

IMMUNE RECONSTITUTION IN PEDIATRIC STEM-CELL TRANSPLANTATION

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1. ABSTRACT

Hematopoietic stem-cell transplantation (HSCT) has successfully been used to cure many pediatric disorders. However, the immunologic alterations associated with transplantation result in profound immunodeficiencies in the transplant recipient, resulting in significant infectious morbidity and mortality. The precarious process of immune reconstitution in the transplant recipient is neither instantaneous nor complete, but influenced by multiple factors such as graft-versus-host disease (GVHD), conditioning regimen, patient age and underlying disease. Studies in pediatric HSCT have revealed unique attributes of immune recovery in pediatric transplant recipients. Future studies addressing these findings are needed to complement novel immunotherapies emerging from the field of transplant immunology.

2. INTRODUCTION

Transplantation has markedly impacted the treatment of a vast array of pediatric diseases, including metabolic, immunologic, and hematologic/oncologic disorders (1-4). No longer is the transplant candidate limited to receive his or her own stem cells, for blood-forming stem cells can be harvested from another individual's bone marrow or peripheral blood and even from banked unrelated umbilical cord blood (5). As transplantation expands in its application towards disease treatment, the field of transplantation immunology likewise continues to reveal reasons for transplantation's successes and failures. Specific to this field is post-transplant immune reconstitution, a complex process marked by the gradual recovery of innate and adaptive immune responses in the transplant recipient (6,7). Not only important in graft-versus-host disease (GVHD) and anti-tumor effects,

such immune recovery is also critical in reestablishing the recipient's ability to effectively respond against pathogens.

This review of immune reconstitution in the pediatric stem-cell transplant patient has three provisions. First, it gives an overview of the immune system as it pertains to hematopoietic stem-cell transplantation (HSCT). Second, this review describes immunologic alterations pertinent to the HSCT patient. Lastly, it provides a general overview of the kinetics of immune reconstitution after HSCT and reviews studies specific to pediatric post-transplant immune reconstitution.

2.1. The immune system: A brief review

The transplant candidate possesses a complex immune system that contains an innate or natural arm and an adaptive or acquired arm (8). Although having different constituents, these two systems converge functionally, providing a complementary surveillance mechanism that discriminates between self and non-self. These two systems also share similar development, for all immune cells originate from a common stem cell through a process known as hematopoiesis (9,10). The first step towards stem-cell differentiation commits the cell to a myeloid or lymphoid progenitor status. With the influence of various cytokines, chemokines and stromal-cell surface adhesion molecules, these progenitor cells ultimately develop into functionally mature cells ranging from red blood cells and platelets to neutrophils and T-cells.

Working towards general elimination, the innate immune system acts as a non-specific front-line defense against foreign antigen (Ag). Composed of cellular and humoral components, innate immunity functions without

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retaining immunologic memory. Cellular constituents include phagocytes (neutrophils, monocytes, macrophages), dendritic (DC) and natural killer (NK) cells. While phagocytes opsonize and destroy antigen via oxidative mechanisms (11), NK cells apply major histocompatibility complex (MHC)-independent cytotoxicity against infection and tumor (12,13). Dendritic cells function as potent Ag-presenting cells (APC), activating T-cell responses. Alternately, complement comprises the humoral portion of innate immunity and provides linkage between the innate and adaptive immune systems (14-16).

Unlike its natural counterpart, the acquired immune system develops Ag-specific memory for what it eliminates (17). Subdivisions of this system include cell-mediated (T cell) and humoral (B cell) immunity. The Ag-presenting cell (APC) is an important accessory to adaptive immunity, for it processes and combines antigen with MHC for presentation during T-cell activation. One example is the dendritic cell, the most potent activator of naïve T-cells (18) whose emerging functions include anti-tumor applications (19).

Humoral immunity involves Ag-specific immunoglobulin (Ig) or antibody (Ab) production. The five antibody classes include IgG, A, M, E, and D with IgG and IgA having four and two subclasses, respectively. Immunoglobulin production can be either T-cell dependent or T-cell independent. The former results in an IgG anamnestic response, while the latter produces a more ephemeral IgM response.

