Naïve T cell Depletion in Stem Cell Transplantation

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Conflict of interest:

MB has received compensation from Miltenyi Biotec (manufacturers of CD45RA immunomagnetic beads) for presentations at conferences and corporate symposium. Miltenyi Biotec are contributing to the funding of a clinical trial, (NCT 03779854; 16-NTCD), a Pediatric Transplantation and Cellular Therapy Consortium (PBMTC) multicenter randomized controlled trial of naïve T cell-depleted peripheral blood stem cell transplantation on which MB serves as the principal investigator. MB is also a Founder and Scientific Advisory Board member of HighPassBio, and a Scientific Advisory Board member of Orca Bio.

Abstract

Allogeneic hematopoietic stem cell transplantation (HCT) is curative in many patients with advanced hematopoietic malignancies. Donor T cells facilitate engraftment, protect against opportunistic pathogens and residual disease, but can also cause graft-versus-host disease (GVHD), with significant morbidity and mortality. Complete T cell depletion can substantially reduce GVHD rates but can also delay immune reconstitution and increase rates of opportunistic infections and relapse. Murine models have shown that naïve T cells (T_N) consistently cause severe GVHD, while memory T cells (T_M) cause milder or no GVHD and have critical graft-versus-tumor function. Informed by experiments performed in murine models of HCT, clinical trials are being conducted to evaluate T_N-depleted peripheral blood stem cell (PBSC) grafts. These trials are showing very low rates of chronic GVHD and of serious acute GVHD in the HLA-matched HCT setting, with lower frequencies of opportunistic infections than after fully T cell-depleted HCT and no apparent increase in relapse rates. Randomized clinical trials are ongoing, comparing standard unselected HCT with T_N-depleted PBSC and other promising GVHD-reduction strategies. Correlative laboratory studies will clarify how anti-tumor function is retained in T_N-depleted HCT and inform strategies to further augment GVL in patients at a high risk of relapse. T_N-depletion of donor lymphocyte infusions and of haploidentical stem cell grafts are also being investigated.

BLOOD ADVANCES TALK

Background and conceptual underpinnings of naïve T cell depletion

- Severe acute and chronic graft-versus-host disease (GVHD) cause significant morbidity, mortality, disability, and social handicap following allogeneic hematopoietic stem cell transplantation (HCT).
- T cells are central to GVHD pathogenesis (1). Specifically, donor T cells are activated by antigen presenting cells (APC), mainly in the lymph nodes draining the intestinal tract, then proliferate and migrate to epithelial tissues where they release cytokines and mediate direct cytotoxicity.
- In HLA-mismatched transplantation, donor T cells recognize alloantigen in several forms, including complexes of mismatched HLA molecules and peptide, and minor histocompatibility (H) antigens (2). In HLA-matched transplantation, donor T cells recognize minor H antigens, which are polymorphic peptides presented with HLA molecules on cell surfaces (3). Most minor H antigens arise from non-synonymous polymorphisms that produce single amino acid changes, which alter peptide processing, binding, or TCR contact. High-avidity TCR in the donor repertoire can recognize the recipient minor H antigens as foreign.
- Although donor T cells cause GVHD in HCT, they also facilitate engraftment, protect against opportunistic pathogens and mediate an immune attack on residual malignant cells, known as graft-versus-leukemia (GVL) or graft-versus-tumor (GVT).
- It is well established that complete T cell depletion (TCD), for example by $CD34^+$ cell selection, can substantially reduce rates of acute and chronic GVHD (4, 5). However, pan-TCD can also delay immune reconstitution and increase rates of opportunistic infections and associated complications such as EBV-post-transplantation lymphoproliferative disease (PTLD) (5). In some reports, pan-TCD was also associated with an increased relapse rate, particularly among patients with more advanced disease pre-HCT (4, 5). As a result of these complications, the HCT field moved away from pan-TCD and researchers began developing more selective T cell depletion approaches.
- It was recognized that different T cell subsets might have different potentials to mediate GVHD.

