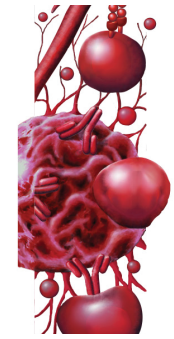
Editorial

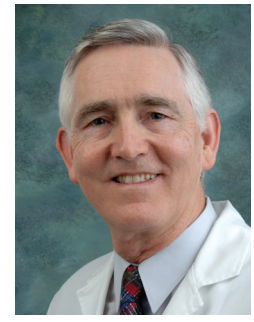
Immunotherapy



Epstein–Barr virus-specific adoptive immunotherapy: a new horizon for multiple sclerosis treatment?

"The beneficial effect of Epstein–Barr virus (EBV)-specific adoptive immunotherapy in this first patient with progressive multiple sclerosis [16] provides supportive evidence for a pathogenic role of EBV, and of decreased CD8$^+$ T-cell immunity to EBV, in the development of multiple sclerosis."

Keywords: adoptive immunotherapy • B cell • CD8 T cell • Epstein–Barr virus • multiple sclerosis • treatment



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Multiple sclerosis (MS) is a common chronic inflammatory demyelinating disease of the CNS causing progressive disability and affecting 2.5 million people worldwide. Usually, the disease has a relapsing–remitting course, with repeated neurologic episodes, each of which is followed by partial or complete recovery and a period free of new symptoms. Most patients with relapsing–remitting MS eventually develop secondary progressive MS, in which there is progressive deterioration independent of relapses. In approximately 10% of patients, MS follows a primary progressive course, with a progressive neurologic deterioration from the onset, sometimes with superimposed relapses. Currently, there is no effective disease-modifying therapy for progressive MS.

Over the last 30 years, there has been increasing evidence that Epstein–Barr virus (EBV) has a role in the pathogenesis of MS [1,2]. EBV infection appears to be present in 100% of MS patients when two independent methods are used to determine EBV seropositivity [3]. Prospective studies have shown that primary EBV infection occurs on average 5.6 years before the onset of MS [4] and that high titers of serum IgG antibodies to EBV nuclear antigen-1 (EBNA1) increase the risk of developing MS [5,6]. Infectious mononucleosis also increases the risk of MS [7].

In 2003, the novel hypothesis was proposed that human chronic autoimmune diseases, including MS, are caused by EBV infection of autoreactive B cells, which accumulate in the target organ where they produce pathogenic autoantibodies and provide costimulatory survival signals to autoreactive T cells that would otherwise die in the target organ by activation-induced apoptosis [8]. It also postulates that the accumulation of EBV-infected autoreactive B cells in the target organ is due to a genetically determined defect in the elimination of EBV-infected B cells by the cytotoxic CD8$^+$ T cells that normally keep EBV infection under tight control. The hypothesis makes predictions that have subsequently been verified, namely: the presence of EBV-infected B cells in the brain in MS [9,10]; a beneficial effect in MS of rituximab, which kills B cells, including EBV-infected B cells [11]; decreased CD8$^+$ T-cell immunity to EBV in MS [12]; and EBV infection of autoreactive plasma cells in the synovium in rheumatoid arthritis [13]. It also predicts that boosting CD8$^+$ T-cell control of EBV by vaccination or by adoptive immunotherapy will prevent and successfully treat chronic autoimmune diseases.

AdE1-LMPpoly is a novel recombinant adenovirus vector encoding multiple CD8$^+$ T-cell epitopes from three EBV latent proteins, namely EBNA1, latent membrane protein (LMP) 1 and LMP2A [14]. Adoptive immunotherapy with autologous T cells expanded *in vitro* with AdE1-LMPpoly increases survival in patients with metastatic nasopharyngeal carcinoma, a disease in which the carcinoma cells are infected with EBV and express EBNA1, LMP1 and LMP2A [14]. Because EBV-infected B cells in the brain in

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MS express the same three EBV proteins [9,15], adoptive immunotherapy with AdE1-LMPpoly may be an effective way to increase the number of CD8$^+$ T cells available to eliminate EBV-infected B cells from the CNS in MS. Recently, we reported the first use of adoptive immunotherapy with AdE1-LMPpoly to treat a patient with MS [16].

The patient was a 42-year-old man with secondary progressive MS. His first attack of MS occurred in 1994 when he was IgG seropositive for EBNA and EBV viral capsid antigen but IgM seronegative for viral capsid antigen, indicating past infection with EBV. The course of his MS was relapsing–remitting until 2004, when it became secondary progressive. From 2000 to 2008, he was treated with IFN-β-1b. Since 2008, he had been unable to walk or transfer himself. By 2012, intention tremor was progressively limiting the use of his hands and he had a flexion contracture of the right knee. The proportion of EBV-specific CD8$^+$ T cells in his blood was below the tenth percentile in healthy EBV carriers and he carried HLA-A2 and HLA-B7, which are restricting elements for several of the EBNA1, LMP1 and LMP2A epitopes in AdE1-LMPpoly. He also had the general CD8$^+$ T-cell deficiency and an increased CD4:CD8 ratio typical of MS [17].

