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Natural Killer Cells: Tolerance to Self and Innate Immunity to Viral Infection and Malignancy

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Abstract

Natural killer (NK) cells are lymphocytes whose ability to identify and kill virally infected and malignant cells while sparing normal cells was poorly understood until the late 1980's and the introduction of the "missing self' hypothesis. According to this hypothesis, downregulation of major histocompatibility complex (MHC) class I molecules during viral infection or malignant transformation triggers NK activation (1). Since this hypothesis was first proposed, much has been learned about NK cell surface receptors, their role in the molecular basis of missing-self recognition, and the mechanisms underlying NK cell tolerance. In this review, we will discuss these mechanisms, as well as their relevance to viral infection and tumor immunity and stem cell transplantation.

NK CELL RECEPTORS: KIR AND Ly49 FAMILIES

Unlike T and B lymphocytes which employ rearranged antigen-specific receptors, NK cells express germline-encoded receptors comprised of both inhibitory and activating forms. The principal NK cell receptors in humans are the killer cell immunoglobulin (Ig)-like receptors (KIRs), members of a polymorphic receptor family within the immunoglobulin superfamily. The main NK cell receptors in mice are the C-type lectin-like molecules belonging to the Ly49 family. In both humans and mice, NK cells also express a lectin-like receptor heterodimer consisting of CD94 coupled with members of the NKG2 family. Interestingly, the KIR molecules are type I integral membrane proteins whereas the lectin-like receptors have a type II membrane orientation.

Most MHC-specific inhibitory NK cell receptors recognize MHC class Ia molecules, while CD94/NKG2 recognizes the MHC class Ib molecule Qa-1 in mice (HLA-E in humans), which presents signal peptides from MHC class Ia molecules, thereby achieving recognition of MHC class Ia expression indirectly (2). Constitutively expressed on healthy cells, class I ligands of inhibitory receptors are often downregulated during pathologic states such as viral infection or transformation (3, 4). The immunoreceptor tyrosine-based inhibitory motif

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(ITIM) in the cytoplasmic tails of the inhibitory receptors associates with the cytoplasmic tyrosine phosphatase SHP-1, but may also recruit other signaling molecules (5).

In a notable example of convergent evolution, inhibitory human KIR receptors and mouse Ly49 receptors are functionally analogous, despite being different in their structure and membrane topology (6). In addition to MHC class I ligand binding and ITIM-mediated inhibition, common features include constitutive expression on unstimulated NK cells, stochastic expression on overlapping NK subsets (7), expression of one or more inhibitory receptors by an individual NK cell (8), encoding by germ-line clusters of polymorphic genes (9, 10), and close sequence and structure homology to ITIM-negative activating receptors. Structurally similar in the extracellular regions to the corresponding inhibitory receptors, the activating KIR and Ly49 receptors have short cytoplasmic tails and associate with DAP12 or other immunoreceptor tyrosine-based activation motif (ITAM)-containing signaling molecules (2). While identified ligands include MHC class I-like molecules, the ligands for many activating receptors remain unknown. For the activating receptor NKG2D, where the ligands are known, some constitutively expressed ligands can become upregulated in response to stress or other stimuli, resulting in “induced-self” in diseased cells and enhanced NK cell activation (11).

Integration of inhibitory and activating receptor signals determines the NK cell response to a target cell. In detection of missing self-MHC on a target cell, a decreased or absent inhibitory signal allows the activation signal to dominate, triggering cytokine production and/or cytotoxicity. Conversely, high expression of an activating ligand on a target cell can result in NK cell activation despite normal MHC expression, although in general, inhibitory signals typically predominate over activating signals.

NK CELL SELF-TOLERANCE: EARLY MODELS

Over the past two decades, several models have been proposed to explain NK cell function and tolerance to self. The “missing self” hypothesis described how NK cells preferentially kill target cells lacking MHC class I expression (1); the “at least one” model proposed that every NK cell expresses at least one inhibitory receptor for self-MHC; and the receptor calibration model suggested that differential expression of NK receptors, depending on host expression of cognate MHC class I ligand, translates to differences in functional response. Despite some evidence in mice and humans supporting each of these models, the models could not fully explain certain acknowledged findings: NK cells from MHC-deficient mice are hyporesponsive (12) and not hyperactive or auto-reactive; NK cells expressing receptors for which the host is lacking the MHC class I ligand are also hyporesponsive and not auto-reactive; and a significant fraction of the NK repertoire expresses no known receptors for self-MHC and yet are hyporesponsive.

