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REVIEW

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Hematopoietic Stem Cell Gene Therapy as a Treatment for Autoimmune Diseases

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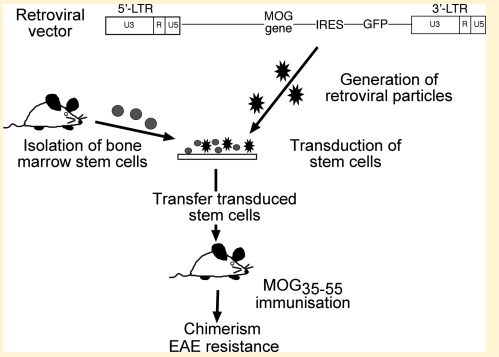
ABSTRACT: A key function of the immune system is to protect us from foreign pathogens such as viruses, bacteria, fungi and multicellular parasites. However, it is also important in many other aspects of human health such as cancer surveillance, tissue transplantation, allergy and autoimmune disease. Autoimmunity can be defined as a chronic immune response that targets self-antigens leading to tissue pathology and clinical disease. Autoimmune diseases, as a group of diseases that include type 1 diabetes, multiple sclerosis, rheumatoid arthritis and systemic lupus erythematosus, have no effective cures, and treatment is often based on long-term broad-spectrum immunosuppressive regimes. While a number of strategies aimed at providing disease specific treatments are being explored, one avenue of study involves the use of hematopoietic stem cells to promote tolerance. In this manuscript, we will review the literature in this area but in particular examine the relatively new experimental field of gene therapy and hematopoietic stem cell transplantation as a molecular therapeutic strategy to combat autoimmune disease.



KEYWORDS: autoimmune disease, immune tolerance, experimental autoimmune encephalomyelitis, gene therapy, bone marrow transplantation

AUTOIMMUNITY

Autoimmune diseases affect approximately 5−6% of the population and can be generalized as resulting from a chronic adaptive immune response that is fueled by self-antigens and leading ultimately to clinical disease. Over 60 autoimmune diseases have been defined and include diseases such as type 1 diabetes (TID), multiple sclerosis (MS), systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).[1] For each of these there is a well-defined and highly specific immune response associated with the disorder such that, for example, for T1D, the insulin secreting beta cells within the pancreatic islets of Langerhans are destroyed, leading to insulin deficiency and the need for insulin replacement.[2] Multiple sclerosis on the other hand is an autoimmune mediated disease that attacks the myelin sheath that surrounds and insulates axonal neurons of the central nervous system.[3,4] This can result in a range of motor, sensory, visual and cognitive system dysfunctions.[5,6] Contrary to general perception, autoimmunity is not restricted to the elderly. Type 1 diabetes can occur in the very young, and the mean age of onset for MS is 20−40 years of age. Therefore as a group, autoimmune diseases represent a major health and lifestyle burden on our society, and there are no cures for them. While there is a lot of activity in devising new treatments and therapeutics for autoimmune diseases, treatments are far from perfect and are associated with side effects, with the key issues being toxicity and specificity. For many autoimmune diseases, corticosteroids are still the major form of treatment, but in the aim of controlling immunity to self, the ability to combat pathogens is also compromised.

IMMUNITY AND IMMUNE TOLERANCE

The basis of immunity involves the ability to recognize foreign bodies and mount appropriate offensive action. This is predominantly in the form of specialized receptors that have evolved or generated with the ability to identify pathogen-associated structures. These can simplistically be divided into the innate and adaptive arms of the immune system. In the innate immune system, these receptors are generically termed pathogen recognition receptors (PRRs) and have evolved to recognize defined structure on pathogens and collectively termed pathogen associated molecular patters (PAMPS). Pathogen recognition receptors can be found on a large range of innate immune cells including macrophages, dendritic cells and granulocytes. The targets of these receptors are often structures or molecules that are unique to the pathogens and important for survival such as terminal mannose residues that are characteristic of bacterial but not mammalian glycoproteins and double stranded RNA found in many viruses but not in mammalian cells.[7,8]

The adaptive immune response, as the name suggests, is more aligned with generating a series of defense mechanisms that are

