Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 5248–5252, July 1990
Immunology

Homogenous junctional sequence of the V14$^+$ T-cell antigen receptor α chain expanded in unprimed mice

(Vα14 gene/bone marrow chimera)

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Communicated by Susumu Tonegawa, April 2, 1990

ABSTRACT **Nucleotide sequences of VJ (variable-joining) junctional regions of V14$^+$ α-chain T-cell receptor genes show that most Vα14$^+$ T cells use one α chain (Vα14Jα281 with a one-nucleotide N region, which is frequently used in keyhole limpet hemocyanin-specific suppressor T-cell hybridomas) in unprimed mice. Moreover, the frequency of this α-chain expression was >1.5% of the total α chains found in laboratory strains, including B10 congenic mice. This is about 10^4 times higher than was expected. The V14J281 α-chain expression was relatively low but was significant in CD4$^+$/CD8$^+$ immature thymocytes and became quite high in mature single-positive T cells, implying that this α chain is selected during T-cell maturation. V14J281 expression increased with time after birth and reached a maximum at around 5 weeks of age. The ligand seems to be a self molecule and to be present in laboratory strains but to be absent in a wild mouse, *Mus musculus molossinus*, because bone marrow chimeras clearly showed that bone marrow cells derived from *Mus musculus molossinus* negative for this α chain raised V14J281-positive T cells in a C57BL/6 environment. The above results suggest that there are some selection mechanisms for this cell type other than those for conventional $\alpha\beta$ T cells and also that the homogenous VJ junction of the V14J281 α chain plays a pivotal role in the selection of the T cell and its ligand reactivity.**

Thymus-derived lymphocytes (T cells) recognize antigens in the context of the polymorphic parts of major histocompatibility complex (MHC) class I or class II molecules by virtue of the heterodimeric $\alpha\beta$ T-cell receptor (TCR), which is found on the vast majority of mature T cells in the thymus and peripheral lymphoid tissues (1, 2). A second TCR, $\gamma\delta$ TCR, has also been identified (3, 4) and found to be relatively abundant in some adult mouse organs and among fetal thymocytes.

The T-cell repertoire seems to be generated by two selection mechanisms during T-cell development in the thymus (5-7). (*i*) Only T cells bearing receptors that will be able to participate in the recognition of nominal antigen plus self MHC molecules in the periphery are selected. This positive selection occurs in the presence of self MHC but in the absence of the nominal antigen. (*ii*) T cells that react strongly with self MHC molecules or MHC plus self antigen [i.e., H-Y or minor lymphocyte stimulatory (Mls) antigen] present in the thymus are thought to be eliminated (negative selection).

Recent estimates of the potential diversity for $\alpha\beta$ and $\gamma\delta$ TCRs have suggested that both TCR repertoires are almost the same size (8). Diversity of TCRs depends primarily on the heterogeneity mediated by somatic recombination of the variable (V) and joining (J) gene segments. Moreover, the N region generated by insertion or/and deletion of nucleotides in VJ junctional regions greatly contributes to the generation of TCR diversity. Perhaps amino acid residues in these regions are analogous to a third hypervariable region and thus are directly involved in ligand binding (8). In contrast to the diversity observed in $\alpha\beta$ TCRs, some $\gamma\delta$ TCRs show striking homogeneity in VJ junctional region sequences (9–11), suggesting that the ligand specificity, functions, or selection mechanisms of these T cells are different from those of $\alpha\beta$ T cells.

We provide evidence herein that some T cells use a homogenous α chain (V14J281). This seems to be a consequence of selection and expansion of this T-cell type, because its frequency is >1.5% of the total α chains in the peripheral lymphoid organs. Moreover, studies using bone marrow chimeras suggest that the ligand is an unidentified self molecule. The results indicate that the homogenous VJ junctional region of the V14J281 α chain, in particular, plays a crucial role in the selection of this type of T cell and its ligand reactivity.

MATERIALS AND METHODS

Animals and Cell Lines. Pathogen-free C57BL/6 mice were purchased from Shizuoka Experimental Animal Co., Hamamatsu, Japan. A wild Japanese mouse strain, *Mus musculus molossinus*, was established and maintained by K.M. Other strains, including B10 congenic lines used, were also maintained by K.M. A thymoma cell line of AKR origin, BW5147, and a keyhole limpet hemocyanin (KLH)-specific suppressor T-cell (Ts) hybridoma (BW5147 × C57BL/6 Ts; 34S-281) used in the present studies have been described (12, 13).

