EUROPEAN HEMATOLOGY ASSOCIATION

Balancing graft versus leukemia and graft versus host responses

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Take-home messages

- Specific targeting of the alloimmune response towards hematopoiesis of recipient origin results in GVL with no or limited to GvHD.
- Due to the high susceptibility of hematopoietic cells for recognition by T cells, limiting the magnitude and diversity of the alloimmune response can result in GVL with limited GvHD.
- Limiting inflammation *in vivo* may result in skewing the alloimmune response towards specific GVL reactivity.

Introduction

The main therapeutic effect of allogeneic hematopoietic stem cell transplantation (alloSCT) is control of the disease by the graft versus leukemia/lymphoma (GVL) reactivity mediated by an alloimmune response of donor T cells recognizing hematopoietic cells from the recipient including the malignant population.[1] However, alloreactive T cells recognizing polymorphic antigens on non-hematopoietic tissues may also result in the development of graft-versus-host disease (GvHD). Therefore, as expected, complete removal of T cells from the graft or in vivo purging of T cells will abrogate GvHD but also GVL. Fortunately, hematopoietic cells usually reside in tissues relatively readily accessible to T cells, and hematopoietic cells are relatively susceptible to recognition by T cells due to their high expression of HLA class I, sometimes HLA class II, and expression of adhesion and costimulatory molecules. A variety of clinical observations has illustrated that GVL reactivity can occur in the presence of limited GvHD.

Current state of the art

The balance between GvHD and GVL depends on many factors. Both reactivities are dependent on genetic polymorphic differences between donor and recipient, and therefore disparity for HLA alleles as well as disparity for minor histocompatibility antigens (MiHA) are dominant factors in this balance. If donor and recipient are fully HLA identical, donor anti-recipient alloimmune responses are the result of differences in presentation of polymorphic peptides presented in the groove of HLA molecules (defined as MiHA), since thymic selection has excluded recognition of non-polymorphic peptides in the context of self HLA molecules.[2] T cells are not exposed to non-self HLA alleles during thymic selection, and therefore any peptide presented in the context of non-self HLA molecules may theoretically be immunogenic for T cells. [3] Since the peptidome presented in HLA molecules contains at least 10 times more monomorphic peptides than polymorphic peptides, frequencies of T cells capable of recognizing non-polymorphic peptides in alloHLA alleles are a magnitude higher than of T cells recognizing MiHA.[4]

Following HLA identical sibling transplantation, the development of donor anti-recipient immune responses mimics the development of T cell responses against other nonself antigens like viral antigens. If the donor has not been exposed to alloantigens by transfusions or pregnancy, T cell responses recognizing MiHA are likely to be present in the naïve and not in the memory T cell compartment.[5] As a consequence, the T cell response has to be provoked by activated dendritic cells (DC) (Table 1).[6] These DC will present endogenous peptides and will cross-present antigens picked up during inflammation and from damage tissue. Since DC are cells derived from the hematopoietic system, donor T cell responses provoked by patient DC are likely to react with the hematopoietic compartment of the recipient.[1] This reaction is identical to the GVL effect. Donor T cells may also react with MiHA derived from broadly expressed genes presented in DC, and with MiHA from damaged tissue in which the DC reside. The more tissue damage, inflammation and danger signals, the greater and more diverse the alloimmune responses.[7] We recently demonstrated in a clinically defined model of development of alloimmune responses, i.e. preemptive donor lymphocyte infusion (DLI) following T cell depleted alloSCT, that the balance

between GvHD+ GVL versus GVL only is highly influenced not only by the antigen specificity of the immune response, but more significantly by the magnitude and diversity of the T cell response against MiHA.[8] This may explain why dosing and timing of T cell infusion may dictate relative GvHD or relative GVL. An alloimmune response directed against DC under limited inflammatory circumstances can result in a restricted GVL reactivity. In contrast, under highly inflammatory circumstances with significant tissue damage in organs where the DC reside, highly diverse immune responses with major magnitude will take place resulting in GvHD, with organ specificity depending on the site of inflammation and tissue damage.[9] These results may also explain why delayed DLI may lead to more specific GVL reactivity, and why late after transplantation higher doses of T cells can be infused with a lower likelihood of GvHD.[10]