Regardless of antibody type, each immunoglobulin is produced by a plasma cell that undergoes a common maturation or ontogeny beginning in the bone marrow and ending in the plasma (20). Originating from a pre-B cell, the activated B cell can function as either a dormant memory cell or an Ab-secreting plasma cell. Antigen-induced differentiation occurs at the stage of the mature B cell with antigen exposure causing specific antibody secretion.

The T-cell receptor (TCR) repertoire determines T-cell specificity (8). The TCR is a heterodimer of one alpha-beta or gamma-delta subunit combined with CD3, a cluster of differentiation antigen composed of invariable gamma, delta, and epsilon subunits and variable zeta and eta subunits. This surface CD3 forms the contact site for and transduces the signal from binding the MHC-associated peptide from an antigen-presenting cell. The resultant T cell is then activated, functioning in the capacity of either a CD4+ or CD8+ lymphocyte. CD4+ cells associate with class II MHC, while CD8+ cells associate with class I MHC. Most T cells have alpha-beta subunits and function as helper cells (Th).

Th cells are further divided into Th1 and Th2 cells depending upon cytokine stimulation and production patterns. IL-12 stimulates Th1 cells that target intracellular pathogens through IFN-gamma and IL-2 production. IL-4 stimulates Th2 cells to produce IL-4, 5, 6, 10, and 13 that work against extracellular pathogens. Th1 cells are

involved in pro-inflammatory autoimmune responses such as graft-versus-host disease (21), while Th2 cells are important in allergic responses.

T-cell ontogeny differs from B-cell ontogeny based upon T-cell repertoire generation (22,23). Like their B-cell counterparts, T-cell progenitors originate from a common hematopoietic stem cell in the bone marrow. However, they then migrate to the thymus and develop into double-positive (CD4+CD8+) thymocytes. Within the thymus these double-positive cells undergo positive or negative clonal selection depending upon weak (positive selection) or strong (negative selection) interactions between TCR and MHC/self peptide. The T-cell repertoire simultaneously develops along with thymocytes, originating from commonly shared genes and maturing into antigen-specific receptors via random rearrangement, fusion, and deletion of junctional gene peptides. Therefore, the end result of migration and maturation is an extensive repertoire of thymocytes awaiting antigen exposure to determine their proliferation or elimination.

Once positively selected, the double-positive thymocytes differentiate into CD3+CD4+ or CD3+CD8+ cells and emigrate from the thymus co-expressing CD45RA molecule on their surface. As they encounter peripheral antigen, these single-positive cells convert to functional CD45RO phenotypes, either CD4+CD45RO+ or CD8+CD45RO+. Thus, T cells expressing surface CD45RA are considered immunologically naïve, while those expressing CD45RO are considered memory/effector cells.

2.1.1. The hematopoietic stem-cell transplant patient: An immunologic alteration

The goal of allogeneic stem-cell transplantation is replacing a hematologic, immunologic, or metabolic deficiency in the recipient with normally reconstituted hematopoiesis and immunity from the donor graft. Ablating the recipient's immune system with chemotherapy and/or radiation therapy achieves immunologic neutralization in the recipient before stem-cell transplantation. However, this preparative regimen also renders the recipient highly susceptible to infection due to profound and extensive immunosuppression (24). In particular, high-dose chemotherapy causes severe lymphocyte relative to phagocyte depletion (25) with subsequent CD4+ recovery being thymic-dependent (26).

The type of transplant and the presence of graft-versus-host disease (GVHD) also influence transplant immunity and subsequent infection risk. Although having less GVHD, autologous transplants are associated with higher incidences of tumor recurrence than allogeneic transplants that have higher incidences of GVHD and infection (27). Histoincompatibility between donor and recipient increases risk of GVHD (28), whereas transplant T-cell depletion (TCD) reduces GVHD but increases infection and leukemic relapse risks (29). Thus, HLA-mismatched allogeneic transplants have high incidences of graft failure, GVHD and infection.

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Graft-versus-host disease occurs when immunocompetent donor cells incite a dysregulated cytokine and cellular response against immunosuppressed recipient cells (21,29,30). GVHD is divided temporally into acute (within 100 days post-transplant) and chronic (100 days post-transplant) disease (31). Regardless of when it occurs, GVHD impairs immune reconstitution and hematopoiesis (32) and increases infection (33) and mortality risks (4).

