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Reviews

Immune Reconstitution after Allogeneic Hematopoietic Cell Transplantation in Children

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Allogeneic (allo) hematopoietic cell transplantation (HCT) has evolved into a potent curative treatment option for a variety of malignant and nonmalignant diseases. The occurrence of complications and mortality after allo-HCT is, however, still high and is strongly associated with immune reconstitution (IR). Therefore, detailed information on IR through immunomonitoring is crucial to improve survival chances after HCT. To date, information about the reconstituting immune system after allo-HCT in pediatric patients is mostly derived from routine standard-of-care measurements. More profound knowledge on IR may provide tools to better predict and modulate adverse reactions and, subsequently, improve survival chances. Here, we provide an overview of IR (eg, immune cell subsets and circulating chemokines/cytokines) after allo-HCT in children, taking into account different cell sources and serotherapy, and discuss strategies to enhance immunomonitoring. We conclude that available IR data after allo-HCT contain limited information on immune cell families (mostly only generic T, B, and NK cells), which would improve with more detailed information on reconstituting cell subsets or effector cell functionality at earlier time points (<1 month). In addition, secretome data (eg, multiplex cytokine/chemokine profiles) could add to the understanding of IR mechanisms and cell functionality and may even provide (early) biomarkers for individual disease outcome, such as viral reactivity, graft-versus-host disease, or graft-versus-leukemia. The present data and suggestions for more detailed, standardized, and harmonized immunomonitoring in future (pediatric) allo-HCT studies will pave the path to "precision transplantation:" an individualized HCT approach (including conditioning), based on detailed information on IR and biomarkers, aiming to reduce transplantation related mortality and relapse, and subsequently improve survival chances.

INTRODUCTION

Allogeneic (allo) hematopoietic cell transplantation (HCT) has evolved into a potent curative treatment option for a variety of malignant (eg, leukemia, lymphoma, multiple myeloma) and nonmalignant diseases (eg, primary immunodeficiencies, hemoglobinopathies, lysosomal storage diseases). Risk factors associated with this procedure may, however, lead to severe and life-threatening conditions. They involve transplantation-related mortality (TRM), infections, the occurrence of relapse (in case of malignancies), acute graft-versus-host disease (aGVHD) and chronic complications, such as chronic graft-versus-host disease (cGVHD) [1-3]. These complications are mainly due to a poor and prolonged lymphopenic state, as illustrated in Figure 1, and immune dysregulation as a result of cytokine fluctuations caused by conditioning [4-6].

Furthermore, strategies to reduce the probability of GVHD, such as in vivo T cell–depleting therapy with serotherapy, such as antithymocyte globulin (ATG) and alemtuzumab, have major impact on immune recovery [7-11] and, thus, affect the risk of relapse or infections. In particular, the unpredictability of the effects of conditioning and additional therapies on immune reconstitution (IR) in individual patients challenges survival outcome after allo-HCT therapy.

More profound knowledge on how this myriad of variables impacts IR after allo-HCT may provide tools to better predict and modulate adverse reactions and, subsequently, improve survival chances. Moreover, IR is dependent on age-related physiological aspects (eg, thymic function, hormones) and, therefore, demands separate study in adults and

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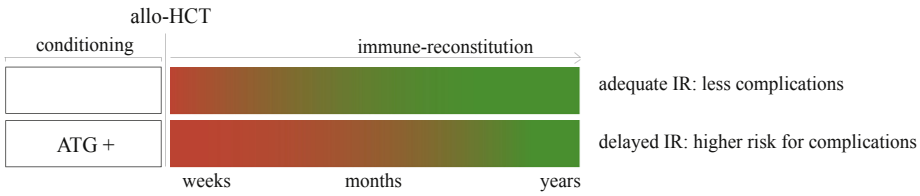

Figure 1. Delayed immune reconstitution after conditioning with ATG is an important factor in risk for allo-HCT related complications. After conditioning before to allo-HCT, and transplantation, immune cell numbers have to recover to normal/reference values of healthy controls. When ATG is added, immune reconstitution is delayed putting the patient at higher risk for transplantation-related complications and mortality.

children. In the present review, we focus on IR, regarding immune cell subsets and secretome after allo-HCT in children, related to the application of different cell sources (bone marrow [BM], peripheral blood [PB], and umbilical cord blood [UCB]) and serotherapy with ATG and discuss strategies to improve IR monitoring.

IMMUNE RECONSTITUTION AFTER HCT

Allogeneic hematopoietic cells can be derived from BM, PB, or UCB. Although recovery of innate immune cells generally takes weeks to a few months, complete reconstitution of adaptive immunity is often delayed, typically from months to even years. Detailed information on IR in pediatric patients is largely lacking, especially during the first month(s) after allo-HCT (Tables 1 and 2, detailed information on studies reviewed in Table 3).

The data presented here report on immune reconstitution up to values previously reported as bottom ranges of reference values. We previously found that time to reach $.5 \times 10^9$/L circulating $CD4^+$ T cells is a strong predictive marker for probability on relapse [69]. Studies incorporating more in-depth analyses of (T) cell subtypes may even better discriminate patients at risk for transplantation-related adverse effects and relapses and which immune profile would be associated with higher overall survival chances.

Reconstitution of the Innate Immune System

Neutrophils

Neutrophils are the first cells that recover after HCT (Table 1) and are used as early validation of engraftment. In children, neutrophils recover around 20 days after allo-HCT (defined as the first day with more than 500 cells/μL for 3 subsequent days) for all cell sources. Neutrophil function (eg, chemotaxis, phagocytosis, superoxide production, and killing of bacteria) lags behind and only reaches normal levels after 2 months [42,43].

