Eur. J. Immunol. 1995. 25: 857–862

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T cell receptor Vβ repertoire in mice lacking endogenous mouse mammary tumor provirus

When endogenous mouse mammary tumor virus (MMTV) superantigens (SAg) are expressed in the first weeks of life an efficient thymic deletion of T cells expressing MMTV SAg-reactive T cell receptor (TcR) Vβ segments is observed. As most inbred mouse strains and wild mice contain integrated MMTV DNA, knowing the precise extent of MMTV influence on T cell development is required in order to study T cell immunobiology in the mouse. In this report, backcross breeding between BALB.D2 (*Mtv-6, -7, -8* and *-9*) and 38CH (*Mtv*$^-$) mice was carried out to obtain animals either lacking endogenous MMTV or containing a single MMTV locus, *i.e. Mtv-6, -7, -8* or *-9*. The TcR Vβ chain (TcR Vβ) usage in these mice was analyzed using monoclonal antibodies specific for TcR Vβ2,Vβ3, Vβ4,Vβ5,Vβ6,Vβ7,Vβ8,Vβ11,Vβ12 and Vβ14 segments. Both *Mtv-8*$^+$ mice and *Mtv-9*$^+$ mice deleted TcR Vβ5$^+$ and Vβ11$^+$ T cells. Moreover, we also observed the deletion of TcR Vβ12$^+$ cells by *Mtv-8* and *Mtv-9* products. *Mtv-6*$^+$ and *Mtv-7*$^+$ animals deleted TcR Vβ3$^+$ and Vβ5$^+$ cells, and TcR Vβ6$^+$,Vβ7$^+$ and Vβ8.1$^+$ cells, respectively. Unexpectedly, TcR Vβ8.2$^+$ cells were also deleted in some backcross mice expressing *Mtv-7*. TcR Vβ8.2 reactivity to *Mtv-7* was shown to be brought by the 38CH strain and to result from an amino acid substitution (Asn → Asp) in position 19 on the TcR Vβ8.2 fragment. Reactivities of BALB.D2 TcR Vβ8.2 and 38CH TcR Vβ8.2 to the exogenous infectious viruses, MMTV(SW) and MMTV(SHN), were compared.
Finally, the observation of increased frequencies of TcR Vβ2$^+$,Vβ4$^+$ and Vβ8$^+$ CD4$^+$ T cell subsets in *Mtv-8*$^+$ and *Mtv-9*$^+$ mice, and TcR Vβ4$^+$ CD4$^+$ T cells in *Mtv-6*$^+$ and *Mtv-7*$^+$ mice, when compared with the T cell repertoire of *Mtv*$^-$ mice, is consistent with the possibility that MMTV products contribute to positive selection of T cells.

1 Introduction

The mouse mammary tumor virus (MMTV) is a type B retrovirus that has integrated at numerous sites in the mouse genome. As many as 30 *Mtv* proviral loci have been identified and mapped in laboratory mouse strains [1]. Recent studies demonstrated that an open reading frame (ORF) in the 3′ long terminal repeat (LTR) of *Mtv* encodes superantigens (SAg) [2–10]. SAg, associated with class II MHC molecules, are known to bind specifically particular V regions of the T-cell antigen receptor β chain (TcR Vβ) (for review see [11, 12]). The specificity of the interaction of MMTV SAg with TcR Vβ domain is thought to be determined by the highly polymorphic COOH-terminal region of the ORF molecule [7, 8, 13, 14]. Through this unique reactivity to TcR Vβ, endogenous MMTV SAg are known to mediate clonal deletion of SAg-reactive T cells during their intrathymic development, and therefore, constitute a major element shaping the mature T cell repertoire of the mouse [15, 16].

