An Invariant T Cell Receptor α Chain Is Used by a Unique Subset of Major Histocompatibility Complex Class I-specific CD4+ and CD4−8− T Cells in Mice and Humans

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Summary

The mouse thymus contains a mature T cell subset that is distinguishable from the mainstream thymocytes by several characteristics. It is restricted in its usage of T cell receptor (TCR) Vβ genes to Vβ8, Vβ7, and Vβ2. Its surface phenotype is that of activated/memory cells. It carries the natural killer NK.1 surface marker. Furthermore, though it consists entirely of CD4+ and CD4−8− cells, its selection in the thymus depends solely upon major histocompatibility complex (MHC) class I expression by cells of hematopoietic origin. Forced persistence of CD8, in fact, imparts negative selection. Here, we have studied the TCR repertoire of this subset and found that, whereas the β chain V-D-J junctions are quite variable, a single invariant α chain Vα14-J281 is used by a majority of the TCRs. This surprisingly restricted usage of the Vα14-J281 α chain is dependent on MHC class I expression, but independent of the MHC haplotype. In humans, a similar unusual population including CD4−8− cells can also be found that uses a strikingly homologous, invariant α chain Vα24-JQ. Thus, this unique Vα-Jα combination has been conserved in both species, conferring specificity to some shared nonpolymorphic MHC class I/peptide self-ligand(s). This implies that the T cell subset that it defines has a specialized and important role, perhaps related to its unique ability to secrete a large set of lymphokines including interleukin 4, upon primary stimulation in vitro and in vivo.

The development and function of the minor subset of CD4−8− double negative (DN1) TCR-α/β+ T cells and their relationship to the mainstream CD4+ or CD4−8+ T cells have been elusive issues (1). In the mouse thymus, a set of mature CD4+ (2-7), but not CD8- (4), cells has recently been described that is strikingly similar to the DN thymocytes because it shares the membrane expression of activated/memory and NK markers and the restricted use of Vα8, Vβ7, and Vβ2 TCR-β chains. This population has the unique potential to secrete a large set of lymphokines, including IL-4, upon primary stimulation in vitro (2, 3, 7) and in vivo (8), thus its physiological role may be to influence the Th1/Th2 differentiation of an immune response (9). It is surprising that the selection element for both these CD4+ (4) and DN (4, 10) cells has been shown to be an MHC class I molecule presented by cells of hematopoietic origin. In addition, and equally surprising, was the finding that such selection is largely CD8 independent and that the persistence of CD8 instead imparts negative selection on these cells (4).

Based on these findings, we suggested that this subset of T cells might express a particular set of TCRs with a level of affinity for the MHC class I ligand that was sufficient for CD8-independent positive selection, the negative selection threshold for CD8+ cells being reached as the TCR level increased later during the developmental process, after the CD4+8+ double positive (DP) precursor stage (4).

We have thus analyzed the TCR repertoire used by these cells. We show here that their Vα usage is even more restricted than their Vβ usage. In fact, they use a single invariant α chain, Vα14-J281, whereas their Vβ8, Vβ7, and Vβ2 TCR-β chains have diverse V-D-J junctions. Moreover, this α chain is virtually identical to a human α chain, Vα24-JQ, that is expressed as an invariant chain by a related subset of DN T cells. Thus, the restricted repertoire of these cells is not likely to be due to an interaction with some putative superantigens, but instead to the recognition of a restricted set of MHC class I-peptide ligands that is conserved in both mice and humans.

Materials and Methods

Mouse. 7-9-wk-old, specific pathogen-free C57BL/6, MHC-I− (β2-microglobulin− [B2m−]) (11), originally provided by Dr. R.
Jaenisch (Massachusetts Institute of Technology, Cambridge, MA) and backcrossed eight times to C57BL/6, B10 (H-2b), B10.D2 (H-2b), B10.A (H-2b), B10.BR (H-2b), B10.M (H-2), B10.RIII (H-2b), B10.S (H-2b), B10.Q (H-2b), B10.SM (H-2b), B10.P (H-2b), and B10.PL (H-2b) mice were obtained from the National Institute of Allergy and Infectious Diseases barrier facility (Bioqual, Frederick, MD). MHC-II- (Aa b-) mice, backcrossed four times to C57BL/6 were obtained from GenPharm International (Mountain View, CA). CD8.1 transgenic mice (13) were obtained from C57BL/6) were obtained from GenPharm International (Mountain View, CA). MHC-II- (Aa b-) mice backcrossed four times to B10.BR.

