Immune Mechanisms Underlying the Beneficial Effects of Autologous Hematopoietic Stem Cell Transplantation in Multiple Sclerosis

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Published online: 9 September 2011
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Abstract A recent phase I/II clinical trial drew serious attention to the therapeutic potential of autologous hematopoietic stem cell transplantation (AHSCT) in multiple sclerosis. However, questions were raised as to whether these beneficial effects should be attributed to the newly reconstituted immune system per se, or to the lymphoablative conditioning regimen-induced immunosuppression, given that T-cell depleting combinational drug therapies were used in the study. We discuss here the possibility that both AHSCT and T-cell depleting therapies may re-program alternatively the immune system, and why transplantation of CD34⁺ hematopoietic stem cells may offer AHSCT a possible advantage regarding long-term remission.

Keywords Autologous hematopoietic stem cell transplantation · Lymphoablation · Multiple sclerosis · T cells

Introduction

Autologous hematopoietic stem cell transplantation (AHSCT) recently emerged as a promising alternative approach to treat patients suffering from multiple sclerosis (MS) refractory to classical immunomodulatory and immunosuppressive based-therapies. Its application to treat MS stems from the premise that a dysregulated, autoreactive immune system could be

eradicated and replaced by a new, tolerant one. To date, it has been applied not only for the treatment of MS, but also for other autoimmune diseases including type 1 diabetes, systemic lupus erythematosus (SLE) and systemic sclerosis [1–4]. With respect to MS, patients suffering from inflammation-associated relapsing-remitting MS and treated relatively early in the course of the disease respond better to AHSCT compared to those with long disease duration or those diagnosed with progressive MS. Although potentially hazardous, AHSCT safety has dramatically improved through the years, as the use of lymphoablative regimens has become increasingly prevalent at the expense of myeloablative strategies. Compared to myeloablation, lymphoablation presumably eliminates more selectively lymphocytes and lymphoid progenitors, and therefore minimizes irreversible toxicity to the hematopoietic cellular environment of the bone marrow. As such, it appears to allow for faster recovery and decreases the probability of hematologic and innate immunity complications [5–7].

Although the efficiency of AHSCT in MS is increasingly being validated [8], the immune mechanisms that account for its therapeutic effects are still largely unknown (see Table 1). Two basic hypotheses have been proposed to explain the beneficial effects of AHSCT. First, AHSCT may “reset” the immune system, yielding a novel, tolerant immune system. Tolerance could be achieved through 3 nonmutually exclusive mechanisms [9]: 1) elimination of the pre-AHSCT pathogenic T-cell lineage(s) [10]; 2) shifting of the pre-AHSCT autoreactive T-cell clones toward a tolerant phenotype [11], and 3) restoration of autoimmune inhibitory immunoregulatory effectors [12]. Yet, an alternative interpretation of the AHSCT data suggests that its clinical benefits might be attributed to the ability of the conditioning regimen to severely deplete T-cell populations for an extended time, thus inducing a state of long-lasting immunosuppression (see Table 2). This could be particularly true for AHSCT therapies that use an antibody-

Electronic supplementary material The online version of this article (doi:10.1007/s13311-011-0062-0) contains supplementary material, which is available to authorized users.

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Table 1 Characteristics of the post-AHSCT immune system compared to pre-AHSCT status

Reference	Disease Treated	Conditioning	Follow-up (years)	Primary Findings
Muraro et al. [16] (2005)	MS	Myeloablative	2	↑ Proportion naive CD4 ⁺ T cells of thymic origin ↓ Proportion memory CD4 ⁺ T cells ↑ T-cell receptor repertoire and clonal diversity
Sun et al. [19] (2004)	MS	Myeloablative	1	No significant differences in phenotypic properties of T cells No significant differences in clonal diversity of T cells
Alexander et al. [18] (2009)	SLE	Lymphoablative	4 (median)	↑ Proportion naive CD4 ⁺ T cells of thymic origin ↑ T-cell receptor repertoire diversity B-cell populations normalization Elimination of autoantibodies
Zhang et al. [9] (2009)	SLE	Lymphoablative	2 (median)	↑ CD8 ⁺ FoxP3 ⁺ TGF-β-producing Treg cells ↑ CD4 ⁺ CD25 ^{high} FoxP3 ⁺ Treg cells

AHSCT=autologous hematopoietic stem cell transplantation; MS=multiple sclerosis; SLE=systemic lupus erythematosus; TGF=Transforming growth factor

mediated lymphoablative conditioning regimen. The validity of either hypothesis is currently a matter of debate in the field of MS and autoimmune diseases [13].