Natural killer (NK) cells

NK cells are lymphocytes of the innate immune system, of which different subsets can broadly be characterized by the expression of CD56 and CD16 in CD3-negative cells. However, most studies characterize NK cells by the expression of CD56, with or without CD16, whereas only some studies exclude $CD3^+$ cells [44]. NK cells trigger an immune response when major histocompatibility complex (MHC class I) is absent or low expressed, eg, on stressed cells, while maintaining self-tolerance to healthy, MHC-expressing tissues. In humans, a relatively large family of NK cell receptors for MHC is represented by killer immunoglobulin-like receptors (KIRs).

After allo-HCT, NK cells are generally the first lymphocytes to reconstitute to normal numbers in 1 to 4 months, independent of cell source (Table 1 and [36]). However, no information is available on earlier time points or NK cell subsets and functionality. Low numbers of total NK cells in the first weeks after HCT are associated with low overall survival and higher risk of infection [15,21], underscoring an important role for NK cells in outcome after HCT.

Monocytes

Monocytes are myeloid cells arising from the BM that travel in the bloodstream and differentiate mainly into macrophages in tissues. Their primary functions are phagocytosis and release of pro- and anti-inflammatory chemokines and cytokines in the innate immune response and during the onset of acquired immunity [45,46]. Expression of CD14 and CD16 classifies subpopulations into "classical" $CD14^{++}CD16^-$, "intermediate" $CD14^{++}CD16^+$, and "non-classical" $CD14^+CD16^{++}$ monocytes [47,48]. Although reconstitution data over time after allo-HCT are unavailable, levels of CD16+ monocyte subsets are increased during aGVHD and restored during aGVHD therapy [49]. Moreover, HLA-DR expression on monocytes is decreased before and during sepsis or bacterial infections and in hepatic veno-occlusive disease [49,50]. In contrast, HLA-DR levels are elevated before and during viral reactivation and aGVHD [50]. These findings suggest that monocyte activation reflects acute inflammatory mechanisms, which may help identify HCT patients at risk for complications.

Dendritic cells

Dendritic cells (DCs) are antigen-presenting cells (APCs) that process and present antigen peptides on their cell surface in MHC-I and MHC-II. After internalization of antigen they migrate to the lymph nodes where they stimulate T and B cells to incite an adaptive immune response specific for phagocytosed antigens. Several DC subtypes can be found within the bloodstream: conventional DCs (cDCs), also referred to as myeloid DCs, and plasmacytoid DCs (pDCs). cDCs and pDCs lack lineage defining surface markers, such as CD2, CD3, CD5, CD19, CD20. cDCs are categorized in two subtypes expressing $CD11c^+CD123^-$ with either CD1b/c or CD141 expression, and pDCs with a phenotype of $CD11c^-CD123^+CD303^+$ or $CD304^+$ [51,52].

Data on cDC and pDC reconstitution after allo-HCT in children are only available for BM transplantation (BMT) and levels seem to strongly depend on age and sex [24-26,38,53] (Table 1). These studies show an initial increase in both pDC and cDC early after transplantation. During the first month, cell counts reach levels similar to, or even higher than, healthy control values for pDCs and cDCs, respectively. Thereafter, a significant decline is observed and the DC counts remain low for a considerable amount

Table 1
Post-Allo-HCT IR and Distinctive Markers of Innate Immune Cell Populations in Children

Cell Nos.	Phenotype	Reconstitution Time			References
		BM	PBSC	UCB	
Neutrophils	Forward/side scatter			23 days	[12]
>.5 × 10^9/L		16 days	*15 days*	19 days	[13]
				20 days	[8]
ATG:					
>.5 × 10^9/L		19 days		28 days	[14]
		25 days		22 days	[15]
				18 days	[8]
NK cells	$CD3^-$				
>.075 × 10^9/L*	$CD56^+CD16^{+/-}$	*1 month*			[16]
Reference value*		*3 months*			[17]
Reconstitution*				3 months	[18]
>.1 × 10^9/L		2-3 months		2-3 months	[12]
		1.5 months			[7]
				1 month	[19]
			4 months	4 months	[13]
				2 months	[8]
>.25 × 10^9/L*		1 month			[20]
ATG:					
>.1 × 10^9/L				3 months	[21]
				3 months	[22]
				3 months	[23]
		1.4 months		1 month	[14]
$AUC_{day\ 0\text{-}90}$*		400		500	[15]
>.1 × 10^9/L				2 months	[8]
>.25 × 10^9/L*				1 month	[20]
cDCs (mDCs)	Lin-MHC+CD11c+				
>.02 × 10^9/L	CD123−CD141+/	>2 months			[24]
	CD1b/c+	10-11 months			[25]
ATG:					
Reconstitution*		>12 months			[26]
pDCs	Lin-MHC+CD11c−				
Reconstitution*	CD123+CD303+/	>2 months			[24]
>.01 × 10^9/L	CD304+	8 years			[25]
		>18 months			[7]
ATG:					
Reconstitution*		>12 months			[26]
γδ T cells	γδ-TCR				
'expansion'*		2 months	7 months		[27]

AUC indicates area under the cell-time curve.
To keep reconstitution times comparable, reference values of normal reconstitution time after HCT were used, based on presented data. No data were available for monocytes.
* Divergent reference value/reference value is not based on reference values, due to lack of information in the article.

of time. Although cDCs stabilize to control reference levels within 1 year, pDCs remain significantly lower and approach normal levels toward the end of a follow-up period of 7 years [25].

Associations between pDC reconstitution and the occurrence of GVHD show some conflicting data [38]. Relatively low pDC levels at days 0 to 60 after HCT are associated with moderate to severe aGVHD in children, which is in line with observations in adults [54-56]. On the other hand, high pDC peak levels at days 0 to 60 are associated with higher risk of relapse, whereas high overall levels of both cDCs and pDCs during the first 200 days after HCT are related to higher relapse-free survival (evaluated as the area under the curve of z-score graphs where a high initial pDC level would lead to a reduced z-score over a longer period of time) [38]. As DCs play an instrumental role in the initiation of immune responses and maintenance of homeostasis, the use of the presence and activation state of DC subsets as biomarkers may be included in future studies.