NK CELL LICENSING MODEL

In the “NK cell licensing” model, an NK cell that has engaged self-MHC via MHC-specific receptors is “licensed” to be responsive to subsequent stimuli received via their activation receptors (13, 14). Conversely, NK cells that do not engage self-MHC are termed “unlicensed”. In mice, the MHC-specific Ly49 receptors first identified as inhibitory receptors for effector responses also ironically mediate licensing. The NK cell licensing model thus describes two populations of NK cells tolerant to self-MHC: NK cells licensed for effector function through self-specific MHC class I inhibitory receptors but which maintain self-tolerance through direct inhibition via these same receptors by self-MHC; and unlicensed NK cells which lack self-specific MHC class I inhibitory receptors, cannot engage self-MHC, and are inert to functionally MHC-deficient host cells.

The licensing model was first proposed by Kim et al. in studies examining the responses of freshly isolated NK cells upon target cell-free antibody cross-linking (13). Naïve NK cells were stimulated with plate-bound antibodies against NK cell activation receptors such as NK1.1 (Nkrp1c) expressed on all immature and mature NK cells in C57BL/6 ($H2^b$) mice. Staining for intracellular interferon γ (IFNγ), in conjunction with surface staining for lineage markers and Ly49 receptors, allowed for the assessment of NK cell activation at the single cell level. Only NK cells expressing an inhibitory receptor specific for self-MHC produced IFNγ upon *ex vivo* plate-bound antibody stimulation (15). Thus, $Ly49A^+$ NK cells from MHC-congenic or transgenic mice expressing $H2D^d$, a ligand for Ly49A, were licensed to produce IFNγ upon stimulation; in contrast, $Ly49A^+$ NK cells from mice lacking an MHC ligand for Ly49A, such as mice with the $H2^b$ haplotype, were unlicensed and produce significantly less IFNγ. NK cell production of other cytokines and NK cytotoxicity produced consistent results, suggesting that a receptor specific for self-MHC must be engaged in order for the NK cell to be licensed for functional competence. The licensing model was further supported by a study in mice transgenic for only a single MHC class I molecule: an $H2K^b$-ovalbumin peptide single chain MHC class I trimer on an otherwise MHC class I-deficient background, where the MHC class I trimer is exclusively recognized by the Ly49C receptor (13). Consistent with the licensing model, $Ly49C^+$ NK cells from these mice produced IFNγ upon stimulation with plate-bound anti-NK1.1, but $Ly49C^-$ NK cells did not. Thus, through engagement of its inhibitory receptor with its cognate MHC class I ligand, expressed as self, an NK cell becomes functionally competent to be triggered through its activation receptors, i.e., it becomes licensed.

LICENSING OF HUMAN NK CELLS

Several subsequent studies have demonstrated that licensing also applies to human NK cells. Approximately 10% of human $CD56^{dim}$ NK cells lack all inhibitory receptors for self-MHC (16, 17). Consistent with an unlicensed state, these cells exhibit reduced cytokine production and cytotoxicity in response to stimulation with MHC class I-negative target cells as well as anti-CD16 crosslinking and antibody-dependent cell-mediated cytotoxicity (ADCC). Despite their hyporesponsiveness, they express normal protein levels of perforin and granzyme and can respond to PMA + ionomycin, indicating that they have not lost inherent capacity to execute effector functions. Conversely, NK cells expressing inhibitory receptors for self-HLA-B and -C alleles exhibit robust response to class I-negative target cells. Moreover, NK cells expressing more than one inhibitory receptor for self-MHC demonstrate even higher effector function, implying a dose effect of surface inhibitory receptors for licensing (17).

The functional NK repertoire, therefore, is defined by NK populations expressing an array of inhibitory receptors within the context of self-MHC. A hierarchy of effector function can be discerned, such that NK cells lacking all inhibitory receptors are largely functionally inert; cells exclusively expressing CD94/NKG2A display modest functional competence; cells with a single inhibitory Ly49 or KIR receptor for self-MHC display higher functional competence; while cells with more than one inhibitory for self-MHC display progressively higher functional enhancement. In humans, the NK repertoire tends toward a state of "intermediate inhibition," with cells of lowest and highest functional potency represented in lower numbers compared to cells of intermediate potency (17, 18) . Data from mouse models also demonstrate a correlation between different Ly49-MHC combinations and a diversity of NK functional potencies (19, 20).

