

Review

Immune ablation and stem-cell therapy in autoimmune disease Immunological reconstitution after high-dose immunosuppression and haematopoietic stem-cell transplantation

Jacob M van Laar

Leiden University Medical Center, Leiden, The Netherlands

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Abstract

Studies on immunological reconstitution after immune ablation and stem-cell therapy may yield important clues to our understanding of the pathogenesis of human autoimmune disease, due to the profound effects of function and organization of the immune system. Such studies are also indispensable when linking clinical sequelae such as opportunistic infections to the state of immune deficiency that ensues after the treatment. Much has been learnt on these issues from comparable studies in haemato-oncological diseases, although it remains to be proven that the data obtained from these studies can be extrapolated to rheumatological autoimmune diseases. Preliminary results from pilot studies in various rheumatological conditions not only pointed to clinical efficacy of the new treatment modality but also unveiled marked effects on T-cell receptor repertoires of circulating T lymphocytes, on titres of autoantibodies and T- and B-cell subsets.

Keywords: immune ablation, reconstitution, stem-cell transplantation

Introduction

The emergence of high-dose immunosuppressive therapy (HDIT), followed by autologous haematopoietic stem-cell transplantation (SCT) as a new treatment modality for selected patients with systemic rheumatic diseases is based on a pivotal role of the immune system in the course of such diseases [1*]. The aim is to maximally suppress or ablate the immune system and then rescue the patient from prolonged cytopenias or haematopoietic failure by infusing haematopoietic progenitor cells (CD34⁺ cells). Studies in experimental models of autoimmune disease in particular have lent support to the concept that autoreactive T lymphocytes can be eliminated by (myelo)lymphoablative chemo(radio)therapy, and that the subsequent transplantation of pluripotent haematopoietic stem cells gives rise to the emergence of

naïve T lymphocytes that are tolerant to self-antigens (reviewed in this issue by van Bekkum [2]).

In humans, the pathogenesis of systemic rheumatic diseases is less well understood due to uncertainties regarding the nature of autoantigens, pathogenic roles of specific T and B lymphocytes, and complexity of cell–cell interactions. Study of the reconstituting immune system in relation to the disease course after HDIT and SCT may, however, yield important insights into the pathogenesis of these diseases because of the temporary profound effects of myeloablative or lymphoablative therapy on the immune system. This holds true for autologous SCT in particular, because it entails neither histoincompatibility (with its attendant risk for graft-versus-host disease) nor the use of immunosuppressive drugs to control such histoincompatibility.

Table 1

Determinants of immune reconstitution after HDIT and SCT
Age-dependent thymopoiesis in children versus adults [5**,6*,7,9*,10*,11]
Source of hematopoietic stem cells: allogeneic versus autologous [8*]; bone marrow versus peripheral blood [8*,13,41*,42]
Mobilization protocol: G-CSF alone versus chemotherapy and G-CSF [42]
Conditioning regimen: myeloablative versus intensive immunosuppressive [5*]
Manipulation of graft (<i>ex vivo</i> T-cell depletion or CD34 enrichment) versus <i>in vivo</i> T-cell depletion versus nonmanipulation of graft [5**,16**,17*,18*,19,20**]
Pre- and post-transplant treatment? [43]
Underlying rheumatic disease?

Clinical considerations also stress the need to incorporate monitoring of immune reconstitution in any treatment protocol. It is well appreciated that the risks associated with HDIT and SCT in terms of morbidity and mortality are determined by numerical recovery of bone marrow cellular elements on the one hand, and functional recovery of cellular interactions on the other. These surrogate parameters of immunological reconstitution in turn depend on the treatment protocol employed. Current trials involve regimens that range from HDIT (eg cyclophosphamide) alone to (myelo)lymphoablative conditioning, in combination with SCT and vigorous T-cell depletion [3*]. It can be anticipated that the more intensive the treatment is, the higher the risks, because of a temporary lack of functional T lymphocytes after treatment. Regeneration of adequate T-cell numbers and repertoire diversity is a key element in the recovery of immune competence. In the absence of such recovery, susceptibility to opportunistic infections is increased.

The present review focuses on reconstitution of the various components of the immune system after HDIT and SCT, with emphasis on the determinants of immune reconstitution (Table 1). Special attention is paid to reconstitution of CD4⁺ T lymphocytes, which are considered to be key players in many autoimmune diseases [4].

