

Interdisciplinary Cancer Research 2

Nima Rezaei *Editor*

Cancer Treatment: An Interdisciplinary Approach

 Springer



Immunological Properties of Manipulated Hematopoietic Stem Cell Grafts

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Abstract

Hematopoietic stem cell (HSC) graft manipulations comprise immunologic laboratory techniques designed for negative selection (i.e., reduction of unwanted cells) and/or positive selection (i.e., enrichment of the desired hematopoietic cells), in order to optimize the cellular composition and immunological properties of stem cell grafts and to improve clinical outcomes of hematopoietic stem cell transplantation (HSCT). In this chapter, the technique of different cellular manipulations on HSC grafts has been explained briefly. The immunological properties of each type of manipulation are discussed separately. An update on the conducted clinical trials implementing various manipulative methods and their outcomes are also provided. Apparently, designed or manipulated grafts have provided a safe platform for improving HSCT outcomes.

Keywords

Enrichment · Graft manipulation · Graft-versus-host disease · Hematopoietic stem cell transplantation · Rejection · T cell depletion

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1 Introduction

Hematopoietic stem cell (HSC) graft manipulations comprise immunologic laboratory techniques designed for negative selection (i.e., reduction of unwanted cells) and/or positive selection (i.e., enrichment of the desired hematopoietic cells), in order to optimize the cellular composition and immunological properties of stem cell grafts and to improve clinical outcomes of hematopoietic stem cell transplantation (HSCT).

Successful allogeneic HSCT involves ablation or attenuation of the recipient's blood and immune system by preparative conditioning regimen, ensued by engraftment of donor HSCs in bone marrow niche of the recipient and reconstitution of hematopoietic cell lineages and immune cells (Peci et al. 2022). However, graft rejection, graft-versus-host disease (GvHD), severe infections, and disease relapse remain to be the major complications faced throughout transplant process. Recent progress in understanding the transactions between specific cell subsets in HSC grafts has led to novel strategies of cellular manipulation in transplant platforms, which could allow prevention of graft rejection and GvHD together with increasing graft-versus-tumor/leukemia (GvT/L) effect, more rapid immune reconstitution, and augmented infection protection.

This review aims to summarize the method of different cellular manipulations on HSC grafts and to discuss their immunological properties and clinical effects.

2 Types of Hematopoietic Stem Cell Graft Cellular Manipulation

The main cellular components of an unmanipulated HSC graft include the hematopoietic progenitors, dendritic cells (DCs), monocytes, granulocytes, T and B lymphocytes, natural killer (NK) cells, and so on (Fig. 1) (Biernacki et al. 2020).

Optimization of graft cellular composition has allowed to achieve steady engraftment beyond major HLA barriers, with negligible transplant complications and improved survival rates. In these procedures, anti-HSC, anti-T, and/or anti-B cell antibodies conjugated with superparamagnetic microbeads or immunomagnetic beads cooperate for separating target cells within a strong magnetic separation column (Avecilla et al. 2016).

2.1 Depletion (Targeted Negative Selection)

Depletion of different cellular elements in HSC graft was previously based on agglutination and elimination of unwanted cells (Aversa et al. 1994; Soiffer et al. 1997). Not attaining good manufacturing practice (GMP) standards and being resource-intensive led to introduction of a more efficient immunomagnetic system (CliniMACS system, Miltenyi Biotec, Bergisch-Gladbach, Germany). In this structure, magnetically labeled cells are held in the separation column, while other

