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REVIEW

Natural Killer Cells in Allogeneic Transplantation: Effect on Engraftment, Graft- versus-Tumor, and Graft-versus-Host Responses

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Natural killer (NK) cells are effectors of the innate immune system and recognize cells transformed by viruses or neoplasia. Their response to "missing self" signals was described 3 decades ago, but the recent discovery of a panoply of activating receptors has made it clear that NK cell reactivity arises from a combination of inhibitory and activating signals. Successful clinical exploitation of NK cell reactivity was demonstrated in allogeneic transplantation for acute myelogenous leukemia from HLA-haploidentical donors when matched donors were not available. Multiple clinical studies have since attempted to use NK reactivity in the setting of both HLA-matched and -mismatched transplantation, with varying results. This review summarizes the heterogeneous clinical results and explains them based on a succinct description of NK cell biology.

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INTRODUCTION

Alternative donors are required for allogeneic hematopoietic cell transplantation (HCT) when suitable matched donors are not available in a timely fashion. Early studies investigating unmanipulated bone marrow (BM) grafts from mismatched or haploidentical related or unrelated donors demonstrated a high rate of nonrelapse mortality (NRM) resulting from graft failure, graft-versus-host disease (GVHD), or delayed immune reconstitution, and showed an association between increasing HLA disparity and worse prognosis [1-5]. Approaches designed to circumvent these hurdles included increasing peritransplantation immunosuppression, increasing the dose of hematopoietic stem cells [6,7], and manipulating the graft through T cell depletion (TCD) [8] or positive selection for $CD34^+$ hematopoietic stem cells [9,10]. A recent review of these studies concluded that GVHD in haploidentical transplant recipients can be ameliorated by TCD, but at the cost of increased relapse and delayed immune reconstitution [5]. Using a reduced intensity conditioning regimen before mismatched or haploidentical transplantation was found to be associated with high rates of engraftment and low treatment-related mortality (TRM) when combined with in vivo or ex vivo TCD [11-13]; however, these approaches were again complicated by relapse of malignancy and delayed immune reconstitution.

Pioneering work from Perugia, Italy, highlighted a remarkable effect of donor natural killer (NK) cells in reducing relapse after TCD haploidentical transplant for acute myelogenous leukemia (AML) [14-16]. This group reported that allogeneic, alloreactive NK cells promote engraftment and the graft-versus-tumor effect, whereas they reduce GVHD. Relapse-free and event-free survival outcomes were significantly better in patients exhibiting killer immunoglobulin-like receptor (KIR) ligands that were mismatched with those from their donor. The publication of these provocative data spurred numerous studies attempting to document the beneficial effects of NK cell alloreactivity; however, these studies'design, methods, and thus results have been very heterogeneous. Examination of a nonexhaustive list of these studies (Table 1) suggests that the following factors may be important in maximizing NK cell alloreactivity: high stem cell dose, extensive TCD, no GVHD prophylaxis, and myelogenous malignancy as the target. Other possible reasons for the disparate findings include the differences in the definition of NK cell alloreactivity (phenotypic vs genotypic; mismatch algorithm), donor source (related vs unrelated;

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Table 1. Selected Clinical Trials Investigating NK Alloreactivity

