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MOUSE CD1-SPECIFIC NK1 T CELLS: Development, Specificity, and Function

Albert Bendelac, Miguel N. Rivera, Se-Ho Park, and Jessica H. Roark

Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544; e-mail: abendelac@molbiol.princeton.edu

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ABSTRACT

NK1 T cells are a specialized population of α/β T cells that coexpress receptors of the NK lineage and have the unique potential to very rapidly secrete large amounts of cytokines, providing early help for effector cells and regulating the Th1 or Th2 differentiation of some immune responses. NK1 T cells express a restricted TCR repertoire made of an invariant TCR α chain, Vα14-Jα281, associated with polyclonal Vβ8, Vβ7, and Vβ2 TCR β chains. NK1 T cells recognize the products of the conserved family of MHC class I–like CD1 genes, apparently in the absence of foreign antigens. Thus, this novel regulatory pathway, which straddles the innate and the adaptive immune systems, is unique in that its activation may not require associative recognition of antigen. Here, we review the specificity and function of mouse NK1 T cells, and we discuss the relationship of this lineage to mainstream T cells and NK cells.

INTRODUCTION

Over the past 10 years, three independent sets of studies contributed to the identification of the NK1 T cells, an unusual set of T cells with many unique features (1–3). Taniguchi and colleagues, focusing on KLH-specific suppressor cells (4, 5), cloned and sequenced an invariant TCR α chain, Vα14-Jα281, expressed by suppressor hybridomas (6), which was subsequently detected at a high frequency (0.5% to 1%) in thymus and spleen extracts (7, 8). Another line of studies was sparked by the observation that a small set of mature thymocytes did not express the CD4 or CD8 coreceptors (double negative, DN), preferentially utilized TCR Vβ8 gene products (9, 10), and expressed high levels of

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CD44, of the NK1.1 molecule usually associated with the NK lineage (11–13), and of Ly6C (14). A similar set of cells was subsequently recognized among mature single positive T cells (15–18).

The link between these two sets of observations came from a third line of study, aimed at defining developmental steps in the functional differentiation of thymocytes (19–21), in which we found a set of thymic CD4 cells that, unlike the bulk of naive peripheral T cells, were able to secrete Th1 and Th2 cytokines upon primary stimulation. Further studies characterized a small population of IL-4 and IFN-γ producing CD4 and DN thymocytes that combined all the features separately reported in the above investigations, i.e. they expressed NK1.1, Ly6C, and high levels of CD44 (16, 18, 21, 22), as well as a TCR repertoire consisting of an invariant Vα14-Jα281 TCR α chain and a restricted but polyclonal set of Vβ8, Vβ7, and Vβ2 TCR β chains (23). In addition, we showed that the NK1 T cells were specific for CD1 (24–26), a family of non–MHC encoded, MHC class I–like proteins that are conserved in mammals (27), and humans were found to have a similar T cell subset, with a homologous TCR α chain, Vα24-JαQ (23, 28–30).

In this review, we discuss the development, specificity, and function of mouse NK1 T cells.

PHENOTYPE AND TISSUE DISTRIBUTION OF NK1 T CELLS

Surface Phenotype of NK1 T Cells

NK1 T cells consist of CD4 and DN cells (60%/40%), both of which stand out by their activated T cell surface phenotype Thy1^{hi}CD5^{hi}B220^{neg}HSA^{lo}CD44^{hi} CD45RB^{hi} LECAM-1^{lo}Ly6C^{hi}3G11^{lo}6C10^{lo}, and their expression of NK surface markers, NK1.1^{int}3A4^{int}IL2Rβ^{int}CD69^{int} CD16^{lo}Ly49A (15%) Ly49C (60%) (defined in the C57BL/6 mouse). Upon culture with high doses of IL-2, they upregulate NK receptors, particularly CD16, and express B220 (31). Eighty-five percent of NK1 T cells express an invariant TCR α chain, Vα14-Jα281 and either Vβ8 (55%, mainly Vβ8.2), Vβ7 (14%), or Vβ2 (7%). There are very few CD8 NK1 cells, particularly with Vα14-Jα281$^+$ TCRs (18, 23). A smaller population of related cells exists that may not express all the surface markers or use the invariant Vα14-Jα281 α chain, while still being NK1.1$^+$ and CD1-specific (24), or that may use the invariant TCR α chain, while not expressing NK1.1.

Other cell types that include DN $\alpha\beta$ T cells and that do not express NK1.1 or the NK1 TCR repertoire will not be covered here; their ligand and function are unknown at present. They include a minor population of DN $\alpha\beta$ T cells in

normal lymph nodes and liver (32, 33), a bone marrow "natural suppressor cell" that suppresses GvH disease (34, 35), and the DN $\alpha\beta$ T cells that accumulate in lpr$^{-/-}$ mice. Gut IEL contain a prominent population of NK1.1-negative $\alpha\beta$ T cells that do not use Vα14 but do express some NK receptors and functions (36). In humans, a majority of $\alpha\beta$ TCR$^+$ DN PBL (usually more than 90%) do not use Vα24-JαQ (28, 29).

Although NK1.1 is the surface marker most tightly associated with NK1 T cells, only three common mouse strains (B6, NZB, and SJL) express a form of NK1.1 seen by the antibody PK136 (it is not clear at the moment which of the other strains express an allelic form unrecognized by the antibody and which express NKR-P1 gene products at all). Another antibody, thought to recognize all Vα14$^+$ cells (37, 38), actually stains less than 20% of Vα14-Jα281$^+$ NK1 T cells in the thymus (39) and fails to stain Vα14-Jα281$^+$ T cell hybridomas or preparations of thymic, splenic, and liver cells enriched for Vα14-Jα281$^+$ cells (A Bendelac, R Fairchild, R Locksley, HR MacDonald, personal communications), suggesting that its recognition might be limited to a subset of Vα14-dependent idiotypes.

Other markers must therefore be used to identify NK1 T cells in NK1.1-negative strains. Although virtually all NK1 T cells are IL2Rβ^+ TCR^{int} (40, 41), this staining combination is not specific because 20% of peripheral CD8 cells (which also express lower levels of TCR than do CD4 cells) are IL2Rβ^+ (A Bendelac, unpublished observations). In the thymus of B6 mice, high levels of CD44 are tightly associated with Vα14 and NK1.1, but in peripheral lymph nodes and spleen they are found on mainstream activated/memory CD4 and CD8 cells, which are more frequent than NK1 T cells. For other mouse strains (such as BALB/c), CD44 is also expressed at high levels on most mainstream T cells. Ly6C is tightly associated with the NK1 T cell phenotype in the thymus but is also expressed in many other T cells in the periphery, and it is not expressed in strains such as NOD. Therefore, in peripheral tissues, NK1 T cells can be identified in the B6 background by the expression of NK1.1 and a bias in Vβ8 usage or, in other backgrounds, by IL2Rβ, a bias in Vβ8 and a CD8-negative phenotype.

