Increased Interleukin 4 and Immunoglobulin E Production in Transgenic Mice Overexpressing NK1 T cells

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Summary

Natural Killer (NK)1.1$^+$ (NK1) T cells are a specialized subset of α/β T cells that coexpress surface receptors that are normally associated with the NK cell lineage of the innate immune system. On recognition of the conserved, major histocompatibility complex class I–like CD1 molecule, these cells are able to release explosive bursts of interleukin 4 (IL-4), a cytokine that promotes the T helper type 2 (Th2) effector class of an immune response. A unique feature of their T cell receptor (TCR) repertoire is the expression of an invariant TCR α chain, Vα14-Jα281, and of a restricted but polyclonal set of Vβ gene families, Vβ8, Vβ7, and Vβ2. Here, we show that transgenic expression of this TCR α chain during thymic development is sufficient information to bias the differentiation of mainstream thymocytes towards the NK1 developmental pathway. It markedly increases the frequency of cells with the NK1 pattern of T cell differentiation and also has drastic consequences for the selection of the Vβ repertoire. Transgenic CD4 cells exhibited a 10–100-fold increase in IL-4 production on mitogen stimulation in vitro and in vivo, and baseline levels of the Th2-controlled serum immunoglobulin isotypes, IgE and IgG1, were also selectively elevated in vivo.

Unlike mainstream α/β T cells, NK1 T cells acquire, during thymic development, the unusual property of secreting IL-4 on primary stimulation, and of expressing receptors normally associated with the NK lineage such as NK1.1 (reviewed in references 1–3). Their positively selecting ligand appears to be the conserved MHC class I–like molecule CD1, expressed on cortical thymocytes (4, 5) rather than the polymorphic MHC restriction elements expressed on thymic epithelium (6–9), and their resulting TCR repertoire is severely limited. More than 85% of NK1 T cells express an invariant TCR α chain, Vα14-Jα281 (10), and a limited set of TCR Vβ chains (Vβ8,7 and 2) (8, 11–15) which, together, generate specificity for CD1 in the apparent absence of foreign antigens (4, 5). NK1 T cells are found among mature HSA^{low} thymocytes as single positive CD4$^+$8$^-$ (the main IL-4 producer) and double-negative (DN)[1] CD4$^-$8$^-$ cells, but they are remarkably absent from the CD8$^+$4$^-$ subset (8, 10). Humans have a similar set of T cells that use the invariant TCR α chain Vα24-JαQ, and Vβ11, the homologues to mouse Vα14-Jα281 and Vβ8, and humans also express a homologous CD1 ligand, the CD1d molecule (10, 16, 17). Thus, NK1 T cells seem to belong to an ancient cell type, straddling the innate and adaptive immune systems, whose semi-invariant α/β TCRs and conserved CD1 ligand predate the speciation of mouse and man.

Their high frequency in peripheral lymphoid tissues (0.5–1% of spleen and LN cells), and their ability to explosively release IL-4 in response to CD1 induction on APC might strongly shift the effector class of immune responses towards Th2 (4, 18, 19). Indeed, NK1 T cells are essential for generating the polyclonal Th2 and IgE response that is induced upon intravenous injection of goat anti–mouse IgD antibodies (20). This can have profound consequences on subsequent immunizations. For example, preimmunization with myelin basic protein coupled to mouse anti–rat IgD antibody induced myelin basic protein–specific Th2 cells and protected rats from subsequent attempts to induce experimental allergic encephalomyelitis (EAE) (21). In addition, NK1 T cells are severely deficient in SJL (22) and nonobese diabetic (23) mice, two strains which are conspicuously susceptible to Th1-mediated autoimmune diseases, EAE and diabetes, respectively. This raises the possibil-

[1] *Abbreviations used in this paper:* DN, double negative; EAE, experimental allergic encephalomyelitis.

ity that the NK1 T cells normally function to suppress or deviate tissue-destructive Th1 responses.

Since CD1 is constitutively expressed by cortical double-positive $CD4^+8^+$ thymocytes, the cell type that appears to positively select the NK1 T cells (5), it is available for recognition by developing thymocytes that express a CD1-specific TCR. We therefore used a transgenic approach to investigate the consequences of expression of a Vα14-Jα281$^+$ TCR on thymocyte development. We generated transgenic mice expressing the rearranged Vα14-Jα281 transgene under the control of an endogenous Vα promoter, the Vα11 promoter, and the Ig enhancer (24). The TCR α chain transgene was tested alone, without a TCR β chain transgene, to determine the extent to which it determines the NK1 developmental fate of mainstream thymocytes. Our results indicate that expression of the canonical Vα14-Jα281$^+$ TCR α chain has drastic consequences on the selection of TCR β chains of the Vβ8 and Vβ7 families as well as on the NK1 differentiation and function of thymocytes. In addition, transgenic mice exhibited a major increase in their potential to produce IL-4, as well as in their baseline levels of Th2-controlled isotypes, in particular IgE.

