Commentary

NK1.1$^+$ T Cell Receptor-α/β^+ Cells: New Clues to Their Origin, Specificity, and Function

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Although NK cells and T cells share a number of biological functions including cytotoxicity and lymphokine secretion (1), they appear to differ fundamentally in the structure of their antigen receptors. Whereas T cells are known to recognize foreign antigens via their CD3-associated heterodimeric α/β or γ/δ TCR, the antigen recognition structures used by NK cells are still in the process of being elucidated. In this respect, current evidence (2–5) suggests that NK cells express polymorphic (but nonrearranging) receptors that belong to the NKR-P1 and Ly-49 families in mouse and the recently cloned p58 family in humans. The nature of the ligands recognized by NK cells is controversial, although there is functional evidence that both Ly-49 and p58 receptor families may mediate negative signals upon engagement of particular MHC class I alleles (2–5). In contrast, NKR-P1 receptors appear to bind to a heterogeneous group of carbohydrate ligands (6), thereby triggering effector functions such as cytolysis (6, 7).

NK and T cells probably arise from related but distinct developmental lineages (8). Whereas the majority of α/β or γ/δ T cells depend upon the thymus for their development, NK cells develop normally in athymic (nude) mice. Furthermore, recombination-deficient mutant (RAG$^{-/-}$ or SCID) mice have normal levels of NK cells despite the complete absence of α/β or γ/δ T cells. Nevertheless, it is likely that both NK and T cells arise from a common "lymphoid committed" precursor cell, since both lineages (as well as B cells) are absent in mice lacking the ikaros transcription factor, whereas other hematopoietic cells develop normally in these mutant mice (9).

In view of the apparently distinct ligands recognized by NK cells and T cells, it is of considerable interest that a subset of lymphocytes sharing receptor structures common to both lineages has been identified in mice (10–12), rats (13), and humans (14, 15). In the mouse, the best-defined such population coexpresses the α/β TCR and NK1.1 (16) (a member of the NKR-P1 family), although other subsets of mouse TCR-α/β cells expressing members of the Ly-49 family have been described (17, 18). This commentary will focus on NK1.1$^+$ TCR-α/β^+ (hereafter referred to as NK1$^+$ T) cells and, in particular, will summarize recent insights into their origin, specificity, and function.

Phenotype and TCR Expression of NK1$^+$ T Cells

The surface phenotype of NK1$^+$ T cells is dramatically different from that of normal α/β T cells. As summarized in Table 1, NK1$^+$ T cells express a number of markers usually associated with an activation/memory phenotype (but absent from naive T cells) such as CD44^{high}, CD45RB^{low}, and CD62L^{low}. In addition, NK1$^+$ T cells express the IL-2R β chain (CD122), which is normally present on conventional NK cells but not T cells. Whereas most mature α/β T cells express either CD4 or CD8 coreceptors, NK1$^+$ T cells are almost exclusively either CD4$^+$ or CD4$^-$8$^-$.

Another unique feature of NK1$^+$ T cells relates to their TCR expression. In comparison with normal α/β T cells, NK1$^+$ T cells express approximately threefold lower surface density of TCR-α/β and have often been referred to in the literature as TCR "intermediate" (TCR^{int}) cells. Furthermore, the TCR usage of NK1$^+$ T cells is heavily biased for both α and β chains. Thus, most NK1$^+$ T cells use one of only three Vβ domains (Vβ8.2, Vβ7, or Vβ2) (19), and a single Vα domain (Vα14) is highly overrepresented among NK1$^+$ T cells but rarely used by conventional α/β T cells (20). The latter finding explains the earlier puzzling observations of Taniguchi and co-workers (21), who identified a subset of peripheral T cells expressing an invariant Vα14-Jα281 rearrangement with no junctional diversity.

Relationship of NK1$^+$ T Cells to TCR^{int}, Invariant Vα14, and lpr/gld T Cells

An important (but not yet totally resolved) issue is the relationship of NK1$^+$ T cells to other unusual TCR-α/β subsets. As noted above, both TCR^{int} and invariant Vα14 T cell subsets have been described. Although all NK1$^+$ T cells are clearly of a TCR^{int} phenotype, it has not been possible technically to address whether other (NK1.1$^-$) subsets of TCR^{int} cells exist. In this issue of the journal, Sato et al. (22) provide a partial solution to this problem. By taking advantage of the expression of IL-2Rβ on all TCR^{int} (but not conventional TCR^{high}) cells, these authors show that NK1.1 defines only a subset of the TCR^{int} population. Further analysis of CD4/8 phenotype indicates that most NK1.1$^+$ TCR^{int} cells are CD4$^+$ or CD4$^-$8$^-$, whereas NK1.1$^-$ TCR^{int} cells are mainly CD8$^+$. It should be noted that the CD8$^+$ TCR^{int} subset, while resembling NK1$^+$ T cells in terms of surface phenotype, has not yet been defined in terms of TCR usage or function.