> "These beneficial effects of EBV-specific adoptive immunotherapy in our patient can be explained by the killing of EBV-infected B cells in the CNS by the adoptively transferred CD8$^+$ T cells."

This treatment was approved by the Royal Brisbane and Women's Hospital Clinical Ethical Review Group and through the Special Access Scheme (category B) of the Australian Government Therapeutic Goods Administration. With informed consent, we collected 400 ml of blood and expanded his EBV-specific T cells by *in vitro* stimulation with AdE1-LMPpoly and IL-2 [14]. After expansion, 38.46% of CD8$^+$ T cells but only 0.22% of CD4$^+$ T cells reacted to the LMP peptides. The EBV-specific T cells were returned to the patient intravenously at fortnightly intervals. To reduce the risk of aggravating CNS inflammation, we chose an initial dose of 5×10^6 T cells, which was only 25% of the median dose used for nasopharyngeal carcinoma [14], and escalated the dose gradually over the following three infusions to 1×10^7, 1.5×10^7 and 2×10^7 cells.

The treatment was successfully completed without significant adverse effects. In particular, there were no fevers, influenza-like symptoms or malaise. Following the treatment, he experienced a reduction in fatigue and painful lower limb spasms, an improvement in cognition and hand function, and increased productivity at work. These improvements were sustained up to the time of the latest review, 21 weeks after the final T-cell infusion, when neurological examination demonstrated increased voluntary movement of his lower limbs. Following treatment the frequency of circulating EBV-specific CD8$^+$ T cells increased and there were decreases in intrathecal IgG production and disease activity on magnetic resonance imaging of the brain.

These beneficial effects of EBV-specific adoptive immunotherapy in our patient can be explained by the killing of EBV-infected B cells in the CNS by the adoptively transferred CD8$^+$ T cells. The EBV-encoded LMP2A and LMP1 proteins targeted by the transferred CD8$^+$ T cells are crucial in allowing EBV-infected B cells to multiply and mature into memory B cells and plasma cells capable of producing large amounts of antibody. LMP2A and LMP1 mimic the antigen-activated B-cell receptor and the activated CD40 receptor, respectively [18,19]. While the EBV-infected autoreactive B cells in the brain may be driving the autoimmune attack on the brain by producing pathogenic autoantibodies and providing costimulatory survival signals to autoreactive T cells [8], the autoimmune process itself could promote the survival, proliferation and differentiation of the EBV-infected autoreactive B cells by releasing CNS antigens and giving CD4$^+$ T-cell help, which would complement the B-cell receptor and CD40 receptor signaling already provided by LMP2A and LMP1, respectively [20] – that is 'double signaling'. This could lead to a vicious circle wherein EBV-infected autoreactive B cells promote autoimmunity, which in turn promotes EBV infection in the CNS. Such extensive double signaling through the B-cell receptor and CD40 pathways in the target organ of patients with chronic autoimmune diseases could be a relatively new experience for EBV in its 40 million years of coevolution with primates. Further research is needed to determine whether EBV-infected B cells and plasma cells in the MS brain are autoreactive, as has recently been shown for EBV-infected plasma cells in the synovium of patients with rheumatoid arthritis [13].

The adoptive transfer of EBV-specific CD8$^+$ T cells in MS is not without risk. The transferred T cells could aggravate inflammation in the CNS and actually worsen MS, either through cross-reactivity between EBV and CNS antigens or through bystander damage [2]. A Phase I clinical trial is needed to determine the safety of EBV-specific adoptive immunotherapy in a larger number of patients with progressive MS. In view of the potential risk of aggravating CNS inflammation, this therapy should probably not be tried yet in patients with relapsing–remitting MS for which a number of disease-modifying therapies are already available.

Another important question is how long any beneficial effect of EBV-specific adoptive immunotherapy in MS is likely to last. Because the therapy does not correct the generalized CD8+ T-cell deficiency that could underlie the impaired CD8+ T-cell immunity to EBV in MS [2,17], it is likely that EBV-specific CD8+ T-cell immunity may eventually wane again after the initial increase from immunotherapy. If such a decrease is accompanied by worsening of MS, consideration should be given to administering a further course of EBV-specific adoptive immunotherapy.

The beneficial effect of EBV-specific adoptive immunotherapy in this first patient with progressive MS [16] provides supportive evidence for a pathogenic role of EBV, and of decreased CD8+ T-cell immunity to EBV, in the development of MS. In addition, our study has implications for the treatment of other chronic autoimmune diseases where EBV also has a pathogenic role [8].

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