Treating autoimmune diseases: is stem cell therapy the future?

Experimental investigations and serendipitous clinical reports had indicated that hematopoietic stem cell transplantation (HSCT) could represent a new powerful procedure for treating severe autoimmune diseases (SADs). Although the therapeutic potential of allogeneic transplantation appeared theoretically potentially curative, the almost paradoxical results by van Bekkum and his group indicated that the autologous procedure also had outstanding therapeutic potential. The European and American experience includes by now an estimated number of autologous transplants for SADs of well over 1000 cases. The December 2008 issue of *Autoimmunity* contains the most recent information about the various autoimmune diseases treated with autologous and allogeneic HSCT. This perspective, rather than reporting on already published clinical trials, focuses on the main questions connected with autologous ASCT – that is, its safety, its currently explored advantages and disadvantages versus the best available nontransplant procedures and its mechanism of action. Allogeneic transplants for non-coincidental diseases are still too few to attempt to draw conclusions, but its scientific and ethical utilization relies uniquely in the pursuit of cure.

KEYWORDS: hematopoietic stem cell transplantation ■ severe autoimmune diseases

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Stem cell therapy for the treatment of severe autoimmune diseases (AD) – generally as hematopoietic stem cell transplantation (HSCT), both allogeneic and autologous, but also more recently as gene therapy-assisted autologous HSCT [1–4] – has become one of the hottest areas of clinical immunology. It has been developing consistently in the last decades, and has generated "excitement and promise, as well as confusion and, at times, contradictory results in the lay and scientific literature" [5]. However, there are two way of addressing the matter. The utilization of any kind of stem cells to promote regenerative medicine [6] must be distinguished from the purpose of suppressing (or eradicating) autoimmune cellular and humoral autoaggression, whether organ or non-organ specific. As already pointed out [7], these two key areas of contemporary investigative medicine must be addressed singularly, and only the second will be discussed in this perspective. This does not mean that both areas are not tightly connected, since supplying new pancreatic β-cells to patients with Type 1 diabetes, whether by islet cell transplantation or by reprogramming pancreatic acinar cells to boost islet cell numbers [8], cannot resolve the disease if the autoimmune aggression is not eliminated [9]. There are some clinical entities where both effects coincide. The most appropriate example is aplastic anemia and some of its minor variants (pure red cell aplasia and pure white cell aplasia), in which allogeneic HSCT both suppresses autoimmunity and provides new hematopoietic stem cells (HSCs) [10]. In all the other autoimmune conditions, this double effect has not been demonstrated conclusively.

The utilization of HSCT, overwhelmingly of the autologous modality, has been growing impressively in the last few years [11–17], and is still increasing steadily [18,19]. Autologous HSCT (ASCT) relies on an extensive debulking of the autoaggressive immune system, that may also be obtained by immunosuppression alone [20], followed by the re-infusion of the patient's HSCs (commonly identified as $CD34^+$ cells). A resetting or re-education of the immune system has been postulated [21–24]. By contrast, the allogeneic procedure is based on the substitution of the faulty immune system with a new healthy one (even if the utilization of related, HLA-identical donors may result in exposure to an autoimmune-prone genoma), that is supposedly capable of eradicating the autoimmune clones by means of the classical combination of high-dose immunosuppressive therapy and a graft-versus-autoimmunity (GVA) effect [25,26], which will be discussed later. Whether this last intervention will be capable of achieving the Holy Grail of self tolerance [27] is yet to be established [28],

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given the complexity of the pathogenesis of ADs, including the neo-antigenicity of altered 'self' proteins [29] and some paradoxical post-transplant relapses notwithstanding full donor chimerism [30–32]; this will also be discussed later.

A brief historical recapitulation

Two streams of research, experimental and clinical, are at the origin of the increasing utilization of HSCT, autologous and allogeneic, for severe autoimmune diseases (SADs) [33]. The first animal studies demonstrated that the transfer of spleen and/or whole marrow cells to immunosuppressed mice (by means of antilymphocyte globulin and irradiation) could reproduce murine lupus [34,35]. Similarly, lymphocytes from patients with a variety of ADs, when transferred to transgenic knock-out mice, reproduced the original human diseases. The culprit cells were demonstrated to be stem or lymphoid progenitors [36,37]. The next and more important step was to ascertain whether healthy HSCs were capable of curing experimental ADs [38]. Human blood stem cells (SCs) were capable of suppressing antibody production in the lupus mice [39] – perhaps this was the first demonstration of a curative effect by xenogeneic HSCT. More recently, it has been elegantly demonstrated that the non-myeloablative transplantation of purified allogeneic HSCs not only prevented, but also induced stabilization or reversal of lupus symptoms in New Zealand black mice [40]. Durable mixed chimerism was also efficacious [41–44], a point that will be discussed later. A further experimental improvement is the intra-bone injection of HSC [45], which is being translated into the clinic [46].

