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REVIEW

Gene therapy in autoimmune, demyelinating disease of the central nervous system

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Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS), where suspected autoimmune attack causes nerve demyelination and progressive neurodegeneration and should benefit from both anti-inflammatory and neuroprotective strategies. Although neuroprotection strategies are relatively unexplored in MS, systemic delivery of anti-inflammatory agents to people with MS has so far been relatively disappointing. This is most probably because of the limited capacity of these molecules to enter the target tissue, because of exclusion by the blood–brain barrier. The complex natural history of MS also means that any therapeutic agents will have to be administered long-term. Gene therapy offers the possibility of site-directed, long-term expression, and is currently being preclinically investigated in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. While some immune effects may be targeted in the periphery using DNA vaccination, strategies both viral and nonviral are being developed to target agents into the CNS either via direct delivery or using the trafficking properties of cell-carrier systems. Targeting of leucocyte activation, cytokines and nerve growth factors have shown some promising benefit in animal EAE systems, the challenge will be their application in clinical use.
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The Disease Processes in CNS autoimmunity

Multiple sclerosis (MS) is the major demyelinating disease of the central nervous system (CNS), which is associated with blood–brain barrier (BBB) dysfunction and mononuclear cell infiltration of the white matter. These lesions expand to leave a trail of demyelination and axonal loss that impair neurotransmission, and produce a spectrum of troublesome symptoms.[1,2] Although MS may follow a variety of unpredictable courses, the typical feature is relapsing–remitting neurological attacks with intermittent and variable recovery followed by a progressive phase where disability continues to steadily worsen over time.[1,2] MS is a complex polygenic trait, but so far the only identified susceptibility loci are within the major histocompatibility complex (MHC).[1,3] These antigens are involved in immune-recognition and underscore the pathological and clinical evidence that disease is due to the activities of the immune system (Figure 1). The current thought is that MS occurs because of an autoimmune attack of CNS myelin and oligodendrocytes, which is probably triggered by viral or other environmental microbes.[4–6]

The autoimmune theory is further supported by the observation that similar clinical and histological disease can be induced in a variety of animals by induction of autoimmunity to a number of myelin antigens, including proteolipid protein (PLP), myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG).[1,4,5,7] These animal models develop experimental allergic encephalomyelitis (EAE).[7] Here, it is clear that autoreactive T cells provide the organ-specificity of disease.[8] They initiate a dynamic cascade of proinflammatory and regulatory events that ultimately produce a myelinotoxic microenvironment with eventual axonal destruction[1,2,9–16] (Figure 1). Despite some limited success, disease is poorly managed and there is a real need for effective treatment.[1,2] Dissection of EAE has played an integral part in defining immune effects that may occur in MS (Figure 1) and, particularly, the development of the Th1/Th2 paradigm, which is integral to the design of many therapeutic approaches to autoimmunity.[17] Myelin-specific Th1 cells clearly transfer disease in rodents and non-human primates and inhibition of the generation of Th1 responses inhibits disease development.[18–21] Furthermore, generation of myelin-reactive Th2/Th3 cells can suppress the emergence of pathogenic Th1 activity in EAE.[19,22–25] However, the therapeutic use of Th2 cytokines (IL-10, TGFβ) or use of cytokine agents with an anti-inflammatory profile (IFNβ, TNFα receptor p55-Ig) in MS patients has so far been mostly disappointing.[26–30] CNS inflammation is shielded from the systemic circulation by the activities of the BBB,[31] which even if compromised during disease still provides a barrier relative to peripheral sites.[9] In addition, excessive systemic cytokine release or inhibition will activate homeostatic feedback systems that may further limit clinical utility, and the widespread expression of cytokine receptors may lead to unwanted side-effects becaue of activities outside the CNS.[27] Owing to the chronic nature of the disease, therapy will need to be prolonged.[1,2] Gene therapy offers the potential to deliver

