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Evolution of Vα14 TCR Gene Family in Mice

RIEKO KANNO,[*1] YOKO MIZUTANI,[*1]
NARUYA SAITOU,[*2] HARUHIKO KOSEKI,[*1]
YASUHIKO MAKINO,[*1] YOSHIHIRO ADACHI,[*1]
TAKESHI AKASAKA,[*1] MASAMOTO KANNO,[*1]
TOSHIHIKO SHIROISHI,[*2] KAZUO MORIWAKI,[*2]
AND MASARU TANIGUCHI[*1]

Center for Neurobiology and Molecular Immunology, School of Medicine, Chiba University, Chiba 260[*1] *and National Institute of Genetics, Mishima 411,*[*2] *Japan*

The repertoire of T cell antigen receptor (TCR) is constructed in the thymus and is believed to be extremely large ($\sim 10^{15}$) based on the calculation by Davis and Bjorkman (*1*). The TCR repertoire is generated by the tissue-specific DNA rearrangement events mediated by somatic recombination of the variable (V) and joining (J) gene segments. Moreover, the N-region generated by insertion and/or deletion of nucleotides in VJ junctional regions greatly contributes to the generation of TCR diversity.

TCR is composed of α and β polypeptide chains which recognize antigens in conjunction with the products of the major histocompatibility complex (MHC) class I and class II molecules. Therefore, T cell receptors, unlike immunoglobulin (Ig) molecules which can bind free antigens, recognize both antigens and self MHC molecules. This phenomenon is called the MHC restriction of antigen recognition and is acquired during T cell development in the thymus.

The TCR repertoire is further shaped by two selection mechanisms during T cell development in the thymus. (a) Only T cells bearing receptors that will be able to recognize nominal antigen plus self MHC in the periphery are selected. This positive selection occurs in the absence of nominal antigen. (b) T cells reacting strongly with self MHC or self MHC plus antigen (self) present in the thymus (self-reactive repertoire) are eliminated. This is negative selection. By these mechanisms, the majority of self reactive T cells are eliminated in the thymus, and thus only a small percentage of developing thymocytes emerges from the thymus and comprises the functional peripheral T cell repertoire. Despite the clonal elimination of self reactive T cell repertoire in the thymus, autoreactive T cells are, in fact, present in the periphery but do not develop autoimmune diseases, suggesting that there is a mechanism for induction and maintenance of self tolerance in the periphery.

In the TCR repertoire, we found that V14$^+$ TCR α-chain possesses unique characteristics (*2-4*). (a) Most of the Vα14 TCRs expressed in the periphery are encoded by the gene of Vα14Jα281 with a one-base N-region. (b) In all laboratory mouse strains, the invariant Vα14Jα281 TCR is expressed on 2-3% of peripheral T cells. (c) The Vα14Jα281 TCR recognizes self molecules encoded by three genes on Chromosome 1, Chromosome 6, and Chromosome 15. (d) Although the invariant Vα14 T cells are autoreactive, they are not eliminated, but are positively selected in peripheral tissues.

According to the unique characteristics of Vα14 T cells, we assume that the Vα14Jα281 T cells have unique functions other than those of helper and cytotoxic T cells. In fact, the decrease of V14J281 TCR α-chain expression strongly correlates with the development of some autoimmune disease status, such as systemic lupus erythematosus (SLE) or lymphoproliferative disorders. MRL/*lpr* mice develop lymphoproliferative and systemic autoimmune diseases at the age of 20 weeks. The expression of Vα14Jα281 TCR decreases to a level of 1/30 as that of the age-matched control mice while no change is observed in the levels of other TCR, such as Vα14$^+$ TCR associated with other Jα or Jα281$^+$ TCR with other Vα. Therefore, the decrease of Vα14Jα281 TCR expression in *lpr* mice is correlated with development of the diseases. It is thus likely that Vα14Jα281 T cells play a decisive role in the development of autoimmune diseases. Here, we summarize our recent results of molecular genetic analyses on the Vα14 TCR gene family and discuss its evolutional aspects.

