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Multiple Sclerosis Associated with a Broad Repertoire of T Cell Receptors That Are Specific to Epstein-Barr Virus

Is infection with the Epstein-Barr virus the long-debated cause of multiple sclerosis? A new study has now added to the mounting evidence supporting this hypothesis: it analyzed the T cell receptor β-chain repertoires of 1,395 MS patients and found more TCRβ sequences specific to the virus in MS patients than in healthy individuals. These findings are consistent with the hypothesis that MS is associated with an ongoing immune response to the Epstein-Barr virus that goes awry, leading to central nervous system damage. They also seem to be independent of differences in genetics or early environmental factors.

By Nicola Donelan

Original research: Tilman Schneider-Hohendorf, Lisa Ann Gerdes, Béatrice Pignolet et al.; Broader Epstein-Barr virus-specific T cell receptor repertoire in patients with multiple sclerosis. *J Exp Med* 7 November 2022; 219 (11): e20220650. doi: https://doi.org/10.1084/jem.20220650

Subjects: Autoimmunity, Infectious disease and host defense, Neuroinflammation

New Ground article reviewed by: Heinz Wiendl and Nicholas Schwab

Multiple sclerosis is a chronic inflammatory disease of the central nervous system. Its devastating effects are due to demyelination in the brain and spinal cord mediated by a malfunctioning immune system. In a recent pivotal study, data from millions of army recruits monitored over a 20-year period, reported in Science in January 2022 (see here: https://www.science.org/doi/10.1126/science.abj8222), showed a clear link between developing multiple sclerosis (MS) after being infected with Epstein-Barr virus (EBV), while no such association was found for other ubiquitous viruses such as cytomegalovirus. The same study showed increased evidence of central nervous system (CNS) damage following EBV seroconversion – the moment in which EBV-specific antibodies appear in the blood serum –, a tell-tale sign of EBV infection leading to MS pathogenesis.

While the definitive cause of MS remains elusive, the abovementioned study and many others

continue to build evidence that infection with Epstein-Barr virus (EBV) plays a causal role in its pathogenesis: no other pathogen is so strongly linked to it.

However, almost everyone over the age of 20 is infected with EBV. So what exactly leads to the actual onset of the disease? To find explanations, researchers are focusing on the role of the immune system in mediating it. In MS pathogenesis, cell-mediated immunity plays an important role, and a major class of T cells known as cytotoxic T cells or CD8+ T cells is definitely involved. These cells, which target infected or damaged cells, form the major T cell population in inflammatory lesions found in the brains of MS patients. It is also known that MS relapses are associated with an influx of T cells in the CNS. Additionally, past studies have demonstrated the selective enrichment of EBV-specific CD8+ T cells in the cerebrospinal fluid (CSF) of MS patients, i.e., they hinted at a localized T cell response to infection with EBV, while enrichment of CD8+ T cells that target other ubiquitous viruses was not observed.

Studying T cell specificity against Epstein-Barr virus in MS

In their recently presented study "Broader Epstein-Barr virus-specific T cell receptor repertoire in patients with multiple sclerosis," published in the Journal of Experimental Medicine, researchers sought to characterize how T cell specificity against EBV is altered in MS. To this end, the team, led by Heinz Wiendl, Nicholas Schwab and Roland Liblau, studied the T cell receptor (TCR) repertoires – the entirety of T cell receptors on the surface of T cells in a given individual – of a large cohort of 1,395 MS patients and 887 controls, plus 35 pairs of twins discordant for MS.

The researchers limited their study to analyzing only human leukocyte antigen serotype A (HLA-A*02)-positive individuals. HLA-A*02 is the most prevalent major histocompatibility complex (MHC) class I allele family in humans. It determines the expression of MHC class I membrane proteins, which presents antigens to the T cell receptor of CD8+ T cells; this is referred to as an MHC-class I-restricted interaction.

