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Positive selection of invariant $V_\alpha14^+$ T cells by non-major histocompatibility complex-encoded class I-like molecules expressed on bone marrow-derived cells

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ABSTRACT **$V_\alpha14^+$ T cells are a unique subset expressing an invariant T-cell antigen receptor α chain encoded by $V_\alpha14$ and $J_\alpha281$ gene fragments with a 1-nt N region. Most invariant $V_\alpha14^+$ T cells develop in extrathymic organs, independent of thymus, and expand at a high frequency in various mouse strains regardless of major histocompatibility complex (MHC) haplotype. In this paper, we show that the positive selection of invariant $V_\alpha14^+$ T cells requires a β_2-microglobulin-associated MHC class I-like molecule not linked to the MHC on chromosome 17. This was determined by linkage analysis on DNA from recombinant mice generated by crossing a C57BL/6 mouse with a wild mouse, *Mus musculus molossinus*, that is negative for invariant $V_\alpha14$ TCR expression. However, the peptide transporter TAP1 is not necessary for positive selection of invariant $V_\alpha14^+$ T cells, indicating the direct recognition of the MHC class I-like molecule without peptide by the invariant $V_\alpha14$ TCR. Further, experiments with bone marrow-chimeric mice show that invariant $V_\alpha14^+$ T cells in the periphery are selected by bone marrow cells, suggesting a unique lineage of $V_\alpha14^+$ T cells differentiated through a selection process distinct from that of conventional $\alpha\beta$ TCR^+ T cells.**

During T-cell development in the thymus, immature thymocytes expressing $\alpha\beta$ T-cell antigen receptors (TCRs) differentiate into mature thymocytes by two selection mechanisms: positive selection and negative selection. These mechanisms involve interaction between immature thymocytes and thymic stromal cells expressing the major histocompatibility complex (MHC) molecules bound with self peptides (1). Experiments using bone marrow-chimeric mice or TCR-transgenic mice have revealed that positive selection depends totally on radiation-resistant thymic epithelial cells (1, 2). On the other hand, a certain cell type in the thymus is positively selected by bone marrow-derived hematopoietic cells (3, 4). In particular, the origin of the thymic stromal cells involved in positive and negative selection is controversial (5).

In previous studies (6–8), most $V_\alpha14^+$ T cells were found to express an invariant TCR encoded by $V_\alpha14$ and $J_\alpha281$ gene segments with a 1-nt N region. Because the N region corresponds to the third base of the triplet code of glycine, the amino acid in the VJ junction of the $V_\alpha14J_\alpha281$ TCR is always glycine. This invariant $V_\alpha14^+$ TCR develops and expands in the peripheral lymphoid organs independent of the thymus at about 2–3% of total $\alpha\beta$ TCRs in various strains with different MHC haplotypes (6–8), implying that invariant $V_\alpha14^+$ T cells consist of a unique T-cell population and are selected by different mechanisms from conventional $\alpha\beta$ T cells in the thymus. Therefore, we have investigated the mechanisms of positive selection of invariant $V_\alpha14^+$ T cells in the periphery.

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MATERIALS AND METHODS

Mice. Specific pathogen-free (SPF) C57BL/6 and C57BL/10 (B10) mice were purchased from Shizuoka Animal Center (Hamamatsu, Japan). A wild Japanese mouse strain, *Mus musculus molossinus* (M.MOL-MSM), was established and maintained by Kazuo Moriwaki and his group (9). Germ-free BALB/c mice were maintained at Yakult Central Institute for Microbiological Research and kindly supplied by Teruo Yokokura. β_2-microglobulin (β_2m) gene-deficient mice (β_2m-mutant mice) were originally established by Zijlstra *et al.* (10) and maintained in our laboratory. Mice lacking class II MHC molecules ($I\text{-}A_\beta$-mutant mice) were described by Cosgrove *et al.* (11). Both β_2m-mutant and $I\text{-}A_\beta$-mutant mice were derived from embryonic stem cells of the 129/Sv strain that have the $V_\alpha14.2$ allele at the TCR loci. In some experiments, β_2m-mutant and $I\text{-}A_\beta$-mutant mice were backcrossed eight times with C57BL/6 ($H\text{-}2^b$) mice, which have the $V_\alpha14.1$ allele, under SPF conditions in our facility. TAP1 gene-deficient mice (TAP1-mutant mice) were provided by Susumu Tonegawa and colleagues (12); their (C57BL/6 × 129)F_3 progeny, which have the $V_\alpha14.2$ allele derived from the 129/Sv strain, were used for experiments.

