

Induction of antigen-specific tolerance through hematopoietic stem cell-mediated gene therapy: the future for therapy of autoimmune disease?

Miranda A. Coleman¹ and Raymond J. Steptoe¹

¹The University of Queensland Diamantina Institute, The University of Queensland, Brisbane, AUSTRALIA.

Address correspondence to:

R.J. Steptoe

UQ Diamantina Institute

The University of Queensland

Princess Alexandra Hospital, Level 4 R Wing Building 1,

Woolloongabba, 4102 Queensland, AUSTRALIA

Phone: +61 7 3176 5393

Fax: +61 7 3176 5946

Email: r.steptoe@uq.edu.au

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Abstract

Based on the principle that immune ablation followed by HSC-mediated recovery purges disease-causing leukocytes to interrupt autoimmune disease progression, hematopoietic stem cell transplantation (HSCT) has been increasingly used as a treatment for severe autoimmune diseases. Despite clinically-relevant outcomes, HSCT is associated with serious iatrogenic risks and is suitable only for the most serious and intractable diseases. A further limitation of autologous HSCT is that relapse rates can be high, suggesting disease-causing leukocytes are incompletely purged or the environmental and genetic determinants that drive disease remain active. Incorporation of antigen-specific tolerance approaches that synergise with autologous HSCT could reduce or prevent relapse. Further, by reducing the requirement for highly toxic immune-ablation and instead relying on antigen-specific tolerance, the clinical utility of HSCT could be significantly diversified. Substantial progress has been made exploring HSCT-mediated induction of antigen-specific tolerance in animal models but studies have focussed on primarily on prevention of autoimmune diseases. However, as diagnosis of autoimmune disease is often not made until autoimmune disease is well developed and populations of autoantigen-specific pathogenic effector and memory T cells have become well established, immunotherapies must be developed to address effector and memory T-cell responses which have traditionally been considered the key impediment to immunotherapy. Here, focusing on T-cell mediated autoimmune diseases we review progress made in antigen-specific immunotherapy using HSCT-mediated approaches, induction of tolerance in effector and memory T cells and the challenges for progression and clinical application of antigen-specific 'tolerogenic' HSCT therapy.

Key Words: autoimmune disease, tolerance, haematopoietic stem cell transplantation, gene therapy

1. Autoimmune Disease

Autoimmune diseases afflict approximately 5-8% of the world's population [1] and are generally categorized as systemic or organ-specific. In systemic autoimmune diseases pathogenic effects are widely disseminated, affecting a range of target organs or tissues. Pathogenesis is complex but can often involve antibody-mediated target organ damage such as that mediated by anti-nuclear antibodies in systemic sclerosis and systemic lupus erythematosus (SLE). Organ-specific autoimmune diseases, on the other hand, typically result from T-cell-mediated autoimmune processes where CD4⁺ and/or CD8⁺ T-cell responses are directed against an often limited number of antigens expressed within specific tissues. This results in damage generally confined to specific target organs or tissues. Classic examples of organ-specific autoimmune diseases are Type 1 Diabetes (T1D), multiple sclerosis (MS), Graves' disease, autoimmune gastritis, and Addison's disease. In these diseases, proteins expressed by pancreatic beta cells, myelinated oligodendrocytes, thyroid exocrine, gastric paracrine cells or adrenal glands respectively are targeted. Because of the unique functions of the target tissues attacked, organ-specific autoimmune diseases are particularly debilitating and can be life-threatening. For example, destruction of the insulin-secreting islet beta cells in T1D is fatal without exogenous insulin replacement. Demyelination and scarring of axons in MS leads to progressive paralysis, loss of thyroid hormone-producing cells in thyroiditis to metabolic disorders and destruction of gastric parietal cells in autoimmune gastritis to an inability to absorb vitamin B12 resulting in pernicious anaemia. While T cells drive pathogenesis, other leukocytes including macrophages have been implicated as key mediators of target tissue destruction [2].

2. Failure of immunological tolerance underlies autoimmune disease

Immunological tolerance is essential for immune homeostasis and controls responses to self-antigens and innocuous environmental antigens while permitting appropriate responses to pathogens. Such is the importance of immune tolerance that several mechanisms have co-evolved. Central tolerance occurs in the thymus and bone marrow, respectively, for T cells and B cells. In

the thymus, developing thymocytes undergo a series of 'testing' processes including selection on the basis of effective recognition of self-Major Histocompatibility Complex (MHC) (positive selection) and TCR affinity with self-MHC/antigen (negative selection). Thymocytes with high affinity for 'self' (potentially pathogenic) are deleted, a proportion of cells of intermediate affinity are recruited into the CD4⁺CD25⁺FoxP3⁺ regulatory T cell (Treg) pool and those with minimal self-reactivity exit into the peripheral naive T cell pool. However, negative selection is not absolute and T cells with specificity for autoantigens frequently escape the thymus. A series of secondary, fail-safe, peripheral tolerance processes exist which control autoreactive T cells that escape central tolerance. Peripheral tolerance is comprised of 'dominant' mechanisms mediated by, for example, Treg that limit the function of T-cells and other leukocytes [3], or 'recessive' processes that cell-intrinsically limit function T-cell function. Autoreactive T-cells encountering antigen-presenting cells (APC) presenting cognate antigen in the periphery or in secondary lymphoid tissues in the absence of inflammation are induced to die (deletion) or are rendered unresponsive (anergic) [4]. The importance of such 'recessive' peripheral tolerance mechanisms has been highlighted in studies showing that autoreactive T cells are commonly found in healthy individuals [5, 6]. In particular, dendritic cells play a key role in mediating peripheral tolerance and have been identified as ideal tools for induction of tolerance in transplantation and autoimmune disease [7, 8] .

Interaction of genetic determinants and environmental influences can lead to tolerance breakdown and development of destructive autoreactive T-cell or B-cell responses in susceptible individuals. Genetic determinants of disease susceptibility have been long investigated and recent GWAS studies are proving a powerful tool for defining implicated immunological pathways. MHC alleles are major risk determinants for systemic and organ-specific autoimmune diseases. For example, 50% of the genetic susceptibility underlying T1D [9] is determined by the presence of a 'susceptible' MHC haplotype and MHC haplotype is an important determinant in other autoimmune diseases including MS and RA [10, 11]. Non-MHC genes also contribute to the

genetic autoimmune diseases risk with single nucleotide polymorphisms (SNP) in *CTLA-4*, *PTPN22* and *IL-2* genes, for example, implicated as contributory factors to several prominent autoimmune diseases. *CTLA-4* polymorphisms have been associated with T1D, MS, SLE and RA and recent evidence suggests a potential role for IL-2 in susceptibility to SLE and T1D. The contribution of these genetic determinants to autoimmune disease susceptibility can be readily explained mechanistically by their impact on pathways of central or peripheral tolerance. For example, CTLA-4 is a negative regulator of T cell function that binds to co-stimulatory molecules and has a central role in maintaining peripheral tolerance [12]. *PTPN22* encodes the protein tyrosine phosphatase non-receptor type 22 that modulates T cell receptor signalling [13] and is crucial in setting the threshold for thymic negative selection. IL-2 is a T-cell cytokine crucial for survival and function of CD4⁺CD25⁺FoxP3⁺ regulatory T cells and perturbations of IL-2 homeostasis mediated through, for example, reduced production contribute to SLE and T1D [14, 15]. Some immunotherapeutic approaches may be able to bypass genetic 'deficiencies' such as these by strengthening alternate tolerance pathways

Although genetic influences underlying autoimmune disease are being elucidated, it is the interaction between environmental influences and genetic susceptibility that controls disease emergence. The importance of environmental influences is evidenced by the massive surge in autoimmune disease incidence that has occurred over the last 70 years, far too fast to be explained by changing genetic influences [16]. Pinpointing environmental factors that influence the development of autoimmune disease has proven difficult, although infection and dietary triggers are the current frontrunners. For example, molecular mimicry has been implicated in T1D since the discovery of a shared determinant between the Coxsackie B virus P2-C protein and GAD65 [17] and a role for Epstein Barr virus in MS has also been proposed [18]. Changes in lifestyle and environmental exposures associated with the development of the 'modern' lifestyle appear to be an important driver of autoimmune disease. Although direct evidence supporting a role for most environmental influences in autoimmune disease development is lacking, the influence of smoking

on RA development through generation of citrullinated antigens is perhaps the most convincing [19] and smoking has also been implicated in MS and SLE [20, 21]. In a similar manner to genetic determinants, targeting tolerance pathways may be an important approach to overcoming environmental influences that drive autoimmune disease in susceptible individuals.