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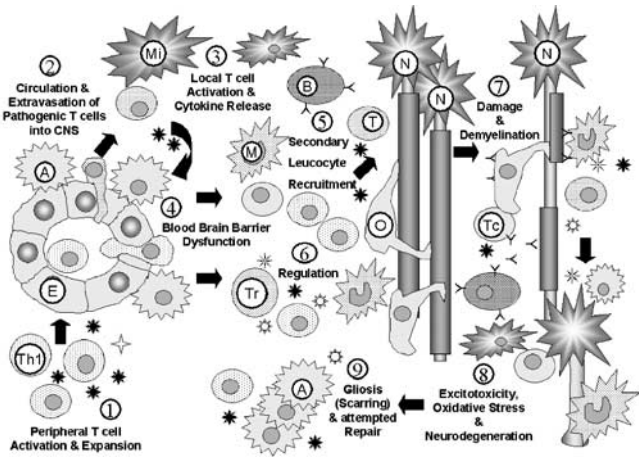

Figure 1 *Multiple disease processes occurring in EAE and MS. T cells (T) are expanded in peripheral lymphoid tissues and once activated they upregulate adhesion molecules (Eg CD49d), immunological receptors and migrate into tissues. If they enter the CNS and encounter antigen presenting microglial (Mi) cells within the perivascular space, they are activated to release proinflammatory cytokines such as IL-1, TNFα, IFNγ, which activate CNS endothelia (E) to upregulate adhesion molecules (CD54, CD105) and chemokines (MCP-1, MIP-1α) that facilitate BBB breakdown. This allows a secondary wave of recruitment of monocytes and T and B cells into the perivascular space, and the lymphocytes and macrophages (M) then migrate out into the parenchyma. These produce a variety of cytokines (stars) and they will have proinflammatory and regulatory activities, some of which will be produced by regulatory T cells (Tr, such as Th2/Th3 cells). These will act on infiltrating cells and resident glia and the outcome will depend on the balance of proinflammatory and regulatory molecules. The proinflammatory lesion promotes myelin destruction and death of oligodendrocytes (○). This may occur through oxidative stress, cytokine attack, for example, membrane TNFα and possibly cytotoxic T cells (Tc). Myelin-specific antibodies (Y) will cause destruction of myelin through complement-mediated lysis or by opsonization to promote macrophage engulfment of myelin. Demyelinated nerves must redistribute ion channels to promote some form of normal function and these may be particularly vulnerable to oxidative stress, and excitotoxic damage through glutamate release either from the nerves themselves or from the infiltrating cells and neurons (N). These slowly die and cause the irreversible accumulation of disability. During the process of damage repair, processes are generated which promote development of glial progenitors and remyelination. However, this is eventually abortive and the CNS is scarred by an astrocytic gliosis, which provides a barrier to further repair.*[1]

agents long-term, especially within the CNS, to multifocal lesions.

Gene therapy in autoimmune demyelinating disease

The key issues for any successful gene therapy approach is the nature of the vector and definition of targets. Gene therapy has not yet been attempted in MS, but there have been a number of studies in EAE that have invariably shown some level of efficacy at inhibiting the disease (Table 1), although in many cases this has only been an amelioration rather than elimination of disease.[32–64] As the majority of the CNS is postmitotic, this puts constraints on the nature of the vector that can be used, and to date administration of plasmid DNA,[32–41] viral infection,[42–53] and retrovirally transduced cell (RVC)-carriers[47,54–63] have been investigated in EAE (Table 1). These have largely focused on inhibition of the immune response either applied centrally to target the local pathological events within the CNS or peripherally administered to inhibit: initial sensitization, the activities of circulating cells or perivascular events in areas of local BBB breakdown. In addition, some studies have attempted to promote repair or inhibition of the demyelination process.[40,44,49,57,61] Cytokines are dynamically expressed as lesions evolve and resolve[12–16] and are of major importance in the development and control of autoimmunity.[12] Many studies in EAE focus on the use of knockout mice.[22,24,65] However, in these mice there is cytokine redundancy, compensation and sometimes lethality due to developmental effects. Exogenous gene delivery provides a useful tool to probe the biology of disease in 'physiologically normal' adult animals. Importantly, it also provides a route for therapy, particularly as gene delivery of cytokines can be shown to be more efficacious than bolus protein delivery.