Tissue Distribution

NK1 T cells seem to have strong preferences for particular tissues, accounting for 20–30% of liver and bone marrow T cells, 10–20% of mature (HSA^{low}) thymocytes, and 0.5%–1% of splenocytes (18, 42–44). Their absolute numbers however, are relatively similar, in the range of 1 million cells per organ. They are rarer (0.1–0.5%) in peripheral lymph nodes. NK1 T cells are virtually absent from gut IEL (42), but their frequency in the lamina propria and Peyer patches is unknown.

Inside tissues, little is known about the location of NK1 T cells, although some observations suggest that they occupy selected areas of functional interest. For example, in MHC II KO mice, the few residual CD4 cells, 30% of which are NK1 T cells, are clustered in lymph node B cell follicles, rather than dispersed in the T cell areas (45). In the irradiated thymus, which is enriched in NK1 T cells, $CD44^{hi}$ cells are observed in the medulla (46).

SPECIFICITY OF NK1 T CELLS

TCR Repertoire

Several findings indicate that the NK1 TCR repertoire is strongly selected at the amino acid level by its specificity for CD1. First, the invariant TCR α junctional regions, which can be made out of the genomic sequences by deletion of two or three nucleotides, display greater nucleotide trimming plus addition of N nucleotides in 40% of NK1 T cells, but they conserve the canonical amino acid length and sequence (23). Second, mice deficient in β2M, which express lower levels of CD1, have a very reduced population of T cells expressing NK1.1 in the thymus, in the spleen, and in the liver (18, 42, 44, 47, 48), and no detectable invariant Vα14-Jα281 rearrangement by PCR (18, 23).

The contribution of the TCR β chain is less evident. The most prominent β chain families associated with Vα14-Jα281 are, in order of frequency, Vβ8.2, Vβ7, Vβ8.3, Vβ2, and Vβ8.1. The CDR3 junctions are variable. We found that 9 out of 12 possible Jβ segments, with diverse length and amino acid sequences, were utilized by a panel of 23 $V\alpha14^+$ hybrids (23). Similar results have been found in humans (30). Nevertheless, NK1 T cells cannot indiscriminately use Vβ chains from these families because mice made transgenic for a particular Vβ8.2 (49) or a Vβ8.1 (50) TCR β chain had decreased numbers of NK1 T cells, which in fact coexpressed endogenous Vβ8.2 and Vβ7 chains. In humans, 99% of $V\alpha24^+$ DN PBL cells in four individuals and 50% in a fifth used Vβ11 (using FACS analysis with antibodies against Vα24 and Vβ11), a homologue to mouse Vβ8 (30). These results leave the question of whether the selection of TCR β chains is driven by their specificity or by pairing constraints with Vα14.

Recent analysis of transgenic mice expressing a Vα14-Jα281 transgene suggests that the driving force is specificity (51). First, transgenic mice bred to the TCR Cα KO background, to ensure exclusive Vα14-Jα281 expression, exhibited two distinct and prominent sets of DN T cells: the $NK1.1^+$ cells with a bias in TCR Vβ usage, and a second set expressing no NK receptor and no bias in Vβ usage. These latter DN T cells are commonly observed in TCR transgenics, irrespective of the specificity of the TCR or the MHC haplotype of the host; it is believed that they represent $\gamma\delta$ lineage cells artifactually

expressing an $\alpha\beta$ TCR (52, 53). Whatever the origin of these cells, their unbiased TCR β repertoire shows that Vα14 can pair with many Vβ families. In addition, unlike the NK1 T cells, these NK1-negative, Vβ-unbiased cells persisted in β2M-deficient Vα14-Jα281 transgenic mice, indicating that they were not selected for specificity to CD1. Second, we observed that the transgenics exhibited a selective 50–90% depletion of CD8 cells bearing Vβ8 or Vβ7, suggesting that most Vβ8 and Vβ7 chains can pair with Vα14 and confer CD1 specificity, which, in the case of CD8 cells, induces negative selection (18, 23). Altogether, these results imply that selection of Vβ families in NK1 T cells occurs on the basis of their contribution to recognition of CD1 and that most of the TCR β CDR3 junctions of Vβ8 or Vβ7 chains are compatible with CD1 recognition by the NK1 TCR.

CD1 Recognition

The restriction and conservation of the NK1 TCR repertoire suggested that NK1 T cells see a conserved ligand. Studies with β2M KO and MHC II KO mice indicated that the development of both CD4 and DN NK1 T cells required the expression of β2M but not MHC II, and that β2M expression was needed on bone marrow–derived but not on radioresistant T cells (18, 42, 47, 48). Normal numbers of NK1 T cells were generated in lethally irradiated double MHC KO mice reconstituted with MHC II–deficient fetal liver cells (25). Finally, TAP-deficient mice normally selected Vα14-Jα281$^+$ cells (54). This portrait of the NK1 T cell ligand fit that of CD1, a non-MHC encoded, MHC class I–like gene family that is conserved across species, is expressed by cortical thymocytes rather than by epithelial cells of the thymus, in a β2M-dependent but TAP-independent fashion (55–58).

The specificity of NK1 T cells for CD1 was firmly established by our finding that T cell hybrids, obtained by fusion of purified thymic NK1 T cells with TCR $\alpha\beta^-$ BW 5147, responded to normal thymocytes, which constitutively express CD1, and that an anti-CD1.1 antibody blocked the response. Furthermore, these hybridomas as well as fresh, purified NK1 T cells responded to an ICAM/B7.1 transfected fibroblast line infected with a CD1.1 recombinant vaccinia virus (24). A variety of CD1.1-transfected mouse, rat, hamster, or human lines can also stimulate NK1 T cell hybrids (S-H Park, JH Roark, A Bendelac, unpublished results).

CD1.1 and CD1.2

The mouse has two CD1 genes, CD1.1 and CD1.2, which span 18 Kb of chromosome 3, with an exon/intron structure typical of MHC I. They are 95% homologous at both the nucleotide and amino acid level, and they are related to the CD1d human isotype (55). Rat has only one CD1 gene, which is slightly

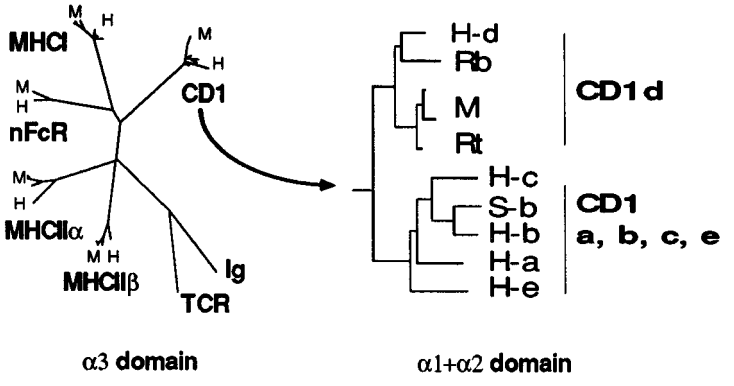

Figure 1 Phylogeny trees of the $\alpha 3$ or $\alpha 1 + \alpha 2$ domains of mouse (M), human (H), rat (Rt), and sheep (S) CD1 amino acid sequences.

more similar to CD1.1, particularly in the 3′ untranslated region (59). Thus, it appears that mouse and rat do not have the CD1a, b, c, and e isotypes found in humans, and that mouse has duplicated its CD1.1 gene. Evolutionary trees indicate that, like the MHC I–like newborn Fc receptor gene, CD1 is as far from MHC II as it is from MHC I, suggesting that the four families separated from a common ancestor before or during the mammalian radiation some 80–100 million years ago. It is also apparent that two ancient sub-families of CD1 genes exist, CD1a-b-c-e and CD1d (Figure 1).