Cell Preparation and FACS® Sorting. Mature mouse thymocyte subsets were purified after one-step killing at 37°C with J11d.2 (anti-heat stable antigen [HSA]) mAb and rabbit complement followed by centrifugation over a density gradient (Lympholyte; Cedarlane, Hornby, Canada) and by three-color staining (as indicated in figure legends, using antibodies obtained from Pharmingen, San Diego, CA) for cell sorting with a FACStar plus® (Becton Dickinson & Co., Mountain View, CA) equipped with an argon dye laser. After sorting, cell samples were divided into duplicates and digested for genomic DNA analysis or extracted for mRNA analysis as described below.

Human PBL were isolated from healthy volunteers after centrifugation over Ficoll gradient (Ficoll-Paque; Pharmacia, Uppsala, Sweden), and treated with a cocktail of anti-CD19, CD14, CD4, and CD8 coated paramagnetic beads (Miltenyi Biotech, Bergish Gladbach, Germany) and passed through magnetic columns (magnetic cell separation [MACS] system; Miltenyi Biotech) to enrich for DN T cells. The cell preparation was subsequently stained with anti-CD8-PE (Amac, Inc., Westbrook, ME), anti-TCR-α/β-FITC (T Cell Sciences, Inc., Cambridge, MA) and biotinylated anti-CD4 (Caltag Laboratories, San Francisco, CA), followed by streptavidin-RED613 (Caltag Laboratories) and FACS® sorted to purify TCR-α/β® DN PBL.

T Hybridoma Generation. CD44+ mature thymocytes were obtained from 9-wk-old C57BL/6 mice after depleting thymocytes with J11d.2 (anti-HSA) and 3.155 (anti-CD8) mAbs plus rabbit complement, removing 3G11 and leukocyte cell adhesion molecule 1-positive cells with biotinylated mAb 3G11 (7E5, ME14), and streptavidin-coated paramagnetic beads (Miltenyi Biotech) using the MACS system, staining with anti-CD5-APC, anti-CD44-FITC, and anti-CD4-PE, and sorting CD5+CD44+CD4+ and CD5+CD44+CD4− (DN) cells. 105 cells of each subset were stimulated, in the presence of 4 × 106 γ-irradiated (30 Gy) low density (Per-55% fraction) spleen cells, with soluble anti-TCR-α/β TCR-α, β, BW5147 thymoma cells using standard procedures (15, 16). Reaction mixture was sampled through oil and transferred onto avidin-coated microtiter plates containing 95 μl of TE buffer for 16 h at 4°C, followed by Streptavidin-PE. TCR-α/β+CD44+CD4+ and TCR-α/β+CD44−CD4− DN cells were sorted and plated into 96-well plates, using an autoclone unit, at 2 × 104 cells/well amplified with a Vβ14-J281 primer pair for 46 cycles before sequencing of the PCR products, nested primers on Cβ: CCAAGCAGCAAGGTGACTCCT and Cα: CTCGGTGAGCTGACTGTC. For human PBL samples, the primer pair used for PCR amplification was Vβ24: CACAAAAAAGCTTCTGACACA and Cα: GCCCACAGACTGTTGCTCTTG; sequencing was performed using a nested Cα: TAGGGAGACAGACGTGTGGCACTGACA and Cβ: ACTGTCGGCTGACTGCACA.

Nuclear Acid Preparation. DNA was prepared by lysing 2 × 104 (unless specified otherwise) cells in 10 mM Tris-HCl, pH 9, 50 mM KCl, 2 mM MgCl2, 0.1% Triton X-100, 0.5% Tween 20, and 100 μg/ml Proteinase K at 56°C for 2 h and at 95°C for 20 min.

Total RNA was extracted with the RNAzole kit (TelTest, Austin, TX) and ethanol-prior to with addition of 5 μg of glycojen (Boehringer, Mannheim), and resuspended in 20 μl of Diethylpyrocarbonate (DEPC) water. Reverse transcription was carried out as described (16): briefly, 5 μl of RNA was denatured for 5 min at 65°C, quickly chilled on ice, and incubated in 20 μl reverse transcription buffer (100 mM KCl, 20 mM Tris-HCl [pH 9.0 at 25°C], 7 mM MgCl2, 1 mM dNTP, 2 mM dithiothreitol, 100 μM hexanucleotides, 5 U/reaction RNase inhibitor, and 4 U/reaction of avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI). Incubation was for 10 min at room temperature and 1 h at 42°C. Reverse transcription was stopped by incubation at 95°C for 5 min and at 99°C for 1 min.