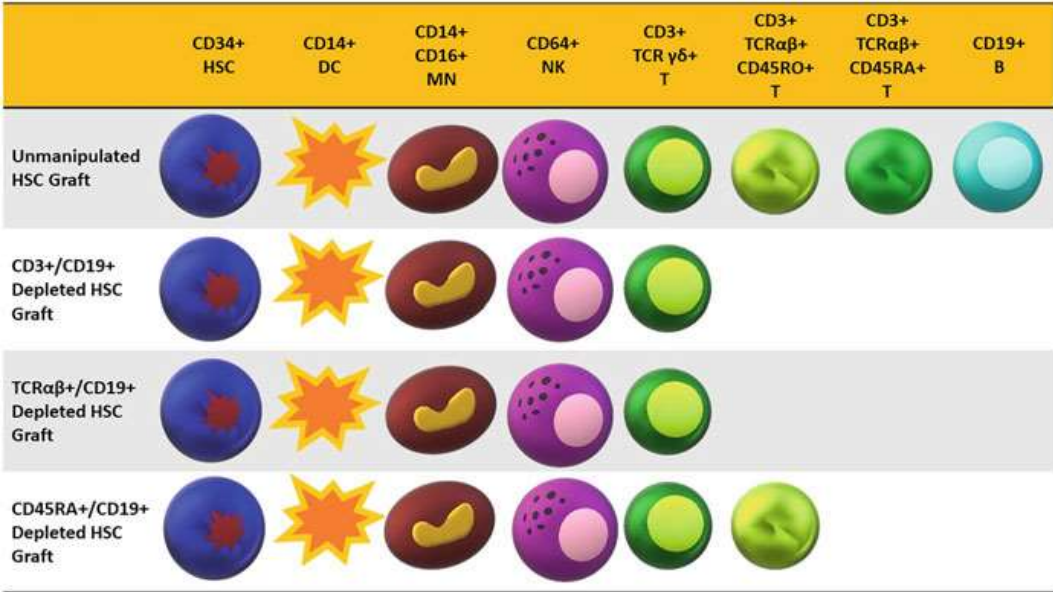


Fig. 1 Cellular composition of unmanipulated and manipulated hematopoietic stem cell grafts. *B* B lymphocyte, *DC* dendritic cell, *HSC* hematopoietic stem cell, *MN* monocyte, *NK* natural killer, *T* T lymphocyte, *TCR* T cell receptor

non-labeled cells stream into the column and are stored up in a collection bag eventually. Likewise, non-labeled cells are let out from the separation column and cumulated in the negative fraction bag (Fig. 2) (CliniMACS 2020).

2.1.1 CD3+ T Cell Depletion

Cluster of differentiation (CD) 3 is a multimeric protein complex, which functions as a T cell co-receptor, expressed with T cell receptor (TCR) α/β or TCR γ/δ heterodimers, crucial for T cell ontogeny (Smith-Garvin et al. 2009). CD3 antigen is a reliable marker for identification of normal T cells and also T cell neoplasms (Salvadori et al. 1994).

Depletion of CD3+ T cells could be conducted by CliniMACS LS or DTS tubing set (Schumm et al. 2013). By this method, a 4.15-log depletion of T cells is reported (Lang et al. 2014).

TCRαβ+ T Cell Depletion

TCRαβ is a heterodimer receptor comprised of the two α and β transmembrane glycoprotein chains, expressed on the majority of peripheral blood T cells. TCRαβ has a variable region which is involved in antigen recognition (CliniMACS 2020).

By means of magnetic microbeads conjugated to a murine monoclonal biotinylated anti-TCRαβ antibody together with an anti-biotin antibody (Fig. 3), TCRαβ+ T cells could be removed from the graft (CliniMACS 2020).

Efficacy of T cell depletion (TCD) with TCRαβ reagent is satisfactory (4.7 log) and is shown to be more effective than CD3+ depletion (4.0 log) and comparable to CD34+ enrichment (4.6 log) (Carreras et al. 2019). By implementation of TCRαβ+