Gamma delta (γδ) T cells

Gamma delta T cells represent about 5% of the whole T cell population, residing predominantly in epithelial tissue [55]. Their T cell receptor (TCR) is composed of γ and δ chains instead of the conventional α and β glycoproteins, which makes them easy distinguishable using flow cytometry. Gamma delta T cells seem to act independent of MHC and are able to recognize nonpeptide antigens that are up-regulated by stressed cells. Different from NK cells, γδ T cells can differentiate into memory cells that readily act against infections [57]. This gives them a broad array of action that includes tumor surveillance, regulation of innate and adaptive immune responses, and tissue maintenance [58]. Importantly, γδ T cells are suggested to facilitate engraftment after allo-HCT and IR, exert graft-versus-leukemia (GVL) responses, and have antiviral activity without exacerbating GVHD (reviewed in [9]).

After BMT in pediatric patients, γδ T cell reconstitution is faster and results in higher cell numbers (60 days) compared with after PB stem cell transplantation (PBSCT)

Table 2
Post-Allo-HCT IR and Distinctive Markers of Adaptive Immune Cell Populations in Children

Cell Numbers	Phenotype	Reconstitution Time			References
		BM	PBSC	UCB	
NKT cells >200 type I NKT cells/10^6 T cells*	$CD3^+CD56^+$	1 month			[20]
ATG: >200 type I NKT cells/10^6 T cells*				1 month	[20]
T cells >1.5 × 10^9/L* Reference value* 80% p5-value* Reconstitution*	$CD3^+$	*24 months* *>12 months* 13 months		 11.7 months	 [16] [17] [28] [18]
>.5 × 10^9/L† >1 × 10^9/L		2-3 months 12-15 months		2-3 months 12-15 months	[12] [12]
>.5 × 10^9/L† >1 × 10^9/L				1.5 months 7 months	[19] [19]
>.5 × 10^9/L† >1 × 10^9/L			*6 months* *>6 months*	3 months >6 months	[29] [29]
>.5 × 10^9/L† >1 × 10^9/L			*8 months* *24 months*	4 months 12 months	[13] [13]
>.5 × 10^9/L† >1 × 10^9/L				2 months 9 months	[8] [8]
ATG: >.5 × 10^9/L† >1 × 10^9/L				9 months 10 months	[22] [22]
>.5 × 10^9/L† >1 × 10^9/L				8 months 11 months	[23] [23]
>.5 × 10^9/L† >1.5 × 10^9/L*		3.2 months 9.3 months		6.3 months 9.3 months	[14] [14]
>.5 × 10^9/L† >1 × 10^9/L				6 months 12 months	[8] [8]
Naïve T cells Reference* (CD4) Reference* (CD8)	$CD3^+CD45RA^+/CD45RO^-$ $(CD62L^+CCR7^+/CD27^+) + CD4^+/CD8^+$	 *>12 months* *>12 months*		 	 [17] [17]
>.25 × 10^9/L† >.5 × 10^9/L		6 months 9 months		 	[7] [7]
>.25 × 10^9/L† (CD4) >.5 × 10^9/L (CD4)			*12 months* *24 months*	8 months 12 months	[13] [13]
>.25 × 10^9/L† (CD4) >.5 × 10^9/L (CD4)				2 months 8 months	[8] [8]
>65% of CD4*		12 months			[20]
ATG: >.25 × 10^9/L† >.5 × 10^9/L				8 months 10 months	[23] [23]
>.25 × 10^9/L† (CD4) >.5 × 10^9/L (CD4)				8 months > 12 months	[8] [8]
>65% of CD4*				12 months	[20]
Memory T cells Reference* (CD4) Reference* (CD8) >.5 × 10^9/L* >23% of CD4*	$CD3^+CD45RO+(CD62L+/-)$	 *>12 months* 3 months 1 month	 *24 months* 	 24 months 	 [17] [17] [13] [13]
ATG: >.5 × 10^9/L* >23% of CD4*				24 months 1 month	[23] [20]

(*Continued on next page*)

Table 2
(*continued*)