3. Current treatments for autoimmune disease

Immunosuppressive drugs are prescribed to treat many autoimmune diseases in an attempt to dampen the immune response. However, blanket immunosuppression is accompanied by a host of negative side effects, including increased susceptibility to serious infection and risk of malignancy. Recently, monoclonal antibodies and other ‘biologics’ have shown great promise for treatment of autoimmune diseases and offer a more specific, though not entirely targeted, approach than blanket immunosuppression. For example, blockade of TNF- α signalling with the antibodies infliximab and adalimumab or the decoy receptor etanercept are commonly used to treat rheumatoid arthritis and Crohn’s disease while the anti-CD20 antibody rituximab has also recently been used to treat rheumatoid arthritis and shows promise in T1D. These approaches, although refined, are still associated with unwanted side-effects and do not specifically target disease-causing cells, although some emerging therapies may be more specific [22]. This has prompted the quest for antigen-specific treatments that solely target disease-causing antigen-specific immune effector cells.

4. Bone marrow and haematopoietic stem cell transplantation

Bone marrow transplantation (BMT) has become widely used as a therapy for some haematological and solid tumours. BMT or hematopoietic stem cell (HSC) transplantation (HSCT) is typically performed using a procedure where BM or HSC are harvested from an MHC non-identical donor (allogeneic) or the patient (autologous), patients are ‘conditioned’ and BM/HSC are infused (**Figure 1**). For tumour therapy, high doses of radiation and/or chemotherapy are typically used to ensure maximum possible reduction of tumour burden and

allogeneic HSCT is used in order to capitalise on a strong graft vs leukemia or graft vs tumour effect in order to clear residual disease. The conditioning used is also immuno- and myeloablative thereby preventing immune rejection of transferred cells and facilitating engraftment of donor HSC. BMT/HSCT is also used to treat genetic disorders such as sickle cell anemia, thalassemia and severe combined immunodeficiency disorder (SCID), where defects in the hematopoietic compartment are severely debilitating or life-threatening. Conditioning regimens are similar to those used for allogeneic HSCT. Allogeneic HSCT carries significant risk of GVHD which occurs in up to 60-80% of cases (30-40% of MHC-matched transplants) and leads to significant morbidity and mortality. Clearly such a risk is acceptable only for the most serious or life-threatening illnesses. Typically, approximately 2×10^6 CD34⁺ cells/kg body weight is the minimum requirement for reliable engraftment. Early in development of BMT procedures, whole BM was harvested but 'mobilisation' of HSC using granulocyte colony stimulating factor (G-CSF) or G-CSF and cyclophosphamide is as effective, less invasive and is now widely used [23-25]. Sufficient HSC can usually be harvested using a standard mobilisation regimen of 10µg/kg/day G-CSF for 4 days prior to leukapheresis [25].

5. Haematopoietic stem cell transplantation for autoimmune disease

The principles developed for HSCT in tumour therapy or genetic deficiencies have been adapted for therapy of autoimmune disease. It was reasoned that as myeloablative conditioning is also highly immuno-ablative it could be used to deplete disease-causing leukocytes and, after autologous HSC re-infusion for recovery of hematopoiesis, a new immune repertoire devoid of activated pathogenic specificities would develop [26]. An inherent component of this rationale is that, as the environmental trigger that elicited disease may have passed, autologous HSCT would facilitate an 'immunological reset' to alleviate or cure disease. Preclinical studies with allogeneic BMT or BMT of BM expressing protective MHC alleles provided early evidence that complete resistance to disease could be achieved by full or mixed allogeneic chimerism [27, 28] and provided proof-of-principle that autoimmune diseases could be 'cured' from within the

hematopoietic compartment. Although effective in animal models, allogeneic or mismatched HSCT remains a serious procedure with substantial risks so could only be considered for the most serious diseases. Autologous HSCT, however, avoids GVHD and other risks associated with allogeneic or MHC-mismatched HSCT, making it more applicable to clinical use for autoimmune diseases. Syngeneic and autologous HSCT was tested in animal models and found effective in EAE and experimental arthritis [29-31].

Between the initial pilot study in 1995 [32] and 2011, over 700 people with MS have undergone autologous HSCT [33]. Approximately 70% of treated patients remain free of disease progression 3 years post-transplant based on expanded disability status scale (EDSS) scores [34]. Autologous HSCT can be life-saving for patients with malignant MS [35] and is more effective in cases of relapsing-remitting MS than primary progressive disease [36, 37]. The potential of HSCT for refractory cases of RA was demonstrated in 1996 when a wheelchair bound patient received an autologous bone marrow transplant and demonstrated a subsequent reduction in Ritchie Articular Index (RAI) from 61 pre-transplant to just 7 at 6 months post-transplant [38]. Since then, a meta-analysis of data from 15 centres where 76 RA patients were treated with autologous HSCT showed two-thirds of patients had positive outcomes but, unlike MS, responses were mostly transient with disease remission lasting 6 months to 2 years. Despite this, patients who were previously unresponsive to conventional disease modifying anti-rheumatic drugs (DMARDs) showed renewed sensitivity to treatment after relapse [39]. Autologous HSCT in SLE patients significantly reduces SLE disease activity index (SLEDAI) scores, complement C3 and C4 levels, antinuclear and anti double stranded DNA antibodies and increases renal function [40]. Resolution of soft-tissue calcification has also been reported [41]. Interestingly, recent post-transplant analyses have revealed CD8⁺ Treg are significantly increased in SLE patients post-transplant and these are proposed to play a central role in restoration of tolerance post-transplant [42]. More recently, autologous HSCT has been tested in T1D and treatment of 15 recently-diagnosed T1D patients showed encouraging results [43]. Pancreatic beta-cell function improved

in all but one patient as measured by C-peptide production and haemoglobin A_{1c}. Anti-GAD antibodies were reduced and insulin independence was maintained from 1 to 35 months.

Continuation of the study culminated in a report on 23 patients, including long-term follow up of the 15 original patients and 20 of the 23 patients experienced insulin-free periods representing significant and prolonged disease amelioration [43]. No mortality was reported, however, several incidences of late-onset endocrine dysfunction were documented, including a case of Grave's disease, autoimmune hypothyroidism, and transient hypergonadotrophic hypogonadism.

Interestingly, preservation of islet beta-cell function is superior to that achieved with other, more conventional immunotherapies currently or recently trialled for T1D [44].

Treatment related mortality (TRM) and other toxicities associated with the procedure remains a limiting factor for widespread application of HSCT as a routine treatment for autoimmune disease. G-CSF and Cy are associated with harmful side effects such as nausea, vomiting and hair loss and, interestingly, this is exacerbated in patients with autoimmune diseases. A potentially serious complication is disease flare induced by G-CSF in MS, SLE, and RA [45, 46]. Conditioning regimes for HSCT aim to ablate or suppress the immune system prior to HSC-mediated immune recovery. Myeloablative or non-myeloablative conditioning regimes can be utilised, with the difference lying in the intensity or dose of conditioning agents administered. A direct association of conditioning intensity with the incidence of TRM was shown in a study of 473 patients with severe autoimmune disease carried out by the European Group for Blood and Marrow Transplantation (EBMT). Patient selection has also been identified as a contributing factor to TRM [47]. The more advanced a patient's disease, the higher the risk of a poor outcome. Some toxicities such as gut damage have been addressed with increased use of non-myeloablative conditioning. Progress in recent years has seen a significant decrease in TRM rates for clinical trials of all autoimmune diseases from 12% in 2001 to 5% in 2010 [48].

Despite the encouraging results obtained, significant risks and side effects along with a substantial relapse rate in some diseases must be addressed before autologous HSCT becomes more widely applicable. Notwithstanding that low-intensity conditioning is associated with reduced risk of complications and mortality, HSCT is currently not a cure for autoimmune diseases and still represents a non-specific highly immune-ablative treatment. Combinational approaches of HSCT with tolerance-inducing therapies may address the incidence of relapse and enable the widespread application of such a treatment.