Following alloSCT with 'matched' unrelated donors (MUD), donor and recipient are usually matched for HLA class-I alleles. Therefore, alloimmune responses against HLA class I restricted antigens behave in principle similar to following HLA identical sibling alloSCT. However, since only HLA-ABC, -DR and -DQ but not HLA-DP are usually taken into account in HLA matching, 80% of donor-recipient pairs will have disparity for HLA-DP alleles. This results in a significant increase of the possibilities of alloimmune responses.[11,12] Since in the absence of inflammation, HLA class-II expression is mainly restricted to cells of the hematopoietic system, allo-HLA-DP responses under non-inflammatory circumstances may lead to a specific GVL reactivity.[13] However, under inflammatory circumstances HLA class-II is upregulated on non-hematopoietic tissues, which may result in an amplification of GvHD.[14] Since allo-HLA responses are T cell responses which can also reside in the memory compartment, the threshold of activation may be significantly lower, and young donors with a lower diversity of the memory T cell repertoire may provoke less GvHD (Table 1). Thus, in unrelated transplants the balance between the development of GvHD and GVL is less predictable.

Following multiple major HLA allele mismatching between donor and recipient, the amplitude and diversity of the immune response will greatly increase, and the alloreactive T cells are likely to be present both in the naïve and the memory T cell repertoire, unless umbilical cord blood (UCB) is used as source.[15,16] Following UCB transplantation, frequencies of alloreactive T cells recognizing the mismatched HLA alleles will be high, but since the UCB T cells will all be naïve, the threshold of activation will be relatively high, and activated DC's are probably necessary to provoke the immune response. This may explain in part the low intensity of GvHD following UCB transplantation. When adult major HLA mismatched donors are used in haplo-identical transplantation, significant T-cell depletion is necessary to reduce the incidence of severe GvHD.[17] This can be performed by complete or partial T-cell depletion of the graft, by in vivo T-cell depletion of the graft using antibodies like anti-T cell globulin (ATG) or alemtuzumab, or by *in vivo* removal of the majority of rapidly activated allo-HLA reactive T cells by the administration of cyclophosphamide post-transplant.[18] Rapidly proliferating T cells will then be depleted, resulting in lower magnitude and diversity of the alloimmune response, resulting in less GvHD. Hopefully, not too many alloreactive T cells will be depleted thereby also removing the GVL reactivity. Obviously, it will be difficult to precisely orchestrate these immune responses. If all T cells are removed from the graft or *in vivo*, the GVL response may completely depend on the presence of alloreactive NK cells.[17] Especially myeloid malignancies have been reported to be susceptible to recognition by alloreactive NK cells capable of killing these cells in the absence of inhibitory self HLA molecules.

Table 1. Dependency of alloreactivity on recipient professional antigen presenting cells.

Donor T cell source	Alloreactivity present in		Alloreactivity dependency on recipient dendritic cells
	Memory compartment	Naive Compartment	
HLA identical sibling	--	++	++
Matched unrelated donor (10/10 HLA match)	+	++	+/-
HLA-mismatched donor/Haplo identical donor	++	++	---
Umbilical cord blood	---	++	+

Future perspectives

Future improvements of alloSCT will depend on successful manipulation of the immune response post-transplant. Approaches after HLA identical transplantation may include the use of relatively purified stem cell populations, in combination with the memory T cell compartment from the donor which contains mainly pathogen specific T cells[19] or the use of purified pathogen specific T cells followed by postponed administration of naïve tumor reactive T cells. Following HLA mismatched transplantation, major depletion of the broad anti-HLA repertoire from both the memory and naïve T cell compartment may be necessary. This may be performed by *in vitro* or *in vivo* T-cell depletion using antibodies, or by the use of cyclophosphamide post-transplant. However, also after HLA mismatched transplantation reintroducing antitumor reactivity may be necessary. This may be performed by specifically targeting HLA molecules with limited tissue expression like HLA-DP or HLA-DQ under non-inflammatory circumstances, or by the use of other alloreactive immune cells like alloreactive NK cells. *In vivo* manipulation of the magnitude of the immune response by tempering inflammation or tissue damage may help to direct the immune response towards more specific GVL reactivity.