DOMINANT EXPANSION OF T CELLS BEARING INVARIANT Vα14Jα281 TCR IN THE PERIPHERY

By RNase protection assay, we found that the invariant Vα14Jα281 TCR expression was about 0.5% of total TCR α-chains in thymus and 2.0% in spleen (*3*). The diversity of TCR α-chains is generated by the combination of >100 Vα and 100 Jα gene segments as well as the N-regions and is calculated to be 10^8. Calculations show that the frequency of one particular TCR expression is $1/10^8$. Thus, the homogenous Vα14Jα281 TCR expression is estimated to be more than 10^4-10^6 times higher than expected.

The nucleotide sequences of cDNAs amplified by reverse transcription-polymerase chain reaction (RT-PCR) technique confirmed the data obtained by RNase protection assay. Twelve of 13 (92%) cDNA clones revealed the invariant Vα14Jα281 sequence with a single nucleotide N-region. As the N-region was the third base of the codon GGX, the VJ junctional regions of all the cDNA clones were translated into a glycine residue. It is speculated that the VJ junction is important for recognition of the ligand, and also that the predominant expression of invariant Vα14Jα281 TCR is due to the selection and clonal expansion which occurs even in unprimed mice, because in the neonatal stage the Vα14 genes are associated with Jα other than Jα281 and become homogenous in the adult.

EXTRATHYMIC DIFFERENTIATION OF HOMOGENOUS Vα14Jα281 T CELLS

Analysis of the tissue distribution of T cells bearing the invariant Vα14$^+$ TCR showed that the frequency of the invariant Vα14Jα281 TCR expression was about 0.5% in thymus, 2-3% in spleen, 6% in bone marrow, and 12% in liver. The data suggest the possibility that the homogenous Vα14$^+$ T cells are selected in extrathymic sites. To test this possibility, we carried out PCR on RNA from spleen of nude (*nu/nu*) and athymic (TXB) mice that had been thymectomized, x-irradiated, and bone marrow reconstituted. Most (19/19 nude and 27/29 TXB mice) productive Vα14$^+$ cDNA showed the invariant Vα14Jα281 sequence with the one-base N-region of euthymic type. Moreover, the frequency of the homogenous Vα14 TCR expression was 0.9-1.5% of total α-chains as estimated by quantitative PCR in nude mice and athymic mice (*4*). The results indicate that positive selection takes place in a thymus-independent fashion.

We attempted to investigate possible extrathymic sites for Vα14$^+$ T cell

differentiation. Two experiments were carried out. First, we PCR-amplified and sequenced the Vα14 cDNA, and detected nonproductive Vα14$^+$ TCR sequences at a high frequency in Peyer's patches (PP, 37.4%), intraepithelium of small intestine (IEL, 45%), liver (30%), bone marrow (BM, 35%), and thymus (18%), but not in spleen (*5*). Since the random nature of VJ joining leads to a considerable proportion of rearrangements being nonproductive at the site of T cell differentiation, the results strongly suggest that organs with nonproductive sequences are the sites for extrathymic development of Vα14Jα281 T cells. Second, the extrathymic Vα14 T cell development was also confirmed by demonstration of signal sequences in the excised circular DNA generated during the TCR rearrangement process. To amplify potential signal joints in the circular episomal DNA, two sets of primers were designed for double step PCR in opposite outward orientations in the unrearranged germline locus in such a way that no DNA amplification was possible. Only when circular episomal DNA products are created by the formation of a signal joint, the PCR primers would amplify fragments carrying two heptamer signal sequences 3′ of the Vα14 and 5′ of Jα281 gene. We successfully detected the PCR products with Vα14Jα281-mediated signal sequences in the nuclear DNA samples from IEL, PP, BM, and liver of nude mice, whereas Vα1.1Jα281 TCR rearrangement which is known to be mainly generated in the thymus was not detected in these peripheral tissues (*5*). The PCR products showed typical recombination signal sequences, in which reciprocal heptamer sequences were joined together in a head to head fashion followed by 12 or 23 spacers and nonamers identical to the flanking sequences of Vα14 and Jα281. These results are direct molecular evidence for the extrathymic development of Vα14Jα281 T cells.

