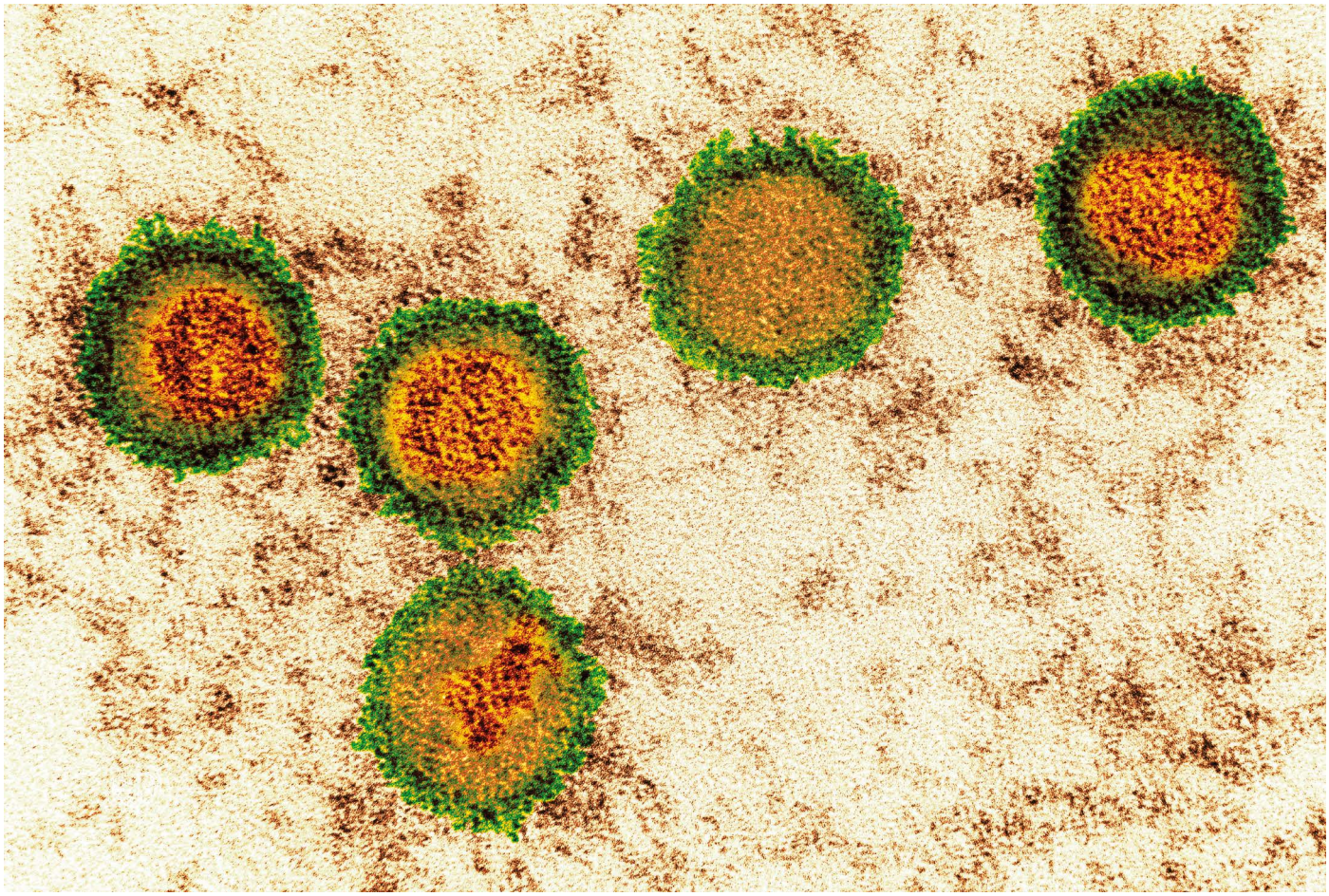

DR GOPAL MURTI/SPL

A small number of people infected with Epstein-Barr virus (pictured) go on to develop multiple sclerosis years later.

THE LONG-TERM CONSEQUENCES OF VIRAL INFECTION

Researchers suspect that viral infections can trigger multiple sclerosis and other chronic conditions. Could vaccines prevent that — and how can researchers find out? **By Asher Mullard**

In a sprawling facility in Silver Spring, Maryland, the US Department of Defense (DoD) has amassed a hoard of epidemiological treasure. Walk-in freezers each the size of a basketball court hold 72 million vials of blood serum meticulously tracked and sorted into cardboard boxes stacked nearly 4 metres high. Technicians pull on winter coats and gloves for 20-minute trips into these −30 °C deep freezers. The vials they bring out hold untold riches.

