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Lymphoid Reconstruction and Vaccines

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ABSTRACT

Allogeneic HSCT is the most reliable, robust, and effective cell-based biotherapy currently available to pediatric and adult patients with hematologic malignancies. The central role of donor-derived lymphocytes in mediating an effective antitumor effect, preventing and controlling opportunistic infections, and causing GVHD is well documented in animal experiments and human trials. The profound lymphopenia after conditioning regimens coupled with molecular tools to distinguish host versus donor cells provides investigators a window into immune recovery after allogeneic HSCT. Serial analyses of T cell subsets linking immunophenotype with function have revealed the kinetics of donor-derived T cell recovery after allografting and provided insights into ways the immune system can be manipulated to augment the graft-versus-tumor (GVT) effect without inducing GVHD. As this review demonstrates, investigators are not limited to being passive observers of this immune reconstitution; rather, we have an opportunity to shape the allografted T cells repertoire to selectively augment immune function.

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KEY WORDS

Hematopoietic stem cell transplantation • Immune reconstitution • Adoptive immunotherapy •
Vaccination • Lymphodepletion

IMMUNE-BASED THERAPIES TO SUPPLEMENT ALLOGENEIC HSCT, AN EXAMPLE OF COMBINATION IMMUNOTHERAPY

Recipients of allogeneic HSCT benefit from a GVT effect in general and a graft-versus-leukemia (GVL) effect in particular, due to donor-derived T cells targeting miHAs selectively expressed by cells or cell subsets of a recipient's hematopoietic system [1]. This observation supports the rationale of infusing miHA-specific T cells after allogeneic HSCT to augment the GVL-effect. If the tissue distribution of the miHA is confined to the malignant cells, then this adoptive immunotherapy should not cause aGVHD. However, the development of cellular immunotherapy with effector cells of defined specificity and function is challenging and is not yet widely available. In contrast, antibody and cytokine therapies have already been successfully tested and used in treatment regimens for a range of human malignancies.

It is routine practice for patients with hypogammaglobulinemia to receive intravenous Ig after allogeneic HSCT. As antigen-specific mAb therapy has been incorporated into chemotherapy regimens, so too have oncologists incorporated these passive immunotherapy approaches into transplantation conditioning regimens. For example, rituximab is employed in conditioning regimens not just to cytoreduce malignant B cell burden [2] but also to deplete normal B cells leading to a reduction in the risk for GVHD [3]. Building on the potential of CD20-directed mAb therapy to selectively cytoreduce patients before HSCT, investigators have successfully used iodine 131 tositumomab and ytterbium 90 ibritumomab in myeloablative doses in blood and BM transplantation protocols for high-risk patients [4-8].

Cytokine therapy has also been used widely to support allogeneic HSCT. For example, supraphysiologic dosing of G-CSF is used to mobilize donor HSCs and as prophylaxis in an attempt to improve myeloid engraftment, although the latter may significantly add to the economic burden of HSCT [9,10]. The infusion of low doses of recombinant human IL-2

is tolerated after successful allografting, but has a checkered history when used as an immunomodulator because it has not been associated with an improvement in rates of relapse [11,12]. However, there is a resurgence of the use of this cytokine, especially at low doses. In particular, recombinant human IL-2 is being used in vitro to help propagate clinical-grade T cells and NK cells for adoptive immunotherapy and in vivo as a surrogate for a deficient endogenous Th response to help sustain proliferation of adoptively transferred CD8$^+$ T cells and to improve the survival of infused NK cells.

DAY 0—NOT JUST ONE DAY

Allogeneic HSCT is evolving from a field where all cell therapy was provided on "day 0" (the day marking infusion of HSCs) to that where a continuum of therapies is applied to meet patient needs and minimize attendant toxicities. The period after completion of conditioning therapy to engraftment of functional lymphocytes is a unique opportunity for immunotherapists. Thus, although day 0 and infusion of the allograft is a signature event, there are additional day 0s signifying infusion of immune cell-based products, such as antigen-experienced lymphocytes, NK cells, and APCs.

T CELL DEPLETION—ARE WE THROWING OUT THE GOOD WITH THE BAD?

