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Treatment of severe autoimmune disease by stem-cell transplantation

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Transplantation of haematopoietic stem cells — cells capable of self renewing and reconstituting all types of blood cell — can treat numerous lethal diseases, including leukaemias and lymphomas. It may now be applicable for the treatment of severe autoimmune diseases, such as therapy-resistant rheumatoid arthritis and multiple sclerosis. Studies in animal models show that the transfer of haematopoietic stem cells can reverse autoimmunity, and several mechanistic pathways may explain this phenomenon. The outcome of ongoing clinical trials, as well as of studies in patients and animal models, will help to determine the role that stem-cell transplantation can play in the treatment of autoimmune diseases.

Most patients with autoimmune disease have a near-normal life expectancy. Nevertheless, some patients suffer severe, therapy-resistant progressive autoimmunity. Haematopoietic-cell transplantation (HCT) is a potential therapy for people with such severe refractory diseases. HCT involves the administration of haematopoietic stem cells (HSC), which are self-renewing and capable of giving rise to all mature haematopoietic cell types and possibly to some non-haematopoietic cell types. The HSC phenotype is characterized by a lack of cell-surface lineage markers and the expression of CD34 in humans. For the sake of simplicity, in this review we use the general term HCT to encompass the transplantation of mobilized peripheral blood cells and bone-marrow cells, both of which are enriched for HSC, as well as to describe purified HSC. The inclusion of other cells besides HSC, especially lymphocytes, in an HCT product, has considerable potential to influence the outcome of HCT.

The recipient is prepared for the transplant by potent immunosuppressive treatment, usually by chemotherapy and/or radiotherapy. This may then be followed by the transfer of autologous haematopoietic cells (auto-HCT; cells harvested from the recipient before patient conditioning (Fig. 1)) or allogeneic haematopoietic cells (allo-HCT; cells harvested from donors rather than the recipient (Fig. 2)) to restore the host immune system. This procedure can cure autoimmune disease in experimental animal models and is now being explored in human clinical trials. So far, auto-HCT has been generally preferred over allo-HCT because of the increased toxicity and potential for rejection in allo-HCT. The risk of graft-versus-host-disease (GVHD) in allo-HCT, which arises from the attack of donor allogeneic T cells on recipient alloantigens, is associated with significant morbidity and mortality. Nevertheless, allo-HCT has been associated with durable complete remission in a small number of patients treated for malignant diseases with coincidental autoimmune disorders. Most recently, several groups have applied non-myeloablative or reduced-intensity conditioning protocols for allo-HCT to the induction of transplantation tolerance and re-establishment of self-tolerance in animal models of autoimmune disease. Although these recent advances have made allo-HCT less toxic, it is an intensive procedure that most probably will not replace current pharmacological treatment for less severe, conservatively treatable autoimmune diseases.

In this article, we review the mechanisms by which HCT might induce tolerance to allo- and autoantigens and discuss recent advances that provide hope for better treatment of severe refractory autoimmune disease. The potential for tissue regeneration using this process is also discussed.

Animal models provide the rationale for HCT

Susceptibility to autoimmune diseases appears to reside in haematopoietic cells. Transplantation of bone-marrow cells from lupus-prone NZB mice into lethally irradiated non-susceptible strains induced the lupus syndrome[1]. This transfer of immune diseases with haematopoietic cells was subsequently confirmed for many autoimmune diseases, including other murine models of systemic lupus erythematosus (SLE), experimental autoimmune encephalomyelitis (EAE), adjuvant arthritis, antiphospholipid syndrome and type 1 diabetes[2]. Resistance to autoimmune diseases could also be transferred to susceptible strains by haematopoietic cells. In mice, HCT from disease-resistant allogeneic animals (allo-HCT) prevented the development of SLE, type 1 diabetes, EAE and other autoimmune diseases[3,4].

Although the prevention of autoimmunity might some day be clinically feasible, at the moment we cannot predict such diseases accurately enough to justify the use of toxic preventive treatments. Unfortunately, animal studies show that preventing the onset of autoimmunity is much easier then reversing established disease. As of 2004, for example, more than 195 methods for preventing or delaying the onset of type 1 diabetes in non-obese diabetic (NOD) mice had been identified[5]. But only a few therapeutic approaches have been effective in reversing established SLE and type 1 diabetes in mice, one of which is allo-HCT[6,7].

In animals, syngeneic HCT (that is, HCT from a genetically identical donor) can be used instead of auto-HCT. For simplicity we will refer to syngeneic HCT as auto-HCT. Although allo-HCT was reliably effective in reversing autoimmunity in animals, auto-HCT did not successfully reverse type 1 diabetes or SLE. However, varying levels of disease remission were achieved following auto-HCT in models of myasthenia gravis[8], adjuvant arthritis[9] and EAE[10]. Allo-HCT was superior in the therapy of EAE. Auto-HCT was most successful at early dis-