RNase Protection Analysis. Total cellular RNA was isolated from adult and neonatal thymus and spleen of unprimed mice by homogenization in 4 M guanidinium isothiocyanate and by ultracentrifugation through a 5.7 M CsCl cushion (14). RNase protection analysis was performed as described (15). Restriction fragments containing TCR α-chain V, J, and constant (C) region cDNAs obtained from the KLH-Ts hybridoma 34S-281 that encompass a part of the 5′ untranslated region of the full-length V14J281 and the 5′ side of the Cα region were subcloned into Gemini plasmids (Promega Biotec), and ^{32}P-labeled RNA probes were prepared by transcription of linealized plasmids. In some experiments, RNA probes were prepared by using genomic DNA encompassing the rearranged V14J281 α-chain gene segments isolated from hybridoma 34S-281. Predicted protection bands are indicated in the figures. Full-length probes were eluted from 4% poly-

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Abbreviations: V, variable; J, joining; MHC, major histocompatibility complex; TCR, T-cell receptor; KLH, keyhole limpet hemocyanin; Ts, suppressor T cell; C, constant; PCR, polymerase chain reaction; Hmt, histocompatibility maternally transmitted.

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acrylamide/7 M urea sequencing gels, as described (13). Hybridization was carried out at 35°C for 12 hr and then samples were treated with RNase at 14°C for 30 min. RNA fragments protected were analyzed in a 4% polyacrylamide/7M urea sequencing gel that was electrophoresed at 1000 V for 2 hr.

Polymerase Chain Reaction (PCR) and Sequencing. Total RNA isolated was also used for the PCR (16). The first-strand cDNA synthesis was initiated with 20 μg of RNA, avian myeloblastosis virus reverse transcriptase (Seikagaku Kogyo, Tokyo), and a Cα-specific primer. After alkaline hydrolysis of RNA and ammonium acetate/ethanol precipitation, the first-strand cDNA was subjected to 30 cycles of PCR amplification with Vα14- and Cα-specific primers. Temperatures were 72°C for polymerization, 95°C for denaturation, and 55°C for primer annealing. Amplified cDNA was digested with *Eco*RI and cloned into pUC118. After screening with ^{32}P-labeled Vα14 probe, positive colonies were isolated, and both strands of the inserts were sequenced by the dideoxynucleotide chain-termination method. Oligonucleotides used in the amplification were 5′-TCTAGAATTGTGTCCTGAGACCGAGGATC-3′ (Cα primer) and 5′-TCTAGAATTCTAAGCACAGCACGCTGCACA-3′ (Vα14 primer). The *Eco*RI linker sequences are underlined.

Bone Marrow Chimeras. Bone marrow chimeras were prepared as described (17). Briefly, 12-week-old C57BL/6 mice were γ-irradiated with 1200 R (1R = 0.258 mC/kg) from a ^{137}Cs source and then reconstituted with 1×10^7 anti-Thy-1.1- or anti-Thy-1.2-treated allogeneic or syngeneic bone marrow cells from *M. musculus molossinus* (Thy-1.1) or C57BL/6 (B6) (Thy-1.2). They were kept for 6–8 weeks and then used for experiments. Thymocytes from the chimeras were analyzed for their Thy-1 phenotypes by using a fluorescence-activated cell sorter (FACS IV, Becton Dickinson) and monoclonal anti-Thy-1.1 or anti-Thy-1.2 antibodies. More than 95% of thymocytes in *M. musculus molossinus* → C57BL/6 chimeras expressed the Thy-1.1 antigen of donor (*M. musculus molossinus*) origin. We also confirmed that V14$^+$ α-chain mRNA in chimeric mice was of *M. musculus molossinus* origin. There is a one-base difference between these two strains at a nucleotide position 1199 (thymidine in C57BL/6 and cytidine in *M. musculus molossinus*), ^{1173}TATCGGTTGTTTTTTGTTTTTTTTTT^{1199}TTTGCGGGTTTATT^{1212}, where position 1199 is thymidine in C57BL/6 (18). We amplified V14$^+$ α-chain cDNA by the PCR with primers specific for Vα14 and for the 3′ untranslated region of α-chain mRNA (oligonucleotide used was 5′-TACACACAGTAGCAGGAGGG-3′, which corresponds to the complementary nucleotide sequence from position 1273 to position 1292). Seven V14$^+$ α-chain cDNAs cloned from chimeric mice were sequenced and all were found to have a cytidine at position 1199.