References

1. Falkenburg JH, Jedema I. Allo-reactive T cells for the treatment of hematological malignancies. Mol Oncol 2015;9:1894-1903.
2. Griffioen M, van Bergen CA, Falkenburg JH. Autosomal minor histocompatibility antigens: how genetic variants create diversity in immune targets. Front Immunol 2016;7:1-9.
3. Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T cells. Annu. Rev. Immunol. 2003;21:139-76.
4. Hassan C1, Kester MG, de Ru AH, et al. The human leukocyte antigen-presented ligandome of B lymphocytes. Mol Cell Proteomics 2013;12:1829-43.
5. Bleakley M, Otterud BE, Richardt JL, et al. Leukemia-associated minor histocompatibility antigen discovery using T-cell clones isolated by in vitro stimulation of naive CD8+ T cells. Blood 2010;115:4923-33.
6. Reddy P, Maeda Y, Liu C, et al. A crucial role for antigen-presenting cells and alloantigen expression in graft-versus-leukemia responses. Nat Med 2005;11:1244-9.
7. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet 2009;373:1550-61.
8. van Bergen CA, van Luxemburg-Heijs SA, de Wreede LC, et al. Selective graft-versus-leukemia depends on magnitude and diversity of the alloreactive T cell response. J Clin Invest 2017;127:517-29.
9. van der Zouwen B, Kruisselbrink AB, Jordanova ES, et al. Alloreactive effector T cells require the local formation of a proinflammatory environment to allow crosstalk and high avidity interaction with non-hematopoietic tissues to induce GvHD reactivity. Biol Blood Marrow Transplant 2012;18:1353-67.
10. Eefting M, de Wreede LC, Halkes CJ, et al. Multi-state analysis illustrates treatment success after stem cell transplantation for acute myeloid leukemia followed by donor lymphocyte infusion. Haematologica 2016;101:506-14.
11. Petersdorf EW1, Malkki M, O'hUigin C, et al. High HLA-DP Expression and Graft-versus-Host Disease. N Engl J Med 2015;373:599-609.
12. Fleischhauer K, Beelen DW. HLA mismatching as a strategy to reduce relapse after alternative donor transplantation. Semin Hemato. 2016;53:57-64.
13. Rutten CE, van Luxemburg-Heijs SA, Halkes CJ, et al. Patient HLA-DP-specific CD4+ T cells from HLA-DPB1-mismatched donor lymphocyte infusion can induce graft-versus-leukemia reactivity in the presence or absence of graft-versus-host disease. Biol Blood Marrow Transplant 2013;19:40-8.
14. Stevanovic S, van Bergen CA, van Luxemburg-Heijs SA, et al. HLA class II upregulation during viral infection leads to HLA-DP-directed graft-versus-host disease after CD4+ donor lymphocyte infusion. Blood 2013;122:1963-73.
15. Amir AL, D'Orsogna LJ, Roelen DL, et al. Allo-HLA reactivity of virus-specific memory T cells is common. Blood 2010;115:3146-57.
16. Melenhorst JJ, Scheinberg P, Williams A, et al. Alloreactivity across HLA barriers is mediated by both naïve and antigen-experienced T cells. Biol Blood Marrow Transplant 2011;17:800-9.
17. Mancusi A, Ruggeri L, Velardi A. Haploidentical hematopoietic transplantation for the cure of leukemia: from its biology to clinical translation. Blood 2016;128:2616-2623.
18. Robinson TM, O'Donnell PV, Fuchs EJ, Luznik L. Haploidentical bone marrow and stem cell transplantation: experience with post-transplantation cyclophosphamide. Semin Hemato. 2016;53:90-7.
19. Bleakley M, Heimfeld S, Loeb KR, et al. Outcomes of acute leukemia patients transplanted with naive T cell-depleted stem cell grafts. J Clin Invest 2015;125:2677-89.