What then is the role of unlicensed cells, which can be identified in humans and mice and represent approximately 10% of all NK cells? Responding poorly to stimulation with MHC class I-negative target cells or plate-bound antibody, they can still produce IFNγ when

stimulated with PMA + ionomycin with similar frequency to self-MHC-specific NK cells. Furthermore, there are no specific differences in developmental markers between licensed and unlicensed cells, suggesting that unlicensed cells are not simply immature. Studies in mice have shown that *in vivo* poly-I:C (polyinosinic:polycytidylic acid) treatment or *in vitro* culture in IL-2 can circumvent the effect of licensing, inducing unlicensed populations to respond to stimulation (13, 21). The ability of cytokine stimulation *in vitro* to reverse the hyporesponsiveness of unlicensed cells suggests similar effects on NK cells may occur *in vivo* during the inflammatory states of viral infection or malignancy. Indeed, NK cells in mixed wild-type: beta(2)-microglobulin-deficient (β2m(−/−)) chimeric mice are initially tolerant of MHC class I-deficient host cells, but readily reject host β2m(−/−) bone marrow after infection with murine CMV, suggesting that the cytokine environment in the inflammatory state associated with viral infection can break established NK tolerance (22). The role of NK cells in viral infection and the relevance of breaking tolerance to self in hematopoietic stem cell cell transplantation are discussed in greater detail in later sections.

For licensing to occur, a self-MHC-specific receptor must engage self-MHC. Moreover, it is known that the self-MHC-specific receptor itself is responsible for directly signaling the licensing event. Gene transfer of intact and mutant Ly49A receptors into hematopoietic stem cells in bone marrow reconstitution studies have shown that the receptor can confer licensing not only when the cognate ligand is present in the host, but also when the ITIM region in the receptor is intact (13). How the ITIM mediates licensing remains unknown, however, but it appears that the ITIM can recruit more than just phosphatases because recent studies indicate that ITIM-dependent inhibitory signaling results in the formation of macromolecular complexes mediated by scaffolding proteins (23). It is possible that there are differences in these macromolecular complexes during licensing.

Other than the requirement of physical interaction between MHC class I with inhibitory NK receptors for licensing to occur, where it occurs and with which accessory cell(s) it occurs remain unclear. Several findings suggest that NK cell licensing occurs during maturation in the bone marrow: expression of inhibitory receptors occurs early in NK cell development (24) and coincides with the acquisition of effector capacity (25, 26); NK cells with self-MHC-specific receptors proliferate at a higher rate than NK cells without self-MHC-specific receptors (13); and BM NK cells undergo proliferation before reaching full developmental maturity (24). While no single cell type appears to be responsible for NK cell licensing, both hematopoietic and non-hematopoietic cell elements have been found to play a role, with bone marrow stromal cells likely contributing (27–30). Other studies suggest that NK cells can also mature in the thymus but this appears to apply to a subset of NK cells that is just beginning to be studied (31, 32).

OTHER CONTRIBUTORS TO NK SELF-TOLERANCE

Other molecular and cellular elements may affect the mechanisms underlying NK cell licensing and self-tolerance. In mice, it has been reported that *cis*-interaction between the MHC-specific receptor with self-MHC on the NK cell itself can occur. These interactions likely have consequences in the conformation of Ly49 homodimers affecting the accessibility for MHC class I binding in *trans* (33) and may affect recruitment of intracellular signaling molecules (34), ultimately leading to modulation of NK cell function. The requirement for simultaneous engagement of multiple activation receptors to achieve full NK stimulation provides another layer of protection from auto-aggression (35). The only apparent exception to this requirement is CD16 (FcγRIII), which binds ligand via soluble IgG antibody, whose production by B-cells is subject to its own strict regulation (36). Furthermore, auto-regulation mechanisms upon NK activation include upregulation of

inhibitory receptor expression (37), endocytosis and degradation of the activation receptor NKG2D (38), and induction of cross-tolerance of other activation pathways (39, 40).

