Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 7518–7522, September 1991
Immunology

Dominant expression of a distinctive V14$^+$ T-cell antigen receptor α chain in mice

(variable region 14 genes/homogeneous variable–joining region junction/wild mice/extrathymic-positive selection)

HARUHIKO KOSEKI*, HIDEFUMI ASANO*, TSUNEYOSHI INABA*, NOBUMOTO MIYASHITA†, KAZUO MORIWAKI†, KIRSTEN FISCHER LINDAHL‡, YOKO MIZUTANI*, KENJI IMAI*§, AND MASARU TANIGUCHI*¶

*Division of Molecular Immunology, Center for Neurobiology and Molecular Immunology, School of Medicine, Chiba University, Chiba, Japan, 280; †National Institute of Genetics, Mishima, Shizuoka, Japan, 411; and ‡Howard Hughes Medical Institute, Departments of Microbiology and Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75235-9050

Communicated by Kimishige Ishizaka, April 24, 1991

ABSTRACT **A distinctive variable region 14-positive (V14$^+$) α chain (V$_\alpha$14$^+$) of the T-cell antigen receptor is predominantly expressed in multiple mouse subspecies. The V$_\alpha$14 family has two members, V$_\alpha$14.1 and V$_\alpha$14.2, which differ by only three amino acids at positions 50–52. Based on the *Eco*RI restriction fragment length polymorphism of the gene encoding V$_\alpha$14, mice can be divided into three groups: type I with an 11.2-kilobase (kb) fragment, type II with a 2.0-kb fragment, and type III with the 2.0-kb and 11.2-kb fragments. Usage of V$_\alpha$14–J$_\alpha$281, where J$_\alpha$281 is an α-chain joining segment, with a one-base N region dominates at the level of 0.02–1.5% of α chains in all laboratory strains, *Mus musculus castaneus*, and *Mus musculus domesticus* but not in *Mus musculus molossinus*, *Mus musculus musculus*, and *Mus spicilegus* samples. The preferential V$_\alpha$14–J$_\alpha$281 expression seems to be due to positive selection because the V–J junctional region is always glycine, despite the ability of the V$_\alpha$14 gene to associate with J$_\alpha$ other than J$_\alpha$281. As V$_\alpha$14–J$_\alpha$281 expression is independent of known major histocompatibility complex antigens, including H-2, TLA, Qa, and HMT, the selecting ligand must be a monomorphic molecule of the mouse, expressed in a subspecies-specific manner. Additional observations, such as the expression of homogeneous V$_\alpha$14–J$_\alpha$281 in athymic mice, suggest that the positive selection of V$_\alpha$14$^+$ T cells occurs extrathymically.**

The mouse T-cell antigen receptor (TCR) $\alpha\beta$-chain heterodimer recognizes antigens in conjunction with polymorphic self-molecules encoded by the *H-2* major histocompatibility complex (MHC) (1). T cells thus express two functional specificities. As some *H-2* congenic mice differ in their dominant TCR usage, it is obvious that MHC molecules play decisive roles in the selection and generation of the TCR repertoire (2). However, the recognition of certain cell-surface antigens does not appear to be subject to such rigid MHC restriction (3–8) because they involve an MHC restriction molecule of low polymorphism; for instance, the HMT antigen is a monomorphic class I MHC molecule in inbred strains (9) and acts as a T-cell restriction element (10).

Homogeneity of TCR $\gamma\delta$ chains has been reported by several groups. Asarnow *et al.* (11), Lafaille *et al.* (12), and Sim and Augustin (13) have demonstrated that some TCR $\gamma\delta$ chains have homogeneous sequences. The restriction molecules for some $\gamma\delta$ TCR are less polymorphic than for $\alpha\beta$ TCR and are encoded in the *Tla* region (14–16). Thus, homogeneous TCR expression may be correlated with monomorphic rather than polymorphic MHC restriction elements.

In our previous studies, we identified a unique TCR gene encoding the α-chain variable region 14 (V$_\alpha$14) as a new family‖ and found that the homogeneous V14$^+$ α-chain is expressed in various *H-2* congenic mice at a high frequency (17, 18). The results suggested that the molecule selecting this TCR is monomorphic (18). To examine this point in detail, we have investigated the frequency of V14$^+$ α-chain usage in various strains and subspecies of wild mice. We provide evidence here that the homogeneous V$_\alpha$14–J$_\alpha$281 is selected according to rules partly distinct from those currently accepted for the selection of TCR repertoire in the thymus (19–22).

