

The Therapeutic Potential of Gene Therapy in Multiple Sclerosis Treatment

Prepared for: Sharon Ahlers, Lecturer
School of Engineering
Cornell University

Prepared by: Alaina Steck
School of Engineering
Cornell University

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Abstract

Autoimmune disorders belong to a diverse class of diseases whose clinical complexity and unknown causes make them difficult to treat. Gene therapy has recently emerged as a therapeutic that promises to overcome several drawbacks that plague current immunosuppressive treatments. This review examines possible gene therapy approaches to treat multiple sclerosis (MS), a demyelinating autoimmune disease, and concludes that gene therapy using anti-inflammatory cytokine treatments and synthetic controlled release systems should be further investigated for use in treating MS. The available literature indicates that the specificity, safety, and sustained effects of gene therapy approaches can be enhanced through the use of an appropriate vector, and that controlled-release systems such as polymeric microspheres (such as those manufactured with poly(lactide-co-glycolide) (PLGA) and comparable polymers) are particularly well-suited for this purpose.

Executive Summary

Autoimmune disorders are complex and poorly understood diseases which occur when the immune system goes haywire and destroys the host's own tissues. As no one knows the root cause of these diseases, treatments are usually palliative rather than curative, and must be based on the little information that clinicians have on autoimmune pathology. Current treatments rely on intensive steroid regimens that produce general immunosuppression and severe side effects. This review examines the possible applications of gene therapy in a single autoimmune disease, multiple sclerosis (MS), and evaluates the feasibility of using a synthetic, controlled-release gene delivery system to carry out this treatment.

Although gene therapy is still in experimental stages, it has progressed to clinical trials in several cases and promises to emerge on a large-scale basis. Gene therapy, which regulates the expression of defective genes to mediate its effects, has been shown to be largely safe, specific, and long-lasting. Yet this technology suffers from two major setbacks. Many commonly-used gene delivery vehicles (e.g., viruses) are inherently immunogenic, leading to adverse reactions and limited effectiveness; and, of the non-immunogenic gene therapy systems available, many suffer from relatively low efficiency.

Fortunately, selecting a more appropriate gene delivery system can solve these two problems. Micron-scale polymer beads, known as microspheres, have been in development for two decades. These microspheres are not only biocompatible, which makes them safer than viral vectors, but also are completely biodegradable. Such systems can be targeted to specific tissues and can deliver DNA over an extended period of time, which significantly enhances treatment efficacy. Using this delivery system in MS treatment schemes could substantially increase the effectiveness of experimental gene therapy treatments. Although such an application has not yet been tested, it deserves investigation to fully elucidate its potential.

1 Introduction

Autoimmune disorders belong to a diverse class of diseases whose clinical complexity and unknown causes make them some of the most difficult diseases to treat. Affecting 3-5% of the U.S. population,¹ autoimmune diseases are marked by a breakdown of the body's ability to distinguish host tissue from foreign, pathogenic agents. The resulting attack on "self" cells and organs can cause either organ-specific failure or systemic destruction, depending on the type of autoimmune reaction. Clinicians treating these chronic diseases face a great challenge: they must curb the haywire immune response while keeping the non-destructive arm of the immune system functional. Currently, doctors rely on delicately-balanced steroid regimens that produce general immunosuppression and curb the assault on self tissues, but which create severe side effects and increase susceptibility to infection.^{1,2}

Such challenges are driving researchers to seek more effective treatments. As scientists begin to better understand autoimmune etiology and genetics, gene therapy is rapidly emerging as a prospective therapeutic, given its potential to do the following:

- ▶ provide sustained, long-term benefits;
- ▶ target defective genes or gene products;
- ▶ *specifically* modulate immune responses; and,
- ▶ repair or partially alleviate existing damage.¹⁻³

Despite rapid advances, gene therapy is still not a fully-developed technology. It is limited primarily by safety concerns, inadequate knowledge of how genetic factors contribute to disease, and low efficacy.⁴ Current understanding of gene manipulation does allow researchers to produce palliative treatments, however, so gene therapy proves useful even in diseases with unknown genetic links. The observed problems with the efficiency of gene delivery can in part be tackled by using synthetic controlled release systems, which will be discussed in Sections 3.4 and 4.2 below.

