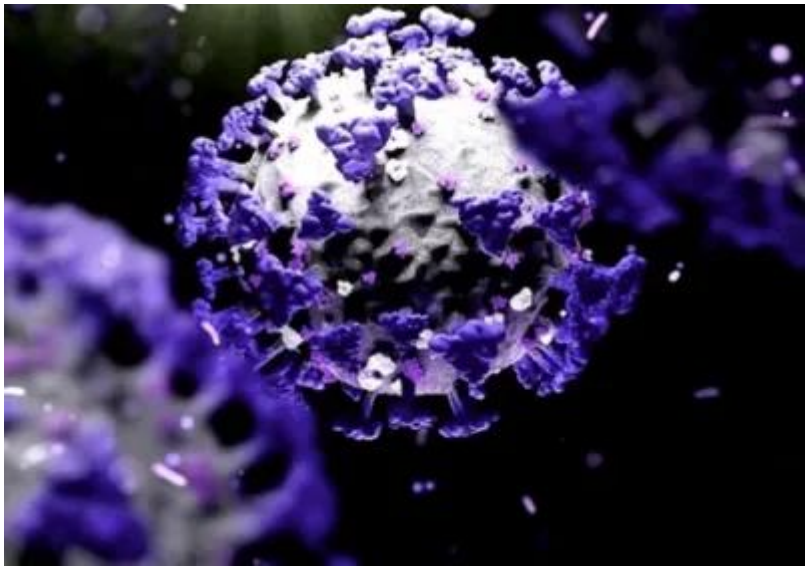
Human endogenous retroviruses (HERVs) are relics of ancient infections, characterized by an RNA intermediate reverse-transcribed into a double-stranded DNA (dsDNA). This dsDNA, called a provirus, can integrate into the host cell's genome. Because of such endogenization and further fixation in the human population, HERVs have been vertically transmitted to offspring in a Mendelian fashion, constituting up to ~8% of the human genome. A consortium of authors conducted this study to investigate whether severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) induces the expression of HERV-W or HERV-K envelope (ENV) proteins (HERV-W ENVs) in cultured peripheral blood mononuclear cells (PBMCs) and plasma of severe COVID-19 patients and *postmortem* tissues obtained from patients deceased from severe acute COVID-19.

HERVs are roughly divided into three classes: Class I comprises gamma-retroviruses, including HERV-H, and HERV-W, Class II comprises beta-retroviruses, including HERV-K and Class III comprises foamy viruses, such as HERV-L and HERV-S. HERV-K is the most active and intact group of endogenous retroviruses within the genome of primates. HERV genome consists of four essential genes (gag, pro, pol, and env). The env gene encodes ENV protein, which was shown to be neurotoxic.

Usually, most HERVs are epigenetically silenced or silenced by a mutation. However, they may be activated under certain conditions, including irradiation, chemical exposures, or exogenous viral factors. When activated by a specific infectious agent, HERVs with pathogenic activity can cause clinical manifestations corresponding to the tissue in which they are expressed. They are considered "dormant enemies within", and some have significant immunopathogenic and/or neuropathogenic effects *in vitro* and *in vivo.*

HERV-W group has been extensively investigated for its putative role in several diseases, such as cancer, inflammation, and autoimmunity (for example, in multiple sclerosis). Despite considerable interest in the link between HERV-W expression and the pathogenesis of human diseases, no conclusive correlation has been demonstrated. Grandi N, Tramontano E. Viruses. 2017 Jun 27;9(7):162. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5537654/

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. Its genome encodes four structural proteins, namely the spike (S), envelope (E), nucleocapsid (N), and membrane (M) protein. The S protein appears to be a major pathogenic factor that contributes to the unique pathogenesis of SARS-CoV-2. Two host-cell factors are important for SARS-CoV-2 viral entry into many cell types: angiotensin-converting enzyme 2 (ACE2), which is bound by S-protein, and transmembrane serine protease 2, which cleaves S-protein, allowing this binding to take place.