Bone Marrow-Chimeric Mice. These mice were prepared as described (13). In brief, 8- to 12-week-old 129/Sv or B10 mice were irradiated (9.0 Gy, ^{137}Cs) and then reconstituted with 10^7 anti-Thy-1.2-pretreated bone marrow cells from 8-week-old β_2m-mutant homozygous or heterozygous mice. The mice were kept for 8 weeks under SPF conditions and used for experiments. The C57BL/10-type $V_\alpha14.1J_\alpha281$ or 129-type $V_\alpha14.2J_\alpha281$ TCR mRNA of donor or recipient origin can be clearly distinguished by RNase protection analysis.

RNase Protection Analysis. The analysis was performed on total cellular RNA as described (6). Two types of antisense RNA probes transcribed from $V_\alpha14.1J_\alpha281C_\alpha$ cDNA or $V_\alpha14.2J_\alpha281C_\alpha$ cDNA were used. To determine the frequency of invariant $V_\alpha14$ TCR, the radioactivity in the $V_\alpha14J_\alpha281C_\alpha$, $J_\alpha281C_\alpha$, and $V_\alpha14$ bands was measured by automated densitometry (Bioimage analyzer BAS2000, Fuji) and expressed as a percentage of that in the total C_α-region transcripts.

Linkage Between Expression of Invariant $V_\alpha14J_\alpha281$ TCR and the Genotype of MHC on Chromosome 17. (C57BL/6 × M.MOL-MSM)F_1 mice were backcrossed with male M.MOL-MSM mice and their progeny were used for experiments. Genomic DNA (20 μg) extracted from kidney was digested with *Eco*RI (New England Biolabs). Restriction fragment analysis was then carried out by genomic DNA blot with cDNA probes for the $I\text{-}A_\beta$ (14) and Hmt (15) genes. The genotypes of MHC in individual BMF1 × M.MOL-MSM recombinant mice were divided into two types: BMF1, with two bands derived from both C57BL/6 and M.MOL-MSM, and M.MOL-

Abbreviations: β_2m, β_2-microglobulin; MHC, major histocompatibility complex; SPF, specific pathogen-free; TCR, T-cell antigen receptor.

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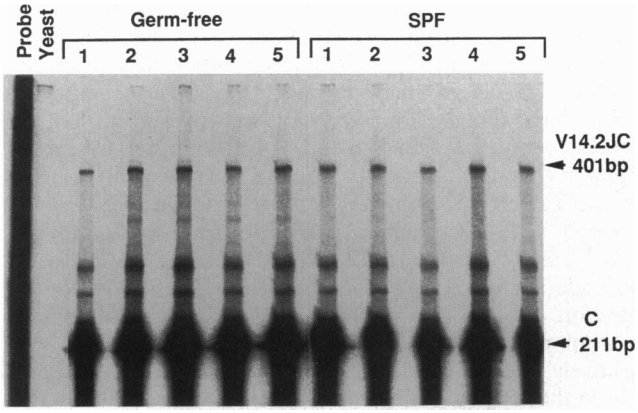

FIG. 1. RNase protection of invariant $V_\alpha14J_\alpha281$ TCR mRNA in germ-free and SPF BALB/c mice. Total RNA (50 μg) from the spleens of five 8-week-old mice was examined with a $V_\alpha14.1J_\alpha281C_\alpha$ probe which detects a 401-bp protected band of $V_\alpha14.2J_\alpha281$ mRNA of BALB/c origin. Soluble yeast RNA was used as a negative control. The VJC/C ratio was calculated on the basis of the radioactivity of the bands measured by automated densitometry and is expressed as a percentage of total C_α-containing transcripts: 1.1% (lane 1), 1.4% (lane 2), 1.9% (lane 3), 1.3% (lane 4), and 1.6% (lane 5) in germ-free BALB/c mice and 1.3% (lane 1), 1.3% (lane 2), 1.5% (lane 3), 1.7% (lane 4), and 1.6% (lane 5) in SPF BALB/c mice. The average VJC/C ratios in germ-free and SPF BALB/c mice were 1.46% and 1.48%, respectively.

MSM, with only the M.MOL-MSM band. At the same time, the expression of invariant $V_\alpha14J_\alpha281$ mRNA was examined by RNase protection analysis. The correlation between the genotype of MHC and the expression of invariant $V_\alpha14J_\alpha281$ mRNA was compared.