To date only scarce data on immune reconstitution exist in patients with rheumatic disease, most of whom have been treated with (myelo)lymphoablative chemo(radio)therapy and autologous SCT obtained by mobilization of peripheral blood stem cells. In contrast, many data have been gathered in patients with haematological or oncological disease treated with HDIT alone or HDIT followed by either autologous or allogeneic SCT. These studies involved different diseases and heterogeneous treatment protocols with regard to source of stem cells (blood versus bone marrow), and mobilization and conditioning

regimens. Thus, the data generated in these studies should be interpreted with the understanding that the overall effect of a treatment protocol on immune reconstitution is the sum of various interventions. However, some basic principles seem to govern reconstitution of the immune system after HDIT (with or without SCT), most notably pertaining to T and B lymphocytes [5**].

T- and B-lymphocyte reconstitution

Theoretically, the T-lymphocyte population after HDIT and SCT can be regenerated with T lymphocytes from four sources: from expansion of T cells that are reinfused along with the stem cells; from transfused or residual stem cells through a process of thymic education, comparable to what happens in early childhood; from stem cells through an extrathymic pathway; and from residual memory T cells that survive the conditioning regimen.

Obviously, in case of HDIT alone, reconstituting T lymphocytes can only be derived from either endogenous stem cells or mature T lymphocytes that have survived the treatment. Age (which is associated with diminished thymopoiesis) and the extent of T-cell depletion (by *in vivo* or *ex vivo* manipulation of the graft or by [myelo]lymphoablation) are the major determinants of the relative contributions of these sources to the recovery of T-lymphocyte population after HDIT and SCT [5**]. This is reflected mainly in the CD4⁺ lymphocyte compartment, as assessed by immunophenotyping of circulating T cells. Naïve CD4⁺ T lymphocytes bear the CD45RA isoform, whereas memory CD4⁺ T lymphocytes express the CD45RO isoform. The level of naïve (CD45RA⁺) CD4⁺ cells drops rapidly after HDIT (whether followed by SCT or not) and recovers slowly, whereas levels of memory (CD45RO⁺) CD4⁺ and CD8⁺ cells recover more rapidly, and sometimes even 'overshoot' [6*,7,8*]. As a result, a prolonged T-cell subset imbalance with inverted CD4:CD8 ratios is found that can last for longer than a year, depending on the protocol.

These differential effects are explained by differences in maturation pathways for T-cell subsets. In principle, reconstitution of the naïve T-cell compartment is achieved by thymus-dependent and thymus-independent pathways. The development of naïve CD4⁺ T lymphocytes from stem cells has been shown to be mainly thymus dependent. The observation of an inverse correlation between the size of the thymus and the level of the CD4⁺ cells in the peripheral blood after HDIT (without SCT) supports the notion that CD4⁺ development depends on residual thymus function [9*]. Additional evidence was recently obtained by T-cell receptor (TCR) recombination excision circle analysis [10*], which showed that thymic output of immature naïve CD4⁺ T lymphocytes decreases rapidly early in life, and stabilizes at a low level after adolescence and until old age. In contrast, levels of circulating memory CD4⁺ T lymphocytes

phocytes and of CD8⁺ T lymphocytes are not severely affected by HDIT and SCT. This is because thymus-independent maturation pathways dictate the regeneration of CD8⁺ T lymphocytes comprising both extrathymic lymphopoiesis from haematopoietic precursors and peripheral expansion of mature CD8⁺ T cells, especially the CD8⁺CD28⁻ subset [6*].