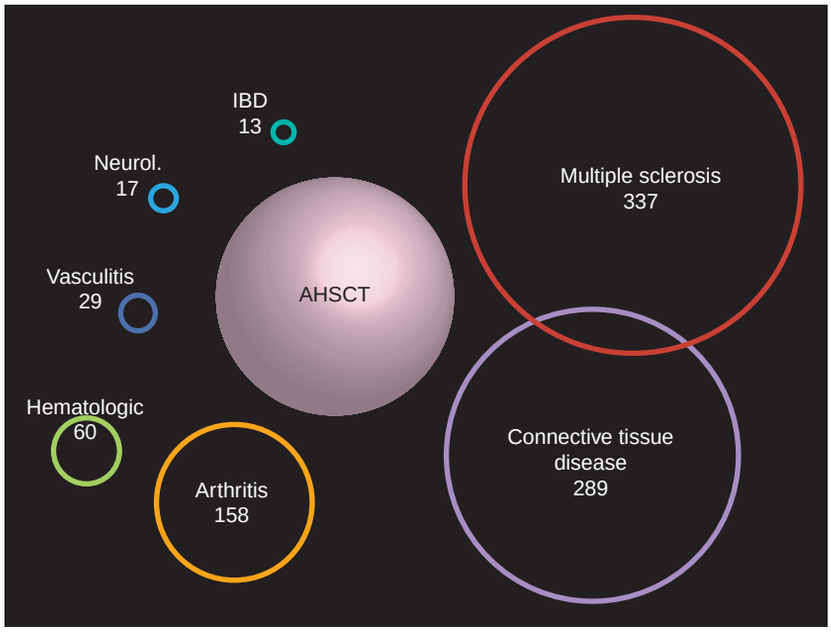

Figure 1. Autologous hematopoietic stem cell transplants registered by the Working Party of the European Group for Blood and Marrow Transplantation at the end of 2007. Multiple sclerosis is still the main indication. AHSCT: Autologous hematopoietic stem cell transplantation; IBD: Inflammatory bowel disease.

The resolution of experimental ADs by means of healthy, compatible allo-SCT was to be expected considering the overwhelming genetic predisposition of inbred strains of mice [47,48], which differs from the intricacies of human ADs in which there is a complex relationship between genetic, environmental and regulatory factors [49,50] and where impaired mechanisms of thymic selection interact, still in poorly elucidated ways, with genetic factors [51]. As previously mentioned, a GVA effect has been postulated [25,26] and theoretically dissected by six different mechanisms [13], with immune-mediated abrogation of autoreactive clones in the foreground. In practice, donor-derived immune cells are capable of mediating an anti-autoimmune effect either specifically, or as part of a more general alloimmune reaction. In an elegant study in experimental autoimmune encephalomyelitis (EAE), it was demonstrated that active alloreactivity was associated with the greatest GVA effect [52]. Whether a similar effect can be translated to the clinic is unknown, since it is too early to be confirmed [53–55] and will be discussed later. A second stream in favor of allo-SCT came from the clinical observation of patients affected by coincidental diseases – that is, patients with ADs who have developed a hematologic malignancy for which they received an allo-SCT and were ultimately cured of both diseases [56]. There were even cases in which allo-bone marrow transplant (BMT) transferred the AD of the donor to the recipient, but cured the latter of their former AD [57].

The rationale for an apparently paradoxical procedure such as autologous HSCT, in which the patient's immune cells (despite varying degrees of HSC depletion *in vitro* and/or *in vivo*) are administered back to them, came from the pioneering studies by van Bekkum and his group [58], who were able to cure EAE and adjuvant arthritis, using both models of human multiple sclerosis (MS) and rheumatoid arthritis (RA), by means of autologous ('pseudoautologous') HSCT. These unexpected results considerably strengthened the philosophy of ASCT for human ADs, even though it was pointed out later that in animal models, the abnormality of the antigen-induced type seems to reside

in immunocompetent T/B cells, but not in the HSCs, and therefore, ASCT may be curative, whereas in spontaneous ADs, new, unaffected HSCs were necessary to achieve a cure [38]. Anyway, the utilization of ASCT is now widely accepted for treating severe, refractory ADs, as will be discussed presently.

