Clinic Rev Allerg Immunol (2015) 49:163–176
DOI 10.1007/s12016-014-8451-x

Gene Therapy for Autoimmune Disease

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Published online: 3 October 2014

Abstract Advances in understanding the immunological and molecular basis of autoimmune diseases have made gene therapy a promising approach to treat the affected patients. Gene therapy for autoimmune diseases aims to regulate the levels of proinflammatory cytokines or molecules and the infiltration of lymphocytes to the effected sites through successful delivery and expression of therapeutic genes in appropriate cells. The ultimate goal of gene therapy is to restore and maintain the immune tolerance to the relevant autoantigens and improve clinical outcomes for patients. Here, we summarize the recent progress in identifying genes responsible for autoimmune diseases and present examples where gene therapy has been applied as treatments or prevention in autoimmune diseases both in animal models and the clinical trials. Discussion on the advantages and pitfalls of gene therapy strategies employed is provided. The intent of this review is to inspire further studies toward the development of new strategies for successful treatment of autoimmune diseases.

Keywords Gene therapy · Autoimmune disease · Animal model · Clinical trial · Delivery vectors

Abbreviations

AAV	Adenoassociated virus
APL	Altered peptide ligand
APN	Adiponectin
BAFF	B cell-activating factor belonging to the TNF family
BDNF	Brain-derived neurotrophic factor
BMSCs	Bone marrow stem cells
CIA	Collagen induced arthritis
$cPLA_2\alpha$	Cytosolic phospholipase A2
CNS	Central nervous system
DRP	DNase-resistant particles
EAE	Experimental autoimmune encephalomyelitis
EDC	1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide
GA	Glatiramer acetate
MOG	Myelin oligodendrocyte glycoprotein
MBP	Myelin basic protein
MS	Multiple sclerosis
PBMC	Peripheral blood monocyte
PLGA	Poly(lactic-co-glycolic acid)
PLP	Proteolipid protein
RA	Rheumatoid arthritis
shRNA	Small hairpin RNA
siRNA	Small interfering RNA
TLR	Toll-like receptor
Tregs	Regulatory T cells
VEGF	Vascular endothelial growth factor

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Introduction

The breakdown of tolerance, together with chronic and often intermittent inflammation, are the underlying physiological mechanisms responsible for autoimmunity [1–4]. Since the first description of multiple sclerosis by Jean-Martin Charcot in 1868, continued progress in the understanding of the etiology, natural history, epidemiology, and pathological mechanisms of autoimmunity have allowed clinicians and researchers to develop effective therapeutics [5–12]. Nevertheless, one of the major challenges with autoimmune diseases is the translation of principles gathered from animal models into strategic and efficient therapies.

Evidence from immunological studies demonstrated that autoimmune diseases are the result of a pathogenic interaction between immunological players. In other words, the cytokine milieu, antigen presenting cells, T cells, B cells, and effector

cells all participate in shaping local microenvironments that skew toward evolving cellular autoimmune responses and the destruction of self-tissues [13–20]. Deciphering the interactions between these players in the development of organ-specific autoimmune responses has largely depended on animal model studies, which are also used for preclinical trials. This is exemplified in the dnTGFBRII and the xenobiotic 2-octynoate-BSA induced mouse models of primary biliary cirrhosis, in which studies from cytokine knockout mice have dissected the complexity of the cytokine milieu in autoimmunity and provided clues for successful therapy [21–28]. While other approaches such as the inhibition of B cell functions and autoantibody production [29], depletion of B cells [30, 31], suppression of T cell proliferation/activation [14, 32], alteration of B cell signaling [33], and inhibition of inflammatory cytokine production [29, 34] are still being evaluated as potential interventions against autoimmunity, gene therapy is a novel alternative strategy undergoing investigation.

Gene therapy is a medical technique, which functional genes are introduced into the body to treat genetic disorders. It usually involves the inactivation or replacement of defective gene in the target cells. The success of gene therapy in ameliorating disease processes and clinical symptoms in animal models has made it an attractive approach in the treatment of human autoimmunity [35]. Subsequently, recent work in preclinical studies has largely focused on the safety, efficacy, and ultimately, feasibility of their application in the corresponding human disease [22, 25, 36, 37]. This process is also greatly facilitated with the development of gene delivery vectors, and herein, a detailed discussion of the delivery vectors used in gene therapy for autoimmune diseases and their characters will be discussed. Furthermore, results from current animal model studies and human clinical trials on two prototypical autoimmune diseases: (a) Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS) (Table 1) and (b) collagen-induced arthritis (CIA) as a model for rheumatoid arthritis (RA) (Table 2) will also be discussed. Emphasis will be placed on the current status of gene therapy modalities developed from these models and the outlook for gene therapy in autoimmune diseases.

Delivery Systems in Gene Therapy

In order for gene therapy to be successful, the safety and effectiveness of gene delivery must be ensured, allowing the transgene to reach target cells and be expressed [38]. Hence, extensive efforts have been put forth in the design of gene delivery systems. Various types of genetic materials are used in gene therapy such as double-stranded DNA, single-stranded DNA, oligonucleotides, and plasmid DNA. An ideal gene delivery vehicle should have the following criteria: (a) It must not interact with any vascular endothelial cells; (b) the therapeutic gene must reach its target cells without any biodegradation; (c) it must be small enough to pass through the cell membrane; and (d) it must be stable enough to reach the nucleus and be expressed. The major strategies used in DNA delivery include the use of viral and non-viral carriers, each system having its pros and cons (Table 3) [39].