Preparation of T-Cell Subsets. For preparation of CD4$^+$/CD8$^+$ immature T cells, thymocytes were stained with both phycoerythrin-conjugated anti-CD4 (GK1.5) and fluorescein isothiocyanate-conjugated anti-CD8 (83-12-5) and fractionated by using a FACS IV. The purity was >95%. To prepare CD4$^+$/8$^-$ and CD4$^-$/8$^+$ single-positive cells, thymocytes were treated twice, either with anti-CD8 or anti-CD4 and then with rabbit complement, and they were further stained with fluorescein isothiocyanate-conjugated anti-CD8 or phycoerythrin-labeled anti-CD4. They were then sorted by using a FACS IV. The purity of single-positive thymocytes was >90%. For preparation of single-positive splenic T cells, immunoglobulin-negative spleen cells "panned-out" with rabbit anti-mouse immunoglobulin-coated Petri dishes (95% pure) were treated with anti-CD4 (GK1.5) or anti-CD8 (83-12-5) and then with complement. The purity was >90%.

RESULTS AND DISCUSSION

Predominant Expression of the V14$^+$ TCR α Chain with Homogenous Junctions in Unprimed Mice. To understand selection and clonal expansion of T cells under physiological conditions, it would be advantageous to analyze the TCR repertoire in naive mice. However, because of the tremendous diversity in TCR repertoires, this may be a very complicated task. The complexity of the α-chain locus has made analysis of this chain particularly difficult, as there are >100 Vα and 80 Jα gene segments (8, 19). To overcome these problems, we used the RNase protection assay (15) and the PCR (16) and investigated the frequency of a particular α chain mRNA in the thymus and spleen.

The degree of Jα diversity associated with a particular Vα14 gene was analyzed by PCR with primers specific for Cα and Vα14, the Vα gene originally isolated from the KLH-Ts hybridoma 34S-281 [Vα14 was originally called Vα281 (12)]. We then amplified and sequenced Vα14$^+$ transcripts of unprimed adult C57BL/6 mice. Thirteen cDNA clones were randomly isolated from two PCR experiments (B6S1 and B6S2 in Fig. 1) and all were found to be in-frame. The sequences of cDNA clones showed that 12 of 13 cDNAs utilized the same VJα segments with a one-nucleotide addition for the N region (Fig. 1). As the N region was the third base of codon GGX, the VJ junctional regions of all cDNA clones formed a glycine residue. Codon preference in the N region was not observed. There was only one exception that used different Jα and N regions. Similar findings were also observed in the thymus. Three of four functional V14$^+$ α chains were the same as those in the spleen (Fig. 1). These findings suggest that the VJ junction is important for binding to or recognition of the ligand.

