An Invariant T Cell Receptor $\alpha$ Chain Defines a Novel TAP-independent Major Histocompatibility Complex Class Ib-restricted $\alpha/\beta$ T Cell Subpopulation in Mammals

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Summary

We describe here a new subset of T cells, found in humans, mice, and cattle. These cells bear a canonical T cell receptor (TCR) $\alpha$ chain containing hAV7S2 and AJ33 in humans and the homologous AV19-AJ33 in mice and cattle with a CDR3 of constant length. These T cells are CD4$^-$CD8$^-$ double-negative (DN) T cells in the three species and also CD8$^{aa}$ in humans. In humans, their frequency was $\approx 1/10$ in DN, 1/50 in CD8$^+$, and 1/6,000 in CD4$^+$ lymphocytes, and they display an activated/memory phenotype (CD45RA$^{lo}$CD45RO$^+$). They preferentially use hBV2S1 and hBV13 segments and have an oligoclonal V$\beta$ repertoire suggesting peripheral expansions. These cells were present in major histocompatibility complex (MHC) class II– and transporter associated with antigen processing (TAP)-deficient humans and mice and also in classical MHC class I– and CD1-deficient mice but were absent from $\beta_2$-microglobulin-deficient mice, indicating their probable selection by a nonclassical MHC class Ib molecule distinct from CD1. The conservation between mammalian species, the abundance, and the unique selection pattern suggest an important role for cells using this novel canonical TCR $\alpha$ chain.

Key words: invariant T cell receptor $\alpha$ chain • CD4$^-$CD8$^-$ T cells • humans • cattle • mice

B and T lymphocytes display a wide repertoire of antigen receptors, made by random recombination of V, (D), and J segments and trimming/ addition of nucleotides at the junctions between these rearranged segments (1). Besides the mainstream lymphocytes, three different types of cell subpopulations have been described that have a limited repertoire diversity: the B1 B cell subset (2, 3), some $\gamma/\delta$ T cell subpopulations (4), and the TCR $\alpha/\beta$ NK1 T cells (5). Restricted repertoires seem to define discrete lymphocyte subpopulations at the frontier between innate and adaptive immunity, as these selected repertoires may allow the presence of a high frequency of preexisting cells reactive against phylogenetically conserved antigens (6, 7). Alternatively, cells with such a restricted repertoire may play an immuno-regulatory role or another physiologic function, such as the wound healing, that results from the secretion of keratinocyte growth factor (8) by $\gamma/\delta$ dendritic epithelial cells (DEC)\cite{9} recognizing self-ligands (9).

B1 cells, which appear early during embryonic life, use recurrent VDJ combinations with few “N” additions (10), and seem to be selected by endogenous ligands (11). Similarly, some $\gamma/\delta$ T cell subsets appear early during ontogeny and use peculiar V-J combinations without N additions (12). These recurrent sequences seem to be favored by enzymatic constraints linked to the recombination process, such as homologous region–guided recombination (4).
Cells bearing these invariant γ/δ TCRs seem to be also selected by endogenous ligands as, in mice deficient for these segments, they are replaced by cells expressing other Vγ/Vδ combinations but similar clonotypic motifs (13).

NK1 T cells use an “invariant” α chain (mAV14A18 in mice [14], hAV24A18 in humans [14, 15]) with a CDR3 of constant length paired with a limited number of Vβ segments (BV251, 751, and 852 in mice, BV1151 in humans). NK1 T cells display peculiar phenotypic characteristics: they are CD3 fascination, CD4++, or CD4–CD8– (DN), and have both activation (CD44® and CD69®) and NK markers (NKR1P1, Ly49) (5). In vivo development of NK1 T cells requires the MHC class Ib CD1d molecule (16), whose expression is dependent on the NKR1P1 receptor (22). However, their true in vivo functions are still enigmatic, though there is evidence that they may be involved in the Th1/Th2 cascade polarization (23), in IL-12-induced tumor rejection (24), and in autoimmunity (25-27).

Mainstream α/β T lymphocytes express either CD8 or CD4 accessory molecules and respond to peptidic antigens presented by MHC class I or class II molecules. The absence of accessory molecules in the DN T cell subpopulation suggests that these cells may recognize high-density ligands (such as α-galactosylceramide) in a C1d-restricted fashion (18, 19) as well as some exogenous zebrafish glycosylphosphatidylinositol (GPI [20]), but the exact endogenous ligand(s) presented physiologically by CD1d have not yet been identified. The most salient feature of NK1 T cells is their ability to secrete very rapidly large amounts of IL-4 in a primary response (21). They can also secrete large amounts of IFN-γ, especially when triggered through their NKR1P1 receptor (22). However, their true in vivo functions are still enigmatic, though there is evidence that they may be involved in the Th1/Th2 cascade polarization (23), in IL-12-induced tumor rejection (24), and in autoimmunity (25-27).

Human Cell Preparations. Heparinized blood was obtained from healthy donors or from MHC-deficient patients, and PBMCs were purified by Ficoll-Hypaque (Amersham Pharmacia Biotech) gradient centrifugation. Cells were frozen in liquid nitrogen until use.

Bovine Cell Separations. Bovine blood was provided by M. N. N. from the GENA Laboratory, Institut National de la Recherche Agronomique (INRA, Jouy en Josas, France). After Ficoll-Hypaque separation, PBLs were labeled with anti-CD4 and/or anti-CD8 mAbs, were obtained from PharMingen. Anti-CD4–FITC and -Tricolor (TC), anti-CD8–FITC and -PE, Anti-CD27–FITC and -TC, anti-CD4–FITC and -PE, and anti-CD3–TC were purchased from Caltag Laboratories. Anti-CD1d–FITC (Diaclone) recognizing an epitope distinct from the anti-CD4 magnetic beads was also used. Streptavidin–Red 613 was purchased from GIBCO BRL. Streptavidin–PE and -TC were obtained from Caltag Laboratories.

In mice, anti-CD4 (RM4-4 or RM4-4A–PE, anti-CD8α or anti-CD8β–FITC, biotinylated anti-CD4, and anti-CD8α, were obtained from Immunotech; anti-CD27–FITC and anti-CD1d–TC were purchased from Pharmingen. Anti-CD8α–FITC and -TC, anti-CD4–FITC and -PE, and anti-CD3–TC were obtained from Caltag Laboratories.

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Human and rodent cells are recognized by NK1 T cells. Indeed, most NK1 T cells recognize some glycolipids (such as α-galactosylceramide) in a C1d-restricted fashion (18, 19) as well as some exogenous zebrafish glycosylphosphatidylinositol (GPI [20]), but the exact endogenous ligand(s) presented physiologically by CD1d have not yet been identified. The most salient feature of NK1 T cells is their ability to secrete very rapidly large amounts of IL-4 in a primary response (21). They can also secrete large amounts of IFN-γ, especially when triggered through their NKR1P1 receptor (22). However, their true in vivo functions are still enigmatic, though there is evidence that they may be involved in the Th1/Th2 cascade polarization (23), in IL-12-induced tumor rejection (24), and in autoimmunity (25-27).