Finally, certain cell-cell interactions provide accessory activation signals to NK cells while others impart yet an additional tier of protection from auto-aggression. Most likely through *trans* presentation of IL-15, dendritic cell-mediated NK activation and recruitment are important in tumor control as well as NK response to malaria and viral infection (41–43). Less well-studied is the activation of NKT cells, which can trigger NK cell cytokine production. In contrast, regulatory T cells can suppress NK cell activation, possibly via expression of membrane-bound TGFβ, leading to NK downregulation of the activating receptor NKG2D. Finally, the NK cell population itself may have a regulatory subset, capable of inhibiting IFNγ production and cytotoxic function by other NK cells.

NK CELLS AND HIV

NK cells play an important role in the control of viral infections, largely based on results from studies in mice. Several studies, including recent studies in humans, have shown that NK cells are activated and expand significantly during the early stages of viral infections, and these early NK effectors might limit viral replication during the early phases of infection, prior to the development of antigen-specific adaptive immune responses that eventually allow for the control of the virus.

NK cells use several classes of receptors to monitor for virally infected cells. In humans, these include the KIR receptors, the NKG2 receptors, the natural cytotoxicity receptors family (NCR), as well as several other classes of co-receptors. In addition, the CD16 (FcγRIII) receptor allows NK cells to recognize and eliminate opsonized pathogens or cells. NK cells can mediate their effector function by a number of different mechanisms, including (i) the secretion of antiviral cytokines; (ii) exocytosis of cytoplasmic granules containing perforin and granzyme; (iii) Fas ligand-mediated induction of apoptosis; and (iv) antibody-dependent cellular cytotoxicity (ADCC). In addition, NK cells can directly modulate adaptive immune responses, through the secretion of cytokines and the interaction with dendritic cells.

The first evidence that NK cells play a critical role in the immune response to viruses in humans arose from a seminal case reporting a teenage girl with a complete NK cell deficiency who experienced a sequence of severe viral infections, including varicella, cytomegalovirus pneumonia, and severe cutaneous herpes simplex infection (44). While NK cells were absent, the patient had normal T cells, B cells, and neutrophils, providing direct evidence for a role of NK cells in the control of herpes viral infections in humans. A number of additional studies have subsequently linked genetic defects in NK cell function and development to susceptibility to viral infection (45, 46). The critical role of NK cells in the control of viral infections is further supported by the observation that viruses have devised elaborate mechanisms to subvert immune selection pressure mediated by NK cells, including the active suppression of presentation of the stress molecules that can serve as NK cell ligands on the surface of infected cells, and the expression of molecules that serve as ligands for inhibitory NK cell receptors.

The strongest evidence for a role of NK cell in HIV-1 infection comes from population studies assessing the role of polymorphisms in NK cell receptors on HIV-1 disease progression. The seminal epidemiologic study by Martin et al. was the first to demonstrate that individuals that co-express the activating KIR3DS1 allele in conjunction with HLA class I alleles from the HLA-Bw4 family that encode an isoleucine at position 80 (referred to as HLABw480I) progressed significantly more slowly towards AIDS than individuals that have only one or neither of these two alleles (47). While the physical interaction between the

KIR3DS1 molecule and HLABw480I molecules has not been shown, functional studies have now demonstrated that KIR3DS1+ NK cells can respond potently to HIV-1-infected Bw480I+ CD4+ T cells and suppress viral replication in these Bw480I+ cells (48). Furthermore, studies in HIV-1-infected individuals showed that NK cells derived from individuals that possess a copy of KIR3DS1 responded more potently to HLA class I negative target cells than NK cells from KIR3DS1-negative subjects. Interestingly, while the expression of KIR3DS1 alone was associated with a significantly elevated NK cell response, the NK cell responses were strongest in individuals with both KIR3DS1 and HLA-Bw480I (49). Furthermore, new evidence now indicates that KIR3DS1+ NK cells expand preferentially during acute HIV-1 infection, and persist at elevated levels, but only in subjects that also co-express its putative ligand, HLA-Bw480I (50). Finally, an increased proportion of KIR3DS1 homozygous individuals were identified in persistently HIV-1 negative but highly HIV-1 exposed individuals, suggesting that KIR3DS1 may also be involved in protection from infection (51). Taken together, these epidemiological and functional data support a cooperative interaction between KIR3DS1 and HLA-Bw480I in the NK cell response to HIV infection. The precise mechanism by which KIR3DS1+ NK cells can recognize HLA-Bw480I+ HIV-1-infected cells, and the potential antigens presented by HLA-Bw480I that might trigger this recognition, still remain to be elucidated.