GENETIC POLYMORPHISM OF Vα14 TCR IN MICE

The genetic polymorphisms of the Vα14 TCR gene family have been investigated in various strains of laboratory mice and some wild mice, including *Mus musculus domesticus* (Western Europe), *M. m. castaneus* (Southeast Asia), *M. m. musculus* (Eastern Europe, Russia, Northern China), and *M. m. molossinus* (Japan) (*4*). In laboratory inbred strains, mice can be divided into three groups by *Pst*I-digested restriction fragment length polymorphism (RFLP) using the Vα14 probe: type I with a 3.0 kb (Vα14.1) and/or 1.8 kb (Vα14.3; pseudo gene) fragment possessed mainly by C57 mice (*i.e.*, C57BL/6), type II with a 2.4 kb fragment (Vα14.2)

detected in the majority of laboratory strains except C57 and DBA, and type III with 1.9 kb, 2.2 kb, and 3.0 kb fragments carried only by DBA mice. DBA mice (both DAB/1 and DAB/2) possessed at least three functional Vα14 genes, one of which is identical to Vα14.1 and the other two similar to but significantly different from Vα14.1, Vα14.2, and Vα14.3. We therefore called them Vα14.4 and Vα14.5, respectively. Surprisingly, Vα14.1, Vα14.2, Vα14.4, and Vα14.5 genes of the laboratory strain types are all preferentially associated with Jα281, and T cells expressing invariant Vα14Jα281 with a one-nucleotide N-region dominate peripheral T cells at the level of 2-3% of total α-chains in all the laboratory strains.

Moreover, *M. m. castaneus* and *M. m. domesticus* but not *M. m.*

TABLE 1
Expression of Vα14.1Jα281 and Vα14.2Jα281 mRNA in Various Strains of Laboratory Mice

Strain	Vα14.1 Jα281	Vα14.2 Jα281	H2	Qa2	Tla	Qa1	Q10	Hmt
A/J	–	+	a	a	a	a	+	a
AKR	–	+	k	b	b	b	+	a
A/WySn	–	+	a	n	b	n	+	a
BALB/c	–	+	d	a	c	b	+	a
CBA/J	–	+	k	b	b	b	+	a
CE/J	–	+	k	n	n	n	+	a
C3H	–	+	k	b	b	b	+	a
I/LnJ	–	+	j	n	n	n	+	a
129/J	–	+	bc	a	c	b	+	a
NZB	–	+	d	n	n	a	+	a
PL/J	–	+	u	b	e	a	+	a
RIIIs/J	–	+	r	b	b	c	+	a
RFM/MsNrs	–	+	f	n	n	n	–[a]	a
SJL	–	+	s	n	a	b	+	a
SM/J	–	+	v	a	b	b	+	a
SWM	–	+	d/?	n	n	n	+	a
WB/ReJ	–	+	ja	a	b	b	+	a
DBA/1	+	+[b]	q	a	b	b	+	a
DBA/2	+	+[b]	d	a	c	b	+	a
C57BL/6	+	–	b	a	b	b	+	a
C57BL/10	+	–	b	a	b	b	+	a
C57BR	+	–	k	n	a	a	+	a
B10A	+	–	a	a	a	a	+	a
B10A(3R)	+	–	i3	a	a	a	+	a
B10A(4R)	+	–	h4	a	b	b	+	a
B10A(5R)	+	–	i5	a	a	a	+	a

[a] Deleted.
[b] Vα14.4/Vα14.5 similar to but distinct from Vα14.2.

musculus and *M. m. molossinus* expressed about 0.02% of the invariant Vα14Jα281 TCR in total TCR α-chains. As shown in Table I, the expression of Vα14Jα281 TCR does not depend on the genotypes of MHC molecules on Chromosome 17, which are known to be important in positive selection of TCR repertoires. Thus, the restriction molecule involved in the selection of the Vα14Jα281 TCR T cells appears to be neither a known MHC molecule, such as H2, TLA, Qa, nor HMT that has a monomorphic nature.