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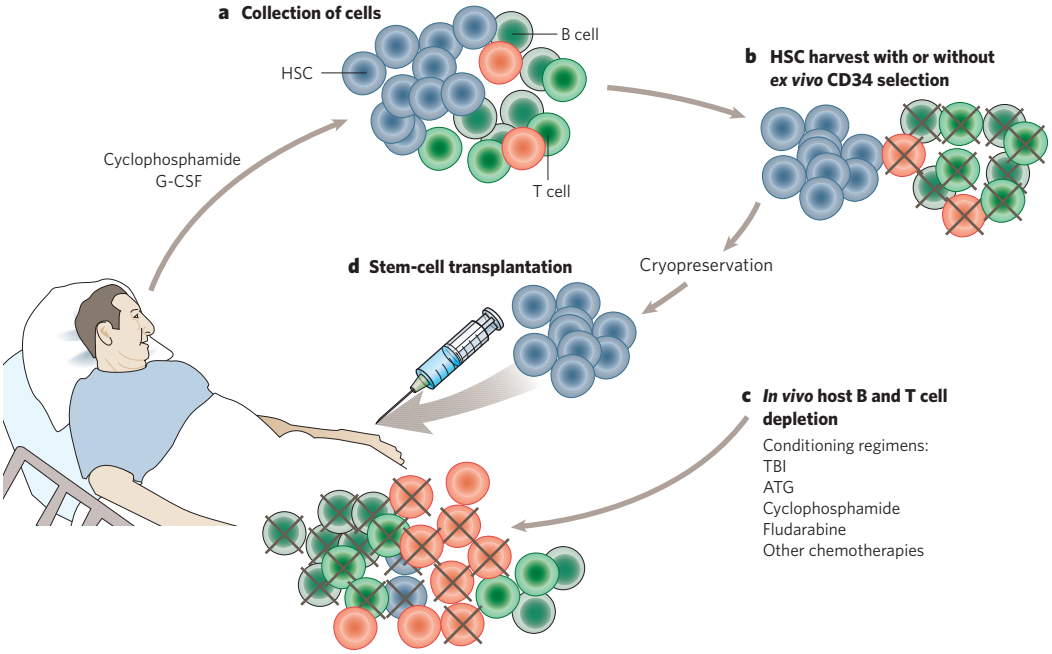

Figure 1 | Autologous HCT as a therapy for autoimmune disease. Red cells represent autoreactive host lymphocytes; green cells represent non-autoreactive host lymphocytes; blue cells represent HSC. The steps in autologous HCT include the following. **a**, Stem-cell collection: HSCs are either harvested from the bone marrow under anaesthesia or collected after mobilization to the peripheral blood by treating the patient with growth factor (for example, G-CSF) and/or chemotherapy (for example, cyclophosphamide). **b**, HSC harvest, with or without *ex vivo* selection using antibody specific for CD34 (a marker of HSCs): efforts may or may not be made to deplete T and B cells from the marrow or mobilized peripheral blood cells. This is usually achieved by HSC positive selection on the basis of CD34 expression. The HSC preparation is cryopreserved. **c**, *In vivo* lymphocyte depletion: various conditioning regimens with the goal of minimizing the burden of autoreactive B and T cells are given to patients. These include combinations of chemotherapeutic agents, total-body irradiation (TBI) and *in vivo* lymphocyte depletion by antibodies, such as anti-thymocyte globulin (ATG), and these treatments often deplete host HSC as well. **d**, Stem-cell transplantation: after conditioning, the cryopreserved HSC preparation is thawed and returned to the recipient to reconstitute haematopoiesis.

ease stages; nearly complete reversal of disease was achieved, but no effect was observed at more advanced stages[11,12]. Also, increased intensity of conditioning therapy correlated positively with the success of auto-HCT[11].

Several observations in animal models of auto- and allo-HCT suggest that these approaches to the therapy of refractory autoimmune disease will be beneficial for patients. First, the partial clinical success of high doses of chemotherapeutic agents, such as cyclophosphamide, for the treatment of autoimmune diseases hinted that even greater success might be achieved with more intensive immunoablative treatments. The major dose-limiting side effect of these treatments is haematopoietic suppression, and this can be reversed by HCT. Given the complications of GVHD and graft failure following allo-HCT and the positive animal data described above, auto-HCT is a logical approach for haematopoietic rescue. Second, the superior efficacy of allo-HCT in some animal autoimmune disease models suggested that allogeneic donor T cells eliminate autoreactive host lymphocytes, and therefore mediate a form of immunotherapy termed graft-versus-autoimmunity (GVA). There is no reason to expect that such immune responses are specific for autoreactive lymphocytes. Therefore this immune reactivity is likely to be a graft-versus-host (GVH) response. Third, animal studies indicate that resistance to autoimmune disease resides in and can be transferred by HSC[2–4]. Finally, engraftment of allo-HSC could result in tolerance to donor antigens, permitting acceptance of donor-derived tissue (for example, islets) without chronic immunosuppressive therapy. Thus, animal studies suggest several potentially useful clinical strategies involving HCT.

Complex, inbred rodent models of autoimmunity provide an opportunity to dissect mechanisms and explore therapies in what is essentially a single donor–recipient combination. But to extend observations from these animal models to humans, several factors must be borne in mind: the impact of the complex and varied genetics of humans; the effect of different reagents used and different responses to treatment modalities; the impact of concurrent and complicating co-morbidities in the patient; and the ability to regenerate an immune system after lymphoablative therapy. Thus, therapies that are safe and effective in murine models may be neither safe nor effective in human trials, and subjects for such trials require careful selection.

Mechanisms by which HCT might treat autoimmunity

Several mechanisms for correction of autoimmunity may apply to HCT (Fig. 3). These are unlikely to be mutually exclusive.

Immunomodulation by immunosuppressive conditioning and auto-HCT

Potent immunosuppressive treatments can eliminate autoreactive memory B cells and T cells (Fig. 3a), thus 'resetting' the immune system. In view of the hypothesized role of microbial antigens and additional unknown environmental triggers in promoting autoimmune disease, it is possible that elimination of the existing T and/or B cell repertoire allows the patient's immune system to 'start from scratch' and regenerate normally. Newly developing B and T cells may be introduced to autoantigens and undergo self-tolerization; alternatively, the chance environmental circumstances that led to the initial autoimmunity might never happen again in the patient's lifetime. The concordance rate of autoimmune diseases in identical twins can be used to gauge the expected recurrence rates if all pre-existing autoreactive lymphocytes were ablated before auto-HCT. These are in the range 10–35%, depending on the disease, and suggest that this approach could lead to sustained remissions in most patients if complete ablation of autoreactive lymphocytes could be achieved.