6. Combining HSCT and gene therapy to achieve better outcomes

Although the potential of autologous HSCT for widespread application is currently restricted by transplant-related toxicities, the most significant impediment to high success rates in treated individuals is subsequent disease relapse. Two underlying causes are most commonly proposed to underlie this, i) disease-causing leukocytes are not completely purged by pre-HSCT conditioning and ii) as autologous HSC are transplanted, the immune system is reinstated with the same complement of disease-associated genetic risks. In the latter case, autologous HSCT relies solely on ‘resetting’ the immune system and contributes no ‘active’ tolerance mechanisms to limit disease recurrence depending on whether eliciting environmental influences are still present, the extent to which the disease is genetically-determined, and the influence of the pre-existing disease-associated inflammatory state in the recipient. With these factors in mind, allogeneic HSCT may be more effective but is ethically unacceptable for all but the most serious conditions such as systemic sclerosis [49]. An alternative approach would be to somehow ‘modify’ HSC prior to re-infusion to ensure the presence of ‘active’ disease-inhibitory tolerance processes after HSCT. It is conceivable that several approaches could be used that replace ‘susceptibility genes’ or engender (re-)instatement of antigen-specific tolerance to disease-causing autoantigens. It is envisaged that the latter approach would purge or silence only disease-causing leukocytes while leaving the remainder of the immune system intact. If exploited effectively, this could reduce toxicities and lead to more efficacious treatments.

Beginning in the 1990's the importance of dendritic cells and other APC types in central and peripheral tolerance began to be recognised and DC were soon proposed as potential tools for limiting pathogenic T-cell responses [50]. In fact, it was shown that expression of the disease-eliciting autoantigens H⁺/K⁺-ATPase or proinsulin targeted to APC could prevent development of autoimmune gastritis and autoimmune diabetes in relevant animal models [51, 52]. Since then it has been widely demonstrated that enforced expression of auto- or neo-antigens, expressed ubiquitously, leads to antigen-specific tolerance to the expressed antigen [53, 54]. A more focussed approach, however, is to genetically-target antigens to APC and extensive studies from our group and others [55, 56, 4] demonstrate the robustness of this approach. In these settings, antigen-specific tolerance occurs through both central and peripheral tolerance pathways [57] including deletion, induction of unresponsiveness and Treg induction. Whether tolerance mechanisms differ when antigen is targeted to APC is not clear but may be a moot point as ubiquitous expression also leads to expression in 'tolerogenic' APC. Enforcing antigen expression in APC is sufficient to overcome inherent genetically-determined tolerance defects in autoimmune-prone mice [51, 52, 58, 59]. Capitalising on this knowledge, Steptoe and colleagues [60] progressed this approach when they demonstrated that transfer of 'gene-engineered' HSC encoding proinsulin transgenically-targeted to MHC class II APC completely prevented subsequent development of autoimmune diabetes in the spontaneously-diabetic NOD mouse. This led to the proposal that HSC genetically-manipulated *ex-vivo* could form an effective 'tolerogenic' therapy for T-cell mediated autoimmune diseases in humans [60]. Similar to the demonstrations that highly-purified HSC could be used, bulk transgenic BM encoding H⁺/K⁺ ATPase was shown similarly to prevent autoimmune gastritis [61]. Unpublished studies from our laboratory have shown that BMT / HSCT from mice expressing APC-targeted antigen replicates in recipients the antigen-specific tolerance seen in donors thereby providing a mechanistic basis for concluding that disease prevention is achieved through induction of antigen-specific T-cell tolerance.

Transgenic 'gene-engineered' HSC can be used to explore therapeutic approaches in animal models, but for human application another approach must be used. Fortuitously, development of retroviral and lentiviral vectors that could effectively transduce HSC paved the way for gene therapy employing HSC. Subsequently, clinical trials have shown the effectiveness of this approach for overcoming genetic deficiencies that manifest within the hematopoietic compartment such as X-linked severe combined immunodeficiency (X-SCID) and adenosine deaminase (ADA) deficiency. The technology used for such 'restorative' gene therapy can also be harnessed to target antigen expression to APC [62-64].

Early studies investigating the use of HSC gene therapy for tolerance induction showed that BM engineered to encode alloantigen through retroviral transduction induced macrochimerism and allo-tolerance [65, 66]. Subsequently, transfer of BM transduced by viral vectors to encode autoantigens was found to prevent autoimmune disease development in a variety of animal models. Xu and colleagues [67] showed that in conjunction with myeloablative irradiation, BM transduced to encode phospholipid protein (PLP) prevented induction of EAE by PLP immunisation. Similarly, transduction of BM using retrovirus encoding either an immunodominant epitope of myelin oligodendrocytic glycoprotein (MOG) or whole MOG has been shown to lead to loss of T-cell responsiveness to the expressed determinant(s), prevention of anti-MOG antibody development and inhibition of EAE induction in response to immunisation with the encoded antigen [68, 69]. Overall, these studies using disease models elicited by specific antigens or antigen-specific TCR transgenic T cells show that induction of tolerance after antigen-encoding BMT limits T-cell responses specific for the expressed protein or determinant(s) to prevent autoimmune disease elicited by immunisation with the targeted antigen.

Autoimmune diseases are typically directed at a range of autoantigens. The studies detailed above show that responsiveness to single disease-related autoantigens can be inhibited and HSCT

used to reinstate a repertoire tolerant to a single antigen. They do not, however, address whether enforced expression of a single antigen protects from T-cell responses elicited by antigens other than that targeted for enforced expression. In NOD mice, development of autoimmune diabetes occurs spontaneously without the need for immunisation against pancreatic β -cell antigens. In this spontaneous disease, insulin appears to be the primary autoantigen to which diabetogenic T-cell responses are initiated and other T-cell responses against other β -cell autoantigens are recruited by 'determinant spreading' as disease progresses [59, 70]. In NOD mice, transgenic expression of proinsulin targeted to APC prevents development of responses to insulin, other β -cell antigens and spontaneous diabetes [52, 58, 71]. Similarly, diabetes development is prevented by transfer of insulin-encoding HSC to young NOD mice [60] but not by transfer of non-engineered HSC. In an extension of this approach, it was shown that BM transduced with a retroviral vector encoding proinsulin expressed under control of the endogenous viral LTR inhibited development of insulinitis after transfer to young (3-4 week-old) female NOD mice when analysed 8 weeks later. However, diabetes development was not assessed in these studies [72]. These NOD mouse studies provide bona-fide evidence that using 'gene-engineered' HSCT to induce 'tolerance' to a key autoantigen prevents the unfolding of a spontaneous disease where a highly diversified pathogenic repertoire is ultimately responsible for target tissue destruction. While most studies have focussed primarily on T-cell responses, transfer of virally-transduced BM cells can also prevent antigen-specific antibody production in murine models [73, 74] and in non-human primates [75]

An alternative approach to the induction of antigen-specific tolerance to disease-specific autoantigens could be to replace genetic alleles that promote disease susceptibility with those that provide a protective effect or to enforce expression of 'protective' genes. One example of this approach might be to replace the 'susceptibility' variable N-terminal repeat (VNTR) of the insulin gene which drives low intrathymic expression of insulin and is associated with increased T1D incidence with the VNTR allele that drives higher expression of insulin and is associated with reduced T1D incidence. However, an easier solution would be to enforcedly express (pro)insulin

in a tolerogenic fashion as described above. In humans, T1D is tightly associated with inheritance of a 'susceptibility' allele of the MHC class II (HLA-DQ) β chain that lacks a charged amino acid at position 57 (β 57-non Asp) [76]. While not the sole determinant of susceptibility, disease progression is increased by homozygosity for β 57-non Asp alleles. In NOD mice, I-A^{g7} (the mouse homolog of the β 57-non Asp HLA-DQB1) is required for diabetes development. Congenic or transgenic expression of an alternative I-A β -chain or I-E (mouse homolog of HLA-DR which NOD do not normally express) prevents diabetes [77] through a mechanism that appears associated with altered T-cell selection [78]. Transfer of BM cells transduced to encode either β 57-Asp containing I-A β -k or I-A β -d to young (5-6 wk-old) prediabetic NOD mice using myeloablative (1050 cGy) total body irradiation (TBI) subsequently prevents spontaneous diabetes development [79]. Reduced T-cell responses to islet antigens and increased negative selection of a diabetogenic T-cell specificity were demonstrated after transfer of BM encoding protective I-A β chains [79]. A potential drawback of this approach could be that an expressed, protective I-A β acts as the equivalent of an alloantigen and whether this approach would be safer than allogeneic HSCT in humans is unclear. Gene-therapy approaches using HSC could be supplemental to approaches aimed at local manipulation of autoimmune inflammation [80, 81]