RESULTS

Molecule Recognized by Invariant $V_\alpha14^+$ T Cells. To investigate the effect of foreign antigens on the dominant expansion of invariant $V_\alpha14^+$ T cells, the level of expression of invariant $V_\alpha14^+$ mRNA was compared in germ-free and SPF BALB/c mice (Fig. 1). No significant difference in the expression of $V_\alpha14J_\alpha281$ TCR mRNA was observed between the two groups. This result indicates that invariant $V_\alpha14^+$ T cells expand without stimulation by foreign antigens, suggesting that the molecule recognized by invariant $V_\alpha14^+$ T cells is a self molecule.

Requirement of a MHC Class I-Like Molecule for the Selection of Invariant $V_\alpha14^+$ T Cells. To investigate the restriction molecule required for the selection of invariant $V_\alpha14^+$ T cells, we examined the expression of $V_\alpha14J_\alpha281$ mRNA in β_2m-mutant and I-A_β-mutant mice (Fig. 2). Invariant $V_\alpha14J_\alpha281$ TCR mRNA was expressed at a similar frequency in both $\beta_2m^{+/-}$ heterozygous mutant mice and wild-type littermate control mice. However, in $\beta_2m^{-/-}$ homozygous mutant mice, which do not express MHC class I molecules, very little $V_\alpha14J_\alpha281$ TCR mRNA was detected (Fig. 2*A*). In contrast to β_2m-mutant mice, $V_\alpha14J_\alpha281$ TCR mRNA was detected at a similar frequency in I-$A_\beta^{-/-}$ homozygous mutant mice, which do not express MHC class II molecules, as in heterozygous mutant mice or wild-type littermate control mice (Fig. 2*B*). These findings indicate that MHC class I, but not class II, molecules are essential for the expression of invariant $V_\alpha14J_\alpha281$ TCR.

Recognition of the Vacant MHC Molecule by the Invariant $V_\alpha14^+$ TCR. A peptide transporter is necessary for antigen presentation through MHC class I molecules (16). To investigate the requirement of MHC peptide for the recognition of invariant $V_\alpha14^+$ T cells, we also examined the expression of invariant $V_\alpha14J_\alpha281$ TCR mRNA in mutant mice lacking the peptide transporter TAP1 (Fig. 2*C*). The expression of invariant $V_\alpha14J_\alpha281$ mRNA was at normal levels in $TAP1^{-/-}$ mice. This result indicates that TAP1, an endogenous peptide transporter, is not necessary for recognition and selection of invariant $V_\alpha14^+$ T cells, whereas a MHC class I molecule is indispensable.

