Frontiers in Nephrology: The Varied Faces of Natural Killer Cells in Transplantation—Contributions to Both Allograft Immunity and Tolerance

Joshua N. Beilke* and Ronald G. Gill†

*Department of Microbiology & Immunology, University of California, San Francisco, San Francisco, California; and †Barbara Davis Center, University of Colorado Health Sciences Center, Denver, Colorado

ABSTRACT

Natural killer (NK) cells are recognized for providing an important early innate immune response to viral and bacterial pathogens and for the surveillance of stressed and transformed autologous cells. However, with the exception of a pronounced role in allogeneic hematopoietic stem cell rejection, it has been challenging to ascribe the precise roles for NK cells in reactivity to tissue and solid-organ transplants. In general, NK cells initiate a rapid, proinflammatory environment that is conducive to many forms of effective immune host defense. This reactivity is often considered deleterious to allograft survival because NK cells are implicated in promoting both acute and chronic graft injury. However, more recent findings indicate that NK cells can also play a surprisingly profound role in allograft tolerance induction. This duality of function requires a reconsideration of the nature and consequence of NK cell reactivity during the allograft response. This review focuses on the differing “faces” of NK cells, especially the unexpected role of NK cells in allograft tolerance induction.


NK CELL RECOGNITION: BEYOND THE “MISSING SELF” CONCEPT

Unlike the rearranged antigen-specific receptor genes that are used by the adaptive immune system, natural killer (NK) cells use a variety of germline-encoded gene products for their recognition armament and so are categorized as part of the innate immune system. For quite some time, the primary paradigm describing NK cell killing was the notion of “missing self,” especially regarding deficient autologous MHC class I expression. That is, it is clear that the absence or reduction of MHC class I molecules permits NK cell activation by transformed or infected cells. However, during the past few years, it has become clear that NK cell recognition involves a broad spectrum of both inhibitory and activating receptor-ligand interactions. Both the activation and inhibition of NK cells predominantly involve interactions with a series of MHC class I and class I–like molecules. Thus, whereas classical MHC class I expression provides the major source of inhibitory signals to NK cells, another series of more unusual, inducible class I–like molecules serve as signals of cellular stress, transformation, and infection.

Many NK receptors are encoded within the NK complex found on mouse chromosome 6 and human chromosome 12p13.2. Several excellent reviews have focused on these receptors and ligands and so are not discussed in great detail. However, there are key concepts regarding NK receptors that should be highlighted. It is important to note that NK cell reactivity is an integrated response controlled by both activating and inhibitory signals (examples of such receptor-ligand interactions are summarized in Table 1). NK cell inhibition is achieved by a series of receptors, such as much of the Ly49 family in mice (although some Ly49 receptors are activating) and the killer cell Ig-like receptors found on human NK cells. The reduction in self MHC class expression by transformed or infected autologous cells or by MHC-disparate allogeneic cells accounts in part for the “missing self” response by releasing such inhibitory stimuli. However, studies in the past several years have identified another series inducible MHC class I–like stimulating ligands that interact with NK activating ligands, such as NKG2D. Taken together, the absence of self is not sufficient to promote NK cell killing but also requires the expression of appropriate activating ligands on the target cell. It will be an ongoing challenge to dissect the contribution of particular activating and inhibitory NK cell receptors to allograft immunity and tolerance.

Because NK reactivity is affected by both positive and negative signals, NK cells can kill self targets despite the pres-
Inhibitory

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIR2DL1 (CD158a)</td>
<td>HLA-C</td>
</tr>
<tr>
<td>KIR2DL2/3</td>
<td>HLA-C</td>
</tr>
<tr>
<td>KIR3DL1</td>
<td>HLA-B</td>
</tr>
<tr>
<td>KIR3DL2</td>
<td>HLA-A</td>
</tr>
<tr>
<td>NKG2A (CD94)</td>
<td>HLA-E</td>
</tr>
</tbody>
</table>