Reference	Disease	Number of patients	Conditioning	TCD	Graft source and composition	NK alloreactivity*	GVHD prophylaxis	Engraftment failure	Acute GVHD grade II +/Chronic GVHD	Infection	TRM	RFS	OS	Benefit from NK alloreactivity?
Haploidentical														
Ruggeri et al., 2007 [16]	AML	112	MA	Ex vivo	PB; 15 × 10^6/kg CD34, 3 × 10^4/kg CD3	1, 2, 3	0	6% vs 10%	10%, NS/NR	38% fatal infections	43%	67% vs 18%	NR	Yes
Leung et al., 2004 [86]	AML, ALL; pediatric	36	NR	$CD34^+$ selection	PB; < 3 × 10^4/kg CD3	1, 2, 4	0	NR	NR/NR	NR	NR	13% vs 54% relapse rate	NR	Yes, for relapse, "missing ligand" model
Lang 2004 [134]	Various	63	MA	CD34 or CD133 selected + ATG	PB; 19.5 × 10^6/kg MNC, < 2.5 × 10^4/kg CD3	1	0	17%	7%/13%	17% fatal infections	27%	NR	42%	No (equivalent to historical matched unrelated donor)
HLA-identical related														
Hsu et al., 2005 [47]	AML, CML, ALL, MDS	178	MA	Ex vivo	BM; 9× 10^5/kg CD3	2	Yes	0%	NS/NS	NR	NR	× 0.41 relapse (AML, MDS)	× 0.52 risk	Yes
Cook 2004 [135]	Various	220	MA/RIC	NR	NR	1, 2	Yes	NR	NS/NR	NR	NR	NS	31.6%) vs 56.1% (4 years)	No; worse survival for myeloid patients with C2/C2 and KIR2DS2 donor
Unrelated														
Giebel 2003 [136]	Various	130	MA	ATG	BM; 4.3 × 10^8/kg MNC	1	Yes	0% vs 4%	0% vs 15% (grade III-IV/NS	NR	6% vs 40%	Relapse 6% vs 21%	87% vs 48% (4.5 years	Yes
Kroger et al., 2006 [104]	AML, CML, ALL, MDS	142	MA	ATG	PB/BM	1, 2	Yes	0%	NS/NS	Increased	× 2.2 risk if alloreactive	× 3 relapse risk (activating KIR)	× 0.5 unless donors are KIR haplotype A	No; ligand/ligand model
Cooley et al., 2008 [59]	AML	448	MA	No	PB/BM	1, 2	NR	NR	NS/× 1.5 risk if activating haplotype	NR	NS	× 2 RFS (activating KIR)	× 1.5 with higher number of activating KIR (3 years)	Y for donors with group B KIR haplotype
Davies 2002 [137]	Various	175	MA	Ex vivo, minority	BM; 2 × 10^8/kg MNC	1	Yes	NS	NS/NR	NR	NR	9%-12% at 5 years (NS)	NS (whole group); × 0.5 (myeloid)	No

Farag 2006 [138]	AML, CML MDS	1571	MA	Minority ex vivo TCD	BM	1	NR	NS	NS/NS	NR	× 1.95 risk	NS	× 0.5 risk	No
Yabe et al., 2008 [132]	Various	1489	MA	ATG, minority	BM	1, 2	Yes	NR	× 1.7 risk; increased with 2DS2 gene in donor/NR	NR	NR	NS	× 1.93 risk	No (worse GVHD)
Miller 2007 [139]	AML, CML MDS	2062	NR	NR	NR	1	NR	NR	44% vs 30% (late-phase CML)/NR	NR	NR	× 0.54 relapse in early disease	NR	Yes, for early myeloid disease
Bornhauser et al., 2004 [99]	AML, CML, MDS	118	MA	ATG	PB/BM; 4 × 10^6/kg CD34	1	NR	10%, NS	46% vs 69%, NS/NS	NR	NR	Relapse 60% vs 35%	NS	No

RFS indicates relapse-free survival; OS, overall survival; MA, myeloablative conditioning; RIC, reduced-intensity conditioning; ATG, antithymocyte globulin; MNC, mononuclear cell; haploA, KIR haplotype A; C2, HLA-C2 alleles; NS, no significant difference; NR, not reported.
*Model used to define NK alloreactivity:
1, ligand/ligand (HLA typing of donor and recipient).
2, receptor/ligand (KIR genotyping or phenotyping of donor).
3, specific cytotoxicity assay of donor NK cells against recipient cells.
4, nonspecific cytotoxicity assay of donor NK cells against NK-susceptible targets (eg, K562 cell line).

haploidentical), disease state at transplantation, and ethnicity. Grafts from both BM or peripheral blood (PB) sources have been effective, but KIR-mismatched grafts may be associated with worse survival in nonmyeloablative transplants from unrelated cord blood (UCB) [17]. Recent evidence also suggests that mothers may be a superior haploidentical donor source [18].

The aforementioned approaches rely on the development of donor hematopoietic stem cell–derived NK cells in the host. Adoptive therapy using mature NK cells from haploidentical donors has been attempted, but a significant antileukemia effect has been difficult to demonstrate [19-22].

In an attempt to explain the heterogeneous outcomes of studies documenting NK alloreactivity, in this review we present a brief update of NK cell biology, describing NK cell development, activation, receptor types, effector functions, and models of alloreactivity. We then summarize the current understanding of the role of NK cells in mediating and modulating engraftment as well as the graft-versus-host and graft-versus-tumor responses.

