

* CHAPTER 14

Immunotherapy post-transplant

* 14.2 Adoptive cellular immunotherapy to harness post-transplant alloreactivity

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1. Introduction

The bidirectional allorecognition responses taking place after HSCT between donor and recipient are responsible for the major complications of the procedure: graft failure and graft versus host disease (GvHD), but also for one of its unique advantages, the graft versus leukaemia (GvL) effect (1).

During the past decades the evidence of this anti-leukaemia effect exerted by donor lymphocytes and responsible in part for the success of allogeneic transplantation has led to a change in the classic definition of HSCT as rescue treatment after myeloablation to the more refined one of cellular immunotherapy. This has led in turn to the introduction of reduced intensity conditioning (RIC) regimens as platforms for host versus graft tolerance and subsequent use of donor lymphocytes to exploit the GvL effect, aiming to achieve long-term remissions with low transplant related toxicity. But unfortunately even using reduced intensity conditioning regimens, HSCT is still associated with severe complications due to alloimmune reactions, precluding a wider use of this technique.

2. Alloreaactions post-HSCT

Alloreactivity after HSCT is a complex process that involves donor T-cells and NK cells interacting with specific recipient target-tissues. This immune response is mediated both by direct lymphocyte-target cell interaction and by cytokines. Alloimmune responses after HSCT are responsible for three major transplant events that determine success or failure of the transplant: engraftment, GvHD, and GvL effects.

2.1. Engraftment/rejection

In allogeneic HSCT, graft rejection is predominantly mediated by residual host T-cells with anti-donor specificity. In conventional HSCT donor T-cells usually counteract this host-versus graft effect. However in T-cell depleted HSCT and in non-myeloablative transplants the incidence of graft failure is increased. In order to achieve a long-lasting engraftment in these settings significant immunosuppression is needed to suppress the recipient immune system and allow the incoming donor cells to grow and eventually to predominate. The use of highly lymphotoxic conditioning regimens (including anti-CD52, fludarabine or anti-thymocyte globulin, amongst others), better post-HSCT immunosuppression and better graft quality have much reduced the incidence of graft failure.

2.2. Graft versus host disease (GvHD)

GvHD remains the major cause of mortality and morbidity after allogeneic HSCT and precludes the more extensive use of this procedure. Acute GvHD (aGvHD) is primarily

a T-cell mediated event where the infused donor T-cells recognise recipient antigens presented by recipient-APCs, leading to tissue damage. This systemic alloimmune response is amplified by the conditioning regimen, especially radiation-based ones, which initiate a proinflammatory cytokine storm including TNF- α and IL-1. A decrease in aGvHD incidence can be obtained eliminating the donor T-cells from the graft, but this approach is associated with a higher incidence in relapse and recurrent life-threatening infections.

Chronic GvHD (cGvHD) is a pleiotropic syndrome with similarities to autoimmune diseases such as Sjogren syndrome or scleroderma. The pathophysiology of cGvHD is fundamentally distinct from that of aGvHD and still poorly understood, in part due to a lack of a good animal model. Although donor T-cells are critical in the initiation of cGvHD, it is now established that the effector pathways involve cells of myeloid lineage and fibrogenic cytokines such as TGF- β .

2.3. GvL/graft-versus-tumour effect

The graft versus leukaemia or tumour effect (GvL/GvT) refers to the donor anti-host response directed against the leukaemia/tumour cells remaining after the conditioning treatment. GvL accounts for the main advantage of allogeneic HSCT over autologous HSCT. The first evidence for GvL in humans was a report of increased risk of relapse in those patients not developing GvHD after HSCT (2). The separation of these two mirrored graft versus host responses, GvHD and GvL, still remains the holy grail of stem cell transplantation. The GvL reaction may be directed against a leukaemia-specific targets or minor histocompatibility antigens (mHag) differentially expressed on haematopoietic cells such as HA-1, HA-2, HB-1 and BCL2A1 (3). Donor T-cells are the primary effectors targeting mHags expressed by leukaemia cells as well as normal tissues, thus contributing to both GvHD and GvL. Although some experimental data suggested that CD8⁺ cells were the major effectors of GvHD, some recent studies imply that CD4⁺ cells alone are sufficient to induce a GvL response (4). Recently, alloreactive NK cells have also emerged as effectors of GvL in haploidentical transplants. The mechanism by which allorecognition occurs during the GvL response has not been extensively investigated, although it has been reported that donor APC are not required for the GvL response whereas host APC are necessary (5, 6). Several studies have suggested a differential use of cytolytic pathways by GvHD and GvL effectors. The former would preferentially use the Fas-FasL pathway (specially in target-organ GvHD) whereas GvL activity by CD8⁺ T-cells is mainly dependent on perforin-mediated cytotoxicity. Alternative GvL cytotoxic pathways like TRAIL (TNF-related apoptosis-inducing ligand) with selective activity for malignant target cells have also been proposed.