MATERIALS AND METHODS

Mice and Cell Lines. C57BL/6CrSlc (*H-2^b*), C3H/HeSlc (*H-2^k*), BALB/cCrSlc (*H-2^d*), AKR/NSlc (*H-2^k*), CBA/NSlc (*H-2^k*), and DBA/2CrSlc (*H-2^d*) mice (6 wk old) were purchased from the Shizuoka Animal Center, Hamamatsu, Japan. All other inbred strains, such as C57BL/10SnJ (*H-2^b*), B10.A/SgSnJ (*H-2^a*), DBA/1J (*H-2^q*), CE/J (*H-2^k*), A/J (*H-2^a*), A/WySnJ (*H-2^a*), RIIIS/J (*H-2^r*), SWM/Ms (*H-2K^d/H-2D$^?$*), RFM/MsNrs (*H-2^f*), WB/ReJ-W (*H-2^{ja}*), 129/J (*H-2^{bc}*), I/LnJ (*H-2^j*), PL/J (*H-2^u*), SM/J (*H-2^v*), SJL/J (*H-2^s*), NZB/BINJ (*H-2^d*), and C57BR/cdJ (*H-2^b*) mice, were maintained by K.M. Wild mice, such as *Mus spicilegus*, *Mus musculus domesticus* (SK/Cam from U.K., M. DOM-PGN 1 and M. DOM-PGN 2 from Canada and M. Dom-Blg from Bulgaria), *Mus musculus musculus* (M. MUS-NJL from Denmark and M. MUS-BLG 1 from Bulgaria), *Mus musculus castaneus* (M. Cas-Bgr from Indonesia), *Mus musculus* (M. sub-Bjn and M. sub-Shh 1 from China and M. sub-Swn 1 from Korea), and *Mus musculus molossinus* (M. MOL-MSM and M. Mol-Kgs from Japan), were also maintained by K.M. *M. m. castaneus* (CAS) haplotype congenic mice with either C57BL/6 or C57BL/10 background, such as B10.CAS3/Kfl, B10.CAS3(R1)/Kfl, and B6.CAS3(R10)/Kfl, were bred in the colony of K.F.L. BW5147 and a keyhole limpet hemocyanin (KLH)-specific suppressor T-cell (T$_s$) hybridoma (BW5147 × C57BL/6 KLH-T$_s$) have been described (23, 24).

RNase Protection Assay. Total cellular RNA was isolated (25), and RNase protection analysis was performed as described (18, 26). To determine the frequency of V14$^+$ α chains, the radioactivities of V–J–constant (C) region, V, and

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TCR, T-cell antigen receptor; V, variable; C, constant; J, joining; MHC, major histocompatibility complex; KLH, keyhole limpet hemocyanin; T$_s$ cell, suppressor T cell; C$_\alpha$, V$_\alpha$, and J$_\alpha$, α-chain C, V, and J regions; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

§Present address: Max Planck Institute of Biophysical Chemistry, Göttingen, F.R.G.
¶To whom reprint requests should be addressed.

‖The sequences reported in this paper have been deposited in the GenBank data base (accession nos. D90229 for V$_\alpha$14.1 and D90230 for V$_\alpha$14.2).

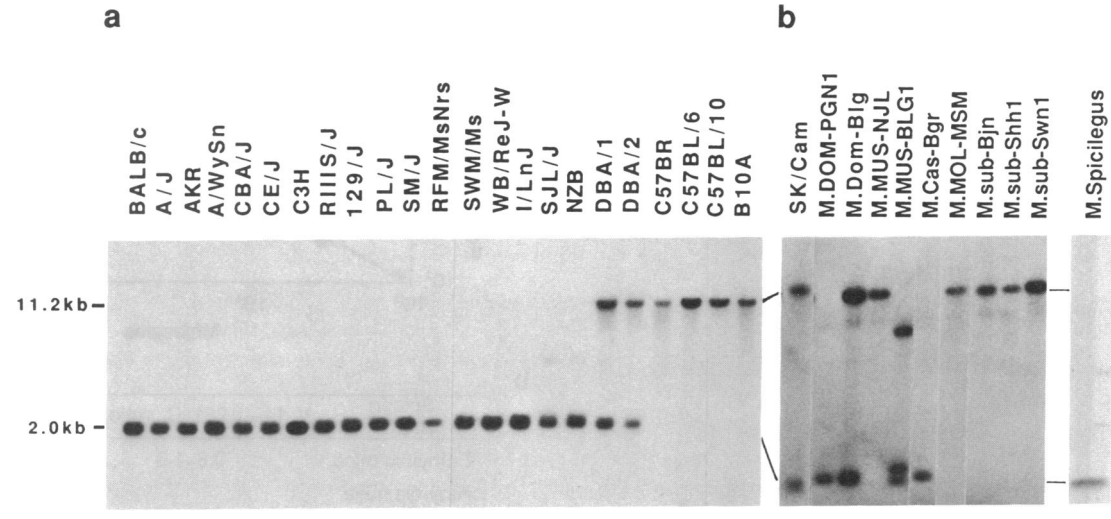

FIG. 1. Southern blot analysis of genomic DNA from inbred and wild mice. DNA (15 μg) from laboratory strains (*a*) and wild mice (*b*) were digested with *Eco*RI and loaded onto each lane. DNA blots were hybridized with the V_α14.1 probe.

J–C bands protected were measured by an automated densitometer (Bio Image Analyzer: Fujix BAS2000, Fuji Film, Tokyo) and expressed as a percentage of total α-chain C region (C_α) transcripts.