EVOLUTION OF Vα14 GENE FAMILY

For further analyses of the genetic polymorphism of Vα14 TCR genes and their evolutionary significance, we PCR-amplified and sequenced Vα14 genes of various *M. musculus* subspecies and *Mus* species. Among four different wild mouse subspecies, three of them, *M. m. domesticus*, *M. m. musculus*, and *M. m. molossinus*, possessed the Vα14.1 type gene which is exclusively carried by C57 and DBA groups among laboratory mouse strains; while most *M. m. castaneus* carried the Vα14.4 of the laboratory mouse strain DBA (Fig. 1). There are, however, several exceptions. For example, *M. m. domesticus* possessed two other Vα14 genes (M. m. dom-2 and M. m. dom-3 in Fig. 1). The dom-2 Vα14 is similar to the type Vα14.5

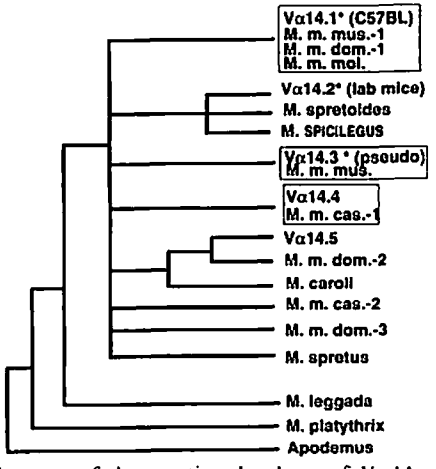

Fig. 1. Schematic dendrogram of the genetic relatedness of Vα14 genes in *Mus* and *Mus* subspecies. These data are based on the nucleotide sequences of Vα14 gene family.

gene while the dom-3 Vα14 is rather unique. A certain type of *M. m. castaneus* Vα14 gene (M. m. cas-2) also was somewhat different from the major type of *M. m. castaneus* of Vα14.4 type.

Analyses of protein variations at 16 loci by Taylor (*6*) have shown that most laboratory strains might derive from *M. m. domesticus*, but the C57 group of mice has been shown to carry an Asian wild mouse component. *M. m. molossinus* is believed to be a hybrid between *M. m. musculus* and *M. m. castaneus*, giving rise to a unique population in Japan (*7*). In our studies, some C57 mice were found to have a pseudo Vα14 gene, Vα14.3, which is carried by *M. m. musculus*, but not by *M. m. castaneus*. Therefore, our results strongly support the data of Huang *et al.* that some immunoglobulin V genes of C57 mice possess a *M. m. molossinus* component (*8*), and it is likely that Vα14.1 and Vα14.3 carried by C57 mice are of *M. m. molossinus* origin whose genes are derived from *M. m. musculus* but not from *M. m. castaneus*.

Concerning the Vα14 gene family in the European species of *Mus*, *M. spretoides* and *M. spicilegus* are shown to possess a single Vα14 gene similar but not identical to Vα14.2, a major type of Vα14 in laboratory strains. On the contrary, the Vα14 genes in *M. spretus* are different from the other two *Mus* species. The Vα14 gene family in *M. spretus* has three distinct genes which are not found in *M. spicilegus* or *M. spretoides*, and also show little similarity with those of laboratory strains. Thus *M. spretus* has a unique characteristics different from other European species of *Mus*.

Distinct from the European genus *Mus* discussed above, there are different species of the genus *Mus* which mainly inhabit India and Southeast Asia, for example, *M. caroli* and *M. leggada*. These species are known to be at a similar genetic distance from *M. musculus*, but are much more distant from *Apodemus speciosus*. Molecular genetic analyses on Vα14 genes clearly showed that *M. caroli* has a single Vα14 gene similar to that of either a laboratory strain, DBA (Vα14.5, see Fig. 1), or the *M. musculus* subspecies *M. m. domesticus* (*i.e.*, M. m. dom-2). Therefore, the Vα14.5 gene is conserved among members of the genus *Mus*, despite their genetic distance.

M. platythrix is somewhat different based on genetic analysis, but is similar to the genus *Mus* in morphology. The order of dichotomy is suggested to be as follows: starting with *Rattus* around 8–11 million years, *A. speciosus* or *M. platythrix* at intermediate times, then the ancestors of the genus *Mus* with splitting off from *M. leggada* between 1.5 and 3.2 million years. Finally the *M. musculus* subspecies appeared between 0.5 and 1.0

million years (*9*). The Vα14 gene in *M. platythrix* is a single member and is positioned between *A. speciosus* and *M. leggada* on the basis of genetic distance (Fig. 1).