CD1.1 and CD1.2 differ significantly in many respects. First B6 mice (but not BALB/c, AKR, NOD, or 129) carry a point mutation that induces a stop codon at the amino-terminus of the $\alpha 3$ domain of CD1.2 (S-H Park, A Bendelac, unpublished results), which is predicted to prevent its surface expression. Thus, the functional properties of the CD1/NK1 pathway, which have mainly been worked out in the B6 system, must be related to the CD1.1 gene. Another CD1.2 mutation, found in all strains studied so far, induces cysteine-to-tryptophan replacement in the $\alpha 2$ domain (60). This cysteine participates in an intradomain disulfide bond, and its replacement is likely to be disabling, because a similar mutation profoundly impairs surface expression of natural and mutagenized HLA-A molecules (61).

CD1 Expression

Reported sites of constitutive expression of mouse CD1 mRNA or protein include the thymus and liver and, to a lesser degree, the spleen and lung (55, 57, 62, 63). Surface expression of CD1 has been documented by FACS staining on cortical thymocytes (25), and splenic B cells (JH Roark, S-H Park,

A Bendelac, unpublished data). Although staining of gut epithelial cells by an anti-CD1 antibody has been reported (56), little mRNA was detected (57) (D Guy-Grand, P Vassali, personal communication). CD1 expression by such important cell-types as Langerhans cells, dendritic cells, macrophages, and B cell subsets in germinal centers, and the conditions or cytokines that regulate expression, have not been studied yet. It will also be important to clarify the function of CD1.2 because there are some indications that CD1.1 and CD1.2 mRNA expression are differently regulated (55).

In rat, CD1 mRNA has been found in Northern blot analyses of spleen, thymus, liver, heart, kidney, and lung (59); in humans, CD1d is expressed on human intestinal epithelial cells (64). Other CD1 isotypes in human, such as CD1a, b, and c, have restricted sites of constitutive expression on cortical thymocytes, Langerhans cells, subsets of dendritic cells and B cells, and GM-CSF plus IL-4 dramatically upregulate CD1b expression by monocytes (65).

Different CD1 Ligands Recognized by T Cells

There is some evidence in B6 mice, where CD1.1 is the only functional gene, that distinct CD1 species may nevertheless be recognized by T cells. For example, several of our NK1 T cell hybridomas recognize CD1-expressing thymocytes and a variety of CD1-expressing cell lines, but they do not respond significantly to spleen cells (24), even though B cells constitutively express CD1. Conversely, seven CD1-specific T cell hybridomas derived from MHC II KO CD4 splenocytes (none using the canonical Vα14-Jα281 TCR α chain) responded to spleen cells. They appeared to distinguish spleen cells of various mouse strains (66), and two of them failed to react to μMT KO spleen cells, suggesting that they recognized a CD1 ligand uniquely expressed by B cells. Given that CD1 exhibits little polymorphism, it is likely that the hybridomas discriminate between modified CD1 ligands, such as for example peptide-associated CD1.

A CD1.1 Peptide-Binding Motif

Peptides and peptide-binding motifs that bind CD1 have been identified using a random peptide phage display library (67). The general motif included aromatic residues at position 1 and 7, and an aliphatic residue at position 4. Synthetic peptides of optimal 15–20 amino acid lengths were found to bind to CD1.1 with dissociation constants of 10^{-6}–10^{-7} M, and importantly, CD8 cells primed against a peptide pulsed CD1-expressing target could kill in a CD1-, peptide-, and CD8-dependent fashion.

Binding to CD1.1 exhibited features similar to both MHC I and II such as fixed anchor residues and the variable length of overhangs, respectively. Preliminary observations on a crystal of soluble CD1.1 expressed in Drosophila

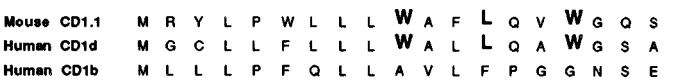

Figure 2 Mouse CD1 and human CD1d leader peptides include the WxxLxxW CD1 binding motif identified by Castano et al (67), suggesting that CD1 may bind its own leader peptide.

cells showed that CD1 had an MHC fold; however, they did not show evidence for bound peptides, suggesting that CD1 is stable in the absence of peptide or that experimental conditions led to a loss of CD1 coligands (I Wilson, personal communication). Indeed, it had previously been suggested that these molecules might be stable in the absence of peptide because CD1 and the neonatal Fc receptor in different species share a conserved proline in the $\alpha 2$ helix region, which is primarily responsible for closing the nFcR groove (68).

A Role for the CD1 Leader Peptide?

In analyzing the CD1 peptide-binding motifs, one of us (MN Rivera) made a surprising observation. A search of the database showed that approximately 0.5% of 50,000 proteins display the WxxLxxW-binding motif. Most importantly, the WxxLxxW motif is included in the leader peptides of mouse CD1.1 and CD1.2 and of human CD1d (Figure 2).

Because surface expression of mouse CD1 occurs in the absence of TAP (58, 66), in which case the ER contains mainly leader peptides (69), the above finding suggests that CD1 molecules may bind their own leader peptides, in a "tongue-biting" manner. This leader peptide may subsequently be removed in a peptide-loading compartment (like the invariant chain CLIP peptide on MHC II), or alternatively, it may stay in the groove to be one of the dominant peptides associated with CD1 and recognized by NK1 T cells. This hypothesis is currently being tested.

Biosynthesis and Intracellular Trafficking of CD1

As mentioned above, a remarkable feature of mouse CD1.1, and human CD1a and b, is their functional expression in cells deficient in proteins involved in peptide loading, such as TAP, DMA/DMB or the invariant chain (18, 58, 65, 66).

Studies of human CD1b have suggested that it can be loaded with exogenous lipid antigens in a chloroquine-sensitive manner, probably in an endosomal compartment (65, 70–72). Moreover, the intracytoplasmic tail of CD1b has a typical endosomal retrieval motif (which is conserved in mouse CD1), suggesting that CD1b may recycle from the cell membrane to the endosome. Indeed, wild-type human CD1b was found to be prominent in the MIIC compartment

and at the cell surface, whereas tail-truncated mutants were markedly decreased in the endosomes (72a).

Thus, in its long, divergent evolution from MHC molecules, CD1 seems to have acquired a unique intracellular biosynthetic pathway, whose elucidation is expected to yield fundamental clues to the biology of the NK1/CD1 system.

Other CD1 Isotypes and CD1-Specific Cells

In mouse, up to 7% of hybridomas derived from MHC II KO CD4 splenocytes were found to be CD1-specific (66), but these used variable, non-Vα14 TCRs. The frequency, phenotype, and function of their cell of origin remain to be determined.