Polymerase Chain Reaction (PCR) and Sequencing. PCR and nucleotide sequencing were carried out as described (18, 27). Oligonucleotides used in the amplification were 5′-TCTAGAATTCTGCCTGAGACCGAGGATC-3′ for a C_α primer and 5′-TCTAGAATTCTAAGCACAGCACGCTGCACA-3′ for a V_α14 primer. Oligonucleotides used for determination of the V_α14.2 sequence were 5′-CCCAAGTGGAGCAGAGTCCT-3′ for the 5′ end and 5′-CCACACAGATGTAGGTGGCA-3′ for the 3′ end of the V_α14 gene. The frequency of V_α14–J_α281 was also determined by a quantitative PCR with 25 cycles of amplification (28). Varied concentrations of the standard cDNA (pTsαVJC) and sample RNA were amplified by PCR with two sets of primers (V14/J281 for V–J and C_α-1/C_α-2 for C_α). Oligonucleotides used were 5′-TAAGCACAGCACGCTGCACAT-3′ for the V_α14 primer, 5′-CAATCAGCTGAGTCCCAGCT-3′ for the J_α281 primer, 5′-CCTCTGCCTGTTCACCGACT-3′ for the C_α-1 primer, and 5′-CAGGAGGATTCGGAGTCCCA-3′ for the C_α-2 primer. The PCR products were detected by DNA blotting with a ^{32}P-labeled 1.1-kb *Eco*RI fragment of pTsαVJC. The radioactivities were measured by the Bio Image Analyzer, expressed in the arbitrary unit of photostimulated luminescence and plotted against template concentrations.

RESULTS

Analysis of Genomic DNA from Inbred and Wild Mice. Laboratory inbred strains could be divided into three groups based on the *Eco*RI restriction fragment length polymorphism (RFLP) with the V_α14 probe (Fig. 1*a*). Type I with an 11.2-kb fragment includes C57BL/6, C57BL/10, and C57BR mice. Most laboratory strains belong to type II with a 2.0-kb fragment. DBA/1 and DBA/2 mice, which carry both 11.2-kb and 2.0-kb fragments, are type III mice. Wild mice had RFLP patterns similar to those observed in inbred mice (Fig. 1*b*). For example, mice from China (M. sub-Bjn and M. sub-Shh 1), Korea (M. sub-Swn 1), and Japan (M. MOL-MSM) revealed the type I pattern. *M. m. domesticus* (M. DOM-PGN 1, M. Dom-Blg, and SK/Cam), *M. m. castaneus* (M. Cas-Bgr), and *M. m. musculus* (M. MUS-BLG 1) showed type II or III patterns. An exception is M. MUS-NJL with the type I pattern. Although *M. spicilegus* is genetically distant (>10^6 yr) from *M. musculus*, it had the type II RFLP pattern. The V_α14 gene family is thus conserved in mouse subspecies.

Nucleotide Sequences of V_α14 Genes. The RFLP analysis suggests that the V_α14 gene family has few members, probably two. We named V_α14.1 gene on the 11.2-kb (type I) and V_α14.2 gene on the 2.0-kb *Eco*RI fragment (type II). We determined the V_α14.2 gene sequence by PCR. Only four nucleotides in the V_α14.2 gene differ from those of V_α14.1 gene at positions 148 (G → C), 150 (C → T), 151 (C → G), and 156 (A → T) (Fig. 2), resulting in three amino acid changes at residues 50–52.

RNase Protection Analysis and Quantitative PCR. We investigated the frequency of the V_α14.2–J_α281 sequence in types II and III mice. Since more than two consecutive nucleotide differences can be detected by the protection assay, the protected length of V_α14.2–J_α281 with the V_α14.1–J_α281 probe is expected to be 401 base pairs (bp) in size, instead of 630 bp for V_α14.1–J_α281, and migration of the V_α14.2 band is expected to correspond to 216 bp rather than 354 bp for V_α14.1. As shown in Fig. 3*a*, C57BL/10 (type I) or BALB/c (type II) showed 630-bp or 401-bp full-length bands, respectively, whereas DBA/2 (type III) possessed both bands.

To determine the frequency of V_α14–J_α281, we compared the radioactivities of bands corresponding to V_α14–J_α281 and total C_α. In all cases, V14.1–J281 and V14.2–J281 α chains were estimated to be 0.5–1.5% of total α chains. The fre-

```
                Q  V  E  Q  S  P  Q  S  L  V  V  R  Q  G  E  15
Vα14.1  CAA GTG GAG CAG AGT CCT CAG TCC CTG GTT GTC CGT CAG GGA GAG
Vα14.2  --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                N  C  V  L  Q  C  N  Y  S  V  T  P  D  N  H  30
Vα14.1  AAC TGC GTC CTT CAA TGT AAT TAC AGT GTG ACC CCC GAC AAC CAC
Vα14.2  --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                L  R  W  F  K  Q  D  T  G  K  G  L  V  S  L  45
Vα14.1  TTA AGG TGG TTC AAA CAG GAC ACA GGC AAA GGT CTT GTG TCC CTG
Vα14.2  --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                T  V  L  V  D  Q  K  D  K  T  S  N  G  R  Y  60
Vα14.1  ACA GTC CTG GTT GAC CAA AAA GAC AAA ACG TCA AAT GGG AGA TAC
Vα14.2  --- --- --- --- C-T G-- --T --- --- --- --- --- --- --- ---
                                H  E  N
                S  A  T  L  D  K  D  A  K  H  S  T  L  H  I  75
Vα14.1  TCA GCA ACT CTG GAT AAA GAT GCT AAG CAC AGC ACG CTG CAC ATC
Vα14.2  --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
                T  A  T  L  L  D  D  T  A  T  Y  I  C  V     90
Vα14.1  ACA GCC ACC CTG CTG GAT GAC ACT GCC ACC TAC ATC TGT GTG G
Vα14.2  --- --- --- --- --- --- --- --- --- --- --- --- --- --- -
```

FIG. 2. Nucleotide sequences of V_α14.1 and V_α14.2 genes. Deduced amino acids are shown as standard one-letter abbreviations. Dashes indicate nucleotides identical to those of V_α14.1.

