Long COVID following mild SARS-CoV-2 infection: characteristic T cell alterations and response to antihistamines

Paul Glynne, Natasha Tahmasebi, Vanya Gant, Rajeev Gupta

ABSTRACT
Long COVID is characterized by the emergence of multiple debilitating symptoms following SARS-CoV-2 infection. Its etiology is unclear and it often follows a mild acute illness. Anecdotal reports of gradual clinical responses to histamine receptor antagonists (HRAs) suggest a histamine-dependent mechanism that is distinct from anaphylaxis, possibly mediated by T cells, which are also regulated by histamine. T cell perturbations have been previously reported in post-viral syndromes, but the T cell landscape in patients who have recovered from mild COVID-19 and its relationship to both long COVID symptoms and any symptomatic response to HRA remain underevolved. We addressed these questions in an observational study of 65 individuals who had recovered from mild COVID-19. Participants were surveyed between 87 and 408 days after the onset of acute symptoms; none had required hospitalization, 16 had recovered uneventfully, and 49 had developed long COVID. Symptoms were quantified using a structured questionnaire and T cell subsets enumerated in a standard diagnostic assay. Patients with long-COVID had reduced CD4+ and CD8+ effector memory (EM) cell numbers and increased PD-1 (programmed cell death protein 1) expression on central memory (CM) cells, whereas the asymptomatic participants had reduced CD8+ EM cells only and increased CD28 expression on CM cells. 72% of patients with long COVID who received HRA reported clinical improvement, although T cell profiling did not clearly distinguish those who responded to HRA. This study demonstrates that T cell perturbations persist for several months after mild COVID-19 and are associated with long COVID symptoms.

INTRODUCTION
As of August 2021, there have been in excess of 200 million cases of COVID-19 worldwide, with more than 4.4 million deaths (https://covid19.jhu.edu/map.html). Not all patients surviving the acute infection recover rapidly and uneventfully. Initial resolution of the acute illness may be followed by a combination of clinical sequelae, including but not limited to pulmonary, neurologic, dermatologic, cardiac, renal, endocrine and autoimmune phenomena, collectively described as long COVID. Importantly, such symptoms may develop following apparent recovery from an initially mild acute illness that did not require medical intervention. It is not uncommon for at least one symptom to persist up to and beyond 7 months.

The majority of patients presenting with long COVID will not have been hospitalized or sustained significant end-organ damage during their initial illness. In contrast to acute COVID-19, it predominantly affects younger patients with few comorbidities, who had relatively mild initial infectious symptoms and who did not come to medical attention until their long COVID symptoms appeared. It is expected to place increasing burdens on healthcare systems, and by delaying the return of these often working people to normal life, it will have wider societal and economic impacts.
Long COVID shares many features with other post-
viral and idiopathic chronic fatigue syndromes. Persistent
alterations both in the numbers of various T cell subtypes
and their biological properties have been reported in such
disorders. In acute COVID-19, perturbations in both B
and T cells are observed irrespective of disease severity. Studies of T cells from individuals who have recovered from
COVID-19 have thus far focused on antigen specificity,
primarily to understand cellular immunity to SARS-CoV-2. Beyond this, a detailed survey of any immuno-
logic sequelae that may persist following clearance of
COVID-19, and none had received prior immunomodulatory medications. Volunteers who had unevent-
fully and rapidly recovered from proven COVID-19 infec-
tion were recruited as controls.

METHODS

Study setting

The Physicians’ Clinic (TPC; part of HCA healthcare UK, the study sponsor) is a private outpatient and diagnostic
center in London.

Study design

This is a prospective observational study of patients previ-
ously diagnosed with mild COVID-19 who have initially
recovered, subsequently developed persistent protean
symptoms suggestive of long COVID. Participants were
recruited between November 2020 and April 2021. None
had previously sought medical attention or ‘treatment’ for
acute COVID-19, none had a history of autoimmunity, and
none had received immunomodulatory medications.

We recruited 49 patients with long COVID (‘symptomatic group’, symptoms >84 days following acute
COVID-19 infection; physician or laboratory diagnosis) to
undergo blood sampling to measure several hematologic
and biochemical variables, and for flow cytometry. The
tests were also offered to 16 volunteer clinician colleagues, all of whom had either PCR or serological evidence of
COVID-19 and who had recovered uneventfully (‘asympto-
matic group’). The age and gender distributions of the
two groups were similar.

