Research investigating links between viruses and Alzheimer's was dismissed for years, but now the evidence is building

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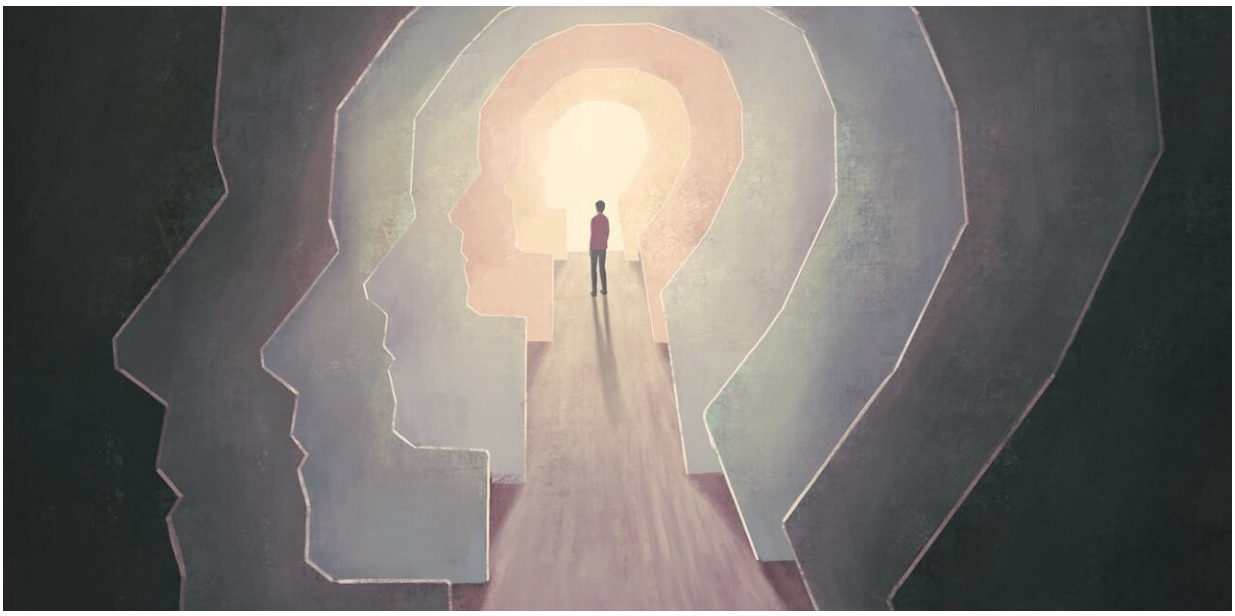

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When I was about seven or eight, I asserted that I wanted to be a scientist, or so my parents told me years later, even though I would have had little idea of what that word meant. In my mind, I was perhaps associating it with making momentous discoveries that were immediately recognized and applauded by the whole world. Soon after, I avidly read Madame Curie, the book by Eve Curie about her mother Marie and how

she overcame poverty and the many challenges faced by women in the late 19th and early 20th century to become a Nobel prize-winning scientist. Marie Curie became my lodestar for the future and thanks to my parents' support and self-sacrifice, I did eventually become a scientist.

Many years later, I found myself confronting what seemed like insuperable odds just as Curie did, though in very different circumstances. I have been an independent researcher since the age of 26 when I completed my Ph.D. My subsequent research in a Cambridge University department on chromatin (a complex of DNA and proteins) went well. Then, after eight years, my husband and I moved to Manchester where the head of the institute where I worked for 12 years decided to end my contract, leaving me jobless and lab-less.

In the decades that followed, my research into viruses as a possible cause of Alzheimer's disease was greeted with much hostility, and almost all my funding applications were refused: a hostility that has continued for 25 years and which has only recently abated, thanks to mounting evidence.

I, along with my tiny research group, survived only through the award of a few small grants from more open-minded charities and companies interested in new approaches. Once I even managed to swap a business class ticket to the US (that was provided for me to speak at a conference) for economy class, so I could use the several thousand-dollar surplus for my lab instead.