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Abbreviations: LTR: Long terminal repeat **MMTV:** Mouse mammary tumor virus **ORF:** Open reading frame **SAg:** Superantigen

Key words: Mouse mammary tumor virus / Superantigen / *Mtv*$^-$ mice / T cell receptor haplotype

All the inbred strains of mouse commonly used for immunological investigation are known to possess several copies of integrated MMTV proviruses. In contrast, the wild-derived inbred mouse strain 38CH was shown not to have DNA hybridizing either with an MMTV envelope-specific probe or with an MMTV LTR-specific probe, indicating that the strain is free of endogenous MMTV [17]. To determine the precise extent of MMTV influence on T cell repertoire in the mouse, [(BALB.D2 × 38CH)F1 × 38CH] mice were backcrossed with 38CH mice. This breeding strategy allowed the segregation of the *Mtv* loci contained in the BALB.D2 genome, *i.e. Mtv-6, -7, -8* and *-9*, and to obtain mice lacking endogenous MMTV or expressing only a single *Mtv* locus. The TcR Vβ usage of these mice was determined by flow cytometry.

2 Materials and methods

2.1 Mice

The wild-derived inbred mouse strain 38CH originated from a couple of *Mus m. domesticus* trapped in Chiarello (Italy). Their offsprings were crossed for 26 generations between brothers and sisters in the animal facilities of the Pasteur Institute. The strain did not hybridize with an MMTV envelope-specific probe nor with an MMTV LTR-specific probe, indicating that it is free of endogenous MMTV [17]. Testing for infectious MMTV particles in mother milk by PCR using MMTV-specific reaction primers did not reveal the presence of exogenous viruses infecting the 38CH strain (H. A.-O., unpublished results). BALB.D2 mice contain four MMTV proviral loci, *Mtv-6,*

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Mtv-7, Mtv-8 and *Mtv-9,* present on chromosomes 16, 1, 6 and 12, respectively. The strain is maintained at the Lausanne Branch of the Ludwig Institute. It was originally obtained from Dr. H. Festenstein, London Hospital Medical College [18]. (BALB.D2 × 38CH)F1 and backcrosses were bred at the Ludwig Institute.

2.2 Monoclonal antibodies (mAb)

The following anti-TcR Vβ fragment mAb were used in this study: B20.6 (rat anti-mouse TcR Vβ2) [19]; KJ25 (hamster anti-mouse TcR Vβ3) [20]; KT4-10 (rat anti-mouse TcR Vβ4) [21]; MR9-4 (mouse anti-mouse TcR Vβ5.1 and Vβ5.2) [2]; FITC-conjugated 44-22.1 (rat anti-mouse TcR Vβ6) [22]; TR310 (rat anti-mouse TcR Vβ7) [23]; FITC-conjugated F23.1 (mouse anti-mouse TcR Vβ8.1, Vβ8.2 and Vβ8.3) [24]; FITC-conjugated F23.2 (mouse anti-mouse TcR Vβ8.2) [24]; KJ16.133.18 (rat anti-mouse TcR Vβ8.1 and Vβ8.2) [25]; RR3-15 (rat anti-mouse TcR Vβ11) [26]; biotinylated MR11-1 (mouse anti-mouse TcR Vβ12) [27]; FITC-conjugated 14-2 (mouse anti-mouse TcR Vβ14) [28].

2.3 Cytofluorometry

For two-color staining, cells (1 × 10^6 per sample) from popliteal, inguinal, axillary, brachial, maxillary and cervical lymph nodes were incubated with 50 μl of anti-TcR Vβ mAb for 30 min at 4 °C. The appropriate antibody dilution was determined before use. After washing with ice-cold PBS containing 2% FCS, cells were incubated with medium alone or with 50 μl of FITC-goat anti-mouse Ig (6 μg per ml; Tago, Burlingame; CA), FITC-goat anti-rat Ig (16 μg per ml; Caltag Laboratories, San Fransisco, CA) or FITC-streptavidin (5 μg per ml; Caltag Laboratories) at 4 °C for 15 min. Then, after washing, cells were incubated at 4 °C for 10 min with 20 μl of rat Ig (1 mg per ml; from Dr. H. Bazin, Brussels, Belgium). PE-conjugated rat anti-mouse L3T4 mAb (0.25 μg per ml; Becton Dickinson, Mountain View, CA) and PE-conjugated rat anti-mouse Ly-2 mAb (4 μg per ml; Boehringer Mannheim, Rotkreuz, Switzerland) were added together on each sample in 50 μl of PBS supplemented with 2% FCS. After a 20 min incubation at 4 °C, samples were washed and analyzed on a FACScan (Becton Dickinson).