In humans, some $CD8^+$ T cell clones from gut IEL recognize self-CD1d-expressing targets (73), and some PBL DN T cell lines recognize self-CD1a and -CD1b (65). PBL DN clones specific for mycobacterial lipids have also been obtained by repeated in vitro stimulation with mycobacterial extracts plus CD1-expressing cells. The TCR repertoire of these cells remains to be investigated. It is not yet clear whether bacterial lipid presentation has been a driving evolutionary pressure for CD1, or whether it is an incidental finding that reflects the hydrophobicity of a presently unidentified self-ligand.

DEVELOPMENT OF NK1 T CELLS

NK1 T cells are virtually absent at birth and regularly accumulate in the thymus and in the spleen and liver, reaching a plateau by 6–8 weeks of life (16, 18, 42). At birth, even putative Vα14 precursors (that might not yet express NK1.1) are very rare, because PCR-generated Vα14-Cα fragments overwhelmingly used non-Jα281 segments in the spleen and thymus, whereas adults mainly exhibit canonical Jα281 junctions (7, 8). In the thymus, continuous BUDR infusion studies suggested that the NK1 T cell compartment has a slow turnover of about 45 days, and that less than 1 to 2% of cells divide every day (A Bendelac, unpublished observations). This does not preclude the possibility that NK1 T cells may divide before they acquire the NK1 phenotype, because NK1 T cell precursors cannot yet be identified phenotypically. Thus, the increased relative frequency of NK1 T cells among mature T cells in the thymus seems to be the result, at least in part, of selective accumulation. A tentative estimate suggests that approximately 20,000 NK1 T cells arise every day to ensure adult steady-state frequencies, whether by self-renewal of mature NK1 T cells pool or by differentiation of new thymic precursors.

The rarity of NK1 T cells at birth does not seem to result from an inability of the newborn immune system to generate them. Indeed, the Vα14-Jα281 canonical sequence is encoded in the germline and does not require TdT-dependent N

additions; both fetal liver (18, 42, 47) and adult bone marrow cells (A Bendelac, unpublished observations) can generate NK1 thymocytes in irradiated recipients. Finally, fetal thymic organ cultures, which include a limited number of fetal thymic precursors, can generate canonical NK1 T cells, i.e. NK1.1$^+$ CD4 and DN, with a bias in Vβ8 and Vβ7 usage (18). However, they are only detected at a late phase in these cultures, in which a large number of fetal thymuses (10–50 in our experiments) are pooled. These observations are most compatible with a model whereby rare precursor cells that randomly rearrange Vα14 to Jα281 undergo strong positive selection, possibly some rounds of cell division, and accumulation in some tissues.

Several questions dominate the problem of NK1 T cell development. Do NK1 T cells develop from a precommitted precursor (for example, an NK precursor) and obey unique rules of development, or do they share early developmental steps with mainstream $\alpha\beta$ T cell precursors and branch off the mainstream differentiation pathway during or after the selection events? What are the CD1-expressing cells that select NK1 T cells in the thymus? How is the expression of the CD4 and CD8 coreceptors regulated; in particular, why do cells specific for CD1, an MHC I–like molecule, end up expressing CD4 or being double negative? Do NK1 T cells undergo negative selection, and why do they appear to be autoreactive?

Lineage Models: Committed vs Mainstream Precursor

COMMITTED PRECURSOR MODEL If there existed a precursor committed to become an NK1 T cell, and if rearrangement were left to chance, there would be thousands of NK1 T cell precursors doomed to die for every cell that achieved the correct Vα14-Jα281 rearrangement (there exist $\approx$100 Vα genes and 50 Jα genes). Although NK1 T cell precursors might implement a genetic program leading to a directed rearrangement, as do $\gamma\delta$ DEC cells (74), the data argue against this possibility. First, the unused α locus exhibits random rearrangements (23, 75). Second, about 40% of NK1 TCRs are trimmed and have N additions so as to reconstitute a canonical amino acid sequence at the V-J junction, suggesting that they have been selected rather than programmed (23). Third, human Vα24-JαQ DN clones exhibit rearrangement at the γ locus (29, 30).

A committed precursor model would predict the existence of a precursor cell that, upon transfer, would preferentially give rise to Vα14 NK1 T cells. Possible candidates would be immature cells that express some of the unusual NK1 T cell surface markers. These putative precursors are likely to be rare, however, since the high frequency of NK1 T cells among mature thymocytes reflects their rate of accumulation rather than production. The model could also be tested using the strategy devised by Raulet and collaborators for $\gamma\delta$

DEC cells (74), whereby the status of a transgenic, truncated Vα14 to Jα281 rearrangement substrate would be monitored in various cell subsets, including NK1 T cells. Indirect clues to the question of lineage may also come in the future from gene KO mice bearing mutations that affect, for example, nuclear factors involved in lineage commitment.

MAINSTREAM PRECURSOR MODEL This model contends that the few mainstream $\alpha\beta$ thymocytes that randomly express a canonical Vα14-Jα281 rearrangement and a Vβ8, Vβ7, or Vβ2 TCR β chain, will recognize their CD1 ligand expressed by cortical thymocytes, branch off the mainstream differentiation pathway, and acquire the NK1 phenotype. About 1 in 15,000 (see above) Vβ8, Vβ7, or Vβ2 expressing immature cells might randomly rearrange a canonical Vα14-Jα281, so that about 1 in 50,000 $\alpha\beta$ TCR-expressing thymocytes would harbor the canonical TCR (about 33% of thymocytes express Vβ8, Vβ7, Vβ2). Among the approximately 50 million $\alpha\beta$ TCR-expressing immature thymocytes produced every day, 1000 cells would have the canonical TCR, i.e. 20 times less than the above estimate of 20,000, a gap that could be filled by an expansion of NK1 T cells after (or during) their selection, for example, if NK1 T cells undergo 4 or 5 rounds of division during their lifetime, or by other mechanisms contributing to improve the frequency of random generation of Vα14 to Jα281 rearrangement.

The model explains why the other α locus and the γ locus of NK1 T cells seem undistinguishable from those of mainstream T cells. One of its predictions is that expression of a Vα14 -Jα281 TCR α chain by mainstream precursors should induce the NK1 phenotype provided that they express an appropriate Vβ. Indeed, mice transgenic for a Vα14-Jα281/Cα construct driven by the Vα11 promoter and the Ig enhancer (a vector that drives expression of TCR genes in mainstream T cells) (76) exhibited a major increase in NK1$^+$ CD4 and DN cells with the expected bias in Vβ usage and in their ability to produce IL-4 in vitro and in vivo (51).

CD1-Presenting Cells in the Thymus

A body of evidence suggests that cortical thymocytes are the source of CD1 presentation for positive selection. First, they are the major cell-type that expresses CD1 constitutively in the thymus, and they stimulate NK1 T cell–derived hybridomas. Second, epithelial cells do not seem to express CD1, and NK1 T cells exist in normal numbers in chimeric mice that do not express β2M on their epithelial compartment but express it on bone marrow–derived cells.