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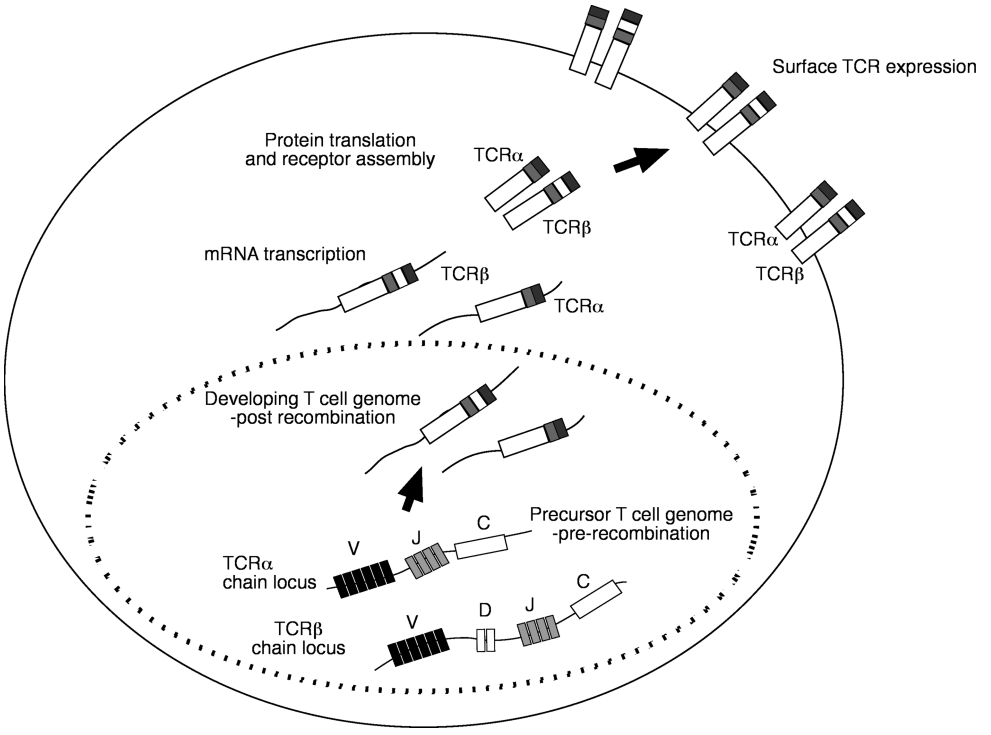

Figure 1. Generation of T cell receptors. During the process of development within the thymus, T cells undergo a process of recombination to generate antigen receptors. T cell precursors do not yet express T cell antigen receptors (TCR). Each chain of the TCR (α and β) is composed of defined regions that are termed variable (V), diversity (D), joining (J) and constant (C). Each of these regions is encoded in the genome, but in the precursor T cells, the V, D, J regions exist as multiple variables. During development within the thymus, within each individual cell, the process of recombination randomly selects V, D, J regions to generate the DNA sequence that now encodes the two chains of the TCR. Following the normal process of mRNA transcription and translation, the two chains of the TCR are generated and assembled where they are transported to the cell surface. Because of this random recombination process, each T cell will generate a unique receptor that is not driven by antigen. These developing T cells within the thymus will then go on to further selection processes before exiting to the periphery. A similar process of recombination also involving V, D, J gene elements to generate B cell antigen receptors also exists but utilizes different genomic regions.

highly specific for a particular structure or pathogen. It also provides the basis of immunological memory that underpins the concept of vaccination and the ability to "remember" previous exposures with resulting quicker and more intense responses. The main effector cells for adaptive immunity are the T and B lymphocytes that generate and display membrane (and secreted) receptors with the capacity for recognizing pathogen even if it has not previously been encountered. This ability stems from the fact that, unlike the receptors associated with innate immunity that are genetically encoded in our genome and inherited through the generation, receptors of the adaptive immune system are generated individually within the developing lymphocytes. This remarkable phenomenon is the result of somatic recombination occurring within each cell that randomly selects and joins together various gene segments encoding the T and B cell antigen receptors which when translated will generate antigen receptors with slightly different binding pockets and thus specificity (Figure 1). For T cells, this occurs within the thymus gland that sits above the heart and for B cells this activity is active within the bone marrow compartment. Because the process of recombination occurs within each developing cell and independently of neighboring cells, the system allows for the generation of a vast array of specificities and estimated to be in the order of 10^{18} and 10^{13} for T and B cells respectively.[9]