But, after years of struggle, there is finally hope for this line of research. An anti-viral trial for Alzheimer's—the first ever—is now taking place at Columbia University. This study is building on the years of work done by my team. Meanwhile, our latest research is looking into the way infectious illnesses increase the risk of Alzheimer's.

Dementia brought home

Career and academic challenges can always be balanced with the help of a support network: a family. I was always lucky with mine. During many of these years, my husband, Shaul Itzhaki, a retired academic who had worked on nucleic acid biochemistry, supported my struggles and never once suggested that I change to a safer, more conventional and non-contentious topic. He was always touchingly happy with any successes I had, and I will always remember our celebratory days when I was awarded a Beit Memorial Fellowship for Medical Research, and later a Newnham College Fellowship, during our years in Cambridge.

Sadly, he died in April 2022, after suffering for about ten years from vascular dementia (a dementia distinct from Alzheimer's disease but with many similar symptoms) and latterly, from a fractured femur that disabled him. The last four or so years were particularly hard to endure as he became increasingly aware of his failing memory. The term "brain fog" is often used in this context, but to me, it seemed more like a mist through which he could very dimly see or perceive what he was struggling to recall; the frustration—desperation, perhaps—that he felt at his inability to grasp, hold, then voice these elusive thoughts was pitiful.

I often took him to talks on topics such as the climate, migration, history and aging, hoping to keep his mind occupied. He seemed to understand many of them, but afterwards, he was quite unable to discuss them, as his memory and ability to speak were declining inexorably. Communication of any type between us was slowly becoming impossible, although he was the person with whom I had once shared my thoughts and hopes, just as he had done with me, and it became particularly sad and unsettling, as we had had so many interests in common. Eventually came the realization that I had "lost" him. It was a bereavement—the loss of him as a person, loss of a mind, not the death of a body; he was existing but not really living.

Another common feature of dementia—sudden changes of mood—affected him during these years. He had been a generally gentle, courteous person. But when, at times, the illness overcame his natural traits, he became violently angry, often for no obvious reason. Part of the problem was that his sense of location had faltered and often during the evenings he became convinced that we were about to leave and go "home" to Manchester, a place we had left in 2013. He would ask repeatedly and anxiously when we had to leave to catch the train to get there. Television programs, even those on historical events, which would have been of particular interest to him, had to be vetted as he lost himself within them. So that after watching one that dealt with, say, the horrors of war, he thought that he was actually living in that frightening world.

Of course, there are so many families going through what my family went through. And there will be many more. That fact has provided one of the main motives for my pursuing my research, despite all the difficulties that have come with it.

Early challenges

During the last five years, studies supporting the idea of a viral role in Alzheimer's disease have greatly increased. Despite this, there is still much opposition to the concept, while many in the field still ignore it.

I am often asked why there has been such hostility. A charitable explanation is that the possible role of a virus in dementia is difficult for others to assess because it straddles two very different topics: virology and Alzheimer's disease. Also, many cannot grasp the concept that people can be infected but not affected (asymptomatic, when the virus resides in the brain without causing symptoms) so they dismiss the data. Either way, I have always stressed that many possible factors lead to Alzheimer's disease—a viral role is just one of them.

My interest in this particular area began, rather unpromisingly, in 1978 when the aforementioned head of institute ended my work contract. The reason he cited was that my research on chromatin, and on the effects of carcinogens on chromatin, was "rather individualistic." I thought this was an extraordinarily inept criticism, as I had generally been acknowledged as being an innovative researcher, and innovation is surely the key to good research. The funding body offered me a post in Glasgow, but that would have meant leaving my husband and children in Manchester.

Luckily, I was immediately given a home in the lab of a medical virologist friend, Richard Sutton. Sutton was an eccentric and pioneering man. He was dogged and wiley, in an endearing way. It was Sutton who first suggested to me the possibility of viral involvement in Alzheimer's disease.