Cell Numbers	Phenotype	Reconstitution Time			References
		BM	PBSC	UCB	
Cytotoxic T cells	$CD3^+CD8^+$				
$>.4 \times 10^9$/L*		*14 months*			[16]
Reference value*		*12 months*			[17]
80% p5-value*		3 months			[28]
Reconstitution*				7.9 months	[18]
$>.25 \times 10^9$/L		2-3 months		2-3 months	[12]
		3 months	9 months		[27]
				8 months	[19]
			6 months	3 months	[29]
			8 months	8 months	[13]
$>.4 \times 10^9$/L*		6 months			[20]
ATG:					
$>.25 \times 10^9$/L				6 months	[21]
				9 months	[22]
				8 months	[23]
		2.8 months		7.7 months	[14]
$AUC_{day\ 0\text{-}90}$*		1000		1000	[15]
$>.4 \times 10^9$/L*				9 months	[20]
T helper cells	$CD3^+CD4^+(CD45^+)$				
$>1.1 \times 10^9$/L*		*24 months*			[16]
Reference value*		*>12 months*			[17]
80% p5-value*		13 months			[28]
Reconstitution*				11.7 months	[18]
$>.2 \times 10^9$/L†		2-3 months		2-3 months	[12]
$>.5 \times 10^9$/L		12-15 months		12-15 months	[12]
$>.2 \times 10^9$/L†		4 months	10 months		[27]
$>.5 \times 10^9$/L		12 months	12 months		[27]
$>.2 \times 10^9$/L†				1 month	[19]
$>.5 \times 10^9$/L				4 months	[19]
$>.2 \times 10^9$/L†			*6 months*	3 months	[29]
$>.5 \times 10^9$/L			*>6 months*	>6 months	[29]
$>.2 \times 10^9$/L†			*8 months*	4 months	[13]
$>.5 \times 10^9$/L			*12 months*	8 months	[13]
$>.2 \times 10^9$/L†				2 months	[8]
$>.5 \times 10^9$/L				12 months	[8]
$>350/mm^3$*,†		9 months			[20]
$>700/mm^3$*		12 months			[20]
ATG:					
$>.2 \times 10^9$/L†				6 months	[21]
$>.5 \times 10^9$/L				12 months	[21]
$>.2 \times 10^9$/L†				9 months	[22]
$>.5 \times 10^9$/L				12 months	[22]
$>.2 \times 10^9$/L†		3-6 months		6 months	[23]
$>.5 \times 10^9$/L		>6 months		8 months	[23]
$>.2 \times 10^9$/L†		6.0 months		5.0 months	[14]
$>.5 \times 10^9$/L		13.3 months		9.3 months	[14]
$AUC_{day\ 0\text{-}90}$*		5000		2000	[15]
$>.2 \times 10^9$/L†		3-6 months		>6 months	[30]
$>.5 \times 10^9$/L		>6 months		>6 months	[30]
$>.2 \times 10^9$/L†				6 months	[8]
$>.5 \times 10^9$/L				12 months	[8]
$>.35 \times 10^9$/L†,*				9 months	[20]
$>.7 \times 10^9$/L*				>12 months	[20]

(*Continued on next page*)

Table 2
(*continued*)

Cell Numbers	Phenotype	Reconstitution Time			References
		BM	PBSC	UCB	
Tregs >4% of CD4*	$CD4^+CD25^+FOXP3^+$	1 month			[20]
ATG: Prop. $CD4^+$ >10*		6 weeks		1-6 weeks	[30]
>4% of CD4*				1 month	[20]
B cells	$CD19^+$ (or $CD20^+$)				
>.1 × 10^9/L $CD20^+$		*14 months*			[16]
80% p5-value*		9-10 months			[28]
Reconstitution*				5.9 months	[18]
>.2 × 10^9/L		12-15 months		2-3 months	[12]
		7 months	4 months		[27]
		6 months			[7]
				2 months	[19]
			12 months	4 months	[13]
				2 months	[8]
>.2 × 10^9/L*		3 months			[20]
ATG: >.2 × 10^9/L				3-6 months	[21]
				6 months	[22]
				3 months	[23]
		6.4 months		3.2 months	[14]
$AUC_{day\ 0\text{-}90}$*		5000		20,000	[15]
>.2 × 10^9/L				6 months	[8]
				9 months	[20]

AUC indicates area under the cell-time curve.
To keep reconstitution times comparable, reference values of normal reconstitution time after HCT were used, based on presented data.
Italics denotes T cell–depleted graft.
* Divergent reference value/reference value is not based on reference values because of lack of information in the article.
† Prereconstitution value to indicate recovery rate/speed.

Table 3
Details of Studies on IR after HCT in Children

Reference	n	Age	Time Points Measured after HCT
[31]*	19	Not specified	During 12 months, frequency not specified
[16]	102	8.1 (1.1-18.4)	1, 2, 3, 4, 9, 12, 18, 24, 36, 48, 60, 72 months
[17]	12	8.9 (2-15)	3 and 12 months
[28]	22	7.3 (1.3-13.2)	1-18 months
[21]	30	6.9 (1-18)	1, 2, 3, 6, 9, and 12 months
[22]	12	4.5 (1.5-13.5)	3, 6, 9, and 12 months
[18]	71	4.0 (0-15)	2-3, 6, 9, 12, and 12-24 months
[12]	46	7.8 (.4-19)	2-3 and 12-15 months
[23]	8	4.6 (1.3-17.2)	3, 6, 9, 12, 24, and 36 months
[32]*	27	8.5 (.2-22)	16 days, and 1, 2, 3, 4, 6, 9, 12, 18, and 24 months
[33]*	117	6.7 (.6-17.9)	1, 2, 3, 6, 9, 12, 18, 24, and 36 months
[34]*	88	7.2 (.6-17)	1-3, 6, 9 months, and 1-5 years
[27]	25	9.8 (1.4-22.2)	60, 120, 180, 240, 300, 365, and >365 days
[24]	43	12 (1.2-19)	Approx. 10, 15, 20, 25, 30, 35, 40, 46, and 60 days
[25]	39	7 (.6-16.2)	17, 20, 24, 32, 40, 62, 68, and 72 days
[35]†	32	9.6 (2.5-23)	Approx. 3-12, 13, 15, 17, 19, 21, 23 weeks, then monthly
[26]	13	11 (3-20)	15-30, 30-60, 60-100, 100-180, and 180-365 days
[14]	226	5.9 (.09-18.7)	1, 2, 3, 6, 9, 12, 18 months, and 2, 3 and 5 years
[7]	51	8 (1-19)	0, 3, 6, 9, 12, and 18 months
[19]	30	1 (.1-12)	1, 2, 3, 6 and 12 months
[36]†	38	2.1 (.8-16.3)	3 and 12 months
[29]	57	6.2 (1-17)	3 and 6 months
[30]	30	5.8 (.3-20.1)	1-6, 6-12, 12-24, and >24 weeks
[15]	103	4.9 (.1-21)	Every 2 weeks during first 90 days.
[13]	76	9 (5-14)	4, 6, 12, and 24 months
[37]*	56	6.9 (.3-13.5)	1, 3, 6, 9, and 12 months
[8]	127	3.2 (.1-22.7)	1, 2, 3, 6, and 12 months
[20]	40	10.8 (.5-20)	1, 2, 3, 6, 9 and 12 months
[38]†	45	10 (5-15)	Not specified
[60]†	102	10.5 (.6-25.2)	Not specified
[40]*	110	7.8 (.4-18.6)	1, 2, 3, 6, and 12 months
[41]†	13	2.4 (.4-8)	1, 2, 3-5, and >5 years

* Not in Table 1 or 2 as no distinction between HCT sources/ATG conditioning was made.
† Not in Table 1 or 2 because of incompatible data, but described in text.