Materials and Methods

Transgenic and Mutant Mice. Vα14-Jα281 and Vα8-Jα37 gene segments were PCR-amplified from genomic DNA of the CD1 specific T cell hybridoma DN32D3 (10) and the IA^d/rabbit Ig-specific T cell clone CDC35, respectively (25), and cloned into a TCR α shuttle vector containing the Vα11 endogenous promoter and the Ig enhancer as described (24). Linearized constructs were injected into B6 fertilized eggs, and transgenic founders screened by tail DNA PCR with specific primers.

C57BL/6, and C57BL/6.Cα null mice (TCR α knockout [26], backcrossed nine times to B6) were purchased from The Jackson Laboratory (Bar Harbor, ME) or Taconic Farms, Inc. (Germantown, NY) and raised under specific pathogen-free conditions. Vα14-Jα281 transgenic mice with a homozygous mutation of the endogenous Cα genes were generated by crossing transgenics to Cα mutants, selecting for offsprings that expressed α/β TCR^{high} blood cells without using endogenous Vα gene products (detected using a combination of anti- Vα2, Vα8 and Vα11 antibodies).

FACS® Analysis/Sorting. Thymocyte and spleen cell suspensions were three- or four-color stained using combinations of antibodies conjugated to tricolor (anti-CD4; Caltag Laboratories, South San Francisco, CA), red 613 (anti-CD8; GIBCO BRL Life Technologies, Gaithersburg, MD), FITC, and PE (or biotin followed by PE-conjugated streptavidin [Caltag]), and analyzed using a modified FACScan® (Becton Dickinson and Co., Mountain View, CA). FITC-conjugated anti–pan-TCRβ (H57), anti-CD3ε (500.A2), or anti-CD8 (53.6.7), biotin-conjugated anti–pan-TCRβ (H57), Vβ7 (TR310), Vβ6 (RR4.7), Vβ10 (B21.5), Vβ14 (14-2), Vα2 (B20.1), Vα11 (RR8-1), Vα8 (B21.14), PE-conjugated anti-NK1.1 (PK136) and CD5 (53-7.3), and APC-conjugated anti-CD8 (53.6.7) were purchased from PharMingen (San Diego, CA). Biotinylated F23.1 anti-Vβ8 was produced in the laboratory. FACS® sorting was performed using a Vantage (Becton Dickinson and Co.) equipped with dual (argon and dye) lasers.

Cell Preparations and Cytokine Production Assays. T cell–enriched spleen cells were obtained after MACS (Miltenyi Biotec, Bergish Gladbach, Germany) depletion of Y3P anti-IA^b positive cells (>90% TCR α/β$^+$). T cell subsets were purified (>98% pure) from T cell–enriched preparations by FACS® sorting after staining with anti-CD4-FITC, anti-CD8-APC, and CD5-PE. 4×10^5 enriched T cells or 10^5 subsetted cells were stimulated in vitro with anti-CD3 antibody (2C11, 1 μg/ml) in the presence of 10^5 2,000-rad irradiated, T cell–depleted spleen cells (pretreated with anti-CD4 (RL172.4), anti-CD8 (3.155), and anti-Thy1(J1J) anti-