OVERVIEW OF NK CELL BIOLOGY

NK cells circulate in the PB and lymphogenous organs, licensed and ready to engage target cells. After a short engagement, they are able to kill unless stopped by an inhibitory signal. NK cells develop in the BM and migrate to the PB, spleen, lymph nodes, and other tissues (eg, lung, liver, or uterus). As a component of the innate immune system, NK cells play a role in surveillance against transformed and virally infected cells [23]. NK cells compose 2% to 18% of the mononuclear cells in human PB [23] and have a turnover rate of approximately 14 days [24]. In humans, NK cells have traditionally been defined as expressing CD56 with or without CD16, without expressing T cell markers (CD3, T cell receptors). Level of CD56 expression further subdivides human NK cells into 2 broad groups, the $CD56^{dim}CD16^{bright}$ subset, which composes 90% of PB NK cells and has cytotoxic function, and the $CD56^{bright}CD16^{-}$ subset, which cooperates with dendritic cells (DCs) and T cells in lymph nodes to secrete interferon (IFN)-γ and promote adaptive immune responses. The $CD56^{dim}CD16^{bright}$ subset expresses major histocompatibility complex (MHC) class I allele–specific KIR, as well as the CXCR1 and CX3CR1 chemokine receptors, whereas the $CD56^{bright}CD16^{-}$ subset expresses the CD94/NKG2A receptors along with lymphogenous organ homing markers, such as CCR7, CD62L, and CXCR3. Recent evidence suggests that the $CD56^{bright}$ differentiates into the $CD56^{dim}$ subset [25]. In the mouse, NK cells are defined as $CD3^{-}$ $NK1.1^{+}$ or $DX5^{+}$ cells, and mature NK cells are further

subdivided into functionally disparate $CD11b^{+}CD27^{bright}$ and $CD11b^{+}CD27^{dim}$ subsets [26]. The $CD11b^{+}CD27^{bright}$ subset is highly cytotoxic, localized in the lymph nodes, and interacts with dendritic cells, whereas the $CD11b^{+}CD27^{dim}$ subset has a higher stimulatory threshold and is found in the spleen and PB. Recent evidence suggests that NKp46 may be a unifying marker of NK cells across both species [27].

NK Cell Development

NK cells arise from common lymphogenous progenitors in the BM in a process requiring signaling through the Flt3-, c-kit–, and gamma chain–associated receptors [28]. Further development, peripheral expansion, and survival depends on cytokine stimulation through the interleukin (IL)-2/IL-15 receptor [29,30]; an indirect effect through osteopontin in the microenvironment also has been postulated [31]. The transcription factors Ets-1 and PU.1 are important in early NK cell development, whereas Gata-2 and T-bet play a role in maturation, and CEPγ, MEF, and MITF are responsible for cytotoxicity and cytokine production in mature NK cells [28].

An NK cell's ability to respond to stimulation is related to the strength of the inhibitory signal received during its development, a process termed "licensing" or "education" [32,33]. Thus, NK cells without self-specific KIR are likely to be hyporesponsive, although evidence from mouse studies suggests that they still may retain the ability to react robustly when stimulated by cytokines or by a suitably potent antigen [34,35]. The acquisition of Ly49 receptors in mice and KIR in humans may be regulated by the cytokines IL-15 and IL-2, respectively [36,37].

NK Cell Activation

After infection or inflammation, NK cells are recruited to tissues under the control of the chemokine receptors CCR2, CCR5, CX3CR1, and CXCR3 [28]. Resting human PB or mouse splenic NK cells have poor cytotoxic potential and require activation, either by direct cell-to-cell contact and receptor recognition or by the action of cytokines. Type I interferon secretion by plasmacytoid and myelogenous DCs [38], DC *trans*-presentation of IL-15 [39], and $CD4^{+}$ T cell production of IL-2 in the lymph node [40] activate NK cells, leading to translation of a preexisting pool of granzyme B and perforin mRNA [41] and secretion of these effector molecules, whereas IL-12 and IL-18 lead to increased IFN-γ secretion by NK cells [42]. In contrast, transforming growth factor-β secreted by regulatory T cells [43] is a negative regulator of NK homeostasis and induces down-regulation of natural cytotoxicity receptor (NCR) families [28,43].