Viruses commonly used as carrier vectors include retroviruses, adenovirus, adenoassociated virus (AAV), lentivirus, and herpes simplex virus. In using a virus as a gene transfer vector, its molecular properties need to be engineered so that the pathogenic portion is removed and replaced by the therapeutic transgene while the infectious components remain intact to allow infection of host cells. Viral vectors are commonly used because of its ability to deliver the transgene efficiently. Additionally, viruses are relatively more cost-effective, can be easily stored, transported, administered, and scaled-up for widespread use. Different viruses will infect different types of cells, which give researchers some control over where their therapeutic genes are expressed. Although they are very efficient, viral vectors are innately immunogenic and thus present safety concerns for patients, particularly those whose immune systems are compromised. In addition, the tedious procedures needed to obtain a workable titer and the size constraint of the transgene packaged have limited its use in gene therapy. Retroviral vectors are devoid of any retroviral genes and result in long-term expression due to their ability to integrate into the chromosome. Their major disadvantage is the fact that they only transduce dividing cells. Recently developed lentiviral vectors do transduce non-dividing cells, but there are concerns regarding the safety of these vectors. Adenoviral vectors generally contain many adenoviral genes, although “gutless” vectors in which all coding sequences have been deleted have been developed. Adenoviral vectors transduce non-replicating cells very efficiently, although expression is short-lived. This transient expression is primarily due to the immune response against residual adenoviral genes or the transgene in early generation vectors and likely results from the deletion of sequences that stabilize the DNA in cells for the gutless vectors [40]. AAV vectors are devoid of any AAV genes and can transduce non-dividing cells. Their transductions have resulted in long-term expression, although it is unclear whether the episomal or the integrated vector is more important for the sustained transgene expression. Unfortunately, production of workable titer of AAV vector can be laborious [41].

Non-viral vectors consist of plasmids that can be propagated in bacteria and oligonucleotides that can be synthesized chemically. Plasmids can transfer a therapeutic gene into a cell while oligonucleotides are usually aimed at inhibiting the expression of endogenous genes. Transfer of non-viral vectors into cells is inefficient, and the effect is generally transient. These vectors do not carry the risk of generating wild-type viruses through recombination [38]. Compared to viral vectors, non-viral vectors generally do not elicit immune responses and can be prepared in high quantities at low cost.

Table 1 Gene therapy in animal models of multiple sclerosis

Target	Delivery vector/ inhibitor*	Experimental model	Study results	References
Myelin-Ag	PLG-PEMA	SJL/J	Reduction in monocytes/macrophages, pDCs, and microglia. Reduction in numbers of IFN-γ and IL-17A cells	[63, 64]
IL-4	HSV-1	C57Bl/6	Increase IL-4 and chemokine to recruit Tregs ($CD4^+CD69^-CD25^+Foxp3^+$) with suppressant function	[57]
BDNF	BMSC	SJL/J	Reduce apoptosis by both caspase-dependent and caspase-independent mechanisms	[56]
TGFβ1	Retrovirus	SJL×BALB/c	Production of TGF-β1	[53]
NR4A2	Retrovirus	C57Bl/6	Augment promoter activities of IL-17 and IFN-γ genes by siRNA silencing of NR4A2 transcription factor	[60]
IL-10	Plasmid DNA	LEW.1AV1 rat	Suppress macrophage activation in tissue	[35]
7ND	Plasmid DNA	LEW.1AV1 rat	Inhibit macrophage infiltration into CNS a decoy by chemokine	[58]
GA	Plasmid DNA	C57Bl/6	Increase proliferation, differentiation, and survival of OPCs	[86]
GA	HSV-1	C57Bl/6	Induce production of sIL-1Rα and diminishes production of IL-1β	[105]
VEGF	Adenovirus	Dark Agouti rat	VEGF blockade	[59]
$MOG_{40–55}$	BMSC	C57Bl/6	Induce central tolerance and regulatory T cells	[55]

PV-267 small molecule inhibitor, *7ND* decoy chemokine, *GA* glatiramer acetate, *HSV* herpes simplex viral vector, *RCV* retrovirally transduced cell vector, *OPC* oligodendrocyte progenitor cell, *NGF* nerve growth factor, *NR4A2* transcription factor, *BDNF* brain-derived neurotrophic factor, *BMSC* bone marrow stem cells $MOG_{40–55}$ the peptide 40–55 of the myelin oligodendrocytic glycoprotein, *VEGF* vascular endothelial growth factor

However, their low transfection efficiency has limited their applications [42]. Non-viral DNA delivery systems are classified into two groups: physical methods and chemical methods. The principle of physical gene therapy systems is to use physical forces to weaken the cell membrane and allow cells to take up DNA by diffusion. Methods such as electroporation, gene gun, ultrasound, and hydrodynamic injection are commonly used [43]. The underlying principle for chemical delivery methods is to package DNA either by (a) electrostatic interaction between anionic DNA and polycations or (b) encapsulating it with biodegradable polymers or by absorbing it. The electrostatic interaction between anionic DNA and polycationic lipids or polymers will form a positive nanosize DNA delivery complex which facilitates cellular internalization and hence improves transfection efficiency [44]. DNA encapsules are primarily nanometric spherical structures of hydrolytically degradable polymers containing DNA [45]. Although DNA encapsulation provides good DNA protection and can potentially regulate DNA release, the presence of organic solvents and high temperatures during encapsulation may destroy the transgene [46]. DNA adsorption is primarily a combination of electrostatic interaction and encapsulation where positively charged polymers are absorbed onto a biodegradable surface to which DNA can be electrostatistically linked. This method drastically improves DNA bioavailability and loading efficiency while eliminating the harsh encapsulation process that may degrade DNA. However, DNA can be subjected to enzyme degradation because of their exposure on surfaces [47].

Multiple Sclerosis: Gene Therapy in the Experimental Autoimmune Encephalomyelitis Model

Multiple sclerosis (MS) is an autoimmune neurological disease characterized by inflammation of the central nervous system (CNS) with subsequent demyelination and axonal damage. The etiology of MS is poorly understood. Nevertheless, results from murine models and immunotargeted therapies in human patients support that its pathology is immune mediated [10, 48, 49]. This is exemplified by experimental autoimmune encephalomyelitis (EAE), which is a commonly used murine model for MS. Two murine models of EAE exist in studying the immunological, histopathological, and clinical course of MS. They are (1) active EAE induced by active immunization with myelin, myelin peptides, myelin proteins (e.g., myelin oligodendrocyte glycoprotein $(MOG)_{33–55}$), and non-myelin antigens (e.g., the proteolipid protein $(PLP)_{191–151}$), and (2) adoptive transfer of EAE induced by intravenous injection of myelin-reactive CD4 T cells into naive mice. $MOG_{33–55}$ induces a chronic progressive EAE, and $PLP_{191–151}$ triggers a relapsing-remitting EAE in mice. These models are also extensively used to identify novel therapeutic targets in MS, with the goal of improving current therapy regimens and developing alternatives.