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NK1 T cells thus far represent the only known T cell subset that has conserved its TCR, its specificity, and its functional features between rodents and primates, suggesting that they may play an ancient and important physiological role (18). Here, we report on the existence of another phylogenetically conserved population of T cells in mammals, defined by the expression of a novel canonical TCR α chain comprising the hAV752 and AJ33 elements in humans and the homologous AV19 and AJ33 elements in mice and cattle. Vα7/19-Jα33+ T cells show an oligoclonal TCR β chain repertoire and are selected by a p2m-dependent but TAP-independent molecule that is neither a classical MHC class I molecule nor CD1d. The conservation between species of T cells displaying this limited repertoire and their unusual restriction pattern suggest that this population may serve a function complementary to that played by NK1 T cells.

Materials and Methods

Antigens. In humans, the following antibodies, anti-CD28–PE, anti-CD45RA–PE, anti-CD45RO–PE, anti-CD57–FITC, anti-CD56–PE, anti-CD1a, b, or c (30-32).

Cells bearing these invariant γ/δ TCRs seem to be also selected by endogenous ligands as, in mice deficient for these segments, they are replaced by cells expressing other Vγ/Vδ combinations but similar clonotypic motifs (13).

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obtained using paramagnetic beads and the VarioMacs system (Miltenyi Biotec). In brief, cells at 10⁷/100 μl were resuspended in PBS, 0.5% BSA, 5 mM EDTA and incubated with anti-CD4 paramagnetic microbeads, washed once, resuspended in 500 μl of the same buffer, and passed through an R column. Eluent was collected as the CD8⁺ fraction. After thorough washes of the column, the eluate was collected as CD4⁺ fraction. In these conditions, the CD4⁺ fraction was ~95% pure.

Oligonucleotides. All primers and probes were obtained from Genosys Biotechnologies and used without further purification. Primers were FITC conjugated. The following primers have been described previously (14): mAV14, mAJ18, mAC-5', mACV, mAC-3', hACV, hAC-5', and hAC-3'. The following primers were used (m, bo, and h stand for murine, bovine, and human; p is for probe; in and out are for the inner and outer primer, respectively, in the case of nested PCR): mAV19, CACTTCT-GAGCCGCTCGAA; mAJ33, biotin-TTAGCTTGGTCCAGGAGCC; pmAV19, GCTTCTGACAGCTCCAG-FITC. The mouse-specific Vp and Vα primers were slightly modified from Casanova et al. (34). The human Vβ primers were derived from Puisieux et al. (35) as modified by Martinon et al. (36). The other human primers were as follows: hAV7S2, biotin-CCTG; hAV24out, AJ33out, and as positive control AV24 and AV18Out. The reaction resulting mix was diluted fivefold, and aliquots were amplified in a second PCR reaction with an AV7S2/AJ33 primer pair for 40 cycles. The specific amplification was characterized by 17 Vβ-specific PCR reactions, and the resulting product was sequenced as described below.

Sequencing. Polyclonal sequencing was performed after amplification for 42 cycles with hAV7S2, boAV19, or mAV19 and either AJ33 or Cα primer pairs. The amplifications were purified using the Wizard PCR clean-up system (Promega Corp.) and sequenced using either a nested primer in the Cα constant region (in mouse) or the same AV19 or AV7S2 primer(s) and a mixture of 7 Jα primers for 45 cycles. The positive reactions were detected by agarose gel electrophoresis, and the amplitcons were sequenced as described below.

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Quantitative Kinetic PCR. Quantitative kinetic ELISA PCR was carried out as described (37). Polyclonal samples were divided into two aliquots before RNA extraction except in a few instances in which the amount of sorted cells was too low (~2 × 10⁶). Averages are values shown.

In the most recent experiments, an ABI prism 7700 apparatus was used instead of the ELISA assay to monitor the amount of ampiclon during the PCR. This apparatus is based on the 5'-3' nuclease activity of Taq polymerase (38), which allows the release of a fluorescent reporter during the PCR. A probe labeled with both a reporter and a quencher dye is spiked into the PCR mix at the beginning of the reaction. The sequences of the Taqman probes used in this study are the following: hCA, mGATGTGCAAAACGCCTTCAACACAG; hAV7.2, mTGAAAGACTCTGCTTACTGCCTTGTGC; mAC, mTCCTCCAAATGATGGCCCGAAACAG; mAV19, mTCCTAGGAGAAGACCTTTACTACCTGT; and mACV, mAACCTCTGCGATGACACT GCCA. Where m preceding each sequence stands for FAM and qp following for TAMRA blocked with a phosphate. When using the ABI prism 7700 apparatus, the following primers were also used: hAV7S2, TCTCTACTGTCAAGGATGTTAC; hAJ33, CACCGGCGCCAGATTAA; hAC-5', ACCCTGAACCTTCCGGT; hAC-3', GCGTAGGAGAAAAGTTGCTT; mAV14, TGGAGATCTACGACCTCCTG; and mAC18, CACAGCTCCTAATGACGCC; mAV19, CCTTCTGAGCCGCCCTAAG; and mAC3', CGTCAACGTGGCCTTCACTACA. In these quantitative kinetic PCR methods, for two samples containing the same amount of C±, a shift of n cycles along the x-axis of the two amplification curves represents an ~1.8-fold difference in the two samples studied.
Results

A previous analysis of TCR α chain repertoire of human DN T cells indicated that besides the invariant hAV24AJ18 TCR α chain, another TCR α chain was frequently expressed that also had a restricted AVAJ usage and recurrent junctional features (39). This α chain, which was encoded by rearranged hAV72AJ33 elements, had a CDR3 of constant length but some variability in the two junctional codons. A GenBank search showed that among the 20 TCR α chains sequenced in cattle, one chain (BOTCRA14) used the homologous Vα and Jα segments with a CDR3 of the same length (40). In mice, no homologous invariant α chain had been described, but the corresponding Vα (mAV19) and Jα (mAJ33) segments display high homology with their human counterparts, as there is only one amino acid difference between the murine AJ33 and its human counterpart (41, 42). Based on these observations, which suggested the existence of another conserved T cell population in mammals, we undertook an extensive analysis of the frequency and repertoire of the T cells bearing this particular combination of AVAJ elements in these three species.

Canonical hAV72AJ33 TCR α Chains, as well as Their Murine and Bovine Homologues, Are Abundantly Expressed in DN T Cells

Homologous hAV72AJ33 or bo/mAV19AJ33 Transcripts Are Enriched in Human, Bovine, and Murine DN T Cells. To determine the frequency and coreceptor phenotype of T cells bearing hAV72AJ33 TCR α chains, PBLs were separated into highly purified α/β DN, CD4\(^+\), or CD8\(^+\) (i.e., CD8αβ\(^+\) and CD8αβ\(^-\)) subsets, and the amount of hAV72AJ33-encoded transcripts in duplicate fractions was quantified by kinetic PCR. Although the amounts of Cα chain were similar in all samples (Fig. 1 A), there was a large shift to the left of the hAV72AJ33 amplification curves for the DN (8.5 cycles) and the CD8\(^+\) fractions (5 cycles) (Fig. 1 B) compared with the CD4\(^+\) fractions, indicating that DN and CD8α\(^+\) cells contained \(\sim\)150–360- and 19–32-fold more hAV72AJ33 transcripts, respectively, than CD4\(^+\) lymphocytes. hAV72AJ33 transcripts were enriched in DN and CD8α\(^+\) T cell preparations from all individuals tested, and on average the enrichment levels of hAV72AJ33 transcripts in DN T cells were much higher than those observed for the NK1 T cell–specific invariant α chain hAV24AJ18 (data not shown).