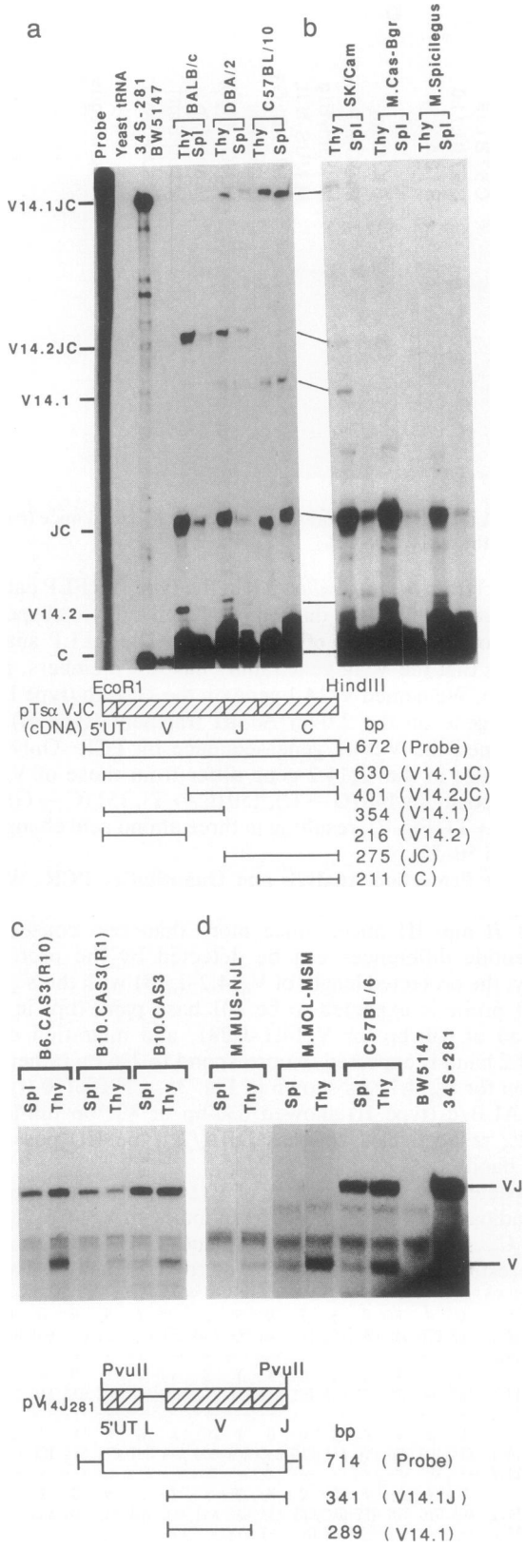

FIG. 3. Predominant expression of V14$^+$ α chains in mice. The levels of V_α14–J_α281 expression were measured by RNase protection. Two probes (17, 18) were used: pTsαVJC cDNA (V_α14.1–J_α281–C_α) in *a* and *b* and pV14J281, a genomic clone (V_α14.1–J_α281), in *c* and *d*. Thymus or spleen RNA (20 μg) was used. Soluble yeast RNA and BW5147 were negative controls. The positive control was RNA from KLH-T_s hybridoma 34S-281. The lengths of the probes are indicated by hatched boxes.