Activating human

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKG2D (CD314)</td>
<td>Inducible MICA, MICB, ULBP</td>
</tr>
<tr>
<td>Nkp30 (CD337)</td>
<td>Unknown</td>
</tr>
<tr>
<td>Nkp46 (CD335)</td>
<td>Viral hemaglutinin</td>
</tr>
<tr>
<td>Nkp44</td>
<td>Unknown</td>
</tr>
<tr>
<td>CD16 (FcγR)</td>
<td>IgG</td>
</tr>
</tbody>
</table>

mouse

<table>
<thead>
<tr>
<th>Receptors</th>
<th>Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK2D</td>
<td>Inducible RAE-1, MULT1, H60</td>
</tr>
<tr>
<td>Ly49D</td>
<td>H-2Dd</td>
</tr>
<tr>
<td>Ly49H</td>
<td>Viral MCMV encoded m157</td>
</tr>
<tr>
<td>CD16 (FcγR)</td>
<td>IgG</td>
</tr>
</tbody>
</table>

NKG2A, murine ULBP-like transcript 1; RAE-1, retinoic acid early inducible-1; ULBP, UL16-binding protein.

| Table 1. Examples of inhibitory and activating receptors on human and mouse NK cells |

NK cells have varied means of enhancing adaptive immunity and probably can contribute to acute allograft rejection. Inhibitory protein. B; MULT1, murine ULBP-like transcript 1; RAE-1, retinoic acid early inducible-1; ULBP, UL16-binding protein.

OVERLAP BETWEEN NK CELLS AND OTHER FORMS OF MHC CLASS I–DEPENDENT IMMUNITY

It is important to emphasize that some of the ambiguity in defining the role of NK cells in allograft immunity and tolerance is due to the overlap of function between NK cells and other types of MHC class I–dependent cells and/or the considerable cross-talk between NK cells and other cells. For example, activated CD8 T cells can express several NK cell–like receptors that can contribute to their effector function. Once activated, human CD8 T cells are capable of tumor cell killing through the NKG2D activating ligand (usually attributed to NK cells) independent of antigen-specific T cell receptor recognition. Also, asialo GM1 is a cell surface marker that often is used to identify and/or target NK cells but is also expressed on an important population of CD8 T cells. These potential NK-like properties of CD8 T cells somewhat blur the traditional distinction between innate and adaptive immunity. Also, another important subset of nonclassical MHC class I–restricted cells, the natural killer T (NKT) cell, shares properties with NK cells. Like NK cells, NKT cells express some NK cell–like receptors, respond rapidly to appropriate stimuli, stimulate DC, and can contribute to antitumor immunity (reviewed by Kronenberg). There are also interactions between NK and NKT cells during the initial innate response to tissue injury or pathogen exposure. However, unlike NK cells, NKT cells express CD3 and a relatively restricted, or “invariant,” T cell receptor repertoire restricted to nonclassical CD1 class I molecules presenting glycolipid moieties. Thus, it is not surprising that it is challenging to distinguish NK cell–specific functions from those of other MHC class I–dependent immune pathways.

CONTRIBUTIONS OF NK CELLS TO ACUTE AND CHRONIC ALLOGRAFT INJURY

Although our own interest has focused on the role of NK cells in tolerance induction, it is important to note the multiple contributions of these cells to both acute and chronic graft rejection. NK cells are widely known as mediators of MHC-disparate hematopoietic stem cell rejection and can constitute an important barrier to T cell–directed tolerance protocols for achieving mixed hematopoietic chimerism. With this notable exception of bone marrow allografts, NK cells are not usually either necessary or sufficient to mediate allograft rejection independent of an intact adaptive immune system. This is graphically illustrated by the findings that tissue and organ allografts are accepted indefinitely in SCID and rag-1−/− mice that are NK replete but T and B lymphocyte deficient. This seemingly ancillary role for NK cells in transplant immunity makes defining their role in rejection difficult. However, there are a number of ways in which NK cells promote the adaptive immune response resulting in both acute and chronic allograft injury (illustrated in Figure 1).

NK cells have varied means of enhancing adaptive immunity and probably can contribute to acute allograft rejection. First, NK cells play an important role in “licensing” antigen-
presenting cells, especially DC, resulting in DC maturation and subsequent T cell activation.21 Also, NK cells provide an early source of IFN-γ that helps tailor the generation of Th-1–like immunity by CD4 T cells.22,23 NK cells also can augment CD4 T cell reactivity by a direct NK:CD4 T cell interaction.24 All of these activities contribute to potentially graft-destructive acute T cell reactivity. Although NK cells are rarely demonstrated to be necessary for the rejection of solid-organ allografts, there are exceptions. It is interesting that NK cells are required to trigger rejection of cardiac allografts in CD28−/− recipients, and this response requires the absence of self MHC expression by the graft,25,26 a classic example of NK reactivity by the missing-self concept.