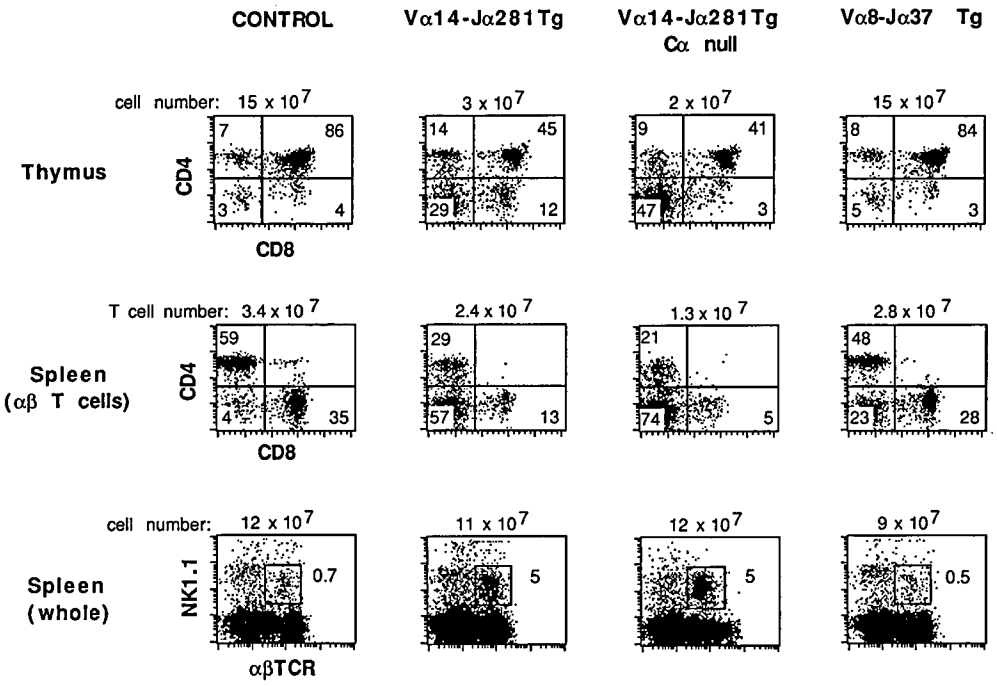

Figure 1. Overexpression of NK1 T cells in Vα14-Jα281 transgenic mice. Thymus and spleen of 6-wk-old Vα14-Jα281 transgenic mice in a normal or in a Cα-null (TCR α knockout) background, transgene-negative littermates, and Vα8-Jα37 transgenic mice (all in a C57BL/6 background) were four-color stained with anti-CD4 tricolor, CD8-red 613, TCR α/β-FITC, NK1.1-PE. The CD4/CD8 dot-plots of spleen cells are gated on TCR α/β-positive cells, to show the population of double-negative $CD4^-8^-$ TCR α/β-positive cells. Whole cell numbers or T cell numbers recovered for each organ are indicated above each dot plot. Similar results were obtained for at least three individual mice in all four Vα14-Jα281 transgenic lines.

bodies plus low toxicity rabbit complement (Cedarlane Laboratories Ltd., Hornby, Canada) for 45 mn at 37°C), in a final volume of a 1:1 mixture of RPMI and Click's media enriched with 10% heat inactivated FCS, 5×10^{-5} M 2-ME, penicillin, streptomycin, and gentamycin (complete medium), in a 96–U-bottomed well microplate. Cytokines released in the supernatant were measured 48 h later using the IL-4-sensitive CT4.S line and the IFN-γ sandwich ELISA as described (27).

For in vivo stimulation with anti-CD3, mice were injected i.v. with 1 μg 2C11 anti-CD3 antibody, and spleen cells removed 90 min later and cultured, without further stimulation, as described (18), at a density of 10^7 cells in 2 ml of complete medium for 2 h to measure cytokine release in the culture supernatant.

Serum Ig Isotype Levels. Serum levels of Ig isotypes were measured by standard sandwich ELISA (IgA, M, G1, G2a, G2b, G3; Southern Biotechnology, Birmingham, AL; IgE; PharMingen). The average level of each isotype in normal mice was set up arbitrarily at 100 U/ml.

Results

Altered T Cell Development and Vβ Usage in Vα14-Jα281 Transgenic Mice. Four distinct founder lines of mice expressing the Vα14-Jα281 transgene were produced in a C57BL/6 background, as well as a control line expressing Vα8-Jα37, a TCR α chain not associated with NK1 T cells (25). Because there exists no antibody specific for Vα14, surface expression of the Vα14-Jα281 α chain was confirmed by showing that transgene expression rescued T cell development in TCR Cα null mice (Fig. 1, third column). Transgene expression was also evident from the phenotype described below.

A common feature of all four Vα14-Jα281 transgene-expressing lines was a fivefold reduction in thymus size, affecting mainly double-positive thymocytes (Fig. 1). This size reduction did not occur in mice expressing the control Vα8-Jα37 transgene, suggesting that a bypass of the double-positive stage of thymic development (28, 29) or a deletion at that stage had occurred as a consequence of the Vα14-Jα281 TCR specificity. A 30% reduction of the number of T cells was also observed in the spleen of Vα14-Jα281 transgenics together with a marked increase in DN α/β T cells (Fig. 1).

In normal mice, both the CD4 and the DN NK1 T cells use mainly Vβ8, Vβ7, and Vβ2 TCR β chains. We have suggested that, coupled with Vα14-Jα281, these β chains allow for high affinity CD1 binding. Indeed, this affinity seems to be high enough that the additional binding contributed by the CD8 coreceptor induces deletion. Thus, normal mice do not have CD8 cells expressing these TCRs and the forced, transgenic expression of CD8 induces deletion of the entire Vα14-Jα281$^+$ NK1 T cell subset (8, 10). Figs. 2 and 3 show that the CD8 single-positive compartment in transgenic mice was selectively depleted of cells expressing the Vβ8 and Vβ7 TCR β chains most commonly used by NK1 T cells whereas control Vβ6$^+$ cells were not deleted (Figs. 2 and 3). The extent of this deletion indicated therefore that a majority of Vβ8 and Vβ7 TCR

