Citation: Cellular Therapy and Transplantation, Vol. 2, No. 6, 2009

Please cite this article as follows: *Rossig C. Graft-versus-Leukemia (GVL) activity in childhood leukemias. Cell Ther Transplant. 2009;2:e.000049.01. doi:10.3205/ctt-2009-en-000049.01*

Submitted: 24 July 2009, accepted: 1 September 2009, published: 7 September 2009

Graft-versus-Leukemia (GVL) activity in childhood leukemias

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Abstract

Allogeneic hematopoietic stem cell transplantation (HSCT) today contributes significantly to the cure of children with high-risk leukemias. The contribution of cellular graft-versus-leukemia (GVL) reactions to the anti-leukemic effects of allogeneic transplantation in pediatric leukemias has not been clarified in detail. Evidence is mainly based on indirect associations of clinical signs of alloreactivity with maintenance of full donor chimerism and relapse-free survival. Therapeutic interventions aimed at deliberately enhancing alloreactive donor cells via early reduction of immunosuppression or administration of donor lymphocytes are limited by the occurrence of graft-versus-host-disease (GVHD). Therefore, an important goal in advancing the use of allogeneic HSCT as treatment for childhood leukemias is the development of therapeutic strategies that induce or augment GVL effects while avoiding GVHD. One potential strategy relies on genetic modification of the receptor specificity of T cells or NK cells to recognize leukemia-associated antigens. Current efforts further focus on an optimal in vivo functionality of therapeutic T cells, including homing to the leukemia microenvironment, persistence, and capacity for specific reactivation.

Keywords: leukemia, pediatric oncology, T cells, immunotherapy, adoptive T cell transfer

Evidence for GVL effects in childhood leukemias

The majority of childhood leukemias have become curable via multi-agent chemotherapy. In subsets of children with high risk molecular subtypes of leukemia, poor response to induction chemotherapy or disease relapse, allogeneic hematopoietic stem cell transplantation (HSCT) today significantly contributes to relapse-free survival [1-3]. Both donor T cells and NK cells within the stem cell graft have been suggested to mediate potent anti-leukemic responses accounting for control of minimal residual disease and maintenance of remission. Unfortunately, data regarding the contribution of GVL activity to clinical disease control is limited by the lack of valid methods for assessing immunological rejection of leukemia cells by donor-derived immune effector cells in vivo. Indirect evidence is provided by correlations of relapse-free survival (RFS) with the extent of HLA compatibility between donor and recipient, or with the occurrence of acute or chronic graft-versus-host disease (GVHD). Furthermore, the effects of tapering immunosuppressive therapy or of administering donor lymphocyte infusions (DLIs) on either RFS or donor chimerism and/or minimal residual disease (MRD) are interpreted as evidence for the anti-leukemic activity of donor T cells.

One example in childhood leukemias is based on a case report demonstrating rapid return of donor chimerism and sustained remission in a patient with relapsed juvenile myelomonocytic leukemia (JMML) after HSCT in response to a single dose of DLIs [4]. JMML is a rare clonal myelopro-

liferative disorder of early childhood for which allogeneic HSCT is the only curative strategy, resulting in event-free survival of about 50% of the children [5]. In a larger series of 21 children who received DLIs for mixed chimerism or relapse after allogeneic HSCT, responses were found in six patients [6]. Five of the responders developed GVHD following DLI, thus arguing against the specific recognition of leukemia-associated antigens and demonstrating the limitations of this strategy by alloreactivity against non-hematopoietic targets.

While a large number of studies have demonstrated an important role of GVL effects in adult patients receiving allogeneic transplants for acute myelogenous leukemia (AML) [7], experience in childhood AML is limited. Among 19 of 81 pediatric AML patients with increasing mixed chimerism, 15 children who received early immunological intervention had an increased probability for event-free survival (pEFS 36%) compared to the 4 patients without intervention (pEFS 0%, $P<0.05$) [8]. In another series of 13 children with high-risk AML in first or second remission, immunotherapeutic interventions after allogeneic HSCT, aiming at inducing limited GVHD, combined with a uniform myeloablative preparative regimen, yielded encouraging early results [9]. Studies in a larger number of children are needed to assess the potential benefits of inducing alloreactivity in childhood AML.