One of the most established MS therapies that have been developed from EAE animal models include natalizumab and glatiramer acetate (GA). Natalizumab has been approved for clinical trials and is a monoclonal antibody that binds to the α4β1 integrin on lymphocytes as well as blocks the

Table 2 Gene therapy in animal models of rheumatoid arthritis

Transgene	Delivery vector	Experimental model	Study results	References
$cPLA_2\alpha$	siRNA (lipoplexes)	DBA/1	Reduce $cPLA_2\alpha$ expression and activity within inflamed joints and lower pro-inflammatory cytokine level (TNF-α and IFN-γ)	[76]
TNF-α	siRNA	C57BL/6J	Reduce both local and systemic inflammation	[74]
IL-1, IL-6, IL-18	siRNA	DBA/1	Silencing single pro-inflammatory cytokines	[73]
shTLR-7	Lentivirus	Sprague-Dawley rat	Lower VEGF, IL-1, and IL-6 levels within synovial tissues	[80]
miR-223	Lentivirus	DBA/1	Reduce bone erosion in ankle joint by attenuating osteoclastogenesis	[106]
BAFF	Lentivirus	DBA/1	Promoting factor for the expansion of Th17 cells	[79]
CTLA-4	IgG fusion protein	DBA/1	Increase $CD4^+CD25^+Foxp3^+$ Tregs and suppress $CD4^+IL\text{-}17^+$ T cells	[107]
APN	Adenovirus	DBA/1	Reduced the number of TRAP-positive cells	[69]
sFlt-1	Adenovirus	DBA/1	VEGF blockade and reduced synovial neovascularization	[70]
CCOL2A1	Plasmid DNA	Wistar rats	Induce a shift of Th1 to Th2 cells and cytokines	[81]

$cPLA_2\alpha$ cytosolic phospholipase A2α, *shTLR-7* short hairpin Toll-like receptor 7, *miR-223* microRNA 223, *APN* adiponectin, *BAFF* TNF superfamily member B cell-activating factor, *CCOL2A1* DNA sequences encoding chicken type II collagen, *sFlt-1* soluble form of vascular endothelial growth factor (VEGF) receptors, *TRAP* tartrate-resistant acid phosphatase

interaction with the integrin ligand (CD106, vascular adhesion molecule-1 (VAM-1)) on endothelial cells [50]. GA is a random polymer consisting of repeated sequences of four amino acids (glutamic acid, lysine, alanine, and tyrosine) and is found in myelin basic protein (MBP) in a specific molar ratio. GA has been shown to have anti-inflammatory and immunomodulatory effects, as well as neuroprotective, neurogenesis, and remyelination properties [51, 52].

As a gene therapy vector, retroviruses are widely studied in order to discover methods of permanently inserting therapeutic genes into encephalitogenic T cells. Upon re-infusing back into EAE mice, the hope is that they will preferentially home to damaged sites within the CNS and release therapeutic molecules. Effector molecules such as interleukin (IL)-4, TGF-β, and nerve growth factors have been delivered by such a method and shown to be effective in ameliorating the disease in EAE mice [53, 54]. Eixarch et al. [55] reported that the transfer of bone marrow cells expressing $MOG_{40–55}$ into EAE mice could induce antigen-specific tolerance and ameliorate symptoms in the established EAE mice, as demonstrated by the lower incidence of anti-$MOG_{40–55}$ antibodies and reduced clinical scores when compared with controls. Furthermore, splenocytes from EAE mice transplanted with MOG-bone marrow cells produced significantly higher amounts of IL-5 and IL-10 upon autoantigen challenge when compared with controls, whereas the frequency of interferon gamma (IFN-γ) and IL-6 specific producing cells was not altered. Recently, brain-derived neurotrophic factor (BDNF), a pleiotrophic cytokine of the neurotropin family, has also been shown to be effective in reducing the severity of EAE [56]. EAE mice receiving bone marrow stem cells (BMSCs) transduced with retroviruses containing the BDNF gene were found to have delayed clinical onset, reduced clinical severity, as well as decreased apoptosis in brain and spinal cord lesions. Immunologically, decreases in the pro-inflammatory cytokines, TNF-α and IFN-γ, and enhanced expression of the anti-inflammatory cytokines, IL-4, IL-10, and IL-11, were found in the CNS of the BDNF transplanted group but not in the control group. Moreover, mice administered with BDNF-engineered BMSCs had reduced demyelination and increased remyelination compared to mice receiving BMSC transfected with an empty vector lacking the BDNF gene.

Gene therapy involving anti-inflammatory cytokines/chemokines is another treatment being investigated in EAE. Sloane et al. obtained their results on anti-inflammatory IL-10 gene therapy by means of injecting a plasmid encoding the rat IL-10 complementary DNA (cDNA) under the control of a hybrid cytomegalovirus enhancer/chicken beta actin promoter intrathecally in a rat EAE model [35]. In another study, the safety and efficacy of intrathecal helper-dependent adenoviral vector-mediated gene therapy in mice and the mechanism induced by IL-4 gene therapy were demonstrated by intracisternal administration of an IL-4-producing HD-Ad

Table 3 Advantages and disadvantages of common gene delivery systems

Vector		Advantages	Disadvantages
Viral vectors	Adenovirus	High titers (10^{12} pfu/mL) High efficiency of transduction in vitro and in vivo Transduces different cell types Transduces both proliferating and non-proliferating cells	Stays episomal Transient expression Packaging cell line necessary Repeated administrations cause immune-related toxicity Possible replication competence No targeting Restricted insert size 4–5 kb
	Adenoassociated virus	Incorporation on human chromosome 19 (wild-type only) to establish latent infection Sustained expression Transduction does not need cell division Small genomic size, no viral genes	No directed targeting Packaging cell line necessary Possible insertional mutagenesis Tedious procedure to produce high titers (10^{10} pfu/mL) Narrow insert size 5 kb
	Herpes simplex virus	Insert size 40–50 kb Neuronal tropism Latency expression Transduce efficiently in vivo Replicative vectors available	Cytotoxic Transient expression, does not incorporate into genome Packaging cell line necessary No directed targeting Relatively low titers (10^4–10^8 pfu/mL)
	Lentivirus	Transduces both proliferating and non-proliferating cells Transduces hematopoietic stem cells sustained expression Realtively high titers (10^6–10^7 pfu/mL)	Safety concerns: from human immunodeficiency virus origin Tedious to manufacture and prepare stock Restricted insert size 8 kb
	Retrovirus	Incorporation into cellular genome Wide-ranging cell tropism Sustained expression Relatively high titers (10^6–10^7 pfu/mL) Bigger insert size 9–12 kb	Low transduction efficiency Packaging cell line necessary Insertional mutagenesis possible Involves cell division for transfection No direct targeting Probable replication competence
Non-viral Vector	Plasmids Oligonucleotides	Relatively safe Causes low immune response Prepared easily, at low cost and in large quantities Stored for long periods due to their stability	Low transfection efficiency limits their use on a large scale