Because a similar invariant α chain had been sequenced once in cattle (40), we examined its distribution within all samples (Fig. 1 A), there was a large shift to the left of the hAV72AJ33 amplification curves for the DN (8.5 cycles) and the CD8α\(^+\) fractions (5 cycles) (Fig. 1 B) compared with the CD4\(^+\) fractions, indicating that DN and CD8α\(^+\) cells contained \(\sim\)150–360- and 19–32-fold more hAV72AJ33 transcripts, respectively, than CD4\(^+\) lymphocytes. hAV72AJ33 transcripts were enriched in DN and CD8α\(^+\) T cell preparations from all individuals tested, and on average the enrichment levels of hAV72AJ33 transcripts in DN T cells were much higher than those observed for the NKT cell–specific invariant α chain hAV24AJ18 (data not shown).

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suggesting that mAV19AJ33-bearing cells were less abundant in mice.

hAV7S2-AJ33, mAV19AJ33, and boAV19AJ33 transripts Display the Same CDR3 Length in the Three Species. To examine the length of the CDR3 regions of the hAV7S2AJ33 transcripts and their homologues in mice and cattle, we carried out polyclonal sequencing of Vα-CA or Vα-JA amplicons obtained from the various lymphocyte subpopulations in these species. As shown in Fig. 2 A, a readable hAV7S2AJ33 sequence was obtained from hAV7S2-CA amplicons derived from DN and CD8α1 samples but not from CD4α1 samples. Therefore, this indicated that in the former but not the latter cells, the hAV7S2 segment was predominantly rearranged to the hAJ33 segment and comprised a CDR3 of constant length. The polyclonal hAV7S2AJ33 sequence displayed some heterogeneity at the VJ junction, in agreement with previous results from Porcelli et al. showing some variability of the two N-encoded amino acids (39).

In cattle, the picture was slightly different, as polyclonal sequencing of boAV19-Cα amplicons demonstrated the predominant presence of the invariant boAV19AJ33 chain in DN cells, but not in CD4+ or CD8+ cells (data not shown). At the VJ junction, the polyclonal sequence derived from DN cells displayed some junctional heterogeneity, indicating also some variability of the two amino acids encoded by N additions. In mice, polyclonal sequencing of the mAV19-Cα amplicons showed a fully readable sequence for the DN but not for the CD8α+ fraction (data not shown). Moreover, the VJ sequence displayed no heterogeneity and corresponded to a “germline” junction without nucleotide trimming or N addition (see also Table

| Cattle | Mouse | Human |
|--------|-------|-------|
|        |       |       |
|        |       |       |
|        |       |       |

Figure 3. High homology of human, cattle, and mouse hAV7S2/AV19-AJ33 α chains. Homologies were scored using the default PAM 250 of ClustalW as implemented in MacVector software (Oxford Technology). Identities are bold and shaded in gray; similarities are light gray. The CDR3 region is boxed.
Using a single cell deposition unit. FACS®-sorted from three different subjects were sorted into PCR plates to hereafter as the "invariant" Vα7/19-Jα33 TCR chain.

This novel canonical TCR CD3 length as shown in the multiple alignment displayed (hAV7S2AJ33, mAV19AJ33, and boAV19AJ33) and constant Vα33 chain. Thus, the actual frequency of CD4+ cells harboring the invariant Vα7-Jα33 chain should be ~1/6,000.

In mice, Vα19-Jα33 cells were less numerous than in humans. Their frequency within α/β DN lymph node cells was 1/56 (Fig. 5 A), compared with ~1/7.5 in humans (Fig. 4 A).

Surface Phenotype and TCR β Repertoire of Vα7/19-Jα33-Bearing Cells

Invariant Vα7-Jα33-bearing cells express memory markers and show a biased usage of hBV2 and hBV13 TCR β chains. In the absence of anti-hAV7S2 or anti-mAV19 antibody, we used the following strategy to characterize the surface phenotype of the population expressing the invariant Vα7-Jα33 chain. α/β DN and CD8α+ cells were depleted or enriched for a given marker by FACS® sorting, and the amounts of Vα7-Jα33 transcripts in the positive and negative fractions were quantified and compared with the amounts of amplified Cα transcripts, in order to take into account the actual frequency of CD4+ cells expressing invariant Vα7-Jα33 chains. Importantly, sequencing of the positive wells at concentrations where only one (sequence 3) corresponded to the invariant Vα7-Jα33 chain.

In hAV7S2 AJ33, single α/β+ DN cells from three different subjects were sorted into PCR plates using a single cell deposition unit. After cell lysis, hAV7S2AJ33 PCR amplification was carried out on genomic DNA with lumino-metry detection of the amplicons. As shown in Fig. 4 A, the assay was sensitive enough to detect a single cell harboring the relevant rearrangement and yielded a frequency of Vα7-Jα33+ cells of ~1/7.4. Using limiting dilution analysis (LDA), the frequency observed for CD8α+ cells was 1/36 (confidence interval: 1/25–1/70; Fig. 4 B). Sequence analysis of amplicons from single cell preparations obtained with DN and CD8α+ cells demonstrated the presence of invariant Vα7-Jα33 transcripts in all cases. A similar analysis on CD4+ cells yielded a frequency of Vα7-Jα33+ cells of 1/1,093 (1/751–1/2,003; Fig. 4 C). Importantly, sequencing of the positive wells at concentrations where the Vα7-Jα33 amplicons were derived from one cell showed that out of six positive wells, two corresponded to a rearrangement of the hAV7S2 to hAJ34 (which were amplified with AV7S2 and AJ33 primers, and the amount of amplicons was quantified by ELISA). (B and C) α/β CD8α+ or CD4+ cells were obtained by FACS® sorting, serially diluted, distributed in a microwell plate at the indicated cell concentration, and processed as above. In C, the amplicons of the six wells indicated (which had a high probability of clonality) were sequenced. The sequences were as follows:

Figure 4. Direct enumeration of AV7S2/AV19-AJ33-bearing cells by PCR–limiting dilution analysis and single cell PCR. (A) DN cells obtained from three human subjects were seeded into microtiter plates using a FACS® single cell deposition unit. After cell lysis, PCR was carried out with AV7S2 and AJ33 primers, and the amount of amplicons was quantified by ELISA. (B and C) α/β CD8α+ or CD4+ cells were obtained by FACS® sorting, serially diluted, distributed in a microtiter plate at the indicated cell concentration, and processed as above. In C, the amplicons of the six wells indicated (which had a high probability of clonality) were sequenced. The sequences were as follows:

1-GCT GTG ATG TCG AT
2-GCT GTG AGA GAG GAT AAC-AJ34-intron-AJ33
3-GCT GTG AGA GAG ATC GGA
4-GCT GNN ACT CGA
5-GCT GTG AGA GAG GAT AAC-AJ34-intron-AJ33
6-GCT GTG AGG GGG GGG GAA AAC-AJ34-intron-AJ33

Only one (sequence 3) corresponds to the canonical AV7S2-AJ33 rearrangement (see text for details). In B and C, frequencies were calculated by maximum likelihood analysis.
account variations in cell number. Fig. 6 displays an example of such an experiment, where we examined whether the CD8 molecules expressed by Vα7-Jα33+ cells were either heterodimeric (αβ) or homodimeric (αα), since CD8αα+ cells represent between 5 and 25% of CD8α+ TCR α/β+ lymphocytes in human PBLs (data not shown). There were ~45-fold more Vα7-Jα33 transcripts in the CD8αα+ fraction than in the CD8αβ+ fraction. This result was confirmed by polyclonal sequencing of the amplicons obtained after amplification with hAV7S2/Cα (V-C) or hAV7S2/hAJ33 (V-J) primers: no readable sequence was obtained from the CD8αβ+ fraction, whereas the invariant α chain was present in the CD8αα+ fraction (Fig. 2 B).

The absence of a readable sequence in the CD8αβ+ fraction using V-J amplification indicates the near absence of the invariant α chain in this fraction. Using this strategy, it could be established that Vα7-Jα33+ cells had mainly a memory phenotype (i.e., CD45R0+CD57−CD56−) and were CD56−CD57−CD28+CD27+ (data not shown).

Despite many attempts using different lymphokine mixtures, we were unable to obtain a large enough number of T cell clones expressing the invariant Vα7-Jα33 chain. Therefore, the TCR β chain repertoire of Vα7-Jα33 chain was directly estimated by single cell PCR analysis. After single cell deposition of DN cells bearing invariant Vα7-Jα33 chain into PCR plates, cells were lysed and a first PCR reaction was carried out using hAV7S2, hAJ33, and a mixture of 29 Vβ and 7 Jβ. This first reaction was then amplified in a second PCR with nested AV7S2/AJ33 primers allowing us to find the bands containing Vα7-Jα33+ cells. Using the reaction mixture of the first PCR corresponding to the positive wells, 24 individual PCR reactions were set up using 1 (or 2) Vβ and a mixture of 7 Jβ primers. This allowed us to get a specific band for 0–2 Vβ which was then sequenced using the relevant Vβ primer. In 9 independent experiments carried out on DN cells obtained from 3 different subjects, the frequency of Vα7-Jα33+ wells ranged between 6 and 16/84. Among the 92 Vα7-Jα33+ wells studied, a β chain could be assigned and sequenced in 37 cases (40%), and we found that there was a heavy bias toward Vβ13 and Vβ2. Of 13 Vβ sequences in donor A, 7 were BV13+ and 3 were BV2+. In donor B, 8/12 were BV13+ and 4/12 were BV2+. In donor C, 7/12 were BV13+ and 2/12 were BV2+. However, despite this biased usage of BV13 or BV2 regions, the TCR β chains derived from Vα7-Jα33+ cells did not show obvious restrictions in βJ usage or in CDR3 length (Table I). Sequence analysis of Vα/Jα junctions confirmed the presence of Vα7-Jα33 chains with the canonical CDR3 length and revealed significant variations in amino acid composition at the two N-encoded positions (Table I). Moreover, the presence of repeated TCR α and β chain junctional sequences in different cells from the same subject cloned in different PCR plates and the fact that these TCR chains were not found in other subjects indicated that this subset had undergone clonal expansion in vivo, an interpretation that is consistent with its memory phenotype (see above). Overall, Vα7-Jα33+ cells appear to use a semirestricted repertoire that is expanded as needed.

TCR β Chain Repertoire of Vα19-Jα33+ Cells. Because the frequency of Vα19-Jα33+ cells is not as high in mice as in

**Figure 5.** mAV19-AJ33+ cells are more frequent in TAP−/− mice. The indicated number of DN T cells from B6 (A) or TAP−/− (B) mice were seeded in PCR microplates, and the presence of the mAV19-AJ33 rearrangement was detected by PCR-ELISA.

**Figure 6.** hAV7S2-AJ33–bearing cells are CD8αα+ or DN. The indicated TCR α/β− fractions were FACS® sorted using a four-color staining with anti-CD4-TC, anti-CD8-FITC, anti-CD8+PE, and anti-α/β TCR-APC. The amounts of Cα (A) or hAV7S2-AJ33 (B) transcripts were quantified by kinetic PCR using the ABI Prism 7700 apparatus (Taqman). This experiment was repeated with cells from another subject with identical results. ΔRn, difference in fluorescence relative units.
humans, the strategy followed to characterize the surface phenotype of human Vα7-Jα33+ cells was not successful in mice. Therefore, to characterize the TCR β chain repertoire of this subset, we took advantage of the fact that Vα19-Jα33+ cells are enriched in TAP−/− mice (see below). We generated T–T hybridomas from DN enriched lymph node cells from TAP−/− mice, and screened for expression of Vα19-Jα33 transcripts. In agreement with the single cell analysis (Fig. 5 B), 16/168 hybridomas studied were Vα19-Jα33+. As shown in Table II, in all cases, these hybridomas carried Vα19-Jα33 chains with the canonical CDR3 length and in all but two cases, the Vα19-Jα33 junction was germline encoded. The Vβ segments used were predominantly BV8− (7/16) and BV6+ (4/16). No restriction in the Jβ repertoire was apparent.

Development and Selection of Vα7/19-Jα33–bearing Cells

Ontogeny of Vα7/19-Jα33–bearing Cells. NK1 T cells are not found in neonates and accumulate after birth (5). Therefore, we examined cord blood lymphocytes for Vα7/19-Jα33 sequences by quantitative PCR and polyclonal sequencing of Vα7-Jα33 amplicons on cDNA obtained from enriched DN/CD8+ or CD4+ fractions. We were unable to find significant amounts of invariant Vα7-Jα33 transcripts in cord blood in the six samples studied. However, significant amounts of this chain could be obtained in young children, albeit in lower amounts than in adults (data not shown).

Similarly, Vα19-Jα33–bearing cells were not found in spleen or thymus of mouse neonates (data not shown). They were present in lymph nodes and blood in large amounts and in smaller amounts in the spleen in the five mouse strains tested, as shown in Table III (top), which displays a summary of the studies examining the tissue distribution of Vα19-Jα33+ cells.