(200 days), whereas no data are available for UCB transplantation (UCBT) (Table 1 and [27]). This difference is probably due to the $CD34^+$ selection of cells from PB, thus depleting γδ T cells and other cells, whereas unmanipulated BM still contains γδ T cells. Moreover, γ δ T cell levels may be decreased after conditioning with ATG before allo-HCT [39].

Unfortunately, these studies do not provide an overview of γδ T cell reconstitution over time and this indicates the need for further evaluation of actual reconstitution. This is further highlighted by findings in adult patients, where normal γδ T cell levels are reached within 1 year in patients without infection, and high γδ T cell counts rapidly after HCT are associated with, and may thus be indicative for, fungal or viral infections [39]. Interestingly, early reconstitution of γδ T cells is also related to better survival chances in pediatric leukemia patients, suggesting a role for these cells in regaining early immune function with an additional GVL effect in case of malignancy [58-61].

Reconstitution of the Adaptive Immune System

NK T (NKT) cells

NKT cells represent a type of T cell with NK characteristics. These cells can be divided into 2 main subsets: type I NKT cells, or invariant/classical NKTs, expressing an invariant TCR-Jα, and type II NKT cells, which have a non-invariant TCR-Jα chain. They express CD3, CD56, and CD161, and most are CD1d restricted for recognition of (glycol) lipids rather than antigen-MHC complexes. Just like NK cells, NKT cell function is regulated by clonally distributed inhibitory receptors termed KIRs that recognize HLA class I allele groups. In general, NKT cells play a role in both the adaptive and the innate immune system and can be activated by both antigen-dependent and -independent pathways. They are able to perform functions attributed to cytotoxic T cells and Th cells, which are direct cytotoxicity and cytokine production. This allows them to exhibit remarkable functional plasticity with both pro-inflammatory (type I NKT cell) and immunoregulatory (type II NKT cell) characteristics. Moreover, NKT cells are associated with anti-tumor effects and could aid in GVL (reviewed in [62]).

Regarding reconstitution after HCT, Charrier and colleagues reported on NKT reconstitution in children, showing that type I NKT levels were normalized already 1 month after BMT and UCBT [20] (Table 2). As early reconstitution of NKT cells in adults is associated with lower probability of aGVHD and higher survival [63], future studies should evaluate NKT reconstitution, also of type II NKT cells, and function after HCT to study their predictive value in children.

T cells

T cells, generally marked with CD3 on their surface, are a family of distinct subsets that use their specific TCR to recognize antigens presented by MHC molecules. T cell reconstitution is dependent on thymic output and homeostatic peripheral expansion (HPE). Thymic tissue is generally destroyed by conditioning regimens, which means that within the first ~6 months, generation of T cells is largely dependent on HPE [64]. HPE is reduced when T cells are removed from the graft before transplantation by ex vivo depletion techniques, during treatment of GVHD with immunosuppressive drugs (mainly steroids), or after serotherapy for conditioning (eg, ATG) [11]. Reconstitution of naïve T cells ($CD45RA^+CD62L^+CCR7^+/CD27^+$) is important for antipathogen and antirelapse immunity after HCT. As the formation of naïve T cells is highly dependent on thymic output, their growing numbers are highly indicative for thymopoiesis [65].

The T cell population can be differentiated into $CD4^+$ Th cells and $CD8^+$ cytotoxic T cells. Th cells show notable plasticity and are able to differentiate into multiple subsets based on cytokine stimulation during MHC class II–dependent priming by APCs. When activated, they start to proliferate rapidly and secrete a set of cytokines that discriminate them as Th1, Th2, Th9, Th17, Th22, or follicular Th cells, each facilitating a different type of immune response. Those responses can be directly toxic to target cells or can stimulate other T cell effector functions or B cell antibody production [66]. After allo-HCT, a rapid but stable CD4 recovery in time seems to be a predictor of higher chance of survival, regardless of cell source. However, a peak of CD4 cells within the first 90 days after HCT, followed by rapid reduction of CD4 levels, is associated with higher mortality [15], probably reflecting a viral or GVHD event.

Cytotoxic $CD8^+$ T cells are antigen-specific immune cells able to attack virally infected cells and tumor cells when antigens are presented in the context of MHC class I. Because of their cytotoxic nature, they are also implicated in transplantation rejection and GVHD. Like Th cells, cytotoxic T cells will disappear after the pathogen is eliminated, although some will remain and form memory T cells. Memory T cell reconstitution ($CD45RO^+$, $CD62L^+$, or $CD62L^-$, central and effector memory populations, respectively) is mainly dependent on T cell differentiation upon activation by an APC. They survive for many years and provide long-term protection against previous encountered antigens (eg, from infections), which is important after HCT.

Despite of the vast variety of subsets within the T cell population and associated distinctive effector functions, most studies fail to differentiate between these cells when studying IR. Subsequently, $CD3^+$ cells are mostly studied as 1 group, with few T cell subsets, including $CD4^+$ and $CD8^+$, $CD45RO^-/CD45RO^+$, but also γδ TCR^+ and $CD25^+FoxP3^+$ regulatory T cells (Tregs) (discussed in other paragraphs). As these cells are implicated in different beneficial or adverse events, more detailed immunomonitoring protocols could provide more insight into the biological mechanisms at play during IR and may provide associations between these mechanisms and clinical effects.