A symptom questionnaire was designed with a binary
symptom grid to initially record the presence (score 1) or
absence (score 0) of the following long COVID symptom
categories: fatigue, constitutional upset (sweats, fever,
arthralgia, myalgia), breathlessness, post-exertional
malaise (PEM), chest pain, neurologic (headaches, neuro-
sensory, brain fog), neuropsychiatric (anxiety, insomnia),
dysautonomia (postural tachycardia), ear, nose and throat
symptoms, gastrointestinal disturbance (food intolerance,
diarrhea, bloating), and dermatological manifestations
(rashes, flushing, urticaria), to give a maximum possible
symptom score of 11. The questionnaires were collated by
one of us (NT) who was not involved in the management of
the patients, and blinded to their clinical details.

Initially, patients were offered supportive care only. However, as the study progressed, all patients were offered
empiric treatment trials with a combination of H1 (lorata-
dine 10 mg two times per day or fexofenadine 180 mg
two times per day) and H2 (famotidine 40 mg once daily or niza-
tidine 300 mg once daily) receptor antagonists (HRAs) for
a minimum of 4 weeks as part of their ongoing care. Of the 49
long COVID-19 study participants, 26 patients consented
to try HRA. Between 4 and 16 weeks after starting treat-
ment, both HRA-treated and untreated patients were asked
to grade their symptoms as now absent, identical, better,
or worse (online supplemental figure 1). In this analysis,
‘absent’ or ‘better’ scored 0, and ‘identical’ or worse, 1.

Flow cytometry and additional laboratory measurements

This was performed on peripheral blood collected in EDTA.
The Beckman Coulter TQ-prep whole blood lysis system
(BS3328, Beckman Coulter) as described previously. This
is in routine diagnostic use in our clinical practice, and
normal ranges had been previously established (in 2018)
in healthy adults with normal automated blood counts for
accreditation purposes. Analysis of primary flow cytometry
data was in Kaluza C (Beckman Coulter) and was under-
taken by one of us (RG), who was blinded to clinical infor-
mation collected and collated by others. The gating strategy
and phenotypes analyzed are shown in online supplemental
figure 2. An automated full blood count was performed on
every specimen in parallel to flow cytometry. T cell popula-
tions were quantified as a percentage of total cellularity, and
absolute numbers calculated from the corresponding total
white cell count. Single cell antigen density was recorded as
median fluorescence intensity (MFI).

Statistical analysis

Comparison of clinical data from the asymptomatic and
symptomatic groups was by Mann-Whitney t-test. Compari-
on of response to HRA was by Wilcoxon matched pairs
signed-rank test. All flow cytometry fluorescence data and
numbers of T cell subpopulations were assumed to lie in
non-Gaussian distributions, and analysis of variance was by
Patient characteristics

Forty-nine patients with a diagnosis of long COVID were recruited. Twenty-five had either PCR or serological evidence for COVID-19. The remainder had suffered their acute illnesses at the start of the pandemic, when PCR testing was not widely available in the UK, and had not subsequently returned positive serology tests. Only one had been vaccinated at the time of enrollment into the study. We recruited 16 individuals known to have had acute COVID-19 infection but who had recovered rapidly and uneventfully to serve as asymptomatic controls. The majority in this group are healthcare professionals; all received at least one vaccination dose (Pfizer) at the time of referral and participation in the study. Patients with long COVID were relatively young (mean 43; range 25–63 years) and showed a female preponderance (29/49; 60.4%). Seventeen out of 49 patients with long COVID had a history of atopy, which is in keeping with reports that atopy is predictive of mild acute disease.14

Routine blood tests

These were undertaken at first presentation to our clinic and included full blood count, erythrocyte sedimentation rate (ESR), C reactive protein (CRP), D-dimer, renal and liver function tests. The results were within the normal range in almost every case. Two out of 49 patients had mildly elevated CRP (NR=<5 mg/L) and 4 out of 49 had a mildly elevated ESR (NR=<15 mm/hour). Circulating total and differential leucocyte numbers were normal in all patients; one patient had an incidental borderline normocytic anemia.

Treatment with HRA

At first presentation, symptoms were categorized using the symptom grid. Twenty-six patients (16 female, 10 male, mean age 44 years) were treated empirically with HRA, and 23 (14 female, 9 male, mean age 41) either declined HRA or were not offered them because they were first seen before HRA treatment became part of our practice. All patients were offered standard supportive care and advice to control symptoms (NICE guideline NG188: https://www.nice.org.uk/guidance/ng188/RCGP/SIGN guidelines).