The precise relationship between NK1$^+$ T cells and invariant Vα14 cells has been more difficult to ascertain because of the absence of reliable antibodies against Vα14. Using semiquantitative PCR, Lantz and Bendelac (20) recently demonstrated a marked enrichment of Vα14$^+$ cells in CD44^{high} CD4$^+$ thymocytes (corresponding to the CD4$^+$

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Table 1. *Phenotype and TCR Repertoire of NK1⁺ T Cells, Conventional T Cells, and lpr/gld T Cells*

Marker	NK1⁺ T	Naive T	Memory T	*lpr/gld* T
NK1.1	+	–	–	–
CD4/8	CD4⁺ or CD4⁻8⁻	CD4⁺ or CD8⁺	CD4⁺ or CD8⁺	CD4⁻8⁻
B220	–	–	–	+
CD44 (Pgp-1)	High	Low	High	High
CD45RB	Low	High	Low	High
CD62L (Mel 14)	Low	High	Low	+/–
CD24 (HSA)	Low	Low	Low	+/–
CD122 (IL2-Rβ)	High	Low	Low	Low
TCR–CD3	Intermediate	High	High	Intermediate
TCR Vβ	2, 7, 8.2	All	All	All
TCR Vα	14	All	All	All
Clonal deletion (endogenous superantigen)	No	Yes	Yes	Yes

subset of thymic NK1⁺ T cells), and most T cell hybrids derived by fusion of this population to the BW5147 thymoma used the invariant Vα14-Jα281 rearrangement. Thus, it appears likely that most NK1⁺ T cells (but few conventional T cells) use Vα14; however, it is not known whether other TCR^{int} subsets (such as the CD8⁺ TCR^{int} population described above) are likewise enriched in Vα14⁺ cells.

Mice homozygous for the *lpr* or *gld* mutations (which are defective in functional Fas or Fas ligand, respectively) develop autoimmune syndromes accompanied by peripheral expansion of a unique subset of CD4⁻8⁻ TCR-α/β cells (23). These *lpr/gld* T cells resemble NK1⁺ T cells in that they are TCR^{int} and express certain activation markers such as CD44^{high}; however, they differ phenotypically from NK1⁺ T cells in that they express the B cell marker B220 and fail to express NK1.1 (Table 1). Furthermore, *lpr/gld* T cells and NK1⁺ T cells differ fundamentally in their TCR repertoires (Table 1). Whereas NK1⁺ T cells use a highly restricted subset of Vβ and Vα chains, *lpr/gld* T cells have a much more diverse repertoire (although Vβ8 is somewhat overrepresented). Moreover, *lpr* and *gld* T cells are subjected to deletional tolerance in the presence of endogenous mouse mammary tumor virus superantigens, whereas NK1⁺ T cells in general are not. Hence, it appears that *lpr/gld* T cells are not related to the NK1⁺ T lineage.

Developmental Origin of NK1⁺ T Cells

The developmental origin of NK1⁺ T cells is controversial. Historically, a T cell subset corresponding phenotypically to NK1⁺ T cells was first identified among CD4⁻8⁻ thymocytes (24, 25), although it was not appreciated for some time that these cells actually express NK1.1 (11). Subsequently, NK1⁺ T cells were observed in other lymphoid organs such as spleen (10) and bone marrow (12) as well as in liver (26). NK1⁺ T cells resident in the thymus appear to develop in situ, since cells of the NK1.1⁺ TCR-α/β⁺ phenotype can be generated in fetal thymus organ cultures established from 14-d mouse embryos (27). Furthermore, neonatal thymus grafts implanted in congenitally athymic (nude) mice can give rise to small numbers of NK1⁺ T cells in recipient bone marrow and spleen (28), suggesting a thymus-dependent origin for at least some peripheral NK1⁺ T cells. Nevertheless, NK1.1⁺ TCR-α/β⁺ cells are readily detectable in extrathymic tissues such as bone marrow (12), spleen (29), and liver (30) of nude mice. Thus, it appears that NK1⁺ T cells can be of either thymus-dependent or -independent origin. This conclusion is compatible with the finding that NK1⁺ T cells require expression of an endogenous ligand on hematopoietic cells (but not specialized epithelial cells) for their development (26, 27, 31).