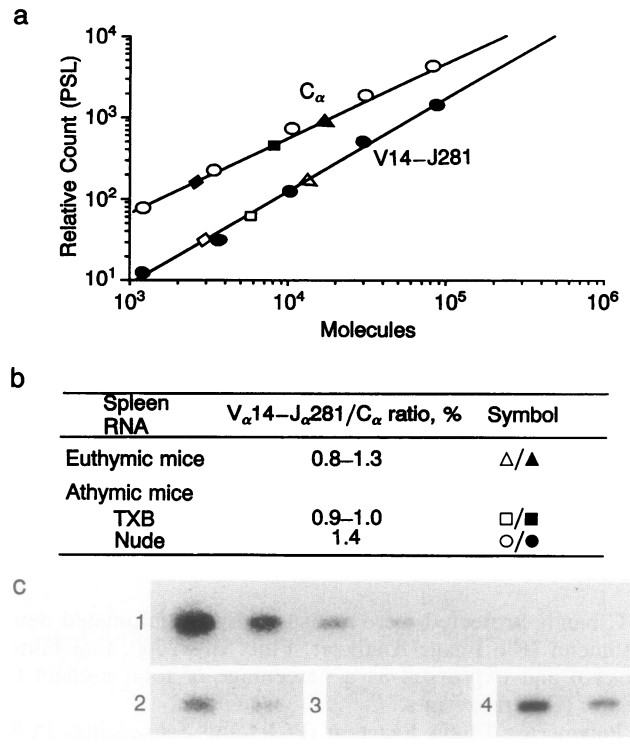

FIG. 4. Quantitative PCR of V_α14–J_α281 and C_α transcripts in euthymic and athymic spleen. (*a*) Arbitrary unit of radioactivity [photo-stimulated luminescence (PSL)] of PCR products amplified by two sets of primers, V/J (△, □, ●) and C_α-1/C_α-2 (▲, ■, ○), were plotted against template concentrations (1:2 serial dilutions of 10^5 copies) of the standard cDNA (pTsαVJC). The ratios of V_α14–J_α281 to C_α were calculated from the radioactivities of sample PCR products, BALB/c (△, ▲), TXB (□, ■) and nude (◇, ◆) according to the standard plots. (*b*) V_α14–J_α281/C_α ratios based on PCR. (*c*) DNA blots with the pTsαVJC probe: 1, standard V_α14–J_α281 (from 10^5 copies); 2, V_α14–J_α281 PCR products of 3.8 μg and 1.3 μg of TXB RNA; 3, V_α14–J_α281 PCR products of 18.8 μg and 6.3 μg of RNA of anti-Thy-1-treated bone marrow cells (BM); 4, C_α PCR products of the same BM samples as in blot 3. Note that V_α14–J_α281 was undetectable in BM, while significant amounts of C_α, probably a truncated form, were detected.

quency was also confirmed by quantitative PCR, demonstrating that the ratios of V_α14–J_α281 to C_α were 0.8–1.3% in spleen (Fig. 4). These results agree with those from the protection assays.

The 630-bp and/or 401-bp bands were also detected at significant, albeit lower, levels (0.02–0.04%) in some wild mice, such as M. Cas-Bgr and SK/Cam but not in *M. spicilegus*, *M. m.* M. MUS-NJL, and M. MOL-MSM (Fig. 3 *b* and *d*). Similar results were also observed in other wild mouse stocks; V_α14–J_α281 expression is positive in M. DOM-PGN 2 but not in M. Mol-Kgs. The results are summarized in Table 1. It is obvious that the combination of V_α14 with J_α281 is preferentially selected and dominates in most mouse subspecies, suggesting that the ligand is monomorphic in nature.

The two *Hmt* alleles, *Hmt*^{a} and *Hmt*^{b} (29), were recently mapped between *Tla/Qa-1* and *Tpx-1*, distal to the *H-2* complex on chromosome 17 (29). All laboratory strains and some wild mice, such as *M. m. domesticus*, carry the *Hmt*^{a} allele, while the *Hmt*^{a} and *Hmt*^{b} alleles segregate in *M. m. castaneus* (30). As all laboratory strains and *M. m. domesticus* were positive for V_α14–J_α281 (Fig. 3), the V_α14–J_α281 expression appeared to correlate with *Hmt*^{a}. We therefore examined V_α14–J_α281 expression in mice congenic for *Hmt*, such as B10.CAS3 with a *M. m. castaneus* (CAS3) MHC segment, including CAS3 *H-2* through *Hmt*^{b}; B10.CAS3(R1) carrying CAS3 *H-2* to *Qa-2* but not *Hmt*^{a}; and B6.CAS3(R10) of

Table 1. Expression of V14$^+$ α-chain transcripts in mice

Mice	Organ	V14.1–J–C	V14.1	V14.2–J–C	V14.2	J–C
C57BL/6	Thy	0.70	0.45	—	—	1.93
	Spl	1.24	0.50	—	—	2.33
BALB/c	Thy	—	—	0.69	0.63	3.60
	Spl	—	—	1.45	0.64	2.24
DBA/2	Thy	0.55	0.19	0.73	0.51	1.79
	Spl	0.85	0.15	1.33	1.00	3.80
SK/Cam*	Thy	0.02	0.12	0.02	0.30	2.30
	Spl	0.04	0.12	0.03	0.01	1.40
M. Cas-Bgr	Thy	—	—	0.02	0.35	2.80
	Spl	—	—	0.03	0.21	2.20
M. MOL-MSM†	Thy	UD	0.17	—	—	1.00
	Spl	UD	0.31	—	—	2.70
M. MUS-NJL	Thy	UD	0.44	—	—	1.37
	Spl	UD	0.39	—	—	1.58
M. spicilegus	Thy	—	—	UD	0.23	2.80
	Spl	—	—	UD	0.23	1.20

RNase protection assay on RNA from thymus (Thy) and spleen (Spl) (see Fig. 3). The intensities of bands (630 bp for V14.1–J–C; 354 bp for V14.1; 401 bp for V14.2–J–C; 216 bp for V14.2; 275 bp for J–C) protected were measured and expressed as a percentage of C_α. UD, undetectable (<0.005%).

*M. DOM-PGN 2 (*M. m. domesticus*) showed similar V14$^+$ α-chain expression patterns.

†Another *M. m. molossinus* stock, M. Mol-Kgs, gave similar results.

