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REVIEW

Role of NK and NKT cells in solid organ transplantation

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Summary

In the context of solid organ transplantation, the exact interactions between the innate and adaptive alloimmune response have not yet been fully explored. In this transplant setting, natural killer (NK) cells have emerged as a particular focus of interest because of their ability to distinguish allogeneic major histocompatibility complex (MHC) antigens and their potent cytolytic activity. Based on this observation and its potential clinical relevance, NK cells have recently been shown to participate in the immune response in both acute and chronic rejection of solid organ allografts. Numerous experimental and clinical studies demonstrate that NK cells determine transplant survival by rejecting an allograft not directly but indirectly by providing bystander effects. In addition, NK cells are influenced by immunosuppressive therapies such as calcineurin inhibitors or steroids. As NK and natural killer T (NKT) cells have also been shown to play a profound role in allograft tolerance induction, this review summarizes the major findings to highlight the functional role of these lymphocyte subsets, which may constitute an underestimated mechanism affecting graft outcome in solid organ transplantation.

Introduction

The progression of improved immunosuppressive therapies has significantly reduced the risk of acute rejection of solid organs post-transplantation. However, the development of chronic rejection and therefore restricted graft survival in the long term still remain a serious problem in transplant medicine. Both antigen-dependent risk factors (*human leukocyte antigens*, HLA) and antigen-independent risk factors (e.g. donor brain death or ischemia reperfusion injury, IRI) result in inflammation and tissue injury, and therefore play a critical role in the initiation of chronic graft failure. For instance, following IRI, oxidative stress induces cytokine and chemokine upregulation, which might enhance the recruitment of recipient-derived immunocompetent cells capable of mediating tissue injury directly or indirectly. An emerging concept in the field of transplantation research is, therefore, to reveal the linkage between innate and adaptive immunity. The immune function of natural killer (NK) cells has been well described during viral infections or tumor development, where either the downmodulation or the induction of NK cell receptor ligands results in NK cell alloreactivity. Although a function for alloreactive NK cells in preventing graft-versus-host disease (GvHD) in the setting of bone-marrow transplantation (BMT) has been reported, the exact mechanisms by which NK and natural killer T (NKT) cells contribute to the rejection or acceptance of solid organs still remain unclear.

Biology of natural killer and natural killer T cells

Natural killer cells

Natural killer cells are large granular cytotoxic lymphocytes that represent a fundamental component of the innate immune system. They are derived from $CD34^+$ hematopoietic progenitor cells (HPCs) [1] and, once released, they comprise roughly 5–20% of lymphocytes in the spleen, liver, and peripheral blood and are present at lower frequencies in the bone marrow, thymus, and

lymph nodes [2]. They were originally identified by their ability to kill certain tumor target cells spontaneously *in vivo* and *in vitro* without sensitization [3,4], and this killing was not restricted by the target cell's expression of major histocompatibility complex (MHC) molecules [3,5]. NK cells are not only an important source of innate immunoregulatory cytokines, but they also possess direct or natural cytotoxic activity against virus-infected, leukemic, and other tumor cells. They also mediate the antibody-dependent cellular cytotoxicity (ADCC) of targets through FcγRIII (CD16), a receptor that binds the Fc portion of antibodies [6,7]. In general, the traditional phenotype defining human NK cells is characterized by the absence of the T cell receptor complex (TCR, CD3) and expression of CD56, the 140-kDa isoform of neural cell adhesion molecule (NCAM) which is also found on a minority of T cells [8,9]. Based on their CD56 receptor expression density, human NK cells can be distinguished as $CD56^{dim}$ or $CD56^{bright}$ NK cells. In peripheral blood, the majority (>90%) are $CD56^{dim}$ demonstrating high expression of CD16, while the remaining 10% are $CD56^{bright}$ NK cells characterized by almost no or dim expression of CD16 [10]. Whereas $CD56^{dim}$ NK cells are regarded as the classical cytotoxic NK cell subset, $CD56^{bright}$ NK cells display marginal cytotoxic capacity, but produce high amounts of cytokines, including IFNγ and TNFα, indicating a primary role in immunoregulatory function (Fig. 1). In contrast, murine NK cells can be identified by using antibodies against the NK1.1 molecule [11,12], the pan-NK marker, DX5 [13], or antibodies directed against the asialo GM1 (ASGM1) surface molecule [14].

Natural killer T cells

In contrast to NK cells, NKT cells comprise a very heterogeneous group of T cells. They were originally characterized in mice as cells that express both a TCR and NK1.1 (CD161c in humans) [15]. The most studied and best-characterized NKT cell population in mice and humans is referred to as type I NKT cells, or iNKT cells. These NKT cells express a TCR formed by the rearrangement of the Vα24 gene segment (Vα14 in mice) to the Jα18 gene segment. iNKT cells recognize glycolipid antigens presented by the nonpolymorphic MHC class I-like molecule CD1d [16]. However, several studies have identified subsets of CD1d-dependent T cells that either express or do not express the invariant Vα24-Jα18 (Vα14-Jα18 in mice) TCR and/or CD161 (NK1.1 in mice). Although all subsets have been referred to as NKT cells, they probably represent functionally distinct cell types [17]. iNKT cells are found with the highest frequency in the liver and the bone marrow of mice, with significant numbers also in the thymus, spleen, and peripheral blood. In humans, the frequency of iNKT cells is usually much lower and a high degree of variability between individuals has been reported [18]. In mice, iNKT cells are exclusively $CD4^+$ or $CD4^-CD8^-$ double negative (DN), while they can be $CD8^+$ in humans [19].

