Resetting the immune system with immunoablation and autologous haematopoietic stem cell transplantation in autoimmune diseases

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ABSTRACT
Over the past 20 years, immunoablation followed by transplantation of autologous haematopoietic stem cells (ASCT) has emerged as a promising treatment option for patients with severe forms of autoimmune diseases (ADs) that insufficiently respond to standard immunosuppressive or novel biologic treatment. Meanwhile, mechanistic studies have provided the proof-of-concept that the long-term, treatment-free remissions achieved by ASCT are associated with the eradication of the autoreactive immunologic memory and a fundamental reconfiguration of the immune system. The latter comprises regeneration of naive B cells and a stable thymic reactivation with re-emergence of thymus-derived naive T cells, including Foxp3+ regulatory T cells, with new antigen receptors, i.e. immune reset. In this article, we discuss mechanistic studies that investigated how such immune renewal after ASCT may rewire a faulty immune system in ADs into a self-tolerant state, to induce long-term remissions.

Introduction
Antigen-specific T cells and long-lived plasma cells (PCs) that persist long after pathogen clearance are responsible for immunologic memory, providing protective immunity to subsequent challenge to the original microbe. In systemic autoimmune diseases, however, such immunologic memory could potentially contribute to the chronicity of autoimmunity and/or flare induction (1). In fact, we could recently demonstrate that long-lived PCs, unlike short-lived PCs, are refractory to cyclophosphamide or B cell depleting therapies (2, 3), and their adoptive transfer from lupus-prone mice (NZB/W) into immunodeficient RAG1−/− mice could induce chronic lupus manifestations, including immune complex nephritis (4). Current immunosuppressive and novel biologic treatments may suppress or attenuate autoimmune responses as long as they are applied, but do not target the autoreactive immunologic memory and cannot switch off the underlying mechanisms to induce therapy-free remission, i.e. cure. In contrast, the general concept of autologous haematopoietic stem cell transplantation (ASCT) is that an extensive immune re-education after eradicating the autoreactive immunologic memory with high-dose chemotherapy, using agents such as anti-thymocyte globulin (ATG), would restore immunologic self-tolerance to induce sustained remissions (5, 6).

Based on experimental data from animal models (7, 8), immunoablation followed by ASCT is performed since 1996 and was shown to induce long-term, treatment-free remissions for several ADs (9-12). To date, more than 2100 cases of ASCT for ADs have been reported to the European Group for Blood and Marrow Transplantation (EBMT) Working Party for Autoimmune Diseases (ADWP), and controlled randomised trials recently demonstrated superiority to standard treatment in the major indications, such as systemic sclerosis (SSc) (13) and Crohn’s disease (14).

The present review focuses on the reconstitution of various components of the immune system following immunoablation and ASCT and how such immune reconfiguration may rewire a chronic autoimmune system into a self-tolerant state, to mediate long-term remissions in ADs.

Imunoablation largely depletes the (autoreactive) immunologic memory
Reduction of autoantibodies and vaccine-induced antibodies.
Depletion of the autoreactive immunologic memory after ASCT is best

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S-53
reflected by the reduction of serum autoantibodies in systemic autoimmune diseases such as systemic lupus erythematosus (SLE) and SSc. In contrast to B cell depletion therapies with rituximab, where autoantibody titres mostly persist after treatment (15), immunoablation has been demonstrated to significantly reduce or even normalise autoantibody titres in such disease. For example, single-centre phase II clinical trials for SLE from the Northwestern University Chicago and our group demonstrated that serum anti-double-stranded (ds)DNA antibodies completely normalised in responding patients whereas they were persistently present before ASCT under chronic immunosuppression, and serum titres for antinuclear antibodies (ANA) were significantly reduced or became negative (12, 16). Notably, depletion of the immunologic memory was not restricted to the autoreactive memory. In addition, vaccine-induced antibody titres were largely extinguished in serum after immune reset (16). This drastic ablation of humoral memory suggests that the ATG used for immunoablation directly targets the PCs secreting such antibodies. In line with this notion, previous in vitro experiments indicated that polyclonal rabbit ATG directly targets PCs via complement-mediated lysis and apoptosis (17) and were able to stain autoreactive PCs with ATG and demonstrate that PCs disappeared from the bone marrow after immune reset (16).

Different transplantation protocols are being employed in the treatment of ADs, varying from lymphodepletion alone, e.g. with cyclophosphamide, followed by ASCT or with additional in vivo T cell depletion using ATG or monoclonal antibodies, such as Campath, or ex vivo graft manipulation using CD34-selection to minimise the risk of re-infusing autoreactive lymphocytes. Depending on the transplantation regimens used, immunologic memory may either reside, especially if no in vivo depletion is performed, or re-infused with the stem cell graft in case of transplantation of unmanipulated stem cell grafts with potential implications on clinical outcomes. Indeed, data from clinical trials indicated that lymphodepletion alone, as performed e.g. for rheumatoid arthritis (18), and use of unmanipulated stem cell grafts after in vivo depleting conditioning regimens for SLE were associated with a higher relapse incidence post-transplantation (10), indicating that a more stringent immune depletion is associated with more favourable outcomes after ASCT.