C57BL/6 genotype, except for the chromosomal segment containing CAS3 *H-2D* to *Hmt^b*. As shown in Fig. 3*c*, these mice all showed the 341-bp full-protection band at the same levels as those of C57BL/10 [the ratios of V_α14–J_α281 to V_α14 are 5.0 in B10.CAS3, 3.0 in B10.CAS3(R1), 4.1 in B6.CAS3(R10), 2.5 in C57BL/6, and 3.9 in C57BL/10], suggesting no relation between *Hmt* and V_α14–J_α281 expression.

V–J Junctional Sequences of V14.2$^+$ α Chain. To confirm the data in Fig. 3, we investigated the V–J junctional sequences of V14.2$^+$ α chains by PCR. Most V_α14.2$^+$ cDNA clones (37 of 42) from BALB/c spleen (20 of 21 of BCS) and thymus (17 of 21 of BCT) had the same sequences composed of J_α281 with a one-base N region, a recombination-generated DNA element, while 5 of 9 V_α14.2$^+$ cDNA clones from neonates (NBS) were associated with other J_α than J_α281 (Fig. 5*A*), strongly indicating positive selection. These patterns were the same as those of the V14.1$^+$ α chain in type I mice (18). PCR analysis on wild mice also confirmed the protection data shown in Fig. 3. Only one of eight functional cDNA clones had the typical V_α14–J_α281 in *M. m. castaneus*, while no V_α14–J_α281 sequences were detected in *M. m. musculus* and in *M. m. molossinus* (data not shown). Thus, V_α14–J_α281 is not selected in some mouse subspecies.

Selection of V14$^+$ α Chain Without Thymic Influence. The above results suggested that the dominant expression and homogeneous V–J junctions of V_α14–J_α281 are the results of positive selection. As V_α14–J_α281 becomes more dominant in the periphery with time after birth [ref. 18 and neonatal (NBS) vs. adult (BCS) in Fig. 5], the selection and expansion of the V_α14$^+$ T-cell repertoire may occur outside the thymus.

To test this possibility, we sequenced V14$^+$ α-chain cDNA from athymic spleen of nude and adult thymectomized, bone marrow-reconstituted (TXB) (Fig. 5*B*). Most (19 of 19 nude and 27 of 29 TXB mice) of in-frame V14$^+$ α chains showed the one-nucleotide V–J junctions with J_α281 typical of euthymic mice. Moreover, the frequency was 0.9–1.4% of total α chains in TXB and nude mice as estimated by quantitative PCR (Fig. 4*b*), although V14$^+$ α chains were not detected in bone marrow cells before transfer (Fig. 4*c*). These results indicate that positive selection can take place in a thymus-independent fashion.

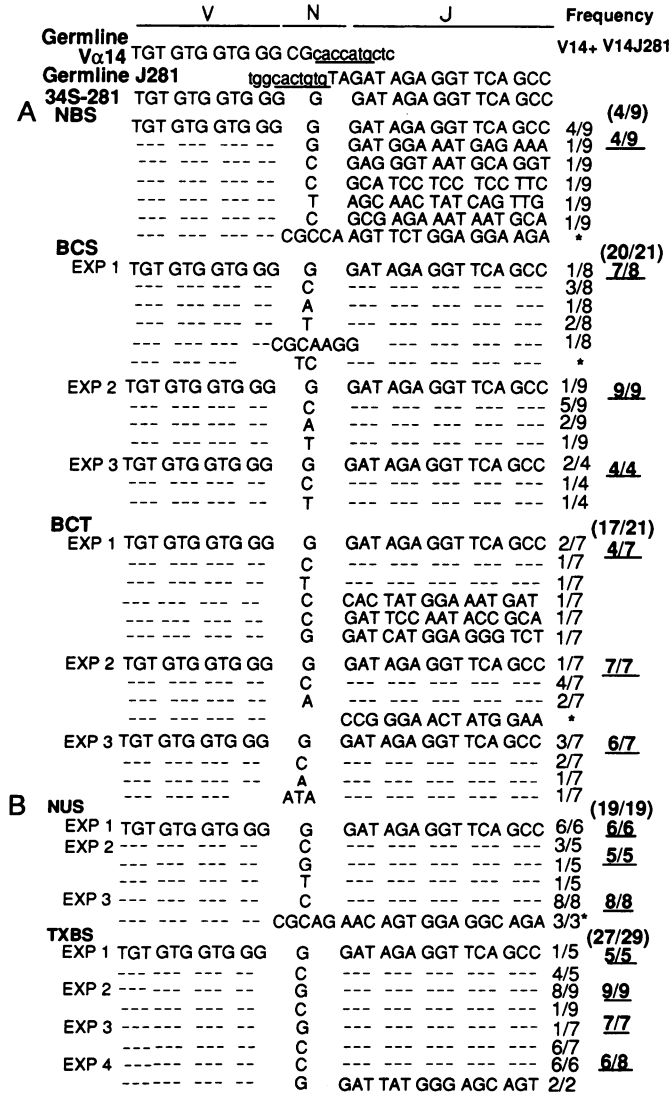

FIG. 5. V–J junctional sequences of V14.2$^+$ α-chain transcripts. PCR data in each experiment were obtained from different batches of RNA from newborn spleen (NBS) or adult spleen (BCS) or thymus (BCT) of BALB/c mice (*A*) and RNA from spleen of BALB/c *nu/nu* (NUS) or C57BL/6 mice that had been thymectomized 6 wk previously, γ-irradiated (10.63 Gy of ^{137}Cs γ-rays), and reconstituted with syngeneic bone marrow cells (1×10^7) that had been pretreated twice with anti-Thy-1.2 and rabbit complement (TXB) (*B*). Nucleotide sequences are aligned with the partial V and J sequences of the 34S-281 hybridoma. Germ-line V–J junctions with heptamer sequences underlined are shown. Identical bases are indicated by dashes. cDNA clones with other J_α than J_α281 are tentatively assigned N regions, and those with asterisks indicate out-of-frame sequences. The frequencies of homogenous V_α14–J_α281 are listed in the right margin.