The role of thymic dendritic cells and macrophages is not yet elucidated because it is not known whether they express CD1. SCID mice reconstituted with β2M-deficient fetal liver cells failed to generate NK1 T cells even though

their dendritic cells were predominantly β2M positive (25). This observation suggests that dendritic cell presentation does not select NK1 T cells, and that presentation by cortical thymocytes is necessary. It is not yet known however if dendritic cell presentation of CD1 is required to generate or expand NK1 T cells. B cells do not appear to be required because μMT KO mice do generate NK1 T cells (25).

Coreceptor Expression by NK1 T Cells

NK1 T cells are found as $CD4^+$ or DN cells, whereas CD8 cells are virtually absent, a remarkable feature for cells that recognize an MHC class I–like molecule.

In humans, NK1 T cells can be identified among PBLs by their coexpression of Vα24/Vβ11 TCRs and CD56, an NK-associated receptor (C Prussin, personal communication). They are mainly DN or $CD8^{low}$ cells, and in some cases also include CD4 cells. Interestingly, the $CD8^{low}$ cells express CD8α/α rather than CD8α/β, and therefore do not have a true CD8 phenotype. Thus, NK1 T cells appear to follow relatively similar rules of coreceptor expression in the different species.

A key to understanding coreceptor expression by NK1 T cells is whether CD1 binds CD8 (as does MHC I), or CD4 (like MHC II), or neither. Although CD1 does not display the canonical α3 domain residues that mediate CD8 binding in other MHC class I molecules, there is strong indirect evidence that CD1 binds CD8 and not CD4. CD8 lines exist that recognize peptide-loaded mouse CD1.1 (67) or human CD1b (65), and both are blocked by anti-CD8 antibody. In vivo anti-CD8 treatment from birth increased the relative frequency of $V\beta7^+$ CD4 and DN NK1 T cells (although it did not impair the selection of the bulk of NK1 T cells), whereas anti-CD4 treatment did not alter NK1 CD4 cell development. Similar results were obtained with CD4 and CD8 KO mice, and CD4 KO mice expressing a human CD2 transgene under the control of the mouse CD4 promoter generated normal amounts of $CD2^+$ NK1 T cells, confirming that CD4 is not necessary to generate $CD4^+$ NK1 T cells. CD8 binding was also suggested by the fact that CD8 transgenic mice, where CD8 was constitutively expressed on all mature T cells, lost all Vα14-Jα281 NK1 T cells (18, 23).

In terms of thymic selection pathways, these results also indicate that CD8 is not absolutely required for the selection of most NK1 T cells and that its persistence at a late stage of thymocyte development, when TCR is upregulated, induces negative selection of CD1-specific Vα14-Jα281 cells. As discussed earlier, this view was also confirmed in a different system, using Vα14-Jα281 TCR α chain transgenic mice. In these mice, $NK1^+$, Vβ-biased CD4 and DN T cells became a prominent subset, whereas the CD8 compartment was depleted of Vβ8 and Vβ7.

Do NK 1 T Cells Undergo Negative Selection?

It may seem surprising that NK1 T cell–derived hybridomas respond strongly to the very CD1-expressing thymocytes that drove their positive selection. Did they escape negative selection?

It has been reported that hybridomas derived from mainstream T cells also responded with high frequency to nurse cells expressing their positively selecting ligands (77), so this response may be related to a lower activation threshold in hybridomas rather than to a higher threshold for negative selection of NK1 T cells. Similarly, the fact that fresh NK1 T cells responded to CD1.1 recombinant vaccinia infected cells may also be related to the very high levels of CD.1.1 expression achieved in this system.

The deletion of Vα14-Jα281 CD8 cells suggests that NK1 T cells can undergo negative selection in the thymus. In addition, mice injected with the Vβ8-specific staphylococcal superantigen SEB from birth exhibited deletion of V$\beta 8^+$ NK1 thymocytes (49). In contrast, a proportion of CD4 and DN V$\beta 8.1^+$ NK1 T cells persisted in an Mls^a positive background, whereas mainstream thymocytes included 0% V$\beta 8.1^+$ cells (49). As it is known that the α chain influences reactivity to Mls (78), it could be argued that pairing with Vα14-Jα281 may confer a lower reactivity to Mls^a. In addition, the fact that NK1 T cells express lower levels of CD4 and TCRs than do mainstream cells may also explain why the Mls reactive subset is not completely deleted.

Altogether, these results indicate that NK1 T cells are not impervious to negative selection. Whether they have a higher threshold for tolerance induction remains an open question. One may envision, as discussed below, that the NK1 phenotype, which includes the expression of NK receptors that can dampen the effects of TCR signaling (79–83), is precisely induced on cells that reach borderline (close to deletion) affinity for their thymic ligand.

Signaling Pathways Mediating the Thymic Selection of NK1 T Cells

H-2^b TCR transgenic mice expressing a mainstream TCR with high affinity (CD8 independent) for K^b generated NK1.1^+ DN T cells, whereas mice expressing another anti-K^b TCR, with lower affinity (CD8 dependent), did not (84). Thus, a TCR originating in a mainstream T cell was able to induce a differentiation similar to that imposed by the Vα14-Jα281 TCR, if it encountered its high-affinity ligand in the thymus. A related scenario may be operative in the subset of DN $\alpha\beta$ T cells bearing an anti-HY transgenic TCR that develop in males (85). In that case, the ‘DN’ cells actually expressed low levels of CD8, as well as activation markers such as CD44.

One common feature of all these cells may be that they express TCRs that see their nominal ligand (the ligand that activates them as mature cells) during thymic development. It has indeed been argued that mainstream positive selection is achieved through recognition of ligands (antagonists, partial agonists) that engage the TCR in a way that is qualitatively different from the nominal ligands (agonists) of mature cells. Positive selection by agonist ligands has been obtained in a few cases, but it induced a distinct phenotype, which included lower levels of CD8 and the loss of killer function (86–88). This concept implies that distinct signaling pathways may be elicited by the different types of ligands. It is remarkable therefore that selection of NK1 T cells appeared to proceed normally in the face of the complete disruption of the Ras/Raf/Mek/MAP kinase cascade that is involved in mainstream positive selection, but which may be dispensable for negative selection (89).

Another common feature in these examples may be that selection is mediated by nontypical APCs, which could conceivably elicit distinct signaling cascades. Indeed, bone marrow–derived rather than epithelial cells drive the selection in at least two cases, NK1 T cells and anti-K^b TCR transgenic cells.

In summary, the NK1 phenotype may be induced in mainstream precursors through unusual signaling pathways recruited by either the type of TCR/ligand interaction (high affinity, or agonist) or the type of ligand-presenting APC involved.

Other mutant mice have provided information on the NK1 T cell developmental pathway. For example, NK1 T cells, which coexpress both CD3 ζ (of T cell lineage) and Fcγ (of NK lineage) (31, 90, 91), behaved like mainstream T cells in that they persisted in Fcγ KO mice and disappeared in CD3 ζ KO mice (91).

Models of Thymic Development of NK1 T Cells

Models of thymic NK1 T cell development are depicted in Figure 3. Vα14-Jα281 DP bind to CD1 on their fellow cortical thymocytes and are signaled through a Ras/Raf/Mek/MAP independent pathway. They modulate CD4, CD8, or both coreceptors. CD8 cells are subsequently deleted, and CD4 and DN cells survive because most of them can see CD1 without CD8. DN cells may also derive from CD8-expressing cells that downmodulate CD8 and therefore escape negative selection. NK receptor induction could be a late event that results in dampening TCR-mediated signaling to rescue NK1 T cells from negative selection.