The degree of immune depletion following immunoablation may also vary depending on the type of the underlying AD. According to previous data (19) and our own observations, the reduction of antinuclear and vaccine-induced antibody titres is less significant in SSc compared to SLE patients and a recent study demonstrated a persistence of pulmonary hypertension (PH)-related specific antibody titres, such as anti-endothelin-receptor antibodies, in SSc patients after immune depletion (20), suggesting a resistance of bone marrow PCs to ATG for so far unknown reasons.

Depletion of (autoreactive) memory T cells following ASCT

In autoimmune diseases, pathogenic T cell functions are mediated by autoreactive memory effector T cells; their elimination is therefore presumed to ameliorate autoimmune inflammation. In line with this notion, Sun and colleagues (21) demonstrated largely reduced T-cell responses to myelin basic protein (MBP) in the reconstituted immune system in MS patients after ASCT compared to pre-treatment. Furthermore, recent data demonstrated that a CD161high pro-inflammatory CD8+ T cell mucosal-associated invariant T (MAIT) subset, which was recently connected to MS immunopathology, could be depleted from peripheral blood following immunoablation and ASCT, while they were maintained at similar frequencies under treatment with interferon β (22).

Even with the most intensive nonmyeloablative-conditioning regimen used for ASCT, a complete eradication of the existing immunologic repertoire may not be feasible. Since experienced memory T cells reside in the bone marrow or secondary lymphoid organs (23), which are hardly to access for immunologic analyses, their fate after immunoablation may only be determined indirectly. With this regard, lessons from vaccination studies in paediatric patients with juvenile arthritis and SLE revealed that the immunologic memory to a neoantigen is lost in most patients after immunoablative pre-treatment, yet memory to a recall antigen boosted before bone marrow harvest is only lost in part of the patients (24).

Immune reconstitution after immunoablation and ASCT for autoimmune disease

Reconstitution of the innate immune system

Following engraftment, the first phase of immune reconstitution is characterised by a significant though transient increase in the proportion of CD56bright natural killer (NK) cell subsets in various autoimmune diseases (19, 22) with a similar kinetic observed in studies investigating immune reconstitution in patients receiving ASCT for malignant diseases (25). Data on the recovery of neutrophil, dendritic cell and innate lymphoid cell (ILC) subsets after ASCT for ADs are scarce and merit further investigations. Here, especially ILC could be of particular relevance in the immune reconstitution for ADs, given their regulatory functions as indicated by their ability to prevent graft-versus-host disease (GvHD) after allogeneic stem cell transplantation for haematologic malignancies (26).

T cell reconstitution

Recurring T cells after immunoablation may theoretically derive from four different sources: (1) from expansion of residual T cells that either survived the conditioning or (2) are reinforced along with the stem cells, (3) from haematopoietic stem cells residing after the conditioning or (4) from transduced CD34-selected stem cells through a process of thymic education. Although dissecting the role of residual vs. transplanted stem cells as source for T cell regeneration is only feasible in allogeneic stem cell transplantation using chimerism analyses, immunologic
studies investigating the T cell reconstitution after autologous transplantation provided important insights into T cell regeneration. After the initial lymphopenia following immunosuppression, T cell recovery occurs at different paces in different lineages and subsets (27). During the first months of immune reconstitution, numeric recovery after ASCT for ADs is predominantly by CD45RO+ CD27- effector memory CD4+ and CD8+ T cells with increased proliferative history and activation status (22), and highly restricted T cell receptor (TCR) repertoire usage (16, 19, 29). The initial expansion of mature T cells could be induced by homeostatic peripheral proliferation driven by low-affinity antigens during lymphopenia (30), which may pose the risk that such lymphopenia-driven proliferation might induce autoimmunity, as demonstrated in animal models (31). However, we and others found no evidence of clonal expansion of autoreactive T cells early after immune reset for SLE upon in vitro stimulation with SLE-associated autoantigens, such as nucleosomes or SmD1, (16, 32). Rather, we showed that expanded memory T cells reacted to virus-specific antigens during infections that are commonly observed in the early phase of immune reconstitution. Expansion of virus-specific T cells was recently confirmed by next generation sequencing (NGS) analyses after immune reset for MS, predominantly within the CD8+ T cell compartment (28).