2.4 Southern blotting

The TcR Vβ alleles contained in 38CH mice were determined by Southern blot analysis of genomic DNA digested by Eco RI or Hind III and hybridized with probes specific for Vβ1, Vβ3, Vβ6, Vβ8, Vβ10, Vβ13, Vβ15, Vβ17 and Vβ19 genes as previously described [29].

2.5 Polymerase chain reaction

Screening of the backcrosses issued from our breeding for the presence of integrated *Mtv* proviral genes was carried out by taking advantage of the size polymorphism displayed by the 3′ untranslated sequence of the provirus LTR [30]. PCR amplification using common LTR-specific primers spanning this length polymorphism, allowed the identification of particular *Mtv* loci based on the size of the amplified fragments. The reaction was performed as described previously [30].

Oligonucleotides used to amplify TcR Vβ8.2 gene were the 5′ oligo RM56 (TGCTCCCAAGATGGGCTCCA) and the 3′ oligo RM57 (CCTATTTCCTTTCTGTGCAGA), and oligonucleotides used to amplify TcR Vβ10 gene were the 5′ oligo RM75 (TGTTTTCCAGACTCCAAACTA) and the 3′ oligo RM76 (GTCCTCCGGCTCTACAGACTT). DNA (1 μg) was boiled for 2 min in the presence of the oligonucleotides (10 μM). The PCR conditions were 1 min at 52 °C, 1 min at 72 °C and 1 min at 92 °C for 40 cycles and for 10 min at 72 °C in 20 μl of PCR buffer containing 20 mM Tris-HCl (pH 8.5), 50 mM KCl, 2 mM $MgCl_2$, 0.01% gelatin, 0.2 mM dNTP (in some experiments, [α-^{32}P] ATP was added), 1 U of Taq polymerase (Perkin-Elmer Corporation, Pomona, CA). The reaction was performed with a DNA thermal cycler (Perkin-Elmer Corporation).

2.6 Gene cloning and sequencing

TcR Vβ8.2 PCR products were size-fractionated on 1% agarose gel and purified using the Geneclean II kit (BIO 101, La Jolla, CA), according to the manufacturer's protocol. PCR products were cloned using the pT7Blue T-Vector kit (Novagen, Madison, WI). Recombinant plasmids were isolated and used as templates for dideoxy sequencing by using the Sequenase Version 2.0 kit (US Biochemichal Corp., Indianapolis, IN) with RM56 and RM57 oligonucleotides as reaction primers.

2.7 *In vivo* assays for superantigen reactivity

To identify *Mtv*$^-$ mice issued from our backcrosses expressing 38CH TcR genes from those expressing BALB.D2 TcR genes, we took advantage of the different TcR Vβ10 haplotype expressed by 38CH (TcR Vβ10^a) and BALB/c (TcR Vβ10^b) strains (this report). TcR Vβ10a gene has an Ava II site in position 81 [31]. Screening of our colony was thus carried out by PCR amplification of the TcR Vβ10 gene using specific primers (see above), followed by Ava II digestion of the amplified products. Size fractionation of the digested PCR products allowed us to identify mice homozygous for the 38CH TcR genes.

38CH TcR gene-homozygous *Mtv*$^-$ mice, along with BALB/c mice, were injected into the hind footpad with 10 μl of mouse milk containing MMTV(SW) [14] or MMTV(SHN) [32] particles disolved in PBS (1/100). Four days later the cells from the draining popliteal lymph node were isolated. Cells were then immunolabeled as described above and analyzed by cytofluorometry.