	Vα14	N	Jα	
Germline Vα14	TGT GTG GTG GG	CGCACCATGCTC		
Germline Jα281		TGGCACTGTGTA	GAT AGA GGT TCA	
34S-281	TGT GTG GTG GG	G	GAT AGA GGT TCA	
C57BL/6 adult spleen				
B6S131	TGT GTG GTG GG	G	GAT AGA GGT TCA	Jα281
B6S141	--- --- --- --	G	--- --- --- ---	
B6S151	--- --- --- --	G	--- --- --- ---	
B6S111	--- --- --- --	G	--- --- --- ---	
B6S271	--- --- --- --	G	--- --- --- ---	
B6S132	--- --- --- --	A	--- --- --- ---	
B6S250	--- --- --- --	A	--- --- --- ---	
B6S227	--- --- --- --	T	--- --- --- ---	
B6S230	--- --- --- --	T	--- --- --- ---	
B6S113	--- --- --- --	C	--- --- --- ---	
B6S249	--- --- --- --	C	--- --- --- ---	
B6S270	--- --- --- --	C	--- --- --- ---	
B6S112	--- --- --- --	CGAG	GGC GGA AAT GAG	
C57BL/6 adult thymus				
B6T1	TGT GTG GTG GG	A	GAT AGA GGT TCA	Jα281
B6T3	--- --- --- --	C	--- --- --- ---	
B6T5	--- --- --- --	C	--- --- --- ---	
B6T4	--- --- --- --	CGCT	TGG AGT GCA GGG	
B6T6	--- --- --- --	CT	TCA TGG ATA GCA	*
C57BL/6 neonatal thymus				
B6NT1	TGT GTG GTG GG	C	GAT AGA GGT TCA	Jα281
B6NT12	--- --- --- --	G	--- --- --- ---	
B6NT2	--- --- --- --	CGGA	AGC AAT GCA AAG	
B6NT3	--- --- --- -	CC	GAA TTA TGG GAG	
B6NT5	--- --- --- --	C	GCG GGG AGC AGT	
B6NT9	--- --- --- -	AG	GAT CTT CCA ATA	
B6NT8	--- --- --	ATAA	CAC CAA TAC AGG	+

FIG. 1. Sequences of Vα14$^+$ transcripts from unprimed C57BL/6 spleen and thymus. Sequences are aligned with the partial V and J region sequences from the KLH-Ts hybridoma 34S-281 and a break is identical at the V-J junction with the putative N regions. Germ-line Vα14 and Jα281 sequences are also illustrated at the top, in which heptamer and nonamer sequences are underlined. Identical bases are indicated by a dash. Transcripts indicated by markers have an out-of-frame sequence (*) or a stop codon in the N region (+).

The usage frequency of the V14J281 α chain was also investigated by RNase protection assay. As shown in Fig. 2*a*, a fully protected VJC band of 630 base pairs (bp) was detected in the thymus and spleen. Densitometric analysis demonstrated that the usage frequency of the V14J281 α chain was >0.7% in the thymus and 1.5% in the spleen of the total α chains. Therefore, this α-chain expression is $>1 \times 10^4$ times higher than was expected from the calculation of Davis and Bjorkman (8), demonstrating that the potential diversity of the TCR α chain was 5×10^5. As the proportion of VJ combinations in PCR-amplified cDNA reflects the actual level of these transcripts prior to amplification (20), it is clear that the T cells bearing this α chain are actively selected and expanded even under unprimed conditions.

Ligand of the V14J281 α Chain. To investigate the ligand of the V14J281 α chain, we made bone marrow chimeras between C57BL/6 and a wild Japanese mouse strain, *M. musculus molossinus* (*M. musculus molossinus* → C57BL/6) (21). The *M. musculus molossinus* strain did not express this α chain despite the apparent expression of the Vα14 (289 bp) or Jα281 (Fig. 2*b*). This implies that *M. musculus molossinus* has a defect in the self ligand that binds this α chain, whereas progenitor T cells in *M. musculus molossinus* bone marrow seem to be intact. In fact, the data in Fig. 2*b* clearly demonstrated that in RNase protection experiments RNA from *M. musculus molossinus* → C57BL/6 chimeras had a VJ band (341 bp) that could be fully protected and had an intensity similar to the 341-bp band in C57BL/6. Therefore, *M. musculus molossinus* lacks the ligand for this α chain, which seems to react with a self element expressed in C57BL/6. In addition, the predominant expression of V14J281 α chain was also observed in germ-free mice (data not shown), which strongly suggests that the ligand is not a conventional pathogen.