Inhibitory NK cell receptors

Meanwhile it has become clear that NK cells possess a variety of inhibitory and activating receptors that engage MHC class I molecules, MHC class I-like molecules, and

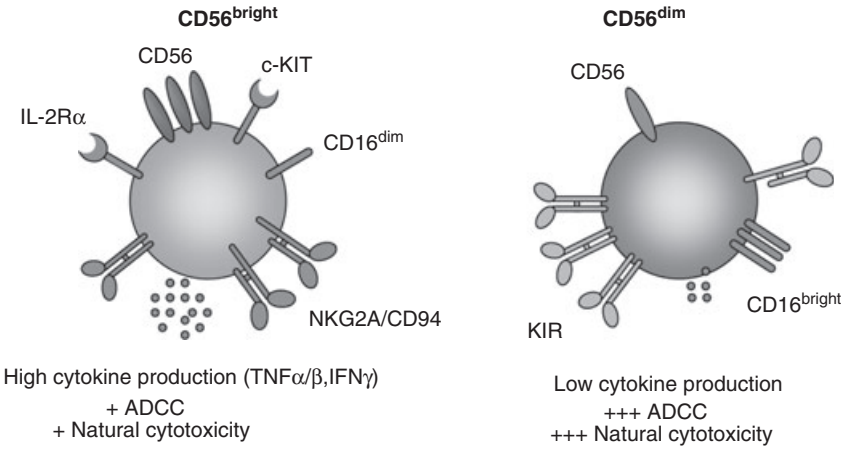

Figure 1 Human $CD56^{dim}$ and $CD56^{bright}$ cells can be distinguished by their receptor repertoire and their effector function. Based on their CD56 receptor expression density, human natural killer (NK) cells can be distinguished as $CD56^{dim}$ or $CD56^{bright}$ NK cells. The majority in peripheral blood are $CD56^{dim}$ demonstrating high expression of CD16, while the remaining 10% are $CD56^{bright}$ NK cells. These cells express only marginal CD16. $CD56^{dim}$ NK cells are regarded as the classical cytotoxic NK cell subset mediating cytotoxic effector functions and antibody-dependent cellular cytotoxicity (ADCC), whereas $CD56^{bright}$ NK cells produce high amounts of cytokines, including IFNγ and TNFα.

molecules unrelated to MHC, and that their cytolytic activity is controlled by the balance between their inhibitory and activating receptors. Thus, NK cells are restricted in engaging potential target cells depending on the expression of their ligands, but this occurs in a very complex fashion that is still not completely understood. Based on the ''missing-self'' hypothesis, NK cells kill target cells that display reduced levels of MHC class I antigens, such as virally transformed or tumor cells [20]. In humans, there are three types of major MHC class I specific inhibitory receptors expressed by NK cells. The human killer-cell immunoglobulin-like receptors (KIRs; Ly49 receptor family in mice) or the immunoglobulin-like transcripts (ILTs) bind classical and nonclassical HLA class I molecules, whereas the C-type lectin heterodimer CD94/NKG2A binds to the nonclassical MHC class I molecule HLA-E (Qa-1^b in mice) [21–23]. Interestingly, the genomic region that encodes KIRs exhibits extensive variability among individuals because of differences in gene content, gene copy number, and allelic polymorphism, thus creating a heterogenous NK cell population in each individual [24,25]. Inhibitory KIRs (e.g. KIR2DL1, KIR2DL2) bind to different HLA class I antigens whose specificity is determined by amino acids in the C-terminal portion of the MHC class I α1 helix [26]. The binding of MHC class I complexes to KIRs or to the heterodimeric CD94/NKG2A receptor initiates inhibitory pathways that can override activating signals [27]. On the other hand, reduced MHC class I expression is not the only requirement for NK cell activation, as overexpression of activating ligands on target cells can also trigger NK cell function (Table 1).

Table 1. Inhibitory and activating NK cell receptors (human and mouse).

Receptor	Ligand
Inhibitory	
Human	
KIR2DL1	Group 2 HLA-C (Asn77Lys80 alleles)
KIR2DL2/3	Group 1 HLA-C (Ser77Asn80 alleles)
KIR3DL1	HLA-Bw4, HLA-A23, 24, 32
KIR3DL2	HLA-A3, -A11
CD94/NKG2A	HLA-E
Mouse	
Ly49A	H-$2D^d$
Ly49C	H-$2K^b$
NKG2A	Qa-1^b
Activating	
Human	
KIR2DS1	Group 2 HLA-C (Asn77Lys80 alleles)
KIR3DS1	HLA-Bw4
NKG2D	MICA, MICB, ULBP-1, -2, -3, -4
NKp30	CMV protein pp65
NKp46	viral hemagglutinins
NKp44	viral hemagglutinins
2B4	CD48
CD16 (FcγRIII)	IgG
Mouse	
NKG2D	RAE-1, MULT1, H60
Ly49D	H-$2D^d$
CD16 (FcγRIII)	IgG

MICA/B, MHC class I-related chain A and B; ULBP 1–4, UL16 binding protein 1–4; RAE1, retinoic acid inducible-1; MULT1, murine ULBP-like transcript.

Activating NK cell receptors

Among activating KIR receptors (e.g. KIR2DS1) [28], NK cells express the C-type lectin homodimer NKG2D, CD16, and natural cytotoxicity receptors (NCRs) including NKp30, NKp44, and NKp46, whereas the latter is selectively expressed on both human and murine NK cells. Additionally, NK cells express a variety of activating co-receptors including 2B4, which may also contribute to NK cell activation [29]. NKG2D binds to cellular stress-induced molecules, including nonclassical MHC class I molecules (MHC class I polypeptide-related sequence A or B, MICA and MICB), and the MHC class I-related UL-16 binding proteins 1–4 (ULBP1-4) [30]. While the ligands for NCRs on tumor cells are still unknown, viral hemagglutinins have been suggested as ligands for the cytotoxicity receptors NKp44 and NKp46 [31,32], whereas CD48 engages 2B4 [33,34]. In summary, these studies indicate that with regard to inhibitory NK cell receptors, NK cells receive activating signals from different receptors to detect a variety of transformation-induced changes [35] (Table 1).