Based on human clinical studies, the purpose of the present discussion is twofold. First, we argue that a third hypothesis (i.e., namely that both therapies alternatively reprogram the immune system) also needs to be considered. Second, we discuss how transplantation of CD34⁺ hematopoietic stem cells (HSC) may be advantageous and critical to the establishment of a nonpathological immune system. It needs to be acknowledged that there are relatively scarce studies being conducted to investigate how the immune system

specifically changes following AHSCT, largely because the application of AHSCT to treat MS is still in its beginnings. However, because MS is an autoimmune disease, it presents characteristics that often closely parallel those of other autoimmune diseases. Therefore, to substantiate the points made in this review, data generated in the AHSCT clinical trials in the context of other autoimmune diseases will be considered when appropriate. In addition, reviews have been written that focus more specifically on the clinical efficiency aspect of AHSCT in MS (for more detail, see Mancardi and Saccardi's [14] article from 2008 and Capello and colleagues' [15] from 2009).

Table 2 Characteristics of the immune system following alemtuzumab pulse

Reference	Follow-up (years)	Primary Findings
Cox et al. [23] (2005)	≤0.5	Lymphopenia ↑ Memory T-cell proportion within CD4 ⁺ T pool ↑ CD4 ⁺ CD25 ^{high} T cells proportion within CD4 ⁺ T-cell pool Proliferation and level restoration of B cell
	1	Normalization of the relative representation of T-cell subtypes Persistent depletion in absolute number of CD4 ⁺ T cells
Jones et al. [33] (2009)	0.25–0.75	Lymphopenia ↑ T-cell proportion responding to self-antigens ↑ T-cell proliferation in response to self-antigens ↑ T-cell passive and Fas-mediated apoptosis
Thompson et al. [36] (2010)	0.25–1	Lymphopenia Restoration and substantial expansion of B cells ↑ Serum B-cell activating factor (BAFF)

AHSCT Mechanism of Action: Immune Reset Hypothesis

In 2005, Muraro et al. [16] provided compelling evidence that AHSCT following myeloablation can reconfigure the immune system. In the peripheral CD4⁺ T-cell population, the proportion of naive CD45RA⁺/CD45RO⁻/CD27⁺ T cells doubled 2 years after AHSCT relative to pretreatment assessment, whereas that of the CD45RA⁻/CD45RO⁺/CD27⁺ central-memory T-cell subgroup decreased significantly by 38%. CD31 expression on CD4⁺/CD45RO⁻ T cells in combination with preliminary data demonstrating an increase in T-cell receptor excision circles indicated a thymic ontogeny as opposed to an expansion in the peripheral T-cell compartment. Considering that thymic output diminishes as an individual ages [17], these data suggest a recovery of thymic functions in mature patients, leading to a rejuvenation of the CD4⁺ T-cell population.

Spectratyping analysis of the hypervariable complementary-determining region 3 (CDR3) encoded within the β chain of T cell receptor (TCR) revealed a widened repertoire diversity within CD4⁺ T cells at the two-year follow-up compared to pretreatment. Sequencing analysis of the complementary determining region 3 at the single T-cell clone level estimated that for a majority of clones, 90% of the complementary determining region 3 analyzed were derived post-transplantation. CD8⁺ T cells also displayed a wider TCR repertoire following AHSCT. Therefore, it appears that through exhaustive clonal renewal, vast TCR rearrangements occur after AHSCT, leading to an augmentation of the T-cell repertoire and clonal diversity.