A powerful immunosuppressive therapy for SADs has been developed at Johns Hopkins University in Baltimore (MD, USA), where such patients are treated with high-dose cyclophosphamide (CY) alone, with an inevitable delay of marrow and blood reconstitution, but the results do not differ significantly from those obtained by ASCT [59,60]. However, the most appealing new approach for a biological control of ADs is the utilization of mesenchymal stem cells [61] that possess several immunomodulatory properties [62], significantly ameliorate graft-versus-host disease (GVHD [63]) and have been considered a valuable therapeutic option for SADs [64,65]. An experimental autoimmune enteropathy was ameliorated by the administration of mesenchymal stem cells [66]. There are great expectations for them, and Phase I/II clinical trials are currently exploring the effectiveness of this kind of cellular therapy [65–68], either associated or not with ASCT. Another interesting approach is based on the idea of achieving antigen-specific tolerance to treat refractory ADs [69], even if translating such therapies from bench to bedside is still mainly theoretical. An approach combining HSCT and transduction of the culprit self-antigens in autologous HSCs in order to achieve central (thymic) tolerance has been developed by Alderuccio and his group [1–3,70], although so far this has only been obtained in animal experiments with organ-specific autoimmune conditions.

Autologous transplantation: progress & questions

In contrast to the long interval that took place between the first allogeneic transplants for animal ADs and translational clinical trials, ASCT trials quickly followed the experimental investigations. It was proposed by myself as a treatment for severe systemic lupus erythematosus (SLE) in 1993 [71] and for ADs in general in 1995 [72]. The first transplants were performed for a connective tissue disease [73] and for severe SLE [74,75]. Following the utilization of ASCT for SADs, treatment grew almost exponentially, so much so that, besides the continually increasing registered transplants in the European Group for Blood and Marrow Transplantation (EBMT) and Center for International Blood and Marrow Transplant Research (CIBMTR) registries, Dominique Farge *et al.* recently conducted a study and analyzed 900 patients [Farge D, Labopin M, Tyndall A *et al.*: Autologous hematopoietic stem cell transplantation (AHSCT) for autoimmune diseases: a 10 years experience from the European Group for Blood and Marrow Transplantation (EBMT) Working Party on Autoimmune Diseases. Manuscript submitted]. Excellent reviews of specific diseases have been published recently [76,77], and a monographic issue of autoimmunity has just been devolved to this theme [19]. Therefore, rather than reviewing these thoroughly published results once again, I shall focus on the most significant and contemporary questions.

Autologous HSCT for ADs has been considered a relatively safe procedure from its inception, but is it becoming safer?

Autoimmune diseases represent an extremely heterogenous spectrum of diseases [78] and, in most of them, severe refractory forms have a poor prognosis and a greatly impaired quality of life. However, one cannot disagree with Burt's recent statement that 'Treatment-related mortality needs to be very low for nonmalignant diseases' [5]. Treatment-related mortality (TRM) reached 12% in the initial EBMT registry [79], decreased to 7 ± 3% in 2005 [80] and finally, did not exceed 5% in the most recent EBMT study including 900 patients [Farge D, Labopin M, Tyndall A *et al.* Manuscript submitted]. In this last study evidence was also found of a clear center effect, indicating that experienced teams who are well acquainted with the multiorgan involvment of SADs produce superior results. In the case of a single disease such as SLE, a collection of 153 patients who underwent transplantation in 30 centers demonstrated a TRM of 7% but, when the results of one center were removed, this decreased to 1.3% [81]. Out of 200 patients transplanted at Northwestern University, Chicago (IL, USA), the TRM observed when nonmyeloablative conditioning regimens were used in 200 patients was 1.5% [82]. This does not mean, of course, that TRM cannot greatly increase in very severe conditions such as advanced scleroderma, since a recent study demonstrated, for the first time, a significant decrease of dermal fibrosis, but at the expense of a 23% TRM. Scleroderma-related organ dysfunction contributed to the seven treatment-related deaths that occurred in the first year after transplantation in this study [83]. In conclusion, the answer to this first question is that ASCT may be

considered reasonably safe when performed by experienced teams, with appropriate conditioning regimens [84] and if the patients are not too disease-compromised. These data need to be counterbalanced by mortality rates from disease progression (9 ± 4% at 3 years despite ASCT in the 2005 EBMT study [80]), and require the adoption of inclusion and exclusion criteria for each category of diseases, although this cannot be detailed here. However, before excluding a severely compromised patient, the encouraging results from Chicago's Northwestern University should be considered, where two SLE patients in dialysis were successfully transplanted and achieved freedom from dialysis [82]. Although the inclusion of patients within approved or investigational protocols is the best policy, it must be realized that, in selected patients with advanced refractory SADs, the decision to perform ASCT will ultimately rely on a combination of clinical acumen, experienced teams and a good patient–doctor relationship.

Which are the most appropriate mobilization & conditioning regimens?

The source of HSCs was initially the bone marrow (BM), but is now the peripheral blood following mobilization procedures to which multiple BM aspirations may be added, if necessary, to reach the desired number of SCs. In the already mentioned EBMT study of 900 patients, the source of HSCs was peripheral blood in 827 cases [Farge D, Labopin M, Tyndall A *et al.* Manuscript submitted]. The most popular mobilizing regimens generally consist of combinations of CY and G-CSF, and have been reviewed recently [84]. Mobilizing regimens incorporating CY (2–4 g/m^2) have the additional, significant advantage of acting as an important therapeutic procedure *per se*. In our own experience of nine SLE patients, complete remission following mobilization with CY 4 g/m^2 was achieved, enabling two of them to dispense from performing the initially programmed ASCT [85]. The similarity of these results with those obtained with the CY-alone protocol are clear [20,59,60].