The role of NK cells in allograft rejection

Although infiltration of NK cells in renal and cardiac allografts has been observed shortly after transplantation, their exact role in solid organ rejection still remains to be clarified [36–39]. NK cell infiltration often occurs before evidence of T cell infiltration and is consistent with the role of these cells as early innate effector cells in response to inflammatory stimuli. Previous studies have shown that IFNγ produced by NK cells during interaction with allogeneic endothelial cells stimulates MHC class I and class II expression, rendering them more susceptible to attack by alloantigen-specific T cells following priming and recruitment to the graft site [40–43]. As it has been reported that rat NK cell-mediated cytolysis of donor target cells *in vitro* is increased in NK cell populations

isolated from recipients of allogeneic heart grafts, we point out that NK cells are not only present but are also activated to effector function following infiltration of solid organ allografts [39]. This NK cell-mediated cytolysis of target cells is further enhanced by adhesion molecule expression and various CC chemokines, including MCP-1 or CX3CL1 (fractalkine), which are induced early following transplantation [44,45]. In a model of acute allograft rejection, CCL3, CXCL10, and CX3CL1 were significantly increased in allografts post-transplantation, suggesting a role for these chemokines in the recruitment of effector cells to allografts. Additionally, IFNγ levels were markedly increased in the serum, indicating that graft-infiltrating recipient-derived NK cells were the major source of this immunoregulatory cytokine [46]. A more recent study suggested a contributory role of IL-15 in graft rejection. Kroemer *et al.* [47] demonstrated that resting NK cells in $Rag^{-/-}$ mice readily reject allogeneic cells, but not skin allografts. However, treatment with an IL-15/IL-15Rα complex resulted in activation of NK cells *in vivo*, expressing an activated phenotype potent in mediating acute skin allograft rejection in the absence of adaptive immune cells.

The original assumption that NK cells do not participate in the rejection of solid allografts was supported by experiments where depletion of NK cells did not result in graft acceptance of skin, heart, or liver allografts [36,48]. In contrast, the final evidence that NK cells play an important role in the rejection of heart transplants is the finding that depletion of NK cells in the absence of CD28 co-stimulation results in markedly prolonged graft survival [49]. These data illustrate that when the co-stimulatory signal is blocked, NK cells play an important role in mediating rejection. Another proof of the functional role of NK cells in allograft rejection has been provided by McNerney *et al.*, who showed that NK but not NKT cells were required for cardiac rejection. NK cells which bear the murine inhibitory receptor Ly49G suppressed rejection, whereas a subset of NK cells lacking inhibitory Ly49 receptors for donor MHC class I molecules was sufficient to promote rejection. Moreover, the authors have been able to show that NK cells promote the expansion and effector function of alloreactive T cells [50]. Although it has been suggested that rejection was independent of the activating receptors Ly49D and NKG2D, treatment with a neutralizing antibody against NKG2D was highly effective in preventing CD28-independent rejection of cardiac allografts in another study [51]. The authors provided evidence that NKG2D ligands were upregulated in heart allografts. These findings were further extended by recent observations made by Zhang *et al.* [52], who showed that renal tubular epithelial cells express NKG2D ligands as a consequence of IRI, thus leading to NKG2D-mediated NK cell killing. The induction of NKG2D ligands (stress-induced MICA proteins) has already been demonstrated on human renal and pancreatic allografts during acute rejection, making it likely that NKG2D ligand induction is relevant for clinical transplantation [53,54].

Both antigen-presenting cells (APCs) and T cells are potential targets of NK cell regulation. NK cells are capable of killing immature dendritic cells (DC) though they can also drive DC maturation. Moreover, NK cells inhibit or promote the activation of alloreactive T cells. In the absence of CD8 T cell activation, Coudert *et al.* demonstrated that NK cells, through their interaction with allogeneic APCs, can quantitatively and qualitatively control allospecific $CD4^+$ T responses *in vivo*. Alloreactivity of host NK cells mediated by missing inhibitory MHC class I ligands expressed by donor APCs resulted in diminished allospecific Th cell responses associated with the development of effector Th cells producing IFNγ rather than type 2 cytokines. In contrast, alloreactive $CD4^+$ T cell priming and Th2 cell development were restored by neutralizing NK cells, supporting the conclusion that NK cell activation reduces alloreactive CD4 T cell priming [55] (Fig. 2). The importance of host NK cell alloreactivity mediated by the absence of self MHC class I molecules on donor endothelium was also shown in the pathogenesis of cardiac allograft vasculopathy (CAV), which requires functional interactions with T cells [56].

KIR–HLA interactions and solid graft outcome – clinical implications

The human killer-cell immunglobulin-like receptors (KIR) are named according to their structural and functional characteristics; i.e. by the number of extracellular Ig domains (2D or 3D) and the type of intracellular tail, mediating either an inhibitory (long [l]) or an activating (short [s]) signal. HLA class I molecules have been identified as ligands for some of the inhibitory KIR molecules (designated 2DL and 3DL). KIR2DL1 binds to HLA-C group 2 molecules (HLA-C2), which have amino acids Asn^{77} and Lys^{80}; KIR2DL2/2DL3 binds to HLA-C group 1 molecules (HLA-C1), which have amino acids Ser^{77} and Asn^{80}; and KIR3DL1 binds to HLA-B and some HLA-A allele products, which contain the Bw4 epitope determined by amino acid positions 77–83 on the α1 helix of the heavy chain. Ligands for activating KIRs (designated 2DS and 3DS, e.g. KIR2DS1) are less well defined, although it has been suggested that they bind to the same HLA-B or HLA-C molecules because of the presence of their homologous codon sequences with inhibitory KIRs in the extracellular domain [57–60] (Table 1).