However, it is not clear whether autoreactive memory T cells, memory B cells and long-lived plasma cells can be completely removed from the haematolymphoid and target organs by any conditioning treatment. Incomplete immunoablation may account for the subopti-

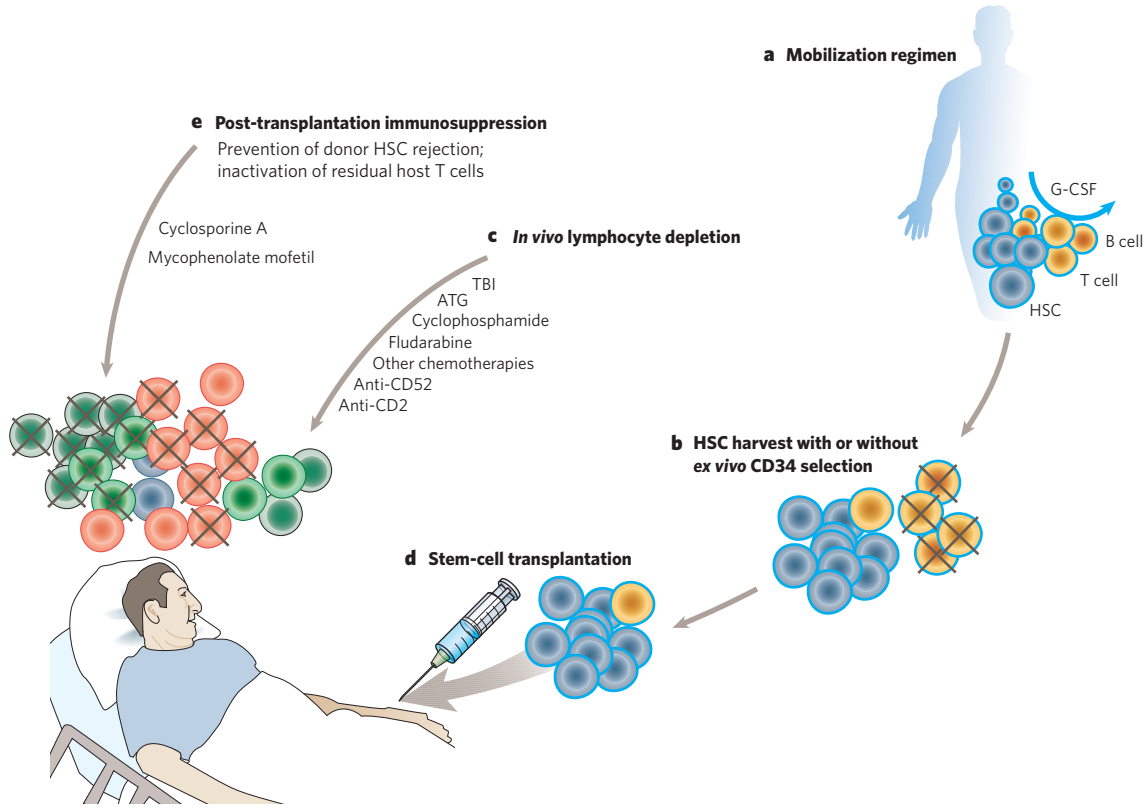

Figure 2 | Allogeneic HCT as a therapy for autoimmune disease. Red cells represent autoreactive host lymphocytes; green cells represent non-autoreactive host lymphocytes; yellow denotes donor lymphocytes; blue cells represent HSC. The steps in allogeneic HCT include the following. **a**, Mobilization regimen: these are similar to the methods employed for autologous HCT, except that cyclophosphamide and other chemotherapies are not used to mobilize stem cells. Mobilization is achieved using the growth factor G-CSF. **b**, HSC harvest, with or without *ex vivo* CD34 selection. This step is similar to that used for auto-HCT. **c**, *In vivo* lymphocyte depletion: this step depletes host T and B cells to prevent rejection and treat the autoimmune disease; it may also be aimed at depleting donor T cells (to prevent GVHD). **d**, Stem-cell transplantation: as for auto-HCT. **e**, Post-transplantation immunosuppression: as prophylaxis against both rejection mediated by residual host T cells and GVHD mediated by contaminating donor T cells, allo-HCT usually requires additional post-transplantation immunosupression with agents such as cyclosporine A and mycophenolate mofetil.

mal responses and high rates of early relapse seen in some clinical trials of auto-HCT (see below). Even with incomplete immunoablation, however, the dependence on multiple cell populations for the maintenance of some autoreactions may lead to improvement in disease. For example, intense conditioning chemotherapy induces severe and prolonged depression of $CD4^+$ T cells, characterized by an inverted $CD4^+$:$CD8^+$ T-cell ratio, a predominance of memory T cells and predominant extrathymic pathways of T-cell recovery[13]. These recovering T cells may exhibit increased susceptibility to apoptosis and altered function[13]. Therefore, altered immune reconstitution and non-specific post-conditioning immunosuppression may result in prolonged disease remission.

In animal models and clinical trials, lymphoablative therapies alone (without HCT) can halt or slow the progression of autoimmune diseases. High-dose cyclophosphamide treatment can reduce the number of cells in the mature immune system, resulting in durable remission in severe aplastic anaemia, Felty's syndrome (characterized by rheumatoid arthritis, splenomegaly and granulocytopenia), autoimmune thrombocytopenic purpura and SLE[14]. This treatment alone is non-myeloablative and does not require HCT for rescue. Although regimens containing cyclophosphamide are highly effective in SLE, they do not achieve satisfactory responses for rheumatoid arthritis. Further studies are needed to evaluate the optimal conditioning regimen for each autoimmune disease as well as the duration and stability of the induced remissions.