3 Results and discussion

3.1 TcR Vβ repertoire

To analyze the TcR Vβ repertoire of our backcross mice, we first determined the TcR Vβ polymorphism existing between 38CH and BALB.D2 mice. 38CH and BALB/c

genomic DNA (BALB/c has the same TcR Vβ haplotype as BALB.D2) digested with Eco RI or Hind III were assayed by Southern blot with DNA probes specific for 11 TcR Vβ families (Table 1), all the TcR Vβ8 members being considered of the same family. As shown in Table 1, the strains exhibited different alleles for TcR Vβ1, Vβ6 and Vβ10 segments. 38CH was also shown to possess TcR Vβ17 and Vβ19 alleles with the same RFLP as in BALB/c, indicating that these segments are non functional.

Screening of the backcross mice issued from our breeding between 38CH and BALB.D2 mice for the presence of integrated *Mtv* provirus was carried out by taking advantage of the size polymorphism displayed by the ORF COOH-terminal sequences of the *Mtv* present in the BALB.D2 genome. PCR amplification using common LTR-specific primers spanning this length polymorphism, allowed the identification of particular *Mtv* loci based on the size of the amplified fragments [30]. Fig. 1 illustrates the segregation of *Mtv* proviral loci observed in the {[(BALB.D2 × 38CH)F1 × 38CH] × 38CH} backcross mice.

Table 1. TcR Vβ haplotypes in 38CH and BALB/c mice[a)]

Vβ domain	Enzyme	BALB/c	SJL	38CH
Vβ10	Eco RI	b	a	a
Vβ1	Eco RI	b	a	a
Vβ8.1 to 3	Eco RI	a	/	a
Vβ13	Eco RI	a	/	a
Vβ6	Eco RI	a	a	b
Vβ15	Eco RI	a	a	a
Vβ19	Hind III	b	a	b
Vβ17	Hind III	b	a	b
Vβ3	Eco RI	a	a	a

a) The allele for each TcR Vβ was determined by Southern blot analysis of genomic DNA digested by the indicated enzyme hybridized with specific TcR Vβ probes as previously described [29].

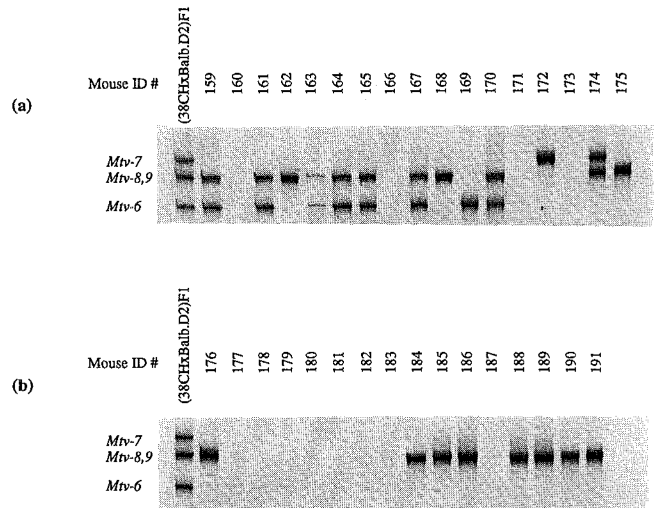

Figure 1. PCR assay to detect endogenous MMTV. Endogenous MMTV LTR display length polymorphisms. PCR with primers specific for LTR and spanning these polymorphisms allows the identification of *Mtv* loci based on the size of the PCR products [30]. Tail DNA isolated from [(BALB.D2 × 38CH)F1 × 38CH] backcross mice (a) and {[(BALB.D2 × 38CH)F1 × 38CH] × 38CH} backcross mice (b) were analyzed in this figure. The ^{32}P-labeled PCR products were size-fractionated on a 6% acrylamide gel and visualized by autoradiography.