They also limited their study to the β chain of TCR proteins, the latter being heterodimers of alpha and beta chains. To analyze and quantify individual TCRβ repertoires obtained from the peripheral blood and CSF of the patient cohorts, only trusted multimer-confirmed TCRβ sequence data from public, curated databases of peer-reviewed studies was used.

MS patients display an altered TCR repertoire against Epstein-Barr virus

The quantification of EBV-specific, MHC-class I-restricted TCRβ sequences was performed in HLA-A*02-positive MS patients as well as in healthy controls. For comparison, TCRβ sequences were also studied for three other viruses: influenza A, SARS-CoV-2, and cytomegalovirus. Using ImmunoSEQ assays, the researchers found that MS patients had broader EBV-specific TCRβ repertoires than healthy donors, even though both populations were infected with EBV. However, when comparing MHC-class I-restricted TCRβ repertoires specific to the other viruses, they found that their diversity was similar in MS patients and in healthy controls.

This result is both novel and complementary to previous findings that EBV-specific responses in MS patients involve CD4+ T cells.

The researchers' next step was to validate their findings. To do so, they used a different immunosequencing method (immunoPETE assays) to sequence TCRβ repertoires from an independent cohort. This cohort consisted of four groups: (A) seven healthy donors, five of them longitudinally sampled before the first and 6 weeks after the second mRNA vaccination; (B) 17 MS patients before as well as 6 and 12 months after anti-CD20 therapy with the monoclonal antibody ocrelizumab; and (C) eight MS patients at two points in time 6 months apart during therapy with the VLA-4-blocking natalizumab. Additionally, the cohort contained (D) 20 samples from patients

with autoimmune encephalitis, added in as neuroinflammatory non-MS controls, to rule out results that were only due to general neuroinflammation.

In the MS patients of this cohort, the researchers confirmed their previous results by also finding a broader EBV-specific TCR repertoire compared to non-MS patients. Some significant epitope-specific differences between MS patients and healthy controls were found as well.

Do genetics and upbringing have an impact on the EBV-specificity of TCRβ repertoires?

The researchers also sequenced and analyzed 35 pairs of monozygotic twins who grew up in identical early childhood environments. In each pair, one twin had MS and the other did not, but both twins were seropositive for EBV. The number of EBV-specific TCRβ sequences was elevated in MS patients compared to their healthy siblings – a finding unique to EBV, and not observed in the twin cohort for the other viruses. This is a powerful result and would appear to be MS-specific, since it can be considered independent of genetics and early environmental factors.

What effect, if any, does MS treatment have on the EBV-specific TCRβ repertoire?

Given that all available treatments for MS patients involve some form of immune suppression, the researchers also studied the impact of these treatments on the TCRβ repertoires. They analyzed the EBV-specific T cell response before and after treatment with antibodies against CD20 and VLA-4 (groups B and C from the validation cohort) and with interferon β. In those receiving anti-VLA-4 therapy there was an increase in EBV-specific TCRβ sequences in peripheral blood after treatment. This was to be expected, since therapy with anti-VLA-4 leads to a sequestration of leukocytes – of which EBV-specific T cells are a subgroup – in the peripheral blood. Testing for the other viruses again showed no significant effect.

These findings correlate well with other studies that found CD8+ T cells in the cerebrospinal fluid and in the brain parenchyma of MS patients. Their presence suggests an ongoing and compartmentalized immune response to EBV in MS.

However, there was no change in the EBV-specific TCRβ sequences in the peripheral blood following anti-CD20 treatment, which reduces the circulation of B cells. This could indicate that B cells in the periphery may not serve as a reservoir for EBV and therefore do not elicit the elevated T cell immune response seen in MS. Further, the antiviral effects expected with interferon β treatment caused no change in the level of EBV-specific TCRβ sequences in the peripheral blood.

What are the characteristics of the altered EBV-specific immune response in MS?