vector in a mouse model of EAE [57]. Mice injected with HD-Ad vector expressing IL-4 showed significant clinical and neurophysiological recovery from chronic and relapsing EAE. The therapeutic mechanism is likely due to increased IL4 in the inflamed CNS areas and the ability of chemokines (CCL1, CCL17, and CCL22) to recruit $CD4^+CD69^-CD25^+Foxp3^+$ regulatory T cells (Tregs). On the other hand, Park et al. examined the effects of a decoy chemokine (7ND) gene therapy, which inhibits the migration of macrophages in acute, biphasic, and chronic EAE in rats [58]. Although the number of CNS-infiltrating macrophages was reduced in all stages of the three types of EAE, the clinical signs of acute EAE and the first attack in biphasic EAE were minimally affected. However, chronic EAE and relapses in biphasic EAE were completely suppressed. To improve the efficacy of anti-inflammatory cytokine and chemokine gene therapy, future strategies should take into consideration the highly diverse pathological mechanisms and growing knowledge of the natural history of MS. In addition to cytokines and chemokines, gene therapy has also been applied to the delivery of other immunomodulatory molecules. For example, Zhu et al. reported that intracerebral delivery of recombinant sFlt-1 (1–3) adenoviral vector into EAE rats significantly reduced disease severity compared with untreated rats. The sFlt-1 (1–3) gene transfer blocked vascular endothelial growth factor (VEGF) and greatly reduced the number of cells that express VEGF and ED1 (CD68) in the CNS and ameliorated the severity of EAE by inhibiting monocyte recruitment [59] .

NR4A2 is a transcription factor that has been found to play a critical role in up-regulating the production of inflammatory cytokines (IL-17 and IFN-γ) in pathogenic T cells and thus triggering the inflammatory cascade [60]. Gene silencing of the orphan nuclear receptor NR4A2 by small interfering RNA (siRNA) effectively reduced IL-17 and IFN-γ production. Furthermore, the silencing of NR4A2 with its specific siRNA

reduced the ability of encephalitogenic T cells to transfer disease in recipient mice. These results suggest that modulation of NR4A2 by specific siRNAs can be effective in MS therapy.

Of the gene therapies discussed so far, viruses act as the common delivery vector. Despite showing great promise in treating MS, adverse reactions and low efficiency are still major obstacles. As a result, researchers began looking for a more appropriate gene delivery system to solve these two problems. Micron-scale polymer beads, poly(lactic-co-glycolic acid) (PLGA), was developed for this purpose [61]. These polymer beads are biocompatible, which makes them not only safer but also keep relative lower clearance of the vehicle from the body than the viral vector. While viruses are only capable of delivering genes in a single dose, these beads can continuously release low levels of genes as they degrade and thus, extend the expression period in the system. At the same time, these polymer beads can be completely degraded into lactic acid and glycolic acid, which then get effectively processed by the body and cause minimal toxicity. In addition, while viruses provide no DNA protection mechanisms once their gene of interest is delivered, PLGA system is able to effectively protect DNA from degradation once inside target cells [62]. Using this method, Hunter et al. reported the success of this long-term protection to MS in their relapsing EAE animal model [63]. Treatment of animals with a standard dose of 1.25 mg of myelin peptide ($PLP_{139–151}$)-coupled particles intravenously was the most effective method at preventing clinical R-EAE symptoms compared to intraperitoneal, subcutaneous, and oral routes. These animals also showed a reduction in the infiltration of inflammatory cells ($CD45^+$ T cell, plasmacytoid dendritic cells, and macrophages/monocytes), cytokine production (IFN-γ and IL-17A), and demeylination in the CNS. Same results were found in and reinforced by Getts' et al. research which used 1 mg of $PLP_{139–151}$-coupled microparticle treatment by IV to the splenic marginal zone and provided evidence to show the importance of the scavenger receptor, MARCO [64]. Furthermore, tolerance induced by peptide-coupled microparticles depends on both the induction of T cell anergy, IL-10, and the activity of Tregs. Collectively, these works highlight the therapeutic potential for using antigen-coupled microparticles to target natural apoptotic clearance pathways by inducing antigen-specific T cell tolerance, while permitting a cost-effective treatment for MS.

Accumulating data from EAE models suggest that gene therapy is a promising approach in the treatment of MS. It should be noted that there are differences in animal species, delivery vectors, and antigens used for immunization. Most importantly, the differences in the disease course, which reflect the clinical subtypes in these studies, need to be considered. It is true that we should interpret the data with caution, but the knowledge gained from these studies provide a significant framework in tailoring potential therapy with respect to disease stages and the underlying pathophysiological process for individual patients. On the other hand, we have to realize that the CNS is recognized as an "immune privileged organ" because of its exceptional functional and anatomical features. Knowledge about the modulation of immune responses within the CNS is very limited. Furthermore, delivery and targeting of therapeutic molecules are often complicated by the presence of the blood-brain barrier. Before treatment of MS in humans with gene therapy, detailed analysis of the following has to be conducted: (1) the potential immunogenicity of gene therapy vectors and therapeutic molecules, (2) the methods for homing vectors, and (3) the timing and level of expression of the therapeutic molecules.