Table I. Vβ repertoire of Human Invariant AV7S2-AJ33 TCR α Chain–bearing Cells

| Vα-Jα69 | Vα-Jα7-Jα33 | Vβ | CDR3 | Jβ |
|---------|-------------|-----|------|----|
| A       | AVROS       | 13.3 | MNKQGKSG  | 2.2 | 3 |
| B       | AVROS       | 13.2 | MNKQGKSG  | 2.1 | 3 |
| C       | AVROS       | 13.3 | MNKQGKSG  | 2.2 | 3 |
| D       | AVROS       | 13.3 | MNKQGKSG  | 2.2 | 3 |
| E       | AVROS       | 13.3 | MNKQGKSG  | 2.2 | 3 |
| F       | AVROS       | 13.3 | MNKQGKSG  | 2.2 | 3 |
| G       | AVROS       | 13.3 | MNKQGKSG  | 2.2 | 3 |
| H       | AVROS       | 13.3 | MNKQGKSG  | 2.2 | 3 |
| I       | AVROS       | 13.3 | MNKQGKSG  | 2.2 | 3 |
| J       | AVROS       | 13.3 | MNKQGKSG  | 2.2 | 3 |
| K       | AVROS       | 13.3 | MNKQGKSG  | 2.2 | 3 |
| L       | AVROS       | 13.3 | MNKQGKSG  | 2.2 | 3 |
| M       | AVROS       | 13.3 | MNKQGKSG  | 2.2 | 3 |
| N       | AVROS       | 13.3 | MNKQGKSG  | 2.2 | 3 |

Table II. Summary of the Mouse Vβ Repertoire of T–T mAV19-Jα33+ Hybridomas

| Vβ | CDR3 | Jβ |
|----|------|----|
| 1. N ot done. | | |

Single TCR α/β+ DN T cells were FACSy sorted into 96-well PCR plates. After screening for the presence of the invariant AV7S2-AJ33 chain, individual TCR α and β chains of the positive wells were characterized and sequenced as described in Materials and Methods. The β chain could be assigned for 40% of the wells. Three independent PCR plates were studied for each subject, as indicated in the last column.

Small caps code for the recombination sequence signal.

b, c, d: identical TCR α and β sequences probably representing a single expanded clone.

fNot done.
DN α/β T cells were purified from the indicated organs in different mouse strains. Expression of the invariant mAV19-AJ33 TCR α chain was estimated by quantitative kinetic PCR and confirmed by polyclonal sequencing (see Materials and Methods, and Figs. 1 and 2). Tissue distribution results are compared with the expression in sorted CD4+ α/β T cells: +++, high expression (>3-cycle shift to the left); +, no increase; −, absence as judged by polyclonal sequencing of mAV19-AJ33 amplicons. Selection results (bottom) in the lymph nodes of the different MHC-deficient mice are compared with the expression in DN cells from B6 lymph nodes using quantitative PCR. All of the results were also confirmed by polyclonal sequencing after mAV19-Cα, or AJ33 amplification as appropriate.

Table III. Strain and tissue distribution, and MHC selection of mAV19-AJ33 T Cells

| Strain     | Haplotype | Lymph nodes | Spleen | PBLs | Thymus | Bone marrow | Liver | IELs | Lamina propria |
|------------|-----------|-------------|--------|------|--------|-------------|-------|------|----------------|
| BALB/c     | H-2d/1-A+ | +++         | +      | +++  | +      | +           | −     | −    | −              |
|            | I-A+/I-E+ |             | +      | +    | +      |             | −     | −    | −              |
| DBA/2      | H-2d/1-A+ | +++         | +      | +    | +      |             | −     | −    | −              |
|            | I-A+/I-E+ |             | +      | +    | +      |             | −     | −    | −              |
| CBA        | H-2k/1-A+ | +++         | +      | +    | +      |             | −     | −    | −              |
|            | I-A+/I-E+ |             | +      | +    | +      |             | −     | −    | −              |
| 129        | H-2b/1-A- | +           | +++    | +    | +      |             | −     | −    | −              |
|            | I-A+/I-E- |             | +      | +    | +      |             | −     | −    | −              |
| C57BL/6    | H-2b/1-A- | +++         | +      | +    | +      |             | −     | −    | −              |
|            | I-A+/1-E- |             | +      | +    | +      |             | −     | −    | −              |
| NUDE B6    | H-2b/1-A- | −           | −      | −    | −      |             | −     | −    | −              |
|            | I-A+/1-E- |             | −      | −    | −      |             | −     | −    | −              |

DN α/β T cells were purified from the indicated organs in different mouse strains. Expression of the invariant mAV19-AJ33 TCR α chain was estimated by quantitative kinetic PCR and confirmed by polyclonal sequencing (see Materials and Methods, and Figs. 1 and 2). Tissue distribution results are compared with the expression in sorted CD4+ α/β T cells: +++, high expression (>3-cycle shift to the left); +, no increase; −, absence as judged by polyclonal sequencing of mAV19-AJ33 amplicons. Selection results (bottom) in the lymph nodes of the different MHC-deficient mice are compared with the expression in DN cells from B6 lymph nodes using quantitative PCR. All of the results were also confirmed by polyclonal sequencing after mAV19-Cα, or AJ33 amplification as appropriate.

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| Strain     | Haplotype | Lymph nodes | Spleen | PBLs | Thymus | Bone marrow | Liver | IELs | Lamina propria |
|------------|-----------|-------------|--------|------|--------|-------------|-------|------|----------------|
| BALB/c     | H-2d/1-A+ | +++         | +      | +++  | +      | +           | −     | −    | −              |
|            | I-A+/I-E+ |             | +      | +    | +      |             | −     | −    | −              |
| DBA/2      | H-2d/1-A+ | +++         | +      | +    | +      |             | −     | −    | −              |
|            | I-A+/I-E+ |             | +      | +    | +      |             | −     | −    | −              |
| CBA        | H-2k/1-A+ | +++         | +      | +    | +      |             | −     | −    | −              |
|            | I-A+/I-E+ |             | +      | +    | +      |             | −     | −    | −              |
| 129        | H-2b/1-A- | +           | +++    | +    | +      |             | −     | −    | −              |
|            | I-A+/I-E- |             | +      | +    | +      |             | −     | −    | −              |
| C57BL/6    | H-2b/1-A- | +++         | +      | +    | +      |             | −     | −    | −              |
|            | I-A+/1-E- |             | +      | +    | +      |             | −     | −    | −              |
| NUDE B6    | H-2b/1-A- | −           | −      | −    | −      |             | −     | −    | −              |
|            | I-A+/1-E- |             | −      | −    | −      |             | −     | −    | −              |

DN α/β T cells were purified from the indicated organs in different mouse strains. Expression of the invariant mAV19-AJ33 TCR α chain was estimated by quantitative kinetic PCR and confirmed by polyclonal sequencing (see Materials and Methods, and Figs. 1 and 2). Tissue distribution results are compared with the expression in sorted CD4+ α/β T cells: +++, high expression (>3-cycle shift to the left); +, no increase; −, absence as judged by polyclonal sequencing of mAV19-AJ33 amplicons. Selection results (bottom) in the lymph nodes of the different MHC-deficient mice are compared with the expression in DN cells from B6 lymph nodes using quantitative PCR. All of the results were also confirmed by polyclonal sequencing after mAV19-Cα, or AJ33 amplification as appropriate.