The role of GVL effects in acute lymphoblastic leukemia (ALL), including childhood ALL, is controversial. Suggestive for a contribution of alloreactivity to sustained remission after allogeneic HSCT was the observation that among 36 children with high-risk or relapsed ALL, relapses occurred only in those receiving transplants from HLA-matched sibling donors (MSD, 8/13), while children after alternative donor HSCT remained disease-free (0/13)2. A similar observation was reported from an independent cohort of 71 children, with a 3-year cumulative incidence of relapse of 55.6± 12.3% for MSD versus 22.0±8.1% for MUD recipients ($P=0.03$) [10]. By contrast, a recent study demonstrated comparable outcomes of MSD (n=41) versus alternative donor transplantations (n=42) in children with ALL receiving an identical TBI-based preparative regimen, with alemtuzumab administered to alternative donor HSC recipients [11]. In summary, no definite conclusions can be drawn regarding potentially superior GVL effects of unrelated grafts and their contribution to the maintenance of remissions. An anti-leukemic effect of chronic GVHD in childhood ALL was suggested by the results of a retrospective analysis of 450 children receiving allogeneic HSCT for hematologic malignancies, demonstrating a significantly reduced relapse probability in patients with chronic GVHD [12]. Importantly, stratifying the analysis by type of malignancy revealed a stronger correlation of chronic GVHD with relapse-free survival in ALL compared to other types of malignancies.

An important goal in advancing allogeneic HSCT for childhood leukemias is the development of therapeutic strategies that induce or augment GVL effects while avoiding clinical GVHD caused by alloreactivity with normal cells. In adults, administration of donor lymphocytes after HSCT has become an established treatment for high-risk myeloid malignancies [13]. The role of DLIs in childhood leukemias is less clear. In a cohort of 23 children with high-risk ALL, transfer of donor lymphocytes to children with decreasing donor chimerism prevented relapse in at least a proportion of patients [14]. However, unselected DLIs fail to rescue the majority of the children, and clinical GVHD remains a significant limitation to increasing cell doses. Efficient strategies for separating leukemia-reactive donor T cells from those responsible for GVHD are not yet available. While selective depletion of alloreactive T cells by an anti-CD25 immunotoxin prior to infusion of donor T cells following T-cell-depleted haploidentical SCT efficiently prevented GVHD [15], 7 of the 16 patients with malignant diseases in this cohort relapsed, arguing against preserved anti-leukemic responses after allodepletion.

Strategies for augmenting GVL effects in childhood leukemia

One means of generating large numbers of leukemia-specific T cells is genetic modification with antigen-specific chimeric receptors (CARs). CARs consist of an antibody-derived single chain Fv domain linked to a cytoplasmic signaling domain, generally derived from the T cell receptor (TCR) ζ chain [16]. Thereby, they redirect the T-cell effector function to a defined surface antigen and allow for the recognition of target cells in an HLA-independent manner. Increased awareness of the importance of co-stimulatory signaling in T cell activation has led to the design of "second-generation" CARs with co-stimulatory signaling components. These optimized CARs indeed promote superior T cell proliferation, persistence and tumor control in vivo [17,18]. Recently, T cells expressing antigen-specific CARs have entered the first clinical trials [19-21]. While CAR gene-modified polyclonal donor T cells can mediate alloreactivity via their native receptors, genetic engineering of T cells with native specificity for a viral antigen may reduce the risk of GVHD while contributing to the reconstitution of virus-specific immune responses following HSCT [22,23].

The specificity and efficacy of the approach depend on the choice of an appropriate target antigen expressed at high densities on the leukemia cells. An attractive target structure for B-cell malignancies, including B-cell precursor ALL of

childhood, is the B-lineage antigen CD19. The specificity of CD19 for the leukemic clone is limited by co-expression on normal B precursor cells and B cells, which will result in a transient defect of B-cell maturation as an undesired side effect of effective CD19-directed immunotherapy. CD19-specific CARs have been shown by various investigators to mediate potent anti-leukemic T-cell responses both in vitro and in vivo [24-26], and their therapeutic efficacy is being evaluated in ongoing clinical studies.

Besides modifying the antigen specificity of T cells, CARs can be used to redirect natural killer cells (NK cells) to leukemia cells. Alloreactive NK cells have been shown to persist in pediatric patients following KIR ligand-mismatched haploidentical HSCT, and are considered as being important players in anti-leukemic cellular immune responses after transplantation [27]. Protocols for the large-scale in vitro activation and expansion of human NK cells for adoptive transfer have been developed [28]. Expression of CD19-specific CARs in human NK cells [29,30] triggers powerful and antigen-specific stimulatory signals, inducing cytolysis of otherwise NK-cell resistant leukemia cell lines and autologous leukemia cells [29,31].

Key issues regarding long-term control of leukemia by cellular immunotherapies are in vivo persistence and functional reactivation of the transferred effector cell populations within the immunosuppressive leukemic microenvironment. Dual-specific T cells with native specificity for a strong viral antigen, e.g. EBV, reengineered to interact with leukemia-associated antigens via CARs, may have superior persistence in vivo and receive potent reactivation stimuli via CARs. Indeed, CAR-transduced EBV-CTLs have been shown to persist for several weeks in the peripheral blood of neuroblastoma patients [21]. Alternative designs of dual-specific T-cells, e.g. carrying a specificity against varicella-zoster virus, might even allow for vaccination strategies to boost and prolong the presence of specific effector cells in vivo [22].