Rheumatoid Arthritis: Gene Therapy in the Collagen-Induced Arthritis Model

Rheumatoid arthritis is a chronic inflammatory disease, which causes progressive deformity and destruction of the joints and thus, leads to disability and even premature death. This condition is characterized by aggressive infiltration of monocytes and T lymphocytes into the synovial lining of joints along with the proliferation of fibroblastic lining cells [65]. Intervening in inflammatory cascades within mice using monoclonal antibodies to cytokines and T cell receptors combined with methotrexate and corticosteroid therapy have formed the basis for current clinical treatments. However, autoimmunity to antibodies and side effects resulting from methotrexate and corticosteroids necessitate the development of alternative approaches, including gene therapy which are being studied using animal models. Although there will be differences in disease mechanisms between experimental animal models and human disease, the former promotes understanding of underlying biology and provide a feasible platform for translation to preclinical study or clinical trial. CIA is an established rodent model of autoimmune polyarthritis with many similarities to human RA. Recent studies on gene therapy have focused on the modulation of immunological mechanisms with the goal of suppressing the levels of pro-inflammatory molecules, the infiltration of activated effector cells, and the clinical symptoms and pathology of arthritis found in this animal [66, 67].

Results from studies increasingly suggest that adiponectin (APN) is an adipocytokine which has protective and anti-inflammatory effects in patients with RA [68]. At the molecular level, APN suppresses TNF-α and IL-6 production by liposaccharide-activated macrophages through the suppression of nuclear factor-kappa B signaling. Ebina et al. [69] demonstrated that treatment of CIA mice with APN-producing, engineered adenovirus suppressed the progression of arthritis; inhibited local deposition of C1q and C3; decreased the deposition of CXCL12 in the synovia; and reduced the infiltration of neutrophils in the cartilage of wrist, knee, and

ankle joints. More importantly, it reduced the number of TRAP-positive cells and ameliorated bone erosion in joints. The angiogenic factor VEGF promotes synovitis and bone erosion in RA, and its expression correlates with disease severity in RA patients and in murine CIA. Using an adenoviral gene delivery system expressing soluble VEGF receptor 1 (sFlt-1), Afuwape et al. demonstrated that adenoviral delivery of human soluble sFLT-1 significantly suppressed disease severity and paw swelling when compared with untreated mice or those receiving the vector alone [70].

siRNA-mediated knockdown of pro-inflammatory cytokines at the messenger RNA level offers an alternative therapeutic strategy in overcoming inflammatory conditions [71, 72]. A number of studies in CIA model have demonstrated that RNAi-based intervention is a promising novel anti-inflammatory therapy for RA. For example, Khoury et al. [73] reported that weekly injections of anti-IL-1, anti-IL-6, or anti-IL-18 siRNA-based lipoplexes significantly reduced the incidence and severity of arthritis and abrogated joint swelling and the destruction of cartilage and bone. When the animals were given a siRNA lipoplex cocktail containing all three siRNAs, the pathologic features of RA, including inflammation, joint destruction, and the Th1 response, as well as overall parameters of RA were improved when compared with the anti-TNF siRNA lipoplex-based treatment. Moreover, Howard et al. showed that knockdown of TNF-α expression in systemic macrophages by intraperitoneal administration of chitosan/siRNA nanoparticles containing an unmodified anti-TNF-α Dicer-substrate siRNA downregulates systemic and local inflammation in mice with CIA [74]. Likewise, Lee et al. found high accumulation at the arthritic joint sites after intravenous injection of poly-siRNA targeting TNF-α with thiolated glycol chitosan nanoparticles two times a week for 7 weeks, and the treatment significantly inhibited inflammation and bone erosion in CIA mice [71].

Similar to the nanoparticles, lipoplexes is a new method developed to deliver siRNA specific in the mononuclear phagocyte system and induce silencing of inflammatory cytokines [75]. Using this approach, Courties et al. was able to downregulate the expression and activity of cytosolic phospholipase A2 (cPLA$_2$α), the enzyme that plays critical role in many inflammatory diseases [76]. Weekly systemic injection of cPLA$_2$α siRNA also significantly reduced the expression of other pro-inflammatory cytokines such as TNF-α and IFN-γ in the CIA model. There was a near complete inhibition of cell infiltration in joints and less paw swelling in the treated animals.

B cell-activating factor belonging to the TNF family (BAFF) is a cell survival and maturation factor for B cells, and overproduction of BAFF is associated with systemic autoimmune disease [77, 78]. Lam et al. showed that gene therapy via the intra-articular injection of replication-deficient lentivirus which expressed small hairpin RNA (shRNA) for BAFF gene silencing inhibited pro-inflammatory cytokine expression, suppressed the generation of plasma cells and Th17 cells, markedly ameliorated joint pathology, and provided long-term suppression of arthritic development in a CIA model [79]. Lentivirus targets dendritic cells in the joint tissue. Thus, gene therapy via local delivery of shRNA by lentivirus possibly inhibits dendritic cell maturation and its role in driving Th17 cell differentiation, all of which restricts the onset and progression of CIA. Similar concept was also applied to deliver lentiviral-medicated Toll-like receptor 7 (TLR7) shRNA gene in a CIA rat model [80]. Overexpression of TLR was observed immunohistochemically in both the lining and sub-lining regions of rheumatoid synovium. After being immunized on days 0 and 7, animals were given two intra-articular treatments on days 7 and 10. On day 16, significant reduction in ankle circumferences, articular index, and radiographic and histological scores was observed. Knockdown of TLR7 gene in this rat model also lowered VEGF concentration, IL-1, and IL-6 levels within synovial tissues.

In searching for an effective therapy that would specifically inhibit inflammatory disease processes while allowing for the effective repair of damaged articular cartilage but avoiding the induction of generalized immunosuppression, Song et al. developed a novel tolerizing DNA vaccine coined pcDNA-CCOL2A1, which contains the chicken type II collagen with deleted N-propeptides and studied its therapeutic efficacy in a rat model of CIA [81]. Their results demonstrated that a single intravenous treatment with pcDNA-CCOL2A1 vaccine alone significantly reduced the severity of disease, footpad swelling, arthritic incidence, and clinical scores, as well as deferred the onset of disease. As such, pcDNA-CCOL2A1 has the potential to be a novel therapeutic agent in RA.