Although there is no direct proof that developing NK1 T cell precursors go through a DP stage, the fact that CD1 specificity relies on TCR α chain expression, which is mainly achieved at the DP stage, would suggest that this should be the case. Circumstantial evidence is provided by the findings that the

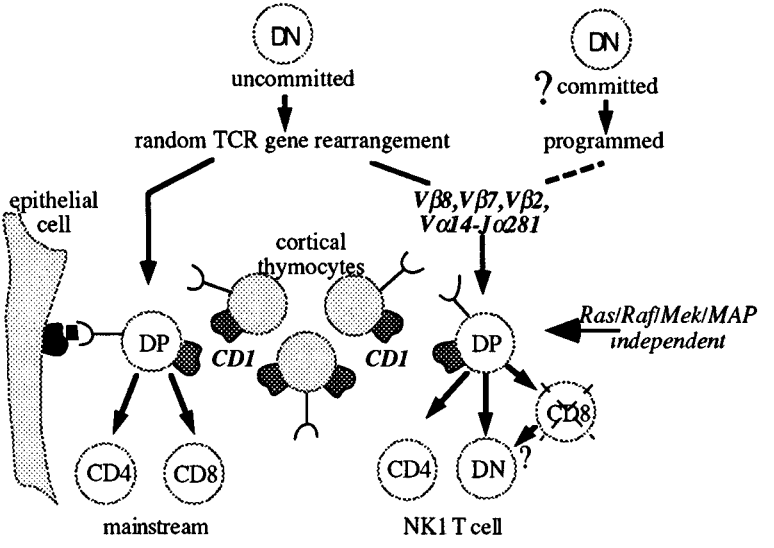

Figure 3 Thymic development of NK1 T cells. NK1 T cells may originate from uncommitted precursor thymocytes that randomly express a CD1-specific Vα14-Jα281/Vβ8, Vβ7 or Vβ2 TCR, and interact with CD1-expressing cortical thymocytes. Coexpression of CD8 induces negative selection. The NK1 phenotype may be acquired in a late maturation event following CD1 recognition. In contrast with mainstream thymocytes, NK1 T cells do not depend on the Ras/Raf/Mek/MAP signaling pathway for their development.

CD8 gene is demethylated among DN NK1 T cells (14) and that CD8-deficient mice have perturbations of the Vβ repertoire of both CD4 and DN NK1 T cells (18).

Is There an Extrathymic Pathway of NK1 T Cell Generation?

Although the fact that positive selection of NK1 T cells does not depend on CD1 presentation by thymic epithelial cells raises the possibility that the thymus may be dispensable for their generation, the thymus does appear to be their main site of development. First, fetal thymic organ cultures generate NK1 T cells (18). Nude mice do not express NK1 T cells in their spleen, bone marrow, or liver (11, 13, 43) (A Bendelac, unpublished observations), and NK1 T cells are generated in nudes after thymic grafting (13). Direct assessment of Vα14-Jα281 rearrangement by quantitative PCR in nudes also shows a drastic 10–100-fold reduction down to background levels (38). Lethally irradiated, thymectomized, and T cell–depleted adult bone marrow reconstituted in mice could generate IL2Rβ^+ TCR^{int} liver lymphocytes, but these cells were almost exclusively CD8$^+$ cells that do not belong to the NK1 T cell population (92).

To search for the site of NK1 T cell development, the signal joint resulting from the circular excision of the intervening sequences between Vα14 and Jα281 was quantified by PCR in different tissues (38). As only one such circle is generated per cell, they are then diluted during subsequent cell division. The tissue with the highest quantity of excision circles per Vα14-Jα281 NK1 T cell is therefore the site of least postrearrangement divisions, which one may assume is the tissue where NK1 T cells are generated (as opposed to the one where they are activated and divide). The study found that bone marrow and liver T cells had 17 times more circles than did thymocytes and concluded that these tissues were the sites of generation and the thymus a site of expansion. However, the study did not take into account the fact that the frequency of Vα14-Jα281 NK1 T cells is far lower among whole thymocytes (0.5%) than among liver and bone marrow T cells (15–30%). After correction, thymic Vα14 NK1 T cells harbor two to four times more excision circles than those in liver or bone marrow, a result that is consistent with the thymus being the main tissue where NK1 T cells are generated.

FUNCTION

From the moment of their differentiation in the thymus (19, 20), "naive" NK1 T cells have a unique set of potent biological properties. NK1 T cells from thymus, spleen, bone marrow, or liver, particularly the CD4 subset, can secrete IL-4 upon primary stimulation. Although they secrete large amounts of Th2 cytokines such as IL4, IL-10, and IL-5, they also secrete Th1 cytokines such as IFNγ and TNFβ (16, 19, 21). Limiting dilution assays suggest that a minimum of 3–5% NK1 CD4 thymocytes are able to secrete IL-4 (21), and ELISPOT assays of liver NK1 CD4 T cells measured a frequency of 20% (93). Whether each NK1 T cell secretes both Th1 and Th2 cytokines (Th0-type cells) or whether two separate functional subsets exist is not yet known.

Although NK1 T cell suspensions stimulated in vitro secrete IL-4 with conventional kinetics, peaking at day 2, their stimulation in vivo by injection of anti-CD3 results in mRNA induction and protein secretion peaking by 1 and 2 hours, respectively (94). In an in vitro splenic fragment culture system, the kinetics of secretion was accelerated, suggesting that some crucial, as yet unidentified, element of the in vivo architecture is able to considerably speed up cytokine mRNA induction and protein secretion.

NK1 T cells also express surface receptors that allow regulatory interactions with other cells. They can induce slow, Fas-mediated killing (95) of cortical thymocytes (which express CD1). They also express CD28, and the rapid in vivo activation of NK1 T cells appears to be partially blocked (50%) by soluble human CTLA4-Ig (94), suggesting that B7 ligands may modulate some aspects of their responses.

The function of the NK receptors expressed by NK1 T cells is just beginning to be studied. NK1 T cells display little YAC killing activity upon primary ex-vivo culture (12), but after short-term cultures in the presence of high doses of IL-2, they upregulated their $CD16^+$ Fc receptors as well as the Fcγ transduction molecule. They mediated redirected lysis through TCR, NK1.1, or CD16 and also killed YAC targets (31, 90). The NK1.1 molecule, which activates the cytolytic function of NK cells (96, 97) and NK1 T cells, could also activate IFN-γ secretion by both cell types (98). IL-12 mediated a similar effect. Remarkably, these modes of stimulation selectively induced Th1 cytokine secretion, since IL-4 was not produced by NK1 T cells in these conditions (98). The expression of Ly-49A, a member of the Ly-49 family of inhibitory receptors, is regulated in vivo, suggesting that it is likely to mediate functional effects. For example, Ly-49A is expressed by 15% of B6 NK1 T cells, but as for true NK cells (99), it is downmodulated in B10.A or B10.BR mice, which express the Ly-49A ligands D^d and D^k (A Bendelac, unpublished data). The type of regulation that inhibitory receptors might exert is illustrated by the reports that TCR-mediated cytolytic activity of some human CTL clones could be inhibited by coengagement of these NK receptors (79, 82), possibly because these receptors activate the HCP phosphatase, which might block TCR-mediated signaling (83). Therefore, NK1 T cells may obey a unique and complex set of "rules of engagement," by which induction of CD1, downmodulation of classical MHC I, and/or expression of particular NK1.1-binding carbohydrates interact to trigger distinct effector functions (Figure 4). This could ensure that NK1 T cell triggering is limited to some cell types or tissues where these conditions are met and that NK1 T cell regulatory functions are modulated to suit the biological nature of the pathogen.