POSITIVE DARWINIAN SELECTION IN THE EVOLUTION OF THE Vα14 GENE FAMILY

Recent studies by Hughes and Nei (*10*) have clearly shown that the rate of nonsynonymous (amino acid altering) nucleotide substitution is significantly higher than that of synonymous substitution in the antigen binding regions, *i.e.*, the CDR3 region of the MHC molecules. They have interpreted these findings as evidence for positive Darwinian selection operating in the immunologically important regions of these molecules.

We have also investigated the ratios of synonymous and nonsynonymous substitution in the Vα14 TCR genes of various mouse species and subspecies. These rates were computed per synonymous site and per nonsynonymous site, respectively. Lu and Nei reported that the number of nucleotide substitutions in the entire V region of T cell receptor $\alpha\beta$ genes is generally 0.58 at the first position, 0.47 at the second position and 0.89 at the third position of the triplet genetic codes (*11*). This pattern of nucleotide substitution is similar to that of most eukaryotic and prokaryotic genes (*12*), suggesting that amino acid substitutions in most TCR V regions are evolutionarily neutral.

In the case of Vα14 genes, on the other hand, more substitutions are found at the first and second nucleotide positions of the codons over the whole V regions, suggesting a high proportion of the nonsynonymous *vs.* synonymous ratios for pairwise comparisons of the Vα14 sequences.

Particularly, the rates of nonsynonymous nucleotide substitution are higher than those of synonymous substitution at the time of the divergence of species (*A. speciosus vs. M. musculus* subspecies or other genus *Mus* and *Rattus vs.* Hamster). For example, nonsynonymous *vs.* synonymous ratios are greatly (≥ 1.0) higher when *A. speciosus* is compared with other genus *Mus* (Table II). This suggests that positive selection occurred at the time *Apodemus* diverged from genus *Mus*. Similar findings are observed between Hamster and *Rattus* (nonsynonymous/synonymous ratio = 0.95), supporting the idea that the positive Darwinian selection is operating at the time of the divergence of some species. This positive selection might not have occurred during the evolution of genus *Mus*, because the ratios of non-

TABLE II
Ratio of Nucleotide Differences of Nonsynonymous and Synonymous Sites

Genes compared	Ratio of nonsyn/syn
Apodemus Vα14 *vs.*:	
M. musculus Vα14.2	1.117
M. leggada Vα14	1.063
M. platythrix Vα14	1.442
M. musculus Vα14 *vs.*:	
M. caroli Vα14	0.220
Rattus Vα14 *vs.*:	
Hamster Vα14	0.953
Human Vα14	0.189

synonymous/synonymous substitutions among this genus are not high but are generally at the level of 0.1–0.5.

These results suggest that the Vα14 gene family is much more affected by the selection mechanisms in its evolution. This is probably because Vα14$^+$ T cells recognize a self molecule and play a role in the negative regulation of the development of autoimmune diseases. It is also possible that a self ligand for Vα14 TCR might undergo critical mutations at the time of the species divergence. On this particular occasion, positive selection mechanisms might operate, resulting in an enhancement of the rate of nonsynonymous substitution.

SUMMARY

The genetic polymorphisms of the Vα14 TCR gene family have been investigated in laboratory mouse strains and some wild mice. Laboratory strains are divided into three groups by RFLP: type I (Vα14.1 and Vα14.3 (pseudo) in C57 group), type II (Vα14.2 in most strains), and type III (Vα14.1, Vα14.4, and Vα14.5 in DBA). All of the Vα14 genes in laboratory strains are preferentially associated with a particular Jα, Jα281. These characteristics have also been observed in some wild mice. Furthermore, sequence analyses of the Vα14 gene family have clearly shown that the ratio of nonsynonymous per synonymous nucleotide substitution is significantly higher at the time of the divergence of species, suggesting that the Vα14 genes undergo positive Darwinian selection at the time of species divergence.

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