In haploidentical BMT, reconstituting NK cells of donor origin develop alloreactivity when their inhibitory

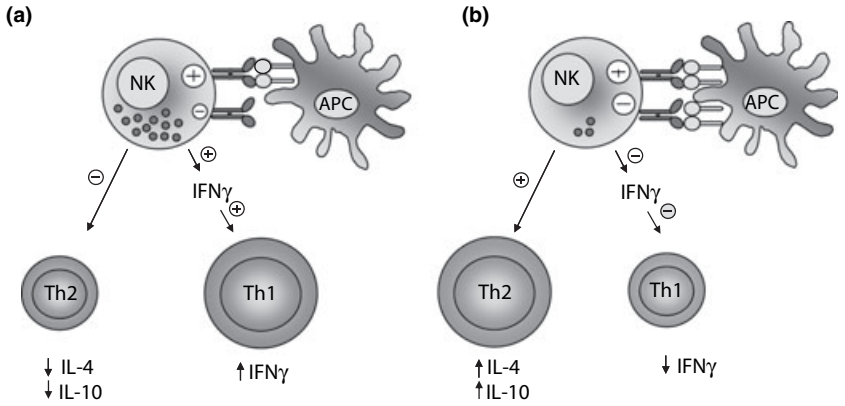

Figure 2 Model of allospecific CD4 control mediated by NK cells and their interaction with donor-derived APC *in vivo*. (a) In the absence of CD8 cells, the lack of corresponding MHC class I ligands for their inhibitory receptors results in NK cell activation, thus leading to the development of a Th1-dominated immune response, whereas the Th2 response is abrogated. (b) The absence of NK cell activation as a result of sufficient inhibitory ligands promotes an alloreactive CD4 response toward a Th2 phenotype. In normal mice, NK cells quickly eliminate allogeneic DCs within the draining lymph nodes, thus limiting alloreactive $CD8^+$ T cell response. NK, NK cell; DC, dendritic cells; Th1, T helper cell type1; Th2, T helper cell type 2.

KIR receptors do not match with HLA-C ligands displayed by recipient cells. Especially in patients with acute myeloid leukemia (AML), alloreactive NK cells protect against the development of GvHD and leukemia relapse [61,62]. On the contrary, the absence of self MHC class I molecules on allogeneic cells of solid grafts would be expected to activate host NK cells to express these functions early following transplantation. Meanwhile, a couple of studies investigating the role of NK reactivity mediated by potential KIR–HLA interactions on solid graft outcome have been performed. In a study of HLA ligand incompatibility between donor and recipient in a large study cohort comprising more than 2757 deceased-donor transplants, no correlation with kidney graft survival could be observed [63]. In contrast, Bishara *et al.* [64] found that recipients transplanted from donors with matching HLA-C groups had significantly fewer rejection episodes in the first year after transplantation, whereas disparities in the HLA-Bw4 epitope did not affect the outcome. It is proposed that the inhibitory receptor KIR2DL1 and its corresponding ligand HLA-C group 2 illustrate a stronger interaction in comparison with KIR2DL2/3 receptors and their corresponding HLA-C group 1 ligand, thus leading to enhanced inhibition of NK cells [65]. These results were supported by observations that NK cells from HLA-C group 1 homozygous subjects positive for the inhibitory receptor KIR2DL3 secreted more IFNγ at earlier time points after infection with influenza A virus than did NK cells from HLA-C group 2 homozygous subjects expressing the inhibitory KIR2DL1 receptor [66]. Recently, we and others demonstrated the potential influence of the HLA-C donor type on allograft outcome. Whereas an HLA-C group 2 homozygous allograft resulted in a significantly reduced risk of acute rejection in kidney transplantation, this observation could not be confirmed in the setting of liver transplantation [67,68]. However, Hanvesakul *et al.* [67] showed that an HLA-C group 2 allele by the donor allograft was associated with less histological evidence of chronic rejection and graft cirrhosis, reduction in graft loss, and an improvement in patient survival at 10 years. Taken together, these studies support the concept that incompatibility in the HLA–KIR ligand interaction dominates NK cell activation *in vitro* and probably *in vivo*. Consequently, donor HLA-C genotype might be a potential determinant of clinical outcome after solid organ transplantation, further underlining the importance of NK cells and their KIR receptors.

Viral infection is a common complication after solid organ transplantation. In the setting of BMT, an association between the number of activating KIR receptors and cytomegalovirus (CMV) reactivation has been illustrated. Additional activating KIR genes in the donor compared with the recipient's genotype correlate with a lower incidence of cytomegalovirus (CMV) reactivation [69]. Similarly, in kidney transplantation a recent paper by Stern *et al.* [70] demonstrated that patients with a KIR A/A genotype, characterized by the presence of only one activating KIR gene, showed a higher rate of CMV infection and reactivation as compared with that of transplant recipients with more than one activating KIR gene (B haplotype), supporting a role for activating KIR in the control of CMV infection after kidney transplantation.

NK cells and immunosuppressive drugs

In general, the influence of different immunosuppressive drugs on NK cell function has aroused particular interest, as it has recently been demonstrated that steroids and calcineurin inhibitors limit the function of IL-2-activated NK cells. For instance, cyclosporin A (CsA) induces a dose-dependent and selective inhibition in the IL-2- and IL-15-induced proliferation of human $CD56^{dim}$ NK in contrast to $CD56^{bright}$ NK cells. NK cells cultured in CsA retained cytotoxicity against the target cell line K562 and, following IL-12 and IL-18 stimulation, CsA-treated NK cells showed more IFNγ-producing cells [71]. In addition, Chiossone *et al.* [72] showed that human NK cells cultured in either IL-2 or IL-15 display different susceptibility to methylprednisolone treatment in terms of cell survival, proliferation, and NK receptor-mediated cytotoxicity. Steroid-induced inhibition of NK-cell cytotoxicity not only resulted in down-regulation of surface expression or function of the activating receptors, but also affected phosphorylation of the ERK1/2 signaling pathway, thus inhibiting granule exocytosis. In contrast, rat NK cells demonstrated robust function in both the absence and presence of cyclosporin and FK506, whereas rapamycin significantly inhibited proliferation and cytotoxicity of NK cells *in vitro*. Experimental investigations *in vivo* illustrated that NK cell numbers remained stable in graft recipients treated with cyclosporin and FK506, whereas there was a significant decrease of NK cells in rapamycin-treated recipients [73].