About the Study and Results

The study investigated whether SARS-CoV-2 can activate the expression of the HERV envelope proteins (ENVs) in cultured PBMCs and plasma from severe COVID-19 patients and *postmortem* tissues obtained from patients deceased from severe acute COVID-19. They also analyzed the expression of HERV-W ENVs in cultured PBMC of healthy donors.

SARS-CoV-2 induces HERV-W ENVs expression in cultures of PBMC from healthy blood donors

PBMCs from healthy blood donors, *in vitro* exposed to SARS-CoV-2, expressed the HERV-W ENVs very early after exposure to the virus, in a manner independent of the ACE2 receptor. The activation of HERVs occurred before the release of IL-6. These findings suggest that another undetermined receptor(s) mediated HERV activation by SARS-CoV-2 and that the induction of the HERV-W ENVs expression was independent of IL-6 production.

The cytofluorometry analysis showed that early expression of HERV-W ENVs in PBMCs of healthy donors was predominantly and strongly induced within the CD3+T cell population, in CD3low T lymphocytes.

PBMCs from healthy donors did not express HERV-K ENV proteins.

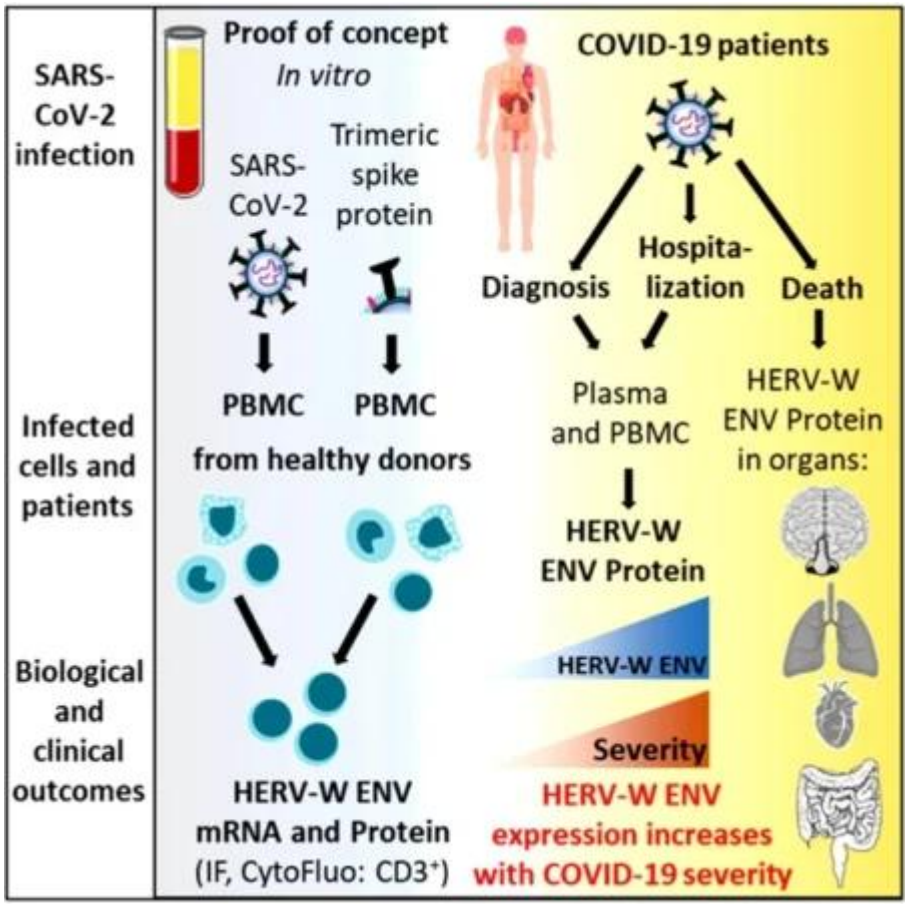


Figure from the original article of Charvet et al. iScience 26,106604 May 19, 2023

SARS-CoV-2 induces HERV-W ENVs expression in plasma and PBMCs of severe COVID-19 patients

All plasma or serum samples from severe COVID-19 patients admitted to the ICU were positive for HERV-W ENV proteins. Furthermore, the mean titer of HERV-W ENVs in plasma progressively increased with disease severity. Accordingly, the authors suggested that HERV envelope proteins could be a potential marker of COVID-19 severity.