NK Receptors

NK cells recognize their targets through inhibitory and activating cell surface receptors. Three main receptor families have been described: KIR, C-type lectin receptors (including NKG2A-E and Ly49 in mice), and NCR (see Table 2). The observation that NK cytotoxicity is triggered by tumor (and other) cells lacking expression of self MHC class I molecules first led to the "missing self" hypothesis and the discovery of the inhibitory receptors [44]; however, it is now apparent that it is the balance of signaling through the different receptors that leads to the final "decision" on NK cell reactivity [45].

The KIR are type I transmembrane molecules belonging to the immunoglobulin superfamily that are encoded on chromosome 19q13.4. They are expressed on γδ CD8 T cells as well as NK cells, and recognize amino acids in the carboxyl terminal domain of the MHC class I α1 helix in specific groups of HLA-A, -B, or C alleles. The specificity of KIR for HLA-C allotypes is determined by allelic dimorphism at residues 77 and 80 of the HLA-C molecule [46]. KIR genetics are of importance for transplantation, because significant diversity occurs at the population level both within and between ethnicities [47]. Furthermore, KIR genes segregate independently of the HLA genes (encoded on chromosome 6), and thus 2 HLA-matched individuals (even if related) may still be KIR-mismatched. An individual's HLA class I genotype dictates which KIR or NKG2A receptor combination occurs as inhibitory

Table 2. Selected Human NK Cell Receptors

Receptor/Gene	Target (Where Known)	Allele Frequency*
Inhibitory		
KIR2DL2/3	HLA-C group 1 (also recognize some HLA-C group 2)	2DL2: 40%-60% 2DL3: 80%-95%
KIR2DL1	HLA-C group 2	90%-100%
KIR3DL1	HLA-A and B with Bw4 motifs at position 77-83 (but not HLA-B1301 or B1302)	90%-95%
CD94/NKG2A	HLA-E	100%
KIR3DL2	HLA-A3/A11	100%
LAIR-1	Collagen	
KIR2DL4	HLA-G	100%
Activating		
KIR2DS4		85%-95%
KIR2DS1	HLA-C group 2	30%-50%
KIR2DS2		40%-50%
KIR2DS3		0-30%
KIR2DS5		20%-40%
KIR3DS1		20%-40%
NKG2D	MICA/B, ULBP	
CD94/NKG2C	HLA-E	
DNAM-1 (CD226)	CD112, CD155	
NKp30	BAT3	
NKp44	Viral hemagglutinin	
NKp46 (CD335)	Viral hemagglutinin	
2B4 (CD244)	CD48	

*From http://www.allelefrequencies.net; accessed December 23, 2008.

receptors on the surface of that person's NK cells, however. Thus, functionally mature NK cells express at least 1 inhibitory receptor for self-HLA, and occasionally as many as 3 or 4 such receptors. Some KIR are more often used as single receptors [48,49].

KIRs may be inhibitory or activating. Recognition of the MHC class I target by inhibitory KIRs lead to phosphorylation of an immunoreceptor tyrosine-based inhibitory motif in their cytoplasmic tail, followed by an inhibitory downstream signal. Activating KIR contain the same extracellular and transmembrane domains as the related inhibitory KIR, but lack cytoplasmic tails. Thus, target recognition by activating KIR leads to an interaction with an adapter protein and activation of alternative downstream signaling pathways [45]. The activating KIR bind MHC class I molecules more weakly than the respective inhibitory KIR [50,51], although the ligands for activating KIR are not well characterized, and it is possible that as-yet unidentified non-HLA proteins serve as their true ligands [52,53]. Based on murine data, the inhibitory signal has been considered the dominant one [54], although this notion has been challenged by recent human data [55].

The mouse homologs of the human KIR are the Ly49 family of receptors, which are structurally distinct type II lectin–related homodimers. Like KIR, this family of receptors recognizes classical MHC class Ia molecules, such as H-2^d and H-2^k in mice, and also occurs in both immunoreceptor tyrosine-based inhibitory and stimulatory forms.

A particular NK cell in mice or humans may express anywhere from 0 to 4 Ly49 receptors or KIR, respectively. The expression of the inhibitory NKG2A receptor varies inversely with the number of KIR genes coexpressed on the cell surface, being highest in cells with no KIR expression [49,56]. Each KIR has a different affinity for its target, although some redundancy exists, such that a single KIR can recognize epitopes shared between different HLA alleles [57]; see Table 2. Further complexity is added by the fact that each MHC class I molecule may be recognized by both inhibitory and activating receptors on the same NK cell.