2 Methods

An extensive literature search using the Medline database was conducted to gather current information on autoimmunity, MS, gene therapy, and controlled gene delivery systems. Medline, which indexes medically-relevant literature, is an appropriate source as this paper focuses on a medical problem and its treatment. Searches using the keywords and subject headings "gene therapy," "autoimmunity," "multiple

sclerosis,” “controlled release,” “DNA,” and “vector” yielded over 445 results. Those articles selected from this pool were either specifically about MS or about gene therapy in autoimmunity. From these articles, pertinent references were selected as additional sources for this review.

3 Results

Since autoimmune diseases vary so widely in pathology and manifestation, it is impossible to choose a representative example. Even within a single disorder such as multiple sclerosis (MS), clinicians find several types of disease progression, each with its own implications for treatment.⁵ Because MS poses particularly challenging and unique obstacles to gene delivery, this particular disease will be the focus of this study. But before we can further discuss gene therapy and begin to develop treatment approaches, we must examine several key aspects of this disease.

3.1 Multiple Sclerosis: a Neurological Autoimmune Disease

As with other autoimmune disorders, no one knows the root cause of MS. The course of the disease is well-studied, and begins when a subset of T cells become autoreactive and attack nervous tissue.

Normally, nervous tissue is “immunologically privileged” and shielded from the rest of the body by the blood-brain barrier (BBB). The malfunctioning cells infiltrate and destroy this membrane, which allows a massive influx of other immune cells and results in neuronal damage and scar formation.⁶ As the immune system is exposed to novel myelin proteins, infiltrating cells stimulate an even more aggressive immune response, which causes demyelination and interruption of nervous transmission,⁷ shown schematically in Figure 1. As the disease progresses, patients experience gross nerve loss, lesions of the spinal cord and brain, and irreversible physical disability.⁸

By far the most prevalent cell type in this arsenal is the macrophage (M ϕ),^{5-7,9,10} which functions not only as an effector cell but also as an antigen-presenting cell (APC).

In the former capacity, M ϕ induce a complement cascade, which destroys target oligodendrocytes as

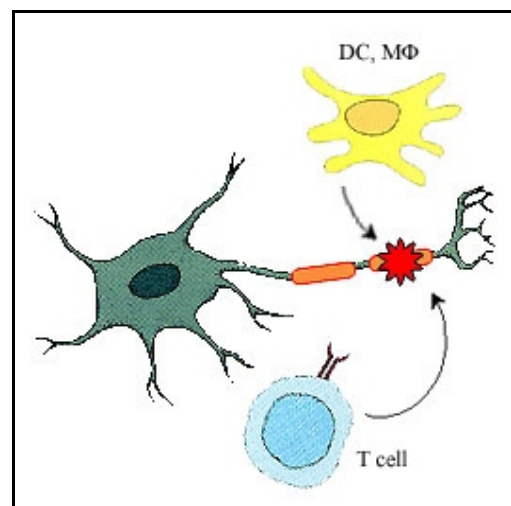


Figure 1. Demyelination in MS. T cells signal macrophages (M ϕ) to attack the myelin sheath surrounding nerve axons. Dendritic cells (DC) exacerbate this process by recruiting additional T cells and M ϕ .

well as neighboring “innocent bystander” cells. As APC, M₁ indirectly propagate tissue destruction by inducing the activation of newly-recruited lymphocytes. In either role, activated M₁ produce IFN- γ , TNF- α , and IL-12, proinflammatory cytokines which serve to activate and recruit additional lymphocytes as well as to enhance M₁ function.^{7,9,11}

A more potent APC is the dendritic cell (DC), which functions solely to activate and maintain immune responses. DC are able to stimulate T cells more effectively than any other known APC, and are thus critical regulators of immune function.^{12,13} In fact, Link et al. propose that DC are even responsible for stimulating the initial autoreactive T cells in MS as well as in other autoimmune disorders,¹⁴ a theory that cannot be undermined. Were DC to definitely mediate such an action, their roles in autoimmunity would be even more significant than those currently known. DC determine whether a T_H1 (inflammatory) or T_H2 (humoral) response predominates, as different types of DC produce either pro-inflammatory (IFN- γ , TNF- α , IL-12) or anti-inflammatory (IL-10, IL-4) cytokines that skew the immune response in one direction.¹⁴⁻¹⁶ This ability to help determine the nature of the immune response is especially noteworthy – several types of autoimmune disorders (including MS) are mediated by an overzealous inflammatory response. Could malfunctioning DC be largely responsible for this result? This and other questions are still unresolved, but it is nonetheless clear that these cells play a critical role in normal as well as abnormal immune function.