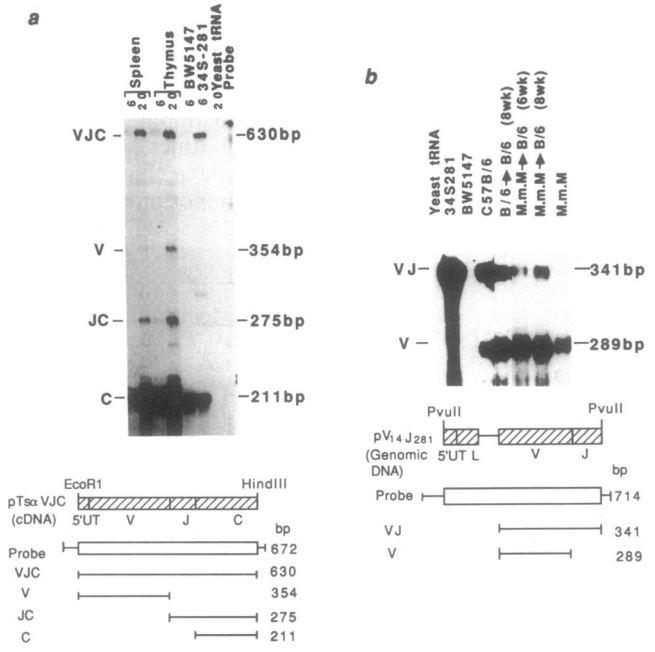
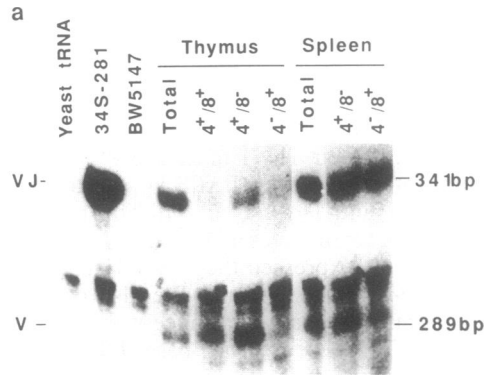


FIG. 2. RNase protection analysis of V14J281 α-chain mRNA levels in C57BL/6 and x-irradiation-prepared bone marrow chimera mice. Two probes were used, pTsαVJC cDNA (*a*) and pV14J281, a genomic clone (*b*). The indicated amounts (6–20 μg) of RNA were analyzed. 34S-281, a KLH-Ts hybridoma; BW5147, a parent for hybridoma; spleen and thymus, respective tissues from adult (8 week old) C57BL/6 mice; C57BL/6, thymus from 8-week-old C57BL/6 mice; M.m.M., thymus of *M. musculus molossinus*; B6 → B6 and M.m.M. → B6, thymus from the respective bone marrow chimera mice. Soluble yeast RNA was also used as negative control. The lengths of the probe are indicated by hatched boxes. Regions of the probes corresponding to V, J, and C sequences are indicated. The ratios of the VJC to Cα transcripts were calculated by scanning densitometer. The V14J281$^+$ α chains detected in the thymus and spleen were 0.7% and 1.5% of total α chains, respectively. Moreover, when the intensity of VJC bands (630 bp) was compared with V bands (354 bp) in *a*, 50% (in the thymus) and 85% (in the spleen) of V14$^+$ α chains used Jα281 segment. (*b*) The RNase protection assay was carried out by using the pV14J281 genomic probe. Note that the intensity of fully protected VJ band (341 bp) in *M. musculus molossinus* → C57BL/6 chimera mice was increased with time after making the chimera. No 341-bp VJ band was found, but a 289-bp V band was detected in 8-week-old *M. musculus molossinus* mice (*b*).

Results obtained by the RNase protection assay were also confirmed by the PCR at the nucleotide level (Fig. 4). We sequenced 11 cDNA clones from double-positive thymo-



b

Subsets	%V14$^+\alpha$-chains		V14J281/V14Jx
	V14J281	V14Jx	
Thymus			
Total	75	25	3.0
CD4+/CD8+	*25*	*75*	*0.3*
CD4+	56	44	1.4
CD8+	80	20	4.0
Spleen			
Total	90	10	9.0
CD4+	80	20	4.0
CD8+	90	10	9.0