Allografts from BM and G-CSF-mobilized peripheral blood have been ex vivo depleted of T cells to broaden the donor pool and application of allogeneic HSCT. Profound T cell depletion (TCD) regimens can be used to safely engraft HSCs from haploidentical donors, but the loss of allogeneic T cells renders the recipients severely immunocompromised and thus vulnerable to infection and relapse. The relative risk-versus-benefit of TCD versus T cell-replete allogeneic HSCT must be weighed for individual patients (Figure 1).

To decrease the threat of TCD, investigators are experimenting with the timely add-back of donor-derived T cells that have been stripped of potential unwanted allogeneic reactivity [13-18]. However, there is a new appreciation that inducing transient lymphopenia may be worth the risk, because the T cell depleted environment may provide a special instance to manipulate the recipient's immune system. This moment comes about due to loss of Treg cells, freeing up of lymphoid "space," and availability of limiting amounts of endogenous cytokines (eg, IL-15 and IL-7). In this environment, adoptively transferred T cells and NK cells can undergo proliferation by taking advantage of the homeostatic control mechanisms that may restore the peripheral pool of lymphocytes [19,20].
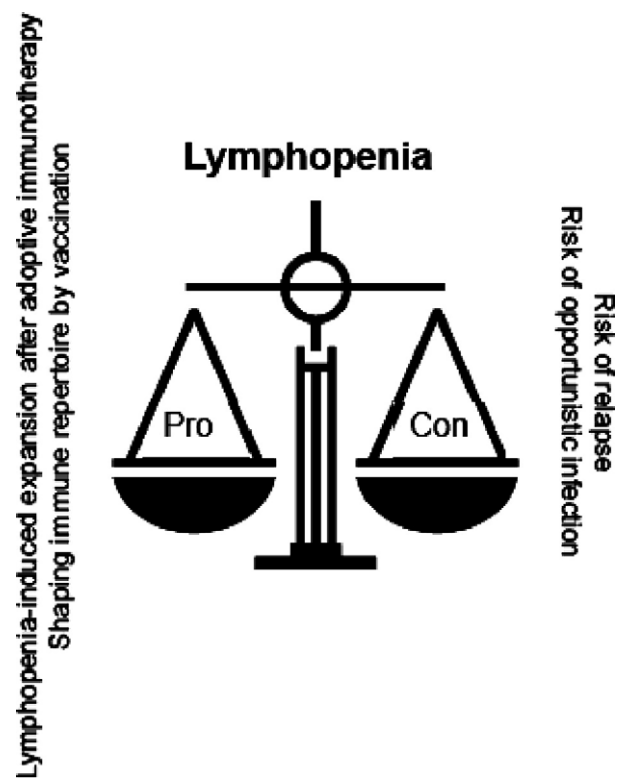

Figure 1. Risk and benefit of inducing lymphopenia after allogeneic HSCT.

This approach has been exploited at the National Cancer Institute for treatment of melanoma using adoptive transfer of autologous melanoma-specific T cells expressing endogenous or introduced melanoma-specific α/β TCRs. These T cells, which have been numerically expanded ex vivo, have successfully treated melanoma tumor deposits when infused after a lymphocyte-depleting regimen of cyclophosphamide and fludarabine [21,22]. The antitumor effect is dependent on (1) potency (using tumor-specific T cells with a defined α/β TCR) and (2) T cell "area under the curve" (measurement of the number of infused antigen-specific T cells over time). Recent data suggest that the recovery of CD8$^+$ T cell response in the setting of lymphopenia requires CD4$^+$ T cells, which may be one reason why adoptive transfer of melanoma-specific CD8$^+$ T cell clones failed to proliferate in vivo [23,24].

Extrapolating these data to allogeneic HSCT, we hypothesize that lymphocyte-depleting preparative regimens may facilitate the therapeutic potential of immunotherapies employing adoptive T cell transfer. Lympho-depletion may also benefit the efficacy for tumor-specific vaccines. This is based on the premise that (1) dose-intensive chemotherapy can lengthen the period of progression-free survival, thus allowing time for a slow-acting therapy such as vaccination to be effective, (2) maximally decreasing the recipient's tumor burden may increase the effectiveness of immunotherapy by mechanisms including decreases

in tumor-induced immunosuppressive effects, and (3) providing tumor antigen exposure after immune depletion in the form of repeat immunizations may take advantage of the antigen-driven peripheral T cell expansion that characterizes immune reconstitution at early time points. The T cell repertoire is then biased toward tumor antigens and antitumor responses at later time points [25].