Fig. 2 Magnetic cell separation system

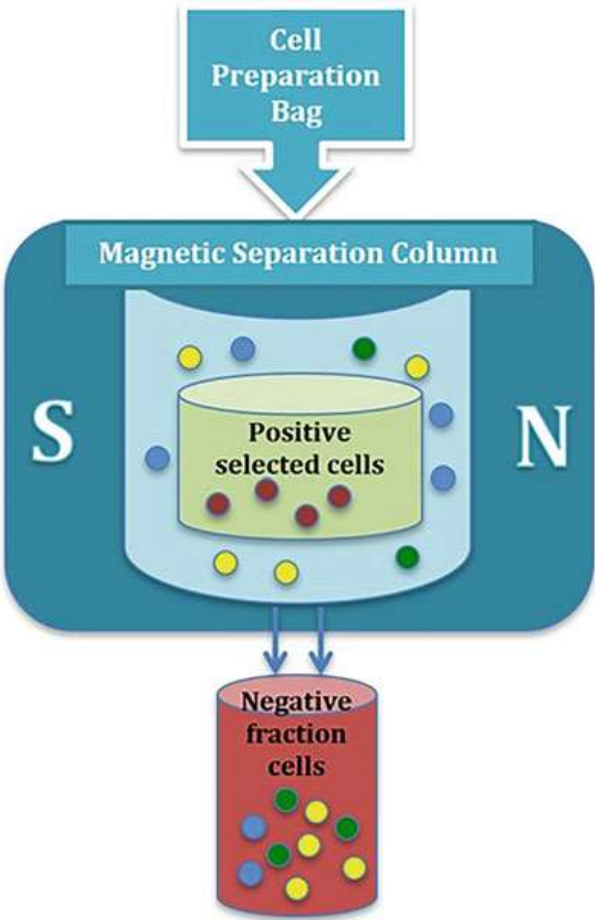
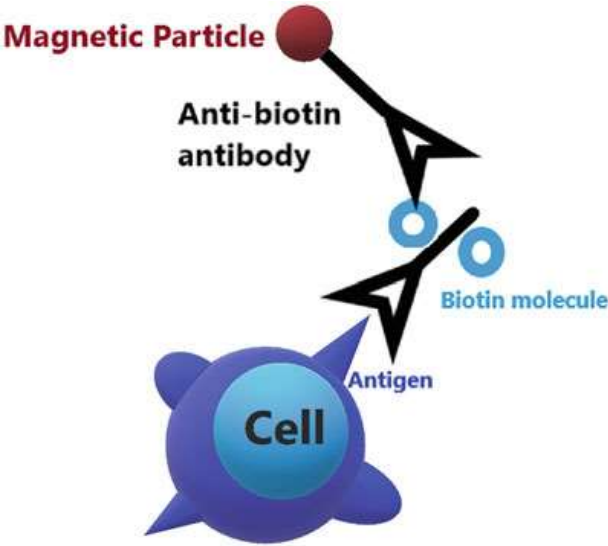


Fig. 3 Structure of TCR $\alpha\beta$ depletion reagent



T cell/CD19⁺ B depletion technique, $0.1\text{--}1 \times 10^5$ $\alpha\beta$ T cells/kg is retained together with the preservation of $\gamma\delta$ T cells and NK cells (Handgretinger 2012).

Naive CD45RA⁺ T Cell Depletion

CD45 is a receptor present in all hematopoietic cells except erythrocytes and plasma cells. CD45 mRNA has six isoforms. The suffix RA and RB indicate the requirement of the amino acid residues, and RO indicates a lack of amino acid residues for the CD45 epitope expression. CD45RA⁺ naive alloreactive T cells are blamed to have a part in GvHD causation (Shlomchik 2007). Thus, their depletion from HSC graft, as a simple and rapid strategy, could lessen the potential complications. In a study after depleting CD45RA⁺ T cell depletion by magnetic bead technology, a median count of CD45RO⁺ T cells from the CD34 negative fraction were recovered (Slatter and Gennery 2017).

2.1.2 CD19⁺ B Cell Depletion

CD19 is a regulatory transmembrane glycoprotein which is involved in B lymphocyte's development and function (Poe et al. 2012; Fujimoto et al. 1998). In all the stages of B cell maturation, from pre-B cells to plasma cells, CD19 is expressed. Therefore, it is considered as a specific surface marker for B cells (Haas and Tedder 2005; Tedder 2009).