The symptom profiles in the treated and untreated cohorts were similar (table 2), the mean symptom burdens were 4.28/11 (range 1–8/11) and 4.91/11 (range 1–10/11). In the treated group, HRA reduced average symptom to 2.68/11 (range 0–6/11) (table 2, figure 1A). The mean time to response was 29.6 days (median 26 days; range 6–89 days). Nineteen patients (72%) experienced a reduction in their symptoms: 5 (20%) reported complete resolution of all symptoms, 13 (52%) reported some improvement, 6 in 3 (14 female, 9 male, mean age 41) either declined HRA or were not offered them because they were first seen before HRA treatment became part of our practice. All patients were offered standard supportive care and advice to control symptoms (NICE guideline NG188: https://www.nice.org.uk/guidance/ng188/RCGP/SIGN guidelines).

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The 23 patients who did not receive HRA were also reassessed between 28 and 119 days after their initial blood tests (median 56 days). Twenty-six per cent reported some spontaneous improvement in their symptoms. One experienced a full recovery, and in five, there was a reduction in some but not all symptoms. However, the majority (15, 65%) reported no change, and 2 (9%) developed new additional symptoms (figure 1B). There was no correlation between SARS-CoV-2 antibody status and spontaneous resolution of symptoms in this group.

T cell compartments

Flow cytometry was performed once as part of the initial blood tests. Despite the time that had elapsed from the acute COVID-19 illness (see table 1, long COVID symptomatic

<table>
<thead>
<tr>
<th>Table 1 Clinical features of study participants</th>
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<tbody>
<tr>
<td>Clinical characteristics</td>
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<tr>
<td>---------------------------</td>
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<tr>
<td>Age range (median)</td>
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<tr>
<td>Female (%)</td>
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<tr>
<td>Ethnicity</td>
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<tr>
<td>White</td>
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<tr>
<td>Asian</td>
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<tr>
<td>Black</td>
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<tr>
<td>Mixed</td>
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<td>Comorbidities</td>
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<td>1 cancer, 1 controlled hypertension</td>
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<td>Allergy or atopy</td>
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<td>Mean days from acute COVID to study testing</td>
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<tr>
<td>Vaccination history at time of recruitment (at least one dose)</td>
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<tr>
<td>SARS-CoV-2 antibodies detected</td>
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<tr>
<td>Long COVID symptoms</td>
</tr>
<tr>
<td>Fatigue</td>
</tr>
<tr>
<td>Constitutional</td>
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<tr>
<td>Breathlessness</td>
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<tr>
<td>Post-exertional malaise</td>
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<tr>
<td>Chest pain (non-cardiac)</td>
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<tr>
<td>Neurologic/neurosensory</td>
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<tr>
<td>Neuropsychiatric</td>
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<tr>
<td>Dysautonomia (postural tachycardia syndrome)</td>
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<tr>
<td>Ear, nose and throat</td>
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<tr>
<td>Gastrointestinal</td>
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<tr>
<td>Dermatological</td>
</tr>
</tbody>
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Kruskal-Wallis H test. Dunn’s multiple comparison test was then used to estimate statistical significance. All statistical analysis, including principal component analysis, was in Prism V9.1 (GraphPad Software, LLC).

RESULTS

Patient characteristics

Almost all patients with long COVID were polysymptomatic (95.8%) as described by others,11 with an average of 4.58/11 typical symptoms (range 1–10). The average symptom duration was 268.9 days (range 87–402) at the time of referral and participation in the study. Patients with long COVID were relatively young (mean 43; range 25–63 years) and showed a female preponderance (29/49; 60.4%). Seventeen out of 49 patients with long COVID had a history of atopy, which is in keeping with reports that atopy is predictive of mild acute disease.14

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Table 2  Symptom profiles of treated and untreated cohorts