Acute GVHD characterized by dermatitis, enteritis and hepatitis can further be divided into afferent (recipient conditioning), amplification (donor T-cell activation), and efferent (cytolytic and inflammatory effectors) phases (34). The conditioning regimen damages host tissues via activating the release of pro-inflammatory cytokines like IL-6, IL-1, and TNF-alpha (35,36). However, conditioning regimens do not possess similar GVH potentials. Comparing cytokine elicitation among TCD- and conventional marrow transplants, Schwaighofer and colleagues (37) found higher IFN-gamma and neopterin production among cyclophosphamide-containing regimens.

Regardless of conditioning regimen, a Th1 type cytokine response with IL-2 and IFN-gamma production results in cytotoxic damage (21,31). Research addressing ways to deter such a Th1 response has provided interesting results. First, the destructive role of IFN-gamma in acute GVHD has been called into question. Murphy and co-investigators (38) have demonstrated reversed cytokine effects in knockout mice with IFN-gamma actually ameliorating and IL-4 accelerating acute GVHD. Thus, cytokines may possess dual roles depending upon when they are secreted. Secondly, redirecting T_{H1} responses towards T_{H2} responses has been successful in reducing incidences of GVHD (39-41). Future research addressing its pathophysiology will provide insights into preventing and treating GVHD (42).

Factors including conditioning regimen, granulocytopenia, and GVHD culminate in predisposing the pediatric-transplant patient to infectious morbidity and mortality (43,44). Resultant host-defense defects are associated with three stages of immunosuppression during transplantation (45,46). The pre-engraftment stage is defined as the time from conditioning-regimen administration until thirty days post-transplant (T+30), when neutropenia and compromised anatomic barriers act as important factors for infection. The post-engraftment stage (neutrophil recovery until T+100) has immune-reconstitution time and incidence of acute GVHD as associated risks, while prevention and presence of chronic GVHD can cause infection during the late-transplant phase of immunosuppression (T+100 until no immunosuppressive therapy or GVHD).

2.1.2. Immune reconstitution in the stem-cell transplant patient: General considerations

Immune reconstitution within the stem-cell transplant recipient is marked by differences in kinetics and specific deficiencies of the innate and adaptive immunity systems based upon intensity of conditioning regimen, type

of graft, and the degree of immunosuppression provided as prophylaxis and/or treatment of GVHD (47). Immune reconstitution in unrelated allogeneic transplant patients may require 12 to 36 months. During this time, immune recovery progresses from a primitive cytotoxic system of NK cells and macrophages to a more sophisticated immunologic surveillance with B- and T-lymphocytes (6,7). In general, innate immunity precedes adaptive immunity and quantitative recovery precedes qualitative or functional recovery.

2.1.3. Overview of recovery

A general chronology of immune reconstitution from earliest to latest cell recovery is phagocytes, NK cells, B-cells and lastly T-cells (7,47,48). However, the functions of the aforementioned cells take longer to normalize than their absolute numbers.

Specifically, quantitative phagocyte recovery occurs by three months post-transplant with neutrophils (~15-45 days) preceding monocytes (~1-2 months) and tissue macrophages (~3 months). Neutrophil chemotaxis and oxygen-dependent bactericidal activity may take as long as four months post-transplant to recover. Evaluating neutrophil function post-transplant, Zimmerli and colleagues (49) found impaired chemotaxis, superoxide production and phagocytic activity in 80% of tested patients with resulting infectious sequelae.

Monocyte function recovers earlier than neutrophil function. Studying cytokine production in six children receiving allogeneic BMT for aplastic anemia or leukemia, Pechumer *et al.* (50) found early monocyte TNF- α and IL-6 production 10 to 14 days post-transplant. Additionally, monocyte function was not adversely affected by chronic GVHD, a notable difference from neutrophil function (49).

NK-cells (CD16⁺CD56^{dim}) appear early after transplant (T+30-50) with antibody-dependent cell-mediated cytotoxicity (ADCC) recovering soon after. Early NK cell-recovery has been attributed to their potential roles in GVHD (51) and graft-versus-leukemia reactions (GVL) (52). Interestingly, a NK-cell subset (CD16⁺CD56^{bright}) with different activation markers and lower ADCC activity than CD56^{dim} cells has been observed post-transplant (53). Whether these cells reflect a distinct developmental stage or perform differently from CD56^{dim} cells remains unknown.