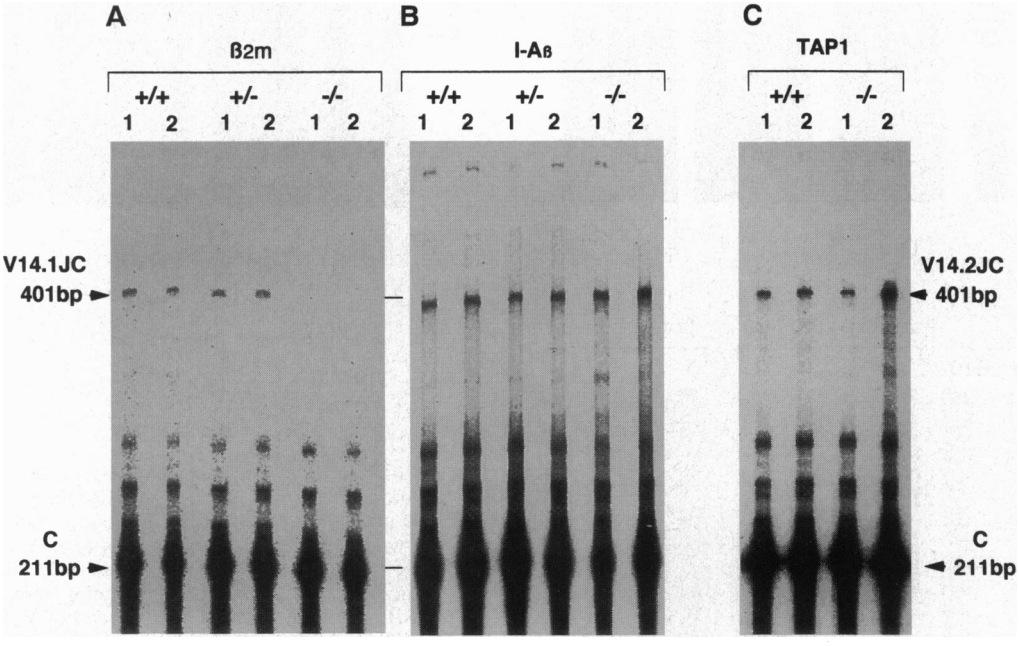

FIG. 2. RNase protection of invariant $V_\alpha14J_\alpha281$ TCR mRNA in β_2m-mutant (*A*), I-A_β-mutant (*B*), and TAP1-mutant (*C*) mice. Total RNA (50 μg) from the spleens of two individual wild-type (+/+), heterozygous (+/−), or homozygous (−/−) mice was examined. In β_2m- and I-A_β-mutant mice, a $V_\alpha14.2J_\alpha281C_\alpha$ probe was used to detect a 401-bp band of $V_\alpha14.1J_\alpha281C_\alpha$ mRNA derived from C57BL/6. In TAP1-mutant mice, a $V_\alpha14.1J_\alpha281C_\alpha$ cDNA probe was used to detect a 401-bp band of $V_\alpha14.2J_\alpha281C_\alpha$ mRNA of 129/Sv origin. The VJC/C ratio was calculated to be 1.9% (lane 1) and 2.1% (lane 2) in $\beta_2m^{+/+}$, 1.5% (lane 1) and 1.7% (lane 2) in $\beta_2m^{+/-}$, and 0% (lanes 1 and 2) in $\beta_2m^{-/-}$ mice; 1.3% (lane 1) and 2.0% (lane 2) in I-$A_\beta^{+/+}$, 0.8% (lane 1) and 1.0% (lane 2) in I-$A_\beta^{+/-}$, and 2.5% (lane 1) and 1.9% (lane 2) in I-$A_\beta^{-/-}$ mice; and 1.6% (lane 1) and 1.4% (lane 2) in $TAP\text{-}1^{+/+}$ and 1.3% (lane 1) and 4.7% (lane 2) in $TAP1^{-/-}$ mice.

Positive Selection of Invariant $V_\alpha14^+$ T Cells by a MHC Class I Molecule Expressed on Bone Marrow Cells. To determine the cell types essential for the selection of invariant $V_\alpha14^+$ T cells, we examined the expression of invariant $V_\alpha14J_\alpha281$ TCR mRNA in bone marrow-chimeric mice produced by transferring bone marrow cells from either wild-type, $\beta_2m^{+/-}$, or $\beta_2m^{-/-}$ mice of 129 origin into X-irradiated B10 mice. In some experiments, B10 bone marrow cells were transferred into β_2m^- mutant mice of 129 origin. Neither $V_\alpha14J_\alpha281$ nor $V_\alpha14^+$ mRNA derived from B10 recipients ($V_\alpha14.1$ type) was detected in any chimeric mice (Fig. 3 *A* and *B*; Table 1), indicating that the lymphocytes in the chimeras had been completely reconstituted with bone marrow cells of 129 donor origin. In 129 $\beta_2m^{+/+} \rightarrow$ B10 or 129 $\beta_2m^{+/-} \rightarrow$ B10 chimeric mice the expression of invariant $V_\alpha14.2J_\alpha281$ mRNA of 129 donor origin was detected at a level of 0.1–0.2%, a level about 1/10th that in B10 control mice (≈1.5%). However, in 129 $\beta_2m^{-/-} \rightarrow$ B10 chimeras no expression of invariant $V_\alpha14.2J_\alpha281$ TCR of donor type was detected (Fig. 3*A* and Table 1). This suggests that invariant $V_\alpha14^+$ T cells develop in the presence of MHC class I-positive bone marrow cells, but not class I-deficient bone marrow cells. These results indicate that invariant $V_\alpha14^+$ T cells are positively selected by bone marrow-derived hematopoietic cells.