NK cells have also been implicated in promoting chronic allograft injury. NK cells even infiltrate syngeneic kidney transplants after ischemic reperfusion injury and may contribute to chronic graft pathology.27 Other studies suggest that NK cells can contribute to chronic allograft vasculopathy, possibly as a result of the missing-self MHC class I expression by allogeneic vascular endothelium on the transplant.28,29 Such NK-dependent injury is IFN-γ dependent,29 a finding that correlates with the requirement of conventional CD4 T cells to mediate acute cardiac allograft rejection by and IFN-γ–dependent mechanism.30 Although it is not yet clear what molecular signals predominate the triggering of NK reactivity to allografts, this response is generally assumed to be harmful to graft survival.

**AN UNEXPECTED CONTRIBUTION OF NK CELLS TO ALLOGRAFT TOLERANCE**

Despite the ongoing correlation between NK cell reactivity and allograft rejection, it has become apparent that NK cells have important regulatory properties31 that can actually facilitate allograft tolerance induction.32,33 We stumbled onto this unexpected property of NK cells by studying the nature of tolerance to pancreatic islet allografts after host treatment targeting either CD154 or CD11a (LFA-1).32 Because CD8 T cells have been shown to constitute a barrier to allograft tolerance induction after co-stimulation blockade,14,34 we set out to study the propensity for tolerance induction in MHC class I–deficient, β-2 microglobulin (β2m) knockout mice. Such animals are deficient in generalized MHC class I–dependent immunity, including CD8 T cells.35 To our surprise, tolerance induction absolutely required an intact host MHC class I pathway in that β2m-deficient hosts were completely resistant to induced allograft tolerance.32 Because NKT cells restricted to nonclassical MHC class I CD1 molecules have been implicated in allograft tolerance induction,36–38 it was possible that this alternate β2m-dependent pathway was required for tolerance. However, NKT cell–deficient CD11-knockout mice were completely amenable to allograft tolerance induction,32 a finding consistent with another similar study.33 NK1.1+ cells that were present in CD11-knockout mice were found in the direct elimination of donor-derived DC that are involved in activating the response. Alternatively, the extent of NK regulation of recipient DC that are involved in "indirect" (host APC-dependent) response33 that accounts for the high frequency of alloreactive cells found in the native T cell repertoire. Thus, NK cells may essentially prune the magnitude of this direct antidonor reactivity by simply eliminating the primary donor-derived DC that are involved in activating the response. Alternatively, the extent of NK regulation of recipient DC that are involved in "indirect" (host APC-dependent) allograft antigen presentation is an important issue that is currently undefined.

Although such allogeneic DC elimination by NK cells can occur, this activity alone probably does not account for the
entire role for NK cells in allograft tolerance. If the sole property of NK cells is to eliminate donor DC via a missing-self response, then semiallogeneic allografts bearing self class I molecules should be protected from such donor DC elimination\textsuperscript{33} and so render the host resistant to tolerance induction. However, we find that recipients can be readily tolerized to (donor × host)F1 allografts,\textsuperscript{32} suggesting that the role for NK cells in tolerance is more complex. An alternate view is that activated T cells themselves may serve as proximal targets of NK cell–mediated regulation (Figure 3). For example, the NK activating ligand MHC class I–related chain A can be induced on stimulated human T cells, serving as a potential target of the corresponding activating NKG2D receptor. Importantly, such cellular “stress” signals on mouse T cells can make them vulnerable to NK killing despite autologous MHC class I expression.\textsuperscript{11} This illustrates the issue raised that NK cells actually integrate both stimulating and inhibitory signals to determine the outcome of the NK response. It is intriguing that this NK killing of autologous stressed T cells was found to be perforin dependent,\textsuperscript{11} consistent with our own findings that tolerance induction to islet allografts was perforin dependent,\textsuperscript{11} suggesting that the role for NK cells in tolerance is more complex.