Local immunogene therapy in CNS autoimmunity

Lesions in MS and EAE occur throughout the CNS[1,2] and as there is limited parenchymal diffusion,[34] this means that the protein would have to be delivered by multiple, invasive injections, which is impractical. This can be overcome by delivery, such that therapeutic agents 'bathe' the CNS through the cerebrospinal fluid (CSF).[64] Currently, CNS delivery of drugs is achieved through the use of osmotic pumps that are expensive and cumbersome.[66] Gene therapy offers particular promise in this area.[64] Where direct comparisons of delivery of inhibitory cytokine and cytokine inhibitory gene vectors have been made, central (CNS) administration exhibits greater efficacy than systemic delivery.[34,46,47,54] Importantly, the nature of the BBB limits not only influx but also importantly egress of molecules from the brain and can, depending on the dose injected,[43,46,47] create a local concentration gradient in the CNS that can achieve local therapy, but does not induce circulating levels that cause peripheral suppression of immune responses.[47,64] Therefore, the CNS may be a unique tissue to deliver potent immunosuppressive agents[43] (e.g. CTLA4-Ig) that may not be tolerated if delivered to other tissue sites because of the unwanted effects of generalized immunosuppression, such as the development of infection.

Injection of naked plasmid DNA, even following incorporation into cationic lipid, exhibits exceedingly low and transient expression in the CNS.[34] In contrast, replication-deficient viral vectors such as adenoviral (AV) and herpes simplex viral (HSV) vectors, which can infect postmitotic cells, can reliably produce secreted protein. Intracisternal[50–53] or intraventricular[46] delivery of viral vectors shows significant and efficient transduction of the ependymal cell layer that surrounds the brain ventricles and spinal canal.[64] The intracisternal (ependymal) delivery also targets the choroidal and leptomeningial cells coating the brain and spinal cord and is therefore a useful target for delivering soluble molecules that consistently reaches all CSF spaces and avoids infection of neurons.[64] Viral vectors can easily produce transgene levels in the range of 10–100 μg/ml within the CSF.[43,47,64] Expression of these vectors vary from a number of days (AV vectors),[43,46,47] to about a month (HSV-vectors),[64,67] which provides sufficient time to undertake experiments in EAE (Table 1). This compares well with the 10–100 ng/ml often produced for months

by retrovirally transduced cell vectors.[47,54] However, the level can be dose-titrated depending on the vector used, and the optimal levels for different cytokines are likely to be varied. Many of the immunological approaches used in gene therapy have evolved around the Th1/Th2 paradigm and IL-4 has been found most consistently to promote inhibition of EAE (Table 1). Furthermore, a HSV-IL4 vector injected into the CNS has been translated from rodents to non-human primates.[48,51,53] This is important in terms of clinical translation as primates have an immune system more closely resembling that in humans and disease often takes some time to develop, as compared to that in rodents.[20] When directly compared, IL-4 is generally more effective than IL-10.[34,42,50,56] However, notably for IL-10, results have been variable (Table 1). The CNS delivery of high-titre adenoviral human IL-10 could inhibit CNS infiltration and importantly the development of relapsing mouse EAE when administered during remission.[46] Interestingly, an adenoviral mouse IL-10 (mIL-10) vector, albeit injected at lower titres, was clinically ineffective despite the production of CSF IL-10 levels that compared well with the levels produced by a retroviral cell vector (RCV-mIL-10) that inhibited the severity of clinical signs.[47] The level of infiltration generally correlates with the clinical severity of EAE.[68] Interestingly, in contrast to the prevention of leucocyte accumulation in the CNS that occurs in most other gene therapy studies, RCV-mIL-10-treated animals exhibited significant CNS infiltration but there was a dramatic shift in the phenotype of the infiltration.[47] This suggests not only the nature of the gene product that can influence outcome, but also the nature of the vector and whether the cell is infected or transfected, possibly through the cytokines they coproduce.[69–71] There have been few cases in current EAE studies (Table 1) where the biological effect was directly attributed to the secretion of the transgene,[38,46,58] which makes it difficult to determine the real therapeutic potential of a particular gene product.

