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Epstein-Barr Virus Might Kick-Start Multiple Sclerosis

Researchers have found that patients with multiple sclerosis (MS) carry a population of immune cells that overreact to Epstein-Barr virus. The virus, which causes mononucleosis and may contribute to some cancers, has long been suspected to play a role in MS. However, the mechanism linking the virus to the disease was poorly understood.

Scientists think that MS—which can cause vision problems, muscle weakness, and difficulty with coordination and balance—is a result of the immune system attacking the body's own nervous system. Not everyone who is infected with Epstein-Barr develops MS, but the results of the new study, published in the June 2006, issue of the journal *Brain*, suggest that some individuals' unusually strong reaction to the virus may trigger the disease. The findings could lead to new therapeutic strategies for better control of the damage caused in this autoimmune disorder.

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The culprit, the researchers say, may be a population of T cells that helps boost other components of the immune system in response to the virus.

"What we discovered in the peripheral blood of the MS patients were T cells that appeared to be primed for action against EBV," said Nancy Edwards, an HHMI-NIH research scholar at the National Institutes of Health (NIH) and co-author of the paper, which was published in advance online.

HHM-NIH research scholars are medical students who are interested in research. They compete for the opportunity to spend a year conducting mentored research in NIH labs. The program is designed to encourage medical students to consider careers as physician-scientists. Edwards, a medical student at Duke University School of Medicine, conducted her research primarily in the laboratory of noted MS researcher Roland Martin at the National Institute of Neurological Disorders and Stroke.

“The susceptibility to acquire MS is inherited, but environmental insults such as viral infections are thought to trigger the disease, and Epstein-Barr virus is one of the leading candidate triggers,” said first author Jan Lünemann, a neurologist and immunologist at The Rockefeller University in New York. “Epstein-Barr virus does not cause MS, but the immune response to this virus is different in MS patients, and our hypothesis is that the altered immune response contributes to the development and progression of the disease.”

Lünemann started the research as a postdoctoral fellow at NIH. He is continuing to investigate the role of Epstein-Barr virus in MS in the lab of Christian Münz, a Rockefeller University researcher who specializes in EBV-specific immune responses.

The people with MS, who are universally infected with Epstein Barr virus, showed increased antibody responses to certain EBV proteins in previous studies, Lünemann said. “Very recent investigations have shown that such enhanced responses occur years before onset of clinical symptoms of MS,” he noted, indicating that EBV plays an important role early in the development of the disease.

“Our aim was to investigate what causes these increased antibody concentrations and if T cell responses to EBV are different in patients with MS,” Lünemann said. He and Edwards focused on one viral protein, called Epstein-Barr virus-encoded nuclear antigen1 (EBNA1).

Epstein-Barr virus usually persists life-long inside immune system B cells and is kept under control by virus-specific T cells. When B cells divide, the virus produces EBNA1 and uses it to slip its own DNA into the new cell. T cells that target EBNA1 are a crucial component of EBV-specific immune responses in individuals without MS.

Lünemann, Edwards, and colleagues began by collecting T cells from 20 untreated patients with MS and 20 volunteers who had been infected by Epstein-Barr virus but did not have the autoimmune disease. They then isolated from each patient the T cells that specifically responded to EBNA1.

A series of experiments revealed a pattern among the EBNA1 T cells in MS patients that was not seen in the healthy volunteers. "We saw a dual effect—not only was there an increased number of EBNA1 responsive T cells, but these T cells proliferated to a greater extent when they were stimulated by antigens," said Edwards.

"We also examined T-cell responses to influenza hemagglutinin, antigens derived from cytomegalovirus, and even EBV antigens other than EBNA1," Edwards said. "The T-cell responses to these were all normal in MS patients, indicating a distinct role for EBNA1 in the disorder."

The team then wanted to determine which portion of EBNA1 the T cells were recognizing. Generally, immune cells recognize one small, specific part on a protein, called an immunodominant region. Earlier evidence had pointed to one end of the protein, so the team decided to focus there. Münz supplied a series of 51 peptides—small segments of the EBNA1 protein—that the team added to T cells from MS patients and healthy controls.

As expected, the T cells in the healthy volunteers activated only in the presence of a specific group of peptides. But, Edwards said, "EBNA1-specific T cells from the MS patients not only increased in frequency, but also recognized a much broader region of the protein, compared to healthy people who carried the EBV virus." Immunologists call this phenomenon epitope-spreading. "This was an interesting and unexpected finding," said Edwards. "At this point, I really believed we had a story."

Finally, the team discovered that the hyper-reactive T cells belonged to the CD4 compartment of memory T cells and that these cells were strong producers of interferon-gamma, an anti-viral protein that shapes immune responses. "Animal research has shown that pro-inflammatory CD4 cells directed against central nervous system antigens can trigger an MS-like disease," said Edwards. "So we knew we were looking at the right population of T cells."

The next step will be to determine how these over-reactive immune cells trigger the destruction of the myelin sheathing that insulates nerve cells. "The

broadened response of the T cells could lead them to recognize and attack cells they aren't supposed to, like brain cells," said Münz. This process, called molecular mimicry, is seen in other autoimmune disorders, such as lupus.

Münz predicted that Edwards, who is returning to Duke University this summer to complete her medical degree, could help bridge the gap between the research worlds of infectious diseases and autoimmune diseases. "It's an exciting new field to which Nancy could greatly contribute," he said.

Whatever the ultimate cause of MS, Edwards finds the therapeutic implications of her work exciting. "For some reason, MS patients chronically accumulate these hyper-responsive T cells," she said. "And if these cells are indeed involved, either directly or indirectly, in central nervous system injury and inflammation, interfering with them could prove effective."