FIG. 3. RNase protection analysis of V14J281 α-chain mRNA in T-cell subsets. RNase protection was performed with the pV14J281 genomic probe as described in Fig. 2*b* and illustrated in *a*. RNA from total thymus (10 μg); CD4$^+$/8$^+$ (10 μg), CD4$^+$/8$^-$ (10 μg), and CD4$^-$/8$^+$ (2 μg) thymocytes; and CD4$^+$/8$^-$ (20 μg) and CD4$^-$/8$^+$ (20 μg) spleen cells; and total spleen cells (20 μg) were used. For a positive control, 10 μg of RNA from 34S-281 was used and for negative controls, BW5147 and soluble yeast RNA were used. The intensity of the VJ band (341 bp) and the V band (289 bp) was measured by densitometer and the percentages of V14$^+$ α chains (Vα14Jα281 vs. Vα14 associated with other Jα) were calculated and summarized in *b*. Note that the amount of RNA of CD8$^+$ thymocytes loaded on the gel was 2 μg so that the bands were faint. However, in CD8$^+$ thymocytes the ratio of VJ (341 bp) to V (289 bp) was 4.0, which is similar to levels of total (3.0) or CD4$^+$ (1.4) thymocytes or of CD4$^+$ or CD8$^+$ splenic T cells (4.0 and 9.0, respectively).

	Vα14				N	Jα				
	G	V	V	G		D	R	G	S	
34S-281	TGT	GTG	GTG	GG	G	GAT	AGA	GGT	TCA	
C57BL/6 CD4+/CD8+ thymocytes										
DP1	TGT	GTG	GTG	GG	G	GAT	AGA	GGT	TCA	Jα281
DP6	---	---	---	--	C	---	---	---	---	
DP10	---	---	---	--	C	---	---	---	---	
DP16	---	---	---	--	G	---	---	---	---	
DP2	---	---	---	G	CC	GGG	CGA	GGC	AAC	Jx
DP5	---	---	---	--	C	GCT	TGG	AGT	GCA	
DP9	---	---	---	--	A	ATA	GCA	TCC	TCC	
DP8	---	---	---	G	C	TTC	ATG	GAT	AGC	*
DP11	---	---	---	--	CG	CGA	GCA	ATA	ACA	*
DP12	---	---	---	G		ATC	TGG	AGG	AAG	*
DP15	---	---	---	--	C	TAA	CAG	TGC	AGG	*

FIG. 4. Sequences of Vα14$^+$ transcripts from CD4$^+$/CD8$^+$ immature thymocytes. Sequences of PCR-amplified cDNAs are aligned with the partial V and J region sequences of hybridoma 34S-281. Identical bases are indicated by dashes. Four of 11 clones use a J segment in V14$^+$ α-chain transcripts that is identical to Jα281 (36%). The clone bearing Vα14 associated with other Jα sequences is indicated as Jx. Transcripts indicated by asterisks have an out-of-frame sequence or a stop codon.

cytes. Four out of seven clones with in-frame junctions (about 50% of V14$^+$ α chain) showed the same sequence as the majority of cDNA clones obtained from thymus and spleen. Seven clones used a Jα other than Jα281, of which four clones were shown to have out-of-frame sequences or a stop codon. Although this particular V14J281 α chain in mature T cells was ≈85% of the V14$^+$ α chains, the expression of the V14J281 α chain in the stage of double-negative immature thymocytes was also significant. In other words, both immature and mature T cells bearing this α chain proliferate and expand even in the thymus. Thus, the mechanisms of selection and expansion of this type of T cells seem to be distinct from those of other αβ T cells.

During ontogeny, V14J281 α chains appeared at 2 weeks after birth, gradually increased, and reached a maximum at 5–8 weeks (Fig. 5). Nothing was detected at fetal day 18 and neonatal stages, by RNase protection assay, whereas expression of the Vα14 and Jα281 genes even in fetal stages was similar to that of an 8-week-old adult mouse. This was also supported by the PCR results (Fig. 1). Only two out of seven cDNA clones were found to possess the V14J281 α-chain sequence. Thus, the majority of V14$^+$ α-chain cDNA clones were associated with other Jα segments at neonatal stages. However, the V14J281 α-chain transcript was dominant in adult mice (8 weeks old) (Fig. 1). Thus, T cells bearing this α chain are indeed selected and expanded in the self environment prior to nominal antigenic stimulations.

Implication for the Homogenous J-Region Sequence of the V14J281 α Chain and Its Functional Roles. The homogeneity of TCR usage has so far been reported by two groups on γδ T-cell subsets. Asarnow *et al.* (9, 10) have demonstrated that the junctional sequences of both γ and δ TCR chains from five Thy-1$^+$ dendritic epithelial cell clones are invariant. Lafaille *et al.* (11) have also shown that the γδ T cells localized in reproductive organs possess the homogeneous (Vγ6Jγ1/Vδ1Dδ2Jδ2) sequences with identical joints. Therefore, the V14J281 α chain is another example of a homogenous TCR on a T-cell subset.