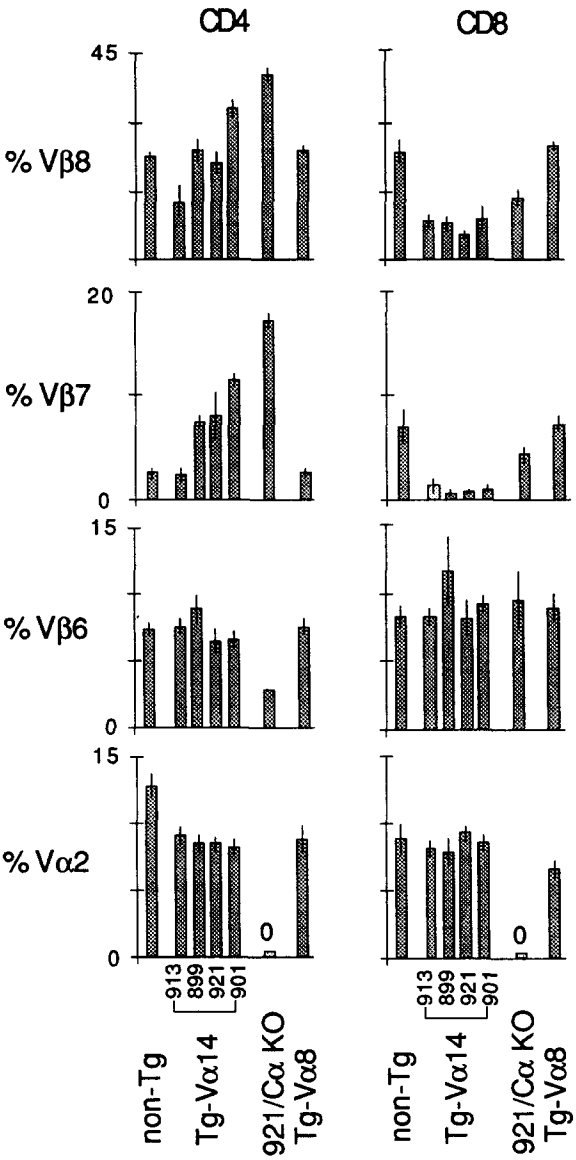

Figure 2. TCR α and β repertoire in Vα14-Jα281 transgenic mice. Thymocytes from 6-wk-old mice of each of the four Vα14-Jα281 transgenic founder lines (913, 899, 901, 921), from the Vα14-Jα281 transgenic 921 line in a Cα-null background, from littermate controls, and from Vα8-Jα37 transgenics were three-color stained with CD4 tricolor, CD8-FITC, and biotinylated anti-Vβ8, Vβ7, Vβ6, Vα2, and pan-TCRβ followed by streptavidin PE. Percentages of Vβ and Vα expressing mature CD4 and CD8 cells are obtained after normalizing to the number of pan–TCRβ-positive cells. Mean ± SD of three to six individual mice per group are represented. Similar results were obtained with spleen cells.

β chains allow for CD1 recognition when paired with Vα14-Jα281.

Vβ usage in the CD4 compartment was variable in different lines of transgenics. The frequency of Vβ7$^+$ cells was increased three- to fourfold in lines 899, 921, and 901, and unchanged in line 913, whereas Vβ8$^+$ cells were increased by 50% in line 901, unchanged in lines 899 and 921, and decreased by 50% in line 913 (Figs. 2 and 3). Such variabil-