3.2 Why MS Presents Particular Challenges to Therapy

MS is known to be a chronic inflammatory disease in which patients exhibit elevated levels of pro-inflammatory cytokines.^{6,7,9,14,16-18} This overabundance of proinflammatory cytokines clearly skews the immune response toward a T_H1 bias, an imbalance that is thought to be the culprit behind many autoimmune diseases in addition to MS.

Traditional therapy relies on the use of drugs that dampen the inflammatory immune response and, as mentioned in Section 1, downregulate the entire immune system and cause highly undesirable side effects. The BBB, however, presents an added challenge to treatment. Its non-polar character prevents large, charged molecules – namely proteins, drugs, and DNA – from passing from systemic circulation into the central nervous system (CNS) and thus prohibits these molecules from acting at the injury site, where they are most needed.¹¹ Furthermore, MS is a chronic disease, and any successful treatment must confer long-lasting protection, a feat that is difficult to achieve even with current treatment regimens.

A fairly recent development in MS treatment involves the administration of IFN- β , an anti-inflammatory

cytokine that serves to inhibit IL-12 production by DC, curb levels of pro-inflammatory cytokines, and reduce the number of autoreactive T cells and M ϕ .^{8,9,11,15,18} IFN- β demonstrates noticeable amelioration of symptoms, but suffers from the same complications discussed above – it requires frequent, chronic administration and is unable to cross the BBB.^{9,19} Such persistent problems compelled researchers to investigate gene therapy applications in autoimmune disease treatments.

3.3 Gene Therapy Approaches to Treating MS

As mentioned previously, gene therapy is particularly well-suited to target autoimmune diseases and to lessen their severity, yet one of the main obstacles to this technology's success is a lack of efficacy. Organisms have evolved to reject foreign DNA, with reason: were there no mechanisms preventing foreign DNA from integrating into the host genome, we would all contain copies of the DNA found in the food we eat (which as one can imagine, would be disastrous). Furthermore, the BBB prevents even free DNA molecules (“naked DNA”) from accessing nervous tissue.

In recent years, researchers have formulated a number of approaches to treat the different facets of MS. The focus, administration, and success of these treatments varies widely. Each mechanism is capable of introducing a range of genes (see Figure 2 for a brief depiction of

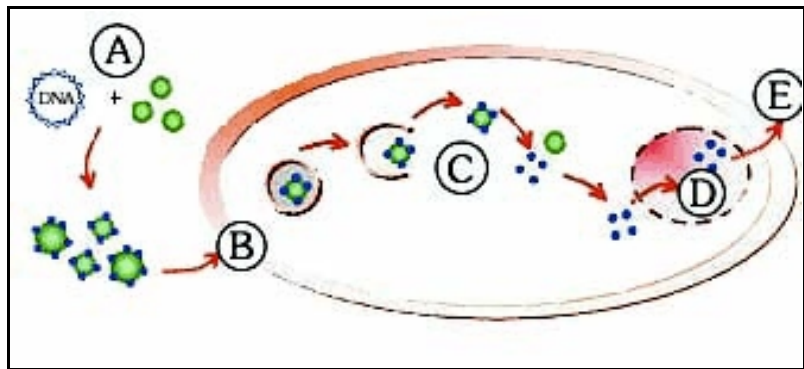


Figure 2. Mechanisms of DNA transfer and expression. (A) Plasmid DNA is incorporated into an appropriate vector system – viruses, polymer spheres, etc. (B) Vectors containing DNA cross the target cell membrane to the cell interior. (C) Plasmid DNA travels to the nucleus as it is released from its carrier. (D) Gene expression begins in the nucleus. (E) The gene product is manufactured by the cell and made available to mediate therapeutic effects. Adapted from Luo and Saltzman, 2000.²⁰