The argument for the role of the cold sore virus, herpes simplex type 1 (HSV1), in Alzheimer's disease was first suggested by American neuropathologist Melvyn Ball in 1984. But he did not pursue the idea in any practical way. Sutton and I carried out what was probably the first convincing experiment seeking the DNA of HSV1 in the human brain. We had predicted that it might be detectable in the brain of immunosuppressed patients because in the absence of an adequate immune system to keep it under control, the virus would be able to multiply. We did indeed find it, and published our results in 1986.

The central concept

HSV1 is mainly transmitted by oral-to-oral contact, causing oral herpes (cold sores). Globally, an estimated 3.7 billion people under age 50 (67%) have HSV1 infection. Most infections are asymptomatic.

Over the years, the supportive data we gathered for the key role of HSV1 in Alzheimer's led me to propose a central concept: that HSV1 is

a major cause of Alzheimer's disease; that in many people, the virus travels to the brain, probably in middle age, and remains present there in latent (dormant) form, but is frequently activated by episodes of stress, head injury, immunosuppression and infections. These "reactivations" lead to productive HSV1 infection and inflammation (and consequent damage to the brain) over the years. The accumulated damage leads eventually to the development of the disease.

The possible role of HSV1, specifically, was proposed for three main reasons. The locations of the damage the virus causes in the brain during the rare but extremely serious acute disease herpes simplex encephalitis (HSE)—caused by HSV1—are precisely the main sites of damage found in the brains of patients with Alzheimer's disease.

The other reasons for implicating HSV1 were that it is very common, affecting at least 80% of the population (in earlier decades more probably 90%), and its ability to remain dormant in the body for years.

These features meet two main characteristics of Alzheimer's disease: that it is all too common, and that it almost always waits until old age to strike its victims. Certain other infectious agents are probably involved too, perhaps individually or in combination, but so far these have been less well studied than HSV1.

The laboratory work

I was offered a more long-term prospect for my research in a department of the University of Manchester's Institute of Science and Technology. The head of the department, John Cronley Dillon, was a larger-than-life character, a bon viveur and art lover, full of novel ideas and wild enthusiasm. He encouraged me to build up a research group (minuscule though it was) and eventually we started the research on HSV1 and Alzheimer's.

It was known that when a person is infected with HSV1, the virus resides lifelong in the peripheral nervous system (PNS)—the part of the nervous system that doesn't include the brain and the spinal cord—in a latent state. It is dormant until it is activated by events such as stress. In 1989 we decided to look for HSV1 in the brain, using the technique of polymerase chain reaction, or PCR. We used PCR to examine DNA extracted from autopsy specimens of Alzheimer's disease patients.