Table III. Strain and tissue distribution, and MHC selection of mAV19-AJ33 T Cells

| Strain     | Haplotype | Lymph nodes | Spleen | PBLs | Thymus | Bone marrow | Liver | IELs | Lamina propria |
|------------|-----------|-------------|--------|------|--------|-------------|-------|------|----------------|
| BALB/c     | H-2d/1-A+ | +++         | +      | +++  | +      | +           | −     | −    | −              |
|            | I-A+/I-E+ |             | +      | +    | +      |             | −     | −    | −              |
| DBA/2      | H-2d/1-A+ | +++         | +      | +    | +      |             | −     | −    | −              |
|            | I-A+/I-E+ |             | +      | +    | +      |             | −     | −    | −              |
| CBA        | H-2k/1-A+ | +++         | +      | +    | +      |             | −     | −    | −              |
|            | I-A+/I-E+ |             | +      | +    | +      |             | −     | −    | −              |
| 129        | H-2b/1-A- | +           | +++    | +    | +      |             | −     | −    | −              |
|            | I-A+/I-E- |             | +      | +    | +      |             | −     | −    | −              |
| C57BL/6    | H-2b/1-A- | +++         | +      | +    | +      |             | −     | −    | −              |
|            | I-A+/1-E- |             | +      | +    | +      |             | −     | −    | −              |
| NUDE B6    | H-2b/1-A- | −           | −      | −    | −      |             | −     | −    | −              |
|            | I-A+/1-E- |             | −      | −    | −      |             | −     | −    | −              |

DN α/β T cells were purified from the indicated organs in different mouse strains. Expression of the invariant mAV19-AJ33 TCR α chain was estimated by quantitative kinetic PCR and confirmed by polyclonal sequencing (see Materials and Methods, and Figs. 1 and 2). Tissue distribution results are compared with the expression in sorted CD4+ α/β T cells: +++, high expression (>3-cycle shift to the left); +, no increase; −, absence as judged by polyclonal sequencing of mAV19-AJ33 amplicons. Selection results (bottom) in the lymph nodes of the different MHC-deficient mice are compared with the expression in DN cells from B6 lymph nodes using quantitative PCR. All of the results were also confirmed by polyclonal sequencing after mAV19-Cα, or AJ33 amplification as appropriate.

Table III. Strain and tissue distribution, and MHC selection of mAV19-AJ33 T Cells

| Strain     | Haplotype | Lymph nodes | Spleen | PBLs | Thymus | Bone marrow | Liver | IELs | Lamina propria |
|------------|-----------|-------------|--------|------|--------|-------------|-------|------|----------------|
| BALB/c     | H-2d/1-A+ | +++         | +      | +++  | +      | +           | −     | −    | −              |
|            | I-A+/I-E+ |             | +      | +    | +      |             | −     | −    | −              |
| DBA/2      | H-2d/1-A+ | +++         | +      | +    | +      |             | −     | −    | −              |
|            | I-A+/I-E+ |             | +      | +    | +      |             | −     | −    | −              |
| CBA        | H-2k/1-A+ | +++         | +      | +    | +      |             | −     | −    | −              |
|            | I-A+/I-E+ |             | +      | +    | +      |             | −     | −    | −              |
| 129        | H-2b/1-A- | +           | +++    | +    | +      |             | −     | −    | −              |
|            | I-A+/I-E- |             | +      | +    | +      |             | −     | −    | −              |
| C57BL/6    | H-2b/1-A- | +++         | +      | +    | +      |             | −     | −    | −              |
|            | I-A+/1-E- |             | +      | +    | +      |             | −     | −    | −              |
| NUDE B6    | H-2b/1-A- | −           | −      | −    | −      |             | −     | −    | −              |
|            | I-A+/1-E- |             | −      | −    | −      |             | −     | −    | −              |
cates that T cells bearing this invariant α chain are probably not selected by a TAP-dependent MHC class I molecule.

Thus, the selecting molecule for the invariant Vα7-Jα33+ cells appeared to be neither an MHC class II nor a classical MHC class I molecule. However, because the defect in MHC class II expression may not be complete and the level of MHC class I expression in TAP-deficient patients is still 1% of normal, no definitive conclusion could be drawn from these studies. Therefore, we turned to the mouse, where carefully controlled studies using well-characterized MHC-deficient mice can be performed.

The selecting ligand for Vα19-Jα33+ cells is β2m-dependent, TAP-independent, and distinct from CD1. The amount of Vα19-Jα33 transcripts was examined in α/β− DN lymph node cells from several MHC-deficient strains. Because the frequency of Vα19-Jα33+ cells and the percentage of DN cells are lower in mice, the differences between positive and negative samples are smaller in mice than in humans. However, despite higher levels of Cα in the β2m−/− samples, there were fewer Vα19-Jα33 transcripts than in control or I-Aβ−/− mice (Fig. 8, A and B). Polyclonal sequencing of Vα19-Jα33 amplicons from β2m−/− samples showed the complete absence of invariant Vα19-Jα33 transcripts. This was consistent with an MHC class I or β2m-dependent class I-like selection of the invariant Vα19-Jα33+ cells. However, the relevant molecule is not a classical MHC class I molecule because the amount of invariant Vα19-Jα33 transcripts was higher in the DN cells from Kβ−/−Dβ−/− mice than in controls, though the Cα levels were lower. These results suggest that the MHC class I selecting molecule is not a classical one and that, in the absence of classical class I molecules, the invariant chain-bearing DN cells are less diluted by mainstream cells. In Fig. 8, C and D, Vα19-Jα33 transcripts were quantified in CD8−, CD1+, and TAP-deficient mice, B6 and β2m−/− mice being used as positive and negative controls, respectively. There was no decrease in the amounts of Vα19-Jα33 transcripts in CD1-deficient mice, indicating that Vα19-Jα33+ cells were not selected by CD1 or a CD1-bound ligand. The curves for TAP−/− samples were actually shifted to the left, indicating an increased frequency of Vα19-Jα33+ cells in TAP−/− mice, in agreement with the result shown in Fig. 5 B where the frequency of Vα19-Jα33+ cells in DN TAP−/− mice was 1/8.5 (i.e., a sixfold increase compared with B6; Fig. 5 A). The Vα19-Jα33 curve corresponding to the CD8−/− deficient mice is similar to that of B6, indicating the presence of Vα19-Jα33 invariant chains in CD8−/− mice. This was confirmed by polyclonal sequencing (data not shown). Altogether, these results, summarized in Table III (bottom), indicate that Vα19-Jα33+ cells do not require CD8 for their selection and that the selecting molecule is a β2m-dependent, TAP-independent molecule distinct from CD1.
Therefore, it is legitimate to wonder why such cells have abundant V~\(\alpha_33\) amounts of C~\(\alpha_33\) lymph node cells were obtained from the indicated mice, and the clonal sequencing.