Quantitative PCR. Quantitative PCR amplification was carried out as previously described (16). 1 μl of cDNA was added to 50 μl of amplification mixture (50 mM KCl, 10 mM Tris-HCl [pH 9.0 at 25°C], 2 mM MgCl2, 0.2 mM dNTP, 0.01% gelatin, and 0.1% Triton X-100) containing 0.25 μM 5′ and 3′ primers with 1.25 U/reaction Taq polymerase and overlaid with mineral oil (Sigma Chemical Co., St. Louis, MO). Temperature was initially at 94°C for 4 min, followed by cycles of 94°C for 45 s, 60°C for 1 min, and 72°C for 1 min in a thermal cycler (model PTC-100; M-J Research Inc., Watertown, MA). At sequential cycle numbers, 5 μl of the reaction mixture was sampled through oil and transferred onto avidin-coated microtiter plates containing 95 μl of TE buffer for quantitation of the amplified products in a liquid-hybridization-ELISA assay with luminometry readings (16).

Single Cell Analysis. Mature thymocytes enriched as described above were stained with anti-CD4-RED613 (GIBCO BRL, Gaithersburg, MD), anti-CD44-FITC, and anti-TCR-α/β-biotin (H57) followed by Streptavidin-PE. TCR-α/β/CD44+CD4+ TCR-α/β/CD44−CD4−, and TCR-α/β+CD44+ DN cells were sorted and plated into 96-well plates, using an autoclone unit, at 1 or 10 cells per well filled with 100 μl of PBS. After centrifugation, PBS was replaced with 10 μl of lysis solution and individual wells amplified with a Vβ14-J281 primer pair for 46 cycles before quantification of the amount of PCR products with a Vβ14-specific probe.

Sequencing. The following polyclonal sequencing of TCR-α chains was performed. After reverse transcription, cDNAs from fresh sorted populations were amplified with Vβ14-Cα (mouse) or
Vα24-Cα (human) primers for 40 cycles, purified (Magiclean; Promega), and sequenced using a nested primer in the constant region and the Cyclist kit (Stratagene, La Jolla, CA) with [33P]dATP. Data were digitalized with a phosphoimager (Molecular Dynamics, Inc., Sunnyvale, CA) and background was subtracted using the National Institutes of Health Image 1.52 software with the vertical ID subtraction procedure.

The following hybridoma TCR-α and β chain sequencing was performed. After characterization of Vα usage by flow cytometry using a panel of anti-Vα antibodies (Pharmingen), Vα-specific PCR amplification was carried out on cDNA using a Cα primer and Vα8-, Vα7-, or Vα2-specific primers. PCR products were cycle sequenced using a nested primer in the Cα region. For TCR-α chains, Vα14-J281 rearrangements were scored by amplifying genomic DNA extracted from 10⁶ hybridoma cells with the Vα14-J281 primer pair and ethidium bromide staining of agarose gel electrophoresis. DNA from eight individual Vα14 + hybridomas was amplified with the Vα14-J281 primer pair and sequenced with the same Vα14 primer as used for amplification.