how this works), which investigators choose according to the aspect of MS they wish to target. One of the most popular experimental approaches uses genetically-engineered cells to reduce disease severity. Cells of interest – either T cells or DC – are removed from a patient's body and cultured in a petri dish, where researchers engineer these cells to produce various therapeutic compounds, usually anti-inflammatory cytokines such as IFN- β , IL-10, or IL-4.^{11,16,21-23} These chimeric cells are then re-introduced into the host, where they function as delivery vehicles for gene products. There is no further transfer of DNA into other cells of the body, which lends an added safeguard to the

procedure. Some cells are even fitted with regulatory promoters to further control gene expression. Frequent administration of a therapeutic is no longer necessary, as the engineered cells only need to be replaced every few months or years. Such *ex vivo* procedures are almost prohibitively expensive, however, require stringent sterility requirements, and cannot be readily implemented on a larger scale.

Some researchers use viruses, gutted of their own nucleic acids and infused with the gene(s) of interest, as a means to get DNA into cells.^{11,24} Viruses are much more cost-effective than cellular vectors and can be easily stored, transported, administered and scaled-up for widespread use. Different viruses (retroviruses, adenoviruses) will infect different types of cells, affording researchers some control over where their therapeutic genes are expressed.²⁴ Although they are very efficient, viral vectors are innately immunogenic and thus present safety concerns²⁵ – an especially important point for any patient, particularly for those whose immune systems are compromised. Adverse reactions can result and rapid viral clearance can hinder delivery of therapeutic genes. Despite these complications, gene therapy systems continue to produce promising results, but gene delivery needs to be optimized if it is ever to reach the clinical sphere.

3.4 Synthetic Controlled Delivery Systems

Engineers have been investigating alternative drug and DNA delivery vectors for two decades. One of the systems that has come from this research uses biocompatible, biodegradable polymers to encapsulate drugs or DNA in micrometer- or nanometer-scale spheres (Figure 3). Appropriately named “microspheres” (or “nanospheres,” depending on their size), these particles degrade in the body over an extended time period that can range from days to months.^{20,26,27} In addition to being easy to formulate and administer, microspheres are very stable and have a shelf life far exceeding that of viral vectors.

But what place does a controlled release system have in gene therapy? A primary advantage lies in the polymers’ malleability. For instance, a microsphere sample encapsulating a therapeutic gene could readily be formulated with a low surface charge and a diameter of as little as 200 nm. Both characteristics would facilitate crossing through the BBB and

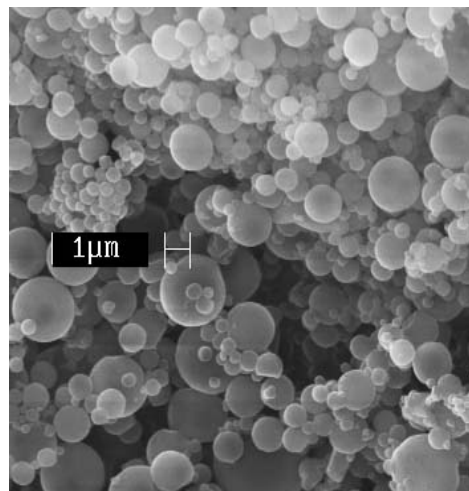


Figure 3. Electron Micrograph of Polymeric Nanospheres. These tiny (0.2 - 5 μm) plastic spheres can be used to deliver drugs and DNA to specific tissues in a controlled fashion. Scale bar = 1 μm . Unpublished image, H. Shen, A. Steck, WM Saltzman, Yale University & Cornell University; April 23, 2003.

enhance entry into target cells, increasing the efficacy of proposed gene therapy treatments.^{26,27} Also, the sustained-release characteristics of such systems allows them to accommodate chronic treatment times.