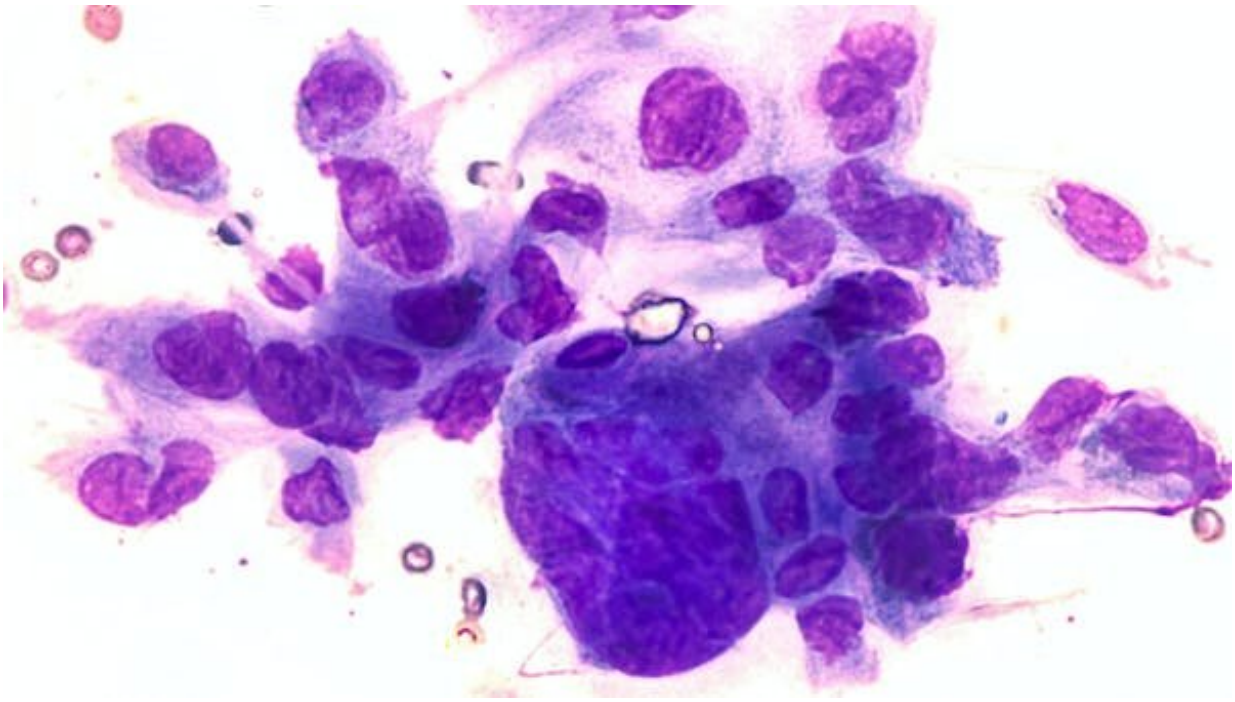
This was the first time PCR, then a new technique, had been used for this purpose. The principle of PCR is to detect a specific sequence in the target DNA by chemically amplifying it, thereby making it vastly more sensitive than the methods used in the previous few studies seeking HSV1 DNA in the brain. However, this method was prone to contamination and could produce spurious data. This meant that my poor Ph.D. student, Gordon Jamieson, spent many frustrating months trying to get it to work satisfactorily. So we were overjoyed when we detected, unambiguously, the DNA of the virus in the brain in 1991.

This was the first microbe to be detected in the human brain (in controls, in the absence of a disease). We were puzzled, though, as to why the virus was present in a high proportion of brains—both control brain specimens (people who had not been diagnosed with Alzheimer's) as well as the brains of patients who had died with the disease. This near equality of prevalence does not undermine the role of HSV1 in Alzheimer's, as some in the field have asserted. Many of the control brains were, in fact, infected with HSV1 but were asymptomatic.

So people can be infected but be asymptomatic, indicating that infection alone is not sufficient to cause disease. A very relevant example is that of cold sores which afflict only a proportion (ranging from 20–40%) of those infected with HSV1. The other 60–80% are asymptomatic. Clearly, another factor determines the degree of damage caused by the virus.

Other supporting factors

That was something we identified in 1997 when we discovered that the virus confers a high risk of Alzheimer's disease when in the brains of people who carry a specific genetic factor. We were extremely excited by this finding, but also apprehensive about adverse reactions of some in the field, as had occurred before when we discovered HSV1 DNA in elderly brains.



Scraping of a skin lesion showing characteristic giant cells in a patient with chicken pox (Varicella Zoster Virus), a type of herpes. Credit: Shutterstock/David A Litman

So we were even more excited when, after I'd suggested examining cold sore sufferers (via a small blood sample), to find what variant of the specific genetic factor they carried, we discovered that it was the same

variant as for Alzheimer's. In other words, the same variant of the genetic factor conferred a risk of damage in the peripheral nervous system, as well as the central nervous system.

Of course, the question arose as to what it is doing, if anything, in the brain. Is it residing there merely as a passenger, doing little or nothing, or does it cause damage?