WHAT IS THE RELATIONSHIP BETWEEN NK CELL AND REGULATORY T CELL REACTIVITY IN ALLOGRAFT TOLERANCE?

There has been a tremendous resurgence in the interest in regulatory T cells (Treg) in the maintenance of allograft tolerance (reviewed by Wood \textit{et al.},\textsuperscript{40} Waldmann \textit{et al.},\textsuperscript{41} and Walsh \textit{et al.}\textsuperscript{42}). Although this discussion largely centers on MHC class I–dependent reactivity, it is important to emphasize that induced allograft tolerance is generally dependent on CD4 T cells. An unanswered question is whether there is a direct connection between NK cell function and Treg activity in the promotion of allograft tolerance. To date, most published evidence favors the contrary notion that there is mutual antagonism between NK cells and Treg.\textsuperscript{43} For example, CD4⁺CD25⁺ Treg generally inhibit NK cell reactivity,\textsuperscript{43} and in transplantation, such Treg can attenuate NK cell–mediated bone marrow allograft rejection.\textsuperscript{44} Thus, although there has been a suggestion that NK cells can alter DC function and promote Treg induction,\textsuperscript{45} there is little evidence that NK cells generally promote Treg or \textit{vice versa}. Rather, we propose that NK and Treg reactivities are temporally distinct processes during allograft tolerance induction. We found that NK cells were required for tolerance induction only during the peritransplantation period. Within 3 wk after transplantation, NK cells were no longer required for long-term allograft survival.\textsuperscript{32} However, there seems to be a requirement for Treg in ongoing maintenance of allograft tolerance. The connection between early innate NK reactivity and longer term Treg activity in tolerance remains unclear. A conservative view is that there is no actual direct interaction between NK cells and Treg in the development of tolerance induction. NK cells may be required early during tolerance induction to restrain DC and/or T cells that can initiate allograft injury, whereas sustained allograft protection is achieved by a subsequent maturation of a regulatory T cell response that is enhanced by the tolerogenic regimen (Figure 4). Thus, it is possible the NK cells do not actually induce tolerance, \textit{per se}, but simply permit allograft survival while a regulatory response develops.
UNEXPECTED ROLE OF PROINFLAMMATORY REACTIVITY IN TOLERANCE: WHEN IS “KILLING” BENEFICIAL TO THE ALLOGRAFT?

The regulatory potential of NK cells is only one facet of a growing body of evidence indicating that several immune pathways that are regarded as graft destructive can also participate in tolerance induction. Generally, the presence of proinflammatory immunity and cytolytic activity clearly correlates with allograft injury. However, it is becoming increasingly apparent that there is also a major regulatory role for many of these same effector cells and molecules, including IFN-γ, perforin, and NK cells. Regarding IFN-γ, there are clear examples where this cytokine can be essential for either CD4 or CD8 T cell–mediated acute rejection and also for chronic rejection. However, there is clearly another side to IFN-γ that can promote allograft survival and tolerance. IFN-γ can actually have an early protective effect on allograft injury. Also, several studies show a major role for IFN-γ in promoting allograft tolerance and may even play an important role in ongoing allograft tolerance activity mediated by Treg.

The cytotytic mediator perforin also demonstrates a marked “duality” in promoting either allograft rejection or tolerance. Although gene expression for the cytotytic mediators perforin and granzymes clearly correlates with graft rejection, perforin is also necessary for allograft survival to otherwise perforin-deficient mice, arguing against an obligate role for perforin expression by conventional T cells for graft survival.

This last point may help to resolve the seeming paradox between both destructive and protective properties of these effector molecules on allograft survival. It is highly likely that these “proinflammatory” pathways are compartmentalized by cell type and/or by anatomic location to promote graft injury or survival, respectively. For example, alloreactive effector T cells expressing IFN-γ and perforin may well mediate acute or chronic graft injury. However, Treg and NK cells may use these same pathways to mediate the regulation of alloimmunity. It will be an ongoing challenge to sort out the opposing roles of these effector pathways in allograft immunity and tolerance and to translate this information into clinically relevant therapeutic strategies to promote allograft survival.

DISCLOSURES

None.

REFERENCES

nodes provides IFN-gamma for T(H)1 priming. Nat Immunol 5: 1260–1265, 2004