- T cells can be divided into a) antigen-inexperienced naïve T cells (T_N) and b) antigen-experienced T cells, including memory and effector T cells which are largely compromised of oligoclonal expansions of T cells specific for pathogens to which an individual has been exposed (6). The antigen-experienced T cell populations can be subdivided into central memory T cells (T_{CM}), effector memory T cells (T_{EM}), effector T cells (T_E) and other subgroups, according to cell surface phenotype, gene expression, metabolic profile and function. For simplicity, I will refer to antigen-experienced T cells as memory T cells (T_M).
- It was hypothesized that T_N would have a greater capacity to cause GVHD primarily because T_M have a more restricted TCR repertoire largely focused on pathogens and logically if HCT donor T cells hadn't previously encountered recipient minor H antigens, there should be few, if any, minor H antigen-specific T_M.
- However, T cell cross-reactivity between HLA-bound epitopes of pathogens (such as EBV) and alloantigens is well recognized (7), and cross reactivity of pathogen-specific T cells against minor H antigens is also possible, although unproven. Moreover, although T_N have an advantage in accessing lymph nodes over some T_M, T_M cells could have other properties that promote GVHD, including faster homeostatic proliferation during lymphopenia, faster activation in response to antigen stimulation and less stringent APC requirements.

- So, the hypothesis was that T_N would cause more severe GVHD, but there were reasons that the converse could be true. Experiments were therefore performed in murine models of bone marrow transplantation (BMT) to test the hypothesis (8-22).

Murine experiments

- The first study to address the potential of T_N and T_M cells to cause GVHD in murine models was performed by Warren Shlomchik's group and published in JCI in 2003(8). Shlomchik and colleagues employed a $CD4^+$ dependent, MHC-matched, minor H antigen mismatched chronic GVHD model. They used donor B10 mice and transplanted bone marrow alone or with a) unseparated splenocytes; b) flow sorted $CD4^+$ T_N cells; or c) $CD4^+$ T_{EM} cells into irradiated Balb/c mice. Mice who received whole splenocytes or T_N cells developed GVHD whereas those that received T_{EM} cells or bone marrow alone

did not. To ensure that this difference was not caused by different T reg content, the investigators performed an additional set of experiments in which T regs were depleted from the T_N and T_{EM} cells before infusion with bone marrow into mice, and they observed that the T reg-depleted T_N still caused GVHD while the T reg-depleted T_{EM} did not. Importantly, in this study it was also demonstrated that by first vaccinating the donor mice, it was possible to transfer immunity to a model antigen in the T_{EM}.

- Multiple subsequent studies were performed by several groups using a variety of murine GVHD models to evaluate the potential of different T cell subsets to cause GVHD (9-21). The models used included a) acute and chronic GVHD; b) MHC mismatched and MHC matched, minor H antigen mismatched, and c) $CD8^+$ and $CD4^+$ dependent. In every study, T_N were demonstrated to have the capacity to cause severe GVHD and in no study did T_{EM} cause GVHD. In some, but not all studies, T_{CM} could cause some GVHD, with similar pathology but a more benign course. For example, in further work by the Shlomchik group, $CD8^+$ T_{CM} could infiltrate the GI tract and the GVHD pathology scores associated with T_{CM} were generally similar to those of T_N (although with a slight trend towards lower severity) but the mice who received T_{CM} regained weight and the GVHD was rarely lethal (15). In addition, the $CD8^+$ T_{CM} could protect the recipient mice from murine CP-CML via recognition of minor H antigens.
- Taken together this body of work in murine models demonstrated that T_N consistently cause GVHD, T_{EM} never do, and T_{CM} can cause a limited form of GVHD and can contribute to a GVL effect.

Pre-clinical human in vitro work and rationale for graft engineering

- Our group studied human T cell responses to minor H antigens (23)
  - We obtained PBMC from siblings, generated monocyte-derived dendritic cells (moDC) from one sibling, isolated T_N and T_M from the other sibling, stimulated the T cells with the moDC and, after 10 days in culture, evaluated the frequency of T cells responsive to target cells of the APC donor and not of the T cell donor.
  - In this assay, which measures the precursor frequency of minor H antigen-specific T cells, minor H antigen-specific T cells were 6-20 times higher in the T_N than the T_M population.
  - Moreover, after further *in vitro* expansion, the T cell lines derived from T_M cells lost their response to minor H antigens, while the T_N-derived lines remained highly cytotoxic.