A variety of conditioning regimens have been utilized, but it could be demonstrated that high-intensity protocols were followed by a lower probability of disease progression, albeit with a higher risk of TRM [80]. The strategy of performing intense immunosuppression without affecting the whole of the hematopoietic system [86] is most generally accepted, taking into account that biologics such as rituximab have a longer immunosuppressive activity than any chemotherapeutic agent. A combination of both strategies, in which 500 mg rituximab is given before and after the regular 200 mg/kg CY protocol ('sandwich technique'), is currently being utilized at Northwestern University, Chicago [87]. Anti-CD20 immunotherapy for the control of relapse following ASCT in patients with RA was already utilized with success by Moore *et al.* [88], and the strategy of using an additional immunotherapy in this area is attractive. Unfortunately, a devastating complication, progressive multifocal leukoencephalopathy (PML) owing to the activation of the John Cunningham virus, has been reported in a disquieting proportion of patients who have been immunosuppressed with biologic agents (natalizumab and rituximab [89]). The first reports of this complication concerned hematological [90] and rheumatological [91,92] patients. However, a recent review reported 52 patients having developed PML, seven of which received HSCT (three allogeneic and four autologous) for lymphoproliferative diseases [93]. Awareness is obviously needed regarding the potential for PML among rituximab-treated patients. Once again, maximal immunosuppression produces greater benefits, but at the same time, it may be associated with unforeseen iatrogenic complications.

What significant changes in the immune system take place following ASCT? Are we really curing autoimmunity?

No other aspect of the ASCT-based procedures has been the object of so much research, controversy, enthusiasm and scepticism. A prolonged depression of CD4$^+$ CD45RA cells is a general finding [94], and takes place following both ASCT and high-dose immunosuppressive therapy alone [20]. The type of immunomodulation that then follows has been called a 'black box' by Muraro and Douek [21], but, thanks to their own [22] and others' investigations [23,24], it is becoming increasingly clear. HDI reduces the population of autoimmune cells to a condition that I have called minimal residual autoimmune disease [54], although, unlike the oncohematologic diseases, molecular analysis, regardless of how ingeniously investigated [95], is generally substituted by the utilization of surrogate biomarkers [96]. While the cure of oncohematological disease requires the eradication of cancer SCs [97,98], a different view may be entertained for ADs. Two basic mechanisms have been postulated.

The first has been defined as a 're-education' of the faulty immune system, obtained by restoring a diverse antigen-specific repertoire through reactivation of the thymic output ('thymic rebound'), which has also been demonstrated to persist, albeit in a lesser measure, in adults [99]. An intriguing strategy for greater thymic regeneration following congenic SCT for mice with EAE consisted of androgen depletion, which significantly ameliorated the results [100]. In myelin-oligodentrocyte glycoprotein-induced EAE in rats, a protective effect was achieved, not only by allogeneic, but also by syngeneic BM grafts, and, surprisingly, also from diseased rats [101]. In a recent, exhaustive immunologic study of ASCT in seven SLE patients, the Berlin group found evidence for an overwhelming regeneration of the adoptive immune system and of the B-cell lineage, that apparently became tolerant to self-antigens [102]. The recurrence of lupus activity observed in three of these patients was accompanied by the development of antinuclear antibodies with new specifities, a finding that suggested the *de novo* development of SLE [103]. The switch from one to another abnormal immune balance had been defined by Shoenfeld as the kaleidoscope of the autoimmune mosaic [104]. The second mechanism is closely related, and consists of the reconstitution of the regulatory T-cell pool following ASCT. T-regs ($CD4^+$ $CD25^+$) expressing the transcription factor Foxp3 are crucial in preventing autoreactivity and restraining autoimmunity throughout life [105]. Elegant experimental and clinical studies have demonstrated the impact of the T regulatory network on the efforts of inducing post-transplant immune tolerance [22,106,107].