Inhibition is not solely MHC class I–mediated. The target of the CD94/NKG2A, B, or C receptor is the nonclassical MHC HLA-E molecule [58]; other inhibitory receptors include NKR-P1A and LAIR-1, which bind to LLT-1 and collagen, respectively [23,51].

The complement of KIR genes on one chromosome comprises a KIR haplotype. KIR haplotypes may vary in terms of gene number and gene content, [51]. Haplotype B is defined as encoding at least one of *KIR2DL5*, *KIR2DS1*, *KIR 2DS2*, *KIR2DS3*, *KIR2DS5*, or KIR*3DS1*, and haplotype A is defined as having none of these loci [59]. Homozygosity for haplotype A is seen in 25% to 30% of Caucasians and 80% of Japanese [59,60], whereas the remainder are heterozygous or homozygous for haplotype B and thus have combinations of activating and inhibitory KIR. Furthermore, KIR genotype correlates with phenotype in only about 75% of cases, because of allelic polymorphism and epigenetic silencing [61-64].

NK cells also may recognize MHC class I–negative targets using the NCR, including NKp30, NKp44, and NKp46, as well as NKG2D, CD16, and DNAM-1. Whereas NKp30 and NKp46 are constitutively expressed by all PB NK cells [65,66], NKp44 is up-regulated in IL-2–activated NK cells [67]. The ligands for the NCR in humans are not well characterized, but NKp30 may recognize BAT3, an intracellular ligand released in exosomes from tumor cells and DCs [68], and also may play a role in regulating DC lysis and maturation [69]. NKG2D plays a role in tumor immunosurveillance [70,71]; its ligands are rarely expressed by normal cells, but are up-regulated in response to cellular stress signals from transformation, viral infection, heat shock, and DNA damage. The ligands for NKG2D include MHC I–related genes A and B (MICA and MICB), as well as UL16-binding proteins (ULBPs) in humans and Rae-1 and H-60 in mice [72,73]. Another receptor with a recently identified role in immunosurveillance is DNAM-1, which recognizes the CD112 and CD155 in both mice and humans [74]. Human NK cells also express CD16, the FCγRIII receptor, which binds the Fc portion of IgG and thus mediates antibody-dependent cellular cytotoxicity [75].

NK Cell Effector Functions

On recognition, an immunologic synapse forms between the NK cell and its target, allowing direct cytotoxicity that is mediated through the perforin, granzyme, Fas/FasL, and TRAIL pathways [76-78], as well as by production of IFN-γ [23]. In addition, it has become clear that the original description of NK cells as "natural killers" spontaneously lysing transformed and virally infected cells encompasses only part of their effector mechanism; NK cells also promote DC maturation through tumor necrosis factor-α and IFN-γ secretion [79] and enhance Th1 polarization in secondary lymphogenous organs [80]. In addition to their viral or tumor targets, NK cells also may kill activated autologous $CD4^+$ cells [81] and thus provide a link between the innate and adaptive immune responses [82,83], with a possible additional role in protecting the host from excessive immune response to pathogens [23].

ALGORITHMS OF NK CELL ALLOREACTIVITY

The 2 major models used to predict NK cell alloreactivity are the "missing self" and "missing ligand" models (see Figure 1). According to the "missing self" model, NK alloreactivity is stimulated when the