We investigated this by examining cerebrospinal fluid (the liquid that bathes the brain) looking for antibodies to the virus. We detected these antibodies in most samples of cerebrospinal fluid, again, consistently, in both Alzheimer's patients and those in the age-matched control groups. This showed that indeed the virus was not just a passive fellow traveler.

We then decided to find if there were direct links between the effects of HSV1 infection and Alzheimer's. Very hesitantly, like explorers in a new continent, we infected human brain cells with HSV1, then stained the cells with antibodies to the specific abnormal proteins seen in Alzheimer's brains—amyloid and tau.

To our surprise and delight we saw accumulations of both types of protein. Also, we found amyloid deposition in the brains of infected mice. However, getting the results published was a Sisyphean task and journal reviewers' comments were often incredulous.

We subsequently used a very complex technique (in-situ PCR) which revealed that in tissue sections of brain, most of the viral DNA was located very specifically within amyloid plaques. This suggested that amyloid might act to cage the virus, thereby inactivating it. All this work provided strong support for a major role of HSV1 in Alzheimer's, and much has since been extended by other labs.

We also discovered that anti-herpes treatment was protective because it

substantially reduced the damage level in the cell cultures we were testing. This further supported a role for the virus in the disease—and pointed to a potential treatment.

A heretic shunned

But a viral role in the development of Alzheimer's was still seen as heretical by many researchers, so our papers continued to be rejected by one journal after another.

For academics, having research published in top journals is often central to keeping your job and career progression because of the perceived value to universities (related to university league table rankings, supposed research quality and performance management).

Similarly, almost all of our grant applications over that 25 years were refused, too, which was even more serious as without funding, the people in my lab couldn't be paid nor materials bought. I was very fortunate in having three successive post-doctoral researchers, Woan-Ru Lin, Curtis Dobson, and especially Matthew Wozniak, who were so dedicated that they were willing to continue to work even when on repeated short-term contracts (sometimes for less than 12 months).

So most of my time was taken up in writing research proposals and filling in application forms, interspersed with writing and submitting articles to journals, and when rejected, trying another. I had to face derision and hostile rants unaccompanied by any meaningful, scientific criticism from reviewers. A typical example was: "This grant essentially centers on a question of belief; are viruses important in Alzheimer's disease, in my view they are not."

Each rejection seemed like the end of the world. It was a heart-stopping moment when opening the envelope or email from the funding body and

scanning the lines in the hope of finding the words, "I'm pleased to tell you …"—though all too often, I found the words, "I regret to tell you." I hid, weeping tears of despair, while a part of my brain questioned whether the work really was nonsensical and whether the ideas were just wild fantasies.

At conferences, I was often shunned by prominent people in the field. My poster presentations were too (posters were the poor man's alternative to giving a talk, a privilege I was rarely given). Although, hearteningly, I found that younger people were interested and excited by the research.

Later, I benefited from the generosity of a colleague, Janusz Kulikowski. Kulikowski was another eccentric who lived an upside-down life, working at night and either sleeping during the day or else amusing himself by lobbing provocative remarks at colleagues. He was really interested in our research, despite working in the totally different field of vision research.

I do realize of course that many others have suffered refusals of grant applications, and I understand how especially heartbreaking it is for those at the start of their career, as it usually means the end of all their hopes and dreams of becoming a scientist. I realize too that I had been exceptionally lucky in being able to do such utterly engrossing work—a continuous, totally fascinating puzzle and challenge—and in having a loving family.

But after each rejection my fear that the work would end was overwhelming. When I did get a grant—any grant—I was elated: the world sparkled. I was so happy and exuberant, not just with the funding but with the fact that some people in the field were supportive of, or at least willing to consider, a possible role for HSV1. I felt so encouraged, vindicated and ready to face any challenge in my work or from fellow

scientists, and brimming over with ideas for new approaches.

Quite often in the later years, some strongly supported our central concept. But there was a huge divide between them and its opponents. And the hostility continues to this day. In 2019, an application by a colleague to a US funding body for a clinical trial of an antiviral for Alzheimer's was refused. I was involved as an adviser because it was based on my lab's research, though I was not an applicant.