T cell reconstitution ($>1 \times 10^9$/L) after allo-HCT without ATG in the conditioning regimen takes about 7 to 12 months in both BMT and UCBT and 6 to 24 months in PBSCT (Table 2). T cell recovery to $.5 \times 10^9$ cells/L can be as early as 1.5 month after UCBT [19], but no differences are apparent between BMT and UCBT [12]. Compared with PBSCT, T cell recovery is faster after UCBT [13,29]. After conditioning with ATG, T cell reconstitution is drastically delayed in particular after UCBT compared with after BMT [14]. These data are in line with the finding that ATG is particularly detrimental for naïve T cells [8], which are more abundant in UCB grafts [67].

Regardless of allo-HCT source, $CD8^+$ T cell reconstitution is faster compared with $CD4^+$ Th cells (2 to 8 months compared with 4 to 12 months, respectively) (Table 2 and [36]), which was also found in adult HCT patients [11,23]. This suggests that HPE of mature cells from the graft is mostly determined by rapid $CD8^+$ T cell reconstitution [68]. $CD4^+$ T cells seem to be affected somewhat more by ATG than $CD8^+$ T cells, for all transplantation sources, which

corresponds with the better predictive value of $CD4^+$ T cell reconstitution for ATG exposure [69].

Naïve T cells seem to recover more quickly (8 to 9 months) than memory T cells (24 months), regardless of transplantation source (Table 2). These findings of memory T cells are, however, based on only a few studies that express a high variability in both time points for sampling and the phenotype of memory subsets, which complicates drawing conclusions for memory T cells. Conditioning with ATG before allo-HCT highly affects naïve T cell recovery, whereas memory T cell reconstitution is not affected. Although UCB almost exclusively contains naïve T cells, memory markers are expressed within 1 month on almost one-half of all T cells after UCBT [19]. Moreover, virus-specific T cells were found 2 months after UCBT, indicating that naïve CB T cells can rapidly be primed and expanded in vivo and, thus, are potential targets for immune adjuvant therapy.

Treg

Tregs have immunosuppressive capacities and are a critical component of both central and peripheral tolerance mechanisms. In general, Tregs inhibit the proliferation and cytokine secretion of T cells, B cells, NK cells, NKT cells, and APCs, and maintain self-tolerance and immune homeostasis [70]. Tregs are identified by a $CD3^+CD4^+CD25^+CD127^{low}$ phenotype [71] with intracellular expression of the transcription factor FoxP3 [72]. They can be subdivided into thymus-derived naturally occurring Tregs and induced Tregs that differentiate from nonregulatory $CD4^+CD25^+$ cells (Tr1 and Tr3) [73].

A limited number of studies described reconstitution of $FoxP3^+$ Tregs after UCBT and BMT, which takes 1 to 6 weeks for both sources, even with ATG in the conditioning [20,30] (Table 2). When comparing UCB, sibling BM, and unrelated BM transplantations, Treg reconstitution after UCBT and sibling BMT is much faster than after unrelated BMT [30]. The results in UCBT may be associated with the finding that fetal T cells more easily develop into Tregs than adult T cells [67]. Together with the lower numbers of effector T cells present in UCB, this could explain why some studies found that GVHD occurs less often after UCBT (despite HLA disparity), compared with other transplantation sources [74,75]. Data in adults showed that low Treg numbers during the first 30 days after transplantation were associated with the occurrence of GVHD [76,77], whereas high numbers of Tregs at day 100 after HCT were also associated with the occurrence of GVHD in another study [55]. Taken together, these studies suggest that Treg numbers are indeed involved in the development and control of GVHD; however, confirmation from other studies in well-defined cohorts for pediatric patients is needed.

B cells

B cells play a vital role in the adaptive immune response, which is highlighted by the finding that slow B cell reconstitution significantly increases the risk of infection [78]. $CD19^+$ pre-B cells differentiate into pro-B cells after immunoglobulin (Ig) heavy chain rearrangement. Subsequently, rearrangement of the light chain leads to differentiation into immature B cells. Maturation of B cells occurs when they come into contact with antigens, after which they express immunoglobulins (IgM and IgD). Mature B cells can be further divided into subpopulations by the presence of CD21 and/or CD27 [79]. In pediatric allo-HCT studies, however, most information is reported on $CD19^+$ B cells alone.

Overall, B cell reconstitution seems to be affected by HCT source and use of ATG. Without ATG conditioning, B cell reconstitution after UCBT is earlier (2 to 3 months) compared with after BMT (>6 months) or PBSCT (4 to 12 months) (Table 2). The faster B cell recovery after UCBT may show similarities with the B cell developmental pattern in babies with levels that rapidly increase within the first 5 months after birth [80]. B cells of children who received ATG before UCBT needed 3 to 6 months for reconstitution, defined as $.2 \times 10^9$/L. B cell reconstitution time is also influenced by steroid treatment of acute and chronic GVHD, leading to fewer B cell precursors, which may be ascribed to a lower Th cell activity [81]. Memory B cell reconstitution is not reached until 2 years after HCT, where switched memory B cells ($CD19^+CD27^+IgD^-IgM^-$) were restored earlier and better than IgM memory B cells ($CD19^+CD27^+IgD^+IgM^+$) in a cohort of primary immune deficiencies [41]. The fact that low levels of B cells are associated with the increased incidence of infections and major complications [11,36,78] underscores the importance of an early and functional B cell reconstitution.