Several authors have reported using the polymer poly(lactide-co-glycolide) (PLGA) to manufacture biodegradable microspheres.^{13, 26-29} PLGA is particularly attractive, as it is biocompatible, approved by the FDA for use in humans, and breaks down completely into lactic acid and glycolic acid, two compounds found naturally in the body. PLGA-based systems can be easily manipulated to tailor the rate and release of the encapsulated substance, be it a drug, protein, or plasmid DNA,^{26,29,30} and some of these systems already have been used successfully to deliver drugs and proteins to the CNS.^{30,31}

4 Discussion

Understanding the cause and progression of a disease is the key step in designing effective treatments and cures. Genomic studies have revealed that the course and severity of MS are closely related to the genetic makeup of the patient⁵ – yet another piece of evidence that supports the use of gene therapy to treat this disease. But as the *cause* of MS is left mostly to speculation, researchers have focused many of their efforts on characterizing MS pathology and on determining the major factors that contribute to disease progression. Some researchers highlight an imbalance in immune responses and suggest focusing on the restoration of that balance to ameliorate the disease,¹⁹ while others finger T cells^{9,21,24} or APCs^{14,22} as culprits worth targeting.

4.1 Affecting T_H Cell Function by Targeting APCs

As mentioned previously, MS is regulated largely by T_H1 cells, so treatment schemes often seek to modulate the activity of this subset. The anti-inflammatory cytokine IFN- β has demonstrated clinical effectiveness and is a currently-used treatment for patients with relapsing-remitting MS (RRMS).^{8,11} This immunomodulator significantly reduces T_H1 cell numbers and lessens the frequency and intensity of MS attacks; however, it must be administered subcutaneously every other day, has a short half-life, and suffers limited efficacy due to the BBB.⁹

A logical step in designing approaches for gene therapy is to introduce an IFN- β -coding plasmid into host cells, effectively creating a long-term supply of this cytokine. Here is where the issue of tissue- and cell-specific targeting comes into play. If we are to present the host system with an usually high quantity of a specific cytokine, we must ensure that the patient experiences localized, efficient treatment with

minimal side effects. A promising tactic lies not in directly targeting the T_H1 cells themselves, but in targeting M and DC. These types of APCs are particularly attractive because they are potent primary activators of T cells and are capable of infiltrating the CNS and migrating to the site of injury.¹⁴ They have naturally high phagocytic rates and readily take in particulate matter (including gene carriers), so targeting these cells is quite feasible. Some researchers have already shown mildly effective treatments mediated by *in vivo*-transfected APC.³² As discussed, bioerodable PLGA systems are capable of providing and enhancing the delivery characteristics just mentioned – localized, sustained treatment along with cell-specific targeting.

4.2 Bioerodable Systems as Gene Carriers for MS Treatment

Several researchers have demonstrated that DC engulf microspheres both *in vitro*¹³ and *in vivo*.²⁹ Since PLGA microspheres are particulate, they indirectly “target” phagocytic cells such as DC and M, but researchers can increase sphere specificity even further by manipulating the spheres’ surface properties. Particle size, surface charge, types of functional groups, and polymer composition all contribute to differential cell uptake.^{13,27,29,30} By optimizing sphere properties specifically for DC and/or M, researchers can increase the efficiency with which these cells internalize PLGA particles and express the encapsulated genes.

Externally introduced genes, whether delivered via viral or synthetic vectors, are not permanently expressed in the host, although transient gene expression has been observed for up to 19 months. The advantage that PLGA systems have in this respect lies in their prolonged degradation and release profiles. While viruses are only capable of delivering genes in a single dose, microspheres continuously release low levels of DNA as they degrade, extending DNA delivery for months and enhancing prolonged expression. The biocompatibility of PLGA systems also ensures relatively low clearance of the vehicle from the body as compared to viral vectors. And while viruses provide no DNA-protection mechanisms once their cargo is delivered, PLGA microspheres have been shown to effectively protect DNA from degradation once inside target cells, further enhancing gene delivery.

PLGA systems show great promise to aid the developing field of gene therapy. Gene therapy is able to safely, specifically, and effectively mediate immune responses over the long term, characteristics that are enhanced by the use of appropriate vectors such as synthetic controlled delivery systems. These technologies show great promise in the treatment of autoimmunity, and should be further examined to fully elucidate their potential.