Are these changes sufficient and stable enough to guarantee a rebuilding of the immune system, substantially configured in a way that is less likely to redevelop autoimmunity [22]? In a study of autotransplanted MS patients, the T cells recognizing myelin basic protein were indeed initially depleted by immunoablation, but were then rapidly expanded from the reconstituted T-cell repertoire in 12 months [108]. More recently, an early recovery of CD4 T-cell receptor diversity was found after 'lymphoablative' conditioning and autologous CD34 cell transplantation in systemic sclerosis (SSc) patients, suggesting that the treatment is not completely T-cell ablative (or, more generally, immune SC-ablative), and thus is not ultimately curative [109]. Finally, in a comprehensive recent study analyzing original and pooled data from autotransplanted MS patients, Mondria *et al.* [110] found not only the already known persistence of CSF oligoclonal bands in 88% of the reported cases, but also the persistence of the soluble lymphocyte activator CD27, thus concluding that complete eradication of activated lymphocytes from the CNS had not been established, notwithstanding an intensive immunosuppressive regimen including ATG, CY and total-body irradiation in two fractions of 5 Gy a day at days 1 and 2 [111]. These patients pertained to the rapid secondary progressive form of MS. The importance of long-lived plasmacytes in this context will be briefly discussed in the following section. Our own clinical experience has included late (and very late) relapses, in a way that suggested a recapitulation of the natural history of lupus [54,85]. Therefore, whether pressing the reset button will turn out to be immunologically curative still requires extensive clinical studies with

Table 1. Controversial issues concerning the autologous hematopoietic stem cell transplantation immunological effects in autoimmune diseases.

Believers	Unbelievers
Immune re-education Muraro P *et al.* (2006) [21] Abrahamsson S *et al.* (2008) [22]	**HD CY alone** Brodsky RA, Jones RJ (2008) [60]
Immune resetting Muraro P *et al.* (2009) [17]	**Expansion of original T repertoire** Sun W *et al.* (2004) [108]
Immune regulation Van Wijk F *et al.* (2008) [24]	**Recovery of CD4 T-cell receptors** Storek J *et al.* (2008) [109]
Regeneration and tolerization Alexander T *et al.* (2008) [102]	**Persistence of oligoclonal bands in CSF in MS patients** Mondria T *et al.* (2008) [110]
Reconfiguration Alexander T *et al.* (2009) [103]	–

AD: Autoimmune diseases; ASCT: Autologous hematopoietic stem cell transplantation; HD CY: High-dose cyclophosphamide; MS: Multiple sclerosis.

very long follow-ups. The most significant data both in favor and against complete immune re-education are summarized in Table 1.

What type of benefit, if any, does ASCT confer to severe, progressive, relapsing–refractory ADs?

In a recent, provocative editorial commenting on the utilization of ASCT for SADs, and more specifically, for the rheumatic diseases, Illei has posed the question whether 'the glass is half full or half empty' [112]. We have already given a tentative answer to this question [113], but I shall try to be more specific here.

The effects of ASCT may be conveniently divided into two phases: the early suppression of ongoing immunoinflammatory events, and the later resetting of the autoimmune clock [22], which is closely related to the length and grade of remission. The first effect is clearly due to the immunosuppressive conditioning regimens and is proportional to the dose intensity [80], independently from HSC rescue [114]. No sophisticated dynamics occur here besides the well-known combination of immunosuppression and abrogation of attending inflammation. This first effect is responsible for its dramatic disease-arresting ('nosostatic') properties, which have been observed in practically all actively aggressive SADs and, most demonstratively, in SLE [81,85]. This change occurs in the aggressive phases of disease, where ASCT may well be the most potent salvage therapy available. A clear distinction of the diverse sensitivity to ASCT according to the phases of disease has recently been made by Shevchenko *et al.* [115], who divided the transplant strategies for MS into 'early', 'conventional' and 'salvage-late' procedures. Among the many examples of this early, dramatic therapeutic effect are, besides the cancellation of systemic symptoms, the almost immediate clearance of inflammatory urinary sediments in lupus nephritis [116], the rapid improvement of nailfold capillaroscopy in SSc [117,118] and the early abrogation of gadolinium-enhancing lesions in MS [119,120]. Intermediate changes may include the striking disappearance of diffuse calcinosis in a child with overlap connective disease [121] and the regression of dermal fibrosis in patients with severe scleroderma [83,122].

The impact of ASCT on SADs in the long run has been discussed in several contributions, but the most important study is by far the already mentioned EBMT analysis [Farge D, Labopin M, Tyndall A *et al.* Manuscript submitted]. Independent of the heterogeneity of the clinical material, progression-free survival, which may be considered as the most accurate estimated outcome of a therapeutic procedure, was 43% at 3 years. The difficulty of obtaining a molecular evaluation of remission has already been discussed. Be that as it may, three features emerge: first, that in the overwhelming majority of patients, no authentic immunological cure may be realistically expected; second, that dramatic remissions occur, which may be life-saving and even long term; third, that even in the case of relapse, the utilization of conventional therapies, to which the patients were formerly refractory, is generally possible. The recent EBMT study [Farge D, Labopin M, Tyndall A *et al.* Manuscript submitted] confirmed that the original diagnosis was the most relevant prognostic factor.

Is ASCT the best available treatment for SADs?