New evidence for the extrathymic origin of at least some NK1⁺ T cells is presented by Sato et al. in this issue (22). In particular, these authors show that NK1⁺ T cells (and other TCR^{int} cells) develop selectively in the liver and spleen of thymectomized irradiated F_1 mice reconstituted with parental or syngeneic bone marrow cells. In control (euthymic) irradiated mice, both NK1⁺ T and conventional (TCR^{high}) T cells develop in these organs; however, the development of normal T cells is delayed relative to NK1⁺ T cells. The bone marrow precursors of NK1⁺ T cells in this adoptive transfer system express Thy-1 but not the TCR–CD3 complex. The findings of Sato et al. (22) are reminiscent of earlier reports in which TCR-α/β⁺ cells were shown to be generated in vitro from bone marrow precursors (32–34); however, a very recent study indicates that most TCR-α/β⁺

cells produced in short-term bone marrow cultures from Thy-1$^+$ TCR-α/β^- precursors are in fact NK1.1$^-$ (35).

If NK1$^+$ T cells do indeed arise extrathymically, it should in theory be possible to demonstrate activity of the recombinase-activating genes (RAGs) (which are essential for TCR rearrangement) in NK1$^+$ T precursor cells in the corresponding tissues. In this regard, RAG-1 and RAG-2 messenger RNA have been detected previously by PCR in liver mononuclear cells (36); however, no RAG-1 transcripts could be detected in liver by Northern blot in a subsequent study (37). Interestingly, Sato et al. (22) find that RAG-1 transcripts are expressed at much higher levels among liver mononuclear cells of reconstituted radiation bone marrow chimeras than in normal mouse liver. These data are consistent with an increased rate of extrathymic TCR-α/β rearrangement in NK1$^+$ T cell precursors in the chimeric animals.

Ligand Specificity of NK1$^+$ T Cells

As mentioned above, most NK1$^+$ T cells express a TCR with a limited β chain (Vβ8.2, Vβ7, or Vβ2) and virtually invariant α chain (Vα14-Jα281) usage. It therefore seems likely that the ligand(s) for this TCR will be highly restricted (or even monomorphic). Several experiments in vivo using gene-targeted "knockout" mice have provided clues as to the potential ligands that control NK1$^+$ T cell development. Thus, it is clear that NK1$^+$ T cells in thymus (27, 31, 38) or liver (26), as well as the related Vα14$^+$ peripheral T cell subset (39), fail to develop in β_2-microglobulin (β_2-m)–deficient mice, suggesting that a β_2m-associated (i.e., MHC class I or related) molecule is a component of the ligand of the NK1$^+$ T cell receptor. Further insight into the nature of this putative ligand comes from recent analysis of mice deficient for the peptide transporter-associated protein 1 (TAP-1). In these mice, which lack conventional MHC class I molecules (H-2K, D and L), Vα14$^+$ T cells (and hence, presumably, most NK1$^+$ T cells) develop normally (39). Taken together, the data with mutant mice suggest that NK1$^+$ T cells recognize a β_2m-associated molecule that does not require peptides transported by TAP-1 for its assembly and surface expression. Interestingly, there are at least two well-characterized molecules that fulfill these criteria: the thymus leukemia antigen (40) and CD1 (41). In this context, Taniguchi and coworkers have identified a wild mouse strain in which Vα14$^+$ T cells do not develop. In F_2 backcross analysis, this defect segregates as a single recessive gene that is unlinked to the MHC (39). If, indeed, the absence of Vα14$^+$ cells in these wild mice reflects a defect in the ligand responsible for NK1$^+$ T cell development, then it follows that the thymus leukemia antigen (which is closely linked to the MHC) cannot be the relevant ligand.

The case favoring CD1 as a potential ligand for NK1$^+$ T cells has been greatly strengthened by very recent analysis of the specificity of NK1$^+$ T hybridomas. Thus, Bendelac et al. (42) demonstrated that several hybridomas bearing the invariant Vα14-Jα281 rearrangement could be stimulated to produce lymphokines in vitro after exposure to CD1-expressing thymocytes or fibroblasts infected with a vaccinia construct containing the mouse CD1 gene. Furthermore, this stimulation could be inhibited by mAbs to either the TCR or to CD1. Weaker reactivity of freshly isolated normal thymic NK1$^+$ T cells to CD1-expressing fibroblasts could also be demonstrated. These data provide the first convincing evidence that CD1 is a component of the physiological ligand recognized by the TCR on NK1$^+$ T cells. It will be of great interest to determine whether CD1 can associate with peptides or other nonpeptidic moieties to stimulate NK1$^+$ T cells. In this respect, it is known that some human TCR-α/β^+ clones can recognize mycolic acid (a mycobacterial cell wall product) in association with CD1b (43).