While viral vectors are excellent for the transfer of gene products into the CNS, there may be consequences of such direct infection. The CNS exhibits some characteristics of 'immune privilege' that allow potentially immunogenic allogeneic cell donors or viral vectors to be delivered and ignored, thus allowing long-term CNS expression.[9,31,72,73] However, once the peripheral immune response to the vector/transgene is primed, lymphocytes can cross the BBB, enter the CNS and eliminate the gene vector/product, which can cause additional damage, unless the gene agent protects the host cell.[73–75] Furthermore, within the context of an inflammatory disease such as MS or EAE once initiated, the pre-existing lesions and BBB dysfunction mean that the immune system is resident and active in the CNS and thus the probability that potentially immunogenic vectors will be recognized is enhanced.[1,2,9] The immunogenic nature of first generation adenoviral and vaccinia virus vectors is now recognized and 'gutless' vectors have been constructed, which will limit this potential and aid longer-term expression.[75] Using RCV, the nature of the infected cell is controlled and primary cultures and conditionally (temperature-sensitive) immortalized cells have been used as biological pumps,[47,54] and have been therapeutically active in relapsing disease.[54] These RCV can be fully characterized *in vitro* prior to *in vivo* analysis. Initial studies utilized fibroblasts,[54] as these cells can easily be obtained from any recipient. However, CNS-derived cells are more likely to survive in the CNS microenvironment and could also contribute to the repair process. Future studies may consider the use of astrocytes, oligodendrocytes or their precursors, stem cells or Schwann cells.[76–78] These latter cells can invade the CNS in both EAE and MS and induce remyelination.[1] These cells produce peripheral myelin, which lacks PLP and MOG that are found in CNS myelin,[1] and thus may be useful as it may not simply replace the target antigen for the immune response. However, there are major obstacles to migration of cells through the CNS, particularly following the generation of gliotic scars.[79,80] Clinical Schwann cell transplantation is being examined in MS and likewise, cellular-based biological pumps have been examined in other human CNS diseases. While implantation into the CNS raises safety concerns, such as uncontrolled growth, if the therapeutic activity comes from the action of a biological pump, then it is possible to encapsulate the cells.[81] This allows even the use of xenogeneic tissue that can be implanted intrathecally via lumbar puncture into the CSF space. Cells are then maintained within the semipermeable membranes to release their products.[81] Importantly, these could be removed should untoward effects occur.

Systemic immunogene therapy in CNS autoimmunity

Systemic gene therapy in EAE has been used in a number of instances, but has typically been applied before or during initial sensitization (Table 1). In these instances, the probable major target is the initial lymphocyte activation that occurs in lymphoid tissue, rather than events within the CNS. Systemic administration of cytokine DNA plasmids has on the whole exhibited no or marginal therapeutic effects (Table 1). Injection of plasmid DNA exhibits poor transduction efficiencies and when used successfully, this has used multiple injections often in regenerating (muscle) tissue.[32,33,35–40] The regulatory sequences of plasmid DNA is important for efficacy[40] and these (eg CG repeats) can modulate cytokine production *in vivo* and can either suppress or worsen EAE, probably because of a secondary effect on sensitization.[69,70] As an alternative approach to achieving local CNS delivery, the migratory potential of primed T lymphocytes has been harnessed and thus allows for systemic delivery.[8] The majority of T cells within inflammatory lesions are probably not CNS-specific but are secondarily recruited to the inflammatory site because of their adhesion molecule and chemokine receptor phenotype.[10] These cells will be targeted to lesional areas and will be activated/maintained locally if they have specificity for myelin or other CNS antigens (Figure 1). T cells can be expanded, infected with replication-deficient retroviral constructs and selected *in vitro* prior to their systemic delivery.[55–61] These can even be tracked using coexpression of a marker protein and can be shown to enter lymphoid tissues as well as the CNS.[8,82] Through the local production of antagonizing molecules *in situ* they can then inhibit neighbouring pathogenic cells via a bystander effect through release of immunosuppressive and neuroprotective growth factors (Table 1)[55–61]

Early studies used T-cell hybridomas as cell vectors and showed disease ameliorating potential, but these