Persistence of gene-modified T cells was further shown when the transfer of gene-modified T cells was preceded by a lymphodepletive regimen [32], which was attributed to a favorable homeostatic cytokine environment and by elimination of regulatory T cells. Selective depletion of regulatory T cells by the CD25-specific antibody denileukin difitox in melanoma patients enhanced the efficacy of a tumor vaccine; however, responsive patients also had severe autoimmune manifestations [33]. Therefore, selective elimination of regulatory T cells in patients after allogeneic HSCT, will likely induce or augment clinical GVHD—again illustrating the dilemma of obtaining a favorable balance between GVL and GVHD.

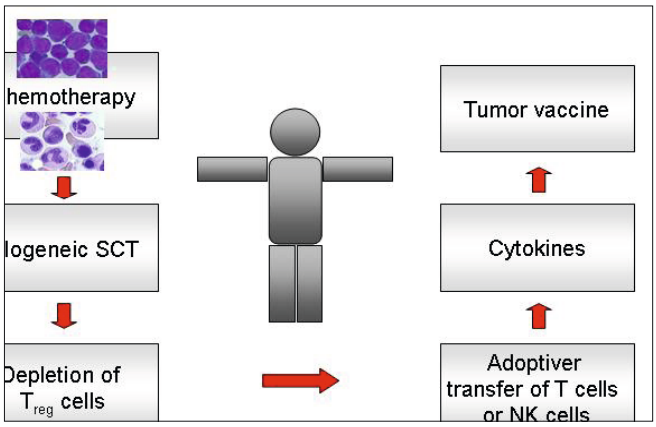

Figure 1. Implementation of immunotherapeutic strategies into the treatment of high-risk childhood leukemia.

Conclusions

Specific cellular immunotherapeutic strategies may prove effective in preventing relapse in children who cannot be cured by allogeneic HSCT alone. Using molecular minimal residual disease monitoring, patients with the highest risk of relapse can now be identified prior to HSCT [1], thus providing a platform for evaluation of the efficacy of immune-based strategies. Besides maximal reduction of leukemia bulk prior to cellular therapy, selective depletion of regulatory T cells, as well as generation of an optimized cytokine milieu and subsequent boosting of anti-leukemic immune responses by vaccination may contribute to generating therapeutic GVL effects (Figure 1).

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Please cite this article as follows: *Rossig C. Graft-versus-Leukemia (GVL) activity in childhood leukemias. Cell Ther Transplant. 2009;2:e.000049.01. doi:10.3205/ctt-2009-en-000049.01*

Ссылка: Cell Ther Transplant. 2009;2:e.000049.01. doi:10.3205/ctt-2009-en-000049.01

Реакция «трансплантат против лейкоза» (РТПЛ) при лейкозах у детей

Клаудиа Россиг

Резюме

Трансплантация аллогенных гемопоэтических клеток (алло ТГСК) вносит существенный вклад в лечение детей, больных лейкозом с высокой вероятностью рецидива. Роль и влияние реакции «трансплантат против лейкоза» (РТПЛ) на течение лейкоза у детей при аллогенной трансплантации не имеет детального объяснения. Эффективность в этих ситуациях основывается главным образом на связи клинических признаков наличия аллореактивности со стабильным полным донорским химеризмом и выживаемостью пациентов на фоне отсутствия рецидивов. Терапевтические подходы, специально направленные на усиление аллореактивности донорских клеток посредством ослабления иммуносупрессивных воздействий или введения лимфоцитов донора, ограничены возможностью развития реакции «трансплантат против хозяина» (РТПХ). Поэтому важной задачей на путях более широкого внедрения трансплантации аллогенных гемопоэтических стволовых клеток для лечения лейкозов у детей является разработка лечебной стратегии, которая позволит усилить РТПЛ и одновременно избежать РТПХ. Одна из таких стратегий подразумевает генетическую модификацию специфических рецепторов Т-клеток или НК-клеток, которая позволила бы распознавать антигены, ассоциированные с лейкозом. Современные разработки сфокусированы на дальнейшей оптимизации in vivo функциональной активности Т-клеток, в том числе их расселения («хоминга») в лейкозном микроокружении, длительного пребывания и способности к специфической реактивации в этой среде.

Ключевые слова: лейкоз, детская онкология, Т-клетки, иммунотерапия, адоптивный перенос Т-клеток

Ссылка: Cell Ther Transplant. 2009;2:e.000049.01. doi:10.3205/ctt-2009-en-000049.01