Cotransfused or residual memory CD4⁺ T cells also expand during the first months after transplantation [11,12*]. The peripheral expansion of mature T lymphocytes observed after HDIT (with or without SCT) has been attributed in part to the encounters with viruses, and is reflected by the elevated expression of activation markers CD25, CD38 and most notably human leucocyte antigen-DR [13,14]. The wave of activated memory CD4⁺ T lymphocytes subsequently subsides due to increased susceptibility to apoptosis [15*]. In the autologous transplant setting it is difficult to determine the precise origin (cotransfused or residual) of this early expanded T-cell pool. Data from studies in recipients of T-cell-depleted allogeneic bone marrow transplant (BMT) [11,12*,16**] indicate that the early post-transplant T-cell compartment has a mixed origin, with T cells derived from both transferred donor T cells and surviving host T cells. In contrast, in patients who underwent unmanipulated BMT, only few recipient T-cell clones were detected, and most were of donor origin. It is likely that similar competing mechanisms apply in the autologous setting. In keeping with this is the observation that recovery of total numbers of circulating CD4⁺ T lymphocytes after HDIT and SCT is related to the number of T lymphocytes present in the graft. T-cell depletion or CD34⁺ stem-cell enrichment of a graft leads to delayed CD4 lymphocyte recovery, and CD4 lymphocyte recovery occurs more rapidly after autologous peripheral blood SCT than after autologous BMT because higher numbers of lymphocytes in the former graft [13,16**,17*].

Whatever the origin of these early memory T cells, the initial expansion of a limited number of mature T-cell clones results in a skewed oligoclonal repertoire, whereas subsequent diversification of TCR repertoires after T-cell-depleted SCT is dependent on the capacity of the thymus to generate naïve thymic emigrants [18*,19]. Molecular analysis of TCR repertoires by CDR3 size spectratyping has revealed differences in T-cell populations isolated before and after transplantation with CD34⁺-enriched autologous peripheral blood stem cells, possibly in response to antigenic stimuli or to a stochastic process [20**].

Analogous to repopulating T lymphocytes, post-transplant B lymphocytes are derived from either cotransfused or residual B cells, or from transplanted or residual stem cells, depending on the treatment regimen employed. The documented transfer of humoral immunity in allogeneic transplantation suggests that differentiated antigen-

selected B cells in the graft can be a significant contributor to post-transplant B cells, whereas residual B cells have also been demonstrated to contribute [5**]. Finally, immunophenotyping data lend support to a role of stem cells as a source of post-transplant circulating B cells. After an initial drop in circulating CD19⁺ and CD20⁺ mature B cells, B cells emerge with an undifferentiated phenotype. In the case of CD34⁺-selected SCT, an overrepresentation of CD5⁺CD19⁺ B cells can be detected during the early post-transplant period, but the significance of this is not well understood [20**]. Although the regeneration of B cells after transplantation has been interpreted as a recapitulation of foetal ontogeny, this has been disputed on the basis of structural differences in B-cell receptor repertoires [21].

Functional effects of immune ablation and stem cell therapy

HDIT and SCT not only affects numbers and repertoires of T and B lymphocytes, but induces functional changes as well. Defective T-cell proliferative responses to mitogens, and TCR-crosslinking antibodies may partly be caused by high numbers of monocytes that are present in growth factor-mobilized peripheral blood-derived SCT, or by type 2-associated cytokines [22–24]. Defective *in vitro* production of IL-2, IL-10 and IFN- γ , but not of IL-4, by peripheral blood mononuclear cells during the first 6 months after HDIT and SCT has also been demonstrated, although the data on IL-10 are conflicting [25,26]. An early expansion of a memory CD4⁺CD7⁻ T-cell subset with preferential IL-4 production has been detected after HDIT and SCT, but not after HDIT alone [27]. The *in vivo* degree of T-cell incompetence after HDIT and SCT is difficult to gauge exactly *in vitro*, however. Deficiencies in humoral responses after HDIT and SCT are attributed both to decreased T-cell help and intrinsic B-cell defects.

Immunological monitoring in patients with systemic rheumatic disease

Studies on immune reconstitution after HDIT (with or without SCT) in patients suffering from severe rheumatological conditions have only recently started to emerge [28]. These conditions predominantly include rheumatoid arthritis (RA), systemic lupus erythematosus, systemic sclerosis and juvenile chronic arthritis. Different protocols are being employed to treat these conditions, varying from HDIT alone to (myelo)lymphoablation followed by autologous SCT and *in vivo* T-cell depletion with antithymocyte globulin or Campath (LeukoSite Inc, Cambridge, MA, USA) monoclonal antibody or *ex vivo* graft manipulation [3*].

The aim of graft manipulation is to minimize the risk of re-infusing autoreactive T lymphocytes. To this end, the graft is purged of T cells by monoclonal antibodies or enriched for CD34⁺ stem cells. In the latter case, monocytes,

natural killer cells and B cells are *de facto* depleted from the graft also.