3. Adoptive cellular immunotherapy post-transplant

3.1 Donor lymphocyte infusions

The best demonstration of the immunotherapeutic effect of alloreactive T-cells is the successful use of DLI for the treatment of relapsed leukaemia after allogeneic HSCT. This is particularly true in the case of CML, whereby post-HSCT relapses can be effectively treated with escalating DLI. Using this approach, around 60–70% of patients re-enter even molecular remission. Other haematological diseases e.g. myeloma and lymphoma, have also been shown to have DLI sensitivity, although to a lesser extent. Variability in the susceptibilities of different types of leukaemia is thought to relate, at least in part, to differences in leukaemic-cell growth kinetics and in GvL effector mechanisms. Unfortunately DLI still involves a risk of GvHD in up to 50% of patients, which can be reduced using an escalating dose approach, delaying the DLI or eliminating CD8 cells from the infusion (7). Another less common complication after DLI is pancytopenia, mainly reported in patients treated in full-blown relapse and with very reduced donor haematopoiesis. The role of DLI not only as a treatment for relapse but also as pre-emptive treatment in RIC transplants is being investigated.

3.2. Suicide-gene-transfected donor T-cells

Another potential solution to alloreactivity from unmanipulated T-cells is to transduce the donor T-cells with a suicide gene, so that they can be eliminated if GvHD occurs. The herpes simplex virus 1-thymidine kinase (HSV-TK) gene has been used in several clinical trials without significant acute toxicity and showing dissociation of GvL from GvHD. However, the results are still conflicting and some safety issues need to be addressed before this strategy is consolidated in clinical practice. Other gene modification approaches like timed induction of genes involved in T-cell apoptosis, like FAS or caspase 9, to treat GvHD are currently being investigated.

3.3. Regulatory T-cells

Among the major mechanisms involved in transplant tolerance, i.e. central deletion, clonal anergy and suppression/regulation of donor-reactive T-cell clones, the latter has proven to be pivotal in the maintenance of tolerance. Several types of regulatory T-cells have been described, such as $\alpha\gamma$ T-cells, NKT-cells, CD8⁺ and CD4⁺ T-cells. The later can be divided into two groups, those CD4⁺ regulatory cells that exert their action secreting inhibitory cytokines like IL-10 or TGF- β such as Tr1 and Th3 cells, and the recently described subset of CD4⁺ regulatory T-cells expressing the IL-2

receptor α -subunit (CD25), which exert their suppressive function in a cell-to-cell contact manner. The CD4⁺CD25⁺ or regulatory T-cells (T_{reg}) subset is considered to be a crucial population in preserving peripheral tolerance not only to autoantigens but also to foreign antigens, and their role in transplantation tolerance is being increasingly acknowledged.

3.3.1. Naturally occurring CD4⁺CD25⁺ Tregs

CD4⁺CD25⁺ regulatory T-cells were first described in murine systems in 1995 by Sakaguchi et al. (8). They demonstrated that this small thymic derived subset was crucial in preventing autoreactivity, as those mice depleted from these cells developed severe systemic autoimmune disease. In humans T_{regs} account for 1–2% of circulating CD4⁺ lymphocytes. In contrast with their murine counterpart human T_{regs} account mainly for those CD4 with intense CD25 expression (CD4⁺CD25^{high}). T_{regs} co-express preferentially other surface markers like GITR (glucocorticoid-induced TNFR-related protein), CD62-L, CTLA-4 or CD152 or CD45RO. However, none of these markers is specific of T_{regs} and can be also up-regulated in activated T-cells. The most specific T_{reg} marker available to date is the forkhead transcription factor FOXP3, which has proven to be central in the development and function of CD25⁺ T_{regs} in both mice and humans. Although it is known that T_{regs} exert their suppressive activity in a cell-to-cell contact manner, the exact mechanism remains to be elucidated.

In mouse models T_{regs} have shown to prevent and control GvHD in HLA-mismatched transplants when co-infused with conventional T-cells in a 1:1 ratio. In tumour-bearing mice the infusion of allogeneic T_{regs} alone was unable to exert a GvL effect and notwithstanding the absence of GvHD those animals died of disease progression. However some studies have demonstrated that co-infusion of allogeneic T_{regs} and effector cells prevented GvHD while preserving GvL, suggesting a distinctive pathway of cell killing for those populations (9).

The role of T_{regs} in clinical HSCT remains unclear and trials to address this are ongoing. If as in mouse models they demonstrate to be crucial in preventing GvHD then the infusion of *ex vivo* expanded T_{regs} could be a plausible treatment for this life threatening condition, although recent publications indicate that in humans T_{regs} may have a short life-span after infusion. If on the other hand T_{regs} are found to suppress anti-tumour immune responses in the transplant setting then their depletion from the DLI product could be advisable to reach higher remission rates.