An influence of NK cells after antibody therapy has been illustrated in patients with multiple sclerosis treated with a humanized monoclonal antibody directed against the anti-IL2Rα chain (Daclizumab). Application of Daclizumab was associated with a significant expansion of $CD56^{bright}$ NK cells, thereby limiting the survival of activated T cells in a contact-dependent manner [74]. Moreover, Rituximab, a therapeutic monoclonal antibody (anti-CD20) frequently applied for the treatment of B cell lymphoma, increases the killing frequency of both resting and IL-2-activated NK cells *in vitro* [75]. Our own observations illustrate that patients treated with rabbit polyclonal antithymocyte globulin (rATG) as induction therapy were found to demonstrate a significant decrease of $CD3^{-}CD56^{+}$ NK cells within the peripheral blood lymphocytes following the first day post-transplantation, and this effect persisted until day 11 post-transplantation. *In vitro* investigations showed that rATG and alemtuzumab (anti-CD52) induce rapid apoptosis in NK cells and a strong induction of inflammatory cytokines, which is exclusively mediated via the binding of the IgG1 Fc part to the low-affinity receptor for IgG, CD16 (FcγRIII) [76]. Taken together, these findings suggest an altered function of NK cells as a consequence of various immunosuppressive therapies. As NK cells are functionally relevant for the effective clearance of opportunistic viral infections and anti-tumor activity, this should be considered in defining the optimal treatment dosage in clinical settings.

Functional aspects of NK and NKT cells in mediating allograft tolerance

There are several studies published indicating that alloreactivity of NK cells may not only play an important role in influencing allograft rejection but may also affect the induction of tolerance. A crucial role for host MHC class I-dependent NK cell reactivity for allograft tolerance could be demonstrated in mice either by inducing co-stimulation blockade using CD154-specific antibody therapy or by targeting cellular adhesion by blocking LFA-1 [77]. In this model of islet transplantation, tolerance induction was shown to require host expression of both MHC class I^{+} and $NK1.1^{+}$ cells, but was independent of $CD8^{+}$ T cell-dependent immunity. Additionally, CD154-specific antibody-induced allograft tolerance was demonstrated to be perforin-dependent, as perforin-competent NK cells were sufficient to restore allograft tolerance in perforin-deficient recipients [77]. The functional aspects of NK cell reactivity have been further demonstrated in a skin transplant model. Skin allografts contain a subset of APCs which is usually destroyed by the host NK cells. But in the absence of NK cells, surviving donor APCs migrate to the host lymphoid and extralymphoid sites. They directly stimulate the activation of alloreactive T cells which were more resistant to co-stimulatory blockade treatment, thus preventing stable skin allograft survival [78]. These observations were further supported by a model of $CD4^{+}$ T cell-mediated allogeneic skin graft rejection, where the absence of host NK cell alloreactivity was characterized by enhanced expansion of alloreactive effector T lymphocytes, including Th2 cells in the rejected tissues. It was demonstrated that host NK cells expressing the $H\text{-}2^{d}$-specific activating receptor Ly49D were recruited within draining lymph nodes and rapidly eliminated allogeneic $H\text{-}2^{d}$ DCs through the perforin pathway, thus regulating alloreactive $CD4^{+}$ T-cell responses in $CD8^{+}$ T cell-deficient C57BL/6 ($H\text{-}2^{b}$) recipients [79]. In summary, these data suggest that host NK cells, through their killing of allogeneic APCs, limit the persistence of donor-derived DCs, therefore contributing to the induction of transplant tolerance (Fig. 3).

The first data showing that Vα14 NKT cells are required for the induction of tolerance were provided by Ikehara *et al.* Administration of an anti-CD4 mAb allowed islet xenografts to be accepted by C57BL/6 mice,

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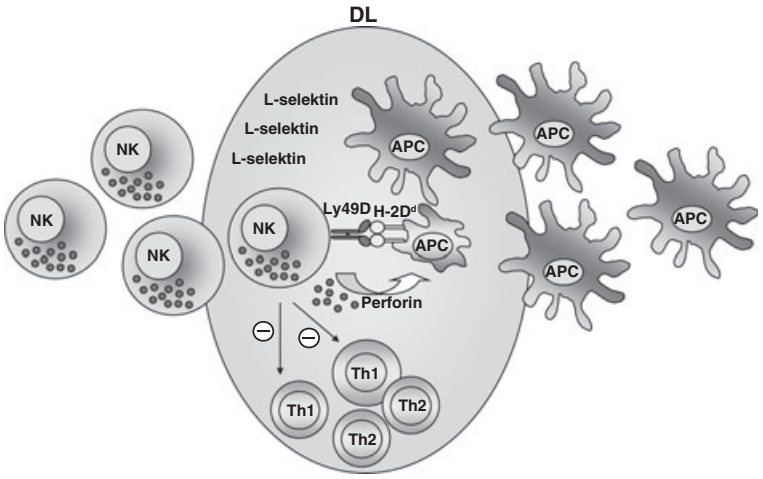

Figure 3 NK cells regulate alloreactive T cell responses *in vivo* through direct killing of allogeneic DCs. Following transplantation, donor-derived DCs migrate to the draining lymph nodes where they induce L-selectin-dependent recruitment of NK cells from the periphery. Migrating NK cells, positive for the activating Ly49D receptor which is specific for H-2^d MHC molecules, kill allogeneic DCs (H-2^d) through a perforin-dependent mechanism, thus limiting alloreactive T cell responses. DL, draining lymph node; DC, dendritic cells; NK, NK cell; Th1, T helper cell type1; Th2, T helper cell type 2.

with no need for immunosuppressive drugs. This effect was associated with NKT cells, as rat islet xenografts were rejected in Vα14 NKT cell-deficient mice, despite the anti-CD4 mAb treatment [80]. Similarly, in models in which tolerance was induced against cardiac allografts by blockade of LFA-1/ICAM-1 or CD28/B7 interactions, long-term acceptance of the grafts was observed only in wild-type but not in Vα14 NKT cell-deficient mice. Adoptive transfer with Vα14 NKT cells restored long-term acceptance of allografts in Vα14 NKT cell-deficient mice [81]. The functional role of CD1d-reactive NKT cells was further emphasized by the finding that NKT cells from transplant-tolerant recipients of cardiac allografts produced higher levels of IL-10, which is required for the maintenance of tolerance. DCs from wild-type tolerant recipients but not NKT cell-deficient recipients showed a higher IL-10-producing profile, a more immature phenotype, and a tolerogenic capability, indicating a novel regulatory mechanism of transplant tolerance mediated by NKT cells [82].