Together the studies performed in EAE and diabetes models demonstrate that the immune system can readily be reset by HSCT using gene-engineered HSC and that reconstitution with a repertoire specifically tolerant to pathogenic autoantigen(s) prevents disease onset. However, for clinical application, an approach to interrupt autoimmune disease progression is sought. Attempts to inhibit disease progression in mouse models have met with mixed success. When Xu et al used myeloablative irradiation (900cGy) conditioning they found transfer of PLP-encoding BM led to an approximately 50% reduction in the mean clinical score of PLP-induced EAE but efficacy was increasingly impaired as EAE progressed (12 days after disease onset) [67]. Chan and colleagues [68] used the same dose of irradiation but incorporated administration of depleting anti-CD4 antibody after transfer of MOG-encoding BM and showed that disease progression was

interrupted and re-induction of disease by MOG immunisation prevented. Together this suggests that during disease progression, immune ablation was required to permit reinstatement of a 'tolerant' repertoire after HSCT and this provided an equivalent 'preventative' effect to that seen for HSCT prior to initial antigen priming. Extending this, Tian and colleagues showed by using immune-depleting myeloablative TBI, that transfer of BM retrovirally transduced to encode protective β 57-Asp-containing I-A β molecules abrogated recurrence of diabetes in NOD mice after syngeneic islet transplantation [82], again most likely through a 'preventative' effect. In keeping with the possible requirement for immune ablation for effectiveness, non-myeloablative approaches to BMT using antigen-encoding BM are less efficacious than myeloablative approaches and as disease progresses the effectiveness of this approach appears to wane [67, 69].

The findings that antigen-encoding BMT/HSCT can induce 'preventative' tolerance and, when combined with immune ablation, prevent disease re-induction represent a substantial step forward for the potential of HSCT as a 'cure' for autoimmune diseases. Incorporation of the 'gene-therapy' steps required for 'antigen-specific immunotherapy' ('tolerogenic HSCT') could be readily incorporated into current HSCT practice with few additional risks, but with the profound potential to minimise or eliminate disease relapse. This would achieve a distinct 'active tolerance' mechanism into the HSCT procedure to purge potentially pathogenic autoreactive T-cell repertoires thereby preventing the key immunological driver underlying disease relapse. While studies to date indicate a high degree of potential for incorporation of 'tolerogenic gene therapy' into HSCT protocols, a number of hurdles that need to be the focus of current research efforts are still present.

7. Challenges for moving forward with combined HSCT and gene therapy

Studies of HSC-mediated 'tolerogenic gene therapy' in mouse models have primarily used myeloablative, immunoablative treatments or a combination of both to achieve engraftment of

transferred HSC and to 'purge' the repertoire of pre-existing pathogenic T-cells. However, the long period required for a 'new' immune system 'tolerant' to the targeted (auto)-antigen(s) to be regenerated along with immune depletion has inherent drawbacks. Not only is there an extended period of immune suppression but protective memory T-cell specificities such as those generated through immunisations are lost. Thymic involution from adolescence onwards has also traditionally been considered an impediment to 'full' immune reconstitution of highly diverse T-cell repertoires after immune ablation or HSCT in adults. Recent evidence, however, suggests the thymus maintains more T-lymphopoietic activity than traditionally thought. A more ideal approach, if possible, might be to exploit the inherent ability of 'tolerogenic' APC for peripheral tolerance induction to purge the T-cell repertoire of undesirable specificities. In this scenario, development of autoantigen-expressing APC could silence pre-existing populations of autoantigen-specific T cells through one or more tolerance mechanisms such as deletion, induction of unresponsiveness or induction of Treg. Successful exploitation of such an approach is yet to be demonstrated experimentally, but adoptive transfer studies performed by our laboratory [83, 84, 4] indicate possible success for such an approach. If this proved possible, then theoretically, tolerance could be achieved using 'gene-engineered' HSCT in the absence of any immune ablation. This is certainly the goal for antigen-specific immunotherapy and would dramatically extend the clinical utility of HSCT.

Effector and memory T-cell responses develop early in the prodromal, or pre-clinical, phase of autoimmune diseases and typically, autoimmune diseases are identified only after such actively pathogenic effector and memory T-cell populations have become established. In humans with T1D effector cells serve as a reliable predictor of disease progression [85, 86]. They continue to expand as disease progresses and ultimately form memory populations that persist in long-standing diabetics [87, 88], long after β -cell function is lost. Any 'cure' must therefore comprise an approach to terminate such pathogenic effector and memory T-cell responses either to permit

preservation of target tissues or to facilitate their regeneration or replacement. Given that progression of some autoimmune diseases (e.g. T1D) to end-stage disease can be predicted, a 'window of therapeutic opportunity' exists where interventions that terminate effector and memory T-cell responses could be employed to prevent further disease progression. Herein lies the largest apparent hurdle for immunotherapy of autoimmune diseases. Upon antigen stimulation, naïve T cells rapidly undergo terminal differentiation to effector and memory T cells. During this process naïve Ag-specific T cells lose their highly malleable differentiation potential and become committed memory and effector populations. Compared to naïve T cells, effector and memory T cells are more sensitive to antigen stimulation, exhibit faster response kinetics, reduced dependence on costimulation and can respond to lower affinity ligands. Memory T cells may be more resistant to induction of apoptosis than naïve cells through increased expression of antiapoptotic molecules [89]. This fully differentiated nature and reduced requirement for costimulation has led to the belief that memory T-cells are resistant to inactivation or tolerance induction. Indeed, memory T cells have been shown to provide a potent barrier to transplantation tolerance induction [90, 91] and may be resistant to regulation [92], conventional immunosuppression [88] and myeloablative conditioning regimens [93-96]. This is in line with observations that T cell ablation (e.g. anti-CD4) is required in addition to myeloablative conditioning to maximise the potential of antigen-encoding BM once disease has commenced [68, 97]. Exciting new data from our laboratory has shown that, unexpectedly, effector and memory T cell responses can be effectively terminated when antigen is targeted to APC [98-100]. This suggests the possibility that gene-engineered HSCT might be useful for induction of antigen-specific tolerance to pathogenic T-cell specificities even in established or progressing autoimmune diseases. Such an outcome would give hope for the ultimate goal of an antigen-specific therapy where immune ablation is not necessary to eliminate pre-existing differentiated pathogenic effector and memory T-cells.

A major advantage of tolerogenic HSCT over other possible immunotherapeutic approaches is that once engrafted, 'engineered' HSC will continue to give rise to APC with enforced autoantigen expression. Thus, as long as donor engraftment levels remain stable it may be possible to attain life-long tolerance due to the constant inactivation of any new autoreactive T-cells that develop. In fact, continued expression of the targeted antigen may be required to maintain tolerance induced by gene-engineered HSC [101], much in the same way it is required to maintain tolerance in transgenic mice [98]. Achievement of 'life-long tolerance' could be a distinct advantage of immunotherapy using gene-engineered HSC over other approaches where immunotherapeutic benefits can be transient.

Experimental studies of tolerogenic HSCT in mouse models have employed almost exclusively non-targeted antigen expression, where 'ubiquitous' promoters drive expression in all cell types. It remains unclear whether 'therapeutic' autoantigen expression should be targeted to APC or if widespread expression under ubiquitous or endogenous viral promoters would be equally, or possibly, even more effective. Based on mouse models, 'targeting' is not required to achieve tolerance and HSC engineered to encode ubiquitously-expressed antigens can lead to induction of tolerance in recipients e.g. [54]. Which APC, if any, might be most effective for induction of therapeutic tolerance is also poorly-defined. Although DC clearly play an important role in maintaining T-cell tolerance they are primarily specialized for induction of immunity raising the question of whether other APC types may more effectively induce tolerance. Evidence has existed for some time that, under certain conditions, B cells in particular, promote peripheral tolerance induction [102, 103] and more recent data has shown a role for B cells in peripheral inactivation of naïve CD8⁺ T cells [63] and possibly memory CD4⁺ T cells [104]. B cells have been effectively exploited for 'engineered' Ag expression and tolerance induction in many studies [105, 63]. We have shown 'rapid inactivation' of effector and memory CD8⁺ T cells when antigen is widely expressed through different APC types [99]. Alternatively, delivery of allo-antigen by transfer of T cells can effectively induce allo-tolerance [106]. In a single study using retrovirally-transduced

BM it was found that targeting MOG to CD11c⁺ cells somewhat reduced disease development, but the effect was not as profound as when MOG expression was controlled (i.e. ubiquitously expressed) by the endogenous retroviral LTR promoter [107].