With the rapid advancement in science and technology, increased research has been undertaken to confirm the validity of gene therapy in RA animal models. Even though short-term morbidity and mortality rates are low in RA patients, clinical symptoms can eventually become severe and affect multiple organ systems. Furthermore, epidemiology studies and prospective data analysis have revealed multiple risk factors, such as environment, hormone, genetics, and infection, are associated with RA. In this section, we discussed diverse and promising gene therapies for RA. There are however limitations to these strategies, both in the laboratory and clinical settings. For example, certain biological molecules, such as cytokines, may need to be systematically delivered at toxic concentrations in order to achieve an effective local response due to their short half-lives. Additionally, the vectors or promoters used to transduce or regulate transgene expression can often lose their immunogenicity after a period of time. Similar transient effects arise using the plasmid DNA approach because of its ability and the efficacy to incorporate into the genome of cells. It is still much too early to conclude which method is the most efficacious. The search for a safe, effective,

and low-cost treatment is always the goal of a scientist. Novel strategies such as gene therapy can be strategically designed by focusing on modulating the risk factors in experimental animals with the goal of preventing the onset of RA in high-risk individuals. With the numerous potential therapeutic targets we have discovered (or will discover), the pathological mechanisms of autoimmunity remain elusive. Further experimental and clinical studies to better understand the various components of gene therapy strategies so that ideal will facilitate the design of treatment regimens for patients with RA.

Gene Therapy Clinical Trials in Autoimmune Diseases

Conventional biotherapies for autoimmune diseases are limited by transient efficacy, severe side effects associated with large dosages, and frequent relapses [82, 83]; gene therapy has become the next potential intervention for patients because of its target-specific and easy-to-control characteristics. Gene therapy in animal models has proven to be reliable and effective in treating several autoimmune diseases [55–59]. In recent years, significant progress has been achieved in the areas of viral vector design as well as other delivery vehicles to reduce the immune response to transgenes. These advancements have led to several successful clinical studies, in which we will focus again on MS and RA (Table 4).

Two DNA vaccines have developed for treating patients with multiple sclerosis. One, named BHT3009, encodes full-length MBP [84, 85]. In a phase II study, BHT3009 was administered at weeks 0, 2, and 4 and then monthly by subcutaneous injection. Although this treatment did not alter the relative risk for relapse or initial time to relapse, there was a reduction in new CNS lesions. The reduction in lesion formation was most prominent in patients that had high concentrations of antibodies targeting MBP, whereas patients with low anti-MBP titers had a similar rate of lesion formation as placebo-treated controls. Although further studies are needed to confirm the potential utility of BHT3009, the existence of a patient population that may not be responsive to BHT3009 supports the need for other therapeutic modalities.

Myelin reactive T cells have been known to play a critical role in the neuropathogenesis of MS [86]. Two human clinical trials using an ex vivo enriched source of myelin reactive T cells which were attenuated by irradiation, as the treatment agent to sensitize the immune system in MS patients. The phase IIb placebo-controlled Tovaxin study involved 150 relapsing-remitting MS participants [48]. The result showed a favorable safety profile for Tovaxin; however, no statistically significant clinical or radiological benefit was identified. Prior disease-modifying treatments might be the cause for reducing Tovaxin treatment and the study power in this study. The second T cell vaccination study involved 24 relapsing progressive MS patients [49]. One year after treatment, 94.1 % of the patients stayed relapse-free during the year and was reduced by 89.6 % even for the patients with any relapse during the year of study in the T cell vaccination group, as compared to the placebo-treated group.

Besides broad-based immune suppression, studies from the induction of prolonged, antigen-specific tolerance provide evidence for treating autoimmune diseases from a different perspective. In the EAE model, single IV injections of autologous cells, such as splenocytes or peripheral blood monocytes (PBMC) coupled with encephalitogenic myelin peptides, blocked epitopes from spreading at an early stage and thus, induced tolerance [87]. One major advantage of this antigen-specific therapy is that tolerance can be induced to multiple epitopes using a cocktail of myelin peptides at the same time. In a phase I clinical trial, antigen-specific tolerance is induced through carrier cells, which are pulsed with antigens in the presence of the chemical cross-linker 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) [88]. As a catalyst, EDC links the free amino acid and carboxyl groups via the formation of a peptide, thereby producing peptide-coated cells and serve as highly tolerogenic carriers. In this trial, escalating dose from 1×10^3 up to the target dose of 3×10^9 cells were given to MS patients. All patients tolerated the infusion of high doses ($>1\times10^9$) of PBMC-EDC coupled with seven different autologous myelin peptides at weeks 2, 6, and 3 months and exhibited a decrease in myelin-specific T cell responses after therapy. The patients with low disease activity showed no occurrence of new T2 lesions or contrast-enhancing lesions, whereas new lesions were detected in patients with more active disease after treatment. Even antigen-specific approaches to generate tolerance have shown to be effective in animal models and been previously tested in clinical trials for MS patients. On the downside, one of the major safety concerns involving antigen or antigen-derived therapies is the risk of developing severe hypersensitivity reactions. The administration of altered peptide ligand (APL) prevented EAE induction in animal models but failed in clinical trials with MS patients. Severe systemic hypersensitivity reactions observed in 13 of 142 subjects after subcutaneous administration of APL of $MBP_{83–99}$ halted phase II clinical trials [89]. In another study that was halted, intradermal administration of CGP77116, an APL of $MBP_{83–99}$, worsened symptoms in patients with MS because at least two patients exhibited increased immune responses to $MBP_{83–99}$ [90]. Antigen-specific therapy appears to induce tolerance with less toxic results and without bystander suppression effects. However, it requires ex vivo isolation of autologous immune cells which leads to subsequent possible adverse side effects. Therefore, contingent on future investigations, antigen-specific therapy may be a feasible option in treating MS and other autoimmune diseases.