**Discussion**

Invariant V~\(\alpha_7.2\)-J~\(\alpha_33\) TCR ~\(\alpha\) chain and its murine and bovine homologues were found in three different mammalian species and, thus, define a new phylogenetically conserved ~\(\alpha\) chain repertoire. In humans, DN and CD8~\(\alpha\)~+~ cells bearing invariant V~\(\alpha_7.2\)-J~\(\alpha_33\) chain accumulate after birth and become quite abundant, as they represent ~0.1–0.2% of all human PBLs. Therefore, it is legitimate to wonder why such cells have not been previously reported. This may be due to the lack of a stringent V~\(\beta\) repertoire restriction, or to the difficulties in growing clones expressing this invariant chain (our unpublished observations), together with methodological limitations linked to TCR ~\(\alpha\) chain repertoire analysis (e.g., lack of allelic exclusion [46]). However, this invariant ~\(\alpha\) chain had already been described in DN cells by Porcelli et al. [39] and, among the 317 random CDR3 sequences reported by Moss and Bell [47], 4 (1.2%) had a sequence corresponding to the V~\(\alpha_7.2\)-J~\(\alpha_33\) invariant ~\(\alpha\) chain. In the same study, this canonical sequence was not found in cord blood samples or in CD4~+~ cells, but represented 3/40 (7.5%) CDR3 sequences derived from CD8~\(\alpha\)~+~ cells. This age and cell distribution is in agreement with our own measurements (data not shown). In cattle, this CDR3 was found in 1/20 TCR ~\(\alpha\) chains randomly sequenced [40]. In mice, random cloning of TCR ~\(\alpha\) chains revealed one example of this invariant V~\(\alpha_19\)-J~\(\alpha_33\) ~\(\alpha\) chain [48]. It should be stressed that as shown in Fig. 3, the AJ33 segments are quasi-identical in the three species. The mouse and human ~\(\alpha\) loci are entirely homologous, with the same genomic organization and an average similarity of 71% including the ~\(\alpha\) coding sequences [41, 42, 49]. The AJ33 is the most similar ~\(\alpha\) segment between mouse and human sequences with only one amino acid difference; the two other most similar human/mouse pairs (AJ23 and AJ24) have four and three amino acid differences, respectively [41]. This quasi identity between mouse and human AJ33 segments suggests a strong selective pressure for these segments.

In humans and cattle, the TCR ~\(\alpha\) chain displayed some junctional diversity, whereas in mice a genomic sequence without trimming or N additions was found in most cases (Table II). However, a murine sequence made by trimming and reconstitution of the canonical sequence through N additions was found in two hybridomas (11F1 and 4H1 in Table II), indicating that the TCR in mice is selected at the protein level. In humans, the invariant TCR ~\(\alpha\) chain was associated with a limited number of V~\(\beta\) segments (mostly hBV2 and hBV13), and the same TCR with the same nucleotide sequence was present in different cells from two subjects, suggesting oligoclonal expansions. Despite this combinatorial restriction, TCR ~\(\beta\) chain diversity of invariant V~\(\alpha_7.2\)-J~\(\alpha_33\)~+~ cells remained extensive, since most J~\(\beta\) segments and seven V~\(\beta\)~s were found associated with CDR3s of variable length. In mice, five different V~\(\beta\)~s were observed with a high proportion of mBV6 and mBV8. Importantly, the murine orthologous segments of hBV13 are mBV8 and mBV6 [50–52].

This semi-invariant repertoire selected at the protein level is reminiscent of the repertoire observed in murine epithelial DEC~s and in murine and human NK1 T cells. In DEC~s, an invariant repertoire generated from genomic sequences is selected at the protein level, as the same epitope recognizable by an mAb can be reconstituted by different V~\(\gamma\) segments when the original GV1 and GV2 segments are inactivated by homologous recombination [13]. For NK1 T cells, selection at the protein level was also apparent [14], and the transgenic overexpression of the invariant mAV14AJ18 was sufficient to induce a large increase in the number of NK1 T cells [53].

The restriction element of V~\(\alpha_7.19\)-J~\(\alpha_33\)~+~ cells is probably an MHC class Ib molecule distinct from CD1d, though a ~\(\beta\)2m-derived peptide presented by a "nonclassical" class II molecule cannot be formally excluded. The selecting molecule should be present in both mice and humans. M urine Q~a~1 and human HLA-E might be good candidates, given their structural homology and their ability to bind to homologous CD94/NKG2 receptors in both species [54–56]. However, when using HLA-E tetramers complexed with HLA-G leader sequence–derived peptide [56], we were unable to find any difference in expression levels of the invariant V~\(\alpha_7.19\)-J~\(\alpha_33\) ~\(\alpha\) chain between FACSSorted HLA-E tetramer–positive and –negative (DN plus CD8~\(\alpha\)~+~) ~\(\alpha\)/~\(\beta\)~+~ TCR PBL fractions (data not shown). Moreover, the in-vivo expression of this invariant TCR ~\(\alpha\) chain and its murine and bovine homologues was found in three different mammalian species and, thus, define a new phylogenetically conserved ~\(\alpha\) chain repertoire. In humans, DN and CD8~\(\alpha\)~+~ cells bearing invariant V~\(\alpha_7.2\)-J~\(\alpha_33\) chain accumulate after birth and become quite abundant, as they represent ~0.1–0.2% of all human PBLs. Therefore, it is legitimate to wonder why such cells have not been previously reported. This may be due to the lack of a stringent V~\(\beta\) repertoire restriction, or to the difficulties in growing clones expressing this invariant chain (our unpublished observations), together with methodological limitations linked to TCR ~\(\alpha\) chain repertoire analysis (e.g., lack of allelic exclusion [46]). However, this invariant ~\(\alpha\) chain had already been described in DN cells by Porcelli et al. [39] and, among the 317 random CDR3 sequences reported by Moss and Bell [47], 4 (1.2%) had a sequence corresponding to the V~\(\alpha_7.2\)-J~\(\alpha_33\) invariant ~\(\alpha\) chain. In the same study, this canonical sequence was not found in cord blood samples or in CD4~+~ cells, but represented 3/40 (7.5%) CDR3 sequences derived from CD8~\(\alpha\)~+~ cells. This age and cell distribution is in agreement with our own measurements (data not shown). In cattle, this CDR3 was found in 1/20 TCR ~\(\alpha\) chains randomly sequenced [40]. In mice, random cloning of TCR ~\(\alpha\) chains revealed one example of this invariant V~\(\alpha_19\)-J~\(\alpha_33\) ~\(\alpha\) chain [48]. It should be stressed that as shown in Fig. 3, the AJ33 segments are quasi-identical in the three species. The mouse and human ~\(\alpha\) loci are entirely homologous, with the same genomic organization and an average similarity of 71% including the ~\(\alpha\) coding sequences [41, 42, 49]. The AJ33 is the most similar ~\(\alpha\) segment between mouse and human sequences with only one amino acid difference; the two other most similar human/mouse pairs (AJ23 and AJ24) have four and three amino acid differences, respectively [41]. This quasi identity between mouse and human AJ33 segments suggests a strong selective pressure for these segments.