The delayed immune reconstitution following TCD is considered as a major risk factor for Epstein-Barr virus (EBV)-associated posttransplantation lymphoproliferative disorders (PTLD); thus, CD19⁺ B cell depletion is performed concurrently with the depletion of CD3⁺ or TCR $\alpha\beta$ ⁺ T cells.

Labeled CD19⁺ cells by means of selective CD19 monoclonal antibodies conjugated to superparamagnetic particles pass through and retain in the separation column with strong magnetic gradient (CliniMACS 2020). In one study, following CD19⁺ B cell depletion of HSC graft using the CliniMACS device, a median of 3.4-log (ranging from 2.0 to 4.7) reduction in B cells was achieved (Pira et al. 2016). In another trial, TCR $\alpha\beta$ ⁺/CD19⁺ depletion in haploidentical HSCT setting led to a median of 3.4-log (ranging from 2.3 to 4.5) depletion of B cells (Bethge et al. 2022).

2.2 Inclusion (Positive Selection/Expansion)

2.2.1 CD34⁺ Cell Enrichment

At the very least, HSC grafts should include hematopoietic progenitor/stem cells that engraft in recipient's bone marrow and are proven to be CD34 positive (Notta et al. 2011). CD34 is a transmembrane protein that is expressed on early hematopoietic progenitor cells and is used to identify and isolate HSCs (AbuSamra et al. 2017). CD133 is also used as a marker for detection and enrichment of HSCs (Koehl et al. 2002).

When labeled HSCs by superparamagnetic microbeads conjugated to a CD34 monoclonal antibody pass through a cell separation column within a strong magnetic field, they retain in the column and a high level of CD34⁺ cell enrichment is

achieved (Fig. 2). CD34+ stem cell positive selection was the first manipulative technique employed on HSC grafts in order to decrease T cell count and to minimize the rate of GvHD. After using CD34+ cell enrichment method, a 4.5 log of reduction in T cells is attained, indirectly (Schumm et al. 2006).

2.2.2 Regulatory T Cell Expansion

Regulatory T (Treg) cells are a subgroup of CD4+ T cells which are involved in promoting immune reconstitution post-HSCT, and they also have a role in endorsing immune tolerance and limiting GvHD (Lalfer et al. 2019).

Several protocols for expanding pure functional Treg cells have been proposed which generally include cultivation in the presence of rapamycin, interleukin (IL) 2, and anti-CD3/CD28 beads for 3–4 weeks. Through these methods, the number of Treg cells could be amplified up to 20–200-fold while preserving their immunoregulatory activity (Lavazza et al. 2022).

2.2.3 NK Cell Expansion

Allogeneic NK cells are recognized to have a considerable GvT/L effect without playing a significant role in GvHD development. By using the NK MACS Medium kit (Miltenyi, Bergisch Gladbach, Germany), from healthy donors, NK cells become isolated by negative selection and then could be expanded without feeder cells along with IL-15 and IL-2 supplementation. In this technique, the maximum cell number is reachable within 5 weeks, with a median of 14050 ± 302 million NK cells attained from a single donor (Ye et al. 2020). In another study, after 5 weeks, in a GMP-grade feeder-free medium, expansion of NK cells led to a median of $14,050 \times 10^6$ NK cells and an ultimate median number of 7.59×10^8 CD56+/CD3- cells with 94% purity and viability (Handgretinger and Schilbach 2018).

2.2.4 $\gamma\delta$ T Cell Expansion

Gammadelta ($\gamma\delta$) T lymphocytes are a minor subset of T cells, defined by expression of γ and δ heterodimeric chains of TCR. It is suggested that $\gamma\delta$ T cells have a favorable impact in the rebuild of immune system post-HSCT, and they also have a GvT/L effect. Various methods for $\gamma\delta$ T cell expansion have been established. Coculture of peripheral blood mononuclear cells and $\gamma\delta$ T cells, incubated with zoledronate, IL-15, and IL-2 combined with aminobisphosphonates, has been shown to enhance the proliferation of purified $\gamma\delta$ T cells, and an average expansion of 229000-fold from a single donor could be achieved (Landin et al. 2021).