In considering the possible ligands recognized by NK1$^+$ T cells, it should be emphasized that the potential contribution of the NK1.1 molecule itself to ligand specificity cannot be discounted. Indeed, NK1.1 is a member of the NKR-P1 family of C-type (calcium-dependent) lectins, and there is evidence that at least some members of this family may interact with complex oligosaccharides present on the surface of certain "NK-susceptible" target cells (6). In conventional NK cells, this interaction apparently leads to activation of the cytolytic program (6, 7). Furthermore, both NK cells (2) and activated NK1$^+$ T cells (44) are capable of redirected lysis of Fc receptor–bearing target cells in the presence of anti-NK1.1 antibodies. Collectively, these data raise the possibility that the NK1.1 molecule on NK1$^+$ T cells (or another NKR-P1 family member) may confer specificity for an oligosaccharide ligand and/or a carbohydrate component of a complex ligand (perhaps involving CD1) that is corecognized by the α/β TCR. In the latter case, NK1.1 could be considered as a type of coreceptor molecule that modifies the avidity of TCR–ligand interactions on NK1$^+$ T cells. This speculative model (Fig. 1) is further supported by the fact that the cytoplasmic domains of NKR-P1 family members share a motif (Cys-X-Cys-Pro) that has been shown to be involved in the association of CD4 and CD8 coreceptors with the tyrosine kinase p56lck (2). However, no direct evidence for a molecular association between p56lck and NK1.1 has been reported.

Function of NK1$^+$ T Cells

Because of the lack of precise knowledge of their ligand specificity, the function of NK1$^+$ T cells under physiological conditions has been difficult to address. However, the functional potential of NK1$^+$ T cells has been investigated in vivo and in vitro by triggering their TCR–CD3 complex with mAbs. Such studies have shown a remarkable ability of NK1$^+$ T cells to produce large amounts of cytokines and, in particular, IL-4. Indeed, it is likely that virtually all the IL-4 produced in vitro by anti-CD3–stimulated thymocytes can be accounted for by the NK1.1$^+$ T subset (45, 46), and similar conclusions have been reached for splenic T cells from normal mice injected with anti-CD3 mAbs (47). As pointed out by others (47), this property of NK1$^+$ T cells may have important implications for the development of conventional CD4$^+$ TCR-α/β^+ cells of the Th2 cytokine profile, since, in many systems, Th2 cells arise preferentially (or exclusively)

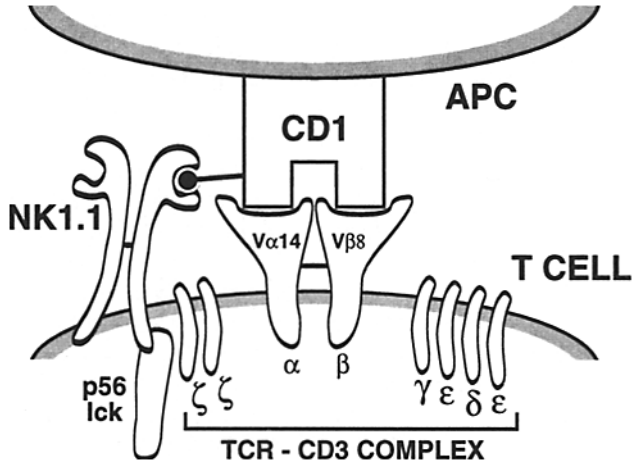

Figure 1. Speculative coreceptor model for CD1 ligand recognition by NK1$^+$ T cells. In this model, the highly conserved TCR-α/β on NK1$^+$ T cells reacts with the nonpolymorphic CD1 molecule. At the same time, the extracellular C-type lectin domain of NK1.1 interacts with a carbohydrate moiety on CD1 to stabilize TCR binding. By analogy with CD4 and CD8 on conventional T cells, NK1.1 could thus signal as a coreceptor via its putative cytoplasmic association with the tyrosine kinase p56lck. It is not known whether the peptide-binding groove of CD1 is occupied, and the putative carbohydrate moiety could be of either endogenous or exogenous origin. Note that NK1.1 is a homodimer and hence could theoretically cross-link TCR–CD1 complexes.

under conditions of stimulation where IL-4 is already present. Indeed, it has been shown recently that CD4$^+$ cells with a CD62L^{low} phenotype (a population that includes NK1$^+$ T cells) are required to direct the in vitro differentiation of Th2 cells from naive CD4$^+$ precursors (48).