- This work suggested that T_N-depletion was likely to reduce minor H antigen-specific T cells and thereby decrease GVHD in humans as well as mice

- We then developed an HCT concept in which we would give stem cells and only T_M to reduce the risk of GVHD compared to unselected donor grafts, and to improve immune reconstitution compared to pan-TCD grafts.

- Human T_N express the high molecular weight form of the CD45 membrane protein tyrosine phosphatase, CD45RA whereas most T_M do not, so if one depletes the $CD45RA^+$ cells then substantial numbers of T_M specific for pathogens should be retained. We therefore developed a clinical scale method for depletion of $CD45RA^+$ T_N using a two-step approach of positively selecting the $CD34^+$ stem cells and taking the CD34-depleted fraction and depleting it of $CD45RA^+$ cells (24). We confirmed that T cells specific for pathogens were retained after $CD45RA^+$ depletion and that they remained functional.

- We've now performed over 150 selection procedures, in preclinical development and clinical trials and been consistently successful in obtaining target cell numbers, specifically $CD34^+$ >5 million/kg, T_M of 10 million T cells/kg, and < 50,000 T_N/kg. In the first clinical trial-which I'll come back to-we confirmed that T cell immune reconstitution in T_N-depleted peripheral blood stem cell (PBSC) transplantation (PBSCT) recipients is similar to unmanipulated BMT and significantly higher than in recipients of $CD34^+$ selected panTCD PBSCT in the first 3 months post HCT (25). Moreover, pathogen specific T cells are transferred with the T_N depleted graft, and expand in vivo in response to low level viral reactivations, implying in vivo function.

Clinical results in human HLA-matched transplantation and donor lymphocyte infusion (DLI)

- In collaboration with Warren Shlomchik, our group conducted the first clinical trial of T_N-depletion of PBSC grafts in HLA-matched PBSCT. This study was published in JCI in 2015 (25). Briefly, T_N-depleted PBSC were administered after myeloablative conditioning to 35 patients with leukemia in remission who had HLA-matched sibling donors. The incidence of acute GVHD was not reduced; however, GVHD in these patients was universally corticosteroid responsive, the grade III acute GVHD rate was low (9%) and grade IV GVHD did not occur. Chronic GVHD was remarkably infrequent (9%) compared with historical rates of approximately 50% with T cell–replete grafts. T_M in the graft resulted

in rapid T cell recovery and transfer of protective virus-specific immunity. Excessive rates of infection or relapse did not occur, and overall survival was 78%.

- We have now completed another two trials of T_N-depleted PBSC that include recipients of HLA-matched unrelated donor PBSC and patients who received less intensive conditioning. Taken together the three studies which have enrolled >140 patients have shown a consistent major reduction in chronic GVHD, very low rates of serious acute GVHD and very encouraging GVHD-free, relapse-free survival. We have also learnt that T_N-depletion is technically feasible in the context of unrelated donor PBSCT and that lower intensity conditioning is sufficient to ensure engraftment of T_N-depleted PBSC.
- None of what I have described is definitive evidence that T_N-depletion reduces chronic GVHD in humans, nor does it shed any light on how T_N-depletion compares to other strategies of GVHD reduction. So, we are now conducting randomized trials that do directly compared T_N-depleted PBSCT with standard unmanipulated HCT and with other promising GVHD-reduction strategies.
    - NCT 03779854 is a Pediatric Transplantation and Cellular Therapy Consortium eight center trial that randomizes children with leukemia between T_N-depleted PBSC and unselected BMT.
    - NCT 03970096 is a multicenter trial for adults or children with leukemia that randomizes patients to one of four arms: a) T_N-depleted PBSC; b) $CD34^+$-selected PBSC; c) PBSC with post-HCT cyclophosphamide (PTCy) and d) PBSC with tacrolimus and methotrexate.
- Several other research groups are also studying T_N-depletion of stem cell grafts or donor lymphocyte infusion (DLI) including groups at Stanford, Duke and St. Jude Children's Research Hospital:
    - Stanford investigators have been studying T_N-depleted $CD8^+$ DLI informed by their work in murine models in Dr. Strobers lab (12, 17) and others in the field. The group published a phase I clinical trial of $CD8^+$ T_N-depleted DLI for patients with recurrent hematologic malignancy after HCT (26). Although it's not possible to draw conclusions about the anti-tumor efficacy of the T_N-depleted DLI because of the nature of the phase I feasibility and safety trial, two of six patients who achieved a CR prior to the cell infusion sustained that CR following the infusion for many months, suggesting efficacy similar to unselected DLI. Importantly, among the 15 patients GVHD was only observed in

