



Review

Gene Therapy for Autoimmune Demyelinating Disease of the Central Nervous System

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Abstract. Gene therapy is currently being explored as a new therapeutic treatment of autoimmune disease. The genetic modification of autoreactive memory T cells (T cell-mediated gene therapy) and autoimmune target tissue (target tissue gene therapy) to produce immunoregulatory cytokines offers a promising way to regulate autoimmunity. Furthermore, regenerative gene therapy offers the possibility of delivering growth factors to damaged autoimmune target tissue as a way of mediating repair. In the current review we discuss the different experimental models that are being used to test the efficacy of gene therapy in that treatment of autoimmune disease. We also discuss the importance of regulating transgene expression to ensure the therapeutic transgene products are delivered specifically to the autoimmune milieu in an antigen-inducible, non-constitutive manner.

Key words: gene therapy; autoimmune disease; myelin; interleukin 10; interleukin 4; cytokines; growth factors.

For years the central nervous system (CNS) was believed to be “an immunologically privileged site”, since cells of the immune system did not cross the blood-brain barrier of the CNS. Upon demonstrating that T cells enter the CNS, it became apparent that the immune system and the CNS have evolved a sophisticated and complex relationship that allows immune cells to enter the CNS to clear foreign viral or bacterial antigens⁴. However, when autoreactive T cells responding to myelin proteins become activated and migrate to the CNS, an autoimmune encephalomyelitis ensues resulting in demyelinated lesions and profound neurologic deficits. The experimental version of this model is called experimental autoimmune encephalomyelitis (EAE), a widely used animal model that mimics many of the clinical and histopathologic features of multiple sclerosis (reviewed in refs. ⁷ and ¹³). Work from a number of researchers has now shown that autoreactive T cells

themselves can be used in a new form of T cell-mediated gene therapy that delivers therapeutic cytokines and regenerative growth factors to autoimmune lesions in EAE.

CD4⁺ T cells have been divided into two different subgroups, Th1 and Th2 cells, based on their production of different cytokines. The pro-inflammatory Th1 cells secrete IL-2, interferon γ (IFN- γ), and tumor necrosis factor β (TNF- β), while the anti-inflammatory Th2 cells secrete IL-4, IL-5, and IL-10. In EAE, myelin-specific T cells of the Th1 phenotype mediate CNS autoimmune demyelinating disease after immunization with myelin proteins or peptides. However, if Th2 T cells are transferred into EAE mice, there is a delay in the onset of EAE and a reduction in its severity. Recently, myelin-specific Th1 T cells have been genetically modified to express anti-inflammatory cytokines, and upon transfer into EAE mice, treat the disease therapeutically.

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SHAW et al.¹² retrovirally transduced T cell hybridomas specific for myelin basic protein (MBP) with a transgene that produced constitutive expression of the anti-inflammatory cytokine, IL-4. Upon transfer into EAE mice, the IL-4 T cell hybridomas mediated a reduction in severity and onset of EAE. Transforming growth factor β (TGF- β) has been shown to reduce the number of relapses in EAE². THORBECKE et al.¹ transduced MBP specific T cell clones with a retrovirus expressing TGF- β . Upon transfer into immunized mice before the onset of EAE, the transduced TGF- β T cell clones were also able to ameliorate EAE.

MATHISEN et al.⁹ transfected an IL-10 transgene into autoreactive memory T cells isolated directly from SWXJ mice immunized with an encephalitogenic peptide of another myelin component, proteolipid protein (PLP). A unique advantage of the IL-10 construct is its regulation by an antigen-inducible IL-2 promoter region, which allows the transfected PLP-specific T cells to secrete IL-10 only when they encounter the PLP antigen. In this way, the researchers created a redundant control system; the transfected T cells would only produce the transgene product upon activation with antigen and the transgene is transfected only into PLP-specific T cells. The IL-10-transfected T cells inhibited onset of EAE and therapeutically altered the course of disease when transferred into EAE mice after initiation of clinical signs. Another important advantage of using normal memory T cells is that the treated mice do not succumb to overgrowth of transferred hybridoma tumor cells.

The work described above demonstrates that T cells can be used as an endogenous delivery system to the CNS for immunoregulatory gene products. However, it is the damage to the CNS that is responsible for the clinical symptoms of autoimmune disease. Other workers have now shown that T cells can deliver neurotrophic factors to damaged nervous tissue. KRAMER et al.⁶ used T cell lines specific for the P₂ protein of peripheral nervous system (PNS) myelin that have been retrovirally transduced to express nerve growth factor (NGF). When transferred into rats, the genetically modified T cells migrated to the PNS and produced a less severe form of experimental autoimmune neuritis (EAN).

Autoreactive T cells may be genetically modified to express transgene growth factors that could mediate the regeneration of tissue damaged during autoimmune attack⁸. Furthermore, transgene constructs employing inducible promoters will allow genetically modified, autoreactive T cells to deliver regenerative growth factors to the CNS in a site-specific and antigen-inducible manner. We have genetically modified autoreactive

T cells to express platelet-derived growth factor (PDGF), a potent growth factor for oligodendrocyte precursor cells. Transfected T cell clones secreted PDGF in an antigen-inducible manner and migrated to the CNS after transfer into EAE mice¹⁵.

While T cells may offer a novel vector for the site-specific delivery of therapeutic and regenerative gene products, the target tissue itself may be genetically modified not only to aid in its own regeneration, but to protect itself from further autoimmune attack. For example, SARVETNICK et al.¹¹ have demonstrated by using non-obese diabetic (NOD) mice, an animal model for type I insulin-dependent diabetes mellitus, that transgenic NOD mice expressing IL-4 in pancreatic β cells are protected against diabetes mellitus.

In the CNS a set of well-characterized progenitor cells called O-2A cells develop into myelinating oligodendrocytes (reviewed in ref. ¹⁰). O-2A cells can be isolated, expanded *in vitro* with PDGF and/or basic fibroblast growth factor (bFGF), and transplanted back into the CNS as a way to repair demyelinated lesions. It has been proposed that O-2A cells be transfected to produce transgene growth factors as a way to increase oligodendrocyte numbers and enhance myelination upon transplantation³. The efficacy of transplanting genetically modified cells expressing remyelinating growth factors has been tested by transplanting *ex vivo*-transfected rat fibroblasts expressing PDGF-A. During spinal cord injury, elevated levels of oligodendrocyte progenitors were detected after recombinant cell injection⁵.

It may be possible to transplant O-2A cells that have been genetically modified to express anti-inflammatory cytokines (IL-4 or IL-10) and thereby mediate their own protection during an autoimmune attack. The transgene design would use promoter regions from myelin-specific genes (e.g. PLP or MBP) to ensure expression is restricted to oligodendrocytes. Upon transplantation, transfected O-2A cells would proliferate, differentiate into myelinating oligodendrocytes, and activate the "protective" cytokine transgene. In fact, the inflammatory milieu in the CNS appears to enhance the survival and migration of oligodendrocyte progenitor cells when transplanted into the spinal cords of EAE rats¹⁴.

In the end, it may be necessary to choreograph treatment by transferring subpopulations of transfected T cells and target tissue stem cells producing different therapeutic and regenerative transgene factors targeting various stages in the development of the diseased target tissue. Together, T cell-mediated gene therapy and target tissue gene therapy may represent novel and

effective approaches for the treatment of autoimmune demyelinating disease of the CNS.

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