3 Immunological Effects of Manipulated Grafts

After allogeneic HSCT, immune reconstitution occurs through several stages. Recovery of innate immunity takes place during weeks to few months after transplant, while the adoptive immunity reconstitution happens within a longer duration (Storek et al. 2008). Considering the importance of prompt immune recovery following HSCT, strategies to improve immune reconstitution post-HSCT, such as

cellular manipulation of HSC grafts, are being more and more employed and have attracted attention.

3.1 Negative Selections

An innovation in the pathway toward successful HSCT was configuring the possibility of GvHD prevention by complete lymphoid cell depletion. However, due to delayed engraftment and immune reconstitution along with higher risk of relapse and infection, more selective depletion methods were brought forward.

3.1.1 CD3+ T Cell /CD19+ B Cell Depletion

T cells (CD3+) are among the main components of HSC graft. Naive T cells (CD45RA+, CD62L+, CCR7+, and CD45RO-) which are produced and released from the thymus proliferate and differentiate into effector T cells (CD45RA+, CD45RO-, CD62L-, and CCR7-) following infection or antigen exposure. After the control of infection, most effector T cells are lost, but a small proportion of them develop into memory T cells (CD45RO+ but CD45RA-), which could be either CD4+ or CD8+ (Mahnke et al. 2013; Farber et al. 2014).

After depletion of CD3+ T cells, although all the abovementioned subtypes of T cells are lost, together with CD34+ progenitors, several graft facilitating cells such as DCs, monocytes, and NK cells remain in the graft (Bader et al. 2011). As the same eliminated alloreactive T cells in charge of GvHD are also advantageous to engraftment and GvT/L effect, following pan-T cell depletion, an increased rate of graft rejection and disease relapse may be observed (Kernan et al. 1989; Ash et al. 1991).

The rationale for co-depletion of CD19+ B cells with CD3+ T cells is that an increased probability of EBV-driven PTLD is mediated by delayed immune reconstitution due to removal of the T cells (Saad and Lamb 2017). After HSCT, EBV-specific T cell responses are weak and impaired, so EBV would be able to apply transforming effects on B cells, leading to proliferation of lymphoblastoid cell lines and oncogenesis of PTLD (Burns and Chaganti 2021). CD19 is expressed by more than 90% of B cell malignancies, so it could act as an attractive target for prevention and treatment of B cell lineage malignancies (Breton et al. 2014).

3.1.2 TCR $\alpha\beta$ + T Cell/CD19+ B Cell Depletion

Double-negative (CD4- and CD8-) thymocytes which are originated from thymus differentiate in to two T cell lineages demarcated by the expression of $\alpha\beta$ or $\gamma\delta$ heterodimers of TCR. Through further development toward double-positivity (CD4+ and CD8+) stage, mature single positive (CD4+ or CD8+) $\alpha\beta$ T cells are produced. However, unlike $\alpha\beta$ T cells, $\gamma\delta$ T cells do not progress through a double-positive stage, and most $\gamma\delta$ T cells remain double-negative (Ciofani and Zúñiga-Pflücker 2010). Hence, CD3+ T cells in peripheral circulation are mostly $\alpha\beta$ T cells (95%) and in a smaller extent are $\gamma\delta$ T cells (5%). The $\alpha\beta$ T cells, as soldiers of adaptive immunity, have a key role in mediating GvHD, while $\gamma\delta$ T cells, as a member of innate immunity, do not seem to have a significant influence on GvHD

pathogenesis (Drobyski et al. 1999; Carding and Egan 2002; Lamb Jr and Lopez 2005). $\gamma\delta$ T cells operate independent from the assistance of MHC molecules, so they are recognized not to trigger alloreactivity.

In an effort to expel the problems of total lymphoid cell depletion, a more selective graft manipulation method based on the specific depletion of TCR $\alpha\beta$ + T cells together with CD19+ B cells has been introduced (Bertaina et al. 2013). Spared $\gamma\delta$ T cells following this method provide a more effective antiviral and antitumoral effect without increasing the probability of GvHD. Relapse rate has been reported to be lower after haploidentical HSCT using TCR $\alpha\beta$ + TCD compared to haploidentical CD3+ TCD (Mehta et al. 2004). Improved survival rates are associated with higher peripheral blood $\gamma\delta$ T cell counts post-HSCT (Lamb Jr et al. 1999).