Results

A Single TCR-α Chain Is Used by CD44hi NK1.1+ TCR-α/β+ Mature Thymocytes. To analyze the TCR Vα repertoire of the CD44hiNK1.1+ TCR-α/β+ thymocytes, we isolated them from the mature thymic population based on their expression of the activated, CD44hi phenotype. Using the few available anti-Vα antibodies, we found that although ~20% of the mainstream (CD44lo) cells expressed one of the Vα2-, Vα8-, or Vα11-TCR-α chains by FACS analysis, virtually none of these Vα chains were expressed among the CD44hi cells (data not shown). These results indicated that CD44hi cells might use a rather restricted set of Vα. To identify these Vα, we compared the expression of different TCR-α mRNAs in CD44hi and CD44lo mature thymocytes by quantitative reverse polymerase chain reaction (RT-PCR) using Vα-specific primers (15, 16). We found (Fig. 1) that Vα14 cDNA was strikingly increased in FACS-sorted CD44hi/CD4+ as well as DN cells, as compared with CD44lo cells, as judged by the difference in the number of amplification cycles necessary to generate similar amounts of PCR products. This increase was estimated to be in the range of 24-fold, using an external standard scale (data not shown). Quantitation of a panel of other Vα mRNAs showed a moderate decrease, usually in the range of two to three fold, as seen in Fig. 1 for Vα11. This relative conservation of Vα11 mRNA in CD44hi cells most likely represents nonallelically excluded and/or out of frame mRNAs (17), since FACS analysis of Vα11 expression showed a 14-fold decrease (0.5% in CD44hi vs 7% in CD44lo cells; data not shown). When the CD44hi cells were sorted according to their Vβ expression, the large increase in Vα14 mRNA was seen in all of the sub-populations studied (Vβ8.2+, Vβ7+, or Vβ8.2–) (Fig. 2), suggesting that TCRs with different Vβ nevertheless use Vα14. In addition, direct polyclonal sequencing from the Cα end of the PCR products obtained with a Cα-Vα14 primer pair, generated a readable sequence for CD44hi but not CD44lo cells (Fig. 3), showing that the Vα14 + CD44hi cells use predominantly one J region, J281, and that they do not display significant heterogeneity in their V-J junction. Experiments where CD44hi-derived products were diluted into CD44lo-derived products before polyclonal sequencing indicated that at least 50% of the amplified products need to

![Figure 1](image1.png)  
**Figure 1.** Increased expression of Vα14 mRNA in mature CD44hi thymocytes. Mature thymocytes (obtained after anti-HSA plus C killing) were stained with anti-CD4-PE, anti-CD44-FITC, and anti-CD8-APC and sorted to obtain the CD44hiCD4+, CD44hiCD4-, and CD44hiDN subsets. 10⁶ sorted cells were divided into duplicate samples of 0.5 × 10⁶ cells and processed for quantitative RT-PCR of Cα, Vα14, and Vα11 mRNAs.

![Figure 2](image2.png)  
**Figure 2.** Vα14 is paired with various Vβ in mature CD44hi thymocytes. Mature CD44hi thymocytes were enriched as described in Materials and Methods and sorted as CD5- (anti-CD5-APC), CD44hi (anti-CD44-FITC) cells, and Vβ8.2 positive or negative (anti-Vβ8.2-biotin plus avidin-PE) or Vβ7 positive (anti-Vβ7-biotin plus avidin-PE) cells. Quantiﬁcation of Cα, Vα14, and Vα11 mRNAs was carried out on duplicate samples of 3 × 10⁶ (Vβ8.2+ and Vβ8.2–) and 7.5 × 10⁶ (Vβ7+) cells and is represented as averaged values at sequential cycles of RT-PCR amplification.
be identical to generate such a readable sequence reaction (data not shown).

To measure the frequency of $V_{\alpha}14$-$J281$ TCR-\(\alpha\) cells in vivo, we sorted CD44\(^{hi}\) cells into 96-well plates at 1 cell/well and amplified genomic DNA with a specific $V_{\alpha}14$-$J281$ primer pair. 64 of 91 and 27 of 79 of the wells were positive in the CD44\(^{hi}\)/DN and CD44\(^{hi}\)/CD4\(^{+}\) sets, respectively (Fig. 4). In contrast, only 1 of 84 wells was positive among the mainstream CD44\(^{lo}\)/CD4\(^{+}\) cells plated at 10 cells/well. Thus, at least 34–70% of the CD44\(^{hi}\) cells had rearranged $V_{\alpha}14$ to J281. Furthermore, because only 55% of the wells scored positive in parallel plates amplified with a $C_\beta$-specific primer pair (data not shown), the frequency of $V_{\alpha}14$-$J281$ rearrangements is probably underestimated by this method because of the technical limitations in handling single cell plated wells.