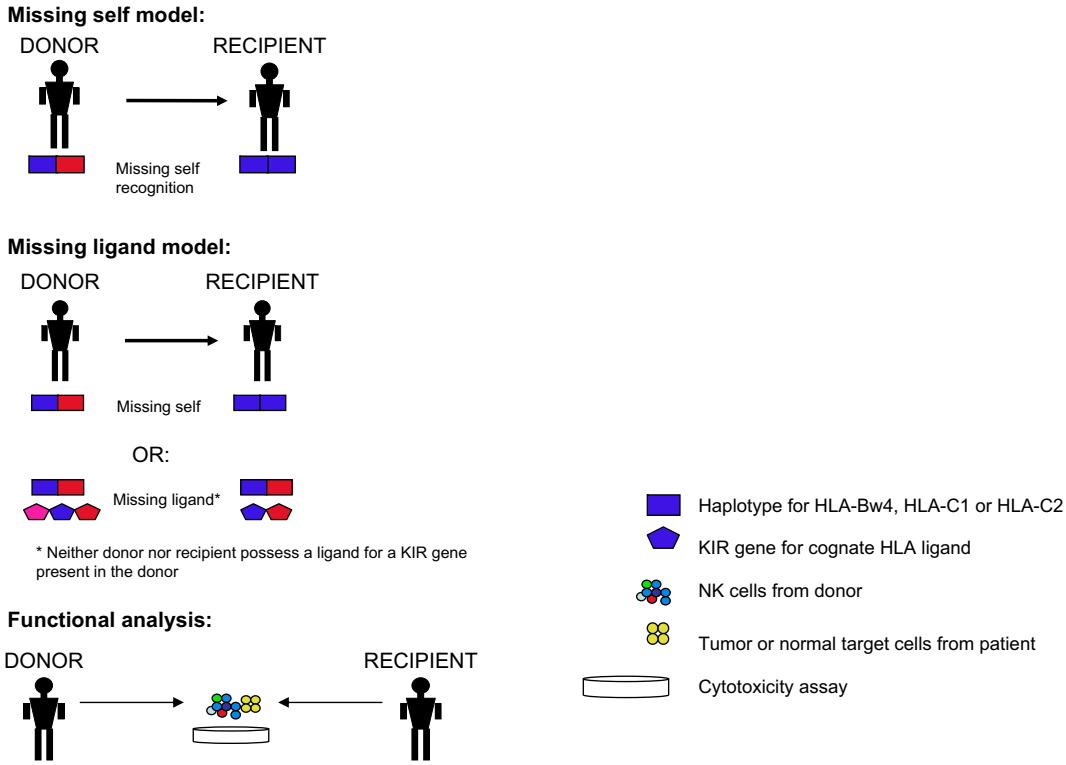

Figure 1. Models used to predict NK cell alloreactivity.

recipient lacks one or more HLA class I alleles present in the donor [84,85]. This was the model used by the Perugia group to predict NK alloreactivity in their transplantation studies. According to the alternative "missing ligand" (also known as the "receptor-ligand") model, NK cell alloreactivity also may occur in donor–recipient pairs matched for KIR and KIR ligands: when there is an extra KIR in the donor for which neither the donor nor the recipient has a ligand, the donor's potentially self-reactive NK cells are anergic in situ, but can trigger an alloreactive effect in the recipient. This finding is based on the observation that most individuals have 3 inhibitory KIR (for HLA-C1 and -C2 and for HLA-Bw4 alleles), but only 1 or 2 HLA KIR ligands on their own cells [47,64,86,87]. This latter model was found to be a better predictor of risk of leukemia relapse by some groups [47,86] but not by others [16], with the discrepancy likely related to differences in age, disease, and conditioning regimens.

Functional analysis may resolve the differences between predictive models and in some cases reveal unexpected findings, as exemplified by recent data reported by several groups. Ruggeri et al. [16] performed functional analysis to identify and quantitate the frequency of alloreactive NK clones against HLA-C–mismatched targets. Alloreactive clones were present in all donors, at a frequency of 8% (± 6%) for HLA-C group 2 mismatches and a frequency of 5% (± 3%) for HLA-C group 1 mismatches. Alloreactive clones were present in only 2/3 of HLA-Bw4–mismatched donors, however. As predicted by their model, Ruggeri et al. [16] found no NK alloreactivity in donor–recipient pairs that were not KIR ligand–mismatched. Fauriat et al. [49] evaluated the frequency of the alloreactive repertoire in donors with the A haplotype (consisting only of inhibitory KIR and *KIR2DS4*) and found that the use of KIR and HLA genotyping alone to predict alloreactivity may significantly overestimate the size of the alloreactive repertoire. They found a wide variability (0 to 62%) in the alloreactivity of the total NK pool in donor–recipient pairs (all of which would have been predicted to be alloreactive by use of genotyping) depending on the extent of KIR ligand expression on theoretical recipients; the alloreactive subset was larger for recipients lacking more than one KIR ligand [49]. Finally, Foley et al. [88] demonstrated that some Bw4 alleles (*HLA-B*1301* and *HLA*1302*) failed to protect targets from *KIR3DL1*-dependent lysis but, unexpectedly, *HLA-A*2402* and *HLA-A*3201* were protective against lysis; these results have implications for donor selection. Up to now, the 1/3 of individuals expressing all 3 KIR ligands (HLA-C1, HLA-C2, and HLA-Bw4) were thought to inhibit NK cells from all donors and thus be unable to benefit from NK alloreactivity [16]. Thus, the aforementioned findings, along with recent work suggesting that activating receptors may occasionally predominate, have important functional ramifications and suggest the need for caution when relying on genotypic predictive models alone.