To obtain cells for auto-HCT before conditioning, stem cells are mobilized from the marrow to the peripheral blood, which permits stem-cell collection without general anaesthesia or bone-marrow harvest. This is achieved by various protocols, many of which involve granulocyte colony-stimulating factor (G-CSF) or cyclophosphamide, a drug that is myelosuppressive but leads to a 'rebound' mobilization of stem cells. Auto-HCT using mobilized peripheral blood stem cells allows strongly immunoablative treatments to be used in patients with refractory autoimmune diseases. Multiple clinical trials of high-dose immunosuppression and chemotherapy with auto-HCT have been initiated in patients with multiple sclerosis, systemic sclerosis, rheumatoid arthritis, SLE and myasthenia gravis[15–18]. These have involved conditioning regimens of varying intensity, variable *in vivo* and *ex vivo* T-cell purging, and variable stem-cell sources and mobilization protocols. Data from phase I/II clinical trials suggest that auto-HCT is feasible and that it may lead to remission of these diseases[15–18]. Although the results vary from disease to disease, over a third of patients have sustained a durable remission, often with no further need for immunosuppressive drugs[18].

Much has been learned about the patient subgroups most likely to benefit from this approach, and we refer the reader to several excellent recent reviews on this topic[16–18]. In some diseases, however, recurrences are relatively frequent[16–18]. Since controversy prevails about the relative importance of T and B cells in the pathogenesis of some autoimmune diseases (such as rheumatoid arthritis), the eradication of memory B and T cells and tolerization of newly developing cells of each lineage may be more relevant to some diseases than others. Although it is clear that toxicity is greatest with high-intensity conditioning regimens and that infectious risks are increased by T-cell purging owing to delayed

immune reconstitution[18], it is not yet clear whether increased conditioning intensity or lymphocyte purging of the HCT (to avoid reinfusion of autoreactive cells) is associated with improved disease outcomes. Clarification of these issues will await randomized phase III trials that compare auto-HCT with standard therapy. These have recently begun in the United States and Europe. Ideally, these data will help to clarify whether the major benefit of such treatments relates to their ability to deplete autoreactive lymphocytes or to re-establish immune regulation that limits autoimmunity. Furthermore, these results will provide proof of efficacy, which is required for the wider application of auto-HCT in various autoimmune diseases.

Immune-mediated destruction of autoreactive T and B cells

Auto-HCT conditioning regimens for the treatment of leukaemia and lymphoma are designed to destroy the maximum number of malignant cells; the elimination of every single malignant cell is not possible. Total elimination and a cure are often only possible using allo-HCT, where total elimination results from a GVH immune response, owing to donor T cells recognizing the alloantigens of the recipient. This is often associated with GVHD — a disease of the skin, intestines and liver. Despite its associated morbidity and mortality, GVHD is linked to enhanced graft-versus-leukaemia (GVL) effects, which reflect immune destruction of residual leukaemic cells, and enhanced engraftment, probably owing to destruction of residual host T cells. A similar beneficial effect has been observed using allo-HCT to treat autoimmunity[19]. Although auto-HCT permits intense host immune suppression, elimination of every resting memory lymphocyte with high-dose chemotherapy and/or radiation is probably not feasible. Complete immune ablation and reliable reversal of autoimmunity may require the GVA effects of allo-HCT (Fig. 3b), as suggested by the superior results of allo-HCT in numerous animal models. However, this benefit comes with the associated risk of GVHD.

The association of GVA with GVHD was confirmed using a meta-analysis of individual patient data[19] and was seen with discontinuation