To analyze the $V_{\alpha}$ and $V_{\beta}$ junctional regions, we generated T cell hybridomas from either CD4\(^{+}\) or DN FACS\(^{\text{®}}\)-sorted mature CD44\(^{hi}\) thymocytes. These hybridomas were representative of the fresh CD44\(^{hi}\) cell population because 24 of 27 expressed either $V_{\beta}8$, $V_{\beta}7$, or $V_{\beta}2$ TCR chains (Table 1). 23 of these 24 (96%) hybridomas had rearranged their $V_{\alpha}14$ segment to J281 confirming that CD44\(^{hi}\) cells use this $V_{\alpha}14$-$J281$ TCR-\(\alpha\) chain almost exclusively. The \(\alpha\) chains from eight of these hybridomas were sequenced and their V-J junctions found to be nearly monomorphic, with a glycine in position 93 and an aspartate in position 94, with a single exception in which a valine replaced glycine 93 (Table 2). Glycine 93 was either coded by a GGC corresponding to the genomic sequence of the V region, or by GGG or GGT, indicating trimming of the 3' end of the V region with at least one N addition. In one instance, aspartate 94 was coded by a GAC instead of a GAT implying that the 5' end nucleotides (TAGAT) of the J region were trimmed and replaced by TGAC. The invariant size of the junctional region and the tendency to conserve the glycine 93–aspartate 94 sequence, despite nucleotide changes, unambiguously indicates that the \(\alpha\) chain CDR3 region is selected at the amino acid level, most probably in order to maintain the specificity of the TCR. In contrast (Table 1), the $V_{\beta}$ chains used many different J regions (10 of the 12 available J\(\beta\) genes) and displayed a large variety of D/N regions of different sizes, sug-
Table 1.  TCR-β Chain V-D-J Junctional Amino Acid Sequences and Frequency of Vα14-J281 Rearrangements in T Cell Hybridomas Derived from DN and CD4+ Mature TCR-α/β+ CD44hi Thymocytes

| Hybridoma | Origin | Vα  | Vα | N/D | Jβ | Jβ | Vα 14-J281 |
|-----------|--------|-----|-----|-----|-----|-----|-------------|
| DN13H1    | DN     | 2   | CTC | KAAGGD | TEVFFG | 1.1 | +           |
| 411D1     | CD4    | 2   | CTCSA | DWEG | SAETLYFG | 2.3 | +           |
| 414A2     | CD4    | 7   | CASS | SDRAD | TGQLYFG | 2.2 | +           |
| 431G11    | CD4    | 8.1 | CASS | RR | SAETLYFG | 2.3 | +           |
| 432F6     | CD4    | 8.2 | CASG | ATGAT | NTEVFFG | 1.1 | +           |
| 431D12    | CD4    | 8.2 | CASGD | AGQGPA | NTEVFFG | 1.1 | +           |
| DN14F9    | DN     | 8.2 | CASG | QQG | NSDQYFG | 1.2 | +           |
| DN32H6    | DN     | 8.2 | CAS  | RE | SNERLFFG | 1.4 | +           |
| 432G7     | CD4    | 8.2 | CASGD | AGTGRVN | NPAPLFG | 1.5 | +           |
| 431A11    | CD4    | 8.2 | CASGD | KG | FRPLYFA | 1.6 | –           |
| 431G5     | CD4    | 8.2 | CASGD | AGG | TGQLYFG | 2.2 | +           |
| DN32D3    | DN     | 8.2 | CASGD | PD1 | QNTLYFG | 2.4 | +           |
| DN31E12   | DN     | 8.2 | CASGD | AWTGSG | QNTLYFG | 2.4 | +           |
| DN14C9    | DN     | 8.2 | CASG | PDWAG | NTLYFG | 2.4 | +           |
| 432B9     | CD4    | 8.2 | CASG | WGG | QDTQYFG | 2.5 | +           |
| 411B10    | CD4    | 8.2 | CASGD | YGERMGGR | QDTQYFG | 2.5 | +           |
| 431F10    | CD4    | 8.2 | CASGD | GLG | QDTQYFG | 2.5 | +           |
| DN32F3    | DN     | 8.2 | CASG | PPLGL | YEQYFG | 2.6 | +           |
| 431E13    | CD4    | 8.3 | CAS  | RDGRGH | TEVFFG | 1.1 | +           |
| 432B8     | CD4    | 8.3 | CAS  | KHEGTAR | APLFG | 1.5 | +           |
| DN13A1    | DN     | 8.3 | CASSD | GWGGA | AETLYFG | 2.3 | +           |
| 411G1     | CD4    | 8.3 | CASSD | AE | DTQYFG | 2.5 | +           |
| 432E4     | CD4    | 8.3 | CASSS | EEVDWG | YEQYFG | 2.6 | +           |
| 432F5     | CD4    | 8.3 | CASDD | PTVP | YEQYFG | 2.6 | +           |