Table 1 Gene therapy vectors used in experimental CNS autoimmunity

Transgene	*Delivery route*	*Gene vector*	*Animal strain tested*	*Inducing antigen*	*Therapeutic effect*	*Clinical efficacy*	*Refs*
IL-1β	Systemic (iv)	Vaccinia virus	SJL mouse	SCH	Prophylactic	+	42
IL-2	Systemic (iv)	Vaccinia virus	SJL mouse	SCH	Prophylactic	+	42
IL-4-Ig	Systemic (im)	Naked DNA	SJL mouse	MBP	Prophylactic	+	35
IL-4	Systemic (im)	Naked DNA	Lewis rat	MBP peptide	Prophylactic	=	36
	Systemic (im)	Naked DNA	SJL mouse	PLP peptide	Prophylactic	=	38
	Systemic (im)	DNA-liposomes	ABH mouse	SCH	Prophylactic	=	34
	Central	DNA-liposomes	ABH mouse	SCH	Therapeutic	+	34
	Systemic (iv)	Vaccinia virus	SJL mouse	SCH	Prophylactic	=/−	42
	Central	HSV-1	BALB/c mouse	SCH	Prophylactic	+	50
	Central	HSV-1	ABH mouse	MOG peptide	Prophylactic	+	48
	Central	HSV-1	ABH mouse	SCH	Therapeutic R	+	51
	Central	HSV-1	*Macaca mulatta*	Myelin	Therapeutic	+	53
	Systemic	RCV-(MBP-T cell)	(PL/J x SJL) mouse	MBP	Therapeutic	+	56,59
IL-6	Systemic (iv)	Vaccinia virus	SJL mouse	SCH	Prophylactic	+	42
IL-10	Systemic (im)	Naked DNA	Lewis rat	MBP peptide	Prophylactic	=	36
	Systemic (im)	DNA-liposomes	ABH mouse	SCH	Prophylactic	=	34
	Central	DNA-liposomes	ABH mouse	SCH	Therapeutic	=	34,47
	Systemic (v)	Vaccinia virus	SJL mouse	SCH	Prophylactic	+	42
	Systemic (iv)	Adenovirus	SJL mouse	SCH	Prophylactic	=	46
	Systemic (in)	Adenovirus	SJL mouse	SCH	Therapeutic	=	46
	Central	Adenovirus	SJL & CSJLF1 mouse	SCH	Therapeutic R	+	46
	Central	Adenovirus	ABH mouse	SCH	Therapeutic	=	43,47
	Central	HSV-1	BALB/c mouse	SCH	Prophylactic	=	50
	Systemic (iv)	RCV-(PLP-T cell)	(SWR x SJL) mouse	PLP peptide	Therapeutic	+	55
	Central	RCV (fibroblast)	ABH mouse	SCH	Therapeutic	+	47
	Systemic (im)	RCV-(MBP-T cell)	(PL/J x SJL) mouse	MBP	Therapeutic	=	56
IFN-β	Systemic (im)	DNA-liposomes	ABH mouse	SCH	Prophylactic	+	34
	Central	DNA-liposomes	ABH mouse	SCH	Therapeutic	+	34
IFN-γ	Systemic (iv)	Vaccinia virus	SJL mouse	SCH	Prophylactic	=	42
	Central	HSV-1	C57BL/6 mouse	MOG peptide	Prophylactic	+	52
	Central	HSV-1	C57BL/6 mouse	MOG peptide	Therapeutic	+	52
TNF-α	Systemic (im)	Naked DNA	Lewis rat	MBP peptide	Prophylactic	=	36
	Systemic (im)	DNA-liposomes	ABH mouse	SCH	Preventive	=	34
	Systemic (iv)	Vaccinia virus	SJL mouse	SCH	Prophylactic	+	42
	Systemic (iv)	RCV-(MBP-T cell)	(PL/J x SJL) mouse	MBP	Therapeutic	−	59,60
p55TNFR-Ig	Systemic (im)	Naked DNA	ABH mouse	SCH	Prophylactic	=	34
	Systemic (im)	DNA-liposomes	ABH mouse	SCH	Prophylactic	+	34
	Central	DNA-liposomes	ABH mouse	SCH	Therapeutic	+	34
	Central	Adenovirus	ABH mouse	SCH	Therapeutic	+	43
p75TNFR	Systemic (im)	DNA-liposomes	ABH mouse	SCH	Prophylactic	=	34
	Central	DNA-liposomes	ABH mouse	SCH	Therapeutic	+	34
	Systemic (ip)	RCV (fibroblast)	ABH mouse	SCH	Therapeutic	+	54
	Central	RCV (fibroblast)	ABH mouse	SCH	Therapeutic	+	54
TGF-β	Systemic (im)	NAKED DNA	SJL mouse	MBP	Prophylactic	+	35
	Systemic (im)	DNA-liposomes	ABH mouse	SCH	Prophylactic	=	34
	Central	DNA-liposomes	ABH mouse	SCH	Therapeutic	+	34
	Systemic (iv)	RCV (PLP-T cell)	(SJL x BALB/c) mouse	PLP peptide	Therapeutic	+	58
GM-CSF	Systemic (im)	Naked DNA	Lewis rat	MBP peptide	Prophylactic	=	36
PDGF-α	Systemic (iv)	RCV (PLP-T cell)	(SWR x SJL) mouse	PLP peptide	Therapeutic	+	47
FGF	Central	HSV-1	C57BL/6 mouse	MOG peptide	Therapeutic	+	49
NGF	Systemic (ip)	RCV (MBP-T cell)	Lewis rat	MBP T cell	Prophylactic	+	61
IP-10	Systemic (im)	Naked DNA	Lewis rat	MBP peptide	Prophylactic	+	39
IP-10	Systemic (im)	Naked DNA	C57BL/6 mouse	MOG peptide	Therapeutic	+	39
MCP-1	Systemic (im)	Naked DNA	Lewis rat	MBP T cells	Prophylactic	+	33
MIP-1α	Systemic (im)	Naked DNA	Lewis rat	MBP T cells	Prophylactic	+	33
MIP-1β	Systemic (im)	Naked DNA	Lewis rat	MBP T cells	Prophylactic	−	33
CAT	Central	Adenoviral	SJL mouse	PLP peptide	Prophylactic	+	44
NTR p75	Systemic (ip)	Antisense	SJL/J mouse	PLP peptide	Prophylactic	+	40
CTLA4Ig	Central	Adenovirus	ABH mouse	SCH	Therapeutic	+	43
TcRVb	Systemic (im)	Naked DNA	PL mouse	MBP	Prophylactic	+	32
PLP epitope	Systemic (im)	Naked DNA	SJL mouse	PLP peptide	Prophylactic	=	38
PLP & IL-4	Systemic (im)	Naked DNA	SJL mouse	PLP peptide	Prophylactic	+	38
MBP epitope	Systemic (im)	Naked DNA	Lewis rat	MBP peptide	Prophylactic	+	36,37
MBP & IL-4	Systemic (im)	Naked DNA	Lewis rat	MBP peptide	Prophylactic	=	36
MBP & IL-10	Systemic (im)	Naked DNA	Lewis rat	MBP peptide	Prophylactic	=	36
MOG	Systemic (im)	Naked DNA	C57BL/6 mouse	MOG peptide	Therapeutic R	=	38
MOG & IL-4	Systemic (im)	Naked DNA	C57BL/6 mouse	MOG peptide	Therapeutic R	+	38
MOG epitope	Systemic (im)	Naked DNA	DA rat	MOG peptide	Prophylactic	+	41