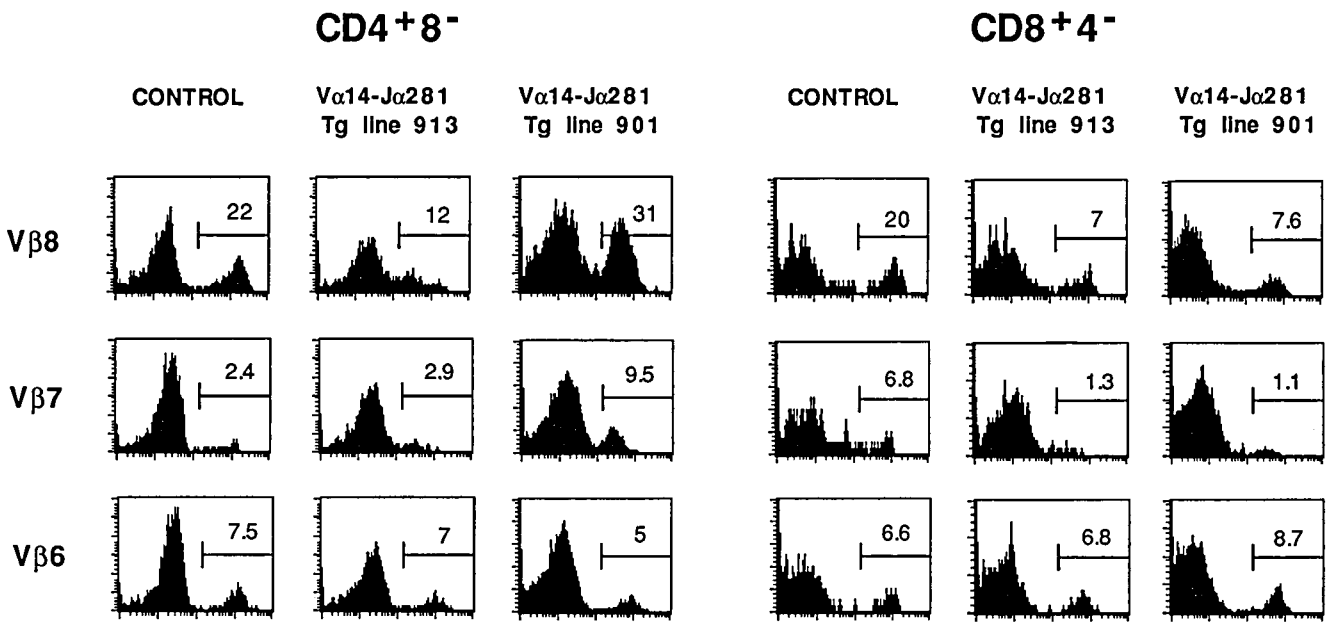

Figure 3. Downmodulation of Vβ8$^+$ and Vβ7$^+$ TCRs on Vα14-Jα281 transgenic CD4$^+$ T cells. The 913 and 901 founder lines illustrated here display a selective downmodulation of Vβ8$^+$ and Vβ7$^+$ TCRs on CD4$^+$8$^-$ thymocytes, which is slightly more pronounced in the deleting 913 line. Vβ6$^+$ CD4$^+$8$^-$ thymocytes display normal levels of surface TCR expression. Note that CD8$^+$4$^-$ thymocytes of both transgenic lines are severely depleted of Vβ8$^+$ and Vβ7$^+$ cells.

ity in frequencies of Vβ8$^+$ and Vβ7$^+$ CD4 cells is likely to correlate with subtle changes in the level or the kinetics of expression of the transgene due to different sites of integration in different lines, resulting in increased positive selection of CD4 cells using Vβ8 or Vβ7 in some cases and negative selection in others. In addition, surface levels of Vβ8 and Vβ7 TCR β chains were found to be selectively decreased in transgenic CD4 cells, from two- to threefold in lines 899, 901, and 921, up to fivefold in the "deleting" 913 line (Fig. 3). This result again mimics the phenotype of normal NK1 T cells which express two to three times less surface TCRs than mainstream T cells, and supports the assumption that high Vβ8$^+$ and Vβ7$^+$ expressors were deleted or that they downmodulated their TCR levels and escaped negative selection.

NK1 T Cell Surface Phenotype. Vα14-Jα281 transgenics also exhibited an increased frequency of T cells expressing surface markers associated with the NK1 phenotype. This was reflected in the net 7- to 10-fold increase in the absolute numbers of NK1.1$^+$ α/β TCR$^+$ cells observed in the spleen of Vα14-Jα281 transgenics (Fig. 1). NK1.1 was expressed on 12–24% of CD4 spleen cells (as well as on 30–60% of DN cells [see below]), but not on CD8 cells (Fig. 4). High levels of CD44, another hallmark of NK1 T cells, were also found on transgenic CD4 cells (Fig. 4) and DN cells (Fig. 5). Results similar to those shown in Figs. 4 and 5 for spleen cells were also observed in mature HSA^{low} thymocytes (data not shown).

DN α/β T cells. A prominent subset of DN CD4$^-$8$^-$ α/β TCR-expressing cells was present in increased num-

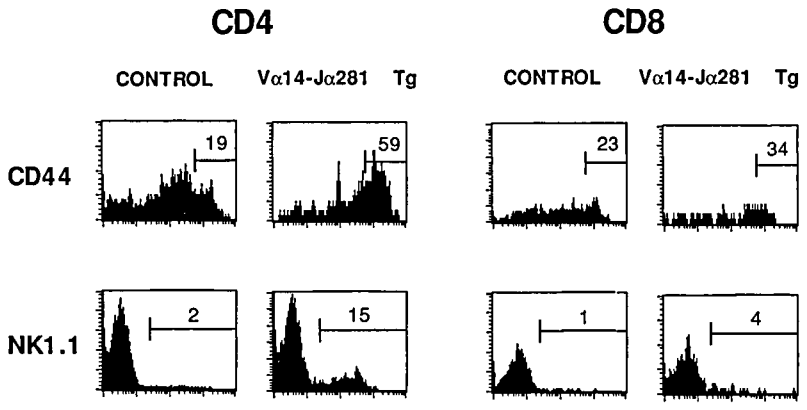

Figure 4. Increased expression of activation/NK receptors by Vα14-Jα281 transgenic CD4$^+$ T cells. Staining with anti-CD44-PE or anti-NK1.1-PE is displayed on gated CD4 and CD8 spleen cells from control and transgenic mice.