References

1. Tarner IH, Fathman CG. The Potential for Gene Therapy in the Treatment of Autoimmune Disease. *Clinical Immunology*. 2002 September; 104 (3): 204-16.
2. Evans CH, Ghivizzani SC, Oligino TJ, Robbins PD. Gene Therapy for Autoimmune Disorders. *Journal of Clinical Immunology*. 2000; 20 (5): 334-46.
3. Tarner IH, Fathman CG. Gene therapy in autoimmune disease. *Current Opinion in Immunology*. 2001; 13: 676-82.
4. Kowalczyk DW, Ertl HCJ. Immune Responses to DNA Vaccines. *Cellular and Molecular Life Sciences*. 1999; 55: 751-70.
5. Lassmann H. Mechanisms of demyelination and tissue destruction in multiple sclerosis. *Clinical Neurology and Neurosurgery*. 2002; 104: 168-71.
6. Steinman L. Multiple Sclerosis: A Coordinated Immunological Attack against Myelin in the Central Nervous System. *Cell*. 1996; 85: 299-302.
7. Miller A, Galboiz Y. Multiple sclerosis: from basic immunopathology to immune intervention. *Clinical Neurology and Neurosurgery*. 2002; 104: 172-76.
8. Grigoriadis N. Interferon b treatment in relapsing-remitting multiple sclerosis: A review. *Clinical Neurology and Neurosurgery*. 2002; 104: 251-8.
9. Furlan R, Bergami A, Lang R, Brambilla E, Franciotta D, Martinelli V, Comi G, Panina P, Martino G. Interferon-b treatment in multiple sclerosis patients decreases the number of circulating T cells producing interferon-g and interleukin-4. *Journal of Neuroimmunology*. 2000; 111: 86-92.
10. Martino G, Poliani PL, Furlan R, Marconi P, Glorioso JC, Adorini L, Comi G. Cytokine therapy in immune-mediated demyelinating diseases of the central nervous system: a novel gene therapy approach. *Journal of Neuroimmunology*. 2000; 107: 184-90.
11. Makar TK, Wilt S, Dong Z, Fishman P, Mouradian MM, Dhib-Jalbut S. IFN- Gene Transfer into the Central Nervous System Using Bone Marrow Cells as a Delivery System. *Journal of Interferon and Cytokine Research*. 2002; 22: 783-91.
12. Goldsby RA, Kindt TJ, Osborne BA. *Immunology*. 4th Ed. New York: W.H. Freeman and Company; 2000.
13. Newman KD, Samuel J, Kwon G. Obalbumin peptide encapsulated in Poly(*d,l*-lactic-co-glycolic acid) microspheres is capable of inducing a T helper type 1 response. *Journal of Controlled Release*. 1998; 54: 49-59.
14. Link H, Huang YM, Xiao BG. Dendritic cells in experimental allergica encephalomyelitis and

- multiple sclerosis. *Journal of Neuroimmunology*. 1999; 100:102-110.
15. McRae BL, Semnani RT, Hayes MP, van Seventer GA. Type I IFNs Inhibit Human Dendritic Cell IL-12 Production and Th1 Cell Development. *Journal of Immunology*. 1998; 160: 4298-4304.
 16. Kim SH, Kim S, Evans CH, Ghivizzani SC, Oligino T, Robbins PD. Effective Treatment of Established Murine Collagen-Induced Arthritis by Systemic Administration of Dendritic Cells Genetically Modified to Express IL-4. *Journal of Immunology*. 2001; 166: 3499-3505.
 17. Calabresi PA, Tranquill LR, McFarland HF, Cowan EP. Cytokine gene expression in cells derived from multiple sclerosis patients. *Journal of Neuroimmunology*. 1998; 89: 198-205.
 18. Croxford JL, Triantaphyllopoulos K, Podhajcer OL, Feldmann M, Baker D, Chernajovsky Y. Cytokine Gene Therapy in Experimental Allergic Encephalomyelitis by Injection of Plasmid DNA-Cationic Liposome Complex into the Central Nervous System. *Journal of Immunology*. 1998; 160: 5181-7.
 19. Hedley ML. Gene therapy of chronic inflammatory disease. *Advanced Drug Delivery Reviews*. 2000; 14: 195-207.
 20. Luo D, Saltzman WM. Synthetic DNA delivery systems. *Nature Biotechnology*. 2000; 18: 33-7.
 21. Correale J, Lund B, McMillian M, Ko DY, McCarthy K, Weiner LP. T cell vaccination in secondary progressive multiple sclerosis. *Journal of Neuroimmunology*. 2000; 107: 130-9.
 22. Chen CC, Rivera A, Ron N, Dougherty JP, Ron Y. A gene therapy approach for treating T-cell-mediated autoimmune diseases. *Blood*. 2001; 97: 886-94.
 23. Tuohy VK, Mathisen PM. T cell design for therapy in autoimmune demyelinating disease. *Journal of Neuroimmunology*. 200; 107: 226-32.
 24. Mathisen PM, Yu M, Johnson JM, Drazba JA, Tuohy VK. Treatment of experimental autoimmune encephalomyelitis with genetically modified memory T cells. *Journal of Experimental Medicine*. 1997; 186: 159-64.
 25. Luo D, Woodrow-Mumford K, Belcheva N, Saltzman WM. Controlled DNA Delivery Systems. *Pharmaceutical Research*. 1999; 16: 1300-8.
 26. Prabha S, Zhou WZ, Panyam J, Labhasetwar V. Size-dependency of nanoparticle-mediated gene transfection: studies with fractionated nanoparticles. *International Journal of Pharmaceutics*. 2002; 244: 105-15.
 27. Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Advanced Drug Delivery Reviews*. 2003; 55: 329-47.