Absolute B-cell numbers start normalizing around three months after transplantation, while immunoglobulin recovery may require several months (i.e., IgM and IgG) to years (i.e., IgA). A notable exception is IgE whose level peaks 3 to 4 weeks after transplantation and has been correlated with acute GVHD (54).

Notwithstanding normalized absolute numbers, immunoglobulin antigen response remains impaired. T-cell dependent responses to neoantigens remain low for at least three months after transplant, while responses to recall antigens are suppressed for at least one year after

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transplant (55). T-cell independent responses take longer to recover usually around one to two years post-transplant (55). Such delays in antibody responses have implications for re-immunization in the HSCT recipient (56).

B-cell reconstitution recapitulates B-cell ontogeny (57). Evaluating humoral immunity in 24 adult transplant patients, Storek *et al.* (58) observed reconstituted B cells as being large in size, undergoing a triphasic recovery in number, and overexpressing CD38, IgM and IgD. They concluded that recipient B-cells originate from maturation-arrested donor stem cells then proceed through humoral ontogeny in the recipient. Additionally, the presence of chronic GVHD was found to hinder this recovery.

Approximately three months post-transplant, T-cells begin to recover. CD8⁺-cell recovery (~3 months) precedes that of CD4⁺-cells (~6 months) resulting in an inverted CD4⁺/CD8⁺ for at least the first six months post-transplant (23,59). As with B-cell reconstitution, chronic GVHD impairs T-cell recovery and prolongs CD8⁺ predominance in the recipient.

Cell-mediated functional recovery does not begin until at least 6 months after transplantation (59). Lymphocyte proliferation assays normalize between 6 to 12 months post-transplant as do T-cell signaling and cytotoxicity in matched-sibling transplant recipients. One reason for T-cell defects may be impaired T-cell activation. Pignata and investigators (60) observed mitogen-activated protein kinase (MAPK) activation failure in 15 of 16 allogeneic BMT patients, postulating a post-translational regulatory defect causing blunted T-cell mitogen response. Using T-cell mitogen proliferation assays to study lymphokine profiles in 27 BMT patients, Schneider *et al.* (61) found decreases in IFN- γ and IL-2 production relative to IL-4. Based upon the mitogens used, the authors hypothesized that the observed lymphokine imbalance resulted from impaired surface-receptor signal transduction in non-immunosuppressed patients or a signaling defect downstream from protein kinase C activation in immunosuppressed patients. Thus impaired T-cell activation may result from different signaling level defects.

Markedly abnormal T-cell receptor (TCR) diversity persists for at least 3 months post-transplant. Aside from myeloablation, age-related thymic involution greatly impairs T-cell immunity thereby restricting naïve CD4⁺ T-cell generation (62). After their elimination in the recipient, T-cells can be replenished via thymic-dependent or thymic-independent pathways. The former results in CD45RA⁺ T-cell generation with a more extensive TCR repertoire than the latter (63). Interestingly, thymic-dependent generation seems more important for naïve helper T-cells than for naïve cytotoxic/suppressor T cells (64). Thus, thymic presence in the post-transplant pediatric patient can potentially produce a more diverse and durable reconstitution deriving from a greater naïve T-cell population than can reconstitution from memory T-cells in the adult patient.

2.1.4. Specific considerations

Studies have addressed the influence of transplant type on immune reconstitution (65,66). Specifically, Keever and colleagues (67) compared immune recovery in T cell depleted (TCD) and conventional bone marrow transplants from HLA-identical sibling donors. Myeloid and lymphoid recovery were not different; however, conventional marrow recipients had greater mitogen-induced immunoglobulin production than TCD-marrow recipients. Additionally, differences in T-cell dependent functions did not significantly correlate into depleted grafts having higher incidences of infection than conventional grafts. Analyzing immune recovery in children, Foot *et al.* (68) also found no significant differences between T-cell depleted and conventional marrow transplants.

Immune recovery after peripheral-blood stem cell transplantation (PBSCT) is faster and more complete than after marrow transplantation (69-71) even when using peripheral stem cells from an unrelated donor (72). Yet, immune reconstitution following PBSCT does have limitations, especially regarding incidence of chronic GVHD (73). Guillaume *et al.* (74) found multiple defects in cytokine production after autologous PBSCT. Likewise, Shenoy and co-investigators (75) found a significant percentage of PBSCT patients (62%) developing cytomegalovirus (CMV) viremia within one year post-transplant presumably due to impaired T-cell proliferation and NK cell-mediated lysis.