To confirm that invariant $V_\alpha14^+$ T cells are preferentially not selected in 129 $\beta_2m^{-/-} \rightarrow$ B10 chimeras, we examined the expression of $V_\alpha14.2^+$ TCR mRNA other than invariant $V_\alpha14J_\alpha281$ TCR in chimeric mice (Fig. 3*B*). A protected $V_\alpha14.2$ band of 129 origin with a length of 354 bp that was associated with J_α other than $J_\alpha281$ was detected at a similar frequency in all three groups of bone marrow chimeras (Fig. 3*B* and Table 1). Since the $V_\alpha14.2$ band was detected even in 129 $\beta_2m^{-/-} \rightarrow$ B10 chimeras, which do not express invariant $V_\alpha14J_\alpha281$ TCR, this result indicates that a MHC class I molecule expressed on bone marrow cells is essential for the selection of invariant $V_\alpha14J_\alpha281^+$ T cells but not for the selection of other $V_\alpha14^+$ T cells. Other $V_\alpha14^+$ T cells may be positively selected by class I molecules on radiation-resistant cells in the recipient or by class II molecules on bone marrow cells of donor origin.

We also confirmed that the expression of MHC class I molecules in the host environment is not necessary for the selection of the invariant $V_\alpha14^+$ T cells (Fig. 3*C*). In B10 $\rightarrow$ 129 $\beta_2m^{+/+}$, B10 $\rightarrow$ 129 $\beta_2m^{+/-}$, and B10 $\rightarrow$ 129 $\beta_2m^{-/-}$ chimeric mice, a 401-bp band of $V_\alpha14.1J_\alpha281$ TCR mRNA of B10 origin was detected at a level similar to that in 129 $\beta_2m^{+/+} \rightarrow$ B10 chimeric mice (Table 1). Although MHC class I molecules