one patient who developed asymptomatic liver GVHD, implying that T_N-depleted DLI may be equally effective and less toxic with less GVHD than standard DLI.

- Similarly, investigators at Duke University have recently published a clinical trial of prophylactic T_N-depleted DLI informed by murine studies performed in Dr. Chao's laboratory (11, 13, 20). In the phase I/II trial, CD45RA-depleted DLI was administered at a median of 113 (76-280) days after a T cell replete HCT with reduced-intensity conditioning including alemtuzumab or thymoglobulin. Of the sixteen subjects treated at different dose levels after the T_N-depleted DLI, one developed acute GVHD and another developed chronic GVHD, supporting the feasibility of this approach (27).
- T_N-depletion of PBSC is being studied in the context of haploidentical HCT at St Jude Children's Research Hospital (28-31). In a recent publication describing preliminary outcomes of the first 50 subjects enrolled in an ongoing clinical trial, the St. Jude investigators report moderately high rates of grade III-IV acute GVHD (around 28%) and chronic GVHD of 26% (31). Interestingly, although the grade II-IV GVHD rate was high, most patients survived the GVHD, and the overall non-relapse mortality among patients in remission at the time of HCT was quite low at 5.6%. This may suggest that even if severe acute GVHD follows T_N-depleted PBSCT in HLA mismatched HCT it may be relatively treatment sensitive. However, the St Jude T_N-depleted haploidentical HCT results do indicate that T_M cells have the potential to cause severe GVHD in HLA-mismatched HCT, possibly due to cross reactivity of pathogen-specific T_M against alloantigens, so further refinement of this approach is required. Of note, in the St. Jude trial a relatively high dose of T_M cells is being given with a median of 76 million/kg, nearly a log higher than we are administering in HLA-matched HCT. It would be of interest to investigate the effect of giving a lower dose of haploidentical T_M cells, and perhaps delaying the T_M infusion for a few weeks after the stem cells, to allow time for resolution of the post-conditioning proinflammatory state. For example, one could give the T_M cells 30 days after the stem cells, rather than one day later in the current protocol.

Conclusions and future directions

- Informed by experiments performed in murine models of HCT, clinical trials are being conducted to evaluate T_N-depletion of stem cell grafts. These trials are showing very low rates of chronic GVHD and

also low rates of serious acute GVHD in the HLA-matched HCT setting, without high frequencies of opportunistic infection such as are found after pan T cell-depleted HCT.

- Overall, the outcomes of T_N-depleted HCT are very encouraging, and the human experience is generally consistent with the results of murine experimentation. Interestingly, in some murine studies acute GVHD was observed when T_{CM} were administered with stem cell grafts but it was rarely lethal. Similarly, in human clinical trials of T_N-depleted HLA-matched PBSC we have not observed a reduction in acute GVHD but it is rarely severe and typically does not evolve to cGVHD, suggesting that T_{CM} may be contributing to the limited acute GVHD in humans also.

- The next critical step is the completion of RCTs comparing T_N-depleted PBSCT with standard unselected HCT, and with other promising approaches to GVHD reduction.