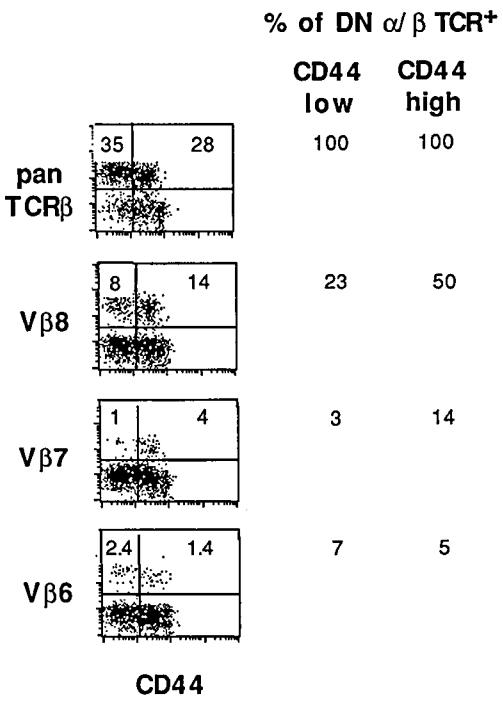

Figure 5. Two populations of DN α/β T cells in Cα null, Vα14-Jα281 transgenic spleens. T cell–enriched spleen cells (obtained after panning out MHC II– and Ig-positive cells) were stained with anti-CD4 tricolor, CD8-red 613, CD44-FITC, and biotinylated anti-TCR antibodies followed by streptavidin-PE. CD44/TCRβ dot plots on gated DN cells are represented. The relative (calculated) frequency of Vβ8, Vβ7 and Vβ6-expressing cells among $CD44^{low}$ and $CD44^{high}$ DN T cells is displayed on the right side of the dot plots.

bers in the thymus and periphery of Vα14-Jα281 transgenic mice (Fig. 1). Although this might be expected from the original association of Vα14-Jα281$^+$ TCRs and DN NK1 T cells (10), it has also been observed that most TCR transgenic mice, irrespective of their TCR specificity, generate a sizable population of DN T cells (30–32). Indeed, our control Vα8 transgenics also harbored a higher frequency of DN α/β T cells (which expressed the Vα8 transgenic chain [not shown]), although this frequency was two times lower than in Vα14-Jα281 transgenics (Fig. 1). Importantly however, the majority of Vα8 transgenic DN cells did not express activation/NK receptors (Fig. 1), nor did they display a biased usage of Vβ gene products (not shown), implying that these unique features are the consequence of particular TCR specificities. A closer examination of the Vα14-Jα281 transgenic DN cells revealed that they could in fact be separated into two distinct subpopulations of approximately equal size, according to their expression of activation/NK receptors (Fig. 5). The $CD44^{high}$ ($NK1.1^{high}$) had a markedly biased usage of Vβ8 and Vβ7 TCR β chains whereas the $CD44^{low}$ subset did not. This could be observed in mice with a homozygous mutation of their TCR Cα genes (Fig. 5), indicating that expression of the Vα14-Jα281 TCR α chain can lead to both Vβ-biased NK1$^+$ and Vβ-nonbiased NK1$^-$ DN phenotypes. This result demonstrates first that the bias in Vβ8 and Vβ7 usage among normal NK1 T cells is not the result of a preferential physical pairing of these TCR β chains with the Vα14-Jα281 TCR α chain, and therefore suggests that these TCR β chains are selected because they contribute affinity to the CD1 ligand. Second, it reenforces the hypothesis that the NK1$^-$ DN T cells observed in "conventional" TCR transgenic mice result from the enforced (transgenic) early expression of an α/β TCR in a separate lineage that does not usually express it, possibly a γ/δ committed precursor, which follows different rules of selection and differentiation (30–32). Interestingly, a population of $CD44^{high}$ IL2Rβ$^+$ NK1.1$^+$ double-negative T cells has recently been observed in an anti-K^b α/β TCR transgenic system (33). As in the anti-CD1 system, the NK1 surface phenotype was ascribed to a higher than usual affinity (CD8-independent) of the anti-K^b transgenic α/β TCR for its thymic ligand during positive selection. Another transgenic line expressing an anti-K^b TCR of lower affinity (CD8-dependent) only produced NK1$^-$ DN T cells.

Endogenous TCR α Chain Usage by Transgenic T Cells. Although we could not directly monitor the frequency of cells expressing the transgenic α chain due to the lack of an anti-Vα14 antibody, FACS® staining for Vα2 (Fig. 2), as well as Vα8 and Vα11 (not shown), which altogether are expressed by ~20% of normal T cells, indicated that the transgenic mice contained 25% fewer CD4 T cells express-

Table 1. *Increased IL-4 Production by Vα14-Jα281 Transgenic CD4 T cells*

	IL-4	IFN-γ
	U/ml	
In vitro, spleen T cells		
Control No. 1	87	20
No. 2	69	18
Transgenic No. 1	7,398	3
No. 2	3,097	4
In vitro, T cell subsets		
Transgenic CD4	1,886	
CD8	1	
DN	261	
In vivo, spleen cells		
Control No. 1	68	1
No. 2	125	1
Transgenic No. 1	1,537	2
No. 2	935	3
No. 3*	1,084	2

Levels of cytokines released in vitro by cells stimulated in vivo or in vitro with anti-CD3. Similar results were obtained with mice from all four transgenic lines.
*TCR Cα-null mouse.