In order to characterize the cellular phenotypes of the CD8+ T cells, the transcriptomes of T cells with EBV-specific TCR sequences were analyzed and compared between healthy individuals and MS patients. To begin with, the T cells from the peripheral blood of healthy controls were analyzed by single-cell RNA sequencing. Then, CD8+ T cells with EBV-specific TCR sequences were shown to belong to different subsets of the effector-memory T cells (TEM) and central-memory T cells (TCM), as evinced by their particular gene signatures. The researchers also distinguished between cells that were specific either against latent (dormant) or against lytic (cell-disrupting) EBV epitopes. Their analysis showed that a higher number of cells specific against latent epitopes belonged to one particular TEM subset, while a lower number belonged to another TEM subset.

Next, the CD8+ T cells from the cerebrospinal fluid of six healthy controls and five MS patients were analyzed for transcriptome differences. As expected, significantly more EBV-specific TCR sequences were found in the cerebrospinal fluid of MS patients than in healthy controls, and noticeably these sequences were mostly directed towards lytic EBV epitopes (36% in healthy donors versus 95% in MS patients). The phenotypes were also distinct between MS and healthy

controls: in the healthy donors group the EBV-specific sequences were mostly part of a particular TEM 1 subset, while in the MS group the CD8+ T cells were evenly distributed between one TCM and two TEM subsets.

These latter results reveal specific characteristics of the immune response in MS. In particular, the findings about TCM cells are very telling, as they are considered to be a hallmark of MS pathology. Since they are also indicative of recent EBV priming, the findings once again indicate an ongoing immune response to EBV in MS.

Outlook

The insights provided by Tilman Schneider-Hohendorf, Béatrice Pignolet, Lisa Ann Gerdes and their colleagues into the altered CD8+ T cell-mediated immune response seen in MS lend further support to the causative role that EBV plays in the pathology of MS. The researchers consider their data "consistent with an ongoing, potentially compartmentalized immune reaction against EBV in MS patients."

There are a few limitations of this study to consider, however, as the authors themselves note. True determination of specificity requires an analysis of paired TCR α/β chains, not just of the TCRβ chain. Schneider-Hohendorf and his co-authors expect, however, that their "results would only get stronger in a paired analysis," given that they could confirm them in the four groups of their independent cohort. Also, it should be noted again that all EBV-specific sequences analyzed were MHC-class I-restricted; therefore, it was not possible to use the findings presented here to evaluate any B cell help by CD4+ T cells, especially in the context of the allele DRB1*15, which has been shown to be the most significant genetic risk factor for MS. Another important limitation to bear in mind is that the current study only quantitatively assessed the T cell repertoire, while it did not show the qualitative nature of the immune response generated.

One of the most likely explanations linking EBV to MS is a persistent EBV infection in the central nervous system that leads to a persistently activated CD8+ T cell response. While the CD8+ response of a normally functioning immune system aims at destroying viruses, it can cause damage to the central nervous system when it malfunctions. This hypothesis is supported by the broader repertoire of EBV-specific CD8+ T cells that are specific to lytic rather than latent EBV epitopes. The authors note, however, that future studies are needed to determine whether this finding is just a byproduct of an aberrant immune response, or if it is actually a driving force behind the characteristic damage to the central nervous system that occurs in MS. ●

The researchers
Nicholas Schwab and Heinz Wiendl are professors at the Department of Neurology with the Institute of Translational Neurology, University Hospital Münster. Roland Liblau is a Professor and Head of the Neuroimmunology team at the Toulouse Institute for infectious and inflammatory diseases, University of Toulouse.

Tilman Schneider-Hohendorf is a researcher at the Department of Neurology with the Institute of Translational Neurology at the University Hospital Münster. Béatrice Pignolet is a researcher at the Toulouse Institute for infectious and inflammatory diseases, University of Toulouse. Lisa Ann Gerdes is a researcher at the Biomedical Center Munich at LMU Munich.

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