3.1.3 Naive CD45RA+ T Cell Depletion

Changes in the expression of CD45 surface molecule occur through the differentiation process of naive T cells toward memory and effector cells (see Sect. 2.1.1.2) (Sallusto et al. 1999). CD45RA is expressed on all naive T cells but is absent on most memory T cells. Depletion of CD45RA+ naive T cells from the graft allows for diminishing the probability of GvHD, while preserving CD45RO+ memory T cells may be effective in prevention of disease relapse and infections (Anderson et al. 2003; Teschner et al. 2014; Bleakley et al. 2014). CD45RO is a suitable marker to characterize the activation status of T cells. As part of adoptive immunity, CD45RO + T cells vigorously activate and proliferate following stimulations and persevere their self-renewal characteristics and stem cell-like multipotency for a lifetime (Woodland and Kohlmeier 2009). These cells also induce their antitumor effect by secretion of granzyme and γ -interferon (Hu and Wang 2017).

Donor lymphocyte infusion (DLI) is commonly used as a way to boost antiviral and/or antitumoral effects posttransplant. However, it could cause a significant risk of inducing severe GvHD. Thus, the depletion of CD45RA+ T cells from DLIs, as a simple and rapid strategy, could lessen the potential complications of DLI (Slatter and Gennery 2017).

3.2 Positive Selections

3.2.1 CD34+ Cell Enrichment

CD34 is a marker almost exclusively expressed on HSC, and its expression is reduced by maturation of hematopoietic cells, becoming entirely absent in fully mature cells (Sidney et al. 2014; McGuckin et al. 2003). CD34+ HSCs have the capacity of proliferation and also differentiation into all cells of the hematopoietic lineage (Servida et al. 1996; Huss 2000). It is suggested that CD34 is able to promote engraftment and homing of HSC by binding to a receptor on endothelial cells of bone marrow (Healy et al. 1995). Enrichment of CD34+ cells has been established to be a safe process that permits fast and durable hematological engraftment. However, following the enrichment technique, due to loss of antigen-presenting cells (APCs)

and mature lymphocytes, impairment of immune reactivity and increase in infections and relapse may occur (Damiani et al. 2003).

In CD34+ selected transplantation, T cell reconstitution completely depends on the generation of naive T cells. Specifically, rebuild of CD4+ T cell repertoire is reliant on production of CD4+ CD45RA+ naive T cells in the thymus (Huttunen et al. 2015; Vescio et al. 1999). It is possible that following the selection procedure, mature committed dendritic progenitors with low CD34 expression drop out and lead to a slower recovery of circulating DCs posttransplant compared to unmanipulated grafts (Damiani et al. 2003).

NK cell recovery does not seem to be impacted in CD34+ enriched HSCT, but as more than 6 to 8 weeks is mandatory for the first appearance of functional alloreactive NK cells after transplant, their advantageous GvT/L effect is somewhat delayed (Locatelli et al. 2013a).

3.2.2 Regulatory T Cell Expansion

Treg cells are capable of suppressing immune responses, sustaining a state of self-tolerance and inhibition of self-directed immunity. In allogeneic HSCT setting, Treg cells have the potential to resolve tissue inflammation and mediate tissue repair. They also promote immune reconstitution and prevent GvHD, due to their immunosuppressive capacities. It has been proven that CD4+/CD25+/FoxP3+ Treg cells are effective in the prevention of conventional T cell-mediated GvHD without hampering GvT/L effect (Edinger et al. 2003; Pierini et al. 2019). They also facilitate donor engraftment by regulating bone marrow microenvironment and boosting donor immune reconstitution after transplant. Adoptive transfer of ex vivo expanded Treg cells within HSCT process could provide a strong GvT/L effect and promote engraftment (Fujisaki et al. 2011; Pierini et al. 2017).