Table 1 (*continued*)

Transgene	*Delivery route*	*Gene vector*	*Animal strain tested*	*Inducing antigen*	*Therapeutic effect*	*Clinical efficacy*	*Refs*
MOG epitope	Systemic (im)	Naked DNA	LEW.1N rat	MOG peptide	Prophylactic	+	41
PLP epitope	Systemic (iv)	RCV (B cell)	(BALB/c x SJL) mouse	PLP peptide	Prophylactic	+	62
MBP-Ig	Systemic (iv)	RCV (B cell)	PL x SJL mouse	PLP peptide	Prophylactic	=	63
MBP-Ig	Systemic (iv)	RCV (B cell)	PL x SJL mouse	MBP peptide	Prophylactic	+	63

Vectors used in EAE studies contained cytokine transgenes: interleukin (IL), interferon (IFN), tumour necrosis factor (TNF), tumour necrosis factor receptor (TNFR), transforming growth factor (TGF), granulocyte macrophage colony stimulating factor (GM-CSF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), nerve growth factor (NGF) and neurotrophin receptor (NTR); Chemokine transgenes: macrophage chemotactic protein (MCP), macrophage inhibitory protein (MIP) and other molecules coding the reactive oxygen species scavenger catalase (CAT), the immunosuppressive CTLA4 immunoglobulin (Ig) fusion protein and myelin antigens. These were administered systemically via the intraperitoneal (ip), intramuscular (im), intranasal (in) or intravenous (iv) routes and were compared with agents injected centrally directly into the CNS, either as naked plasmid DNA, with or without cationic lipids (liposomes) or in adenoviral or herpes simplex viral (HSV) vectors of the transgenes, or were delivered using retrovirally transduced cell vectors (RCV) of fibroblast, B cell, of myelin-specific T-cell origin. These were administered to EAE-susceptible animal strains in either a prophylactic (agent administered before, during or shortly after induction) or therapeutic (shortly before or during development of clinical disease) fashion. In some instances, the gene vectors were delivered in a therapeutic context during relapsing disease (Therapeutic R) and either ameliorated (+), worsened (–) or had no effect of the clinical disease (=).