Strong evidence for NK cell-mediated viral immunity arises from examples of viral adaptation to evade immune pressure. In an effort to evade adaptive T cell immune responses, HIV-1 Nef downregulates the expression of HLA-A and HLA-B molecules on the surface of infected cells (52, 53). However, reduced HLA class I expression can make these cells more susceptible to NK cell lysis through lack of engagement of inhibitory receptor-expressing NK cells. HIV-1 has therefore evolved means to maintain or even upregulate on the surface of infected cells the expression of HLA-C molecules, direct ligands for a number of inhibitory KIRs, and through HLA-E, indirect ligands for the inhibitory NKG2-CD94 complex (54, 55). Thus HIV-1 is able to evade recognition by both CD8+ T cells and NK cells by downregulating HLA-A and HLA-B, while increasing the expression of ligands for other inhibitory NK receptors on the surface of infected cells.

HIV-1 may select for epitopes presented by HLA class I on the surface of infected cells that might either enhance the binding of inhibitory KIRs, or avoid recognition by activating KIRs. Several studies have demonstrated that KIRs can discriminate between peptides presented by a number of HLA class I alleles, including HLA-B27, HLA-Cw3, HLA-Cw4, HLA-Cw7 and HLA-A3/A11, revealing the critical role of specific residues of the HLA-presented peptides in the peptide-specific interaction with KIR (56–62). These data demonstrate that the sequence of the peptide presented by HLA class I molecules can influence the ligation by inhibitory, and potentially also activating KIRs, and thereby influence target cell lysis. This might place significant pressure on the virus at specific residues and therefore drive KIR-dependent viral evolution.

Taken together, there is increasing evidence suggesting that NK cells play an important role in the control of HIV-1 infection, and might impose immunologic selection pressure on the virus. Future studies aimed at identifying the mechanisms by which NK cells recognize HIV-1-infected cells, and how HIV-1 has evolved to evade NK cell mediated immune selection pressure will provide important insights into the correlates of protective immunity in HIV-1, and might help to design immunologic interventions aimed at enhancing NK cell mediated control of HIV-1 replication.

NK CELLS AND HEMATOPOIETIC STEM CELL TRANSPLANTATION

The role of NK cells in allogeneic hematopoietic stem cell transplantation (HSCT) continues to generate interest as more data emerges on the significance of NK cell immunogenetics on

transplant outcomes. NK cells robustly reconstitute the peripheral blood immediately following transplantation, and historical studies have implicated NK cells in suppressing graft-versus-host disease, promoting bone marrow engraftment, and exerting a graft-versus-leukemia effect. More recent studies have described improved HSCT outcomes with infusion of higher $CD56^{dim}/CD56^{bright}$ NK ratios in the allograft and more rapid NK cell recovery immediately following transplant (63, 64). With an increased understanding of how NK receptor-ligand interactions drive NK function, determining the molecular mechanisms underlying these clinical and laboratory observations has become progressively more feasible. In parallel, studies correlating NK immunogenetics with transplant outcomes continue to provide additional insights to the function of activating NK receptors, about which comparatively little is known and whose ligands remain largely obscure.