For Alberto Ascherio, an epidemiologist at Harvard T.H. Chan Medical School in Boston, Massachusetts, the vials have yielded a rare gift in the quest to discover the cause of multiple sclerosis (MS), a disease in which the immune system attacks nerve cells.

Researchers have long suspected a link between MS and the Epstein–Barr virus (EBV), but it has been hard to establish a strong connection, partly because almost everyone gets an EBV infection at some point, most of them harmless. The samples in the DoD's freezers provided an unparalleled chance to explore the link. After analysing data and samples collected from more than 10 million army, navy and air force service members since 1993, Ascherio found that EBV infection increases the risk of MS 32-fold[1].

"I've never seen anything so strong, so black and white," says Ascherio. Smoking increases the risk of lung cancer 15–30-fold.

These results, combined with emerging mechanistic insight into how the virus triggers brain damage[2], are raising the prospect of treating and even preventing MS. A phase I trial

of an EBV vaccine is under way, although it will be years, if not decades, before large trials can shed light on whether vaccines forestall MS.

These advances come at a time when researchers are more interested than ever in what happens months and years after a viral infection. Two years into the coronavirus pandemic, huge numbers of people face lasting symptoms after their initial infection with SARS-CoV-2. Concern over long COVID looms large for both the public and health officials, and funders have poured more than US$1 billion dollars into understanding the biology of this nebulous post-viral condition.

The longer-running effort to understand the causes of MS highlights the problems and promise of untangling the complex relationships between infectious diseases and later chronic conditions. Progress with these investigations can seem slow, but Katherine Luzuriaga, a clinician-scientist specializing in childhood infectious diseases at UMass Chan Medical School in Worcester, Massachusetts, has faith in the steady march of science. "As scientific methods and technologies evolve," she says, "I think we're going to get a lot more insights into post-viral conditions."

Mysterious origins

Researchers have been trying to prove for more than a century that various chronic diseases have roots in infection. Nobel-prize-winning microbiologist Barry Marshall went as far as drinking a slurry of *Helicobacter pylori* bacteria to show that they cause chronic stomach ulcers. Others have proposed that complex diseases from myalgic encephalomyelitis/chronic fatigue syndrome to Alzheimer's disease are linked to certain pathogens, but irrefutable evidence is hard to come by. In some cases, that might be because multiple pathogens and factors are at play; in others, it could be because the relationships aren't real.

This year, the evidence that EBV can cause MS got a boost from two studies[1,2].

MS, a debilitating autoimmune disease, affects around 2.8 million people worldwide. As the immune system attacks nerves in the brain and spinal cord, stripping off their protective myelin sheathing, people with MS experience symptoms including fatigue, numbness, pain, loss of vision and depression. The symptoms worsen over time, and can lead to disability and shortened life expectancy. Drugs can slow the progress of disease, but don't completely prevent symptoms.

Several factors seem to make the immune system misfire and drive MS. The geographical distribution of cases and other data suggest that lack of sunshine and vitamin D have a role. Genetic factors raise the risk, at least a little bit. EBV, first discovered in 1964, has also been suspected since at least the 1970s.

EBV is everywhere: more than 95% of adults are infected[3]. Most infections cause no symptoms, but EBV can trigger an illness called infectious mononucleosis. No one ever fully clears the virus from their body. Yet only a tiny proportion of people develop MS — 0.2% in the United Kingdom, for example. This creates a conundrum for researchers: how can you prove that a near-ubiquitous virus causes an autoimmune disease in an unlucky few?

Ascherio's epidemiological approach was to track the MS and EBV status of military recruits using medical records and the DoD's stored blood-serum samples. He and his team identified 955 individuals who were diagnosed with MS while in the military, they reported in *Science*[1]. Just 35 of these people did not carry EBV at the start of their service, the team showed. All but one had contracted EBV by the time of their MS diagnosis — an infection rate of 97%. By contrast, the infection rate in controls, who did not develop MS, was 57%.

Then, the team measured levels of a protein called neurofilament light chain, a marker of neurodegeneration. After EBV infection, those individuals who went on to develop MS had higher levels of neurodegeneration than did people who did not develop the condition.