Similar observations on naive and central memory T cells, recent thymic emigrants, and TCR repertoire following AHSCT with SLE patients have also been reported by Alexander et al. [18]. Thus, not only do they corroborate the conclusions of Muraro et al. [16], but they also suggest that AHSCT has properties that are disease independent. Note, however, that an early AHSCT investigation did not report the same pre- vs post-AHSCT immune differences. Indeed, in 2004, Sun et al. [19] concluded that the structural and functional phenotypes of reconstituted T cells post-AHSCT were similar to those of pre-transplantation T cells. However, these data were obtained at a 1-year follow-up, whereas the effects in the Muraro et al. [16] and Alexander et al. [18] studies were observed for a span of 2 years following transplantation. Considering that CD34⁺ hematopoietic stem cell (HSC) grafts are not instantaneous, and that important expansion of the peripheral T-cell compartment takes place during the first 6 months following AHSCT [16], a 1-year follow-up period may have been insufficient for assessing an immune system reconfiguration that has yet to reach its equilibrium. Finally, no data regarding the functional phenotype of a rejuvenated immune system were reported

by Muraro et al. [16]. Such data would be valuable, as defects in immunomodulatory mechanisms are potential etiological components of MS.

In 2009, Zhang et al. [9] reported evidence that AHSCT introduces or reintroduces immunoregulatory mechanisms. Although this study was performed with SLE patients, parallels with MS may possibly be drawn, given that both are autoimmune diseases. Patients underwent lymphoablative AHSCT and immune assays were performed on average 33 months after transplantation. Interestingly, immune phenotypes of transplanted patients were compared to those of healthy individuals and those of SLE patients treated with conventional therapies.

Zhang et al. [9] first compared the pre- and post-AHSCT responsiveness of peripheral blood mononuclear cells (PBMC) to SLE-related nucleosomal histone epitopes *in vitro*. In patients with lupus, such stimulation induces a strong interferon (IFN)- γ synthesis in CD4⁺ T cells. After AHSCT, however, IFN- γ production by CD4⁺ T cells is completely abrogated. It is noteworthy that CD4⁺ T cells of patients in clinical remission and treated with conventional approaches still exhibit a propensity for IFN- γ production under similar conditions [20]. In contrast, a significant upregulation of interleukin (IL)-13 synthesis occurred with post-AHSCT CD4⁺ T cells. Interestingly, CD4⁺ T cells from healthy individuals did not display this production of IL-13, raising the possibility that clinical remission may not necessarily imply that typical physiological parameters are restored. In total, these data suggest that AHSCT in the context of SLE shifts the adaptive immune response to relevant antigens from a Th1/inflammatory toward a Th2/anti-inflammatory phenotype.

Extrinsic factors, as opposed to intrinsic ones (e.g. CD4⁺ T-cell sensitivity/responsiveness to the immunogens) mediated these phenotypic changes of CD4⁺ behavior. Indeed, removing CD8⁺ T cells from post-AHSCT PBMC restored the production of IFN- γ in CD4⁺ T cells. In contrast, when post-AHSCT PBMC were stimulated with nucleosomal epitopes in co-culture with CD8⁺ T cells preserved from the pre-AHSCT stage, IFN- γ production remained elevated. Hence, AHSCT appears to reconfigure CD8⁺ T cells toward an immunosuppressive phenotype.

The ability of CD8⁺ T cells to regulate CD4⁺ T-cell proliferation *in vitro* also changed remarkably following AHSCT. Indeed, in the presence of irradiated allogenic antigen-presenting cells, both pre- and post-AHSCT CD4⁺ T cells proliferated roughly 30% more when co-cultured with pre-AHSCT CD8⁺ T cells compared to control conditions. However, co-culture with post-AHSCT CD8⁺ cells inhibited the proliferation of either population of CD4⁺ T cells by 25%. Interestingly, CD8⁺ T cells from patients treated with conventional therapy neither potentiated nor suppressed CD4⁺ proliferation. Finally, whereas proliferation potentiation

by pre-AHSCT CD8⁺ T cells was mediated through a cell contact dependent mechanism, transforming growth factor (TGF)- β and IL-10 produced by post-AHSCT CD8⁺ T cells suppressed CD4⁺ T-cell proliferation.