- One of the important challenges is to understand the GVL responses that are retained in T_N-depleted HCT with a view to further augmenting GVL in patients at a high risk of relapse. Conceivably, lymphocytes mediating GVL after T_N-depleted HCT could include a) T cells originating in the donor T_N population that have escaped depletion, b) antigen-inexperienced T cells that have undergone phenotypic conversion to memory-like T cells in the donor, or c) pathogen-specific memory T cells with cross reactivity against leukemia-associated antigens or minor H antigens (or other alloantigens in HLA-mismatched HCT) respectively. Other lymphocytes subsets involved in GVL after T_N-depleted HCT could potentially include NK cells, which are numerically increased after T_N-depleted HCT. Laboratory studies to understand GVL in T_N-depleted HCT are underway.

References

1. Shlomchik WD. Graft-versus-host disease. Nat Rev Immunol. 2007;7(5):340-52.

2. Koyama M, Hill GR. Alloantigen presentation and graft-versus-host disease: fuel for the fire. Blood. 2016;127(24):2963-70.

3. Bleakley M, Riddell SR. Molecules and mechanisms of the graft-versus-leukaemia effect. Nat Rev Cancer. 2004;4(5):371-80.

4. Pasquini MC, Devine S, Mendizabal A, Baden LR, Wingard JR, Lazarus HM, et al. Comparative outcomes of donor graft CD34+ selection and immune suppressive therapy as graft-versus-host disease prophylaxis for patients with acute myeloid leukemia in complete remission undergoing HLA-matched sibling allogeneic hematopoietic cell transplantation. J Clin Oncol. 2012;30(26):3194-201.

5. Ho VT, Soiffer RJ. The history and future of T-cell depletion as graft-versus-host disease prophylaxis for allogeneic hematopoietic stem cell transplantation. Blood. 2001;98(12):3192-204.

6. Lanzavecchia A, Sallusto F. Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. Science. 2000;290(5489):92-7.

7. Burrows SR, Silins SL, Khanna R, Burrows JM, Rischmueller M, McCluskey J, et al. Cross-reactive memory T cells for Epstein-Barr virus augment the alloresponse to common human leukocyte antigens: degenerate recognition of major histocompatibility complex-bound peptide by T cells and its role in alloreactivity. Eur J Immunol. 1997;27(7):1726-36.

8. Anderson BE, McNiff J, Yan J, Doyle H, Mamula M, Shlomchik MJ, et al. Memory CD4+ T cells do not induce graft-versus-host disease. J Clin Invest. 2003;112(1):101-8.

9. Anderson BE, Zheng H, Taylor PA, Matte-Martone C, McNiff JM, Jain D, et al. Memory T cells in GVHD and GVL. Biol Blood Marrow Transplant. 2008;14(1 Suppl 1):19-20.

10. Zhang Y, Joe G, Zhu J, Carroll R, Levine B, Hexner E, et al. Dendritic cell-activated CD44hiCD8+ T cells are defective in mediating acute graft-versus-host disease but retain graft-versus-leukemia activity. Blood. 2004;103(10):3970-8.

11. Chen BJ, Cui X, Sempowski GD, Liu C, Chao NJ. Transfer of allogeneic CD62L- memory T cells without graft-versus-host disease. Blood. 2004;103(4):1534-41.

12. Dutt S, Tseng D, Ermann J, George TI, Liu YP, Davis CR, et al. Naive and memory T cells induce different types of graft-versus-host disease. J Immunol. 2007;179(10):6547-54.

13. Chen BJ, Deoliveira D, Cui X, Le NT, Son J, Whitesides JF, et al. Inability of memory T cells to induce graft-versus-host disease is a result of an abortive alloresponse. Blood. 2007;109(7):3115-23.

14. Zheng H, Matte-Martone C, Li H, Anderson BE, Venketesan S, Sheng Tan H, et al. Effector memory CD4+ T cells mediate graft-versus-leukemia without inducing graft-versus-host disease. Blood. 2008;111(4):2476-84.

15. Zheng H, Matte-Martone C, Jain D, McNiff J, Shlomchik WD. Central memory CD8+ T cells induce graft-versus-host disease and mediate graft-versus-leukemia. J Immunol. 2009;182(10):5938-48.

16. Anderson BE, Tang AL, Wang Y, Froicu M, Rothstein D, McNiff JM, et al. Enhancing alloreactivity does not restore GVHD induction but augments skin graft rejection by CD4(+) effector memory T cells. Eur J Immunol. 2011;41(9):2782-92.