While this mechanism works well in generating a repertoire of receptors with the potential of recognizing a vast array of structures, the fact that receptors are randomly generated also means the probability that receptors generated can recognize and respond to self-molecules is high. The consequence of activating self-reactive lymphocytes is self-reactivity and the chance of developing autoimmune diseases. To counter this, the immune system has also developed a number of key mechanisms termed immune tolerance aimed at identifying and deleting potentially pathogenic clones or generating cohorts of lymphocytes capable of controlling or suppressing the activity of self-reactive clones that may escape into the periphery and termed peripheral tolerance. The understanding of immune tolerance has challenged immunologists for many decades, but some key findings have emerged that are relevant to this review. I will use the T cell compartment as an example, but there are many excellent reviews on immune tolerance that the readers may wish to read.[10−14] During T cell development within the thymus, it is now clear that exposure to self-molecules or antigens is critical for purging of clones that are highly self-reactive and likely to be pathogenic. This process largely occurs via interaction of the antigen receptors with processed self-molecules and presented by the major histocompatibility complex (MHC) on epithelial cells of the thymic medulla and also by bone marrow derived dendritic cells that have developed within the thymus or have entered from the periphery (Figure 2). A relatively recent finding that has helped explain how tolerance can develop to peripherally restricted molecules has been termed promiscuous gene expression.[13,15−17] Within the

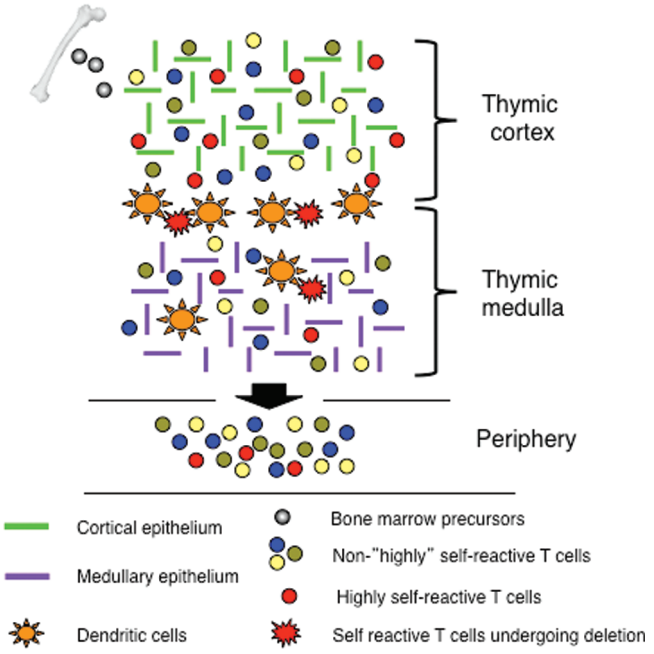

Figure 2. T cell development within the thymus. T cell precursors enter the thymus from the bone marrow and start their development within the thymic cortex with rounds of proliferation and the random generation of surface T cell receptors. This process will by chance generate T cells with receptors that will recognize self-antigens with strong affinity (highly self-reactive) and cells with lower affinity (non-"highly" self-reactive) antigen receptors. As T cells migrate through the thymic cortex, those with properly formed and thus functional receptors as defined by interaction with major histocompatibility complex (MHC) plus peptide are maintained as process labeled as positive selection. As cells move into the thymic medulla, interaction with MHC/self-peptide on medullary epithelium and bone marrow derived dendritic cells is used to select cells with high affinity for subsequent deletion. Cells that exit the thymus will thus be depleted of highly self-reactive clones, however the process is not 100% efficient and cells with potential pathogenic self-reactivity do enter the periphery where they can participate in the development and progress of autoimmune diseases.

thymus, under the direction of known and unknown transcription factors, genes that are normally active in peripheral sites, such as insulin within the pancreas, are also expressed within the thymus. The expressed molecules are not functional *per se* but generated with the sole purpose of being displayed to developing cells to identify those strong binders that could be pathogenic if they escaped into the periphery. A key transcription factor associated with this is the autoimmune regulator element (AIRE).[13,18] In humans and mice, AIRE is predominantly expressed in rapidly turning over medullary epithelial cells of the thymus,[19] and it is estimated that it may control the expression of hundreds to thousands of genes that encode peripheral molecules.[20−22] Humans that have a natural mutation in the AIRE gene[23] develop the autosomal recessive disorder, autoimmune poly-endocrinopathy-candidiasis-ectodermal dystrophy (APECED), otherwise known as autoimmune polyglandular syndrome type 1 (APS-1), which is characterized by the development of a range of autoimmune diseases.[24] A similar phenotype is observed in aire deficient mice following gene targeting, highlighting the evolutionary importance of this mechanism within the immune system to reduce the export of highly self-reactive clones.[20,25,26]