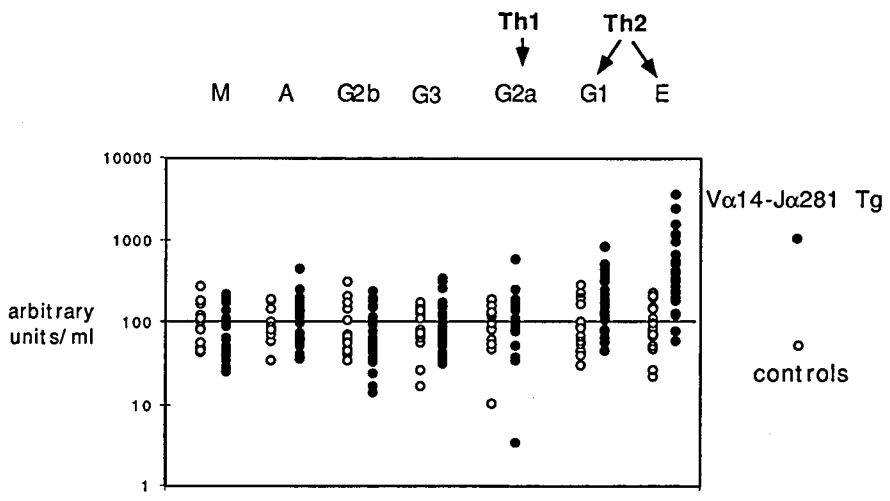

Figure 6. Increased frequencies of Th2-controlled Ig isotypes in Vα14-Jα281 transgenic mice. Serum levels of Ig isotypes were measured by standard sandwich ELISA in 3–5-mo-old Vα14-Jα281 transgenic mice, and littermate controls. Six to eight individual mice were examined for each of the four transgenic lines and all data were pooled, as there was no detectable difference between the different lines. The average level of each isotype in normal mice is set up arbitrarily at 100 U/ml. IgE average levels in Vα14-Jα281 transgenic mice are increased sixfold above controls ($P < 0.001$) and IgG1 are increased twofold ($P < 0.01$).

ing endogenous α chains than control mice. This suggested that at least 25% of CD4 T cells expressed the transgenic α chain on their surface. Conversely, no reduction of endogenous α chain expression was observed on CD8 cells, again supporting the view that CD8 cells expressing high surface levels of the transgenic Vα14-Jα281 α chain were deleted and that the remaining CD8 cells were those which expressed an endogenous α chain capable of outcompeting Vα14-Jα281 for pairing with the TCR β chain. Indeed, mice expressing the Vα14-Jα281 transgene in a Cα-null background exhibited a marked reduction of CD8 cells (Fig.1), whereas their CD4 cells contained an even higher frequency of $V\beta8^+$ and $V\beta7^+$ TCRs (Fig. 2).

Biased IL-4 Production by Transgenic T Cells. To test whether transgenic expression of Vα14-Jα281 also induced an increase in the functional potential to produce IL-4, we measured the levels of cytokines released upon TCR cross-linking with anti-CD3 antibody. In vitro, transgenic T cells secreted ~60 times more IL-4 and 4 times less IFN-γ than transgene-negative littermates (Table 1) or than Vα8-Jα37 transgenic controls (not shown). As in normal mice, the increased IL-4 secretion was mainly contributed by $CD4^+$ T cells (Table 1).

In vivo, we measured the rapid release of IL-4 that is observed within 1 h of TCR engagement, a specific feature of NK1 T cells (18). Table 1 shows that Vα14-Jα281 transgenic spleen cells release >10-fold more early IL-4 than controls. Identical results for the in vitro and in vivo experiments were obtained in all four Vα14-Jα281 transgenic founder lines tested, as well as in TCR Cα-null mice expressing the Vα14-Jα281 transgene.

Increased Baseline Levels of Th2-controlled Ig Isotypes. If NK1 T cells are effective regulators of Th1/Th2 differentiation in normal conditions, one would expect the transgenic mice to express a specific increase of the baseline levels of the serum Ig isotypes that are promoted by IL-4, induced as a result of ongoing immune responses to environmental antigens. As shown in Fig. 6, Vα14-Jα281 transgenic mice of all founder lines had a selective and conspicuous increase of IgE (sixfold above controls on average) and IgG1 (twofold above controls), the two Th2-controlled isotypes (19, 34). The blood levels of other isotypes including IgM, IgA, IgG2a, IgG2b, and IgG3 were unchanged. Control Vα8-Jα37 transgenics, raised under the same specific pathogen-free conditions, did not exhibit changes in IgE or IgG1 levels (not shown).