Table 4 Human clinical trials of gene therapy in autoimmune diseases

Disorder/ Syndrome	Clinical trial	Transgene	Objectives	Results
Multiple sclerosis	A phase I single-center, open-label, dose escalation trial administering PBMC-EDC-coupled with autologous myelin peptides in RR and second progressive MS patients	Myelin peptides	To access the safety, tolerability, and in vivo mechanisms of action of this antigen-coupled cell tolerance regimen.	Six patients with low disease activity showed no increase in new T2 lesion of contrast-enhancing lesions by MRI. No further relapse occurred during the 6-month follow-up period [88].
Multiple sclerosis	A phase IIb placebo-controlled autologous T cell immunotherapy, a pool of cell lines raised against immunodominant peptides derived from MBP, MOG, and PLP (Tovaxin) (NCT 00245622)	Myelin peptides	To evaluate safety, tolerability and efficacy of Tovaxin in patients with relapses and remission multiple sclerosis and clinically isolated syndrome	Evidence clinical efficacy in favor of Tovaxin, as defined by ARR. Further studies in patients with more active disease were advised to increase the likelihood of detecting a clinically meaningful benefit [48].
Multiple sclerosis	A phase I, double-blind placebo-controlled dose escalation study of recombinant T cell receptor ligand (RTL-1000) for treatment of MS	Myelin peptide	To determine the maximum tolerated dose safety and tolerability of RTL-1000 in HLA-DR2^{+} MS patients	Thirty-four subjects who completed the study tolerated 2–60 mg dose of RTL-1000 [108]
Multiple sclerosis	A phase I/II T cell vaccination study, using four injections of attenuated T cell line reactive to nine different myelin peptides, in relapsing progressive multiple sclerosis patients	Myelin peptides	To evaluate the safety and efficacy of T cell vaccination (irradiation-attenuated, autologous anti-myelin T cells) in a double-blind, randomized controlled setting	There was a profound clinical effect on relapses and a beneficial effect on disability, as evidenced by the follow-up expended disability status scores and the performance in the timed 10-m walking test [49].
Multiple sclerosis	An open-label phase I trial using IV infusion of CTLAIg in RR MS patients	CTLAIg	To study the safety and efficacy of CTLAIg in RR MS patients	CTLAIg was well tolerated in patients with mild adverse events. A reduction in MBP proliferation was found within 2 months of infusion and decreased IFN-γ production by MBP-specific lines [109].
Multiple sclerosis	A phase I trial of immunotherapy with BHT-3009 alone or combined with atorvastatin (NCT00103974)	DNA plasmid vaccine encoding MBP	To evaluate the safety of BHT-3009 alone and when combined with atorvastatin (Lipitor) in patients, followed by determining dose of BHT-3009 and regimen for phase II testing	One publication indexed to this study by the NCT number. Using this plasmid, this was the first successful attempt of a DNA vaccine in modulating the autoimmune response in human subjects [84].
Multiple sclerosis	BHT-3009 immunotherapy in RR MS patients (NCT00382629)	DNA plasmid vaccine encoding MBP	To evaluate the effects of BHT-3009 on the occurrence of new gadolinium (Gd) enhancing MRI in RR MS patients	Low dose reached primary end point for reduction of the rate of new enhancing MRI lesions. Induction of Ag-specific immune tolerance was observed in a preselected subgroup of patients [85].
Multiple sclerosis	A double-blinded, placebo-controlled, randomized phase II study of subcutaneous administration of altered peptide ligand of MBP in RR MS patients	Altered peptide ligand of MBP	To evaluate the safety and influence after altered peptide ligand administration (NBI 5788) on magnetic resonance imaging in patients	Adverse effect: induction of non-encephalitogenic Th2 autoimmune response, including the frequency of cells produced IL-5 and IL-13 and the peripheral blood cytokine levels [89]
Multiple sclerosis	A magnetic resonance imaging-controlled, single-center, baseline-to-treatment cross over phase II clinical trial using altered peptides ligands of MBP (CPG77116) in RR MS patients	Altered peptide ligand of MBP	To access the safety, tolerability, and efficacy on MRI and immunological parameters of 50 mg CGP77116 given subcutaneously weekly	No significant clinical benefits were observed. The study was terminated due to adverse side effects from the treatment, in addition three of eight patients experienced exacerbations of disease. [90].
Rheumatoid arthritis	A phase I study transferred human IL-1Ra cDNA to MCPs on RA patients' hand by ex vivo retroviral transduction of	IL-1Ra cDNA retrovirus	To evaluate the safety and feasibility of IL-1Ra gene transfer to human rheumatoid arthritis patients	All subjects tolerated the treatment without adverse effects, and they gave positive RT-PCR signals. Synovia that were recovered

Table 4 (continued)

Disorder/ Syndrome	Clinical trial	Transgene	Objectives	Results
	autologous synovial fibroblasts			from MCP joints of intermediate and high dose subjects produced elevated IL-1Ra [99].
Rheumatoid arthritis	A phase I study transferring IL-1Ra cDNA in an ex vivo, retroviral strategy to patients' joint	IL-1Ra cDNA retrovirus	Subsequent study to confirm the safety and efficacy of IL-1Ra transfer in an ex vivo fashion and expressed intra-articularly	In the short term (1 month), two subjects responded with reduced joint pain and swelling [100].
Rheumatoid arthritis	A phase I dose escalation study of intra-articular administration of tgAAC94 AAV2 vector containing TNFR:Fc fusion gene (NCT00617032)	TNFR:Fc AAV2 vector	To evaluate the safety of intra-articular administration of tgAAC94 vector	Subject died in this study. No other result was provided by the sponsor (Targeted Genetic Corp.). [96]
Arthritis, rheumatoid Arthritis, psoriatic Ankylosing spondylitis	Phase I/II study of repeat intra-articular administration of tgAAC94, a vector containing the TNFR:Fc fusion gene with and without concurrent TNF-α antagonists (NCT00126724)	TNFR:Fc AAV2 vector	To evaluate the safety of intra-articular administration of tgAAC94 in subjects currently taking TNF-alpha antagonists, and the safety of repeat intra-articular administration of tgAAC94	A death occurred in a case report [97]. No result was provided by the sponsor (Targeted Genetic Corp.). Two publications automatically indexed to this study by the NCT number [95, 110].