MISSING SELF IN HSCT

For the purposes of examining the clinical effects of “missing self” by licensed NK cells, perhaps no other clinical setting is more suitable than the HLA-mismatched, or more specifically, KIR-ligand mismatched allogeneic HSCT. In this circumstance, donor-derived licensed NK cells infused into patients lacking the MHC class I ligands present in the donor recognize the absence of donor MHC ligands on potential target cells, leading to a lack of inhibition and lower threshold for activation, enhanced clearance of tumor cells, and improved outcomes for patients. This model was exemplified in studies by Ruggeri et al. (65), who examined 60 high-risk leukemia patients undergoing HLA haplotype-disparate HSCT and identified a missing-self relationship between donor and recipient HLA genotypes predictive of donor NK alloreactivity, culminating in a potential graft-versus-leukemia (GVL) effect (66). In these studies, donor and recipient HLA-B and HLA-C genotypes were segregated according to their inhibitory KIR ligand categories: the HLA-C1 alleles recognized by KIR2DL2 and KIR2DL3 and characterized by Ser77 and Asn80 (including, with rare exceptions, HLA-Cw1, -Cw3, -Cw7, -Cw8, Cw14, and Cw16 allotypes); the HLA-C2 alleles recognized by KIR2DL1 and characterized by Asn77 and Lys80 (including, with rare exceptions, HLA-Cw2, -Cw4, -Cw5, -Cw6, -Cw15, -Cw17, and –Cw18 allotypes); and the HLA-B alleles possessing the Bw4 serological epitope in the $\alpha 1$ domain, recognized by KIR3DL1. [Since this study, HLA-A alleles possessing the Bw4 epitope have also been found to bind the inhibitory KIR3DL1 and confer licensing effect (67, 68).] Among the 60 donor-recipient pairs studied, 20 fulfilled the conditions predictive of missing self, where recipients lacked KIR ligands present in the donor. Thus, NK cells from a donor heterozygous for HLA-C group 1 and HLA-C group 2 ligands and who exhibits the inhibitory KIR receptors for both HLA-C ligand groups, upon transfer into a recipient homozygous for HLA-C group 1, will be released from inhibition by failure of the donor inhibitory KIR to engage its cognate HLA-C group 2 ligand. A similar prediction could be made for recipients lacking the HLA-Bw4 epitope if their donor exhibits a HLA-Bw4 allotype. Accordingly, alloreactive NK clones could only be isolated from patients in the first 3 months following HSCT if the recipient lacked an HLA epitope that was present in the donor (KIR ligand incompatibility in a graft-versus-host vector). Alloreactive NK lysis of recipient PHA-stimulated blasts or B-lymphocyte cell line (BLCL) target cells corresponded with NK lysis of leukemic blasts from the same individuals and could be blocked by competitive incubation with target cells expressing the absent allele group (66). Furthermore, the authors noted that the alloreactive NK clones isolated from patients could only effectively kill AML and CML leukemia target cells, but could not reliably kill ALL target cells, revealing a disparity in NK sensitivity between myeloid and lymphoid leukemias. When the inverse HLA relationship existed between donor and recipient predicting host-versus-graft alloreactivity, graft rejection occurred only in one rare instance. A follow-up study of 112 HLA haplotype-disparate transplants confirmed that AML recipients lacking donor self-specific KIR ligands from KIR ligand incompatible pairs

experienced lower incidence of relapse and improved event-free survival (69). As further evidence of an NK-mediated graft-versus-leukemia effect, transfer of alloreactive NK clones into NOD/SCID mice previously infused with human blastic CML resulted in disease clearance and longer survival compared to leukemic mice that did not receive infusion with alloreactive NK clones. In a mouse model of mismatched bone marrow transplant (F_1 *H-2*$^{d/b}$ $\rightarrow$parent *H-2*^{b} BMT), infusion of alloreactive NK cells permitted a mismatched transplant following reduced-intensity conditioning, surprisingly without development of graft rejection or GVHD. These findings advanced the possibility that donor-derived alloreactive NK cells could prevent lethal GVHD via by eliminating recipient antigen-presenting cells (APCs) responsible for initiating GVHD (65).

Investigations of the missing-self effect in other HLA-mismatched transplant populations have yielded variable results (70, 71), however, indicating that the missing-self impact on HSCT outcome may be most evident in specific disease categories and under specific treatment conditions, such as in vivo T-cell depletion with ATG.

