 2015; 7:123.
263 [9] Minervina, A. A., Pogorelyy, M. V., Kirk, A. M., Crawford, J. C., Allen, E. K., Chou, C.
264 H, et al. SARS-CoV-2 antigen exposure history shapes phenotypes and specificity of
265 memory CD8(+) T cells, *Nat Immunol* 2022; 23:781-790.
266 [10] Shugay, M., Bagaev, D. V., Zvyagin, I. V., Vroomans, R. M., Crawford, J. C., Dolton,
267 G, et al. VDJdb: a curated database of T-cell receptor sequences with known antigen
268 specificity, *Nucleic Acids Res* 2018; 46: 419-427.
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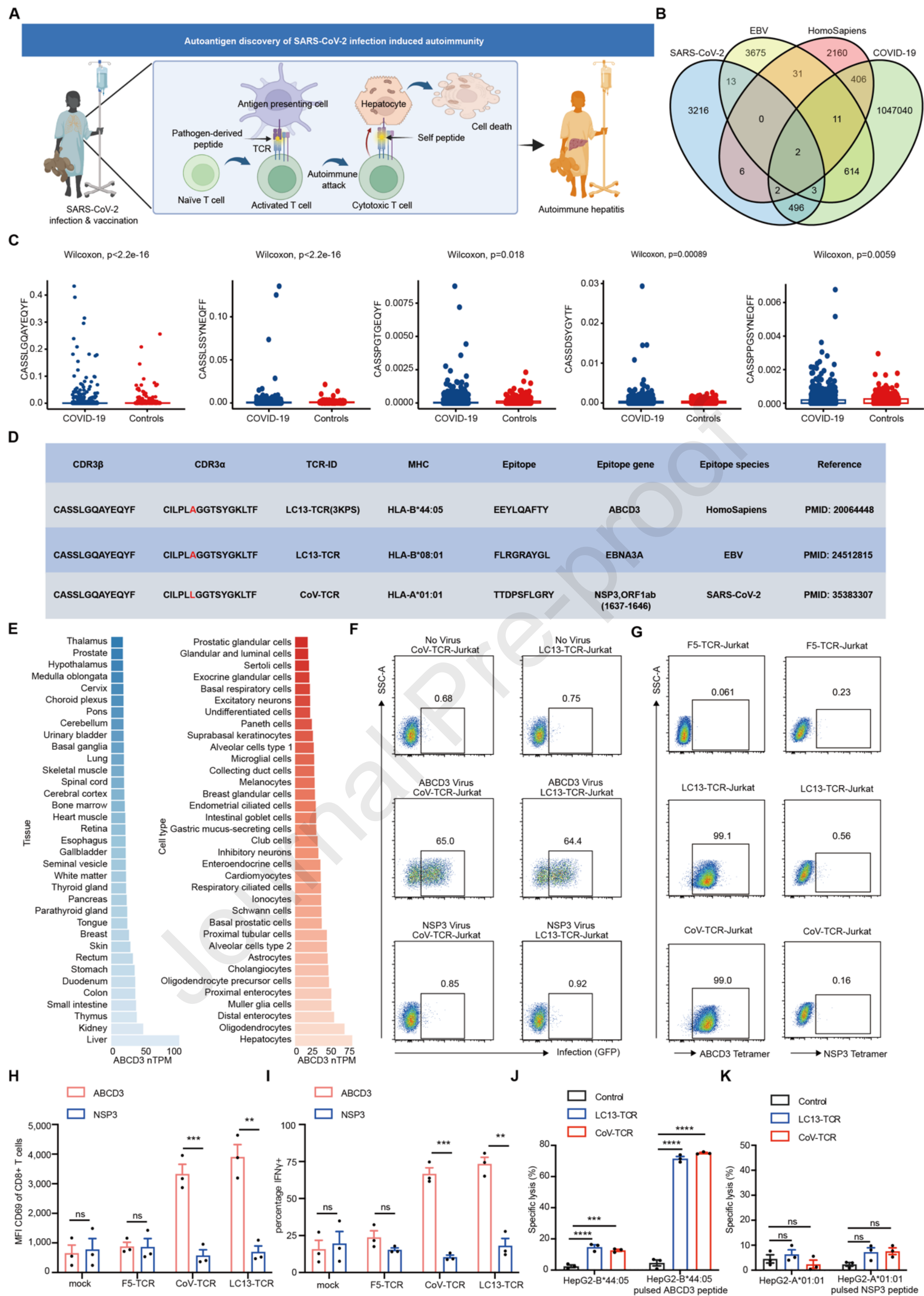

Figure 1. TCR cross-reactivity in COVID-19 patients

(A) The proposed immunological mechanisms underlying COVID-19-related autoimmune-like hepatitis. **(B)** Venn diagram showing the overlap of antigen-specific CDR3β sequence (SARS-CoV-2, human proteome and EBV) obtained from VDJdb