The TcR-Vβ expression on lymph node cells of the backcross mice either lacking endogenous *Mtv* or containing one of the *Mtv* loci brought by the BALB.D2 background, *i.e.* *Mtv-6, Mtv-7, Mtv-8* or *Mtv-9,* was analyzed in the $CD4^+$ and $CD8^+$ populations by flow microfluorometry. The expression of TcR Vβ1, Vβ9, Vβ10, Vβ13, Vβ15, Vβ16, Vβ18 and Vβ20 segments was not tested due to the lack of appropriate mAb. As shown in Tables 2 and 3, mice lacking *Mtv* exhibited a full TcR Vβ repertoire with no apparent deletion of a particular TcR $Vβ^+$ cell subset. The presence of *Mtv-6* correlated with the deletion of TcR Vβ3- and

Table 2. TcR Vβ usage by lymph node $CD4^+$ cells in mice expressing different endogenous *Mtv*

mAb	TcR-Vβ	Percent of $CD4^+$ cells in mice expressing:				
		No *Mtv*[a)] (n = 6)	*Mtv-6* (n = 3)	*Mtv-7* (n = 6)	*Mtv-8* (n = 4)	*Mtv-9* (n = 4)
B20.6.5	2	5.4 ± 0.7	6.1 ± 0.4	8.6 ± 0.3	*9.0 ± 1.4*[b)]	*8.2 ± 0.8*
KJ25	3	4.6 ± 0.2	**0.7 ± 0.5**[c)]	7.5 ± 1.2	5.8 ± 1.1	6.6 ± 0.7
KT4-10	4	5.2 ± 0.7	*8.1 ± 0.6*	*10.5 ± 0.2*	*10.1 ± 0.3*	*9.0 ± 0.4*
MR9-4	5	4.1 ± 0.5	**1.2 ± 0.5**	6.6 ± 0.6	**2.8 ± 1.4**	**1.1 ± 0.3**
44-4-2	6	7.0 ± 0.7	7.1 ± 0.4	**0.3 ± 0.1**	7.7 ± 1.6	8.1 ± 0.8
TR310	7	3.1 ± 0.6	4.1 ± 0.1	**0.6 ± 0.1**	3.6 ± 0.8	2.6 ± 1.0
F23.1	8	24.2 ± 0.9	29.2 ± 3.0	**15.8 ± 2.5**	*32.6 ± 2.8*	*35.1 ± 1.2*
F23.2	8.2	11.0 ± 0.7	14.3 ± 1.2	**4.8 ± 3.8**	*17.6 ± 1.8*	*15.6 ± 0.7*
KJ16	8.1 + 8.2	14.4 ± 1.8	16.2 ± 1.4	**6.4 ± 2.7**	21.9 ± 0.9	24.0 ± 1.5
RR3.15	11	6.5 ± 0.7	5.7 ± 0.5	7.7 ± 0.6	**3.0 ± 0.0**	**0.4 ± 0.3**
MR11.1	12	7.4 ± 0.4	7.6 ± 0.1	10.0 ± 0.5	**1.4 ± 0.4**	**0.1 ± 0.0**
14.2	14	5.9 ± 0.9	6.6 ± 0.0	7.5 ± 0.6	7.1 ± 1.9	7.0 ± 0.3

a) Lymph node $CD4^+$ cells, isolated from Mtv^- mice or from mice expressing only *Mtv-6, Mtv-7, Mtv-8,* or *Mtv-9,* were labeled with a panel of anti-TcR Vβ mAb as described in Sect. 2.3. Samples were examined on a FACScan (Becton Dickinson).
b) Frequencies higher than expected after compensation of MMTV SAg-mediated deletion are shown in italic characters.
c) Values indicating statistically significant deletion are in bold.