Others DN (3) 2-, 7-, 8- – (3)

Table 2. Nucleotide and Amino Acid Sequences of Vα14-J281 Junctions from Eight T Cell Hybridomas Derived from DN and CD4+ Mature TCR-α/β+ CD44hi Thymocytes

| Germline Vα14 | TGT | GTG | GGC | Gcac | GAT | AGA | GGT | TCA | GCC | Germline J281 |
|---------------|-----|-----|-----|------|-----|-----|-----|-----|-----|---------------|
| Vα Sequences  |     |     |     |      |     |     |     |     |     |               |

| 5/8 | TGT | GTG | GGC | Gcac | GAT | AGA | GGT | TCA | GCC |
|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|
|     | C   | V   | G   | D    | R   | G   | S   | A   |     |
| 1/8 | .   | .   | .   | .    | GGG*| GAT | .   | .   | .   |
|     |    |    |    |      |    |     |    |    |    |
| 1/8 | .   | .   | .   | GTA  | GAT | .   | .   | .   | .   |
|     |    |    |    |      |    |     |    |    |    |
| 1/8 | .   | .   | .   | GGT  | GAC | .   | .   | .   | .   |
|     |    |    |    |      |    |     |    |    |    |

* N additions are in bold characters, and are underlined.
suggesting an absence of gross structural constraints in the CDR3 regions. Altogether, these results suggest that the CD44hi NK1.1+ TCR-α/β+ thymocytes do not constitute a distinct lineage using a genetically programmed TCR gene rearrangement (such as, for example, the V3+α/β T cells; 18, 19) but rather that they are selected after recognition by their TCR of thymic MHC-peptide complex(es). It is interesting to note that Vα14-J281 invariant α chains have previously been described in KLH-specific suppressor hybridomas and subsequently reported in various mouse tissues in vivo using RNase protection assays and quantitative PCR analysis (20–22). Although neither the cellular subset that expresses Vα14-J281, nor the associated β chains had been characterized, it is likely that the NK1.1+ TCR-α/β+ population studied by us is the main source of the Vα14-J281 rearrangements identified by these authors. Gut lymphocytes have also been reported to contain cells bearing a modified version of this invariant α chain with a deletion of aspartate 94 (23), emphasizing the importance of the CDR3 region in the selection process and suggesting that a slightly different MHC/peptide ligand is expressed in the gut.

The MHC Ligand. To characterize the MHC ligand required for selection of these cells, we performed a systematic analysis of their TCR repertoire in a panel of MHC congenic B10 mice. Surprisingly, we found the same frequencies of cells bearing Vα8, Vβ7, and Vβ2 positive TCRs, as well as a similar increase of Vα14-J281 rearrangements in all mice tested (Fig. 5). Using MHC knockout mice (11, 12), we confirmed that MHC class I but not II expression is required for the selection of these Vα14-J281+ cells (Fig. 6). We also confirmed that CD4+ but not CD8+ thymocytes include Vα14-J281+ cells and that forced (transgenic) expression of CD8 results in deletion of the Vα14-J281+ cells from the CD44hi thymocyte population (Fig. 6). This suggests that TCRs made of a Vα14-J281 α chain and of a Vα8, Vβ7, or Vβ2 β chain have a particular affinity for their MHC class I ligand and that they bind it in a classical way, allowing the interaction between CD8 and MHC class I (24, 25). Altogether, these data suggest that the putative selecting element for thymocytes may be a nonpolymorphic MHC class I molecule, located either inside or outside the MHC complex region, a hypothesis put forward (4, 10) because, like CD1 or Tla (26, 27), it is selectively expressed on bone marrow-derived but not epithelial thymic cells. Alternatively, though less likely, this ligand could be a nonpolymorphic peptide degenerately presented by polymorphic MHC molecules (28–31).