Moving forward, there are other areas where additional research is required in order to understand how 'gene-engineered' HSC may be best applied as a clinically applicable therapeutic for autoimmune diseases. For example, what level of engineered HSC engraftment (chimerism) is required for effective prevention or termination of T-cell responses or autoimmune disease? We showed diabetes development in NOD mice was effectively inhibited by levels of proinsulin-encoding HSC engraftment as low as 5% [60]. But, in general, most investigations have been performed using much higher levels of gene-engineered HSC-induced chimerism. While low levels of chimerism are effective in 'preventative' approaches where myeloablative conditioning is used and a new 'tolerant' immune system is regenerated, whether such low levels would be effective when attempts are made to reduce the extent of myeloablation or immunoablation in experimental protocols is unclear. It is readily apparent that the levels of 'chimerism' achievable in humans will be dependent on the nature of the 'conditioning' regime used. As attempts are made to reduce the toxicity of conditioning will the dose of HSC transferred be sufficient and will the number of HSC required become a limiting factor? Currently, conditioning-related toxicities are the biggest single challenge limiting widespread application of HSCT for autoimmune diseases. Current conditioning regimes, even 'nonmyeloablative' protocols are designed to be heavily immunoablative and further progress in development of conditioning regimes tailored to the needs of 'tolerogenic' HSCT is required. For transduction of HSC, both retroviral and lentiviral vectors have proven effective in humans. The ability of these vectors to integrate their genetic payload into the host genome is essential for their effectiveness but comes at a cost. Retroviruses may preferentially insert genetic material into oncogenic sites and the detrimental effects of this became apparent in a clinical trial for X-SCID where the therapeutic 'gene' was

inserted in close proximity to the proto-oncogene promoter LMO2, leading to leukemia in some recipients [108]. Lentiviral vectors may be safer in this respect [109] but concerns about recombination with 'endogenous' viruses have dogged attempts for clinical use. A recent trial showed effectiveness of lentiviral vectors in ADA but further studies and follow-up will be required to fully define the comparative safety of each vector type. Development of effective alternative approaches for gene transfer, such as non-viral vectors, while most likely some way off would broaden the clinical utility of HSCT for autoimmune diseases.

8. Summary

Autoimmune diseases afflict a large percentage of the world's population and are both debilitating and life-long. To date, there is no 'cure' for any autoimmune disease and current treatments are riddled with serious side-effects. HSCT has emerged as a potential treatment for a number of autoimmune diseases, but is limited by treatment related toxicities and a high incidence of disease relapse in some settings. A promising way forward is to combine 'tolerogenic' gene therapies with autologous HSCT to provide active tolerance mechanisms and induced permanent disease remission. This approach has the potential to prevent disease relapse, minimise the need for immunoablative conditioning and diversify the application of autologous HSCT-based immunotherapies. Further understanding of how to optimise gene delivery and autoantigen expression will add impetus to application of progress of 'tolerogenic' HSCT. We suggest that targeting autoantigen expression to 'tolerogenic' APC, such as dendritic cells, will provide significant therapeutic benefit and has the potential to antigen-specifically turn-off established pathogenic T-cell responses underlying some autoimmune diseases. This could mark a new era for treatment of immunological disorders especially autoimmune diseases.

Take Home Messages

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| <ul style="list-style-type: none">• Nonmyeloablative conditioning reduces the transplant-related risks of HSCT but preserves host immunity• Clinical trials treating AD with nonmyeloablative HSCT have significant relapse rates• Remaining or re-emerging autoreactive cells need to be dealt with for permanent disease remission• Gene therapy has been used successfully in humans and may be feasibly combined with HSCT to antigen- specifically silence pathogenic autoreactive T-cells |
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References

- [1] Davidson A, Diamond B. Autoimmune diseases. *The New England journal of medicine*. 2001;345:340-50.
- [2] Hutchings P, Rosen H, O'Reilly L, Simpson E, Gordon S, Cooke A. Transfer of diabetes in mice prevented by blockade of adhesion-promoting receptor on macrophages. *Nature*. 1990;348:639-42.
- [3] Tang Q, Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. *Nat Immunol*. 2008;9:239-44.
- [4] Steptoe RJ, Ritchie JM, Wilson NS, Villadangos JA, Lew AM, Harrison LC. Cognate CD4+ help elicited by resting dendritic cells does not impair the induction of peripheral tolerance in CD8+ T cells. *J Immunol*. 2007;178:2094-103.
- [5] Sun JB, Olsson T, Wang WZ, Xiao BG, Kostulas V, Fredrikson S, et al. Autoreactive T and B cells responding to myelin proteolipid protein in multiple sclerosis and controls. *Eur J Immunol*. 1991;21:1461-8.
- [6] Danke NA, Koelle DM, Yee C, Beheray S, Kwok WW. Autoreactive T cells in healthy individuals. *J Immunol*. 2004;172:5967-72.
- [7] Thomson AW, Robbins PD. Tolerogenic dendritic cells for autoimmune disease and transplantation. *Annals of the rheumatic diseases*. 2008;67 Suppl 3:iii90-6.
- [8] Torres-Aguilar H, Blank M J, L.J., Shoenfeld Y. Tolerogenic dendritic cells in autoimmune diseases: crucial players in induction and prevention of autoimmunity. *Autoimmun Rev* 2010;10:8-17.
- [9] Davies JL, Kawaguchi Y, Bennett ST, Copeman JB, Cordell HJ, Pritchard LE, et al. A genome-wide search for human type 1 diabetes susceptibility genes. *Nature*. 1994;371:130-6.
- [10] Jersild C, Svejgaard A, Fog T. HL-A antigens and multiple sclerosis. *Lancet*. 1972;1:1240-1.
- [11] Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum*. 1987;30:1205-13.
- [12] Perez VL, Van Parijs L, Biuckians A, Zheng XX, Strom TB, Abbas AK. Induction of peripheral T cell tolerance in vivo requires CTLA-4 engagement. *Immunity*. 1997;6:411-7.
- [13] Hill RJ, Zozulya S, Lu YL, Ward K, Gishizky M, Jallal B. The lymphoid protein tyrosine phosphatase Lyp interacts with the adaptor molecule Grb2 and functions as a negative regulator of T-cell activation. *Exp Hematol*. 2002;30:237-44.
- [14] Yamanouchi J, Rainbow D, Serra P, Howlett S, Hunter K, Garner VE, et al. Interleukin-2 gene variation impairs regulatory T cell function and causes autoimmunity. *Nat Genet*. 2007;39:329-37.
- [15] Tang Q, Adams JY, Penaranda C, Melli K, Piaggio E, Sgouroudis E, et al. Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. *Immunity*. 2008;28:687-97.
- [16] Gale EA. The rise of childhood type 1 diabetes in the 20th century. *Diabetes*. 2002;51:3353-61.
- [17] Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AJ. Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. *J Clin Invest*. 1992;89:283-92.
- [18] Fournau JM, Bach JM, van Endert PM, Bach JF. The elusive case for a role of mimicry in autoimmune diseases. *Mol Immunol*. 2004;40:1095-102.
- [19] Stolt P, Bengtsson C, Nordmark B, Lindblad S, Lundberg I, Klareskog L, et al. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Ann Rheum Dis*. 2003;62:835-41.
- [20] Costenbader KH, Kim DJ, Peerzada J, Lockman S, Nobles-Knight D, Petri M, et al. Cigarette smoking and the risk of systemic lupus erythematosus: a meta-analysis. *Arthritis Rheum*. 2004;50:849-57.
- [21] Healy BC, Ali EN, Guttman CR, Chitnis T, Glanz BI, Buckle G, et al. Smoking and disease progression in multiple sclerosis. *Arch Neurol*. 2009;66:858-64.
- [22] Mozes E, Sharabi A. A novel tolerogenic peptide, hCDR1, for the specific treatment of systemic lupus erythematosus. *Autoimmun Rev*. 2010;10:22-6.
- [23] Siena S, Bregni M, Brando B, Ravagnani F, Bonadonna G, Gianni AM. Circulation of CD34+ hematopoietic stem cells in the peripheral blood of high-dose cyclophosphamide-treated