17. Dutt S, Baker J, Kohrt HE, Kambham N, Sanyal M, Negrin RS, et al. CD8+CD44(hi) but not CD4+CD44(hi) memory T cells mediate potent graft antilymphoma activity without GVHD. Blood. 2011;117(11):3230-9.

18. Juchem KW, Anderson BE, Zhang C, McNiff JM, Demetris AJ, Farber DL, et al. A repertoire-independent and cell-intrinsic defect in murine GVHD induction by effector memory T cells. Blood. 2011;118(23):6209-19.

19. Li N, Matte-Martone C, Zheng H, Cui W, Venkatesan S, Tan HS, et al. Memory T cells from minor histocompatibility antigen-vaccinated and virus-immune donors improve GVL and immune reconstitution. Blood. 2011;118(22):5965-76.

20. Zhang P, Wu J, Deoliveira D, Chao NJ, Chen BJ. Allospecific CD4(+) effector memory T cells do not induce graft-versus-host disease in mice. Biol Blood Marrow Transplant. 2012;18(10):1488-99.

21. Zhang J, Barefoot BE, Mo W, Deoliveira D, Son J, Cui X, et al. CD62L- memory T cells enhance T-cell regeneration after allogeneic stem cell transplantation by eliminating host resistance in mice. Blood. 2012;119(26):6344-53.

22. Huang W, Mo W, Jiang J, Chao NJ, Chen BJ. Donor Allospecific CD44(high) Central Memory T Cells Have Decreased Ability to Mediate Graft-vs.-Host Disease. Front Immunol. 2019;10:624.

23. Bleakley M, Otterud BE, Richardt JL, Mollerup AD, Hudecek M, Nishida T, 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(23):4923-33.

24. Bleakley M, Heimfeld S, Jones LA, Turtle C, Krause D, Riddell SR, et al. Engineering human peripheral blood stem cell grafts that are depleted of naive T cells and retain functional pathogen-specific memory T cells. Biol Blood Marrow Transplant. 2014;20(5):705-16.

25. Bleakley M, Heimfeld S, Loeb KR, Jones LA, Chaney C, Seropian S, et al. Outcomes of acute leukemia patients transplanted with naive T cell-depleted stem cell grafts. J Clin Invest. 2015;125(7):2677-89.

26. Muffly L, Sheehan K, Armstrong R, Jensen K, Tate K, Rezvani AR, et al. Infusion of donor-derived CD8(+) memory T cells for relapse following allogeneic hematopoietic cell transplantation. Blood Adv. 2018;2(6):681-90.

27. Maung KK, Chen BJ, Barak I, Li Z, Rizzieri DA, Gasparetto C, et al. Phase I dose escalation study of naive T-cell depleted donor lymphocyte infusion following allogeneic stem cell transplantation. Bone Marrow Transplant. 2020.

28. Triplett BM, Shook DR, Eldridge P, Li Y, Kang G, Dallas M, et al. Rapid memory T-cell reconstitution recapitulating CD45RA-depleted haploidentical transplant graft content in patients with hematologic malignancies. Bone Marrow Transplant. 2015;50(7):968-77.

29. Shook DR, Triplett BM, Eldridge PW, Kang G, Srinivasan A, Leung W. Haploidentical stem cell transplantation augmented by CD45RA negative lymphocytes provides rapid engraftment and excellent tolerability. Pediatr Blood Cancer. 2015;62(4):666-73.

30. Triplett BM, Muller B, Kang G, Li Y, Cross SJ, Moen J, et al. Selective T-cell depletion targeting CD45RA reduces viremia and enhances early T-cell recovery compared with CD3-targeted T-cell depletion. Transpl Infect Dis. 2018;20(1).

31. Mamcarz E, Madden R, Qudeimat A, Srinivasan A, Talleur A, Sharma A, et al. Improved survival rate in T-cell depleted haploidentical hematopoietic cell transplantation over the last 15 years at a single institution. Bone Marrow Transplant. 2020;55(5):929-38.