DISCUSSION

In this paper we analyzed the predominant expression of V14$^+$ α chains in mouse subspecies. Based on RFLP with the V_α14 probe, laboratory strains can be divided into types I, II, and III (Fig. 1*a*). Surprisingly, wild mice show RFLP patterns similar to the laboratory strains. For example, mice from Northeast Asia (China, Korea, and Japan) are type I, whereas most other wild mice show RFLP patterns similar to type II or III.

Four major mouse subspecies are differentiated by encoded protein variations at 42 loci based on genetic distance values (31). These subspecies are *M. m. domesticus*, *M. m. musculus*, *M. m. castaneus*, and *M. m. bactrianus*. The

Japanese wild mouse, *M. m. molossinus*, is a subspecies derived from a natural, extensive intercrossing between *M. m. castaneus* and *M. m. musculus*, giving rise to a unique population (32). Most laboratory strains are taken as archetypes of *M. m. domesticus*, but some (the C57 group) have been shown to have a *M. m. molossinus* component. All wild mice from Asia showed the RFLP pattern with the 11.2-kb *Eco*RI band typical of the C57 mice (Fig. 1*b*); the V_α14 probe thus seems useful for analysis of mouse ancestry.

Nucleotide sequencing revealed only three amino acid differences at positions 50–52 between the V_α14.1 and the V_α14.2 genes (Fig. 2). It is intriguing that a majority of V_α14.2$^+$ cDNAs also use the J_α281 gene fragment with a one-base N region (Figs. 3 and 5). The N region forms the third base of a glycine codon. Therefore, the V–J junctions are quite homogeneous at the amino acid level. Moreover, the frequency of V_α14.2–J_α281 is estimated to be 0.5–1.5% of total α chains in all laboratory strains (Table 1). These data were also confirmed by quantitative PCR (Fig. 4*b*). Therefore, both V_α14.1 and V_α14.2 genes that are associated with J_α281 carrying one nucleotide N regions are preferentially selected over other combinations and dominate in the immune system. As V_α14.1 and V_α14.2 genes differ in the region equivalent to the complementarity determining region 2 (CDR2) (Fig. 2), the V–J junctional region must be more important for the selection.

Homogeneous V–J junction and predominant expression of V_α14–J_α281 appear to be caused by positive selection, and the ligand seems to be a self element. Our previous study of bone marrow chimeras (*M. m. molossinus* → C57BL/6) clearly demonstrated that V_α14–J_α281 from *M. m. molossinus* origin (a strain in which V_α14 is not normally expressed with J_α281) is selected and dominates in the C57BL/6 environment (18). As the expression of V14$^+$ α chain is independent of the *H-2* MHC haplotypes but is possibly mouse subspecies specific (Figs. 3 and 5 and Table 1), the molecule involved in this selection seems to be monomorphic rather than polymorphic.

The HMT molecule was considered a candidate for the selecting element. However, we found no correlation between *Hmt* genotypes and V_α14–J_α281 expression (Fig. 3*c*). All the inbred strains express V_α14–J_α281 at high frequency (Table 1). They include various *H-2* haplotypes and several allelic forms of Qb-1 (Q4), Qa-2, Q10 (*H-2^f* mice are negative), Qa-1, and TL antigens. Thus, we see no correlation with any known MHC antigen. However, other, as yet undefined, monomorphic MHC molecules are candidates for the structure selecting this α chain.

The mechanism of positive selection of V_α14–J_α281 seems distinct from that currently accepted for the selection of the $\alpha\beta$ TCR in the thymus (19–22). The results in Figs. 4 and 5 show that the selection can take place at extrathymic sites. Most in-frame V14$^+$ α-chain transcripts from athymic mice were typical V_α14–J_α281, and the frequency was almost the same as those in euthymic mice. The V14$^+$ α chains are heterogeneous in the neonatal stages (Fig. 5). Thus, the dominant V_α14–J_α281 expression is unlikely to be due to the selectivity of the recombinational machinery.

Together with our previous data, these results suggest that V_α14–J_α281 is selected in a thymus-independent fashion and expanded in the periphery. Sim and Augustin (13) have also suggested positive selection of an invariant TCR δ chain at extrathymic sites. This is analogous to V_α14–J_α281, suggesting the existence of similar selection mechanisms for some TCR α and for invariant TCR $\gamma\delta$ chains.

A selective pressure independent of known MHC molecules is the cause for this new type of T-cell selection. It will be important to determine the nature of the selecting agent. Genetic experiments will soon test whether this agent is determined by an MHC-linked gene. It remains to be seen what roles the T cells bearing this homogeneous α chain play in the immune system.