Umbilical-cord transplantation (UCT) has been successfully implemented in treating various diseases (76-78). Immune reconstitution after UCT is comparable to that after peripheral-blood and marrow transplantation (79,80). However, qualities intrinsic to cord blood make it an ideal source of stem cells with a greater potential for immune reconstitution and a lesser potential for GVHD (81). For example, cord blood contains a large naïve lymphocyte population with a polyclonal T-cell receptor repertoire (82). Secondly, not only do umbilical cord blood T cells have decreased cytotoxic proliferation and impaired cytokine production (IL-4, IL-6, TNF- α , IFN- γ) (83,84), but they also possess NK cell potential and activity similar to adult peripheral T cells (84). Thus, lacking the means to produce significant GVHD, umbilical cord T-cells can function in mounting a graft-versus-leukemia (GVL) response. Another possible mechanism for decreased GVHD is reduced umbilical cord T-cell expression of nuclear factor of activated T cells-1 (NFAT1) causing reduced IFN- γ and TNF- α production (85). Together, these results serve as catalysts for future studies addressing additional features unique to banked unrelated cord-blood transplantation.

2.1.5. Immune reconstitution in the pediatric stem-cell transplant

Our knowledge of immune reconstitution is largely based upon observations from adult studies, for studies exclusive to the pediatric transplant patient are infrequent (68,79,86-90). Table 1 summarizes current data specific to immune recovery in pediatric stem-cell transplantation. Most striking are the highly variable

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Table 1. Studies exclusive to immune reconstitution in pediatric stem-cell transplantation.

	Mean or median		Stem-cell transplant	Engraftment (d)						Lymphocyte recovery (mo)				GvHD	Death	Death		
Study	Age (Range)	Type (n)	Manipulation (n)	HLA match (n)	ATG	ANC >500	Plt>50	ALC >500	CD2+ or 3+ CD2+ or 3+	CD56+	CD4+	CD8+	CD19+ or 20+ CD19+ or 20+	CD4+ CD8+	Acute	Chronic	Total	Cause (n)
Study	Age (Range)	Type (n)	Manipulation (n)	HLA match (n)	ATG	ANC >500	Plt>50	ALC >500	CD2+ or 3+ CD2+ or 3+	CD56+	CD4+	CD8+	CD19+ or 20+ CD19+ or 20+	CD4+ CD8+	Acute	Chronic	Total	Cause (n)
Foot <i>et al.</i> [68] 1991	6.7 y (2.4-15.7)	Bone marrow: logeneic (16)	T-cell depletion (12)	N/A	No	N/A	N/A	N/A	12	N/A	9	1	3	6	9	3	4	GvHD (2)
N=19		Autolog us (2) Syngeneic (1)																Relapse (2)
Kurtzberg <i>et al.</i> [79] 1996	6.6 y (0.8-15.1)	Unrelated cord blood	None	6/6 antigen (1) 5/6 antigen (9) 4/6 antigen (11) 3/6 antigen (3)	Yes	22	82	6	N/A	3	N/A	N/A	N/A	7	17	2	13	Graft failure (2)
N=24																		Relapse (2) Infection (7) Other (2)
Locate lli <i>et al.</i> [86] 1996	8.7 y (7-10)	Related cord blood	None	6/6 antigen (3)	No	23	43	N/A	1	1	6	1	2	6	None	None	0	0
N=3																		
Kook <i>et al.</i> [87] 1996	8.1 y (1.1-18.4)	Bone marrow: "Closely matched unrelated" (14) "Partially matched related" (88)	T-cell depletion (102)	N/A	Yes	N/A	N/A	N/A	36	1	36	18	18	12	11	13	N/A	N/A
N=102																		
Godthelp <i>et al.</i> [88] 1999	8.9 y (2-15)	Bone marrow: Matched-related (6) Matched-unrelated (6)	T-cell depletion only for MDS matched-unrelated transplants (3)	N/A	No	N/A	N/A	N/A	3	3	12	3	3	12	5	4	3	Relapse (2) Infection (1)
N=12																		
Giraud <i>et al.</i> [89] 2000	4.5 y (1.5-13.5)	Unrelated cord blood	None	6/6 antigen (0) 5/6 antigen (5) 4/6 antigen (5) 3/6 antigen (2)	Yes	28	82	3	7	3	12	12	6	3	6	2	4	Relapse (1) Infection (3)
N=12																		
Kamani <i>et al.</i> [90] 2000	NBL n=15 3 y (2-18) AML n=15 10 y (2-16)	Autologous bone marrow	NBL: Monoclonal Ab AML: 4 HC	N/A	No	N/A	N/A	4	4	4	12	4	4	12	None	None	0	0
N=30																		