The elevated levels of FoxP3 detected in CD8⁺ T cells following AHSCT, along with the ability of post-AHSCT CD8⁺ T cells to produce TGF- β and suppress CD4⁺ T-cell proliferation and pro-inflammatory activity suggest that a CD8^{TGF- β} Treg subset exists within the CD8⁺ T-cell collective population. Therefore, these CD8^{TGF- β} Treg cells through immunomodulatory mechanisms could be critical to clinical remission in SLE following AHSCT. Whether they are relevant to the remission that follows AHSCT in MS is still unknown, but the possibility is interesting in light of a recent report implicating CD8⁺/CD25⁺/FoxP3⁺ T cells in MS [21].

AHSCT Mechanism of Action: The Immunosuppression Hypothesis

The observations that immune changes accompany clinical remission following AHSCT are correlative. In addition, evidence corroborates the significant and long-lasting immunosuppressive properties of alemtuzumab or rabbit anti-thymocyte globulin (rATG) that have been predominantly used to achieve lymphoablation in AHSCT. Hence, alemtuzumab or rATG may dampen the immune system activity, thus inhibiting its capacity to mount autoreactive responses. Because the alemtuzumab-based therapies are efficient with MS, it adds further support to this hypothesis [22]. Note that because the clinical efficacy of rATG has yet to be demonstrated for MS, this discussion will limit itself to alemtuzumab.

Alemtuzumab, also known as Campath-1H, targets CD52, a glycoprotein present at the surface of most immune cells, including T-lymphocytes [23–25]. One early report suggested that it is present on more than 95% of thymocytes [26]. Importantly, however, its expression on CD34⁺ HSCs is low [27]. Binding of alemtuzumab to CD52 leads to cytotoxicity through different mechanisms, including antibody-mediated cytolysis [28], complement-induced membrane attack complex [29], and apoptosis [30, 31]. The cytolytic effects of alemtuzumab lead to prolonged lymphopenia and a complete reconstitution of T-lymphocyte populations may take as long as 5 years [32].

Characterization of the immune profile in the year following alemtuzumab therapy in MS patients indicated that reconstitution of the different populations of lymphocytes does not evolve at the same rate, and that overall reconstitution proceeds in 2 phases [23]. The early phase occurs during the first 6 months. Initially, trace amounts of CD4⁺ and CD8⁺ cells are detected in the circulation 1 month after the

treatment. As T cells start to recover, and until month 3, the proportion of CD4⁺/CD45RO⁺ memory T cells significantly increases relative to CD4⁺/CD45RA⁺ naive T cells. CD4⁺/CD45⁺/CD25^{high} Tregs largely contribute to this effect; the representation of these cells at 3 and 6 months after alemtuzumab doubled and tripled, respectively, compared to pretreatment levels. In addition, the CD8⁺ T-cell population recovers faster than CD4⁺ T cells in the early phase. Finally, during the late phase T cells ratios among the different T-cell populations re-equilibrated toward pretreatment levels. Note, however, that absolute cell counts are still decreased 1 year after treatment, with the total CD4⁺ and CD8⁺ T-cell populations reaching only 32% and 55% of the pretreatment levels, respectively.

Changes in T-cell properties are also seen at the individual cell level after alemtuzumab administration [33]. *In vitro* T-cell proliferative potential is substantially increased 3 months after therapy compared to pretreatment. This occurs both in response to either unstimulated or myelin basic protein exposure conditions. Furthermore, T cells are also more susceptible to passive and Fas-mediated apoptosis.