Conclusion

A new understanding of the functional role of NK and NKT cells in the context of solid organ transplantation has emerged. Numerous studies demonstrate that both lymphocyte subsets cannot be regarded only as killers, but have been recognized as active players in the process of mediating either rejection or tolerance. In this context, the observed NK alloreactivity mediated by missing MHC ligands seems to remain controversial. Although an activated NK cell status correlates with an inflammatory phenotype and graft destruction, NK killing of donor-derived APCs represents one of the key mechanisms for tolerance induction. Although most of the functional findings about NK cells are derived from experimental data, the studies summarized above suggest an important influence of NK and NKT cells on allograft survival. In the light of the finding that NK cells are indeed influenced by various immunosuppressive drugs, it will be an ongoing challenge to uncover the exact mechanisms of these effector cells to translate this information into clinically relevant therapeutic options.

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References

1. Miller JS, Verfaillie C, McGlave P. The generation of human natural killer cells from CD34+/DR− primitive progenitors in long-term bone marrow culture. *Blood* 1992; **80**: 2182.
2. Lian RH, Kumar V. Murine natural killer cell progenitors and their requirements for development. *Semin Immunol* 2002; **14**: 453.
3. Kiessling R, Klein E, Wigzell H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *Eur J Immunol* 1975; **5**: 112.
4. Herberman RB, Nunn ME, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity. *Int J Cancer* 1975; **16**: 216.
5. Herberman RB, Nunn ME, Holden HT, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors, II: characterization of effector cells. *Int J Cancer* 1975; **16**: 230.

6. Allavena P, Damia G, Colombo T, Maggioni D, D'Incalci M, Mantovani A. Lymphokine-activated killer (LAK) and monocyte-mediated cytotoxicity on tumor cell lines resistant to antitumor agents. *Cell Immunol* 1989; **120**: 250.
7. Landay AL, Zarcone D, Grossi CE, Bauer K. Relationship between target cell cycle and susceptibility to natural killer lysis. *Cancer Res* 1987; **47**: 2767.
8. Lanier LL, Testi R, Bindl J, Phillips JH. Identity of Leu-19 (CD56) leukocyte differentiation antigen and neural cell adhesion molecule. *J Exp Med* 1989; **169**: 2233.
9. Ritz J, Schmidt RE, Michon J, Hercend T, Schlossman SF. Characterization of functional surface structures on human natural killer cells. *Adv Immunol* 1988; **42**: 181.
10. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. *Trends Immunol* 2001; **22**: 633.
11. Sentman CL, Kumar V, Koo G, Bennett M. Effector cell expression of NK1.1, a murine natural killer cell-specific molecule, and ability of mice to reject bone marrow allografts. *J Immunol* 1989; **142**: 1847.
12. Koo GC, Peppard JR. Establishment of monoclonal anti-NK-1.1 antibody. *Hybridoma* 1984; **3**: 301.
13. Moore TA, von Freeden-Jeffry U, Murray R, Zlotnik A. Inhibition of gamma delta T cell development and early thymocyte maturation in IL-7 2 /2 mice. *J Immunol* 1996; **157**: 2366.
14. Charley MR, Mikhael A, Bennett M, Gilliam JN, Sontheimer RD. Prevention of lethal, minor-determinate graft-host disease in mice by the in vivo administration of anti-asialo GM1. *J Immunol* 1983; **131**: 2101.
15. Bendelac A, Rivera MN, Park SH, Roark JH. Mouse CD1-specific NK1 T cells: development, specificity, and function. *Ann Rev Immunol* 1997; **15**: 535.
16. Matsuda JL, Naidenko OV, Gapin L, *et al.* Tracking the response of natural killer T cells to a glycolipid antigen using CD1d tetramers. *J Exp Med* 2000; **192**: 741.
17. Godfrey DI, MacDonald HR, Kronenberg M, Smyth MJ, Van Kaer L. NKT cells: what's in a name? *Nat Rev Immunol* 2004; **4**: 231.
18. Lee PT, Putnam A, Benlagha K, Teyton L, Gottlieb PA, Bendelac A. Testing the NKT cell hypothesis of human IDDM pathogenesis. *J Clin Invest* 2002; **110**: 793.
19. Ishihara S, Nieda M, Kitayama J, *et al.* $CD8^+NKR\text{-}P1A^+$ T cells preferentially accumulate in human liver. *Eur J Immunol* 1999; **29**: 2406.
20. Ljunggren HG, Karre K. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol Today* 1990; **11**: 237.
21. Braud VM, Allan DS, O'Callaghan CA, *et al.* HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 1998; **391**: 795.
22. Karlhofer FM, Ribaudo RK, Yokoyama WM. MHC Class I alloantigen specificity of Ly-49+ IL-2-activated natural killer cells. *Nature* 1992; **358**: 66.
23. Gunturi A, Berg RE, Forman J. The role of CD94/NKG2 in innate and adaptive immunity. *Immunol Res* 2004; **30**: 29.
24. Hsu KC, Chida S, Geraghty DE, Dupont B. The killer cell immunoglobulin-like receptor (KIR) genomic region: gene-order, haplotypes and allelic polymorphism. *Immunol Rev* 2002; **190**: 40.
25. Hsu KC, Liu XR, Selvakumar A, Mickelson E, O'Reilly RJ, Dupont B. Killer Ig-like receptor haplotype analysis by gene content: evidence for genomic diversity with a minimum of six basic framework haplotypes, each with multiple subsets. *J Immunol* 2002; **169**: 5118.
26. Lanier LL. NK cell recognition. *Annu Rev Immunol* 2005; **23**: 225.
27. Natarajan K, Dimasi N, Wang J, Mariuzza RA, Margulies DH. Structure and function of natural killer cell receptors: multiple molecular solutions to self, nonself discrimination. *Annu Rev Immunol* 2002; **20**: 853.
28. Chewning JH, Gudme CN, Hsu KC, Selvakumar A, Dupont B. KIR2DS1-positive NK cells mediate alloresponse against the C2 HLA-KIR ligand group in vitro. *J Immunol* 2007; **179**: 854.
29. Colucci F, Di Santo JP, Leibson PJ. Natural killer cell activation in mice and men: different triggers for similar weapons? *Nat Immunol* 2002; **3**: 807.
30. Raulet DH. Roles of the NKG2D immunoreceptor and its ligands. *Nat Rev Immunol* 2003; **3**: 781.
31. Mandelboim O, Lieberman N, Lev M, *et al.* Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature* 2001; **409**: 1055.
32. Arnon TI, Lev M, Katz G, Chernobrov Y, Porgador A, Mandelboim O. Recognition of viral hemagglutinins by NKp44 but not by NKp30. *Eur J Immunol* 2001; **31**: 2680.
33. Brown MH, Boles K, van der Merwe PA, Kumar V, Mathew PA, Barclay AN. 2B4, the natural killer and T cell immunoglobulin superfamily surface protein, is a ligand for CD48. *J Exp Med* 1998; **188**, 2083.
34. Latchman Y, McKay PF, Reiser H. Identification of the 2B4 molecule as a counter-receptor for CD48. *J Immunol* 1998; **161**: 5809.
35. Eagle RA, Trowsdale J. Promiscuity and the single receptor: NKG2D. *Nat Rev Immunol* 2007; **7**: 737.
36. Heidecke CD, Araujo JL, Kupiec-Weglinski JW, *et al.* Lack of evidence for an active role for natural killer cells in acute rejection of organ allografts. *Transplantation* 1985; **4**: 441.
37. Nemlander A, Saksela E, Häyry P. Are ''natural killer'' cells involved in allograft rejection? *Eur J Immunol* 1983; **13**: 348.
38. Blancho G, Buzelin F, Dantal J, *et al.* Evidence that early acute renal failure may be mediated by CD3− CD16+ cells in a kidney graft recipient with large granular lymphocyte proliferation. *Transplantation* 1992; **53**: 1242.