3.4. NK cells

Both clinical and experimental evidence support a role for NK cells as GvL effectors. Human leukocyte antigen (HLA)-C is now known to be the most important MHC class

I subgroup involved in NK inhibition through killer inhibitory receptors (KIRs). In the setting of HLA-mismatched haploidentical HSCT for AML, donor NK clones fail to encounter their class I inhibitory KIR ligands, resulting in the killing of host leukaemic cells. An advantage of using alloreactive NK cells as anti-tumour effectors is that, unlike T-cells, they do not cause GvHD. Clinical trials to address the role of NK-cell-mediated anti-tumour reactivity in the context of KIR-ligand-mismatched allo-HSCT have obtained promising results (review in (10)).

3.5. Mesenchymal stem cells (MSCs)

MSCs are multipotent cells with capacity to differentiate *in vitro* and *in vivo* into several mesenchymal tissues, such as bone, cartilage and fat. Although bone marrow is the main source for MSCs they can also be isolated from blood, adipose tissue, foetal tissue and cord blood. They play a crucial role maintaining the marrow stroma and they have shown in animal models to enhance engraftment after autologous and allogeneic HSCT. Several studies also support their role in restoring myeloid, lymphoid and megakaryocytic lineages. Importantly, MSCs also disclose strong inhibition of T-cell proliferation to alloantigens *in vitro*. Autologous and allogeneic MSCs disclosed low immunogenicity and are safe to infuse in humans. Thus, MSCs may be used to enhance haematopoietic three-lineage engraftment and to prevent graft rejection and GvHD in HSCT. Their exact mechanism of action is unclear; however, a handful of reports already support the use of MSC to treat refractory GvHD and recurrent graft failure; trials to confirm their role in this setting are ongoing (review in (11)).

3.6. Cellular vaccines

The lymphopenic environment and “enhanced” cytokine milieu that immediately follows HSCT favours the expansion of T-cell clones, supporting the use of post HSCT vaccination. Using specific CTLs directed against tumour proteins (e.g. BCR-ABL, PR1 and WT1) or minor antigens is a plausible way of enhancing the GvL effect after transplant for haematologic malignancies. However, before vaccination can be applied effectively in HSCT recipients it will be necessary to develop methods for selectively preventing aGvHD and thus eliminating the need for post-transplant immunosuppression. The combination of the potent GvL effect of the allograft with a vaccine boost for leukaemia-specific T-cells could prove to be a highly effective strategy to control refractory leukaemias. Moreover adoptive immunotherapy with donor-derived HA-1 CTLs in combination with HSCT could also become an attractive treatment for solid tumours.

Another vaccination approach is DC-based immunotherapy after allogeneic stem cell transplantation, used to enhance GvL reactions without aggravation of GvHD. The

experience so far from phase I/II clinical trials shows the feasibility of DC vaccination using various target antigens like bcr/abl or patient-specific idiotypic proteins, but the clinical benefit is still unclear.

4. Future directions

Adoptive T-cell immunotherapy with DLI is already an established and efficacious treatment of post-HSCT relapse in certain settings. New strategies aiming at more specific responses against relapsed or persistent leukaemias and tumours following HSCT and at inhibiting GvHD are being explored. These include depletion or infusion of selected cell populations, genomic modification and vaccination. However, several studies have already identified difficulties with implementing such strategies including inadequate cell persistence or expansion *in vivo*. A better understanding of the mechanisms involved in the GvL/GvHD immunobiology post-HSCT together with an improved methodology for T-cell expansion and manipulation is crucial to eventually harnessing post-transplant alloreactivity and improving patient outcome.

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Multiple Choice Questionnaire

To find the correct answer, go to <http://www.esh.org/ebmt-handbook2008answers.htm>

- 1. One of the following is considered to date the most specific marker for human regulatory T-cells:**
- a) GITR
- b) CTLA-4
- c) FR4 (folate receptor 4)
- d) FoxP-3
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- 2. Which of the following is true for mesenchymal stem cells (MSC)?**
- a) MSC can only be isolated from cord blood
- b) MSC are highly immunogenic
- c) MSC inhibit T-cell proliferation to alloantigens
- d) MSC have shown to promote graft failure in murine systems
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- 3. One of the following minor histocompatibility Ag (mHAg) expression is restricted to haematopoietic cells:**
- a) HA-3
- b) HA-2
- c) B60/HY
- d) A1/HY
-
- 4. Which subset of lymphocytes has demonstrated to enhance GvL reactions in haploidentical SCT?**
- a) Recipient CD8⁺
- b) $\alpha\gamma$ lymphocytes
- c) Alloreactive NK cells
- d) Tregs

5. One of the following strategies would not be useful to enhance GvL responses:

- a) Infusion of KIR-mismatch alloreactive NK cells
- b) Infusion of *ex vivo* expanded Tregs
- c) DC vaccination
- d) WT-1 peptide vaccination

NOTES