Termed promiscuous gene expression, this phenomenon refers to the fact that, within the thymus, an array of self-antigens are expressed and displayed to the developing T cells via MHC complexes. Those T cells that have, by chance, developed receptors that bind strongly to presented self-antigen are deleted (Figure 2). However, the fact that autoimmune diseases do exist indicates that the process is not absolute and pathogenic cells do escape into the periphery where they may be activated to initiate an autoimmune response. In fact, circulating lymphocytes with self-reactive potential can be found in all individuals and it is the influences of genetic susceptibility and environmental factors that are thought to largely dictate the development of disease. Apart from this central process of tolerance, the immune system has also developed peripheral mechanisms aimed at controlling the extent of self-reactivity and thus autoimmunity.[10,27−29]

GENETIC MANIPULATION OF HEMATOPOIETIC STEM CELLS AND INDUCED IMMUNOLOGICAL TOLERANCE

The ability to genetically manipulate whole animals and isolated cell populations has paved the way to better understand key principles of immunity but has also provided avenues for potential therapeutic strategies. As mentioned above, we now understand that exposure of developing lymphocytes to self-antigens is an important step in the process of immune tolerance. Building upon this, the question that emerged was whether targeting antigen expression to key areas of the immune system would help promote additional tolerance. To this end, we were the first to demonstrate in a transgenic model of the stomach specific autoimmune disease, experimental autoimmune gastritis (EAG), that ectopic expression of the gastric H/K ATPase-subunit (target autoantigen) in MHC class II positive cells could promote robust tolerance and render mice resistant to EAG.[30] The tolerance was antigen and disease specific since the mouse strain used is also susceptible to developing additional autoimmune diseases but these were not altered in our transgenic mice. Similar experiments using key autoantigens associated with other experimental disease models have since been reported including the NOD model for type 1 diabetes (T1D)[31,32] and experimental autoimmune uveitis,[33] supporting the key principle that ectopic expression of antigens can promote immune tolerance. While it has been known for many decades from the observations of Medawar and Billingham in twin calves that the bone marrow compartment can have a profound effect on immune tolerance,[34] today we know that bone marrow derived cells such as dendritic cells can also influence tolerance (as well as the main instigator of immunity) through their key role as an antigen presenting cell.[35−37] The introduction of antigen into dendritic cells followed by transfer to recipient mice can promote antigen specific tolerance. However, the ability to transfer tolerance from transgenic mice ectopically expressing autoantigen to recipient naive mice through bone marrow transfer[38,39] provided the key observation that manipulating the bone marrow compartment and more specifically hematopoietic stem cells (HSCs) alone may be sufficient to induce tolerance and provide an avenue for human translation.[40] This forms the cornerstone of the hypothesis that gene therapy using hematopoietic stem cells can be used to promote autoantigen specific tolerance as a treatment for autoimmune disease.[41−43]

The experimental protocol typically used involves the isolation of bone marrow stem cells from donor mice that are cultured ex vivo and transduced with retrovirus encoding the target antigen.