There is no doubt that ASCT is a powerful therapeutic procedure for SADs, but can it be regarded as the best treatment available, considering the increasing utilization of new pharmacological, immunological and other remedies? Evidence-based medicine requires randomized prospective (Phase III) clinical trials, and these are being actively pursued for SSc (e.g., the Autologous Stem cell Transplantation International Scleroderma [ASTIS] trial in Europe and the Standard Care versus Celecoxib Outcome [SCOT] trial in North America), MS (Autologous Hemopoietic Stem Cell Transplantation for Multiple Sclerosis [ASTIMS], which is probably the most advanced trial), Crohn's disease (Autologous Stem Cell Transplantation for Crohn's Disease [ASTIC]) and SLE (Hematopoietic Stem Cell Transplantation for Systemic Lupus Erythematosus [ASTIL]).

It is clear that this is the only way to obtain a scientifically correct answer. However, there are two points to consider. First, the pace of medical progress is such that by the time that these laborious trials will have reached statistical significance, new agents may have superseded those utilized in the nontransplant arms. Second, in a sizable proportion of these patients, ASCT may heuristically [123] be integrated with other therapeutic interventions, including high-dose immunoglobulins (HDIG), biologicals and, possibly, new 'intelligent' molecules. An example of the utilization–integration of every treatment available is a patient of ours with severe Sjögren's syndrome who developed chronic inflammatory demyelinating polyradiculoneuropathy (another AD that responds

Table 2. Ongoing randomized controlled trials of autologous hematopoietic stem cell transplantation for autoimmune diseases.

Trial	Disease	Country	URL (trial indentifier)
Nonmyeloablative regimen			
ASSIST	Systemic sclerosis	USA/Brazil	www.clinicltrials.gov (NCT00278525)
ASTIL	Systemic lupus erythematosus	Europe	Pending
ASTIS	Systemic sclerosis	Europe	www.astistrial.com
KISS	Chron disease	USA	www.clinicltrials.gov (NCT00271947)
MIST	Multiple sclerosis	USA/Canada/Brazil	www.clinicltrials.gov (NCT00273364)
Myeloablative regimen			
ASTIMS	Multiple sclerosis	Europe	www.astims.org
SCOT	Systemic sclerosis	USA	www.clinicltrials.gov (NCT00114530)

ASSIST: American Scleroderma Stem Cell versus Immune Suppression Trial; ASTIL: Autologous Stem Cell Transplantation International Lupus; ASTIMS: Autologous Stem Cell Transplantation International Multiple Sclerosis; ASTIS: Autologous Stem Cell Transplantation International Scleroderma; KISS: Crohns Immune Suppression versus Stem Cells; MIST: Multiple Sclerosis International Stem Cell Transplant; SCOT: Scleroderma Cyclophosphamide or Transplantation.

to ASCT [124]) and subsequently also severe aplastic anemia, and was successfully treated with a combination of allo-SCT and HDIG, with final complete negativization of anti-Ro and anti-La antibodies.

Allogeneic transplantation: few facts & many questions

More cogently than with the autologous procedure, animal experiments and results from coincidental disease patients [56] had indicated allo-SCT as a powerful instrument to cure autoimmunity. In an International Workshop held in Bethesda, MD, USA in 2005 [125], it was stated that 'the potential for a one-time delivery of a curative therapy is outstanding'. But will it really be so? Many clinical trials are being pursued worldwide, but I shall confine myself only to published material from our personal experience.

Clinical results

A retrospective EBMT study [126] involved 35 patients having received 38 allogeneic transplants for various ADs, both hematological and nonhematological. The donors were identical siblings for 24 patients, matched unrelated donors (MUD) for three, mismatched related for two and syngeneic for three patients. TRM was 22.1% at 2 years and 30.7% at 5 years, while death owing to progression of disease was 3.2% at 2 years and 8.7% at 5 years. Of the 29 surviving patients, 55% achieved complete clinical and laboratory remission, and 24% achieved a partial remission. Other significant case reports will be mentioned subsequently. Anyway, the consensus is that nonmyeloablative, reduced-intensity conditioning regimens should be utilized [127]. A protocol including high-dose $CD34^+$ cell infusions, partial T-depletion and no post-transplantation immunosuppression has been utilized with success in nonmalignant disorders by Elhasid *et al.* [128]. The intriguing protocol utilized in Ahmedabad (Gujarat, India) [129] has not had many chances to be utilized in other centers.

Immunological aspects

The substitution of an immune system that is behaving badly with a normal, healthy one is the rationale of the allogeneic approach, and its successful achievement is the prerequisite for embarking on a treatment that has been saddled with a 30% mortality rate after 5 years [126]. Although it is predictable that TRM following allo-SCT, if further pursued, will probably become lower, what with an improvement of the learning curve and with optimized conditioning regimens, the only legitimate motivation for performing it is to achieve a cure.