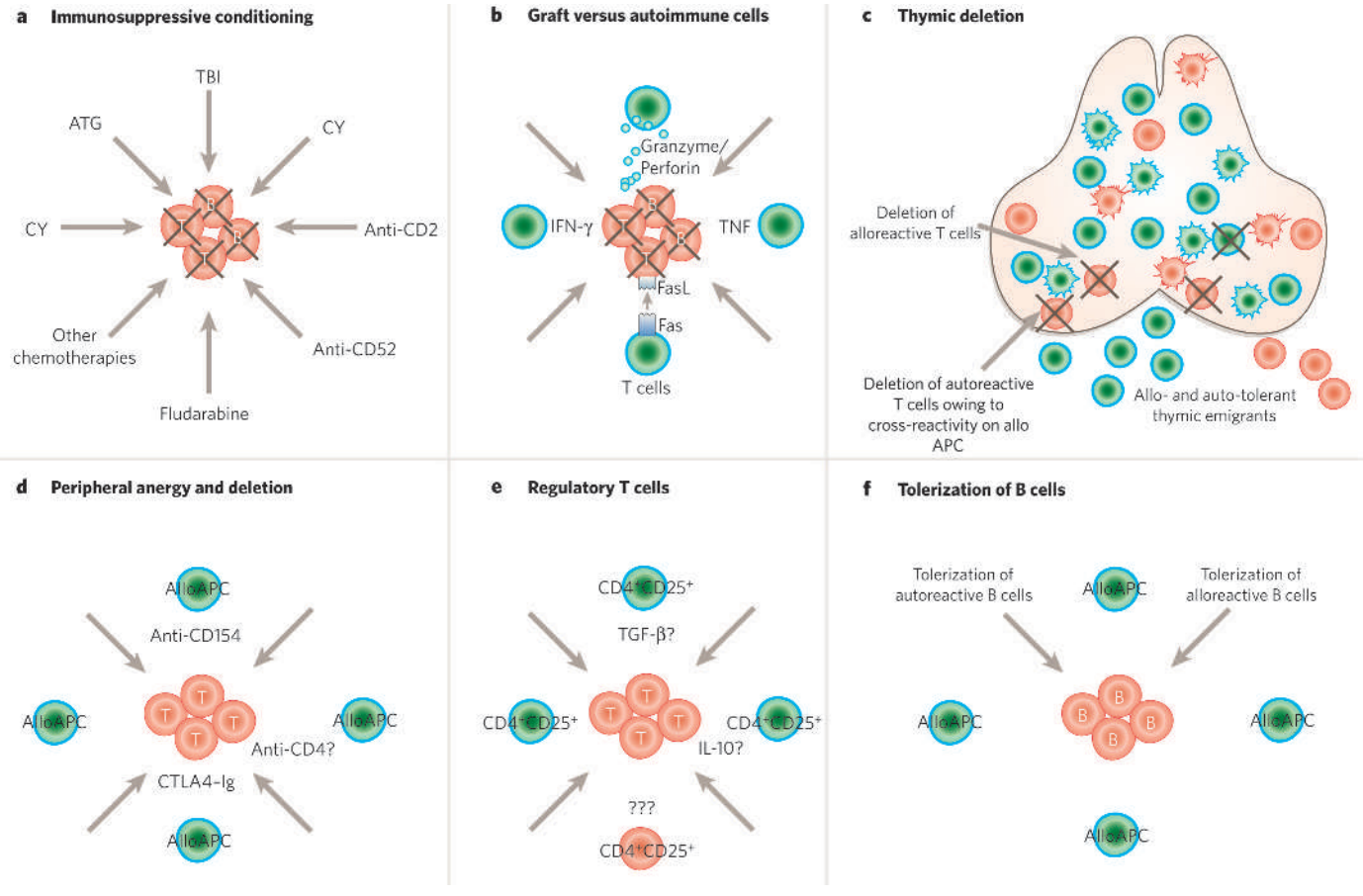

Figure 3 | Mechanisms by which HCT might ameliorate autoimmune diseases. These mechanisms are not mutually exclusive. Donor-derived cells are shown in green and host-derived cells are shown in red. **a**, Immunomodulation by immunosuppressive conditioning: potent immunosuppressive treatments such as total body irradiation (TBI), cyclophosphamide (CY), anti-CD2 antibodies (anti-CD2), anti-CD52 antibodies (anti-CD52), fludarabine and anti-thymocyte globulin (ATG) can eliminate the majority of B and T cells. In addition to alloreactive and autoreactive memory B cells and T cells, other T and B cells are depleted. **b**, Immune-mediated destruction of autoreactive cells (graft-versus-autoimmune host T and B cells). Donor T cells recognize alloantigens and destroy residual memory B and T cells. This mechanism is applicable to allo-HCT only. **c**,Deletion of alloreactive and autoreactive T cells in the thymus. If both donor and host cells contribute to haematopoiesis and to the antigen-presenting cell (APC) pool in the thymus, the new T-cell repertoire generated in the recipient thymus is deleted of T cells recognizing both allo- and autoantigens expressed by haematopoietic cells of both origins. **d**, Induction of anergy and deletion of autoreactive and alloreactive T cells in the periphery. Costimulatory blockade of the CD40–CD154 and CD28–CD80–CD86 pathways in concert with allogeneic BMT can overcome the T-cell barrier to allo-HSC engraftment, which in turn quickly produces donor-specific tolerance and could tolerize cross-reactive autoreactive lymphocytes as well. This mechanism plays a role only in allo-HCT. **e**, Tolerization of peripheral autoreactive/alloreactive T cells by regulatory T cells. $CD4^+CD25^+$ regulatory T cells have been implicated in the maintenance of peripheral tolerance to organ-specific self-antigens as well as alloantigens. The secretion of TGF-β and IL-10 by regulatory T cells has been suggested to mediate this process. Both allo-HCT and auto-HCT could incorporate this mechanism. **f**, Tolerization of autoreactive and alloreactive B cells. In addition to alloantigens, haematopoietic chimaerism could potentially tolerize pre-existing recipient B cells to autoantigens expressed by donor haematopoietic cells. This mechanism plays a role only in allo-HCT.

of immunosuppression in a patient receiving allo-HCT to treat leukaemia[20]. There are two documented case reports of non-myeloablative allogeneic transplants for Evans' syndrome, a disease characterized by combined autoimmune thrombocytopenia and autoimmune haemolytic anaemia. Complete clinical and immunological remission followed GVHD, in one case elicited by donor lymphocyte infusion[21,22].

Although depleting T cells from the HCT product can prevent GVHD, this would be expected to lead to a loss of GVA, similar to the loss of GVL that has been observed with this strategy. Some strategies for separating GVHD from GVL that have been explored in animal models might also favour the achievement of GVA while avoiding GVHD. These include the administration of donor-derived regulatory T cells[23], the direct intra-bone injection of donor bone-marrow stem cells and stromal cells[24] and the infusion of alloreactive natural killer cells[25]. An additional strategy, for which proof of principle has been obtained in pilot clinical trials[26], involves the non-myeloablative induction of mixed chimaerism with a regimen that includes extensive T-cell depletion of the initial HCT. When conditioning-induced inflammation has subsided, donor lymphocytes are then transplanted, which mediate a 'lymphohaematopoietic GVH response'. This GVH response eliminates normal and malignant recipient haematopoietic cells but does not migrate to the GVHD target tissues, the skin, gut and liver, and so GVHD does not occur[27].

Deletion of alloreactive and autoreactive T cells in the thymus

Allo-HCT and full donor reconstitution leads to intrathymic deletion (or tolerance) of newly developing thymocytes that recognize donor antigens, permitting acceptance of donor organs without immunosuppressive therapy. Mixed haematopoietic chimaerism is a state in which HSCs, and therefore multi-lineage haematopoietic populations, of the recipient and the donor co-exist. Mixed haematopoietic chimaerism has several advantages over full allogeneic chimaerism and is being explored in animal models as an approach to achieving organ-graft tolerance. Mixed haematopoietic chimaerism can be achieved by milder conditioning treatments that do not ablate host haematopoietic cells[28], overcoming some of the high treatment-related toxicity that has largely prevented the clinical application of allo-HCT for non-life-threatening diseases. Mixed chimaerism is associated with improved immunocompetence compared with full chimaerism, particularly if the donor and recipient do not share MHC alleles[28]. Mixed but not full chimaeras contain a life-long source of host-type antigen-presenting cells (APCs) that can most effectively present antigens to T cells that are positively selected in the recipient thymus[28] (Fig. 3c).