ENGRAFTMENT AND IMMUNE RECONSTITUTION

Many studies have documented NK cells' ability to mediate rejection of allogeneic BM in murine models, as initially described in the "hybrid resistance" model [89]. This phenomenon has been ascribed to classical "missing self" recognition, but activating receptors, such as NKG2D, may play a role as well [90].

In an extension of these observations, infusion of alloreactive NK cells (in the graft-versus-host direction) into haploidentical mice led to ablation by the NK cells of host hematopoiesis and antigen-presenting cells (14). This may explain the ability of alloreactive NK transplants to facilitate engraftment, as first described by the Perugia group in 2002. Interestingly, a recent update of this data no longer showed a significant impact of NK alloreactivity on rejection [16]. NK cells are relatively radioresistant [91], and the presence of host-versus-graft alloreactive NK cells may increase the risk of graft failure or incomplete chimerism [92,93].

NK cells are the first lymphogenous cells to repopulate after engraftment [94]. Early after HLA-matched HCT, NK cells are $NKG2A^+$ and KIR^-; reconstitution kinetics are variable, and acquisition of a donor-type KIR repertoire may take anywhere from 3 months to 3 years [86,95]. Alloreactive NK cells of donor origin were detectable from 1 to 3 months up until at least 12 months posttransplantation in some studies of haploidentical transplantation [16,55], although another study found that NK cells reconstituting in the haploidentical setting had an immature $CD56^{bright}$ KIR^- $NKG2A^+$ phenotype, and that even putatively alloreactive cells had poor effector function against primary leukemia cells [96]. Reconstitution is adversely affected in the setting of T cell–replete grafts [97,98], and peritransplantation immunosuppression affects NK cell subsets and function [99,100]. In contrast, several groups have reported an association between NK recovery after HLA-identical HCT and improved relapse-free and overall survival (RFS, OS) [101,102].

Increased incidences of infection and infection-related mortality have been noted in several studies [16,103,104]. Impaired immune function is likely related to the extensive TCD required to prevent GVHD, as well as to the contribution of an NK-mediated attack on host antigen-presenting cells. Finally, cytomegalovirus (CMV), a common pathogen in severely immunocompromised patients, has been shown to shape the NK receptor repertoire in healthy donors [105]. Conversely, donor KIR genotype was found to have an effect on CMV reactivation in HCT in some studies [106], but not in others [104].

ANTITUMOR EFFECT

NK cell–mediated rejection of tumor cells occurs through MHC class I–dependent and –independent mechanisms. In vitro cytotoxicity has been demonstrated against many tumor types, including AML and chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), non-Hodgkin lymphoma (NHL), multiple myeloma (MM), T-cell acute lymphoblastic leukemia (ALL), melanoma, renal cell carcinoma (RCC), and neural tumors [107-109]. Preclinical models have convincingly demonstrated efficacy against human AML [14]. Tumor cells exhibit differential sensitivity to NK cytotoxicity because of variegated expression of inhibitory and activating receptors on NK cells [23,71], thus NK-mediated clearance of leukemia cells can be augmented by the blockade of inhibitory receptors [110,111].

Clinical data on NK cell antitumor efficacy is limited mainly to hematologic malignancies. The most impressive and frequently quoted data was published in 2002 and most recently updated in 2007. In total, 112 patients with high-risk AML, of whom 61 were in remission and 51 in relapse, were transplanted with HLA-haploidentical grafts from 51 NK alloreactive or 61 nonalloreactive related donors. In this case, NK alloreactivity was defined by the presence of KIR ligands in the donor, which were absent in the recipient, KIR gene for missing self recognition in the recipient, and alloreactive NK clones against recipient targets. Transplantation from NK alloreactive donors led to a remarkably low relapse rate in patients transplanted in remission (3% vs 47%), and to a superior EFS for patients whether transplanted in remission (67% vs 18%) or in relapse (34% vs 6%). Disease status and transplantation from an NK alloreactive donor were the only independent prognostic factors [16]. A lower relapse rate in patients transplanted in the setting of potential NK alloreactivity has been demonstrated by some groups, but not by others (see Table 1).