MISSING KIR LIGAND IN HSCT

Is donor-recipient class I-mismatching necessary to achieve an NK cell-mediated effect in HSCT? Several studies now indicate that beneficial NK effects are possible even in HLA-matched HSCT where donors possess inhibitory KIR for which neither the donor nor the recipient have the relevant class I ligand (72–75). In a study of 178 patients with AML, MDS, CML, and ALL who received T-cell depleted allografts from HLA-identical siblings, 63% of recipients lacked one or more HLA class I ligands for their donor's inhibitory KIR. Comparison of survival and relapse rates between patients with and without ligand for donor inhibitory KIR revealed that AML and MDS patients with "missing KIR ligand" had significantly improved overall and disease-free survival secondary to lower relapse. Furthermore, patients lacking more than one ligand for donor inhibitory KIR exhibited the greatest benefit in overall and disease-free survival than those who lacked only one, implying a dose-effect of lack of KIR ligand (72). Classically licensed NK cells in this setting were not likely the mediators of the alloreactive effect: because donors and recipients were HLA-matched, the donors also lacked the same relevant class I ligands for their inhibitory KIR, implying that these non-self-specific KIR-expressing NK cells could not be licensed for effector function. A recent study by Yu et al. has clarified the apparent incongruity, demonstrating that in the first 3 months following HSCT, circulating unlicensed NK cells with inhibitory KIR for non-self HLA do indeed exhibit effector function, displaying cytokine response and cytotoxicity toward tumor target cells lacking the class I ligand for the inhibitory KIR (76). In distinct contrast to "missing self" behavior of licensed NK cells tolerized to self-HLA, these studies demonstrate that in HSCT, unlicensed NK cells can circumvent the effects of licensing, break tolerance to self, and function according to a less restrictive "missing HLA ligand" mechanism. Just as CMV infection can circumvent the effects of licensing in mice, conferring effector function to otherwise unlicensed cells, HSCT may provide an in vivo inflammatory cytokine milieu conducive for breaking tolerance to self. The cytokines capable of inducing activation of unlicensed cells in vivo remain unknown, and may be elaborated only in specific transplant conditions, accounting for inconsistencies in demonstrating the missing ligand effect in other transplant populations (69, 77).

Activating Donor KIR Genotype and HSCT Outcome

Studies correlating donor KIR genotype with HSCT outcomes have been informative and may ultimately help to guide donor selection. In a study of 65 HLA-matched sibling HSCT, patients with donors exhibiting genotypes with activating KIR2DS1 and 2DS2 displayed decreased relapse (78), findings supported by a later study demonstrating direct lysis of

HLA-C2 homozygous leukemic blasts by 2DS1+ NK cells (79). In another study examining 448 AML patients, donors with a KIR B-haplotype was associated with higher relapse-free survival, where KIR B-haplotypes are generally characterized by more than one activating KIR (80). Most recently, Venstrom et al. have described a decreased risk for grade II-IV GVHD in patients whose with donors exhibiting the activating KIR3DS1 (81). In findings that may inform laboratory studies of innate immunity to viral infection, different groups have described decreased CMV reactivation with donor activating KIR (82), specifically KIR3DS1 (83). Still other studies have identified other activating KIR with specific combinations of inhibitory KIR-HLA as important forces on GVHD, relapse, and survival. Large-scale multi-center studies are still needed to confirm these findings and codify the exact KIR-HLA relationships and transplant conditions important for exploiting NK reactivity. Identification of the molecular ligands for the activating KIR will clarify the mechanisms underlying these correlative studies.

KIR-HLA AND AUTOLOGOUS HSCT FOR SOLID TUMORS

In settings of inflammation, such as infection or allogeneic HSCT, normally hyporesponsive NK cells can circumvent the effects of licensing and become cytotoxic to hematologic targets lacking the HLA class I ligand. Comparable cytokine conditions may exist after high dose chemotherapy with autologous HSCT, leading investigators to reason that NK cells stimulated to behave according to “missing ligand” may also display anti-tumor effects against solid tumors (84, 85). A recent retrospective analysis of KIR and HLA genotypes in high-risk neuroblastoma patients undergoing autologous HSCT showed that patients lacking HLA class I ligand(s) for autologous inhibitory KIR have improved outcomes (85). Highlighting the importance of innate immunity in solid tumor control, these data not that KIR-HLA immunogenetics may provide a novel prognostic marker for high-risk NB and invite similar studies for other malignancies.

CONCLUSIONS

NK cell receptors and ligands are vital to the mechanisms underlying NK cell tolerance, which can be altered in the setting of viral infection and hematopoietic cell transplantation. Receptor-ligand immunogenetics therefore represents a powerful tool in predicting NK alloreactivity both for hematopoietic and solid tumor treatment outcomes. An understanding of NK immunogenetics and tolerance is essential in designing future therapies involving adoptive NK cell therapy.

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