For Ascherio, this analysis proves that the virus drives the chronic disease, even if more work is needed to find out why only a fraction of infections result in MS. "We've all been brain-washed with the idea that association is not causation. OK, but then give me an alternative explanation for all the data," he says.

"I would have to have infinite money and infinite follow-up to do this trial."

One possible explanation is that a faltering immune system is an early sign of MS, and that viral invaders — including EBV — take advantage of the opportunity to infect. Ascherio failed to find any signs of other viral opportunists in the serum samples, helping to discount this hypothesis. But doubts persist in some quarters. Marshall's critics raised similar arguments against his idea, suggesting that ulcers might create the environment for *H. pylori* infection, rather than the other way around.

Bill Robinson, chief of immunology and rheumatology at Stanford University in California, used to dismiss the EBV–MS hypothesis for this reason. "I was very sceptical that EBV was involved," says Robinson. After spending five years using a battery of immunological techniques to study the antibodies that people with MS make, he has done a U-turn.

During EBV infections, immune-system cells known as B cells pump out antibodies against a protein made by the virus, called EBNA1. That protein happens to share some structural similarities with a protein in the central nervous system called GlialCAM. Over time, some of the B cells can start making antibodies that bind to both EBNA1 and GlialCAM. The result is a friendly fire attack on neurons. Some 20–25% of people with MS carry these trigger-happy antibodies, Robinson and his colleagues reported in *Nature*[2] this year.

"This changes everything. It's been very hard to pin the tail on the donkey. Our work provides a mechanism," says Robinson.

The combination of solid epidemiological data and mechanistic explanation is a compelling sales pitch for the post-viral theory, says Paul Lieberman, a molecular virologist at the Wistar Institute in Philadelphia, Pennsylvania. He was convinced even before the most recent data, but they "push the needle further", he says. The surest way to convince the doubters would be to show that prevention or treatment of EBV wards off MS. "A clinical trial is definitely worth trying," says Lieberman. "It's not totally clear how to do that yet."

Blocking EBV

A first step is to identify ways to keep EBV at bay. In January, Luzuriaga watched as a healthy volunteer walked into a sterile exam room to join a trial of one possible contender, a vaccine called mRNA-1189, made by biotechnology company Moderna in Cambridge, Massachusetts. Moderna hopes to build on the success of its mRNA COVID-19 vaccines by taking on EBV. mRNA-1189 encodes four EBV proteins that might teach the immune system to resist viral infection. Another Moderna vaccine candidate, mRNA-1195, has been designed to help the immune system to control EBV in people who already carry the virus. Two EBV vaccine candidates from the US National Institutes of Health are also approaching clinical trials.

"It's tremendously exciting," says Luzuriaga, a lead investigator on the mRNA-1189 trial.

The aim of the first trials will be to show that these vaccine candidates are safe, and can reduce the burden of infectious mononucleosis. Also known as mono, glandular fever and the kissing disease, this illness causes symptoms including extreme fatigue and fever, and affects 30–50% of people who contract EBV for the first time as teenagers or young adults[4].

Demonstrating that EBV vaccines have a benefit against MS will require much heavier lifting. Jeffrey Cohen, chief of the Laboratory of Infectious Diseases at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland, estimates that a trial would require tens of thousands of young-adult volunteers, followed for up to ten years. Because of the ubiquity of the virus, even screening for volunteers who haven't already had EBV would be a logistical headache.

A prevention trial that begins by vaccinating infants would make screening easier or unnecessary, says Ruth Dobson, a neurologist at Queen Mary University of London. But MS

A nurse visits a woman with multiple sclerosis at her home in the United Kingdom.

LINDSEY PARNABY/AFP/GETTY

typically strikes between the ages of 20 and 40, so researchers would be waiting decades for the results. "I would have to have infinite money and infinite follow-up to do this trial," she says.

For Cohen, the most likely route to robust MS-prevention data is to wait for an EBV vaccine to be approved to prevent infectious mononucleosis, and then track whether recipients develop MS. On average, it takes around ten years for a vaccine to run the clinical-trial gauntlet. After approval, observational data would take many more years to accumulate, but without the practical challenges of a prospective trial. Health authorities such as the US Food and Drug Administration (FDA) might need to mandate post-approval studies, he adds; otherwise, companies would have little incentive to collect the data. "That's a really important lesson here."

In the best case, EBV vaccines will provide long-lasting sterilizing immunity — blocking infection altogether. Vaccines against human papillomaviruses do this, and so prevent cervical cancer. But previous EBV vaccine candidates have not given that level of protection (the same is true for COVID-19 vaccines, which reduce the severity of illness but don't necessarily prevent infection with SARS-CoV-2).