IR after Haploidentical Stem Cell Transplantation

As the use of haploidentical cell transplantation (haplo-HCT) has undergone an evolution over the last decades using various strategies, it will be discussed here separately. Recently, haplo-HCT has renewed interest as a HCT source, with a rising number of haploidentical transplantations being performed in pediatric patients. Historically, haplo-HCT was performed using high-dose $CD34^+$-selected cells (haplo-PBSCT) and ex vivo T cell depletion methods (eg, $CD34^+$ selection) to minimize/prevent alloreactivity. Although GVHD was prevented successfully, high rates of viral reactivation/infections and relapse were noted due to delayed IR [82,83]. After haplo-HCT with $CD34^+$-selected grafts in children, B and T cells (including $CD3^+$, $CD4^+$, and $CD8^+$ T cells) take 4 to 6 years to reconstitute (reviewed in [82]), with no detectable T cell recovery until 5 to 6 months after haplo-HCT [83]. Slow NKT reconstitution (18 months) was associated with high relapse rates [84].

Currently, new options for this HCT source show improved IR potential compared with historic data on $CD34^+$-selected haplo-HCT [85]. The 2 most commonly explored options are $TCR\alpha\beta^+/CD3^+$ T and $CD19^+$ B cell depletion of the graft, and a T cell–replete graft followed by post-HCT cyclophosphamide (day +3, +4) treatment. Only a few studies have evaluated IR after haplo-HCT, showing comparable IR to allogeneic BMT [86,87]. IR after haplo-HCT with a T cell–replete graft followed by post-HCT cyclophosphamide remains to be evaluated in pediatric patients, as no published data are available so far. After haplo-HCT with $\alpha\beta^+$ T and $CD19^+$ B cell depletion of the graft in pediatric patients, generally, a prompt $\gamma\delta$ T cell recovery from graft origin is seen during the first weeks [88,89], NK cell recovery to a reference value of $.1 \times 10^9$/L takes about 1 month, T cell recovery ($>.5 \times 10^9$/L) 3 months, and T cell reconstitution ($>1 \times 10^9$/L), 12 months [85,87,88,90]. However, the functionality of immune cells after haplo-HCT needs further investigation. Further comparisons of IR, as well as functionality, after haplo-HCT with BMT, PBSCT, and UCBT are of interest to evaluate its clinical efficacy as an alternative HCT source.

Table 4
Serum Cytokine Levels and Their Relation to Clinical Outcomes after Allogeneic HCT in Pediatric and Adult Patients

Cytokine	Kinetics	Clinical Relation	Reference
IL-7	↑ at +1 week	Successful T cell depletion ↑	[91]
	↑ at +1 week	T cell recovery ↓	
	↑ at +2 weeks*	II-IV aGVHD ↑* (several days later)	
	↑ at +1 week*	Mortality ↑*	
TNF-α	↑ at +2 weeks	Viral reactivation ↑ (ongoing)	[92]
	- (not present) 1-4 weeks	Viral reactivation ↓ (ongoing)	
IL-6	↑ at +1 week	Viral reactivation ↑ (1-3 weeks later)	[92]
	↑ during 1-4 weeks	GVHD ↑ (≥2 weeks later)	[93]
	↑ during 1-4 weeks	VOD ↑ (≥2 weeks later)	[93]
	↑ at +1 and +4 weeks*	Transplantation-related complications ↑*	[94]
IL-10	↑ at 1 week and 4 weeks*	Transplantation-related complications ↑*	[94]
HGF	↑ during 2-14 weeks	VOD ↑ (≥2 weeks later)	[93]
	↑ at +2 and +3 weeks	II-IV and III-IV aGVHD (visceral) ↑	[95,96]
GM-CSF	↑ baseline	GVHD ↑	[93]
IL-2Rα	↑ at +2 and +3 weeks	Grade II-IV and III-IV aGVHD (skin) ↑	[95,96]
IL-8	↑ at +4 weeks	cGVHD ↓	[95]
IL-17A	↓ baseline*	Systemic cGVHD ↑*	[97]
ST2	↑ at day 28*	II-IV and III-IV aGVHD ↑* TRM ↑*	[98]

HGF indicates hepatocyte growth factor; GM-CSF, granulocyte macrophage colony–stimulating factor; ST2, suppressor of tumorigenicity 2.
* Data from adult patients veno-occlusive disease (VOD).

BIOMARKERS IN THE SECRETOME AFTER HCT

Using cytokines as biomarkers for clinical outcome/complications may be a powerful and yet underutilized strategy for efficient clinical decision making. This may be particularly true in the early stages after allo-HCT where lymphopenia, conditioning-induced tissue damage, and complications (eg, GVHD, infections) may leave specific fingerprints of cytokine/chemokine profiles. Here, we provide an overview of potential biomarkers within the secretome after allo-HCT (summarized in Table 4) derived from studies in children and, due to the limited numbers of studies, adults.

IL-7 is implicated as a biomarker for successful T cell depletion and recovery. As such, increasing IL-7 concentrations may be interpreted as a reflection of delayed immune reconstitution. However, high IL-7 levels were also related to increased mortality and aGVHD [91]. Other potential biomarkers for aGVHD, which can be found at least several days to even weeks before its onset, are high levels of IL-6, granulocyte macrophage colony–stimulating factor, hepatocyte growth factor, and IL-2Rα, the latter 2 being related to visceral or skin aGVHD, respectively [93,95,96]. A high suppressor of tumorigenicity 2 level at day 28 is a strong indicator of grades II to IV or III and IV aGVHD and TRM [98]. High levels of IL-8 are associated with lower probability of cGVHD and low levels of IL-17A predict increased probability of cGVHD [95,97]. Moreover, biomarkers for viral reactivation are again IL-6, which peaks 1 to 3 weeks before onset, but also TNF-α, which is an indicator for an ongoing viral reactivation [92]. Veno-occlusive disease is also related to higher levels of IL-6 and hepatocyte growth factor, which are heightened at least 2 weeks before clinical symptoms occur [93]. High serum IL-6 and IL-10 were found to be early predictors of overall transplantation-related complications [94], wherein IL-10 is suggested to protect the host from transplantation-related complications, including aGVHD [99,100].