If both donor and host cells contribute to haematopoiesis, the new T-cell repertoire generated in the recipient thymus is rendered tolerant of antigens expressed by haematopoietic cells of both origins. Haematopoietic cells most efficiently delete thymocytes with high affinity for self-MHC–peptide complexes, thus ensuring tolerance to the donor and host[28]. Induction of mixed chimaerism prevents the development of autoimmune disease with greater efficiency than auto-HCT does in several animal models. The reason that donor haematopoietic cells can induce tolerance of newly developing autoimmune-prone recipient T cells in these models is not known. It is possible that donor-derived APCs expressing allogeneic MHC–peptide complexes mediate intrathymic deletion of autoreactive host thymocytes with cross-reactive TCRs. However, the observation of similar phenomena in different disease models, using different animal strains, makes this explanation somewhat unsatisfactory.

Genetic susceptibility and resistance to type 1 diabetes are associated with highly polymorphic genes of the MHC. The NOD mouse is homozygous for a unique H-2 haplotype (H-2^{g7}), and the I-A^{g7} allele is an orthologue of the HLA-DQ0302 allele in humans, which confers susceptibility to type 1 diabetes. Although controversial, some studies suggest that MHC class II I-A^{g7} molecules, by binding to self-peptides with low efficiency during thymic selection, permit self-reactive T cells to escape negative selection[29]. The expression of stable 'protective', cross-reactive allo-MHC–peptide complexes on donor HSC-derived APCs may result in specific central deletion of host-derived autoreactive T cells.

Studies with I-A^{g7}-restricted islet-cell-reactive TCR-transgenic mice expressing antidiabetogenic MHC class II haplotypes show that protective MHC class II molecules induce deletion of certain diabetogenic TCRs. This negative selection of autoreactive thymocytes is mediated by thymic bone-marrow-derived cells independently from endogenous superantigens[30]. Intrathymic deletion mediated by 'protective' MHC class II occurs in a transgenic diabetes model involving a diabetogenic TCR from a $CD8^+$ T-cell clone[31], and in NOD mice receiving syngeneic HSC transduced with a protective MHC class II molecule[32]. In another diabetogenic TCR transgenic model in NOD mice, however, protective MHC class II molecules expressed by F_1 mice tolerize diabetogenic $CD4^+$ T cells, not through deletion but through generation of regulatory cell populations[33]. In this case, the thymic epithelium expressed the protective MHC molecules. Non-haematopoietic-cell MHC expression is essential for positive selection of regulatory T cells[33], but this mechanism cannot readily explain the tolerance of autoreactive clones in mixed haematopoietic chimaeras, because the donor MHC is expressed only by haematopoietic cells.

Many of the studies showing prevention of autoimmune disease by induction of mixed chimaerism have involved lethal total-body irradiation and reconstitution with allogeneic plus syngeneic HSCs. The formidable and opposing problems of GVHD and failure of engraftment that have been observed in lethally conditioned humans when extensive HLA barriers are crossed make this approach unlikely to have clinical relevance. A clinically relevant protocol for reliably achieving sustained mixed chimaerism across MHC barriers for reversal of autoimmune disease must specifically overcome the host immune barriers to the donor and be devoid of GVHD risk. Over the past 15 years, several such protocols have been successfully developed in animal models, many of which involve *in vivo* monoclonal antibody treatment to eliminate both donor and host T cells *in vivo*. This outcome has been achieved in a small number of patients receiving a non-myeloablative conditioning regimen involving *in vivo* and *ex vivo* T-cell depletion[26]. However, after lymphoablative conditioning, T cells are regenerated quite slowly by the adult thymus, making non-lymphoablative regimens desirable. More recently, protocols have been developed that substitute a costimulatory blockade for some or all of this T-cell depletion. As discussed below, additional mechanisms must be involved in the tolerization of both pre-existing alloreactive and autoreactive T cells in such protocols.

Anergy and deletion of peripheral auto- and alloreactive T cells

In all normal adults, mature T cells with anti-donor reactivity already exist in the periphery. These cells must be eliminated or inactivated by initial host conditioning to prevent the rejection of the infused donor HSCs. Because the adult thymus is very slow to regenerate T cells after lymphoablative conditioning, it would be desirable to avoid recipient T-cell depletion in regimens for HCT. Instead, blockade of the costimulatory signalling pathways CD40–CD154 and CD28–CD80/CD86 together with allo-HCT can overcome the T-cell barrier to allo-HSC engraftment, which in turn quickly produces donor-specific tolerance[34,35]. Long-term maintenance of this systemic tolerance is achieved by central deletion of alloreactive T cells, the same as in other mixed-chimaerism models[34,35]. However, because this approach does not include complete depletion of peripheral recipient T cells before HCT, other mechanisms must allow acceptance of donor bone marrow and tolerize pre-existing recipient T cells (Fig. 3d). The combination of anti-CD154 monoclonal antibodies and allo-HCT tolerizes existing alloreactive $CD4^+$ T cells in the periphery by anergy followed by deletion, owing to presentation of donor antigens on APCs in the absence of a CD40-mediated activation signal[34]. Donor-reactive $CD8^+$ T cells also undergo deletion in the periphery, even more rapidly than $CD4^+$ T cells in this model[36] (Y. Takeuchi *et al.*, unpublished observations), by mechanisms that are currently under investigation.

A non-myeloablative regimen using costimulatory blockade with

sublethal total body irradiation induced tolerance to allogeneic islets transplanted into NOD mice. However, nearly all haematopoietic cells in these animals were derived from donors[37]. In another model, mixed chimaerism was induced in NOD mice that received MHC-matched allo-HCT. Transplantation of donor islets led to a long-term cure of diabetes, despite the presence of lymphocytic infiltrates in the islet grafts[38].