- patients: enhancement by intravenous recombinant human granulocyte-macrophage colony-stimulating factor. *Blood*. 1989;74:1905-14.
- [24] Serke S, Kirsch A, Huhn D. Mobilization of circulating haemopoietic cells (blood stem cells) by GM-CSF in patients with malignancies. *Infection*. 1992;20 Suppl 2:S100-2.
- [25] Schmitz N, Linch DC, Dreger P, Goldstone AH, Boogaerts MA, Ferrant A, et al. Randomised trial of filgrastim-mobilised peripheral blood progenitor cell transplantation versus autologous bone-marrow transplantation in lymphoma patients. *Lancet*. 1996;347:353-7.
- [26] Ikehara S, Good RA, Nakamura T, Sekita K, Inoue S, Oo MM, et al. Rationale for bone marrow transplantation in the treatment of autoimmune diseases. *Proc Natl Acad Sci U S A*. 1985;82:2483-7.
- [27] Ikehara S, Ohtsuki H, Good RA, Asamoto H, Nakamura T, Sekita K, et al. Prevention of type 1 diabetes in nonobese diabetic mice by allogeneic bone marrow transplantation. *Proc Natl Acad Sci USA*. 1985;82:7743-7.
- [28] LaFace DM, Peck AB. Reciprocal allogeneic bone marrow transplantation between NOD mice and diabetes-nonsusceptible mice associated with transfer and prevention of autoimmune diabetes. *Diabetes*. 1989;38:894-901.
- [29] van Bekkum DW, Bohre EP, Houben PF, Knaan-Shanze rS. Regression of adjuvant-induced arthritis in rats following bone marrow transplantation. *Proc Natl Acad Sci U S A*. 1989;86:10090-4.
- [30] Knaan-Shanzer S, Houben P, Kinwel-Bohre EP, van Bekkum DW. Remission induction of adjuvant arthritis in rats by total body irradiation and autologous bone marrow transplantation. *Bone Marrow Transplant*. 1991;8:333-8.
- [31] Karussis DM, Vourka-Karussis U, Lehmann D, Ovadia H, Mizrachi-Koll R, Ben-Nun A, et al. Prevention and reversal of adoptively transferred, chronic relapsing experimental autoimmune encephalomyelitis with a single high dose cytoreductive treatment followed by syngeneic bone marrow transplantation. *The Journal of clinical investigation*. 1993;92:765-72.
- [32] Fassas A, Anagnostopoulos A, Kazis A, Kapinas K, Sakellari I, Kimiskidis V, et al. Peripheral blood stem cell transplantation in the treatment of progressive multiple sclerosis: first results of a pilot study. *Bone Marrow Transplant*. 1997;20:631-8.
- [33] Fassas A, Kimiskidis VK, Sakellari I, Kapinas K, Anagnostopoulos A, Tsimourto V, et al. Long-term results of stem cell transplantation for MS: a single-center experience. *Neurology*. 2011;76:1066-70.
- [34] Saccardi R, Kozak T, Bocelli-Tyndall C, Fassas A, Kazis A, Havrdova E, et al. Autologous stem cell transplantation for progressive multiple sclerosis: update of the European Group for Blood and Marrow Transplantation autoimmune diseases working party database. *Mult Scler*. 2006;12:814-23.
- [35] Mancardi GL, Murialdo A, Rossi P, Gualandi F, Martino G, Marmont A, et al. Autologous stem cell transplantation as rescue therapy in malignant forms of multiple sclerosis. *Mult Scler*. 2005;11:367-71.
- [36] Samijn JP, te Boekhorst PA, Mondria T, van Doorn PA, Flach HZ, van der Meche FG, et al. Intense T cell depletion followed by autologous bone marrow transplantation for severe multiple sclerosis. *Journal of neurology, neurosurgery, and psychiatry*. 2006;77:46-50.
- [37] Metz I, Lucchinetti CF, Openshaw H, Garcia-Merino A, Lassmann H, Freedman MS, et al. Autologous haematopoietic stem cell transplantation fails to stop demyelination and neurodegeneration in multiple sclerosis. *Brain : a journal of neurology*. 2007;130:1254-62.
- [38] Joske DJ, Ma DT, Langlands DR, Owen ET. Autologous bone-marrow transplantation for rheumatoid arthritis. *Lancet*. 1997;350:337-8.
- [39] Snowden JA, Passweg J, Moore JJ, Milliken S, Cannell P, Van Laar J, et al. Autologous hemopoietic stem cell transplantation in severe rheumatoid arthritis: a report from the EBMT and ABMTR. *J Rheumatol*. 2004;31:482-8.
- [40] Burt RK, Traynor A, Statkute L, Barr WG, Rosa R, Schroeder J, et al. Nonmyeloablative hematopoietic stem cell transplantation for systemic lupus erythematosus. *JAMA*. 2006;295:527-35.

- [41] Mandelbrot DA, Santos PW, Burt RK, Oyama Y, Block GA, Ahya SN, et al. Resolution of SLE-related soft-tissue calcification following haematopoietic stem cell transplantation. *Nephrol Dial Transplant*. 2008;23:2679-84.
- [42] Zhang L, Bertucci AM, Ramsey-Goldman R, Burt RK, Datta SK. Regulatory T cell (Treg) subsets return in patients with refractory lupus following stem cell transplantation, and TGF-beta-producing CD8+ Treg cells are associated with immunological remission of lupus. *J Immunol*. 2009;183:6346-58.
- [43] Couri CE, Oliveira MC, Stracieri AB, Moraes DA, Pieroni F, Barros GM, et al. C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA*. 2009;301:1573-9.
- [44] Skyler JS, Ricordi C. Stopping type 1 diabetes: attempts to prevent or cure type 1 diabetes in man. *Diabetes*. 2011;60:1-8.
- [45] de Vries EG, Willemse PH, Biesma B, Stern AC, Limburg PC, Vellenga E. Flare-up of rheumatoid arthritis during GM-CSF treatment after chemotherapy. *Lancet*. 1991;338:517-8.
- [46] Openshaw H, Stuve O, Antel JP, Nash R, Lund BT, Weiner LP, et al. Multiple sclerosis flares associated with recombinant granulocyte colony-stimulating factor. *Neurology*. 2000;54:2147-50.
- [47] Comi G, Kappos L, Clanet M, Ebers G, Fassas A, Fazekas F, et al. Guidelines for autologous blood and marrow stem cell transplantation in multiple sclerosis: a consensus report written on behalf of the European Group for Blood and Marrow Transplantation and the European Charcot Foundation. BMT-MS Study Group. *J Neurol*. 2000;247:376-82.
- [48] Farge D, Labopin M, Tyndall A, Fassas A, Mancardi GL, Van Laar J, et al. Autologous hematopoietic stem cell transplantation for autoimmune diseases: an observational study on 12 years' experience from the European Group for Blood and Marrow Transplantation Working Party on Autoimmune Diseases. *Haematologica*. 2010;95:284-92.
- [49] Nash RA, McSweeney PA, Nelson JL, Wener M, Georges GE, Langston AA, et al. Allogeneic marrow transplantation in patients with severe systemic sclerosis: resolution of dermal fibrosis. *Arthritis Rheum*. 2006;54:1982-6.
- [50] Steptoe RJ, Thomson AW. Dendritic cells and tolerance induction. *Clin Exp Immunol*. 1996;105:397-402.
- [51] Alderuccio F, Toh B-H, Tan S-S, Gleeson PA, van Driel IR. An autoimmune disease with multiple molecular targets abrogated by the transgenic expression of a single autoantigen in the thymus. *J Exp Med*. 1995;178:419-26.
- [52] French MB, Allison J, Cram DS, Thomas HE, Dempsey-Collier M, Silva A, et al. Transgenic expression of mouse proinsulin II prevents diabetes in nonobese diabetic mice. *Diabetes*. 1997;46:34-9.
- [53] Xu H, Wawrousek EF, Redmond TM, Nickerson JM, Wiggert B, Chan CC, et al. Transgenic expression of an immunologically privileged retinal antigen extraocularly enhances self tolerance and abrogates susceptibility to autoimmune uveitis. *Eur J Immunol*. 2000;27:272-8.
- [54] Steitz J, Soloff AC, Barratt-Boyes SM, Alber SM, Watkins SC, Okada H, et al. Balb/c EGFP mice are tolerant against immunization utilizing recombinant adenoviral-based vectors encoding EGFP: a novel model for the study of tolerance mechanisms and vaccine efficacy. *Mol Immunol*. 2010;47:1149-53.
- [55] Brocker T, Riedinger M, Karjalainen K. Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes in vivo. *The Journal of experimental medicine*. 1997;185:541-50.
- [56] Probst HC, Lagnel J, Kollias G, van den Broek M. Inducible transgenic mice reveal resting dendritic cells as potent inducers of CD8+ T cell tolerance. *Immunity*. 2003;18:713-20.
- [57] Doan T, McNally A, Thomas R, Steptoe RJ. Steady-state peripheral dendritic cells continuously inactivate T cells that escape thymic negative selection. *Immunol Cell Biol*. 2009;87:615-22.
- [58] Jaeckel E, Klein L, Martin-Orozco N, von Boehmer H. Normal incidence of diabetes in NOD mice tolerant to glutamic acid decarboxylase. *J Exp Med*. 2003;197:1635-44.
- [59] Krishnamurthy B, Dudek NL, Thomas HE, Purcell AW, Lew AM, Harrison LC, et al. Proinsulin precedes IGRP in the hierarchy of autoantigens in autoimmune diabetes. *Endocrine Journal*. 2005;52(Suppl.):64.