Investigators have also combined vaccine therapy with adoptive immunotherapy to improve T cell effector function. This has particular appeal to augment cellular immunity after autologous HSCT. Pre-chemotherapy T cells, especially if primed, are very effective for adoptive immunotherapy in conjunction with repeat tumor antigen exposure, when administered after lymphocyte-depleting chemotherapy [26].

IMMUNE RECONSTITUTION AFTER ALLOGENEIC HSCT—RIGHTING THE WRONG

Allogeneic HSCT cures many patients with a history of relapsed or high-risk hematologic malignancies. To broaden the application of allogeneic HSCT, alternative nonsibling donors are often recruited; but preparing the host and/or allograft from these donors typically causes disturbances to the recipient's immune system and the newly allografted recipient is profoundly immunocompromised. Thus, despite reduction in intensity of conditioning regimens and improvements in supportive care, infection remains an important cause of morbidity and mortality after allogeneic HSCT and delayed T cell immune recovery is typically a primary risk factor for death due to infection. When GVHD occurs, the risk of death due to infection is further increased due to direct effects of GVHD on the recovering immune system and the consequences of application of immunosuppressive agents.

Prospective analyses of immune reconstitution parameters reveal a hierarchy of disturbances that are associated with (a) selection of a donor and source of a donor's HSCs, (b) number of infused donor-derived HSCs, (c) ex vivo manipulation of donor-derived HSCs to reduce T cells in general or alloreactive T cells in particular, and (d) use of immunosuppressive medications such as corticosteroids. For example, TCD of the allograft or use of an umbilical cord blood (UCB), particularly a small UCB unit relative to recipient weight, leads to profound delay in recovery of lymphocyte number and disruption of function after allogeneic HSCT. Some maneuvers may shorten this period of functional and numerical lymphopenia, such as (a) infusion of megadoses of donor-derived TCD HSCs, (b) combining two UCB units or ex vivo-expanded UCB units, (c) enhancing thymic function (as proposed by administration of leuprolide), or (d) infusion of donor-derived T cells (in some cases

expressing a conditional suicide gene to eliminate infused T cells in the event of severe toxicity) [27-29].

Prolonged and profound lymphopenia renders the transplanted recipient at high risk for opportunistic infection. Studies of opportunistic viral infections have improved our understanding of the central role T cells have in control of CMV, EBV, varicella zoster virus, adenovirus, and BK virus infections. To prevent and treat these viral infections, investigators have harvested virus-specific memory T cells from donors, expanded them ex vivo, and infused them after allogeneic HSCT [30-34]. As ex vivo cell propagation technology has evolved and our understanding has deepened of what subpopulations of T cells might be the most effective in vivo, investigators have used adoptive immunotherapy of viral disease to advance methods to (a) infuse T cells soon after day 0, (b) adoptively transfer both CD4$^+$ and CD8$^+$ populations of antigen-specific T cells versus CD8$^+$ clones, and (c) exploit selection methods (eg, using paramagnetic beads) for rapid acquisition of minimally manipulated antigen-specific T cells (identified by tetramer binding or T$_c$1 cytokine secretion). Such lymphocytes include cells that are likely less polarized in their ex vivo differentiation into effector T cells and less likely to undergo replicative senescence compared with T cells harvested from ex vivo cultures that have undergone multiple repetitive cycles of stimulation to achieve numeric expansion (and possibly selection of an introduced transgene) [35,36]. These adoptive transfer studies rely on the premise that antigen-specific T cells can be identified in the donor. In the event that the donor is seronegative, then vaccination may be undertaken, primary T cells may be genetically manipulated to achieve redirected specificity, or in vitro priming of antigen-inexperienced T cells may be attempted.