3.2.3 NK Cell Expansion

Donor NK cells not only play a role in assisting engraftment, but they are also crucial players in relapse prevention after transplant (Murphy et al. 1992; Ruggeri et al. 2002, 2007). It has been demonstrated that adoptive transfer of expanded NK cells is a safe procedure and it does not induce GvHD. Even so, this method could prevent the development of T cell-mediated GvHD (Ruggeri et al. 2002). Numerous studies have advocated that higher number of donor NK cells in the graft could reduce GvHD occurrence (Soiffer et al. 1993; Yamasaki et al. 2003; Abrahamsen et al. 2005; Larghero et al. 2007; Sivori et al. 2011; Tanaka et al. 2012; Kim et al. 2016). Alloreactive NK cells eliminate APCs through their cytolytic activity and indirectly suppress T cells' proliferation and T cell-mediated GvHD (Meinhardt et al. 2015). In addition to the aforementioned indirect mechanism, NK cells could also hamper GvHD by lysing activated T cells directly at effector sites (Ghadially et al. 2014). Moreover, in the presence of NK cells, the ratio of donor Treg cells to total donor conventional CD4+ and CD8+ T cells is increased, which makes an immunomodulated environment contributing to suppression of GvHD (Olson et al. 2010). Increased NK cell count early post-HSCT is a powerful determinant of GvT/L effect,

resulting in lower incidence of relapse and better transplant outcomes (Leung 2011; Locatelli et al. 2013b; Pieterse and van der Vlag 2014; Maggs et al. 2017).

3.2.4 $\gamma\delta$ T Cell Expansion

Recently, unconventional T cells and their immunologic function in transplant have attracted attention. $\gamma\delta$ T cells as one of unconventional T cells have the properties of both innate and adaptive immunity (i.e., prompt response and no donor MHC restriction, similar to innate immunity elements, and TCR-dependent activation alike conventional T cells) (Godfrey et al. 2015).

Following allogeneic HSCT, adoptive transfer of expanded $\gamma\delta$ T cells could exert antitumoral and anti-infectious effects. $\gamma\delta$ T cells are capable of identification and reactivity against multiple ligands on tumor cells, and they also have the ability of early sensing the metabolic changes in malignant-transformed cells (Sebestyen et al. 2020). Moreover, $\gamma\delta$ T cells express Fc receptor γ III (CD16) which binds to the Fc portion of immunoglobulins and mediate antitumor effects via antibody-dependent cellular cytotoxicity, similar to NK cells (Lafont et al. 2001). $\gamma\delta$ T cells also employ strong antiviral and antibacterial effects. It has been shown that increased number of $\gamma\delta$ T cells in the early post-HSCT period is associated with less CMV reactivation compared to patients with lower number of $\gamma\delta$ T cell (Park et al. 2018; Gaballa et al. 2019).

4 Clinical Impacts of Manipulated Grafts

Allogeneic HSCT remains to be the best potentially curative option for a diversity of conditions including malignant and nonmalignant disorders (Bertaina et al. 2014). However, due to the absence of an available HLA-matched donor for many patients, a progressive shift toward alternative donors especially HLA-haploidentical relatives has occurred. Nevertheless, significant HLA disparities between recipient and donor trigger development of GvHD and GF, mediated by alloreactive T cell responses (Rocha and Gluckman 2006; Locatelli et al. 2009). In such a case, in vitro TCD of HSC grafts has raised as a safe and efficient method to reduce alloreactivity (Lazzari et al. 2022; Bethge et al. 2022).

Clinical application of CD3+/CD19+ depleted haploidentical HSCT has been reported in patients with hematological malignancies with acceptable GvHD incidence and low TRM, together with comparable survival outcomes to those of conventional HSCT (Bader et al. 2011; Palma et al. 2012; Pérez-Martínez et al. 2012). In a prospective study, in acute leukemia pediatric patients who underwent CD3+/CD19+ depleted allografts from their haploidentical family donors, 88% primary engraftment, 7% grade III–IV acute GvHD, 21% chronic GvHD, and 46% 3-year event-free survival (EFS) were reported (Lang et al. 2014).