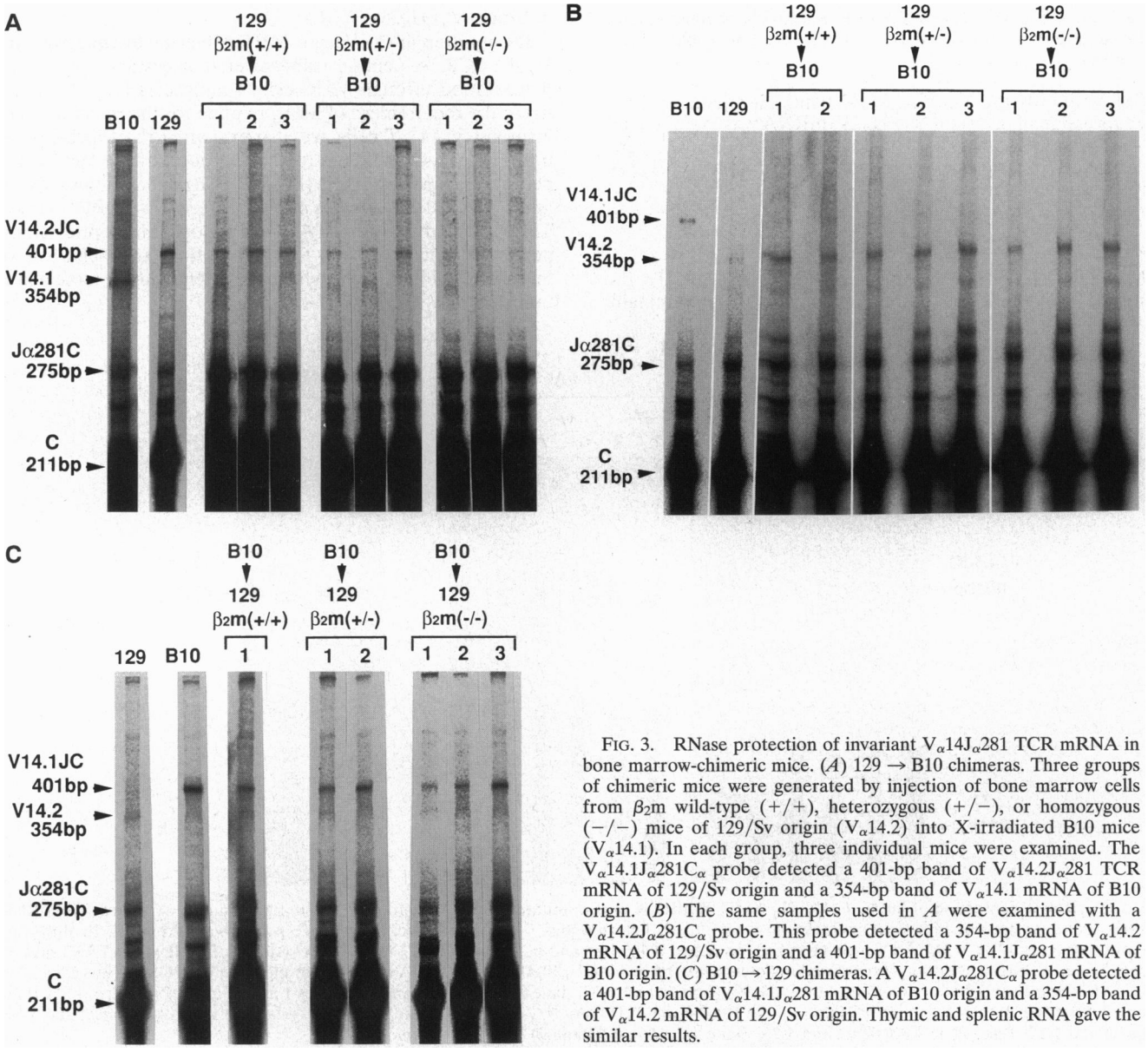

FIG. 3. RNase protection of invariant $V_\alpha14J_\alpha281$ TCR mRNA in bone marrow-chimeric mice. (*A*) 129 $\rightarrow$ B10 chimeras. Three groups of chimeric mice were generated by injection of bone marrow cells from β_2m wild-type (+/+), heterozygous (+/−), or homozygous (−/−) mice of 129/Sv origin ($V_\alpha14.2$) into X-irradiated B10 mice ($V_\alpha14.1$). In each group, three individual mice were examined. The $V_\alpha14.1J_\alpha281C_\alpha$ probe detected a 401-bp band of $V_\alpha14.2J_\alpha281$ TCR mRNA of 129/Sv origin and a 354-bp band of $V_\alpha14.1$ mRNA of B10 origin. (*B*) The same samples used in *A* were examined with a $V_\alpha14.2J_\alpha281C_\alpha$ probe. This probe detected a 354-bp band of $V_\alpha14.2$ mRNA of 129/Sv origin and a 401-bp band of $V_\alpha14.1J_\alpha281$ mRNA of B10 origin. (*C*) B10 $\rightarrow$ 129 chimeras. A $V_\alpha14.2J_\alpha281C_\alpha$ probe detected a 401-bp band of $V_\alpha14.1J_\alpha281$ mRNA of B10 origin and a 354-bp band of $V_\alpha14.2$ mRNA of 129/Sv origin. Thymic and splenic RNA gave the similar results.

Table 1. Expression of invariant $V_\alpha14^+$ TCR in bone marrow-chimeric mice

Chimera	Expression of TCR, %		
	$V_\alpha14J_\alpha281C_\alpha$	$J_\alpha281C_\alpha$	$V_\alpha14$
129 $\beta_2m^{+/+}$ → B10	0.20	1.90	0.62
129 $\beta_2m^{+/-}$ → B10	0.10	1.57	0.61
129 $\beta_2m^{-/-}$ → B10	−0.01*	1.44	0.60
B10 → 129 $\beta_2m^{+/+}$	0.12	0.92	ND
B10 → 129 $\beta_2m^{+/-}$	0.24	0.62	ND
B10 → 129 $\beta_2m^{-/-}$	0.33	0.59	ND

Data are based on the experiments shown in Fig. 3. Means of the individual data for each group were summarized. ND, not determined. *Significant reduction in invariant $V_\alpha14J_\alpha281$ TCR expression ($P < 0.01$, t test).

were not expressed in the recipient 129 $\beta_2m^{-/-}$ mice, the expression of invariant $V_\alpha14J_\alpha281$ TCR of B10 donor origin was normal. These results support the finding that invariant $V_\alpha14^+$ T cells are positively selected by a MHC class I molecule exclusively expressed on bone marrow-derived hematopoietic cells.