Obviously, different regimens will produce different results on immune reconstitution. The data generated thus far seem generally comparable to those obtained in haematological diseases with respect to immunophenotyping of circulating mononuclear cells. The bottom line is that recovery of natural killer cells and monocytes after HDIT and SCT is prompt, but that numerical and functional recovery of lymphoid and immune effector cells occurs more gradually.

In three RA patients, mobilization of haematopoietic stem cells with HDIT and granulocyte colony-stimulating factor (G-CSF) alone led to a drop in the number of circulating CD45RA⁺ naïve T lymphocytes, whereas the number of circulating memory cells was more stable [29]. transient decrease in rheumatoid factor (RF) level was reported. Interestingly, an increase in the level of circulating CD4⁺CD7⁻ T cells appeared to be associated with disease relapse. This T-cell subset is suspected of playing a role in the pathogenesis of RA [30]. It would be of interest to assess the cytokine profile of this subset in these patients, in the light of the above-mentioned preferential IL-4 production of this subset in patients with haematological diseases.

HDIT with unmanipulated SCT led to substantial clinical responses in four RA patients, concomitant with moderate depressions of RF levels in blood [31]. The persistence of RF positivity cannot solely be explained by the putative reinfusion of autoaggressive lymphocytes, because HDIT without SCT had comparable effects [32].

In another study [33**], a patient with RA was treated with a myeloablative conditioning regimen, followed by SCT that was T-cell depleted and CD34⁺ enriched. Enumeration by flow cytometry of CD4⁺ T cells expressing TCRs of different V β families showed a restoration of T-cell diversity compared with the major skewing seen 3 months after transplantation, but with differences in the relative contributions of the families in pre- and post-transplant reperitoires. *In vitro* T-cell proliferative responsiveness and delayed-type skin hypersensitivity to a recall antigen returned to pretransplant levels.

A sustained clinical response was achieved in two out of four RA patients treated with HDIT (cyclophosphamide), antithymocyte globulin and positively selected SCT [34]. RF was only slightly reduced in three patients, but disappeared at 6 months in one of the responder patients. The same treatment in two lupus patients led to an initial decrease in antinuclear antibody titres and antidouble-stranded DNA antibodies, concomitant with a clinical response. In a girl with systemic sclerosis a marked and

sustained improvement in skin score and general well-being was observed 2 years after the transplantation procedure [35]. No correlation was found with the results of *in vitro* proliferation tests and immunophenotyping of circulating mononuclear cells. The persistence of autoantibodies (antinuclear and anti-Scl70) suggested that not all autoimmune clones were eradicated.

Conclusion

The present data do not allow firm conclusions to be drawn regarding the correlation of clinical responses and results of immunological monitoring. At this point it must be recalled that, in RA patients, therapies with anti-CD4 monoclonal antibodies were not very effective, despite prolonged depletion of circulating CD4⁺ T lymphocytes [36]. As a possible mechanism it was shown [37] that IFN- γ producing memory CD4⁺ T lymphocytes were spared by this treatment and that CD4⁺ T lymphocytes persist in the joints. Other studies in RA [38] have also demonstrated lack of correlation between numbers of circulating cells and clinical responses, with persistence of synovial mononuclear cell infiltrates.

From a critic's viewpoint, eradication of autoreactive memory T and B lymphocytes by autologous SCT may be impossible, and allogeneic SCT may be necessary to achieve this goal. A cure may also remain elusive if rheumatic diseases originate in stem-cell defects, as has been mooted for RA and systemic lupus erythematosus [39]. Optimists are supported by the beneficial clinical effects observed thus far. In any case, an extensive search for mechanisms that underlie the remissions observed in many patients, and the relapses that occurred in others seems justified. Whether these mechanisms turn out to be only related to rigorous modulation of the immune system, or whether effects on nonimmune cells are also involved needs to be investigated [40]. It is up to the rheumatologists and immunologists to proceed by close collaboration, preferentially across institutional and national borders, to address the fundamental questions associated with this new treatment modality.

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Author's affiliation: Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands

Correspondence: Dr JM van Laar, MD, Leiden University Medical Center, Department of Rheumatology, C4-R, PO Box 9600, 2300 RC Leiden, The Netherlands. Tel: +31 71 526 3598; fax: +31 71 526 6752; e-mail: jmvlaar@lumc.nl