- [60] Steptoe RJ, Ritchie JM, Harrison LC. Transfer of hematopoietic stem cells encoding autoantigen prevents autoimmune diabetes. *J Clin Invest.* 2003;111:1357-63.
- [61] Murphy K, Biondo M, Toh BH, Alderuccio F. Tolerance established in autoimmune disease by mating or bone marrow transplantation that target autoantigen to thymus. *Int Immunol.* 2003;15:269-77.
- [62] Cui Y, Golob J, Kelleher E, Ye Z, Pardoll D, Cheng L. Targeting transgene expression to antigen-presenting cells derived from lentivirus-transduced human hematopoietic stem/progenitor cells. *Blood.* 2002;99:399-408.
- [63] Werner-Klein M, Dresch C, Marcon IP, Brocker T. Transcriptional targeting of B cells for induction of peripheral CD8 T cell tolerance. *J Immunol.* 2007;178:7738-46.
- [64] Dresch C, Edelmann SL, Marconi P, Brocker T. Lentiviral-mediated transcriptional targeting of dendritic cells for induction of T cell tolerance in vivo. *J Immunol.* 2008;181:4495-506.
- [65] Sykes M, Sachs DH, Nienhuis AW, Pearson DA, Moulton AD, Bodine DM. Specific prolongation of skin graft survival following retroviral transduction of bone marrow with an allogeneic major histocompatibility complex gene. *Transplantation.* 1993; 55:197-.
- [66] Bagley J, Tian C, Sachs DH, Iacomini J. Induction of T-cell tolerance to an MHC class I alloantigen by gene therapy. *Blood.* 2002;99:4394-9.
- [67] Xu BY, Haviernik P, Wolfrain LA, Bunting KD, Scott DW. Bone marrow transplantation combined with gene therapy to induce antigen-specific tolerance and ameliorate EAE. *Molecular Therapy.* 2006;13:42-8.
- [68] Chan J, Ban EJ, Chun KH, Wang S, Backstrom BT, Bernard CC, et al. Transplantation of bone marrow transduced to express self-antigen establishes deletional tolerance and permanently remits autoimmune disease. *J Immunol.* 2008;181:7571-80.
- [69] Eixarch H, Espejo C, Gomez A, Mansilla MJ, Castillo M, Mildner A, et al. Tolerance induction in experimental autoimmune encephalomyelitis using non-myeloablative hematopoietic gene therapy with autoantigen. *Molecular Therapy.* 2009;17:897-905.
- [70] Nakayama M, Abiru N, Moriyama H, Babaya N, Liu E, Miao D, et al. Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice. *Nature.* 2005;435:220-3.
- [71] Krishnamurthy B, Dudek NL, McKenzie MD, Purcell AW, Brooks AG, Gellert S, et al. Responses against islet antigens in NOD mice are prevented by tolerance to proinsulin but not IGRP. *J Clin Invest.* 2006;116:3258-65.
- [72] Chan J, Clements W, Field J, Nasa Z, Lock P, Yap F, et al. Transplantation of bone marrow genetically engineered to express proinsulin II protects against autoimmune insulinitis in NOD mice. *J Gene Med.* 2006;8:1281-90.
- [73] Bracy JL, Iacomini J. Induction of B-cell tolerance by retroviral gene therapy. *Blood.* 2000;96:3008-15.
- [74] Mitsushashi N, Fischer-Lougheed J, Shulkin I, Kleihauer A, Kohn DB, Weinberg KI, et al. Tolerance induction by lentiviral gene therapy with a nonmyeloablative regimen. *Blood.* 2006;107:2286-93.
- [75] Fischer-Lougheed JY, Tarantal AF, Shulkin I, Mitsushashi N, Kohn DB, Lee CC, et al. Gene therapy to inhibit xenoantibody production using lentiviral vectors in non-human primates. *Gene Ther.* 2007;14:49-57.
- [76] Morel PA, Dorman JS, Todd JA, McDevitt HO, Trucco M. Aspartic acid at position 57 of the HLA-DQ beta chain protects against type I diabetes: a family study. *Proceedings of the National Academy of Sciences of the United States of America.* 1988;85:8111-5.
- [77] Lund T, O'Reilly L, Hutchings P, Kanagawa O, Simpson E, Gravely R, et al. Prevention of diabetes in insulin-dependent diabetes mellitus in non-obese diabetic mice by transgenes encoding modified I-A β -chain or normal I-E α -chain. *Nature.* 1990;345:727-9.
- [78] Schmidt D, Verdaguer J, Averill N, Santamaria P. A mechanism for the major histocompatibility complex-linked resistance to autoimmunity. *J Exp Med.* 1997;186:1059-75.
- [79] Tian C, Bagley J, Cretin N, Seth N, Wucherpfennig KW, Iacomini J. Prevention of type 1 diabetes by gene therapy. *J Clin Invest.* 2004;114:969-78.
- [80] Leung PS, Dhirapong A, Wu PY, Tao MH. Gene therapy in autoimmune diseases: challenges and opportunities. *Autoimmun Rev.* 2010;9:170-4.

- [81] Leung PS, Shu SA, Kenny TP, Wu PY, Tao M. Development and validation of gene therapies in autoimmune diseases: Epidemiology to animal models. *Autoimmun Rev.* 2010;9:A400-5.
- [82] Tian C, Ansari MJ, Paez-Cortez J, Bagley J, Godwin J, Donnarumma M, et al. Induction of robust diabetes resistance and prevention of recurrent type 1 diabetes following islet transplantation by gene therapy. *J Immunol.* 2007;179:6762-9.
- [83] Brocker T, Riedinger M, Karjalainen K. Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes in vivo. *J Exp Med.* 1997;185:541-50.
- [84] Probst HC, Lagnel J, Kollias G, van den Broek M. Inducible transgenic mice reveal resting dendritic cells as potent inducers of CD8+ T cell tolerance. *Immunity.* 2003;18:713-20.
- [85] Viglietta V, Kent SC, Orban T, Hafler DA. GAD65-reactive T cells are activated in patients with autoimmune type 1a diabetes. *J Clin Invest.* 2002;109:895-903.
- [86] Arif S, Tree TI, Astill TP, Tremble JM, Bishop AJ, Dayan CM, et al. Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. *J Clin Invest.* 2004;113:451-63.
- [87] Monti P, Scirpoli M, Rigamont IA, Mayr A, Jaeger A, Bonfanti R, et al. Evidence for in vivo primed and expanded autoreactive T cells as a specific feature of patients with type 1 diabetes. *J Immunol.* 2007;179:5785-92.
- [88] Vendrame F, Pileggi A, Laughlin E, Allende G, Martin-Pagola A, Molano RD, et al. Recurrence of Type 1 Diabetes after Simultaneous Pancreas-Kidney Transplantation, despite Immunosuppression, Associated with Autoantibodies and Pathogenic Autoreactive CD4 T-cells. *Diabetes.* 2010;59:947-57.
- [89] Grayson JM, Zajac AJ, Altman JD, Ahmed R. Increased expression of Bcl-2 in antigen-specific memory CD8+ T cells. *J Immunol.* 2000;164:3950-4.
- [90] Valujskikh A, Pantenburg B, Heeger PS. Primed allospecific T cells prevent the effects of costimulatory blockade on prolonged allograft survival in mice. *Am J Transplant.* 2002;2:501-9.
- [91] Adams AB, Williams MA, Jones TR, Shirasugi N, Durham MM, Kaech SM, et al. Heterologous immunity provides a potent barrier to transplantation tolerance. *J Clin Invest.* 2003;111:1887-95.
- [92] Yang J, Brook MO, Carvalho-Gaspar M, Zhang J, Ramon HE, Sayegh MH, et al. Allograft rejection mediated by memory T cells is resistant to regulation. *Proc Natl Acad Sci U S A.* 2007;104:19954-9.
- [93] Steptoe RJ, Stankovic S, Lopaticki S, Jones LK, Harrison LC, Morahan G. Persistence of recipient lymphocytes in NOD mice after irradiation and bone marrow transplantation. *J Autoimmun.* 2004;22:131-8.
- [94] Casorati G, Locatelli F, Pagani S, Garavaglia C, Montini E, Lisini D, et al. Bone marrow-resident memory T cells survive pretransplant chemotherapy and contribute to early immune reconstitution of patients with acute myeloid leukemia given mafosfamide-purged autologous bone marrow transplantation. *Exp Hematol.* 2005;33:212-8.
- [95] Shatry AM, Roopenian DC, Levy RB. Survival and function of MiHA epitope-specific host CD8 T cells following ablative conditioning and HCT. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation.* 2007;13:293-8.
- [96] Dubinsky AN, Burt RK, Martin R, Muraro PA. T-cell clones persisting in the circulation after autologous hematopoietic SCT are undetectable in the peripheral CD34+ selected graft. *Bone Marrow Transplant.* 2010;45:325-31.
- [97] Nikolic B, Onoe T, Takeuchi Y, Khalpey Z, Primo V, Leykin I, et al. Distinct requirements for achievement of allotolerance versus reversal of autoimmunity via nonmyeloablative mixed chimerism induction in NOD mice. *Transplantation.* 2010;89:23-32.
- [98] Kenna TJ, Thomas R, Steptoe RJ. Steady-state dendritic cells expressing cognate antigen terminate memory CD8+ T-cell responses. *Blood.* 2008;111:2091-100.
- [99] Kenna TJ, Waldie T, McNally A, Thomson M, Yagita H, Thomas R, et al. Targeting antigen to diverse APCs inactivates memory CD8+ T cells without eliciting tissue-destructive effector function. *J Immunol.* 2010;184:598-606.
- [100] Nasreen M, Waldie TM, Dixon CM, Steptoe RJ. Steady-state antigen-expressing dendritic cells terminate CD4+ memory T-cell responses. *Eur J Immunol.* 2010;40:2016-25.