A Non-MHC-Encoded Class I-Like Molecule Essential for Selection of Invariant $V_\alpha14^+$ T Cells. We investigated whether the restriction element responsible for the selection of invariant $V_\alpha14^+$ T cells is encoded on chromosome 17, on which MHC molecules and non-classical class I-like molecules, the class Ib molecules, are located. Linkage between the expression of invariant $V_\alpha14J_\alpha281$ TCR mRNA and MHC genotype (I-A_β and Hmt) on chromosome 17 was analyzed (Table 2). The wild Japanese mouse strain M.MOL-MSM does not express invariant $V_\alpha14J_\alpha281$ TCR despite the apparent expression of $V_\alpha14^+$ or $J_\alpha281^+$ TCR, indicating that M.MOL-MSM lacks a selection element for invariant $V_\alpha14J_\alpha281$ TCR (6). As all laboratory strains express invariant $V_\alpha14^+$ TCR at a high level, we generated (C57BL/6 × M.MOL)F_1 (BMF1) × M.MOL-MSM recombinant mice, and the correlation between MHC genotype and invariant $V_\alpha14J_\alpha281$ TCR mRNA expression was examined in each mouse. About half the mice expressed $V_\alpha14J_\alpha281$ mRNA. The MHC genotypes could be divided into two types: BMF1 and M.MOL-MSM. If the positive selection elements of $V_\alpha14J_\alpha281$ mRNA were encoded by genes on chromosome 17, all the BMF1-type mice should express $V_\alpha14J_\alpha281$ mRNA, while none of the M.MOL-MSM-type mice should express invariant $V_\alpha14^+$ TCR. As shown in Table 2, the expression of $V_\alpha14J_\alpha281$ mRNA was not correlated with MHC genotype. This indicates that the gene coding for the positive selection element for invariant $V_\alpha14J_\alpha281$ TCR is not located on chromosome 17.

DISCUSSION

In previous studies, invariant $V_\alpha14^+$ T cells were found to expand mainly in the peripheral organs at a high frequency in all laboratory mice regardless of MHC haplotype and also in some subspecies of wild mice, suggesting that the MHC molecule involved in $V_\alpha14$ TCR recognition and selection is monomorphic in nature (7). Moreover, invariant $V_\alpha14^+$ T cells appear to recognize an endogenous self molecule rather than an exogenous peptide and are thus selected even in germ-free mice as well as in conventional mice (Fig. 1). This indicates that invariant $V_\alpha14^+$ T cells are selected and expand without stimulation by foreign antigens.

Some of the genetic properties of the molecule involved in the selection of invariant $V_\alpha14^+$ T cells have been identified in the present experiments. A β_2m-associated MHC class I-like molecule is essential for the selection of invariant $V_\alpha14$ TCR as defined in β_2m-mutant mice (Fig. 2). However, the gene encoding the MHC class I-like molecule essential for the invariant $V_\alpha14$ TCR selection is not on chromosome 17, which contains clusters of several MHC genes as well as class Ib genes, such as those encoding Qa, Tla, and Hmt. We found no significant correlation between the expression of invariant $V_\alpha14^+$ TCR and MHC or MHC-related genes on chromosome 17 (Table 2). Thus, the restriction element for invariant $V_\alpha14$ TCR is a non-MHC-encoded class I-like molecule. This finding is also supported by the failure of surveys of different mouse strains to establish a link between invariant $V_\alpha14$ TCR expression and specific MHC haplotypes.

Class I-like molecules that are not linked to the MHC have been reported (17–22). These include CD1, the low-affinity IgG Fc receptor of murine neonatal intestine (FcRn), and zinc-α-globulin (ZAG), and undoubtedly there are others yet undiscovered. CD1 is a well-known MHC-unlinked class I-like molecule in human and mouse. In mouse, two genes (mCD1.1 and mCD1.2) located on chromosome 3 have been identified (18). CD1 proteins are monomorphic molecules associated with β_2m, and they have the ability to present antigens. Moreover, they are expressed preferentially on bone marrow-derived cells—i.e., Langerhans cells, dendritic cells, and B cells—and thymocytes themselves (17). Some human $\alpha\beta$ or $\gamma\delta$ TCR^+ $CD4^-$ $CD8^-$ T cells specific for *Mycobacterium* use CD1 molecules as restriction elements (19–21). Thus, CD1 is one candidate for the selecting element of invariant $V_\alpha14^+$ T cells. FcRn is a β_2m-associated molecule that is expressed predominantly in neonatal epithelial cells of the small intestine (22). Although the distribution and expression of FcRn are limited and its ability to present antigens is unknown, FcRn and related molecules could be involved in the selection of invariant $V_\alpha14^+$ T cells.