Table 3. TcR Vβ usage by lymph node $CD8^+$ cells in mice expressing different endogenous *Mtv*

mAb	TcR-Vβ	Percent of $CD8^+$ cells in mice expressing No *Mtv*[a)] (*n* = 6)	*Mtv-6* (*n* = 3)	*Mtv-7* (*n* = 6)	*Mtv-8* (*n* = 4)	*Mtv-9* (*n* = 4)
B20.6.5	2	4.6 ± 0.9	5.3 ± 0.5	8.3 ± 0.3	6.0 ± 0.5	6.2 ± 0.7
KJ25	3	2.4 ± 0.3	**0.6 ± 0.4**[b)]	4.6 ± 0.8	3.8 ± 2.1	4.2 ± 0.7
KT4-10	4	3.9 ± 0.4	4.2 ± 1.0	5.9 ± 0.9	3.6 ± 0.6	5.5 ± 0.3
MR9-4	5	16.3 ± 2.2	**5.3 ± 1.1**	25.3 ± 2.3	**7.4 ± 1.2**	**1.0 ± 0.1**
44-4-2	6	9.0 ± 0.8	10.0 ± 1.0	**0.4 ± 0.3**	10.8 ± 0.4	10.8 ± 1.0
TR310	7	6.7 ± 0.9	7.4 ± 1.2	**0.9 ± 0.2**	6.2 ± 0.3	6.7 ± 0.8
F23.1	8	32.5 ± 1.4	36.9 ± 0.7	**17.0 ± 0.8**	34.3 ± 4.2	41.4 ± 1.1
F23.2	8.2	12.9 ± 1.4	12.5 ± 1.8	**3.5 ± 2.4**	9.8 ± 0.7	13.7 ± 2.2
KJ16	8.1 + 2	23.6 ± 1.9	22.0 ± 3.4	**5.8 ± 1.2**	21.1 ± 0.7	30.3 ± 0.9
RR3.15	11	4.0 ± 0.6	4.7 ± 0.3	7.9 ± 0.7	3.9 ± 0.2	**0.6 ± 0.4**
MR11.1	12	1.9 ± 0.2	2.2 ± 0.5	2.9 ± 0.3	**1.0 ± 0.6**	**0.0 ± 0.0**
14.2	14	3.1 ± 0.8	4.2 ± 0.5	5.8 ± 0.5	4.1 ± 0.1	3.7 ± 0.5

a) Lymph node $CD8^+$ cells, isolated from Mtv^{-ve} mice or from mice expressing only *Mtv-6, Mtv-7, Mtv-8*, or *Mtv-9*, were labeled with a panel of anti-TcR Vβ mAb as described in Sect. 2.3. Samples were examined on a FACScan (Becton Dickinson).
b) Values indicating statistically significant deletion are in bold.

Vβ5-expressing cells and *Mtv-7* with the deletion of TcR Vβ6-, Vβ7- and Vβ8.1-expressing cells, as reported by others [5, 33]. It is known that TcR $V\beta5^+$ and $V\beta11^+$ cells are deleted in mice expressing *Mtv-8* and *Mtv-9* [2, 3, 6]. As amplified *Mtv-8* and *Mtv-9* products cannot be distinguished with our PCR technique, we took advantage of the partial deletion of TcR $V\beta11^+$ cells usually observed in $Mtv\text{-}8^+$ animals [6] (or the absence of deletion in the CD8 subset, Table 3) for distinguishing $Mtv\text{-}8^+$ and $Mtv\text{-}9^+$ backcross mice. It is interesting to note that the different level of deletion promoted by *Mtv-8* and *Mtv-9* products correlates with their level of RNA expression. Günzburg et al. have indeed shown that *Mtv-8* genes in lymphoid cells have a higher level of DNA methylation than *Mtv-9* genes, implying a higher level of expression of MTV-9 products [34]. In our study we found *Mtv-8* and *Mtv-9* as deleting elements of TcR $V\beta12^+$ cells. This deletion was more pronounced than for TcR $V\beta5^+$ and $V\beta11^+$ cells, suggesting a stronger TcR Vβ12-*Mtv-8/9* SAg interaction. Finally, it is noteworthy that we did not observe significant variations in the repertoire of our backcross mice that would result from the segregation of H-2 genes; mice expressing the same *Mtv* products but a different H-2 haplotype (38CH or d) exhibited similar TcR Vβ repertoires.