- [101] Tian C, Bagley J, Iacomini J. Persistence of antigen is required to maintain transplantation tolerance induced by genetic modification of bone marrow stem cells. *Am J Transplant*. 2006;6:2202-7.
- [102] Buhmann JE, Foy TM, Aruffo A, Crassi KM, Ledbetter JA, Green WR, et al. In the absence of a CD40 signal, B cells are tolerogenic. *Immunity*. 1995;2:645-53.
- [103] Townsend SE, Goodnow CC. Abortive proliferation of rare T cells induced by direct or indirect antigen presentation by rare B cells in vivo. *J Exp Med*. 1998;187:1611-21.
- [104] Dalai SK, Mirshahidi S, Morrot A, Zavala F, Sadegh-Nasseri S. Anergy in memory CD4+ T cells is induced by B cells. *J Immunol*. 2008;181:3221-31.
- [105] Melo ME, Qian J, El-Amine M, Agarwal RK, Soukhareva N, Kang Y, et al. Gene transfer of Ig-fusion proteins into B cells prevents and treats autoimmune diseases. *J Immunol* 2002;168:4788-95.
- [106] Tian C, Yuan X, Jindra PT, Bagley J, Sayegh MH, Iacomini J. Induction of transplantation tolerance to fully mismatched cardiac allografts by T cell mediated delivery of alloantigen. *Clin Immunol*. 2010;136:174-87.
- [107] Ko HJ, Chung JY, Nasa Z, Chan J, Siatskas C, Toh BH, et al. Targeting MOG expression to dendritic cells delays onset of experimental autoimmune disease. *Autoimmunity*. 2011;44:177-87.
- [108] Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, et al. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. *Science*. 2003;302:415-9.
- [109] Chang AH, Sadelain M. The genetic engineering of hematopoietic stem cells: the rise of lentiviral vectors, the conundrum of the Itr, and the promise of lineage-restricted vectors. *Molecular Therapy*. 2007;15:445-56.

Figure 1

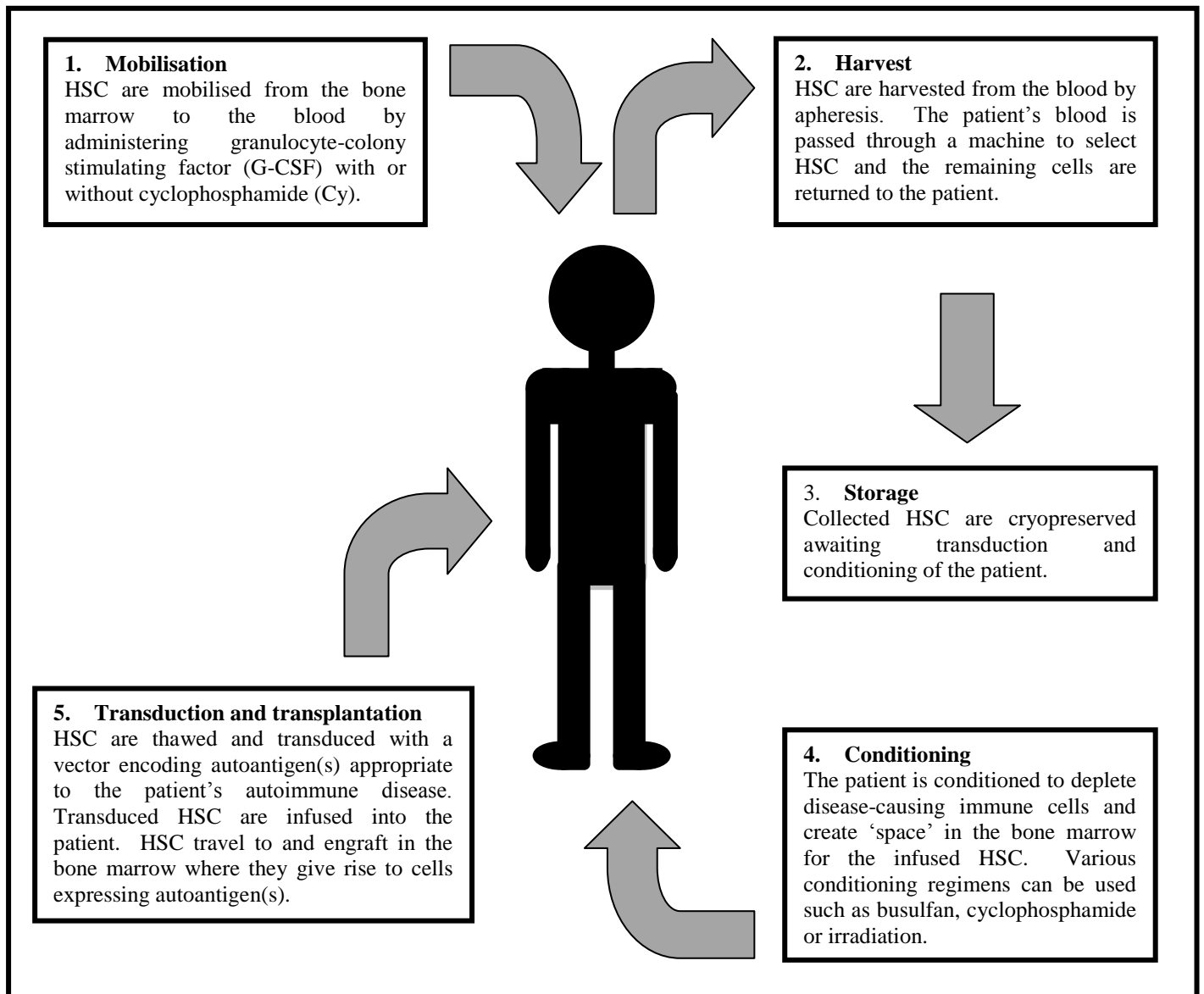


Figure 1. A 'conceptualised' scenario for 'tolerogenic' autologous HSCT. Hematopoietic stem cells (HSC) are harvested using administration of mobilising agents and apheresis. HSC are then stored, then recovered and transduced with a vector encoding appropriate autoantigen(s). Patients undergo mild conditioning before infusion of 'engineered' HSC. Once engrafted in the bone marrow, infused HSC give rise to cells expressing autoantigen(s). The specific cells that express the autoantigens could be determined by choice of the promoter/antigen construct encoded in the transducing vector.