The second phase of T cell reconstitution is characterised by recurrence of thymic-derived T cells. As opposed to peripheral expansion, thymic education is the prerequisite for immune renewal and could provide a pool of naïve T cells with new antigen receptor specificities. Recent studies support a role for de novo regeneration of naïve T cells from the thymus (16, 19, 29, 33), indicated by the co-expression of CD31 and CD45RA in combination with high levels of T cell receptor excision circles (TRECs) that are characteristic for recent thymic emigrants (RTEs) (34, 35). Depending on the conditioning regimen used and the amount of CD34+ stem cells transplanted, RTEs usually recover to normal levels at 2 years post-transplant as demonstrated in studies for MS (29), RA (18), SLE (16) and SSC (19) and mostly exceed pre-transplant levels during the following years even in older adults, indicating a stable thymic reactivation. As expected, such rejuvenation of the CD4+ T cell compartment is associated with the vast diversification of the TCR repertoire in MS (28, 29), SSc (19) and SLE (16). Among recurring T cells, both CD4+ CD25high Foxp3+ (22, 32, 36, 37) and an unusual CD8+ Foxp3+ T regulatory (Treg) subset (32) returned to levels seen in healthy individuals; they expressed markers of RTEs and He- lios (37), a marker of thymic-derived Tregs (38), and displayed a diverse TCR repertoire (37, 39), confirming a reset of the Treg compartment following ASCT. Of note, although Tregs are thought to play a key role in maintaining self-tolerance, their adoptive transfer after immunosuppression together with the stem cell graft did not elicit additional clinical improvement in a murine model of ASCT, but conversely resulted in a delayed reconstitution of the graft-derived T cell compartment. Therefore, Treg therapy should be applied with caution as it may hamper immune renewal (39).

B cell reconstitution

Analogous to T cells, repopulating B cells could either derive from residual B cell clones or originate from haematopoietic progenitor cells following ASCT. Unlike T cell reconstitution, which is characterised by an initial expansion of memory cells, recurring B cells after ASCT predominantly display a naïve phenotype including immature transitional B cells. Naïve B cells returned to normal levels within 12 months post-transplantation as demonstrated in immunologic studies for SLE and SSC (16, 19), whereas the level of memory B cells was diminished for up to three years. This is remarkably in view of the disturbed B cell homeostasis characteristic for SLE, including a relative predominance of IgD CD27+ memory B cells and expansion of CD27high CD20neg plasmablasts in peripheral blood (40). These findings support the notion that the B cell compartment predominantly regenerated from the haematopoietic stem cells rather than from residual B cell clones. Here, quantification of kappa-deleting recombination excision circle (KREC) could - in analogy to TREC assessment - provide a practicable method to investigate the bone marrow output for B cells, as recently demonstrated in patients with acute leukaemia after allogeneic transplantation (41). Apparently, recurring B cells after immune reset for ADs are tolerant to self-antigens, either resulting from a renewed B cell receptor repertoire and/or facilitated by recurring IL-10 producing B regulatory (Breg) cells, although available data are scare to prove this assumption.

Conclusions

There is now accumulating evidence that immunosuppression followed by ASCT not solely exerts prolonged immunosuppressive effects as initially proposed, but rather provides fundamental changes of the immune system that may rewire a chronic autoimmune system into a naïve and self-tolerant state, which was already assumed by the pioneering research of van Bekkum’s group (7). Such re-induction of self-tolerance is achieved by two major principles of ASCT, that is eradication of the pathogenic autoreactive immune repertoire and profound immunologic renewal. In analogy to stem cell transplantation for haematologic malignancies that yields at the eradication of malignant clones, immunosuppression for ADs is performed with the premise to deplete immunologic memory cells that are refractory to conventional immunosuppressive therapies. This notion is supported by serologic data and TCR repertoire analyses that demonstrated disappearance of pre-existing autoantibodies (16) and prominent T cell clones (28) post-transplantation, respectively.

In addition, immunological studies in AD patients treated with ASCT have demonstrated substantial post-transplant modifications of the adaptive
immune system, *i.e.* immune reset. Among the many aspects of immune re-education, a vast diversification of the TCR repertoire (28, 29) and functional renewal of the Treg compartment (37, 39) seem to be most crucial for the re-induction and maintenance of self-tolerance in ADs (Fig. 1). This is the result of a stable thymic reactivation, also referred to as thymic rebound, which represents a form of true thymic hyperplasia that is associated with the continuous influx of RTEs, providing numbers in ASCT-treated patients comparable to those of young children. Although the basic principles of ASCT in ADs have recently been elucidated, important issues remain unresolved from both clinical and scientific points of view. These include the variability associated with type of AD, continued use of immunosuppression and the effect of concomitant use of immunosuppressive or B cell depleting therapies. Furthermore, it remains unclear what immune cells really need to be eliminated prior to ASCT, and what cells are involved in preserving the immune balance during immune reconstitution. It is also unclear whether relapses after ASCT resemble a redevelopment of autoimmunity due to the patient’s genetic predisposition or are alternatively driven by residual autoreactive memory cells that eventually escaped regulatory mechanisms. An improved understanding of these questions is essential to develop ASCT protocols according to disease-specific requirements and to potentially minimise the toxicity of this procedure. Recently, immune monitoring and biobanking of ASCT-treated AD patient sample guidelines have been published (42) that may provide a reliable infrastructure for further collaborative and comparative research studies to investigate these issues.

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S-57