Although ALL cells are generally held to be less susceptible to NK cell mediated attack than AML [14,112], *MLL*-rearranged ALL cells have been shown to be susceptible to NK alloreactivity, with a sensitivity which is proportional to the extent of KIR-ligand mismatch [86]; this effect was also found in AML and myelodysplastic syndrome (MDS) [47]. Clearly, inhibitory KIR are not the only factor permitting antitumor NK alloreactivity. The presence of certain activating KIR genes in the donor may be associated with a lower relapse rate [113], and NKG2D plays a role in AML and CML [114,115].

Barriers to successful NK antitumor activity include tumor bulk and immunoevasion mechanisms [116,117]. Tumor escape from NK attack has been shown to occur through down-regulation on the tumor of activating receptor ligands, such as MICA/B, ULBP, CD112, CD155, and CD48 [112,115,118,119]. Direct contact with AML cells leads to down-regulation of NCR on the NK cells, correlating with worse survival [120]. NKG2D may be down-regulated after exposure to high levels of soluble tumor-derived MICA/MICB

[121]. Finally, an NK cell–suppressive NK subset may be induced by tumor cells [122].

Attempts to counter tumor immunoevasion mechanisms by manipulation and enhancement of NK cell function can be achieved using cytokines or ligation or modulation of inhibitory or activating receptors, as has been reviewed recently [116,123]. Examples of this include blockade of the interaction between KIR2DL1/2/3 and HLA-C molecules as postremission therapy in patients with AML and MM [124], use of bispecific antibodies to direct effectors to lyse otherwise-refractory target cells [125], genetic modification of NK or T cells to express a chimeric NKG2D receptor [126], and down-regulation of myeloma MHC class I molecules using bortezomib [127].

EFFECTS ON GVHD

In a preclinical model, infusion of alloreactive NK cells into haplodentical mouse recipients as part of the conditioning regimen was protective against GVHD after a T cell–replete transplantation [14]. A rationale for this observation may be provided by NK-mediated ablation of host DCs [14], lysis of donor T cells [128-130], and the absence of activating NK receptor ligands on normal nonhematopoietic cells [131].

Clinical results vary, however. Although the initial report from the Perugia group suggested a favorable effect of NK alloreactivity on the incidence of acute GVHD (aGVHD), the updated results no longer showed a significant difference [16]. As can be seen in Table 1, most groups report no significant difference in GVHD rates, and in fact GVHD may be worsened by KIR mismatch or in the presence of some donor-activating KIR genes [21,93,132,133]. Because KIR mismatch correlated with HLA mismatch in some of these studies, T cell–induced GVHD clearly is a major confounder.

CONTROVERSIES AND CONCLUSIONS

Alloreactive NK cells in transplantation can have remarkably favorable effects on relapse and survival, as well as adverse outcomes related to relapse, infection, and GVHD. These discrepancies relate to differences in donor selection, conditioning regimens, extent of TCD, hematopoietic stem cell dose, disease state at transplantation, nature of disease, and algorithm of NK alloreactivity. In broad brushstrokes, it seems the most favorable conditions include a myeloablative conditioning regimen, maximal stem cell and minimal T cell doses, lack of interference with NK expansion by posttransplantation GVHD prophylaxis, and selection of patients with myelogenous disease in remission.

Selecting the most appropriate NK alloreactivity model is of vital importance. Preclinical and clinical results point variously to the "missing self " or "missing ligand" model as the most predictive. NK cell receptor recognition is a complex and incompletely elucidated process, and tests of donor NK cell function against leukemia and patient target cells have sometimes revealed unexpected results [49,55,88]. Thus, functional analysis, including quantitation of the alloreactive educated NK cell pool, should be incorporated in real time into the donor selection process, as should KIR genotyping of the donor. Proper donor selection may be only part of the answer, however; perhaps "tumor selection"—an individualized assessment of tumor cell expression of NK receptor ligands or strategies to increase expression of ligands—could be incorporated into these models.

Hopefully, these major issues will be addressed with well-designed clinical trials. Nonetheless, several unanswered questions will require further biological input:

- Is the observed reduction in relapse because of a persistence of NK immunosurveillance?
- How are donor stem cell–derived NK cells educated in the recipient, and do hyporesponsive NK cells acquire effector function after transplantation?
- What are the ideal conditions for in vivo persistence of NK cells after transplantation?
- Should a particular NK cell subset be preferentially expanded, and if so, by what means?

Despite these unanswered questions, the use of alloreactive NK cells in transplantation continues to be an exciting example of the translation of basic biological principles to clinical medicine.

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