These data indicate that the dynamics over time are extremely important in exploring the possibility of discriminating the predictive value of these mediators as a biomarker (initiation phase) versus the use as a reflection of ongoing adverse events (effector phase). Although this overview shows their potential as biomarkers, the current lack of validated cytokine profile studies in time, combined with the lack of reporting actual serum/plasma concentrations, complicates a comprehensive view on their predictive value in pediatric HCT patients. Harmonized HCT immunomonitoring studies with identical timing and logistics of sampling and standardized analyses methods will provide the necessary in-depth insights into the biology of IR and functionality of regained immune cells, which may result in biomarker identifications to predict and treat complications earlier and more efficiently.

CONCLUDING REMARKS AND FUTURE PERSPECTIVE

Adequate IR after HCT is the most important determinant for survival, as most life-threatening complications (eg, relapses and viral reactivation) are associated with delayed immune function. Historically, it has been suggested that IR after UCBT would be relatively slow because of the low number and immaturity of lymphocytes transferred with the graft, but from the pediatric studies presented here, we conclude that IR after UCBT is rapid and comparable to other stem cell sources. Since UCBT and BMT are most widely used for pediatric allo-HCT, fewer data are available for PBSCT. Reconstitution of NK and overall T cells after UCBT are comparable to those after BMT (Tables 1 and 2), and Treg, B, and Th cell reconstitution seem to be faster after UCBT, compared with after BMT/PBSCT. Moreover, $CD8^+$ cytotoxic T cell reconstitution is faster after BMT [14], but virus-specific $CD8^+$ T cells can be found earlier after UCBT [19]. As such, UCBT has gained ground because of improved conditions in the UCBT setting (eg, higher graft cell counts, better quality of UCB units, and professional storage of grafts in biobanks) as discussed in an elegant review by Gluckman et al. [101]. Another major determinant in IR and the outcome after UCBT is the use of T cell–depleting serotherapy, such as ATG, which has changed over the years.

Interestingly, faster IR after UCBT results in decreased leukemic relapse rates and improved overall survival [33]. This may relate to the generation of a broader T cell repertoire already within 2 months after UCBT [19] and suggests an intrinsic capacity of the developing immune system to generate antitumor specific CTL shortly after UCBT. The presence of T cell–depletion therapy significantly impacts T cell IR and subsequently limits the broadness of TCR repertoires [19]. In the presence of T cell–depleting serotherpy, polyclonality was similar in UCBT and BMT within 2 to 12 months after transplantation [102]. However, UBCT patients showed a more diverse TCR repertoire after 2 years [102]. Altogether, the fast proliferative capacity and subsequent broadness of the TCR repertoire after UCBT may provide an explanation for the reported lower relapse rates after UCBT (in the absence of ATG) compared with after BMT [103,104].

The effect of ATG conditioning on IR of the adaptive immune system, especially T and B cells, is more profound after UCBT than after BMT. Because ATG conditioning is related to higher incidence of viral reactivation [105], some centers have abandoned ATG to ensure early and robust IR after UCBT. Recently, our group investigated optimal ATG exposures before UCBT or BMT, aimed to not affect IR after transplantation but maintain effective T cell depletion at time of transplantation [69]. Optimal exposure after UCBT was found to be lower (<20 arbitrary units [AU]*day/mL) than after BMT/PBSCT (<50 AU*day/mL). In addition, exposure to ATG according to the present regimen was higher in older children, which put these children at increased risk of complications due to delayed IR [106]. This highlights the need for personalized ATG dosing regimens to maintain effective T cell depletion without inhibiting IR after HCT. It also opens up important avenues for additional immunotherapy to skew specific T cell responses after UCBT against previously identified tumor-associated antigens [107] or infectious agents [108,109]. In all, these findings emphasize the requirement of interpreting the results from comparative studies on IR or clinical effects after HCT with different stem cell sources in light of the absence or presence of T cell–depleting serotherapy.

Overall, IR data are mostly derived from routine standard-of-care measurements (T, B, and NK cells), but lack detailed information on reconstituting cell subsets or effector cell functionality. In addition, most studies started evaluating IR not earlier than 1 month, and mostly 2 to 3 months after HCT, whereas more information on immune status at earlier time points may hold early predictive values for HCT outcomes. In addition, secretome data (eg, multiplex cytokine/chemokine profiles) could also add to the understanding of IR mechanisms and cell functionality, and may even provide (early) biomarkers for individual disease outcome, such as viral reactivity, GVHD, or GVL. However, particularly in children, information on the secretome after HCT is lacking and should be incorporated in future studies to evaluate its value for biomarker profiles.

Although sufficient information is available for immune cell families (T, B, NK cells), the lack of both detailed information on IR, regarding subsets and function, and secretome data severely hampers the development of potential biomarkers and standardization of immunomonitoring. Standardization of cell subset identification by flow cytometry, and post-HCT time points evaluated, between centers is required to enable valid data comparison between studies and to facilitate multicenter studies. In addition, detailed information on IR is of importance to achieve the most optimal effect of adjuvant immunotherapies to improve disease control and prevent infectious complications. This will further the development towards “precision transplantation” for patients undergoing allo-HCT: an individualized HCT approach, based on detailed information of IR and biomarkers of each patient. This information enables timely intervention by the physician in case of complications, such as treating an upcoming viral reactivation with prophylactic antiviral therapy. This will reduce the probability of TRM, relapse, and other life-threatening complications, and subsequently improve survival chances.

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