T CELL REGENERATION—IT'S NOT JUST ABOUT NUMBERS

Characterizing the kinetics of the emerging allografted immune system has defined two main pathways of T cell regeneration. In the absence of thymic function, appreciable populations of immunecompetent T cells are generated by peripheral expansion of mature T cells. This peripheral expansion pathway is influenced by antigen and therefore prone to skewing by vaccination. Recovery of thymic function, the route of T cell regeneration after allografting, may be important for the long-term health of the immune system, because thymopoiesis is sufficient to contribute effectively to reconstitution of lost T cell populations.

To understand engrafted T cell function, investigators have employed quantitative tools to show how the TCR repertoire recovers after allogeneic HSCT. Cytokine flow cytometry can assess the number and
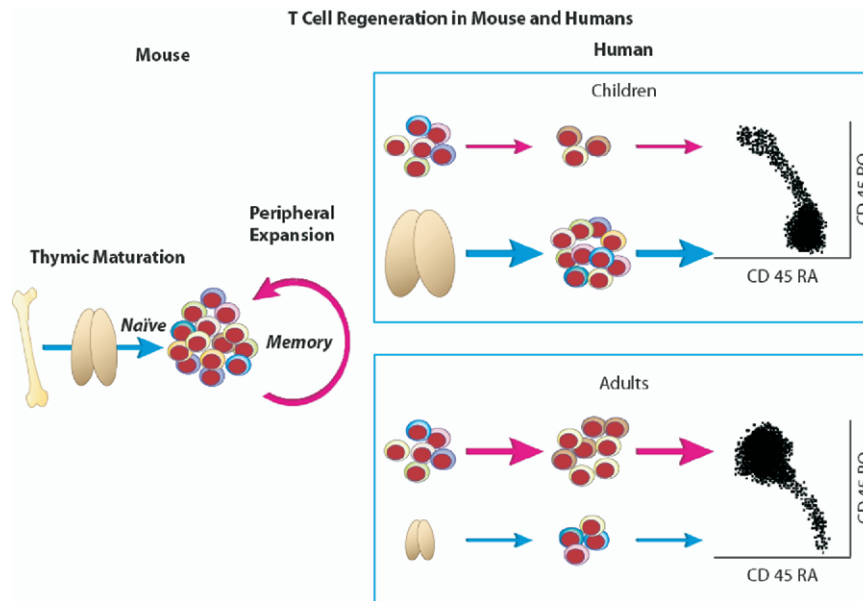
Figure 2. Routes of T cell recovery in peripheral blood after lymphopenia.

function of CD4$^+$ and CD8$^+$ T cells at a single-cell level. This approach can demonstrate how the number, function, and maturation status of antigen-specific T cells correlate with protective immunity to individual pathogens capable of causing disease in recipients (eg, CMV) [37,38]. In addition, we have learned that the thymus, known to be the primary site of lymphopoiesis in childhood, but assumed to be dormant in adults, still functions after HSCT and contributes to T cell immune recovery, even in some adult recipients (Figure 2) [39-41].

Studies correlating T cell function (assessed by cytokine flow cytometry) with TCR repertoire (assessed by binding of tetramer) have led to an understanding that there are discrepancies in recovering T cell populations (eg, after UCB transplantation) between the number of antigen-specific T cells and their function [42]. Thymic function plays a central role in regenerating naive T cell function and most patients can recover thymic function in the weeks to months after allogeneic HSCT. These data support the use of post-thymic interventions (eg, androgen blockade, administration of desired cytokines such as IL-7) to augment immunity after allogeneic HSCT and increase the number and diversity of naive T cells capable of responding to (vaccine) antigens. However, for those patients with impaired thymic function, eg, recipients of UCB transplantation, the delay in thymic recovery may be associated with functional "holes" in the T cell repertoire and increased risk of opportunistic infection. This understanding of immune reconstitution has repercussions for vaccination and there are three potential strategies for optimizing or exaggerating T cell response to tumor-antigens in the setting of allogeneic HSCT: (a) early vaccination after cessation of lymphodepleting chemotherapy to take advantage of antigen-driven T cell reconstitution by peripheral expansion, (b) adoptive transfer during a period of lymphopenia of T cells presensitized to specific antigens of choice or rendered specific to a desired antigen, and (c) late vaccination to take advantage of a new, repaired T cell repertoire that appears with thymic recovery in a subset of patients.