Allo-SCT is traditionally regarded as a 'platform for immunotherapy' [130]. An exhaustive analysis of the mechanisms by which it might cure ADs has been performed by Sykes and Nikolic [13], who have placed the already discussed GVA effect in the foreground. A retrospective study demonstrated, in an analogy to an established pattern in oncohematologic diseases, that there were more relapses of coincidental ADs in patients transplanted for hematologic malignancies with no GVHD than in those who developed it [25]. In a patient transplanted for Evans syndrome, complete remission was achieved only after the development of grade IV GVHD, which did not impede survival [131]. However, this effect could not be detected in the recent EBMT study [126], and much greater clinical material would be necessary to obtain significant evidence.

Efforts have been made, as already attempted in oncohematologic diseases, to separate GVHD from GVA [132]. A potent GVA effect was elegantly demonstrated in rat models of EAE [107]. Clinically, there is a group of patients who had been allotransplanted for SADs in whom donor lymphocyte infusions were necessary to achieve full donor chimerism, which ultimately ensured complete remissions of the SADs [133–136]. However, these results are counterbalanced with others that are in favor of the hypothesis that mixed chimerism might be capable of inducing long-term remissions [137,138].

Further controversial evidence comes from the analysis of relapsed patients. There appear to be two types of relapses. An example of the first type is the report of a failure of allo-SCT to arrest disease activity in a patient with MS having been successfully transplanted owing to coincidental chronic myeloid leukemia [139]. As suggested by the author, the immune response to an abnormal antigen should be expected to continue despite a new, healthy immune system. Even more disquieting are the already mentioned reports of patients with SADs who, having received allo-SCT, subsequently relapsed notwithstanding full donor chimerism. The first and widely acknowledged case was a female patient with RA, who received an HLA-identical transplant because of gold-induced aplastic anemia [30], and the second case concerns another patient with RA and multilple myeloma, in whom the myeloma was cured but the RA relapsed [31]. However, the most demonstrative case of this type of paradoxical relapse is the one of a patient with severe Evans syndrome who was transplanted from his HLA-identical sister, but needed a series of donor lymphocyte infusions in order to achieve full donor chimerism and complete hematologic remission [32]. Unfortunately, this patient relapsed and died with a terminal hemolytic-uremic syndrome 5 years later [33]. The patient was male and had received the bone marrow of his HLA-identical sister. The immunoglobulins (IgG and IgM) eluted from his 100% XX expanded B cells were not the ones eluted from his Coombs-positive cells. It was hypothesized that the autoantibodies might have been secreted by long-lived host plasmacytes surviving in postulated marrow niches [140]. Even allowing for the hypothesis that relapses in donor cells in patients transplanted for leukemia might be less uncommon than they were generally thought to be [141,142], this is still an extremely rare event, having been identified in 14 out of 10,489 transplants in a recent survey [143]. By contrast, three relapses in the much smaller group of autoimmune allotransplanted patients inevitably causes some perplexity [144]. Only further careful investigations will hopefully elucidate this unexpected problem. A synthesis of these controversial results is shown in Table 2.

Syngeneic transplants (a niche event) have been utilized for few patients. Three patients with RA received syngeneic transplants following high-dose immunosuppression. The first was a patient with severe seronegative RA who enjoyed a long-term remission [145]. However a second patient with progressively erosive, rheumatoid factor-positive RA, who was treated with high-dose CY and received an unmanipulated peripheral blood graft (PBSCT) from her identical twin sister, had a poor clinical response associated with serological persistence [146]. An unpublished case is the one of 45-year-old lady with severe seropositive RA who was transplanted in Genoa, Italy, from her identical twin sister on

Table 3. Discrepancies in Allo-stem cell transplantation for autoimmune diseases.

Positive effects	Controversial results
Clear experimental demonstration of a GVA effect Smith-Berdan S *et al.* (2007) [40] Van Wijmeersch B *et al.* (2007) [52]	**Mixed chimerism is beneficial experimentally** Li H *et al.* (1996) [41] Seung E *et al.* (2000) [42] Verda L *et al.* (2008) [44]
Clinical benefit of GVHD in coincidental diseases Hinterberger W *et al.* (2002) [25]	**No benefit from GVHD clinically** Daikeler T *et al.* (2009) [126] Chakrabarti S *et al.* (2001) [137] Burt R *et al.* (2004) [138]
Favorable effects of DLI Marmont A *et al.* (2003) [133] Musso M *et al.* (2004) [134] Hayden PJ *et al.* (2005) [135] Marmont A *et al.* (2006) [136] Loh Y *et al.* (2007) [132]	**Relapses notwithstanding complete donor chimerism** McKendry RJ *et al.* (1996) [30] Tapprich C *et al.* (2003) [31] Marmont A *et al.* (2006) [32]

DLI: Donor lymphocyte infusions; GVA: Graft-versus-autoimmunity; GVHD: Graft-versus-host disease.