In a recent study, mixed chimaerism was established across extensive MHC barriers in overtly diabetic NOD mice with non-myeloablative conditioning involving anti-CD154 monoclonal antibodies. These mice accepted donor allogeneic islets and were thereby cured of established diabetes, despite significant T-cell reconstitution by the NOD recipients[39]. Surprisingly, mixed haematopoietic chimaerism reversed destructive autoimmunity, permitting acceptance of syngeneic islet grafts in established diabetic mice, despite the fact that large numbers of pre-existing NOD T cells escaped depletion with anti-T cell monoclonal antibodies[39] (B. Nikolic and M. Sykes, unpublished data). Thus, in addition to tolerizing newly developing host T cells intrathymically, unknown peripheral mechanisms must tolerize pre-existing autoreactive NOD memory T cells in this model. Without HCT, non-myeloablative conditioning did not induce tolerance to either syngeneic or allogeneic islets, demonstrating that allo-HSC are crucial for tolerization of diabetogenic and alloreactive T cells.

Tolerization by regulatory T cells

$CD4^+CD25^+$ regulatory T cells are involved in the maintenance of peripheral tolerance to organ-specific self-antigens. Their development is regulated by the forkhead transcription factor Foxp3 (ref. 41), and its genetic deficiency leads to a lack of $CD4^+CD25^+$ regulatory T cells and autoimmune syndromes in humans and mice[40,41] (see the review by Rudensky and Kronenberg, page 598). Studies of wild-type and *Foxp3*-deficient mixed chimaerism showed that the gene acts intrinsically to generate regulatory cells only among $Foxp3^+$ cells[42]. However, mixed chimaerism involving HSC expressing and lacking Foxp3 allows for the development of $Foxp3^+$ regulatory cells that can dominantly inhibit autoimmunity. By this mechanism, mixed chimaerism can correct a variety of autoimmune syndromes associated with intrinsic defects in haematopoietic cells and their ability to generate regulatory cells[43] (Fig. 3e).

In contrast to 'haematopioetic-cell-intrinsic' defects in regulatory cell development, it is unlikely that mixed chimaerism can correct autoimmunity caused by thymic epithelial-cell-intrinsic abnormalities. Such defects would include the failure of $I\text{-}A^{g7}$ to positively select regulatory T cells[33], a function performed by the thymic epithelium. This would also apply to defects in *Aire* (autoimmune regulator), a gene expressed by medullary thymic epithelial cells that regulates the intrathymic expression of numerous organ-specific self-antigens[44]. *Aire* mutations in mice and humans are associated with autoimmunity[44,45] owing to the lack of expression of these self-antigens in the thymus. This may result in insufficient central deletion of self-reactive T cells[44] and a failure to generate specific $CD4^+CD25^+$ regulatory T cells[46].

Tolerization of autoreactive and alloreactive B cells

Autoantibodies provide diagnostic and prognostic criteria and play a key role in the pathogenesis of numerous autoimmune diseases, leading to considerable interest in B-cell-directed/depleting therapies for autoimmune diseases. In addition to T-cell tolerance, induction of mixed allogeneic chimaerism also results in B-cell tolerance (Fig. 3f). Using an α(1,3)-galactosyltransferase (GalT) wild-type to GalT-deficient mouse HCT model, non-myeloablative mixed chimaerism tolerizes pre-existing B cells producing natural antibody against the carbohydrate Gal epitope expressed by the donor[47]. Furthermore, mixed chimaerism can also tolerize a class-switched IgG anti-Gal memory B-cell response[28]. The absence of B cells with receptors recognizing Gal in long-term mixed chimaeras suggests a role for clonal deletion/receptor editing in the maintenance of B-cell tolerance[28] (see the review by Goodnow *et al*. for more on mechanisms of tolerance, page 590). However, B cells expressing these receptors take time to disappear, suggesting that anergy is the initial tolerance mechanism (T. Kawahara *et al*., unpublished observations). Thus, mixed chimaerism might tolerize pre-existing recipient B cells recognizing autoantigens that are shared by the donor haematopoietic cells.

Clinical applications of allo-HCT

The substantial risk of morbidity and mortality associated with allo-HCT has generally prevented it from being used to treat patients with autoimmune diseases. Only recently, on the basis of the rationale discussed in the previous sections, encouraging results from animal models, and the development of non-myeloablative conditioning regimens, some investigators have started exploring allo-HCT for the treatment of severe autoimmune diseases. Clinical observations in patients undergoing allo-HCT for the treatment of severe aplastic anaemia or acute leukaemia, who had a coincident autoimmune disease, support this approach. Although most of these patients have achieved remission of the autoimmune disease[15], there are some notable exceptions. Three patients with rheumatoid arthritis, psoriasis and Crohn's disease relapsed despite achieving nearly full donor chimaerism[48,49]. There may be several reasons for these relapses: a lack of GVHD (GVA); ongoing immune destruction by persisting recipient cells; the use of HLA-identical donors; and the possible existence of similar autoimmune-susceptible genes in the donor.

Allo-HCT has recently been applied as a therapy for Evans' syndrome, a disease characterized by combined autoimmune thrombocytopenia and autoimmune haemolytic anaemia. Complete remission of severe Evans' syndrome was achieved following HLA-identical sibling cord blood transplantation, but the patient died nine months after HCT from acute liver failure of unknown aetiology[50]. Recently, allo-HCT followed by donor leukocyte infusion to enhance GVA led to complete remission of Evans' syndrome for 2 years after transplantation, in association with grade II GVHD[22]. In seven patients receiving allo-HCT to treat refractory autoimmune cytopenias, one died of treatment-related complications, one with a transient response died of progressive disease, and five had continuous responses[51].