One reviewer said: "This application is peripherally related to the idea that Human Herpes Virus (HHV) infection could play a role in Alzheimer's disease pathogenesis … the evidence is weak, the supporting data are weak." The second reviewer proclaimed: "The novelty of this approach appears to be quite lacking. The suggestion of latent microbe-based activation by (unknown) factors coincident with a 'deteriorating immune system' as the cause for Alzheimer's seems like hand waving": poetic perhaps, but hardly a brilliant display of scientific disputation. In fact, no adverse comments had ever been supported by any scientific argument, despite a public assertion once by a senior government official that the HSV1/Alzheimer's work had been refuted (though when challenged, he was unable to cite any such article).

Most researchers acknowledge that new, surprising and challenging ideas should be viewed with caution. But ideas should not be dismissed without any deliberation. Perhaps another major reason for the hostility is that many people in the field have been working for several decades on amyloid as a cause, and so are understandably distressed on learning that it might not be a direct cause, except in rare familial cases. This occurs despite our repeatedly stressing that numerous factors contribute to Alzheimer's and amyloid is clearly an important feature.

Exciting developments

But, as the Columbia University study shows, attitudes to the topic of Alzheimer's and HSV1 are slowly, but steadily, improving. Of course, I am very happy about this, for the sake of patients and their caregivers. And I have to admit that recognition of the work on HSV1 is personally gratifying as, like most people, I am heartened to know that my work has achieved something.

I am pleased that the research that I and others are carrying out is now moving forwards in even more exciting directions, including the use of a 3D bioengineered human brain model which, when infected with HSV1, displays many Alzheimer's-like characteristics.

We are now investigating the effects of infectious diseases and a possible role for vaccinations. This follows an explanation I published with my then-senior post-doctoral associate, Curtis Dobson, to account for the finding that certain vaccines decreased the risk of Alzheimer's disease. We suggested that infections might reactivate latent HSV1 in the brain and that vaccines might decrease the consequent risk of Alzheimer's disease by reducing the occurrence of such infectious diseases.

For example, in the case of shingles—which is caused by another type of herpes virus, varicella zoster virus (VZV)—a recent study I carried out with Manchester University epidemiologists showed that vaccination against the disease may protect against the development of Alzheimer's. Two subsequent studies showed the same result. However, much further work needs to be done to elucidate the findings that certain types of vaccine appear to reduce the risk of Alzheimer's.

I, along with researchers at Tufts University, then decided to find out if VZV (which also causes chickenpox) plays a role similar to HSV1 in causing brain damage leading to the development of Alzheimer's.

Our results showed that VZV infection of the cells does not lead to the formation of the main characteristic Alzheimer's features in the brain. However, VZV infection does result in certain other Alzheimer's-like features, including increased inflammation. And—importantly—VZV was seen to reactivate the latent HSV1 infection in the brain model, with the consequent occurrence of Alzheimer's-like characteristics. This is consistent with our suggestion that infections reactivate latent HSV1 in the brain.

The recent evidence that another herpes virus, Epstein Barr, is a cause of another brain disease (multiple sclerosis) strengthens the likelihood of viral involvement in certain other such diseases.

We now plan to find out if other infections cause HSV1 reactivation from latency. If they do, the obvious corollary would be to try to limit infections by vaccination, and by improving standards of hygiene and living conditions—a particular need in developing countries—to reduce microbial transmission.

In addition, we now have some exciting preliminary findings suggesting that percussive brain injury (for example, concussion) can cause HSV1 reactivation. This is a very different type of injury from infection and the results suggest that the virus might be pivotal in the brain's response to diverse types of damage.

This is an exciting field of study and I hope bright young scientists will enter it. Nobody said being a scientist was easy, but with the right encouragement from family, friends and open-minded peers, it is amazing what challenges can be overcome.

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