Recent evidence suggests that a subset of B cells has an active role in the etiology of MS and may potentiate activity of MS-promoting T cells [34]. Reports that administration of antibody Rituximab, which targets CD20 on B cells, is effective at treating MS corroborate this hypothesis [34, 35]. Interestingly, alemtuzumab also substantially affects B-cell homeostasis, and the reconstitution of these cells contrasts dramatically with that of the T cells [36]. Indeed, by month 3 after an alemtuzumab pulse, B cells have not only re-established their pretreatment levels, but continue to proliferate massively and reach 165% of their original pretreatment levels at the 1-year follow-up. This reconstitution is essentially mediated by the generation of immature B cells, as opposed to proliferation of memory B cells.

AHSCT Mechanism of Action: Reconciling Hypothesis

Nonmyeloablative conditioning regimens followed by AHSCT, alemtuzumab-based therapies and possibly rATG-based therapies all have the potential to significantly improve the prognosis of MS patients. As such, it can be expected that clinical experiments with these therapies in the near future will become less aimed at demonstrating their clinical potential per se, and more focused on 1) the investigation of their therapeutic mechanisms, 2) which category of patients benefits the most from them, and 3) how to minimize side effects. Concerning the molecular and cellular mechanisms involved in the benefit resulting from these therapies, whether the theoretical gap existing between nonmyeloablation followed by AHSCT on the one hand, and the alemtuzumab-based therapies on the

other, will remain unclear. Indeed, as both therapies involve lymphoablation, substantial overlap in their physiological effects may exist. The therapeutic effects provided by each approach may arise from a common mechanism, and therefore reconciling hypotheses should be considered. One such hypothesis is that in response to the lymphoablative nature of either therapies, the reconstitution and adaptation of the immune system to the lymphoablative drugs re-programs this system *de facto*, which then becomes either incapable of mounting MS autoimmune attacks of clinical importance, or self-tolerant.

The extent to which AHSCT leads to an immune reset or reconfiguration is not clear. No consensual operational definition of what “immune reset” exactly represents has been proposed. Hence, the lack of such definition can make the use of the term “reset” misleading, as it entails a return to a default state of immune parameters. In reality, this may not necessarily be the case, and demonstrating that the immune system of an MS patient returns to a pre-pathological state is technically challenging. Furthermore, and as previously mentioned, there are differences in the phenotype of a post-AHSCT immune system and that of a healthy individual [9]. One possible explanation for this is that the ontology of the post-AHSCT immune system is influenced by internal and external factors that are likely to be markedly different from those typically operating in early life. Indeed, the physiological parameters of an MS patient (e.g., immune cell ratios, metabolism, and so forth) are different from those of the fetal and infancy stages, and the exposure to environmental factors (e.g., pathogenic agents) that can impact immune development are also different [37]. Of note, persisting T cells also have been detected in patients following either myeloablative or nonmyeloablative conditioning regimen, and these may also influence the development of the reconstituting immune system [38, 39]. Thus, it is not clear at this point whether the immune system is actually “reset” in the true sense of the term. However, the characteristics that the immune system acquires following AHSCT certainly suggest that it has become alternatively programmed.

On the other hand, alemtuzumab does have important effects as previously mentioned on the ratios of different T-cell populations [23]. Thus, the benefits alemtuzumab provides to MS patients may be a consequence of these ratio shifts and not necessarily because T-cell subpopulations to which MS effectors belong are significantly decreased in absolute numbers. Indeed, it is still unknown whether alemtuzumab is effective because the pool of autoreactive T cells is greatly diminished, or because autoreactive mechanisms are altered by immunoregulatory mechanisms, for example provided by CD4⁺/CD25⁺ Tregs which recover relatively fast in number. Note also that a combination of both hypotheses is possible. In 2009, Jones

and Coles [40] similarly proposed that alterations in the overall immune status and not lymphopenia per se were probably critical for the beneficial effects of alemtuzumab in MS.

The immune system of patients in either therapy likely presents similar characteristics in the aftermath of the lymphoablative conditioning regimen or the alemtuzumab pulse cycle. However, divergences emerge with the transplantation of a graft of HSC, which is free of T-cell clones [41]. For instance, cell numbers appeared to recover more rapidly in the Muraro et al. [16] study in 2005 than in the Cole et al. [22] study in 2008. There also appears to be a higher proportion of patients achieving progression-free survival with AHSCT at a 3-year follow-up, although these are still preliminary results [8, 22]. Yet, this lays the ground for a fundamental question: How does transplantation of CD34⁺ HSCs following a lymphoablative conditioning regimen alter the reconstitution and reprogramming of the immune system?