We express our sincere thanks to Dr. T. Sado, Institute of Radiological Science, Chiba for preparation of TXB mice. We also thank Ms. Hiroko Tanabe for preparing and Evan Hermel for reading the manuscript. This work was supported by special coordination funds for promoting science and technology of the Science and Technology Agency, Japan.

1. Zinkernagel, R. M. & Doherty, P. C. (1979) *Adv. Immunol.* **27**, 51–177.
2. Bill, J., Appel, V. B. & Palmer, E. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9184–9188.
3. Burton, R. C., Chism, S. E. & Warner, N. L. (1977) *J. Immunol.* **118**, 971–980.
4. Forman, J. & Flaherty, L. (1978) *Immunogenetics* **6**, 227–233.
5. Fischer-Lindahl, K. (1979) *Immunogenetics* **8**, 71–76.
6. Fischer-Lindahl, K., Bocchieri, M. & Riblet, R. (1980) *J. Exp. Med.* **152**, 1583–1595.
7. Molnar-Kimber, K. & Sprent, J. (1980) *J. Exp. Med.* **151**, 407–417.
8. Wakabayashi, S., Taniguchi, M., Tokuhisa, T., Tomioka, H. & Okamoto, S. (1981) *Nature (London)* **294**, 748–750.
9. Fischer-Lindahl, K., Hermel, E., Loveland, B. E., Richards, S., Wang, C.-R. & Yonekawa, H. (1989) *Cold Spring Harbor Symp. Quant. Biol.* **54**, 563–569.
10. Loveland, B., Wang, C.-R., Yonekawa, H., Hermel, E. & Fischer-Lindahl, K. (1990) *Cell* **60**, 971–980.
11. Asarnow, D. M., Kuziel, W. A., Banyhadi, M., Tigelaar, R. E., Tucker, P. W. & Allison, J. P. (1988) *Cell* **55**, 837–847.
12. Lafaille, J. J., DeCloux, A., Bonneville, M., Takagaki, Y. & Tonegawa, S. (1989) *Cell* **59**, 859–870.
13. Sim, G. K. & Augustin, A. (1990) *Cell* **61**, 397–405.
14. Bluestone, J. A., Cron, R. Q., Cotterman, M., Houlden, B. A. & Matis, L. A. (1988) *J. Exp. Med.* **168**, 1899–1916.
15. Bonneville, M., Ito, K., Krecko, E. G., Itohara, S., Kappes, D., Ishida, I., Kanagawa, O., Janeway, C. A., Murphy, D. B. & Tonegawa, S. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5928–5932.
16. Vidovic, D., Roglic, M., McKune, K., Guerder, S., MacKay, C. & Dembic, Z. (1989) *Nature (London)* **340**, 646–650.
17. Imai, K., Kanno, M., Kimoto, H., Shigemoto, K., Yamamoto, S. & Taniguchi, M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8708–8712.
18. Koseki, H., Imai, K., Nakayama, F., Sado, T., Moriwaki, K. & Taniguchi, M. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5248–5252.
19. Teh, H. S., Kisielow, P., Scott, B., Kishi, H., Uematsu, Y., Blüthmann, H. & von Boehmer, H. (1988) *Nature (London)* **335**, 229–233.
20. Kisielow, P., Teh, H. S., Blüthmann, H. & von Boehmer, H. (1988) *Nature (London)* **335**, 730–732.
21. Sha, W. C., Nelson, C. A., Newberry, R. D., Kranz, D. M., Russel, J. H. & Loh, D. Y. (1988) *Nature (London)* **336**, 73–76.
22. Kaye, J., Hsu, M. L., Sauron, M. E., Jameson, J. C., Gascoigne, R. J. & Hedrick, S. M. (1989) *Nature (London)* **341**, 746–749.
23. Taniguchi, M., Saito, T. & Tada, T. (1979) *Nature (London)* **278**, 555–558.
24. Koseki, H., Imai, K., Ichikawa, T., Hayata, I. & Taniguchi, M. (1989) *Int. Immunol.* **1**, 557–564.
25. Glisin, V., Crkvenjakov, R. & Byus, C. (1974) *Biochemistry* **13**, 2633–2637.
26. Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1984) *Nucleic Acids Res.* **12**, 7035–7056.
27. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230**, 1350–1354.
28. Wang, A. M. & Mark, D. F. (1990) in *PCR Protocols*, eds. Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. T. (Academic, New York), pp. 70–75.
29. Richards, S., Bucan, M., Brorson, K., Kiefer, M. C., Hunt, S. W., III, Lehrach, H. & Fischer-Lindahl, K. (1989) *EMBO J.* **8**, 3749–3757.
30. Fischer-Lindahl, K. (1986) *Curr. Top. Microbiol. Immunol.* **127**, 272–278.
31. Nei, M. (1972) *Am. Nat.* **106**, 283–292.
32. Bonhomme, F. & Guénet, J.-L. (1989) in *Genetic Variants and Strains of the Laboratory Mouse*, eds. Lyon, M. F. & Searle, A. G. (Fischer, Jena, F.R.G.), pp. 649–662.