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LETTER

Association between Epstein-Barr-Virus reactivation and development of Long-COVID fatigue

To the Editor,

Post-viral fatigue after SARS-CoV-2 infection is a major concern in the ongoing COVID-19 pandemic. Up to 10% of patients develop Long-COVID,[1] a syndrome defined by the National Institute for Health and Care Excellence as post-infectious long-term symptoms for more than 12 weeks, which cannot be explained by alternative pathologies.[1] While some Long-COVID symptoms, such as the shortness of breath or chest pain, might be associated with organ damage, the origin of post-COVID fatigue, including debilitating fatigue and impaired memory and concentration, remains unclear. Several viruses were associated with post-viral fatigue, such as Epstein–Barr-Virus (EBV) or influenza viruses. Also, myalgic encephalomyelitis/chronic fatigue syndrome, a WHO defined independent disease, may develop, amongst other triggers, after viral infections. However, the mechanisms leading to post-viral fatigue syndrome in overall and especially in Long-COVID are unresolved.[2] So far, a higher frequency of EBV viremia was observed only in acute severe COVID-19 infections.[3]

In the present prospective study, we analysed whether Long-COVID fatigue may be triggered by SARS-CoV-2 persistence in the gastrointestinal or respiratory tract after acute disease,[2] or by COVID-19-associated reactivation of EBV.

We tested for SARS-CoV-2 RNA in stool and throat washings, and for EBV DNA in stool, throat washings and blood by real-time PCR (EBV) and real-time RT-PCR (SARS-CoV-2) as described before.[4,5] The experimental procedures were performed with permission of the human ethics committee of the Medical University of Vienna (vote number: 2281/2020). All patients provided written informed consent. Samples were collected between 74 and 471 days (median: 235 days) after beginning of acute SARS-CoV-2 infection in 30 Long-COVID patients characterized by persistent fatigue, post-exertional malaise (PEM), autonomic dysfunction and/or orthostatic intolerance. Twenty age- and sex-matched patients, who have fully recovered after the SARS-CoV-2 infection, were recruited for control purposes. Samples were collected between 106 and 571 days (median: 275 days) after beginning of acute SARS-CoV-2 infection. All patients had mild infections, were not hospitalized and were infected between spring 2020 and autumn 2021. We further analysed virus-specific antibodies including SARS-CoV-2 IgA and IgG with commercial ELISA assays (Euroimmun). As Long-COVID patients showed a higher frequency of positive titres for specific EBV antibodies compared with controls in earlier studies,[6] the patients' EBV IgM and IgG were tested by a commercial microarray (ViraMed). Demographic data as well as inclusion and exclusion criteria are depicted in Table S1. More detailed information on the methodological procedures is listed in the Supporting Information.

At time point of sampling, SARS-CoV-2 RNA was detectable neither in throat washing nor stool in any of the study participants by real-time RT-PCR. SARS-CoV-2 antibody titres (IgA and IgG) did not differ between the cohorts (Figure S1). Most study participants had been vaccinated after COVID-19 disease prior to sampling (24/30 in Long-COVID patients, 17/20 in controls). EBV real-time PCR was negative in all blood or stool samples. However, EBV DNA was detected in throat washings in 15/30 (50%) of Long-COVID patients while only in 4/20 (20%) of non-Long-COVID patients who had recovered from their SARS-CoV-2 infection ($p = .0411$). EBV load levels were not significantly different between the two cohorts in EBV-specific real-time PCR positive samples (Figure 1). All patients of both groups, except one patient in the fully convalescent COVID-19 group (SARS-CoV-2 – LC), had past EBV infections, as confirmed by EBV IgG seropositive and IgM seronegative status at the time of investigation, thus, the EBV replication observed was due to EBV reactivation rather than primary infection. EBV specific antibody titres as assessed by microarray in blood were not different between the groups (Figure S2).