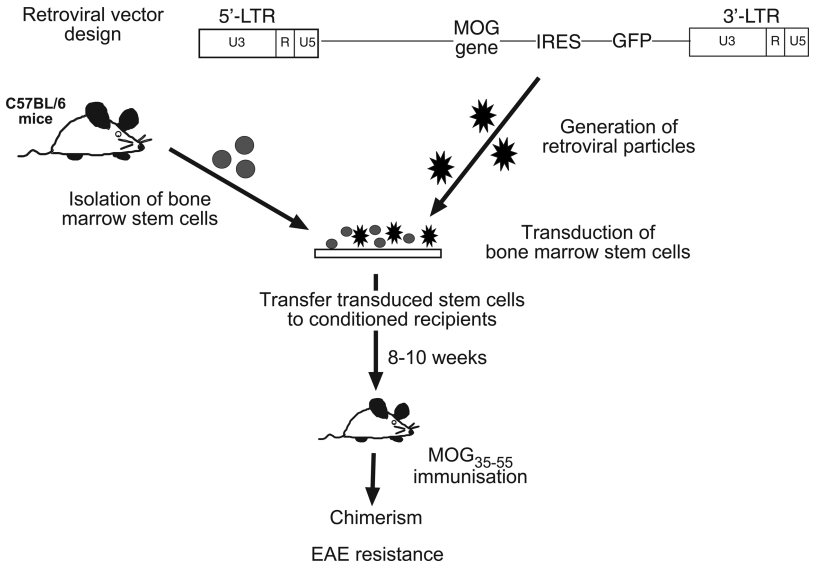

Figure 3. Experimental model. To direct the expression of autoantigen in bone marrow derived cells, retroviral vectors encoding the MOG genes are generated in vitro. Vectors also encode green fluorescent protein (GFP) driven by an internal ribosomal entry site (IRES) to aid in cell tracking and enumeration. Bone marrow cells isolated from donor mice are cultured ex vivo with retrovirus, followed by transfer to preconditioned recipients (total body irradiation). Recipient mice are housed for 8−10 weeks to allow for bone marrow engraftment and reestablishment of the hematopoietic system. At this stage, mice can be analyzed for chimerism or subjected to experimental autoimmune disease induction to assess disease development or protection.

Cells are then transferred to preconditioned recipient mice and left to engraft and reestablish their hematopoietic system, after which they can be analyzed (Figure 3). Unless the retroviral vector is modified, expression is driven by the endogenous retroviral promoter and in all transduced cells and their downstream progeny. In this system, it would include all cells of the hematopoietic system including T cells, B cells and dendritic cells. The degree of antigen that is expressed in this system will be determined by the level of expression within each cell and the overall molecular chimerism within the animal.

We have recently focused on the autoimmune mouse model of experimental autoimmune encephalomyelitis (EAE) that can be induced in mice by immunization with whole myelin or myelin components, and this has proven to be a useful model in understanding the immunology associated with human MS and the development of therapeutics.[44−46] A number of autoantigens associated with the myelin sheath have been identified in MS including myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP).[47] In C57BL/6 mice, EAE can be induced by immunizing with the MOG_{35-55} peptide emulsified in Freund's complete adjuvant. This generates a progressive paralysis ascending from the tail to the forelimbs. This can be easily scored in animals as the disease progresses and provides a relatively accurate measure of the immune pathology within the central nervous system (CNS). Histological analysis can be used to visualize and quantify CNS inflammation and myelin damage, and immunological assays measuring specific T cells and autoantibody reactivity can verify specific immune responses to the various myelin components. Using this model, we have shown that, following the transfer of bone marrow cells transduced with retrovirus encoding MOG as outlined in Figure 3, mice are completely resistant to the induction of EAE.[48] Analysis of mice reveals widespread molecular chimerism in hematopoietic cells including dendritic cells, T cells and B cells, and we have observed dendritic cells within the thymus that have been generated from transduced bone marrow and thus expressing MOG. The longevity of the process is well illustrated by the fact that bone marrow from old (9 month old mice) chimeric mice could transfer immune tolerance when transferred into naive mice, suggesting that long-term life-long tolerance had been established.[48]

While prevention of disease is one thing, our ability to also treat established disease and promote long-term tolerance reinforces the potential for clinical translation. Again using the MOG_{35-55} immunization model of EAE, we were able to demonstrate that mice with established EAE could be induced into remission and immune tolerance.[48] This particular study involved an initial short course of prednisolone to promote remission,[49] after which mice were transplanted with BM transduced with retrovirus encoding MOG. All mice that received MOG BM remained disease free for the initial observation period of 8 weeks. In contrast, control groups that had received normal BM or BM encoding the irrelevant autoantigen gene proinsulin II (associated with type 1 diabetes) relapsed to some extent, although 80% of mice also remained free of EAE features. The observation that BM transplantation alone may be sufficient to reverse disease is reminiscent of the data that is emerging from human trials utilizing autologous hematopoietic stem cell transfer (HSCT) as a means of treating autoimmune diseases including MS.[50−52] However, similar to our observations in animal studies, relapses in human trials are also observed,[51,53,54] and this suggests that robust tolerance has not been achieved and susceptibility to autoimmunity remains. To demonstrate that normal BM transplantation alone does not induce tolerance, we showed that mice receiving normal BM or BM transduced with irrelevant autoantigen that EAE could readily be induced by rechallenge with MOG_{35-55} peptide. In contrast, mice receiving MOG transduced bone marrow remained disease resistant.[48] This key observation supports the notion that the bone marrow transplantation alone will not be sufficient to promote robust tolerance, and additional measures or modifications may improve on the process as it is practiced today.