All hospitalized cases with COVID-19, who were positive for the HERV-W ENVs, had lymphopenia. Importantly, all hospitalized patients had CD3+T lymphocytes positive for HERV-W ENVs, and a significant proportion of CD3low and CD3high T-cells and CD19+B lymphocytes. The authors emphasized that the expression of HERV-W was not previously identified in T-cells in other pathological conditions.

Cytofluorometry analysis of the blood samples from 21 COVID-19 patients demonstrated a significant correlation between the plasma levels of soluble HERV-W ENVs and the percentage of CD3+T cells positive for HERV-W ENVs.

Further analysis in 51 COVID-19 patients showed that patients infected with the Omicron variant had a lower proportion of CD3low T lymphocytes positive for HERV-W ENVs. In contrast, in patients infected with the Delta and Omicron variants, CD19+B lymphocytes positive for HERV-W ENVs were the most prevalent.

There was no difference in the proportion of CD14+monocytes positive for the HERV-W ENVs between hospitalized COVID-19 patients and healthy blood donors.

Sera from 43 patients diagnosed with other diseases were negative for the HERV-W ENVs and the HERV-K ENVs.

HERV-W ENVs expression in postmortem tissues from severe COVID-19 patients

Postmortem immunohistochemistry analysis was performed on the lung, heart, gastrointestinal tract, nasal mucosa, and brain samples from patients deceased of severe acute COVID-19.

In the lungs and heart, circulating microthrombi from the lung blood vessels, cardiac muscle, endothelial cells from numerous small blood vessels of the heart, and pericardial fatty tissue were positive for HERV-W ENVs. According to these results, HERV-W ENVs expression in the lungs and heart was closely associated with COVID-19 pathology, such as vasculitis or intravascular thrombotic process. All tissue samples were negative for HERV-K ENVs.

The SARS-CoV-2 N antigen was detected in epithelial cells but not in alveolar macrophages of the lung samples. The SARS-CoV-2 N antigen was not detected in cardiac tissues.

In the gastrointestinal tract, intestine mucosa and lymphoid tissue next to SARS-CoV-2-positive areas were positive for HERV-W ENVs. SARS-CoV-2 N antigen was found in epithelial cells, the gastric antral mucosa, around submucosal glands of the intestine, and in immune cells from lymphoid tissue associated with mucosa.

In the central nervous system, specimens were taken from the frontal lobe and areas of the olfactory bulb. The HERV-W ENVs were detected in the nasal mucosa, the endothelium of blood vessels, the olfactory bulb, and the microglial cells of the frontal lobe. SARS-CoV-2 N antigen was detected in nasal mucosa and CNS-nasal tissue interface, but not in the

olfactory bulb or the frontal lobe. According to the authors, these findings suggest that viral replication occurred in nasal mucosa, but not in the neighboring CNS areas of the olfactory bulb.

Conclusion

This study has shown that SARS-CoV-2 induces the HERV-W envelope protein expression. It seems that HERV-W ENV proteins are not solely biomarkers of COVID-19 severity or evolution, but also possible pathogenic players that may be involved in the immunopathogenic pathways associated with acute COVID-19 infection and post-acute COVID syndrome.

This article was published in Science.

Journal Reference

Charvet et al. SARS-CoV-2 awakens ancient retroviral genes and the expression of proinflammatory HERV-W envelope protein in COVID-19 patients. iScience 26,106604 May 19, 2023 (Open Access) https://doi.org/10.1016/j.isci.2023.106604