The selection mechanisms of these T cells are largely unknown. Analogous to the homogeneity of γδ TCR of the dendric epithelial cells and reproductive organs, the use of homogenous VJ junctional sequence of the V14J281 α chain in the periphery strongly suggests that the mechanisms for selecting this cell type are different from those for conventional αβ T cells. This unusual selection is also evident in the following results. (*i*) This α-chain expression was higher in spleen than thymus (Fig. 2*a*) and increased with time after

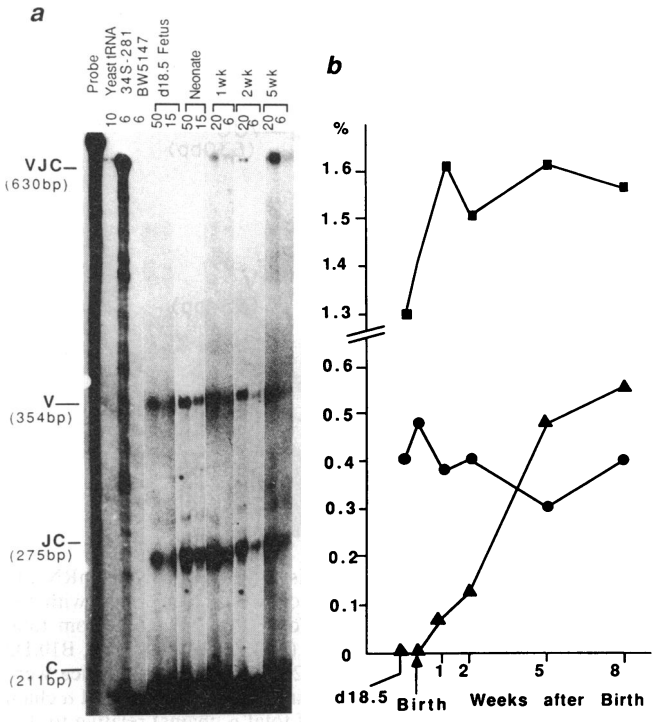


FIG. 5. RNase protection analysis of V14J281 α-chain mRNA levels in thymus at various stages of ontogeny. RNase protection was carried out as described in Fig. 2*a* with the pTsαVJC as a probe. (*a*) The indicated amounts (6–50 μg) of C57BL/6 thymus RNA were obtained from a fetus at day 18.5 of gestation (d18.5 fetus), a neonate, and mice at 1 week old, 2 weeks old, and 5 weeks old. (*b*) The intensity of bands corresponding to VJC (630 bp), V (354 bp), JC (275 bp), and C (211 bp) was calculated by scanning densitometer. The ratios of the VJC-(▲), V-(●), and JC-(■) containing transcripts to Cα mRNA were measured and expressed as percentages of total α chains.

birth (Fig. 5), although the ligand seems to be a self molecule (Fig. 2*b*). (*ii*) A high level of V14J281 α-chain expression was observed in various B10 congenic strains (Fig. 6). This is contradictory to the present hypothesis, because the concept of positive selection for conventional αβ T cells is based on the H-2-dependent selection mechanisms (5–7). (*iii*) The homogenous sequence of this α chain was frequently found in the spleen of athymic mice, suggesting that the positive selection of this cell type is in fact operating in the periphery without thymic influence (unpublished results).

There are several other possible explanations for the homogenous VJ junctional region of this α chain. It could be due to preferential rearrangements analogous to the frequent joining of a heavy chain V segment to the most proximal heavy chain J region as observed in the development of some B cells (22). However, this was not the case here because PCR data using mRNA of neonatal thymocytes showed no preferential association of the Vα14 with Jα281 segment (Fig. 1). In addition, it is also conceivable that the activity of terminal deoxynucleotidyltransferase, exonuclease, or both might be more limited in the V14J281-α-chain-bearing T cells than in the vast majority of other αβ T-cell populations, if limited heterogeneity is not simply due to the difference in cellular selection, as suggested by Lafaille *et al.* (11).