PBMC-EDC peripheral blood monocytes-1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, *MBP* myelin basic protein (MRI), *MOG* myelin oligodendrocyte glycoprotein, *PLP* proteolipid protein, *RR MS* relapsing-remitting multiple sclerosis, *ARR* annualized relapse rate, *Th2* type 2 T helper cells

Rheumatoid Arthritis

Recent advancements in biotechnology and preclinical studies have revolutionized RA therapeutics using biologically-derived immunomodulating compounds such as TNF-α inhibitors, IL-1 blocking agents, and anti-inflammatory cytokines [2, 67, 68]. Clinical trials in RA have shown that administration of TNF-α antagonist is safe, effective, and well tolerated [91, 92]. On the other hand, there are still lingering concerns about anti-TNF therapy, including the re-activation of granulomatous diseases such as tuberculosis, the re-activation of chronic hepatitis B if not given concurrently with antiviral therapy, the increased risk for lymphoma, and the tendency for a worse prognosis in patients with advanced heart disease, as compared to the general population [93]. Data from preclinical gene transfer studies using a recombinant AAV vector containing a human TNF-immunoglobulin Fc fusion gene (rAAV2-TNFR:Fc; tgAAC94) in a rat model for experimental arthritis showed that a single dose of 1×10^{12} DNase-resistant particles (DRP)/mL of tgAAC94 administered via intra-articular injection resulted in the suppression of arthritis in both the affected and contralateral joints [94]. Subsequently, a phase I multi-site double-blind, placebo-controlled, dose escalation study was conducted in the USA and Canada [95]. Fourteen RA patients received randomized, single intra-articular injections of tgAAC94, at a concentration of 1×10^{10} or 1×10^{11} DRP/mL, or placebo into the knee or ankle and were then evaluated by complete blood count, blood chemistry, and urinalysis. In addition, clinical response was evaluated by examining the target joint for tenderness. Notably, intra-articular injections of tgAAC94 were well tolerated and without major safety issues. No adverse effects were associated with the development of anti-AAV2 capsid antibodies. Synovial fluid TNFR:Fc protein was not detected at the doses used. Improvement in tenderness and swelling was noted in both cohorts. In summary, this clinical study showed that gene therapy using intra-articular injections of tgAAC94 is safe and well tolerated up to 1×10^{11} DRP/mL joint volume in subjects with inflammatory arthritis. However, the small number of subjects in this trial has limited its statistical power, and further validating measures for assessing the response to a single joint of local treatment is needed. Nevertheless, data from this phase I study provides the proof of concept for rAAV-mediated immunomodulatory therapy in autoimmune diseases. A further clinical study that evaluated the safety of intra-articular administration of tgAAC94 in subjects currently taking TNF-α antagonists and the safety of repeated intra-articular administration of tgAAC94 (NCT00126724) was completed in May 2009. Patients showed well tolerance with no major safety issues to the treatment. At 12 weeks after injection, reduced swelling in knee joint was noted in 2 out of 11 patients [96]. However, it is worth to mention that a death occurred in a case report [97]. On the basis of its investigation,

the patient's death was due to disseminated histoplasmosis with subsequent bleeding complications and multi-organ failure. Even none of the available data support a conclusion that the gene therapy agent contributed to the patient's death; this case highlighted the risk of opportunistic infections in patients receiving such AAV gene therapy.

IL-1 is a pivotal pro-inflammatory cytokine that has been shown to contribute to the clinical manifestations of RA. The ability of IL-1 to drive inflammation and joint erosion and to inhibit the tissue repair process has been clearly established in vitro and in animal models. IL-1Rα is the IL-1 receptor antagonist that is increased in RA patients [98]. In an ex vivo, retrovirus-based clinical trial, genetically modified autologous synovial fibroblasts with a recombinant retrovirus (MFG-IRAP) that carried the human IL-1R1 cDNA were injected into the metacarpophalangeal joints of nine RA subjects [99]. One week later, the synovia were retrieved from these patients and showed stable transgene expression. Using a visual analog scale for the entire treatment period, a subsequent clinical study with two subjects showed rapid reduction in pain and swelling for both persons involved. Northern Blotting analysis revealed increased expression of IL-1Rα and decreased expression of matrix metalloproteinase-3 and IL-1β [100]. Given these promising data, larger sample sizes with objective outcome measurements in future studies are needed to evaluate the efficacy and feasibility of this protocol.

The Future of Gene Therapy in Autoimmunity

Differences in genetics, epigenetic, and pathophysiology should preclude any conclusion about the therapeutic effects of autoimmunity treatment in humans based solely on animal models. Additionally, negative treatment results in animal models do not automatically exclude positive results in humans. The success of gene therapy in alleviating autoimmune responses in various animal models of autoimmunity holds great promises of similar applications in treating human autoimmunity. However, despite extensive studies in animal models and prudent conceptualization, clinical studies sometimes run into spectacular setbacks, especially when it comes to trials investigating new drugs. Major obstacles that limited the development of gene therapy include (a) gene silencing, (b) insertional mutagenesis which results from random integration of the therapeutic vector genome within the host DNA, (c) phenotoxicity which can result from the overexpression or ectopic expression of the donated gene, (d) immunotoxicity which refers to harmful immune response to either the vector or the transgene product, (e) horizontal transmission of the donated DNA or the risk of vector shedding, and (f) vertical transmission which refers to inadvertent germ line transmission of the donated DNA. Therefore, to date, clinical trials involving gene therapy in autoimmunity is limited to a small number of autoimmune disorders.

However, rapidly expanding knowledge on the structural and functional characteristics of the human genome allows for the development of genome-wide approaches to investigate the molecular circuitry that wires the genetic and epigenetic programs of somatic stem cells. High-throughput approaches are essential to study the transcriptome, the epigenome, and the usage of regulatory elements in the genome. Indeed, genome-wide association studies highlighted approximately 60 risk loci for RA [101], and 48 more new susceptible variants were recently identified for MS [102]. Clinical trials for biomarker identification (NCT01638715, NCT01642706, NCT01835613) may reveal candidate genes that can be targeted in therapeutic designs. Benefits of inducing long-term tolerance via transplantation of "engineered" hemopoietic stem cells (HSC) have been shown in animal models [103]. In a phase I/II clinical study, progression-free survival and reversal of neurological disability at 3 years post-autologous HSC transplantation were shown in relapsing-remitting MS patients [104]. As a result, HSC transplantation stands out above other immunotherapies, given its potential to provide tolerance for life once HSCs possessing modified genes are successfully implanted in the host. These efforts, together with the continuous advancement in cutting edge technologies such as gene silencing and potential use of mesenchymal stem cells, can open new chapters in the treatment of autoimmune diseases.

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