Functions

There are already several well defined systems in which the unique regulatory functions of NK1 T cells can be appreciated.

Vα14-Jα281 TRANSGENIC MICE Transgenic mice expressing a Vα14-Jα281 TCR α chain driven by the Vα11 promoter and the Ig enhancer exhibited a tenfold increase in DN and CD4 NK1 T cells. Their splenic CD4 cells produced 100 times more IL-4 upon mitogen stimulation in vitro and greater than tenfold more of the early IL-4 induced by anti-CD3 injection in vivo. In contrast, the transgenic mice secreted fivefold less IFNγ (51). Their baseline serum levels of Th2-controlled IgE and IgG1 isotypes, which reflect humoral responses to environmental antigens, were elevated sixfold and twofold, respectively, while other isotypes, including the Th1-controlled IgG2a were remarkably conserved. These observations support the hypothesis that NK1 T cells promote Th2 responses. They also show that Th1-controlled IgG2a is unaffected, suggesting that NK1 T cells are not systematically recruited in all immune responses.

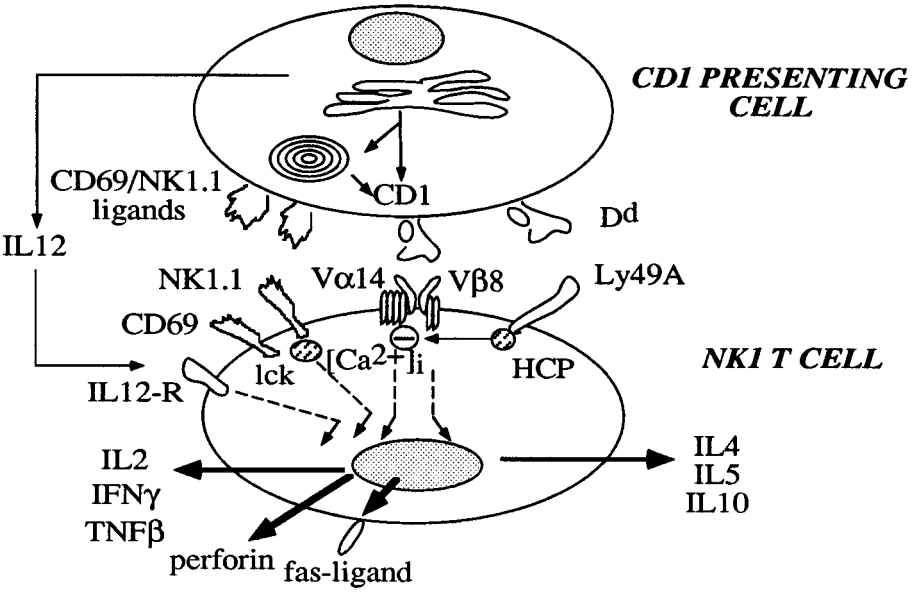

Figure 4 Multiple signaling path ways for NK1 T cell activation. Multiple signaling receptors are likely to operate during NK1 T cell interaction with a CD1-expressing cell. TCR engagement triggers both Th1 and Th2 cytokine secretion, whereas NK1.1 or IL12R may selectively promote Th1 functions, and MHC I-binding Ly49 receptors may block TCR-mediated signaling through the recruitment of the HCP phosphatase.

Indeed, preliminary experiments have shown that, in the face of a 500-fold increase of the IL-4/IFN-γ secretion ratio after mitogen stimulation, these transgenic mice mount normal antigen-specific IFN-γ and IL-4 responses in vitro after KLH/adjuvant immunization.

ANTI-IGD-INDUCED TH2 ACTIVATION The anti-IgD system is a classic CD4 cell–mediated, IL-4-dependent model of polyclonal IgG1 and IgE secretion (100), where i.v. injection of goat anti-IgD antibodies induces an IL-4/IgG1-IgE response. β2M KO mice were severely impaired in their ability to mount the typical response to anti-IgD, while CD8 KO or MHC class II KO mice responded normally (44), pointing to a β2M-dependent cell type as the source of IL-4. Direct proof that NK1 T cells were required was obtained by cell transfer experiments in which injection of B cells and thymic NK1–enriched T cells reconstituted the IgE response in irradiated β2M KO mice (44). Mature thymocytes depleted of NK1 T cells or mature thymocytes extracted from β2M-deficient mice failed to provide help. Importantly, IgE was secreted only

by $\beta 2M^+$ B cells (genetically able to express CD1), suggesting that NK1 T cells recognized CD1 on the surface of B cells (44). This anti-IgD system raised important questions as to how B cells receive help from NK1 T cells. It is unlikely that CD1 presents peptides from goat Ig for recognition by NK1 T cells. IgD cross-linking may induce or hyperinduce CD1, either directly or by recruiting other factors to the B cell surface. Another possibility is that anti-IgD–induced B cells downmodulate surface receptors that are inhibitory to NK1 T cells such as MHC I, thus relieving negative stimuli while maintaining or increasing positive signals through CD1. Although the physiological significance of the anti-IgD system is unknown, the results suggest that NK1 T cells can help B cells in the absence of associative recognition of antigen.

TOXOPLASMA GONDII INFECTION MHC II KO mice can be vaccinated, using an attenuated strain of *Toxoplasma gondii*, to eliminate a subsequent low dose challenge of *T. gondii* through CD8 cell- and IFNγ-mediated activation of macrophages (101). Paradoxically, efficient vaccination was abolished by in vivo treatment with either anti-CD4 or anti-NK1.1, suggesting that the $CD4^+$ NK1 T cells were providing critical help to CD8 cells, allowing them to clonally expand and successfully face a challenge with *T. gondii*. Sorting of CD4 NK1 T cells 7 days after *T. gondii* infection demonstrated that they were actively producing IL-2 but not IL-4 mRNA. Although previous studies have emphasized their Th2-promoting function, one is reminded that NK1 T cells also produce Th1 cytokines. The implication of these results is that NK1 T cells can modulate the type of help that they give to effector cells. It is possible that certain stimuli electively turn on Th1 cytokines and turn off Th2 cytokines, as is suggested by the NK1.1 cross-linking experiment described earlier (98). Since it is unlikely that *T. gondii* antigens are presented by CD1, the parasite might instead modulate the expression of CD1, and/or of ligands for NK receptors, driving NK1 T cells to secrete IL-2 and help CD8 cells.