39. Petersson E, Ostraat O, Ekber H, *et al.* Allogeneic heart transplantation activates alloreactive NK cells. *Cell Immunol* 1997; **175**: 25.
40. Watson CA, Pezelbauer P, Zhou J, Pardi R, Bender JR. Contact-dependent endothelial class II HLA gene activation induced by NK cells is mediated by IFN-gamma-dependent and -independent mechanisms. *J Immunol* 1995; **154**: 3222.
41. Timonen T, Patarroyo M, Gahmber CG. CD11a-c/CD18 and GP84 (LB-2) adhesion molecules on human large granular lymphocytes and their participation in natural killing. *J Immunol* 1988; **141**: 1041.
42. Ayalon O, Hughes EA, Cresswell P, *et al.* Induction of transporter associated with antigen processing by interferon gamma confers endothelial cell cytoprotection against natural killer-mediated lysis. *Proc Natl Acad Sci USA* 1998; **95**: 2435.
43. McDouall RM, Batten P, McCormack A, Yacoub MH, Rose ML. MHC class II expression on human heart microvascular endothelial cells: exquisite sensitivity to interferon-gamma and natural killer cells. *Transplantation* 1997; **64**: 1175.
44. Taub DD, Sayers TJ, Carter CR, Ortaldo JR. Alpha and beta chemokines induce NK cell migration and enhance NK-mediated cytolysis. *J Immunol* 1995; **155**: 3877.
45. Kondo T, Morita D, Watarai Y, *et al.* Early increased chemokine expression and production in murine allogeneic skin grafts is mediated by natural killer cells. *Transplantation* 2000; **69**: 969.
46. Obara H, Nagasaki K, Hsieh CL, *et al.* IFN-gamma, produced by NK cells that infiltrate liver allografts early after transplantation, links the innate and adaptive immune responses. *Am J Transplant* 2005; **5**: 2094.
47. Kroemer A, Xiao X, Degauque N, *et al.* The innate NK cells, allograft rejection, and a key role for IL-15. *J Immunol* 2008; **180**: 7818.
48. Markus PM, van den Brink M, Cai X, *et al.* Effect of selective depletion of natural killer cells on allograft rejection. *Transplant Proc* 1991; **23**: 178.
49. Maier S, Tertilt C, Chambron N, *et al.* Inhibition of natural killer cells results in acceptance of cardiac allografts in CD28-/- mice. *Nat Med* 2001; **5**: 557.
50. McNerney ME, Lee KM, Zhou P, *et al.* Role of natural killer cell subsets in cardiac allograft rejection. *Am J Transplant* 2006; **6**: 505.
51. Kim J, Chang CK, Hayden T, *et al.* The activating immunoreceptor NKG2D and its ligands are involved in allograft transplant rejection. *J Immunol* 2007; **179**: 6416.
52. Zhang ZX, Wang S, Huang X, *et al.* NK cells induce apoptosis in tubular epithelial cells and contribute to renal ischemia–reperfusion injury. *J Immunol* 2008; **181**: 7489.
53. Sumitran-Holgersson S, Wilczek HE, Holgersson J, Söderström K. Identification of the nonclassical HLA molecules, mica, as targets for humoral immunity associated with irreversible rejection of kidney allografts. *Transplantation* 2002; **74**: 268.
54. Hankey KG, Drachenberg CB, Papadimitriou JC, *et al.* MIC expression in renal and pancreatic allografts. *Transplantation* 2002; **73**: 304.
55. Coudert JD, Coureau C, Guéry JC. Preventing NK cell activation by donor dendritic cells enhances allospecific CD4 T cell priming and promotes Th type 2 responses to transplantation antigens. *J Immunol* 2002; **169**: 2979.
56. Uehara S, Chase CM, Kitchens WH, *et al.* NK cells can trigger allograft vasculopathy: the role of hybrid resistance in solid organ allografts. *J Immunol* 2005; **175**: 3424.
57. Martin MP, Gao X, Lee JH, *et al.* Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat Genet* 2002; **31**: 429.
58. Biassoni R, Pessino A, Malaspina A, *et al.* Role of amino acid position 70 in the binding affinity of p50.1 and p58.1 receptors for HLA-Cw4 molecules. *Eur J Immunol* 1997; **27**: 3095.
59. Stewart CA, Laugier-Anfossi F, Vély F, *et al.* Recognition of peptide-MHC class I complexes by activating killer immunoglobulin-like receptors. *Proc Natl Acad Sci USA* 2005; **102**: 13224.
60. Stern M, Ruggeri L, Capanni M, Mancusi A, Velardi A. Human leukocyte antigens A23, A24, and A32 but not A25 are ligands for KIR3DL1. *Blood* 2008; **112**: 708.
61. Ruggeri L, Capanni M, Urbani E, *et al.* Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science* 2002; **295**: 2097.
62. Ruggeri L, Mancusi A, Capanni M, *et al.* Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. *Blood* 2007; **110**: 433.
63. Tran TH, Mytilineos J, Scherer S, Laux G, Middleton D, Opelz G. Analysis of KIR ligand incompatibility in human renal transplantation. *Transplantation* 2005; **80**: 1121.
64. Bishara A, Brautbar C, Zamir G, Eid A, Safadi R. Impact of HLA-C and Bw epitopes disparity on liver transplantation outcome. *Hum Immunol* 2005; **66**: 1099.
65. Fan QR, Long EO, Wiley DC. Crystal structure of the human natural killer cell inhibitory receptor KIR2DL1-HLA-Cw4 complex. *Nat Immunol* 2001; **2**: 452.
66. Ahlenstiel G, Martin MP, Gao X, Carrington M, Rehermann B. Distinct KIR/HLA compound genotypes affect the kinetics of human antiviral natural killer cell responses. *J Clin Invest* 2008; **118**: 1017.
67. Hanvesakul R, Spencer N, Cook M, *et al.* Donor HLA-C genotype has a profound impact on the clinical outcome following liver transplantation. *Am J Transplant* 2008; **8**: 1931.
68. Kunert K, Seiler M, Mashreghi MF, *et al.* KIR/HLA ligand incompatibility in kidney transplantation. *Transplantation* 2007; **84**: 1527.
69. Chen C, Busson M, Rocha V, *et al.* Activating KIR genes are associated with CMV reactivation and survival after