A Subset Similar to the Mouse NK1.1+ TCR-α/β+ Population Exists in Humans. There are some striking homologies between human and mouse TCR usage in DN T cell populations. For example, among the Vβ chains found in human DN lymphocytes (Vβ2, Vβ8, Vβ11, and Vβ13 [32–34], Vβ11 and Vβ13 are closer in sequence to the mouse Vβ8 and Vβ7 chains expressed in CD44hiNK1.1+ mouse thymocytes than to other mouse Vβ (34, and data not shown). Even more striking is the similarity that we found between the α chains.

**Figure 5.** TCR-β and -α repertoire of mature CD44hi thymocytes in MHC congenic mice. (Left) Percentage of Vα8+, Vβ7+, and Vβ2+ cells determined by FACS analysis among CD44hi and CD44lo subsets of B10 (H-2b), B10.D2 (H-2d), B10.A (H-2k), B10.BR (H-2k), B10.M (H-27), B10.RIII (H-2k), B10.S (H-2k), B10.Q (H-2b), B10.SM (H-2k), B10.P (H-2k), and B10.PL (H-2k) mice. (Right) Quantitation of Vα14-J281 rearrangements among FACS-sorted CD44hi and CD44lo subsets of B10, B10.D2, B10.BR, B10.M, B10.RIII, and B10.S mice. Values are averaged from duplicate samples of 2 x 10⁴ cells.

**Figure 6:** Role of MHC and CD8 molecules during the selection of Vα14-J281-positive thymocytes. Vα14-J281 rearrangements are measured as average values from duplicate samples of 2 x 10⁴ sorted cells. (A) CD44hi or CD44lo mature TCR-α/β+ thymocytes obtained from H-2b (MHC-II+) and H-2k (MHC-I−) mice. (B) CD4+, CD8+, or CD44hi mature TCR-α/β+ thymocytes from normal B10.BR mice (note that 24% of the CD4+ and 1.5% of the CD8+ population were CD44hi and CD44lo mature TCR-α/β+ thymocytes from CD8.1 transgenic B10.BR mice.)
Figure 7. The human homologues of mouse V$_{a}$14 and J281 gene products. (A) Alignments of mouse V$_{a}$14-J281 and human V$_{a}$24-JQ. (B) Pustell matrix comparison of V$_{a}$14 and V$_{a}$24, V$_{a}$14 and the closest mouse V$_{a}$ (V$_{a}$15), V$_{a}$24 and the closest human V$_{a}$ (V$_{a}$5.1). In Pustell matrix, each dot represents a similarity above 60% in a window of eight amino acids centered on the x and y coordinates as calculated with the Mac Vector program, using a PAM250 scoring matrix (IBI, New Haven, CT).

Figure 8. The human homologue of mouse NK1.1+$^{+}$ TCR-$\alpha$/$\beta$+ cells. Polyclonal sequencing of V$_{a}$24+$^{+}$ TCR-$\alpha$ chains from human DN and un-separated PBL. TCR-$\alpha$/$\beta$+ DN cells (0.6% of human PBL), were FACS$^{+}$ sorted and compared with whole PBL (80% of which are TCR-$\alpha$/$\beta$+).

It has been reported that V$_{a}$24-JQ is expressed as an invariant (without junctional variability) chain in some human DN PBL and clones (32, 33). According to GenBank, the human V$_{a}$24 has more homology to mouse V$_{a}$14 than any other mouse V$_{a}$ has and the closest protein sequence to mouse J281 is that of human JQ (Fig. 7, A and B). Using the Blast analysis software (35), the homology score between V$_{a}$14 and V$_{a}$24 was 316 whereas the closest other mouse or human V$_{a}$ was at 180 (mouse V$_{a}$15) and 223 (human V$_{a}$5.1), respectively; most of the other V$_{a}$ are below 160 (identity would be 480 and homology scores are considered significant above 50). The closest protein sequence to the mouse J281 recorded in GenBank is that of human JQ (score = 80 and identity is at 107) whereas all other mouse or human J regions have a score below 61. In accordance with the mouse data, we found a readable JQ sequence after direct polyclonal sequencing (from the C$_{a}$ end) of V$_{a}$24-C$_{a}$ PCR amplified products from freshly isolated human peripheral blood DN cells (Fig. 8), confirming their predominant use of JQ without junctional polymorphism. The importance of the junctional region for the selection of this cell population is indicated by the conservation of the unique VVG (or S) DRGGS sequence in the CDR3 of both mice and humans.