Unlike conventional NK cells, NK1$^+$ T cells appear not to lyse NK-sensitive target cells such as YAC-1 when freshly isolated ex vivo; however, they are capable of redirected lysis of Fc receptor–bearing target cells in the presence of antibodies to the TCR–CD3 complex (11). Furthermore, NK1$^+$ T cells (like NK cells) can be induced to kill tumor cells after exposure to cytokines such as IL-2 and IL-4 in vitro (44, 49, 50) or IL-12 in vivo (30). Interestingly, even freshly isolated thymic NK1$^+$ T cells are able to lyse normal cortical (CD4$^+$8$^+$) thymocytes, apparently via the Fas-mediated pathway (51). Since cortical thymocytes express the putative NK1$^+$ T cell ligand CD1 (52), it is possible that NK1$^+$ T cells may play a role in thymic selection or homeostasis.

Are NK1$^+$ T Cells "Suppressor" Cells?

No discussion of NK1$^+$ T cells would be complete without reference to their possible relationship to suppressor cells. Indeed, the invariant Vα14-Jα281 rearrangement that is now known to be characteristic of most NK1$^+$ T cells was first identified as a conserved rearrangement in a panel of KLH-specific suppressor cell hybridomas (53), and other suppressor T cell hybridomas specific for antigens such as DNP use the identical α chain (54). Although the actual mechanism of suppression mediated by such hybridomas is still not well defined, it is tempting to speculate that NK1$^+$ T cells may in general be involved in negative regulatory functions, perhaps facilitated by their cytolytic activity and potential to secrete large amounts of inhibitory cytokines.

A more physiological regulatory function of NK1$^+$ T cells relates to the supression of hemopoiesis. It has been known for many years that irradiated F_1 mice frequently reject parental bone marrow grafts. The phenomenon, known as hybrid resistance, is genetically complex and involves the participation of NK1.1$^+$ effector cells (55). Whereas it was initially assumed that these effector cells are conventional NK cells, several studies clearly implicate NK1$^+$ T cells in hybrid resistance (10, 56). This issue is currently controversial, and it is possible that both NK and NK1$^+$ T cells may be involved in the suppression of hemopoiesis (55).

NK1$^+$ T Cells Expressing TCR-γ/δ

This commentary has focused on the well-established subset of NK1$^+$ T cells that expresses TCR-α/β. However, in this issue of the journal, Arase et al. (57) describe a novel population of TCR-γ/δ^+ NK1$^+$ T cells that is present in very low frequency in normal thymus. Interestingly, TCR-γ/δ^+ NK1$^+$ T cells are increased dramatically in the thymus of CD3-ζ–deficient mice, where, in contrast, mature TCR-α/β^+ cells (including the NK1$^+$ T subset) fail to develop. By analogy to conventional TCR-γ/δ^+ cells present in the gut epithelium (58), it is likely that thymic TCR-γ/δ^+ NK1$^+$ T cells are able to develop in CD3-ζ–deficient mice because the CD3ζ-chain in their TCR complex can be substituted by the homologous FcϵRI γ chain, which normally associates with CD16 to form the high affinity Fc receptor (59, 60). In any event, it will be of considerable interest to further characterize this novel NK1.1$^+$ TCR-γ/δ^+ subset in terms of its origin and function. Given the paucity of information concerning the physiological ligand recognized by conventional TCR-γ/δ cells, it will be particularly challenging to address the specificity of the NK1$^+$ TCR-γ/δ receptor. As a first approach, it will obviously be informative to investigate whether V gene usage is biased in these cells, as is the case for the TCR-α/β^+ NK1$^+$ T subset.

Concluding Remarks

NK1$^+$ T cells represent a fascinating subset of TCR-α/β (and apparently TCR-γ/δ) cells with poorly defined ligand specificity. As outlined here, it appears likely that NK1$^+$ T cells can develop independently in several extrathymic tissues (notably bone marrow and liver) as well as in the thymus. Because of their ability to secrete large amounts of IL-4 upon TCR engagement, NK1$^+$ T cells may play a pivotal role in Th1/Th2 lineage commitment of conventional CD4$^+$ TCR-α/β^+ cells. Furthermore, the potential of NK1$^+$ T cells to mediate target cell lysis under certain conditions may allow them to carry out other regulatory functions, such as the suppression of immune responses or of hemopoiesis. Ultimately, NK1$^+$ T cells may turn out to be bona fide regulatory T cells that can even be discussed openly in public (61).

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