Following TCR $\alpha\beta$ + /CD19+ depleted haploidentical HSCT in malignant disease, a German group has reported promising results including 88% primary engraftment and 15% acute GvHD grades III–IV. Median time to recovery of CD3+, CD4+, and CD56+ cells was significantly faster in patients who received TCR $\alpha\beta$ + /CD19+

depleted allograft compared with historical control group of patients who had received CD34+ selected grafts (Lang et al. 2015).

In a study by Bethge et al., pediatric and adult patients with mostly malignant disorders received TCR $\alpha\beta$ + /CD19+ depleted peripheral blood stem cell transplantation from haploidentical family donors with reduced intensity preparative regimen, and none of the patients developed grade III/IV acute GvHD. Estimated probabilities of 2-year overall survival (OS) and disease-free survival (DFS) were 63% and 50%, respectively (Bethge et al. 2022).

In a single-arm clinical trial conducted by Bleakley et al., all of the high-risk leukemia patients who received naive T cell depleted HSCT engrafted successfully. Grades II–IV acute GVHD were developed in 66% of patients that was similar to T cell-replete HSCT recipients. However, chronic GVHD was less frequent in comparison with T cell-replete transplantation. Rate of infection was not so high due to rapid T cell recovery and transfer of functional memory T cells (Bleakley et al. 2015).

In a trial in pediatric and young adult patients with high-risk hematologic malignancies who received ex vivo T cell depleted haploidentical allogeneic HSCT, an increase in the 3-year OS and EFS was observed in recipients of CD45RA+ depleted grafts compared with historical T cell depleted group (Mamcarz et al. 2020).

In the absence of a fully HLA-matched donor, mismatched and haploidentical HSCT has also been considered for patients with nonmalignant diseases. However, the high rate of GF and GvHD, delayed immune recovery, and severe infections still remain the main obstacles of successful outcome.

In patients with Fanconi anemia, following T cell depleted, CD34+ selected haploidentical HSCT, graft rejection has been reported to be 17%. Cumulative incidences of grades II–IV acute and chronic GvHD were 17% and 35%, respectively, and the 5-year OS and DFS were 83% (95% CI, 62% to 100%) and 83% (95% CI, 62% to 100%), respectively (Zecca et al. 2014).

TCR $\alpha\beta$ + /CD19+ depleted haploidentical HSCT has also been conducted in patients with primary immunodeficiency and has resulted in a high OS (96.7%) (Bertaina et al. 2014). CD45RA+ T cell depleted mismatched HSCT in patients with severe aplastic anemia and congenital amegakaryocytic thrombocytopenia has been associated with rapid myeloid and platelet recovery and no severe infections within the first hundred days post-HSCT (Gasior Kabat et al. 2021).

In the early posttransplant period, immature and poorly functional NK cells predominate, and mature functional donor derived NK cells emerge in the patient peripheral blood several weeks after transplantation (Pende et al. 2009). NK cell reconstitution and its function are mainly impacted by GvHD prophylaxis, especially by posttransplant cyclophosphamide that abolishes majority of mature donor NK cells including alloreactive NK cells (Russo et al. 2018). Accordingly NK cell-mediated GvT/L effect in the early posttransplant period seems non-efficacious to prevent relapse and provides the rationale for adoptive infusion of ex vivo activated NK cells during this time window especially in haploidentical HSCT setting (Stern et al. 2013; Locatelli et al. 2013b). So it seems that the use of fresh or IL-15

stimulated NK cell infusion early after haploidentical stem cell transplant might enhance GvT/L effect without increasing the risk of GvHD (Leung 2011; Moretta et al. 2011; Maggs et al. 2017).

Doubtlessly, future of transplant therapy will involve more cellular manipulative strategies; thus, more clinical trials with larger study population will be needed to establish the optimal interventions and to maximize the advantages of these novel procedures.

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