Discussion

Do NK1.1+$^{+}$ V$_{a}$14+$^{+}$ Thymocytes Originate from Mainstream Thymocyte Precursors or Do They Constitute a Distinct Lineage? Altogether, these results suggest that a unique recombination of one V$_{a}$ and one J$_{a}$ TCR gene, and a corresponding nonpolymorphic MHC class I molecule/peptide(s) for which this TCR-$\alpha$ chain has affinity, have been conserved in both mice and humans. The rare occurrence of such a V$_{a}$-J$_{a}$ recombination and its selection at the amino acid rather than the nucleotide level, suggest that this population does not represent a separate lineage that is genetically programmed to rearrange this particular set of TCR genes. The possibility
that this population is composed of cells that have escaped the “classical” positive selection process and are expanded as mature thymocytes by the recognition of self or environmental antigens is also unlikely, because, if this were the case, one would not expect to see a TCR repertoire that is solely restricted by nonpolymorphic MHC class I/peptide ligand(s). Rather, we favor a model where mainstream thymocyte DP precursors expressing the appropriate Vα14-J281/Vβ8, Vα7, or Vβ2 TCRs recognize nonpolymorphic MHC class I/peptide ligand(s) selectively displayed on the surface of bone marrow–derived thymic cells. They undergo a stochastic down-modulation of CD4, CD8, or both CD4 and CD8. The resulting CD4+ and DN cells, because of their intrinsic (CD8 independent) affinity for the selecting ligands, are rescued at the second stage of the positive selection process, following a model similar to one proposed by Chan et al. (36), whereas the CD8+ cells, after upregulation of their TCR level, reach the negative selection threshold and are eliminated. The particular CD44+NK1.1+ surface phenotype and the unique lymphokine secretion potential of this subset could be related to the particular affinity of the TCRs for the MHC class I molecule and/or the type of APC involved in presenting the selecting ligand.

The MHC Class I Ligand. Two findings suggest that a potential candidate for this conserved MHC molecule may lie in the Qa-1 family of genes. First, anti-Qa-1 CD8+ mouse T cell clones predominantly use the same Vβ chains (Vβ8, Vβ7, and Vβ2) as the NK1.1+ TCR-α/β+ T cells (37). Second, although most MHC genes, whether polymorphic or not, have diverged widely between species, Qa-1 molecules are very homologous to the rat RTBM.1 molecule and share unique features of their peptide binding cleft with both the rat RTBM.1 and the human HLA-E molecules (38). As for the peptides involved in the selection of this cell subset, the evidence that these cells can be obtained in long-term fetal thymic organ culture (4) suggests that they must be self-antigen(s). In line with the observation that tryptic digests of heat shock proteins stabilize the surface expression of Qa-1 (39), one may speculate that the selecting peptides are members of the widely conserved family of stress proteins. Once in the periphery, these cells might respond to upregulated levels of these same peptides or, alternatively, to foreign antigens that are structurally related, in an analogous manner to the γ/δ T cells that respond to heat shock proteins (40).

What Is the Function of the NK1.1+Vα14+ Thymocytes? The conservation through speciation events of such an unusual T cell subset bearing an invariant TCR-α chain associated with the recognition of nonpolymorphic MHC class I/peptide(s) strongly argues for an important, though yet uncharacterized, function for these cells. Since their particular TCRs are unlikely to recognize most foreign pathogens, they could be recruited after upregulation of their self-ligand(s) or recognition of variant, foreign peptides. Alternatively, their reactivation may result from downregulation of the NK-like molecules (or of their ligands), as it was recently suggested that some of these may bind MHC class I molecules and transduce inhibitory signals (41). One potential role for the NK1.1+Vα14+ thymocytes is hinted at by their unique property of being able to produce large quantities of IL-4 upon primary stimulation in vitro (37) and within minutes of TCR cross-linking in vivo (8). Because the early secretion of IL-4 appears to be critical for the generation of Th2-type humoral immune responses over Th1-type cell-mediated responses, the NK1.1+Vα14+ T cells may play a role in determining the Th1/Th2 phenotype of some immune responses to particular pathogens or to self-antigens (9, 42).

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Note added in proof: In the same issue of the Journal, P. Dellabona et al. (44) report a detailed analysis of the human blood Vα24-JQ+ T cells that further demonstrates the similarity to the mouse NK1.1+Vα14-J281+ T cell subset.

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