non-T-cell depleted HLA-identical sibling bone marrow transplantation for malignant disorders. *Bone Marrow Transplant* 2006; **38**: 437.
70. Stern M, Elsässer H, Hönger G, Steiger J, Schaub S, Hess C. The number of activating KIR genes inversely correlates with the rate of CMV infection/reactivation in kidney transplant recipients. *Am J Transplant* 2008; **8**: 1312.
71. Wang H, Grzywacz B, Sukovich D, *et al.* The unexpected effect of cyclosporin A on CD56+CD16- and CD56+CD16+ natural killer cell subpopulations. *Blood* 2007; **110**: 1530.
72. Chiossone L, Vitale C, Cottalasso F, *et al.* Molecular analysis of the methylprednisolone-mediated inhibition of NK-cell function: evidence for different susceptibility of IL-2- versus IL-15-activated NK cells. *Blood* 2007; **109**: 3767.
73. Wai LE, Fujiki M, Takeda S, Martinez OM, Krams SM. Rapamycin, but not cyclosporine or FK506, alters natural killer cell function. *Transplantation* 2008; **85**: 145.
74. Bielekova B, Catalfamo M, Reichert-Scrivner S, *et al.* Regulatory CD56(bright) natural killer cells mediate immunomodulatory effects of IL-2Ralpha-targeted therapy (daclizumab) in multiple sclerosis. *Proc Natl Acad Sci USA* 2006; **103**: 5941.
75. Bhat R, Watzl C. Serial killing of tumor cells by human natural killer cells – enhancement by therapeutic antibodies. *PLoS ONE* 2007; **28**: 326.
76. Stauch D, Dernier A, Volk HD, Pratschke P, Kotsch K. Targeting of Natural Killer cells by rabbit antithymocyte globulin and Campath-1H: similar effects independent of specificity. *PLoS ONE* 2009; **4**: e4709.
77. Beilke JN, Kuhl NR, Van Kaer L, Gill RG. NK cells promote islet allograft tolerance via a perforin-dependent mechanism. *Nat Med* 2005; **11**: 1059.
78. Yu G, Xu X, Vu MD, Kilpatrick ED, Li XC. NK cells promote transplant tolerance by killing donor antigen-presenting cells. *J Exp Med* 2006; **203**: 1851.
79. Laffont S, Seillet C, Ortaldo J, Coudert JD, Guéry JC. Natural killer cells recruited into lymph nodes inhibit alloreactive T-cell activation through perforin-mediated killing of donor allogeneic dendritic cells. *Blood* 2008; **112**: 661.
80. Ikehara Y, Yasunami Y, Kodama S, *et al.* CD4(+) Valpha14 natural killer T cells are essential for acceptance of rat islet xenografts in mice. *J Clin Invest* 2000; **105**: 1761.
81. Seino KI, Fukao K, Muramoto K, *et al.* Requirement for natural killer T (NKT) cells in the induction of allograft tolerance. *Proc Natl Acad Sci U S A* 2001; **98**: 2577.
82. Jiang X, Kojo S, Harada M, Ohkohchi N, Taniguchi M, Seino KI. Mechanism of NKT cell-mediated transplant tolerance. *Am J Transplant* 2007; **7**: 482.