Tolerance induction through HSC chimaerism

Mixed allogeneic chimaerism may induce a reliable and robust form of allo- and autotolerance. If regimens for achieving mixed chimaerism in patients with acceptably low toxicity are developed, this approach might have a role in the treatment of autoimmune diseases. Two centres have published results of their attempts to induce mixed chimaerism for the establishment of allograft tolerance in patients. The Stanford group used a conditioning regimen consisting of total lymphoid irradiation and anti-thymocyte globulin (ATG) followed by transplantation of G-CSF-mobilized HLA-mismatched $CD34^+$ cells and a kidney from the same donor followed by post-transplant immunosuppression[52]. Three out of four patients developed transient chimaerism that was lost within the first three months. Two patients were weaned from all immunosuppression after 12 months. They subsequently developed acute rejection episodes and immunosuppressive therapy had to be resumed[52]. Thus, tolerance was not achieved. At Massachusetts General Hospital, we have used combined kidney transplantation and non-myeloablative HCT from HLA-identical related donors to patients with multiple myeloma with renal failure using a regimen involving peri-transplant ATG, cyclophosphamide, thymic irradiation and a short post-transplantation course of cyclosporine[53,54]. A second protocol involves HLA-haploidentical transplantation to related recipients who do not have malignant disease. Patients are conditioned with cyclophosphamide, anti-CD2 antibody and thymic irradiation followed by combined renal transplantation and HCT from the same HLA-haploidentical donor. Encouraging preliminary results have been obtained (T. Kawai *et al.*, unpublished data).

Another group recently applied a mixed-chimaerism approach to the therapy of severe, refractory rheumatoid arthritis[55]. The patient was conditioned with fludarabine (an antimetabolite, purine antago-

nist), cyclophosphamide, and anti-CD52 (which recognizes T and B cells) and received HLA-matched $CD34^+$ HCT, which resulted in mixed chimaerism among T cells (55% donor $CD3^+$ cells) and myeloid cells (70% donor $CD33^+$ cells). One year after HCT, the patient had suffered from no major infection and had had no acute or chronic GVHD. Remission of rheumatoid arthritis without GVHD or the need for immunomodulative medications was achieved[55].

Although these data are promising, the risk of GVHD and the absence of definitive information on the efficacy of auto-HCT, which lacks this risk, make it unclear what role allo-HCT will ultimately play in the treatment of autoimmune diseases. At present, the substantial mortality and morbidity associated with allo-HCT must be carefully weighed against its therapeutic benefits. The careful and proper choice of prospective patients with refractory autoimmune diseases that are resistant to current pharmacological treatments is essential. It is possible that the therapeutic benefit of auto-HCT could be enhanced by the addition of expanded regulatory cell populations, such as $CD4^+CD25^+$ regulatory T cells, or mesenchymal stem cells, which have potent immunosuppressive properties and have recently been reported to ameliorate severe GVHD[56].

Future directions

The recent extension of the allo-HCT approach from rodents to humans for tolerance induction to alloantigens is highly encouraging. An understanding of the tolerization processes that apply to naive and memory T and B cells will ultimately lead to the development of less toxic, non-myeloablative conditioning regimens. The development of such conditioning regimens will lead to new hope for patients in need of organ transplants and possibly for those with autoimmune diseases. The ultimate role for allo-HCT in the treatment of autoimmune disease will be better defined by results of ongoing auto-HCT trials, and by an improved understanding of disease pathogenesis, the genetic defects underlying different forms of autoimmune disease, and the mechanisms by which HCT may tolerize memory T and B cells.

In particular, the application of allo-HCT followed by transplantation of islets from the same donor has the potential to improve the therapy of patients with type 1 diabetes. Islet transplantation can normalize glucose levels and in theory could prevent some of the complications of diabetes. The immunosuppressive 'Edmonton Protocol' (which includes increased numbers of high-quality islet cells, omission of steroids and immunosuppression with tacrolimus, rapamycin and an anti-interleukin 2 receptor antibody) is a major step forward. Nonetheless, the overall outcome of clinical islet allotransplantation has been somewhat disappointing[57]. After 3–4 years, only 12–25% of transplanted patients were insulin independent[57], possibly because of the allo- and autoimmune responses combined with complications of the drug therapy. In addition, the life-long immunosuppression needed to achieve allograft acceptance is hard to justify in young type 1 diabetic patients, making tolerance-inducing approaches particularly desirable. Tolerance would permit graft survival without the need for continuous immunosuppressive therapy. Successful tolerance protocols would overcome alloresponses to donor islet alloantigens and could prevent recurrent autoimmunity in the donor islets, as suggested by mixed-chimaerism studies in NOD mice with advanced diabetes[39]. Either combined HSC and islets from deceased donors or the combined transplantation of embryonic stem cell[58] or adult stem-cell-derived islets and HSC could ultimately be used to achieve tolerance and cure of type 1 diabetes. In addition, promising approaches to xenotolerance induction, including HCT[59], might ultimately allow the use of xenotransplants (grafts from donors of other species such as pigs) that could provide a solution to the shortage of allogeneic islets.

In addition to tolerization of autoreactive and alloreactive cells, HCT might also allow regeneration of tissues that are destroyed by autoimmune disease. This could theoretically occur owing either to regeneration from endogenous stem cells once autoimmunity is reversed, or because of differentiation of HSCs or other cells in the HCT product into non-haematopoietic tissues. Endogenous islet regeneration has been described following allogeneic HCT in new onset diabetic NOD mice[60], and transplanted haematopoietic cells have been reported to promote endogenous pancreatic regeneration[61,62]. Studies in mixed-chimaeric NOD mice with well established diabetes show, however, that endogenous islet regeneration cannot maintain normoglycaemia and that islet transplantation is necessary to correct the disease[39]. Several groups have reported that HSC can differentiate into hepatic cells, myocardium, kidney cells and other non-haematopoietic cells types, including islet cells[63,64], but it is possible that these studies reflect cell fusion rather than differentiation from cells contained in HCT[65,66]. Although splenocytes have been reported to contain cells that regenerate islets in diabetic NOD mice[67], other groups have not been successful in reproducing these results[68]. HCT leading to sustained mixed chimaerism in well-established diabetic mice does not achieve similar results[39]. Thus, the real potential of tissue regeneration from HSC to cure autoimmune disease is controversial and remains an area of intense investigation. ■

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