Partially protective EBV vaccines could still prevent MS, says Robinson, but their success will depend on how exactly the EBV infection triggers its downstream effects. Does a single bout set off post-viral problems, or do the levels and long-term persistence of the virus make a difference? For EBV–MS — and many other suspected post-viral conditions — these are open questions with big implications.

Antiviral adventure

If viral load and persistence matter, antiviral drugs are another good bet for preventing post-viral conditions. Antivirals that wipe out the hepatitis C virus, for instance, have helped to alleviate the burden of chronic liver disease that the virus can cause.

But for this to work, antivirals need to be great at their job. For now, nothing with enough power to kill EBV is ready for rigorous clinical trials. "We don't have a real antiviral drug to target EBV-infected cells," says Cohen. A few drugs slow the virus's replication, he adds, but not enough to clear it from the body or change the clinical course of infectious mononucleosis.

This might be because the virus has two stages in its life cycle: a lytic phase, in which it replicates like mad; and a latent phase, in which it hides from the immune system. Latent viruses are notoriously challenging to kill: it is hard to jam up the viral machinery when the gears are barely turning.

"I certainly wouldn't say it's going to be easy," says Lieberman, who is developing antivirals that target EBNA1 to take out the latent virus[5]. If the community can unpick the relative contributions of the two phases, and how the latent virus is reactivated, it could open new doors.

Another strategy is to destroy the virus's breeding ground: the B cells. Atara Biotherapeutics in South San Francisco, California, is attempting this, with ATA188, a therapy made from immune T cells engineered to hunt and destroy B cells that harbour EBV.

A phase I/II trial is under way in people who already have progressive MS, in hopes of slowing the progression of disease. Preliminary results are due later this year. "If they see activity, that would mean game on," says Robinson.

But neurological diseases are hard to treat once brain damage has set in. T-cell therapies might fare best when used earlier in the course of disease, but they are an emerging therapy with an uncertain safety profile — an unlikely candidate for a large-scale prevention trial.

Erin Longbrake, a neurologist at Yale University in New Haven, Connecticut, has thought about how to balance the need for early intervention with the side effects a treatment can bring. Her therapy of choice is the FDA-approved MS drug ocrelizumab, which kills B cells to reset the malfunctioning immune system. It was not designed as an antiviral, but by happy accident it knocks out at least some of the EBV reservoir. Because it broadly depletes the immune system, however, treated individuals are at high risk of other infections. It's a high price to pay for someone without a disease.

So Longbrake has been looking for those with most to gain. A tiny subset of people have damaged areas in their brains similar to those caused by MS, but none of the accompanying symptoms. Such lesions are sometimes noticed incidentally on a brain scan. Nearly half of these people will develop MS within ten years of the discovery[6].

"If you told me I had a 50:50 chance of having MS, I'd want to do something about that," says Longbrake. A trial testing whether ocrelizumab can slow the development of MS in 100 people with lesions is recruiting participants.

Researchers are also working to identify people at high risk of other post-viral complications to ease those clinical trials.

Be prepared

It could take decades before an EBV-directed intervention proves to be a way to stave off MS. And although long COVID has renewed broad interest in the lasting effects of infections, every suspected link between a virus and a disease has its own unique and lengthy research journey ahead. For Dobson, the keys to success are preparation and patience. Ascherio's epidemiological advances, for instance, were enabled by the decades of biological samples banked by the DoD — an expensive method that takes years to yield insights. "Biobanks are a really hard sell. And then everybody loves them once all the hard work has been done," says Dobson. Similar disease-agnostic resources — which have been collecting samples throughout the pandemic — will yield insights into the long-term effects of other viruses. Already, the UK Biobank has shown how the SARS-CoV-2 virus can affect brain structures[7].

The long view is needed for clinical trials, too, she adds. Trials have to sign up the right people and find clear ways to measure success — easier for a condition such as MS, which doctors can diagnose and monitor with some precision, than for long COVID, which has no clear clinical definition. "If we don't start thinking about these trials now, we'll be in the same place 15 to 20 years from now."

Asher Mullard is a science journalist based in Ottawa, Canada.

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Clarification

This Feature originally stated that Katherine Luzuriaga is the lead investigator on the mRNA-1189 trial. She is, in fact, the lead investigator only for the UMass Chan part of the trial.

Clarified 6 April 2022