<table>
<thead>
<tr>
<th>Symptom group</th>
<th>Number of patients reporting symptom</th>
<th>HRA treated (26)</th>
<th>Untreated (23)</th>
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<tbody>
<tr>
<td></td>
<td>Initial assessment</td>
<td>Follow-up assessment</td>
<td>Initial assessment</td>
</tr>
<tr>
<td>Fatigue</td>
<td>17/26</td>
<td>12/26</td>
<td>15/23</td>
</tr>
<tr>
<td>Constitutional upset</td>
<td>10/26</td>
<td>7/26</td>
<td>16/23</td>
</tr>
<tr>
<td>Breathlessness</td>
<td>6/26</td>
<td>5/26</td>
<td>9/23</td>
</tr>
<tr>
<td>Post-exertional malaise</td>
<td>12/26</td>
<td>11/26</td>
<td>16/23</td>
</tr>
<tr>
<td>Chest pain</td>
<td>13/26</td>
<td>6/26</td>
<td>6/23</td>
</tr>
<tr>
<td>Neurologic</td>
<td>13/26</td>
<td>4/26</td>
<td>17/23</td>
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<tr>
<td>Neuropsychiatric</td>
<td>7/26</td>
<td>4/26</td>
<td>11/23</td>
</tr>
<tr>
<td>Dysautonomia</td>
<td>4/26</td>
<td>4/26</td>
<td>6/23</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>8/26</td>
<td>5/26</td>
<td>5/23</td>
</tr>
<tr>
<td>Dermatological</td>
<td>9/26</td>
<td>3/26</td>
<td>7/23</td>
</tr>
<tr>
<td>ENT</td>
<td>10/26</td>
<td>8/26</td>
<td>7/23</td>
</tr>
<tr>
<td>Average symptom</td>
<td>4.28±1.70</td>
<td>2.60±1.97</td>
<td>4.91±2.57</td>
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</tbody>
</table>

ENT, ear, nose and throat; HRA, histamine receptor antagonist.

87–408 days, asymptomatic controls 100–404 days), we observed marked perturbations in the numbers of circulating EM T cells (automated total lymphocyte counts were normal).

Twenty-five out of 49 symptomatic and 3 out of 16 asymptomatic participants had CD4 + EM counts that were below the lower limit of the normal range (figure 2A), and receiver operating characteristic (ROC) analysis confirmed that the CD4 + EM count could distinguish the two groups (figure 2B). Forty-three out of 49 patients with long COVID and 14 out of 16 of the asymptomatic group had reduced CD8 + EM counts, which were below the median of the normal range (figure 2C). Although the mean count was lower in the long COVID than in the asymptomatic group, the CD8 + EM count did not distinguish the two cohorts in an ROC analysis (figure 2D). All other T cell compartments, including CD4 + and CD8+ central memory (CM) cells, naïve T cells and TEMRA cells, were within normal limits.

Our flow cytometry antibody panel allowed us to compare the expression levels (antigen densities) of proteins important for regulating T cell function.15 We observed that the antigen density of PD-1 (programmed cell death protein 1, CD279) was significantly increased in both CD4 + and CD8+ CM cells in all participants, although this was more marked in symptomatic patients with long COVID (figure 3A). Intriguingly, CD28 expression was significantly increased in CD4 + CM cells in the asymptomatic group, but not the long COVID group (figure 3B). Expression of both proteins was similar in all other T cell compartments, and expression of CD57 did not vary significantly.

Although both the numbers of circulating EM T cells and combined PD-1 and CD28 antigen density in CM cells distinguished asymptomatic from symptomatic participants (figure 2B, online supplemental figure 3), neither predicted responsiveness to HRA (online supplemental figure 4).

**DISCUSSION**

Most patients with long COVID have mild or asymptomatic initial infections and are often diagnosed months later. We report clinical and immunologic features in 49 such patients, comparing them with an age-matched cohort who made full recoveries from COVID-19. We documented their symptoms, quantified their T cell subcompartments and scored their response to HRA.

The majority were younger females with few comorbidities, although 35% were previously atopic (table 1). Many were polysymptomatic, presenting up to 400 days after initial infection. Our key observations are (1) HRA reduces symptom burden in 72% of patients. (2) CD8 + EM T cells are reduced for up to 400 days following COVID-19 irrespective of symptoms. (3) Long COVID is associated with reduced CD4 + EM T cells. (4) CD4 + and CD8+ CM cell PD-1 levels are increased following COVID-19 (more marked in long COVID). (5) Increased CD4 + CM CD28 levels correlate with asymptomatic recovery from COVID-19.

The gradual response to HRA argues against a classical anti-anaphylactic mechanism of action. T cells express both H1 and H2 histamine receptors,16 and famotidine also desensitizes T cells through beta-arrestin,17 18 which suggests that in long COVID, HRA acts through T cells. All symptoms improved except dysautonomia, suggesting that this arises through another mechanism. Indeed, dysautonomia following other viral infections is associated with autoantibodies to adrenergic and cholinergic receptors,19 and COVID-19 is linked with autoimmune phenomena.19 20