It is plausible that alemtuzumab modulates lymphoid progenitor niches within the thymus by eliminating lymphoid progenitors from existing niches or by creating novel niches to meet the demands for important proliferation to ensure a prompt return for T-cell homeostasis. Note that these possibilities are not mutually exclusive. These niches will replenish in time through the proliferative activity of residual lymphoid progenitors (blood CD34⁺ HSCs), and possibly through dedifferentiation of B cells to uncommitted lymphoid progenitors. The contribution of B cells might be relevant in a context of T-cell lymphopenia, considering the major burst of proliferative activity that they exhibit following depletion and their capacity to dedifferentiate into T-cell progenitors [42]. Among those cellular types, it can be argued that CD34⁺ HSCs have the highest potential for the reconstitution of a homeostatic, nonautoreactive immune system. Indeed, alemtuzumab-surviving lymphoid progenitors may still possess the capacity to generate MS promoting T-lymphocytes, whereas the ontological and cellular history of B cells could have set epigenetic barriers that incapacitate them from generating a homeostatic immune system. Thus, without transplantation, the competitive advantage possessed by endogenous CD34⁺ HSCs over the other cellular populations for vacant lymphoid progenitor niches is relatively small as compared to that obtained after transplantation. This may be a key component of the mechanism responsible for the different, although still preliminary, clinical outcomes between AHSCT and alemtuzumab-pulse therapies.

Conclusion

Despite technical challenges associated with comparing a pre- vs post-AHSCT immune system, the data gathered thus

far from varied clinical trials yield converging evidence that AHSCT fundamentally modifies the immune system in ways that are hardly possible with lymphoablation *per se*. This conclusion, along with the increasing expertise that is being developed around the world with AHSCT therapies allows for a cautious optimism for the use of AHSCT in the treatment of MS.

Acknowledgments The Canadian Institutes in Health Research (CIHR), Neuroscience Canada (Brain Repair Program), and the Multiple Sclerosis Society of Canada support this research.

Full conflict of interest disclosure is available in the [Electronic Supplementary Material](#) for this article.

Disclosure One author (S.R.) holds a Canadian Research Chair in Neuroimmunology.

Competing Interest The authors declare no competing interests.

Appendix

Box 1: Improving AHSCT to treat MS

Although lymphoablative conditioning regimens are safer than myeloablative ones, they still may pose significant health issues. For instance, development of secondary autoimmune diseases can occur following lymphoablation [43], and few cases of autoimmune thrombocytopenia occurred in the study by Burt et al. [8]. To address this issue and decrease the risks of developing secondary autoimmune diseases, rabbit anti-thymocyte globulin (rATG) has now largely replaced alemtuzumab to achieve lymphoablation [8, 44], although this does not completely eliminate the risk to develop secondary autoimmune diseases. Note that rATG administration also appears to significantly alter and/or reprogram the immune system [45].

Finally, as more and more data regarding the different immunological profiles of pre- vs post-autologous hematopoietic stem cell transplantation (AHSCT) therapy are gathered, it is conceivable that they will eventually be used to predict probabilities of remission and anticipate relapses and/or complications. Hence, these data will allow for more efficient treatment management, which should further improve the health condition of multiple sclerosis (MS) patients.

Box 2: Transplantation of Bone Marrow Mesenchymal Stem Cells

A different transplantation strategy for the treatment of multiple sclerosis (MS) consists of the infusion of autologous mesenchymal stem cells, either intravenously or intrathecally, and/or intracisternally. A primary aim of this strategy is to promote the activity of neuronal and myelin

repair mechanisms to restore central nervous system functions, although this procedure also has non-negligible immune-modulatory effects. However, because no myeloablation or lymphoablation is performed with this type of therapy, it does not allow for the elimination of the pathological immune system.

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