DONOR-DERIVED NK CELLS—NATURALIZED KILLER CELLS?

It is now accepted that some patients with myeloid malignancies who are the recipients of haplo-disparate grafts develop an improved GVL-effect when the donor and recipient killer cell Ig-like receptors (KIRs) are appropriately mismatched, leading to donor-derived NK cells being released from inhibition [43,44]. NK cells are part of the innate immune response, and as the first lymphocyte subset to engraft, they have been implicated in contributing to the GVT-effect and suppression of GVHD [45,46]. The question for oncologists is how to (a) reliably predict this NK-mediated effect, (2) broaden the applicability to malignancies other than AML, (c) harness the NK-effect for HLA-matched transplants, and (d) improve the NK cell-versus-tumor effect.

Correlating immunogenetics with NK cell function in vitro and in vivo has improved our understanding of the receptor-ligand barriers that serve to keep NK cell function quiescent. The relative activation state of NK cells is governed as an amalgamation of activating and inhibitory signals that are currently being elucidated. The NK cell inhibitory signals include the inhibitory KIRs, which have specificities for

HLA class I molecules. Thus cells are susceptible to lysis by activated NK cells if the targets lack expression of classical HLA class I molecules. Further, if the target cells in the recipient lack an HLA class I allele present in the donor HLA genotype and the recipient's HLA class I is not a ligand for the KIR on donor NK cells, then the allogeneic donor NK cells can be activated for cytolysis and mediate an anti-leukemic effect, especially if the target cells are AML blasts. The activation of NK cells due to this missing KIR ligand effect is possible because the KIR and HLA genes segregate independently of each other, which results in the phenotype of persons who lack KIR receptors for their respective HLA ligands and persons who lack HLA ligands for their respective KIR receptors. The predictive models of allogeneic NK cell behavior based on immunogenetics has led to the preselection of donors for haploidentical HSCT based on KIR and HLA genotyping. However, these models of donor-recipient genetics (KIR and HLA typing) and phenotype (NK-mediated killing) remain incomplete and this limits the application of NK cell therapy to other malignancies and reliably extrapolating the NK effects outside the haploidentical HSCT setting [47,48].

Because animal models of allogeneic NK cell immunobiology and antitumor effect are generally of uncertain clinical significance, investigators have piloted phase I/II clinical studies to directly assess the role of infused allogeneic NK cells. These trials adoptively transfer haploidentical (CD3-depleted and IL-2 activated) NK cells into lymphopenic patients with hematologic and solid tumors and demonstrated that an antitumor effect is dependent on (a) potency, achieving the appropriate mismatch between donor KIR and recipient classic HLA molecules, and (b) the NK cell area under the curve, sustaining the persistence of infused NK cells after lymphodepleting chemotherapy with an associated increase in endogenous IL-15 and accompanied by infusion of exogenous IL-2 [49]. Oncologists are currently combining haploidentical HSCT and adoptive transfer of activated NK cells to infuse NK cells before or after allogeneic HSCT in an effort to limit relapse rates after HSCT.

THE FUTURE OF COMBINATION IMMUNOTHERAPIES

Profound lymphopenia after allogeneic HSCT can result in death of the recipient from opportunistic infection or relapse due to an incomplete GVT-effect. But it also represents an opportunity. Oncologists are familiar with balancing risk/benefit ratios and recognize the therapeutic power of immunotherapy, and are consequently an ideal group of investigators to advance combination immunotherapies. Investigators are already combining cellular therapy with lymphodepleting chemotherapy and antibody therapy. In the future other combinations will be realized: (a) immunotherapy with gene therapy to introduce new cellular functions or remove impediments to function, (b) adoptive immunotherapy with vaccine therapy to deliver an effector cell population and an immune stimulus, (c) cytokines and antibodies (immunocytokines), (d) antibodies (or immunocytokines) and T cells, using genetic manipulation to introduce mAb-derived chimeric antigen receptors to redirect the specificity of T cells, and (e) antibodies (or immunocytokines) and Fc receptor-expressing NK cells to achieve tumor-specific NK cell-mediated antibody-dependent cellular cytotoxicity. It is anticipated that these active and passive immune-based therapies will be individualized for each patient based on an understanding of immune recovery.

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