Discussion

Our results indicate that expression by mainstream thymocytes of the NK1 T cell Vα14-Jα281 TCR α chain alone has drastic consequences on the positive and negative selection of Vβ chains in CD4 and CD8 cells, respectively, mimicking the CD1-specific TCR repertoire expressed on normal NK1 T cells. The extent of the depletion of Vβ8- and Vβ7-positive CD8 cells indicate that Vα14-Jα281 can pair with a very large proportion of TCR β chains using the Vβ8 and Vβ7 gene families (which are used by 30–50% of the T cell population in most mouse strains), to form a TCR with affinity for CD1, and that the overall avidity of interaction is increased by the CD8 coreceptor. The results imply that, from a developmental (and evolutionary) perspective, one may consider that expression of the Vα14-$J\alpha281^+$ TCR α chain alone is sufficient information to bias the differentiation of a thymocyte towards the NK1 pathway. It remains to be tested, however, whether expression of the Vα14-Jα281 TCR α chain itself is genetically programmed in NK1 T cells, or whether it occurs randomly.

The reduced size of transgenic thymuses and the slightly

different degrees of positive vs negative selection between transgenic founder lines are compatible with the view that Vα14-Jα281$^+$ TCRs have an intrinsically high avidity for their CD1 ligand (8, 10, 35). In addition, the transgenic system, by virtue of its earlier TCR expression, may project an exaggerated picture of the normal selection process. Together with a recent report showing that a transgenic TCR unrelated to the NK1 T cell lineage could also induce the differentiation of DN NK1 T cells, provided that the avidity for its thymic ligand was high (33), our results support the contention that expression of receptors of the NK lineage and differentiation into DN T cells can be imparted to mainstream T cell precursors during their thymic development.

Support for the involvement of a distinct signaling pathway in the induction of the NK1 T cell phenotype comes from recent studies by Aberola-Ila et al. (36) who found that NK1 T cells were normally generated in the face of an ablation of the Ras/Raf/Mek/Map kinase pathway which mediates the positive but not negative selection of mainstream thymocytes. The quality, or the avidity, of the interaction between the developing thymocyte and the ligand-presenting cell, and the resulting differences in signaling, may therefore influence the functional differentiation of thymocytes, as previously suggested in models of positive selection with agonist and antagonist ligands in vitro (37, 38). Altogether, these results raise the intriguing possibility that the interaction between CD1-expressing cortical thymocytes and Vα14-Jα281 TCR-expressing thymocytes activates a distinct signaling pathway that induces downmodulation of CD8 as well as induction of NK receptors, particularly those that may dampen TCR-mediated signaling (39), thus allowing cells to escape negative selection.

An important property of CD4 NK1 T cells, that of rapidly releasing IL-4 on TCR engagement, is also conferred to transgenic CD4 cells, resulting in the secretion of a markedly biased Th2 cytokine profile upon primary polyclonal stimulation in vitro and in vivo. The increased baseline levels of IgG1 and IgE Ig isotypes in Vα14-Jα281 transgenic mice therefore further indicate that NK1 T cells play a physiological role in regulating the Th1 vs Th2 differentiation of immune responses.

On the other hand, it is also remarkable that, in the face of a prodigious increase in potentially available IL-4 at the outset of immune responses, Vα14-Jα281 transgenics conserve normal levels of other isotypes, in particular the Th1-controlled IgG2a. This finding suggests that there exist immune responses, presumably those in which CD1 is not induced, where NK1 T cells are simply not recruited, or perhaps even in which they are inactivated by IL-12, as was recently documented in *Listeria monocytogenes* infection (40). Indeed, preliminary experiments have shown that immunization of Vα14-Jα281 transgenic mice with KLH and RIBI adjuvant (which includes mycolic acid and lipid A) induces antigen-specific T cells that produce IL-4 and IFN-γ in amounts similar to those of normal mice (not shown). We also found that Vα14-Jα281 B6 transgenics recovered from *Leishmania major* infection with virtually the same kinetics as nontransgenic littermates (Scott, P., and A. Bendelac, unpublished results).

Although the range of conditions in which NK1 T cells are recruited to drive the differentiation of helper cells remains to be determined, the fact that NK1 T cells are autoreactive to CD1 and that they have been shown to mediate Th2 help for B cells upon anti-IgD injection, suggests that they may specialize in providing rapid help in a non–antigen-specific way. The induction of CD1, for example, may serve as a clue that triggers NK1 T cells, bypassing the need for associative recognition of antigen by classic T helper cells, thus providing a jump start for the effector response. Future challenges will be to elucidate the precise cellular and molecular pathways that drive the differentiation of NK1 T cells in the thymus, and their recruitment in the course of immune responses.

We thank David Parker for the gift of the CDC35 T cell clone, Mark Davis for the TCR-α shuttle vector, John Cardott and Larry Duckett for developing the four-color FACSCAN®, Andrea Barnes, Anne-Marie Dinkel, and Darren Hasara for managing the mouse colonies, and Bana Cuenod-Jabri and Polly Matzinger for critical comments on the manuscript.

The initial phases of this work were performed at the Laboratory of Cellular and Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health. This work was funded in part by a grant from the Mallinckrodt Foundation (A. Bendelac), and by a Cancer Research Institute award (A. Bendelac).

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Received for publication 18 June 1996.

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