There are at least two possibilities for the ligand specificity of the V14J281 α chain. First, the ligand is a self peptide, as strongly suggested by the chimera experiments (Fig. 2*b*). As KLH-Ts hybridomas all use this α chain (13), it is likely that the self ligand cross-reacts with the nominal antigen KLH. The amino acid in the VJ junctional region is always a glycine (Fig. 1). Thus, it seems reasonable that the specific amino

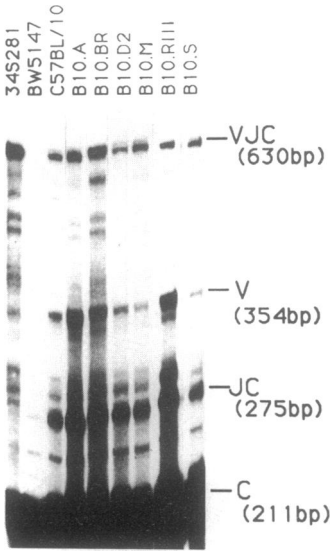


FIG. 6. RNase protection analysis of V14J281 α-chain mRNA in B10 congenic strains. RNase protection was performed with the pTsαVJC cDNA probe as described in Fig. 2*a*. RNA from total thymus (20 μg) of B10 (H-2^b), B10.A (H-2^a), B10.BR (H-2^k), B10.D2 (H-2^d), B10.M (H-2^f), B10.RIII (H-2^r), or B10.S (H-2^s) mice were used. Note that all B10 congenic strains express the V14J281 α chain (630 bp) at a high level (about 1% of total α chains) relative to that observed in C57BL/6 mice.

acid at this position in the α chain would be critical for binding. In fact, the amino acid at position 100 (VJ junctional region) in the β chain determines the antigen specificity in cytochrome *c*-specific T cells (23). If this is the case, other features (such as the β chain) of TCR may discriminate the polymorphic parts of MHC restriction elements. Our studies shown in Fig. 6 in part support this concept, because B10 congenic mice with a different H-2 expressed this α chain at the same level. Similar observations have been reported by Roth *et al.* (24) who found that three H-2-congenic mice (BALB/c, BALB.B, and BALB.K) expressed the identical V58J58-positive α chain originally isolated from clone 2C that was specific for the alloantigen. Therefore, it is likely that the β chain is the determining factor for distinguishing polymorphic MHC determinants.

The second possibility is intriguing. The V14J281 α chain recognizes a "monomorphic MHC," such as the histocompatibility maternally transmitted (Hmt) molecule (25). The Hmt is, however, monomorphic only in the laboratory strains independent of H-2 but has been shown to be polymorphic among mouse subspecies. The two allelic forms of Hmt, Hmt^a and Hmt^b, have been identified. The Hmt^a is invariant in all laboratory strains, whereas a wild mouse strain, *Mus musculus castaneus*, expresses Hmt^b (26). In fact, Fischer Lindahl *et al.* (25) have detected H-2-unrestricted cytotoxic T cells reactive with all Hmt^a mice. This Hmt thus may be a candidate for the molecule selecting the V14J281 α chain. This is unlikely however, because the V14J281 α chain is expressed in *M. musculus castaneus* as well as in all laboratory strains (unpublished results). If other molecules are expressed in a mouse-subspecies-specific manner, they could be candidates for positive selection of the particular subsets of T cells.

The function of V14J281$^+$ T cells has not yet been precisely determined. However, the existence of autoreactive "naturally activated" lymphocytes with a nonlytic suppressive function in normal individuals has been reported (27). It has also been demonstrated that a fraction of T cells with this TCR α chain mediates the regulation of the anti-KLH antibody response (17, 28). One can, therefore, assume that these cell types probably possess regulatory functions. This may be because autoreactive T cells with regulatory activity work as a fail-safe mechanism to control the self-reactive repertoire escaping from negative selection during T-cell development in the thymus.

We thank Dr. Susumu Tonegawa (Massachusetts Institute of Technology) for his help with the RNase protection assay. We are also thankful for the assistance of Dr. H. Kamisaku and Dr. N. Miyashita. This work was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture, and by funds of the Science and Technology Agency (Japan).

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