The peptide transporter TAP1 is not necessary for the positive selection of invariant $V_\alpha14^+$ T cells, despite the fact that a class I-like molecule is essential for selection. Although the recognition mechanism used by invariant $V_\alpha14^+$ T cells is unknown, it is reasonable to discuss possible explanations for the above findings. (*i*) Invariant $V_\alpha14^+$ T cells may recognize a class I-like molecule without a binding peptide. Indeed, small amounts of empty but functional class I molecules seem to be

Table 2. Lack of correlation between the expression of invariant $V_\alpha14J_\alpha281$ TCR mRNA and MHC genotype on chromosome 17 in BMF1 × M.MOL-MSM recombinant mice

Trait	DNA from individual recombinant mouse																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
I-A_β genotype	M	F	M†	M	F	F	F	M	M	?‡	M	F	M	M	F	F	F	M	M
Hmt genotype	M	F	F†	M	F	F	F	M	M	F	M	F	M	M	F	F	F	M	M
$V_\alpha14J_\alpha281$ expression	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Discrepancy	*		*	*				*	*	*		*			*	*	*		

Restriction fragment analysis using I-A_β and Hmt probes was carried out on DNA from BMF1 × M.MOL-MSM recombinant mice. F and M indicate the restriction patterns of BMF1-type or M.MOL-MSM type, respectively. The level of expression of invariant $V_\alpha14^+$ TCR mRNA at >0.1% of total TCR α mRNA was positive (+); <0.1% was classed as negative (−). Asterisks indicate the mice with discrepancy between MHC genotype and invariant $V_\alpha14^+$ TCR expression.

†Crossing over of genes between I-A_β and Hmt loci was suggested.

‡MHC genotype (I-A_β) was not determined by the genomic DNA blot analysis.

present on cell surfaces in TAP1-mutant mice (12). In addition, a γδ T-cell clone specific for a monomorphic class Ib molecule recognizes a Tl molecule without a peptide, and the activation of γδ T cells does not appear to require an antigen-processing pathway (23). If this is the case, invariant $V_\alpha14^+$ T cells may recognize only the framework of a vacant class I-like molecule.

(*ii*) Invariant $V_\alpha14^+$ T cells may recognize a nonpeptide antigen bound with the class I-like molecule. Although this notion is unconventional, it has been proposed that one function of class Ib molecules is the presentation of nonpeptide antigens such as carbohydrates or glycolipids (17, 24).

(*iii*) There may be an alternative antigen-presenting pathway for class I-like molecules. In fact, the antigen-processing pathway of CD1b to autoreactive αβ TCR^+ $CD4^-$ $CD8^-$ T cells is chloroquine sensitive, similar to the pathway of MHC class II molecules (20). In addition, a peptide transporter (TAP)-independent "endogenous" processing pathway exists in TAP-deficient RMA-S cells, where heat shock protein hsp73 has the ability to bind to a certain peptide and work as a transporter (25). Therefore, it is possible that invariant $V_\alpha14^+$ T cells recognize a complex of a self peptide plus a MHC class I-like molecule, rather than a vacant class I-like molecule, in a process like the hsp73-mediated pathway for class I-restricted presentation.

The class I-like molecule essential for invariant $V_\alpha14$ T-cell selection seems to be expressed on bone marrow-derived hematopoietic cells. Since the positive selection of conventional class I- or class II-restricted T cells is evidently conferred by thymic epithelium or fibroblasts, the selection mechanisms for $V_\alpha14^+$ T cells might be distinct from those of conventional T cells (1, 2, 26, 27). As invariant $V_\alpha14^+$ T cells are positively selected in bone marrow, liver, and Peyer's patches even in athymic conditions (7, 8), thymic epithelial cells are not essential for the generation of $V_\alpha14^+$ T cells.

Similarly, Bix *et al.* (3) showed that αβ TCR^+ $CD4^-$ $CD8^-$ mature thymocytes expressing a skewed $V_\beta8$ repertoire required class I expression by hematopoietic cells rather than thymic epithelial cells in their selection process. Therefore, the class I-dependent selection of αβ TCR^+ $CD4^-$ $CD8^-$ thymocytes in the thymus might be similar to that for the $V_\alpha14^+$ T cells in the periphery.

The function of invariant $V_\alpha14$ TCR^+ T cells is still unknown. Our preliminary experiments have demonstrated that a specific decrease in $V_\alpha14^+$ cells is tightly correlated with the development of autoimmune diseases in *lpr* mice and also that the diminution of $V_\alpha14^+$ T cells by anti-$V_\alpha14$ antibody accelerates and augments the production of anti-double-stranded DNA autoantibodies responsible for autoimmune disease development in young *lpr* mice. Therefore, it is important to elucidate the functions and mechanisms of immune responses controlled by invariant $V_\alpha14$ T cells.

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