28. Wang D, Robinson DR, Kwon GS, Samuel J. Encapsulation of plasmid DNA in biodegradable poly(D,L-lactic-co-glycolic acid) microspheres as a novel approach for immunogene delivery. *Journal of Controlled Release*. 1999; 57: 9-18.
29. Walter E, Dreher D, Kok M, Thiele L, Kiama SG, Gehr P, Merkle HP. Hydrophillic poly(DL-lactide-co-glycolide) microspheres for the delivery of DNA to human-derived macrophages and dendritic cells. *Journal of Controlled Release*. 2001; 76: 149-68.
30. Newman KD, Samuel J, Kwon G. Obalbumin peptide encapsulated in Poly(*d,l*-lactic-co-glycolic acid) microspheres is capable of inducing a T helper type 1 response. *Journal of Controlled Release*. 1998; 54: 49-59.
31. Péan JM, Menei P, Morel O, Montero-Menei CN, Benoit JP. Intraseptal implantation of NGF-releasing microspheres promote the survival of axotomized cholinergic neurons. *Biomaterials*. 2000; 21: 2097-101.
32. Chattergoon M, Robinson TM, Boyer JD, Weiner DB. Specific Immune Induction Following DNA-Based Immunization Through In Vivo Transfection and Activation of Macrophages/Antigen-Presenting Cells. *Journal of Immunology*. 1998; 160: 5707-18.

Abbreviations Used in This Paper

MS – multiple sclerosis

T_H1 – T-helper 1

T_H2 – T-helper 2

DC – dendritic cell

M – macrophage

APC – antigen-presenting cell

PLGA – poly(lactic-co-glycolic) acid

DNA – deoxyribonucleic acid

Glossary of Terms

Axon	The extension of a neuron along which outgoing messages are transmitted.
Dendritic Cell	A “professional” antigen presenting cell. Most of these cells are migratory; they are highly phagocytic until mature.
Lymphocyte	An immune cell.
Macrophage	Lymphocyte that can act both as an antigen-presenting cell and as an effector (target-killing) cell. Highly phagocytic until maturation, produce primarily pro-inflammatory cytokines.
Myelin	The protective sheath that surrounds nerve cell axons. It is produced by specialized cells called oligodendrocytes, and is necessary for signal transmission.
Neuron	The type of nerve cell responsible for transmission of signals throughout the body.
Promoter	Component of a plasmid (or genome) that regulates the expression of a given gene by turning gene expression “on” or “off” in response to a signal.
T cell	A subset of lymphocytes that is further divided into T-helper (T_H1 or T_H2) or T-cytotoxic (T_C) cells. So named because they develop and mature in the thymus.
T_C cells	Subset of T cells that kill target cells. Positive for the surface marker CD8.
T_H cells	Subset of T cells that, when activated, primarily produce cytokines to stimulate other immune cells. Positive for the surface marker CD4. Some T_H cells also kill target cells.
T_H1 cells	T cells that produce pro-inflammatory cytokines (such as IFN- γ , TNF- α , IL-12), which support the cell-mediated arm of the immune response.
T_H2 cells	T cells that produce cytokines (including IL-10 and IL-4) that support the humoral (antibody-mediated) immune response.