cells eventually killed the recipient because of unrestricted hybridoma (tumour) growth.[56,59,60] However, this has been reproduced with *ex vivo* cloned T cells.[55,58] In all cases, the T-cell donor cell lines were derived from syngeneic and potentially encephalitogenic T cells (Table 1). All healthy individuals appear to harbour peripheral T cells specific for myelin antigens that can be expanded *in vitro*.[1,83,84] Therefore, it should be feasible to generate terminally differentiated, nonpathogenic lines that can enter the CNS to release their products. This can be achieved through antigenic selection such as expansion of the T cells using altered peptide ligands (APL). APL are amino acid-substituted mimics of myelin proteins that drive protective Th2 responses.[85–87] In EAE, it is clear that autoimmunity to myelin antigens plays an important role in the immune response.[1,18,19] In humans, there is as yet no definitive proof of obligate autoimmunity to myelin,[1,84] but using a bystander approach to create an immunosuppressive microenvironment[25] does not require knowledge of the nature of the pathogenic antigen. However, i*n vitro* preactivation that is a prerequisite for entry of T cells into the CNS[8,18] may induce cytokine release in the blood stream or lymphoid tissues, which may cause side effects. For instance, systemic administration of active recombinant TGFβ in humans causes reversible nephrotoxicity.[27] This may have been avoided if the native TGFβ had been used, as this is typically latent until cleaved at sites of inflammation. It is possible to engineer such cells with suicide genes, so that they can be eliminated should disease-worsening occur.[88]

Systemic vaccination gene therapy in CNS autoimmunity

Recent advances in genomics and proteomics have tremendously increased the list of potential targets in MS[16,17,89] and may even be used to define autoantigenic targets in individual humans possibly for DNA vaccination reverse genomics.[89] A number of approaches aimed at preventing the generation of encephalitogenic T cells have been assessed (Table 1).[32,36–38,40,62,63] In some EAE models such as the PL/J mouse and Lewis rat, the disease is caused by activity of cells with very limited T-cell receptor (Tcr) subtype heterogeneity. Here, the majority of encephalitogenic cells express TcrVb8[90] and prophylactic, systemic DNA vaccination against this Tcr subtype has induced EAE amelioration.[32] While restricted TcrVb heterogeneity may not always occur, even in inbred animals,[91] clinical studies in selected patients with repeated TcrVb peptide injections are already underway and appear safe.[92] Several studies have targeted the vaccinations to myelin antigens to tolerize the immune response.[36–38,62,63] This approach should offer the most specific form of therapy and thus limit potential side effects. This has been shown to be active in disease, either as a secreted fusion protein[62,63] or DNA vaccination,[36–38,40] induced with all of the three major encephalitogenic myelin proteins (Table 1). In EAE it is clear that different strains respond to different peptide epitopes because of the restrictions imposed by the MHC haplotype(s) expressed, and the pathogenic T-cell repertoire expands with disease progression.[1,4] The emergence of new T-cell populations generated as a result of previous neurological insult ('determinant spread') is thought, by many, to drive the development of subsequent relapses.[4] This would limit a simple peptide approach in outbred humans, unless the mechanism of action was bystander suppression, which is not the case in many of the myelin antigen gene therapy studies.[37,40,63] However, recently it has been shown that determinant spread is not necessary for relapse progression.[93] This implies that it may be possible to have impact on disease by tolerizing the dominant response that drives relapses, even if minor potentially encephalitogenic responses are present. Gene vaccination studies demonstrate that delivery of whole myelin protein is active at inhibiting EAE.[38] This suggests that it may be possible to use a cocktail of proteins that could accommodate the majority of epitopes to which a human may respond, especially as the autoantigens in MS are unknown. Furthermore, if bystander suppression can be shown to be the operative mechanism, this may not be necessary. The majority of vaccination studies have only been examined via prophylactic treatment (Table 1). Inhibition of sensitization within lymph nodes can completely prevent the development of disease, but this may not offer any major insight on potential effects,