Taken together, SARS-CoV-2 persistence could not be detected in our study participants up to 10 weeks after infection, but EBV reactivation in the throat was more common in patients with Long-COVID fatigue, also months after acute SARS-CoV-2 infection, compared with convalescent SARS-CoV-2 patients. This suggests that EBV replication

Johanna Rohrhofer, Marianne Graninger, Elisabeth Puchhammer-Stöckl, and Eva Untersmayr contributed equally to this work.

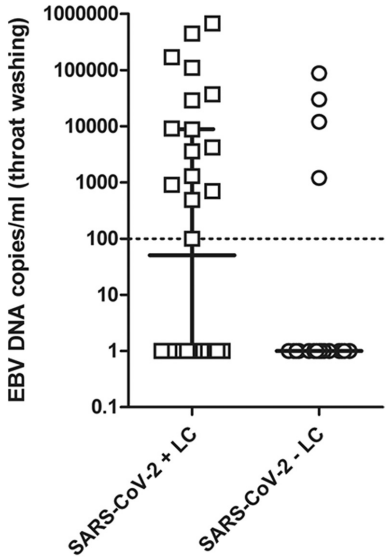

FIGURE 1 EBV DNA copies/ml measured by in-house real-time PCR in throat washing samples of patients after SARS-CoV-2 infection with Long-COVID fatigue (SARS-CoV-2 + LC) and without Long-COVID fatigue (SARS-CoV-2 − LC). Bars indicate median plus interquartile range. Dotted line indicates the detection limit. Statistical analysis was performed by Mann–Whitney *U* test. A *p*-value < 0.05 was considered significant.

may be a co-factor in a sub-group of patients developing Long-COVID fatigue. However, contributing factors, such as HLA-subtype depending on the answers to latent EBV infection, have not been evaluated in this study and represent limitations. Earlier reports of differences in the EBV antibody profile of Long-COVID patients[6] could not be confirmed, possibly due to the small sample size analysed in our study. Further and larger studies are needed to clarify the impact and mechanism of EBV-associated Long-COVID fatigue.

AUTHOR CONTRIBUTIONS

RJ, PSE and UE involved in conceptualization. RJ, GM, LL, GSA, KL and RD involved in methodology. RJ, GM, LL and SJ involved in formal analysis. SM, PSE and UE involved in resources. RJ and GM involved in data curation. RJ, GM, PSE and UE involved in writing original draft preparation. RJ, GM, LL, SJ, GSA, KL, RD, SM, PSE and UE involved in writing—review and editing. RJ and GM involved in visualization. UE involved in supervision and project administration. PSE and UE involved in funding acquisition. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Johanna Rohrhofer[1]
Marianne Graninger[2]
Lisa Lettenmaier[1]
Johannes Schweighardt[1]
Salvatore Alessio Gentile[1]
Larissa Koidl[1]
Davide Ret[1,3]
Michael Stingl[4]
Elisabeth Puchhammer-Stöckl[2]
Eva Untersmayr[1]

[1]*Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria*
[2]*Center for Virology, Medical University of Vienna, Vienna, Austria*
[3]*Division of Macromolecular Chemistry, Institute of Applied Synthetic Chemistry, Vienna University of Technology, Vienna, Austria*
[4]*Facharzt Zentrum Votivpark, Vienna, Austria*

Correspondence
Elisabeth Puchhammer-Stöckl, Center for Virology, Medical University of Vienna, 1090 Vienna, Austria.
Email: elisabeth.puchhammer@meduniwien.ac.at
Eva Untersmayr, Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, 1090 Vienna, Austria.
Email: eva.untersmayr@meduniwien.ac.at

ORCID

Johanna Rohrhofer https://orcid.org/0000-0002-2783-2099
Marianne Graninger https://orcid.org/0000-0002-2152-7798
Larissa Koidl https://orcid.org/0000-0001-9974-7929
Davide Ret https://orcid.org/0000-0002-7461-0923
Elisabeth Puchhammer-Stöckl https://orcid.org/0000-0002-0673-8335
Eva Untersmayr https://orcid.org/0000-0002-1963-499X

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.