278 database with TCR repertoire of COVID-19 patients obtained from ImmuneCODE. **(C)**
279 The incidence of five representative CDR3β sequences in the healthy control
280 individuals and COVID-19 cohorts. **(D)** Annotation of LC13-TCR and CoV-TCR. **(E)**
281 Transcriptional expression levels of ABCD3 in different human organs(left) and
282 different cell types (right). **(F)** Recognition validation via interaction-dependent
283 infection mediated by VSVGmut lentiviruses. **(G)** Experimental validation of the
284 recognition ability of LC13-TCR and CoV-TCR for HLA-B*44:05-restricted self-
285 peptide derived from ABCD3 (EEYLQAFTY) or HLA- A*01:01-restricted peptide
286 derived from NSP3 (TTDPSFLGRY) using tetramer binding assay. **(H)** Expression of
287 CD69 in $CD8^+$ T cells expressing different TCRs co-cultured with ABCD3-B*44:05 or
288 NSP3-A*01:01-expressing HepG2 cells. **(I)** Level of IFNγ in $CD8^+$ T cells expressing
289 different TCRs co-cultured with ABCD3-B*44:05 or NSP3-A*01:01-expressing
290 HepG2 cells. **(J)** Cytotoxicity of LC13-TCR or CoV-TCR T cells against HLA-
291 B*44:05-expressing HepG2 cells and HLA-B*44:05-expressing HepG2 cells pulsed
292 with ABCD3 self-peptide (EEYLQAFTY). **(K)** Cytotoxicity of LC13-TCR or CoV-
293 TCR T cells against HLA-A*01:01-expressing HepG2 cells and HLA-A*01:01-
294 expressing HepG2 cells pulsed with SARS-CoV-2 NSP3 protein peptide
295 (TTDPSFLGRY). The data are presented as percentage-specific lysis. n=3 independent
296 samples. Data are presented as mean±s.e.m and P values are determined by a two-tailed
297 Student's t-test, ns= not significant, $^{**}P < 0.01$ and $^{***}P < 0.001$ and $^{****}P < 0.0001$.
298 Abbreviations: COVID-19, coronavirus disease 2019; TCR, T cell receptor; SARS-
299 CoV-2, severe acute respiratory syndrome coronavirus 2; CDR3β, complementarity
300 determining region 3 of β chain; EBV, Epstein-Barr virus; ABCD3, ATP binding
301 cassette subfamily D member 3; HLA, human leukocyte antigen; NSP3, nonstructural
302 protein 3
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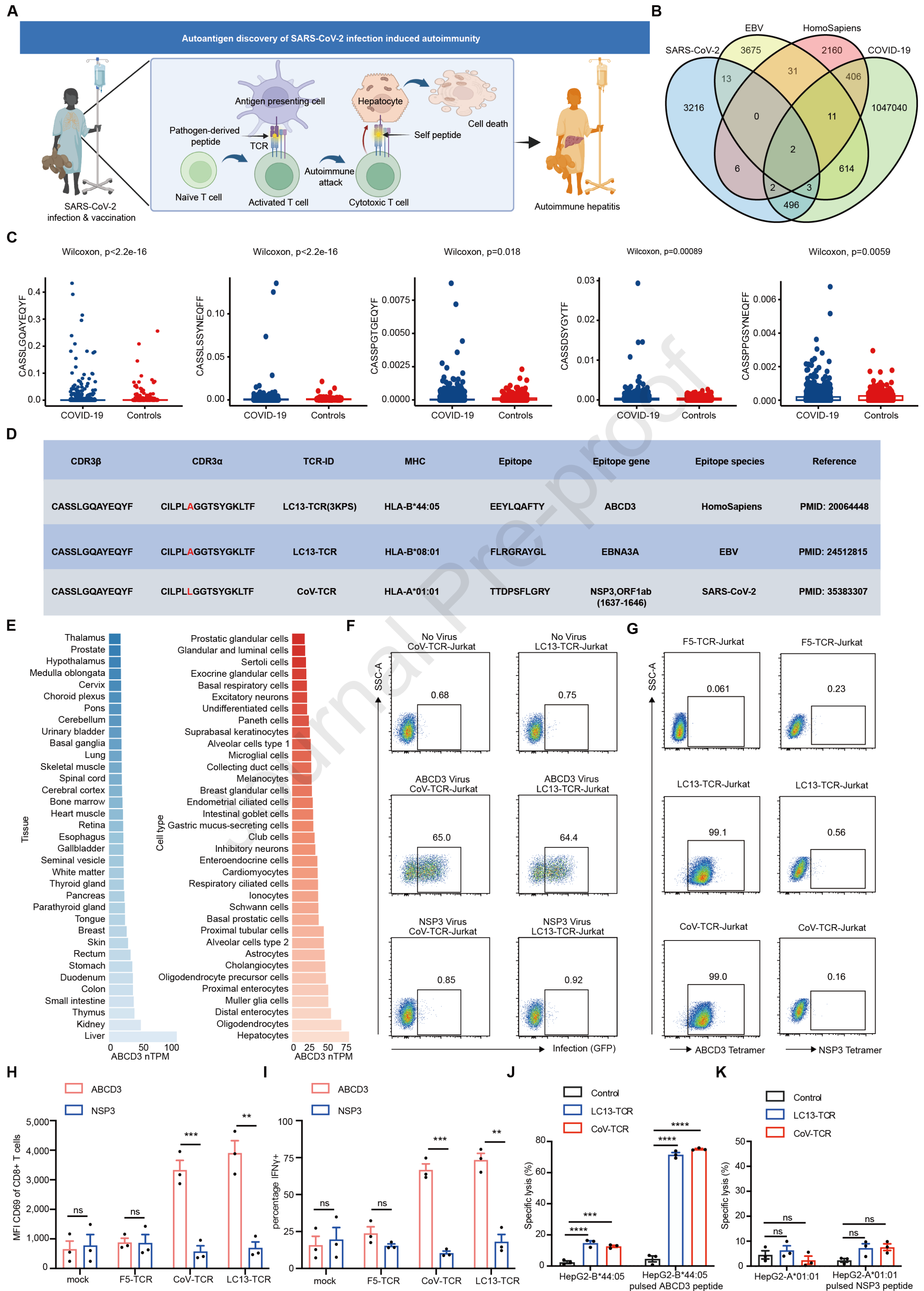

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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