possibly worsening the effects of established CNS disease.[94,95] Further work is required to demonstrate that they are effective in a truly therapeutic context in long-term established disease. However, one study has shown some success in this respect and utilized coadministration of myelin DNA and IL-4 to drive the induction of a Th2 response.[38] In contrast, a previous study reported that coadministration of IL-4 DNA inhibited the efficacy of myelin DNA vaccination.[36] At present DNA vaccination is no more efficient than tolerance induction using a single or short course of native myelin protein.[4,95] Gene delivery is simpler than the production and application of recombinant proteins and is, therefore, an attractive approach. Although acute EAE is T-cell mediated,[1,18] myelin-specific B-cell responses contribute to demyelination in relapsing disease[96] and evidence from rodent and primate studies suggest that a Th2-deviated B-cell response may even exacerbate chronic disease.[97,98] Repeated subcutaneous injections of myelin-altered peptide ligands in MS were halted because of the occurrence of adverse effects.[99,100] These effects were not evident in acute EAE, where essentially all of the gene therapy studies have been performed, but were subsequently shown to occur in rodents following repeated administration of peptide in established relapsing EAE.[97] Therefore, it will be important to demonstrate the efficacy of DNA vaccination in long-established EAE and suggests also that gene expression needs to be controlled in the clinic.

Future prospects for gene therapy in CNS autoimmune demyelination

Although MS is considered to be an immune-mediated disease, immunosuppressive therapies currently fail to inhibit disease progression.[2,26,101] However, these therapies have had positive outcomes in reducing lesion formation and the relapse rate.[2,26,101] This is beginning to underscore the new belief that the major cause of permanent progressive disability is because of neurodegeneration.[102,103] While this occurs early in MS and EAE,[102,103] it is likely that because of inflammation-induced demyelination, a CNS microenvironment is created where the nerves are particularly sensitive to neurotoxic insults, such as glutamate excitotoxcity, oxidative free-radical damage and toxic ion fluxes, and a slow degenerative process that may continue independent of the inflammatory response is triggered.[102,104–107] Although some success has been shown by gene-delivered growth factors that provide trophic support (eg NGF and PDGF. Table 1),[49,57] neuroprotection by any route in CNS autoimmunity, particularly in long-established disease, is essentially unexplored. However, there is evidence from other neurodegenerative systems that gene delivery of growth factors can promote remyelination.[108–110] It is likely that effective therapy will require a combination of agents that target different elements of the disease process (Figure 1). At the moment, monotherapies are largely being investigated and these have concentrated on targeting the immune response.[26] Therefore, perhaps our expectations of a treatment are too high and success will probably only be realized once a combination of therapies are applied. Gene therapy may easily allow multiple products to be delivered that can target different pathways; however, much more work is required to show that these can work and importantly that they are safe. MS is a chronic disease and although the quality of life is reduced, it is not rapidly fatal.[1,2] Therefore, any agents should be used cautiously and safely. It is likely that CNS-directed gene therapy would be clinically developed in other more fatal diseases such as brain tumours where some clinical studies have been undertaken.[111] Gene vectors will need to be inducible and ideally removable, as clinical experience has been found that some animal studies do not translate and treatments may make disease worse.[112,113] Although there is supportive evidence that MS is a Th1-mediated disease,[1,2,114] the Th1/Th2 paradigm is less clear-cut in humans. While Th2 deviation may be part of the action of IFNβ,[115] further studies are warranted, as it is important to determine whether a Th2-deviated response is safe in long-established disease. In 'classical' EAE, while CD4 encephalitogenic cells are Th1, IFNγ has consistently been shown to be protective,[113,116] and thus contrasts with observations in humans.[117,118] However, recently a CD8-mediated disease has been induced in which IFNγ is proinflammatory.[119,120] In human MS, there is evidence for CD8 T-cell expansions within the CNS,[121,122] which is indicative of local immune activity and further studies are warranted in this area as it is important that the models reflect events occurring in human disease. Furthermore, inhibition of TNF activity has consistently demonstrated disease-ameliorating effects in EAE although disease worsening occurred following systemic TNF neutralization in MS.[29] This has also been observed in some EAE systems.[112] Cytokines regulate the immune response and systemic perturbation of the homeostatic balance can induce pro or anti-inflammatory effects. Animal studies suggest that local targeting of proinflammatory cytokines within the target tissue is important, yet this has seldom been attempted in humans and is a challenge for the future. At the moment, gene therapy is a useful experimental tool, which can help dissect mechanisms in experimental disease but it is still in its infancy. While it offers future promise, much has to be done before this can be considered as a therapy in human CNS autoimmunity.

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