In humans and cattle, the TCR ~\(\alpha\) chain displayed some junctional diversity, whereas in mice a genomic sequence without trimming or N additions was found in most cases (Table II). However, a murine sequence made by trimming and reconstitution of the canonical sequence through N additions was found in two hybridomas (11F1 and 4H1 in Table II), indicating that the TCR in mice is selected at the protein level. In humans, the invariant TCR ~\(\alpha\) chain was associated with a limited number of V~\(\beta\) segments (mostly hBV2 and hBV13), and the same TCR with the same nucleotide sequence was present in different cells from two subjects, suggesting oligoclonal expansions. Despite this combinatorial restriction, TCR ~\(\beta\) chain diversity of invariant V~\(\alpha_7.2\)-J~\(\alpha_33\)~+~ cells remained extensive, since most J~\(\beta\) segments and seven V~\(\beta\)~s were found associated with CDR3s of variable length. In mice, five different V~\(\beta\)~s were observed with a high proportion of mBV6 and mBV8. Importantly, the murine orthologous segments of hBV13 are mBV8 and mBV6 [50–52].

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variant Vα19-Jα33 chain was not expressed by three TAP-independent anti-Qa-1 clones provided by J. Forman (University of Texas, Dallas, TX [57]; data not shown). Nonetheless, no definitive conclusions as to the HLA-E/Qa-1 specificity of the invariant TCR can be drawn from these negative results because the invariant TCR may recognize HLA-E complexed with another peptide. Another candidate class I molecule that is widely expressed in both mice and humans is the recently described MR1 molecule (58, 59). Identification of the selecting element is underway by testing reactivity of mouse hybridomas against different cell types and MHC class I/II transfectants.

The expression of CD8αα on Vα7-Jα33+ cells could argue for an extrathymic development pathway (43). However, these cells were not found in nude mice (Table III, top), in human intraepithelial lymphocytes (IELs) (Cerf-Bensussan, N., E. Treiner, and O. Lantz, unpublished results), or in murine IELs or lamina propria lymphocytes (Guy-Grand, D., F. Tilloy, and O. Lantz, unpublished results), and their frequency was not particularly high in mouse bone marrow (Table III, top). Thus, Vα7/19-Jα33+ cells seem to be mainly thymus dependent. In this respect, the fact that we have been unable to find large numbers of these cells in human or mouse thymus (data not shown) does not preclude an intrathymic development. Indeed, if the antigen is not present in sufficient amounts in the thymus, these cells may not accumulate locally and may be diluted out by mainstream α/β T cells in the periphery. The absence of significant numbers of Vα19-J33+ cells in mouse CD8α+ cells (Fig. 1F, and polyclonal sequencing after V-J amplification [not shown]) is in agreement with the extremely low numbers (<0.4%) of CD8αα+ cells in murine blood or lymph nodes (data not shown). The CD8αα expression on some invariant Vα7-Jα33+ cells and its complete absence on Vα19-Jα33+ counterparts is reminiscent of the phenotype of NK1 T cells, which may express CD8αα+ in humans (28) but never in mice. The reasons for such phenotypic differences between mice/cattle and humans as well as the role of CD8αα expression in human PBLs are not clear. There have been contradictory reports concerning the role of homodimeric CD8 in defense mechanisms against broadly distributed pathogens and other populations may exist in other organs in the same way that γδ subpopulations are distributed.

Concerning the development pathway of the Vα7/19-Jα33 cells, in the three species there seems to be no expression of the invariant α chain in the CD8β+ T cells, strong enrichment for this sequence within DN cells (as well as within human CD8αα+ cells), and persistence of the invariant sequence in the CD4+ subset. Indeed, in humans, the frequency of invariant α chain-bearing cells was ~1/6,000 in the CD4+ fraction. Moreover, polyclonal sequencing of Vα7-Jα33 ampiclons from CD4+ cells (Fig. 2A) revealed the presence of canonical CDR3 at a low, though readable, frequency, whereas the canonical CDR3 was undetectable in CD8αβ+ samples (Fig. 2B). In mice and cattle, there was also a small but reproducible shift of the CD4+ amplification curves to the left compared with the CD8αβ+ curves, suggesting some expression of the invariant α chain in murine or bovine CD4+ cells. Accordingly, a readable canonical sequence in Vα19-Jα33 amplicons was detected in the CD4+ but not in the CD8αβ+ fraction in both species (data not shown). The absence of any readable sequence after V-J amplification of CD8αβ+ cells suggests that the CD8αβ+ Vα7/19-Jα33-bearing cells are deleted by negative selection, as are NK1 T cells in CD8 transgenic mice (14). These results are compatible with the hypothesis that Vα7/19-Jα33 cells follow a normal T cell development pathway but that, in contrast with NK1 T cells where the numbers of CD4 and DN NK1 T cells are similar, CD4+ T cells bearing the Vα7/19-Jα33 chain are diluted out by mainstream lymphocytes.

A memory phenotype associated with an oligoclonal repertoire may be related to recognition of either an endogenous ligand or a ubiquitous pathogen. In favor of an endogenous ligand is the finding that invariant Vα7-Jα33+ cells existed in an 18-mo-old child who had not been vaccinated with Calmette-Guérin bacillus (BCG) and in several subjects who had negative EBV and CMV serologies (data not shown). On the other hand, the abundance of Vα7/19-Jα33-bearing cells in humans and cattle contrasting with a lower number of these cells in mice could be related to the clean environment in which laboratory mice are housed. The two hypotheses are not mutually exclusive, as cells selected by an endogenous ligand could be ready to react against a highly prevalent pathogen.

Both NK1 T cells and Vα7/19-Jα33-bearing cells display semi-invariant repertoires (i.e., one monomorphic α chain and a biased Vβ chain repertoire), suggesting that their TCRs may have similar characteristics, probably a high affinity for a selecting ligand by the invariant α chains, as the transgenic overexpression of AV14A18 is sufficient to greatly increase the number of NK1 T cells (53). Together with the similarities in their restricting elements (a TAP-independent, β2m-dependent nonclassical MHC class I-like molecule), the absence of accessory molecule (CD4 or CD8) involvement suggests that they may recognize a high-density ligand that might therefore be a saccharide or a glycolipid.

Are there other invariant TCR α chains defining other T cell subpopulations? We did not find any after measuring the amounts of several other α chains which had been found either in DN T cells (39) or in a "regulatory" subpopulation (65–67; Tilloy, F., and O. Lantz, unpublished observations). However, the definite resolution of this issue awaits a systematic study of the expression of all AV-AJ combinations and other populations may exist in other organs in the same way that γδ subpopulations are distributed.

Concerning the functions of Vα7/19-Jα33+ lymphocytes, future analysis of the mAV19A33 transgenic mice we have made will certainly help address this point. The high frequency of Vα7/19-Jα33+ T cells and their extensive phylogenetic conservation in mammals both argue for an important physiological function. Because Vα7/19-Jα33+ and NK1 T cells are selected by distinct ligands, they may complement each other and act synergistically either in defense mechanisms against broadly distributed pathogens or in immune/nonimmune homeostatic processes.
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