Table 1. Studies That Have Addressed the Induction of Immunological Tolerance Following the Transduction and Transplantation of Bone Marrow Stem Cells

model	gene	expression profile	outcome	ref
autoantigen	MOG	ubiquitous	resistance to EAE induction	48
	PLP	ubiquitous	resistant to EAE induction	67
	proinsulin	ubiquitous	reduced incidence of insulitis in NOD model of T1D	56
	MOG	dendritic cell	delayed onset of EAE	
	MBP	T cells	no effect on EAE development	58
	MOG	T cells	no effect on EAE development	68
neoantigen	LCMV glycoprotein	ubiquitous	immune tolerance induced	69
	green fluorescent protein	ubiquitous	immune tolerance induced	70
	ovalbumin	dendritic cell	immune tolerance induced	57
	ovalbumin	B cell	induction of peripheral tolerance	71
	factor VIII	ubiquitous	immune tolerance induced	72
alloantigen	major histocompatibility	ubiquitous	prevention of diabetes in NOD model of T1D	73,74
allergen	Phl p 5 (*Phleum pratense* 5)	ubiquitous	resistance to allergic response	75

A number of studies have now been published which clearly demonstrate that immune tolerance can be generated to ectopically expressed antigens that are derived from the introduction of transplanted bone marrow stem cells transduced ex vivo with retrovirus encoding antigen. This includes not only autoantigens but also neoantigens, alloantigens and allergens (Table 1) and suggests that the mechanism(s) involved are universal and can be applied to any antigen.

The mechanisms of tolerance associated with ectopic expression of autoantigen following transfer of transduced bone marrow remain to be fully elucidated although the induction of central tolerance within the thymus has been reported in some studies.[48,55] This is most likely the result of increased expression of autoantigen within the thymus by tolerance promoting cells such as dendritic cells. Following BM engraftment, GFP+ dendritic cells can be readily identified within the thymus,[48,56−58] and thus the simplest model of tolerance induction would involve enhanced presentation of autoantigen to developing T cells by autoantigen expressing dendritic cells that have developed from transduced bone marrow stem cells (Figure 2). However the fact that DC targeted expression in our hands did not replicate disease resistance observed with widespread expression from the wild-type retrovirus[58] suggests that other mechanisms within the periphery may also active. There are a number of questions that still remain to be addressed in this model, such as the following: (1) What is the role of ectopic autoantigen expression in peripheral DCs in tolerance induction? (2) Are antigen specific regulatory T cells generated in the thymus and periphery? (3) What role do ectopic autoantigen expressing B cells have in tolerance induction? (4) Are coinhibitory pathways implicated in tolerance induction? Answers to these fundamental questions will provide a comprehensive profile of where and how bone marrow targeted autoantigen gene therapy is able to promote tolerance in an antigen specific manner.

ADDITIONAL AVENUES OF GENETIC MANIPULATION AND IMMUNE TOLERANCE

This study has purposely focused on the genetic manipulation of bone marrow stem cells as a means of promoting tolerance in the context of autoimmune disease. However a gene therapy approach that utilizes defined and differentiated cell populations is also generating potential avenues for immune therapy. This includes cells such as T and B cells or dendritic cells and expressing a wide variety of cargo such as antigen and suppressive cytokines. The detailed analysis of this field is beyond the intended scope of this review, and a number of relevant articles and reviews can readily be found in the literature.[59−66]

SUMMARY

The ability to manipulate or correct specific biological processes through the application of gene therapy provides a powerful tool to treat human disorders. This is evident by the large array of gene therapy trials currently active. The immune system provides an accessible target for gene therapy given the relative ease with which the hematopoietic system can be isolated and transferred from individuals. It is this potential that we have capitalized upon in our studies in autoimmunity that aim to direct tolerance to defined autoantigens. The fact that bone marrow derived cells play an important role in promoting immune tolerance provides us with a way of generating a "Trojan horse" that can infiltrate the immune system and direct tolerance. The fact that tolerance can be generated to a wide variety of molecules and not just autoantigens supports this as a universal process with application across a range of situations where immune tolerance would be beneficial. Our studies in EAE have demonstrated to date that the tolerance generated is robust and long-lived. By targeting the stem cell, we have generated an environment in which the tolerogenic process is self-perpetuating through continuous stem cell self-renewal and generation of bone marrow derived cells that are driving the process. While there is still much to be learned in our models, we believe that the demonstrated potential of gene therapy to treat autoimmunity will continue to drive studies that will ultimately see this tested in humans.

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