recovery times among studies, probably reflecting differences in underlying diseases, conditioning regimens and methods of treatment and prevention of GVHD.

Two major differences between pediatric and adult immune recovery have been discerned. First, the pediatric transplant patient has the advantage of an intact thymopoietic pathway that generates a potentially more diverse TCR repertoire from CD45RA⁺ lymphocytes. Two studies have clearly demonstrated this advantage. Studying regeneration of CD4⁺ T-cells and their associated CD45 isoforms after high-dose chemotherapy, Mackall *et al.* (26) found that younger patients had greater recovery of CD4⁺

T-cells and faster regeneration of CD4⁺CD45RA⁺ lymphocytes than older patients did. They correlated these findings with younger patients' thymic rebound concomitantly demonstrated by chest tomography.

An Austrian study reflected similar findings after allogeneic BMT. Comparing T-cell regeneration in a 15 year-old thymectomized patient (mediastinal sarcoma) with nine other allogeneic BMT patients without thymic manipulation, Heitger and colleagues (64) noted that the thymectomized patient failed to reconstitute CD4⁺CD45RA⁺ T cells but could generate CD8⁺CD45RA⁺ T cells. They concluded that the former required an intact

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thymus while the latter regenerated independently from thymic influence. Together these studies have important clinical implications, including children experiencing less opportunistic infection and more robust immune regeneration than their adult counterparts.

Second, children undergoing transplantation seem to experience faster immune recovery than adults do. Small and investigators (91) evaluated post-transplant lymphoid phenotype and function and infectious complications in 62 consecutive patients receiving related- and unrelated-TCD allogeneic marrow transplants. Recovery rates for NK cells were similar regardless of age. However, CD3⁺, CD4⁺, and CD8⁺ cells and mitogen responses recovered more quickly in children receiving related transplants. Also, infection analysis for unrelated transplants revealed a higher incidence of opportunistic infections in adults (44%) versus children (22%). The authors concluded that adult recipients of unrelated marrow transplants experience more profound T-cell lymphopenia and prolonged lymphocyte recovery than do children. More studies addressing such differences in immune recovery are needed to shed light upon age-specific immunotherapy strategies.

3. CONCLUSIONS

With continued expansion in scope and application, the field of transplant immunology will not only provide answers, but it will also further generate questions pertaining to stem-cell transplantation. Although helpful, studies addressing immune reconstitution have been limited in their focus and design. Specifically, each possesses different variables that can influence recovery; for no two studies have patients with similar underlying diseases, transplants, conditioning regimens, and GVHD prophylaxis and treatment. Therefore, study results may not necessarily be applicable to all transplant patients.

Nevertheless, these studies have significantly added to an increasing understanding of what transpires during immune recovery. Adult studies have revealed how immune reconstitution gradually occurs and what factors augment or hinder its course. Data pertinent to the pediatric transplant patient has yielded an appreciation for the thymus and its role in recovery. Thus, these studies serve as the foundation upon which more knowledge and experience will be built.

One such future direction for transplant immunology is the successful implementation of immunotherapy (92,93). Two examples of innate anti-tumor mediators are natural killer and dendritic cells. Their unrestricted MHC cytotoxicity against tumor cells make NK cells an attractive potential cancer therapy (13,19,94,95). Likewise, the dendritic cells' highly effective antigen-presentation and subsequent T-cell-mediated response has been harnessed to selectively destroy cancer cells (96,97). Together these are potential alternatives to conventional immunosuppressive therapy and offer hope for future achievement of complete and highly selective anti-tumor therapy (98).

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