To our knowledge, this is the first report of a rapid turnaround, routine laboratory test detecting persisting abnormalities in the T cell landscape many months after mild COVID-19. We identify a late, chronic phase of the T cell response, perhaps linked to that reported in acute infection.21 22 CD8 + EM cells are reduced irrespective of symptoms, which at this stage likely reflects the recovery from COVID-19 itself, rather than the development of long COVID. Consistent with this, dominant CD8 + responses in acute COVID-19 correlate with milder disease, suggesting a protective role through suppression of inflammation.7 9
T cell changes specific to long COVID include reduced CD4+ EM cells, and increased PD-1 on both CD4+ and CD8+ CM cells. PD-1 is a coinhibitor, contributing to T cell memory, and is associated with T cell exhaustion in chronic viral infection. Increased CD28, a costimulator essential for signal transduction and T cell triggering, was limited to those who had made an uneventful recovery from COVID-19 and may represent a ‘healthy’ immune response to SARS-CoV-2. Our detection of T cell perturbations so late after infection suggests that SARS-CoV-2 could persist for longer than originally assumed. In this regard, we note preliminary observations of reverse transcription of SARS-CoV-2 RNA and genomic integration in human cells.

Our observations encourage further detailed clinical studies of the potential benefit of HRA and a deeper immunologic analysis of why the T cell landscape is perturbed in long COVID. Furthermore, T cell flow cytometry could be developed to provide a rapid and straightforward diagnostic test for long COVID.
and approved. Patients and volunteers who consented to participate were subsequently fully informed of the analysis results, which were discussed face to face. The Institutional Review Board approval ID is 15:15/12/20.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. All relevant data are included in the manuscript.

Supplemental material This content has been supplied by the author(s).

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REFERENCES


Figure 3 Central memory (CM) T cell programmed cell death protein 1 (PD-1) and CD28 levels distinguish patients with long COVID from the asymptomatic fully recovered group. (A) PD-1 antigen density expressed as median fluorescence intensity (MFI, arbitrary units (AU)) in CD4+ (left) and CD8+ CM cells. CD4 + CM: Healthy volunteers, mean 292 AU, range 246–321 AU. Asymptomatic, mean 313 AU, range 279–381 AU. Symptomatic, mean 313 AU, range 259–383 AU. p values <0.05 indicated Kruskal-Wallis test. CD8 + CM: Healthy volunteers, mean 292 AU, range 249–329 AU. Asymptomatic, mean 344 AU, range 274–477 AU. Symptomatic, mean 352 AU, range 256–451 AU. Asymptomatic, mean 508 AU, range 435–532 AU. Symptomatic: mean 508 AU, range 437–563 AU. CD8 + CM: Healthy volunteers, mean 469 AU, range 420–512 AU. Asymptomatic: mean 499 AU, range 420–512 AU. Symptomatic: mean 508 AU, range 437–563 AU. CD8 + CM: Healthy volunteers, mean 496 AU, range 435–532 AU. Asymptomatic: mean 528 AU, range 453–572 AU. Symptomatic: mean 508 AU, range 437–563 AU. CD8 + CM: Healthy volunteers: mean 469 AU, range 420–512 AU. Asymptomatic: mean 499 AU, range 438–547 AU. Symptomatic: mean 472 AU, range 395–532 AU. Significant p values are indicated.

Correction notice This article has been corrected since it was first published to include the Institutional Review Board approval ID.

Contributors PG, VG and RG conceived the project and co-wrote the manuscript. PG saw the patients and documented clinical symptoms. NT collated clinical and laboratory information. RG performed all flow cytometry analysis. RG and VG undertook all statistical analysis.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval The study protocol, patient consent form, information leaflets and questionnaire were submitted to the host Institutions’ IRB


Patients recruited n=49

Initial long-COVID symptom score, Blood tests n=49

Offered HRA n=43

Accepted n=26

Declined n=17

Presented before HRA entered our practice n=6

HRA Treated n=26

HRA + Supportive care

Untreated n=23

Supportive care only

4-16 weeks

Follow up long-COVID symptom score

Supplemental Figure 1
Flow chart showing study design
Supplemental Figure 2

Gating strategy used to identify T cell compartments
Supplemental Figure 3

Scatter plots showing CD28 and PD1 antigen density in CD4+ (left) and CD8+ EM cells following mild COVID-19. Symptomatic (Long-COVID) and asymptomatic recovered group as indicated. ULN, upper limit of normal. LLN, lower limit of normal.

Supplemental Figure 3
**Supplemental Figure 4**

Principal component analysis (PCA) of CD4+EM, CD8+EM, and CD4+CM and CD8+CM antigen densities of PD1 and CD28 in in long-COVID patients who did (brown circles) and did not respond to HRA (green).
Supplemental Figure 1

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