29 July 2005. The conditioning regimen consisted of 160 mg/kg CY. Both rheumatoid factor and anticyclic citrulline peptide titers decreased significantly (anticyclic citrulline peptide from 234 to 2 IU/ml), but a clinical relapse occured with fever, polyarthritis and elevation of ESR, which required further treatment. However, in the field of autoimmune hematologic diseases, an already splenectomized patient with chronic autoimmune thrombocytopenic purpura went into complete long-term remission following PBSCT [147].

Conclusion & future perspective

Is there, at the time of this writing, sufficient evidence to answer the question of whether stem cell therapy, in the form of HSCT in its various paradigms, is and will be the best present and future therapy for SADs [54,148]? There has been a tendency to place the blame of autoimmunity on faulty immune systems, thus assimilating ADs to the neoplastic lymphoproliferative diseases [149]. However, most ADs result from a combination of faulty immune systems and antigen (target organ) dysfunctions. The distinction between primary and secondary ADs [150], the first being sustained by primary immune defaults and the latter by a predominant antigenic trigger (that may vanish following its removal [151]), has been considered helpful for the evaluation of SCT interventions. However, the interaction between immune system and target organ antigenicity is extremely tight, as exemplified in the classical dispute between genes and environmental factors [49,152] and in the open question of whether autoimmunity results primarily from faulty immune systems or target organ challenge, which has been researched for a long time in Type 1 diabetes [153–155]. The attaiment of an insulin-free diabetes, even if not permanent, following ASCT is an encouraging and illuminating event [9,153].

As already discussed, three points need to be elucidated. The EBMT prospective, randomized trials will soon enable us to answer the question as to whether ASCT is superior, or not, to the best treatment currently available. However, there are at least two considerations to be made. The first is that the protocols utilized in the nontransplant arms appear to me *a priori* weaker than the conditioning regimen utilized in the transplant arm. However, still more important will be the programming of any single patient, in which it is predictable that both programs will become progressively integrated. A good example is the already mentioned 'sandwich' Chicago protocol [82]. In addition, there is evidence of the increasing value of nonhematopietic (mesenchymal) cellular therapy [62–67,156] and of other new agents that are already at disposal or just around the corner.

A word of caution must be given concerning the potential development not only of PML, as already discussed, but also of therapy-related myelodysplasia and leukemia, which must be closely observed for when utilizing alkylating drugs and others [155,157]. Fortunately, such reports have not appeared in this area, and recourse to ASCT in patients with SADs should not be hindered by the fear of late malignant complications, although careful long-term surveillance is mandatory.

Executive summary

- Two different sets of investigations are at the origin of hematopoietic stem cell transplantation (HSCT) for severe autoimmune diseases. The experimental evidence consists of the disease transfer/cure of animal severe autoimmune diseases, such as as murine lupus by means of HSCT, both allogeneic but also, almost paradoxically, autologous.
- The clinical evidence comes from serendipitous reports of patients who were allotransplanted for coincidental diseases and were finally cured of both conditions. The encouraging results of autologous HSCT (ASCT) in experimental autoimmune diseases were enthusiastically translated into human therapy by clinicians hoping to achieve great results without incurring the rigors associated with the allogeneic procedure.
- Well over 1000 ASCT for severe autoimmune diseases have been performed worldwide so far, with multiple sclerosis and connective tissue diseases in the foreground. Transplant-related mortality and morbidity have decreased to well under 5%. A dramatic disease-arresting effect is a constant benefit, but the whole course of the disease appears to be influenced favorably. Profound changes in the autoimmune circuitry have been demonstrated, but no authentic eradication of disease should be realistically expected. Important multicentric, prospective trials are ongoing to compare ASCT with the best-available nontransplant therapies, but it may be argued that in the end, both approaches will be integrated for single patients, and that new agents will possibly alter the present strategies.
- Allogeneic stem cell transplantation is eliciting great expectations, but the burden of higher mortality and morbidity with graft-versus-host disease must be considered, even when making recourse to reduced conditioning regimens. Paradoxical relapses notwithstanding complete donor chimerism have been reported. Further experience is clearly needed, but the early enthusiasm for an attractive one-shot therapy must be considered with realistic evaluation, at least until new significant breakthroughs will be attained.

Great expectations have been associated with allogeneic SCT [13,125,144], but its position is still uncertain. Ongoing trials will hopefully offer some answers to the questions, or provide hope as to whether the total eradication of a faulty immune system will be sufficient, and whether solid evidence exists concerning a clinically exploitable GVA effect. In addition, the persistence of self-antigens [152] and the real significance of the disturbing relapses reported, in spite of full donor chimerism [134], will have to be scrutinized and hopefully clarified. In the meantime, our own strategy has been to perform allo-SCT only in patients with ADs that have developed a comorbid condition requiring it. A watchful attitude appears to be the most judicious policy for now [53–55].

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