EFFECTS OF IL-12 Work on *Listeria monocytogenes* has brought to light an added level of complexity of the NK1/CD1 pathway (93). Upon infection with Listeria, a liver-tropic Th1-inducer, IL-4 secretion by liver lymphocytes, normally the property of NK1 T cells, rapidly disappeared. This effect was blocked by anti–IL-12 injection or mimicked by injection of IL-12, suggesting that IL-12 production by Listeria-infected macrophages (102) could modulate NK1 T cells. Upon systemic administration of IL-12, NK1 T cells may also participate in the clearance of liver metastases of the EL-4 lymphoma (which expresses low levels of CD1, A.B., unpublished observation). This is suggested by a study in which the increased in vitro cytotoxicity of liver mononuclear cells against EL-4 was impaired by anti-NK1.1 or anti-CD3 antibodies (103).

OTHER POSSIBLE FUNCTIONS OF NK1 T CELLS There are a number of other conditions in which NK1 T cells may play a role. However, as is apparent from the following list, the available observations in many instances are only correlative, and there is yet no certainty that the relevant cell subset is the canonical CD1-specific Vα14-Jα281 NK1 T cell.

Autoimmunity

In a screen of laboratory mouse strains, the NK1 T cells of SJL (41) and NOD mice (104) (JH Roark, A Bendelac, unpublished results) were found to be reduced (3–5 fold) in frequency and to exhibit a profound defect in IL-4 secretion. These defects were seen in young as well as in old mice. Other strains with normal NK1 T cell numbers or function included B6, BALB/c, CBA, C3H, and DBA/2. It is quite remarkable that SJL and NOD also share a marked propensity to the Th1-mediated autoimmune diseases, experimental allergic encephalomyelitis (EAE) and diabetes, respectively, raising the possibility that the defect in NK1 T cells may play a role in disease susceptibility.

A quantitative defect in NK1 T cells has also been reported in other autoimmune mouse models such as lpr/lpr and NZB and BW mice (105, 106). Unlike NOD and SJL, this defect is not apparent in young mice and only appears progressively after 5–6 weeks of life, suggesting that it may be secondary to the disease process rather than one of its contributing factors.

In humans, four systemic sclerosis (SS) patients exhibited a decreased frequency of Vα24-JαQ DN PBL (107). Further studies on larger numbers of patients are awaited to confirm this result.

NK1 T Cells in Th2 Responses

The spectacular properties of NK1 T cells in vivo have prompted several investigations aimed at determining the range of conditions in which NK1 T cells are involved. As a surrogate for the eagerly awaited CD1 KO mouse, β2M KO mice offer an environment where CD1 expression and NK1 T cell frequency are reduced. The β2M mutation has been bred into the B6 and BALB/c backgrounds, and mutant mice have so far been shown to mount normal Th2 responses to a variety of stimuli, including *Nippostrongylus brasiliensi*, the egg stage of schistosomiasis, *Leishmania major*, and protein antigens (108, 108a). Although these results may be explained by residual NK1 T cells or other unidentified effects of the β2M mutation, they suggest that several Th2 responses are likely to occur without CD1 or NK1 T cells.

Hybrid Resistance

NK1 T cells have been suggested to be involved in, and in some conditions be the major effector of, hybrid resistance to bone marrow transplantation, a phenomenon traditionally ascribed to true NK cells (109).

Suppression

It is intriguing that the Vα14-Jα281 chain has been associated in the past with so-called "suppressor circuits." Taniguchi and colleagues (4–6) defined two types of KLH-specific H-2^b–restricted suppressor cells, for which they obtained T cell hybridomas from KLH-primed B6 spleen T cells purified on KLH-coated plastic dishes. One hybridoma, 34S-18, produced a soluble suppressor factor (TsF) with KLH-binding activity that induced another hybridoma, 34S-281 ("the inducible acceptor suppressor"), to secrete another TsF with KLH-specific and H-2^b-restricted suppressor activity on a secondary (IgG) anti-DNP PFC assay. 34S-281 was anti-idiotypic in the sense that it could be activated by some anti-KLH monoclonal antibodies. Both hybridomas as well as seven other suppressor hybridomas obtained in a total of three fusions, expressed the invariant Vα14-Jα281 TCR α chain.

Moorhead and Fairchild defined DNP-specific, MHC class I–restricted suppressor cells, and obtained hybridomas that produced a TsF, that upon injection in vivo, suppressed by 50% a recall DTH reaction elicited by skin painting with 2,4-dinitrobenzene sulfonate. One of nine hybridomas used the canonical Vα14-Jα281 TCR α chain and a Vβ8.2 TCR β chain. They suggested that the DNP/K^d-specific TsF spontaneously released by the hybridoma is a soluble form of the TCR itself, because it was eluted from a column of anti-Vβ8 conjugated Sepharose beads (75).

Although present understanding of the cytokine secretion properties of NK1 T cells would support the idea that they promote some types of immune responses, and may therefore suppress others, their involvement in the systems described above remains enigmatic. In particular, the property of recognizing KLH or DNP and the apparent restriction to polymorphic MHC I alleles conflict with the CD1-specificity and restricted TCR repertoire of NK1 T cells.

CONCLUSION

The discovery that mouse NK1 T cells recognize CD1 and mediate unique functions constitutes the first solid evidence that CD1 gene products play an immunological role. The emerging picture of the NK1/CD1 pathway is that of a specialized regulatory component of the immune system which can rapidly provide Th1 or Th2 help to effector cells, be they CD8 cells or B cells, in an antigen-nonspecific manner. Both NK1 T cell activation and the type of cytokines they secrete upon activation may be under the control of innate recognition/decision pathways. For example, conditions resulting in CD1 induction may trigger the NK1 $\alpha\beta$ TCR; downmodulation of classical MHC I may relieve negative stimuli transduced by receptors of the Ly49 type; and microbial carbohydrates may trigger lectin-type receptors that modulate the type of cytokines secreted by

NK1 T cells. Such a system allows for a jump start in life-threatening situations, when waiting for conventional CD4-mediated antigen-specific help would put the host in danger of being overwhelmed by the pathogen. It also allows for innate, evolutionarily selected, hard-wired mechanisms of early Th1/Th2 regulation. This possibility was foreseen some years ago (110) and has received some support with the demonstration that NK cells can enhance Th1 differentiation in response to Leishmania in the C3H mouse strain (111).

A conceptual problem with this type of nonantigen-specific help, however, is that it violates the rule of associative recognition (112), a fail-safe mechanism that is thought to deal with the unavoidable emergence of self-reactive lymphocytes (113). However, putting early help under the control of the innate immune system, which can appreciate the biology of the antigen (i.e. its pathogenic context), is an alternative that has begun to be appreciated (114, 115) and has been formulated in a novel and simple model, the Danger model (116), which contends that normal immune responses start and subside with pathogen-inflicted damage, rather than with the mere recognition of foreign antigens.

It has become apparent in the past few years that many important issues are raised by the CD1/NK1 pathway. Most exciting is the possibility that the NK1 T cell represents a novel type of regulatory T cell, one that straddles the adaptive and innate immune systems. The complexity of its surface recognition receptors, and the potency and range of its functions, are indeed indications that the NK1 T cell may live up to this ambitious role.

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