Because *Mtv* products induce clonal deletion, they must be present in the thymus, and therefore it is possible that they could also play a role in the process of positive selection. Positive selection usually occurs in the thymus to allow maturation of T cells capable of recognising Ag peptides associated with self-MHC products (for review see [35]). If *Mtv-6, -7, -8* or *-9* products are involved in positive selection, the expression of a given *Mtv* locus in our backcross mice would result in an increased frequency of cells expressing a particular TcR Vβ fragment. As seen in Table 1, mice containing *Mtv-8* and *Mtv-9* had a higher percentage of $CD4^+$ cells expressing TcR Vβ2, Vβ4 and Vβ8 fragments than expected after compensation of the deletion of TcR $V\beta5^+$, $V\beta11^+$ and $V\beta12^+$ cells (*i.e.* 16.3% of the T cell repertoire is deleted in $Mtv\text{-}9^+$ mice when compared with Mtv^+ mice and, therefore, each one of the non-deleted TcR $V\beta^+$ cell subsets is expected to increase by 16.3% to compensate this deletion. However, TcR $V\beta2^+$, $V\beta4^+$, and $V\beta8^+$ $CD4^+$ cells in these mice increased by 51.8%, 73.1%, and 45.0% [(% *Mtv-9*/% Mtv^-) × 100], respectively. Similar observations were seen in mice expressing *Mtv-6* and *Mtv-7* for TcR $V\beta4^+$ $CD4^+$ cells. Interestingly, a normal compensation balanced the deletion of *Mtv*-reactive TcR $V\beta^+$ cell subsets among $CD8^+$ cells and no increase in frequency of a particular TcR $V\beta^+$ cell subset was seen (Table 2). The observation of higher frequencies of T cells expressing a particular TcR Vβ fragment in our backcross mice is consistent with a role for MMTV products in positive selection. However, additional experiments are required to test this hypothesis.

A previous study by Liao and Raulet [36] has identified *Mtv-7* (or $Mls\text{-}1^a$) as a possible ligand for the positive selection of TcR $V\beta14^+$ T cells; expression of *Mtv-7* leading to a significant and specific increase in the abundance of TcR $V14^+$ T cells. Analysis of our backcross mice did not show TcR $V\beta14^+$ T cell subset with an increased frequency due to the expression of particular *Mtv* products; suggesting that *Mtv-7* (and *Mtv-6, Mtv-8* and *Mtv-9*) does not participate directly in the process of positive selection of T cells expressing the TcR Vβ14 fragment. The apparent discrepancy between Liao and Raulet's observation and our results could be explained by the different H-2 haplotype expressed by the strains of mouse used in these studies. It is known that class II MHC molecules associate with SAg at the cell surface for presentation to T cells [11, 12]. Some MHC haplotypes present better than others. One could, therefore, propose that 38CH haplotype, that was expressed by the majority of the mice analyzed in this study, would not constitute a good SAg presenter. However, the observation that $H\text{-}2^{38CH}$ products were able to present MMTV SAg in the process of negative selection as efficiently as $H\text{-}2^d$ products, as reported in the present paper (see above), argue against this hypothesis. Moreover, in another experiment, backcross mice that contained *Mtv-7* only and expressed BALB.D2 $I\text{-}E^d$ molecules, did not show increased frequency of $CD4^+$ TCR $V\beta14^+$ T cells (data not shown). Taken together, our data show that in our system, the absence of positive selection of $CD4^+$ TcR $V\beta14^+$ cells by *Mtv-7* is not due to the failure of SAg presentation by MHC class II molecules.

3.2 TcR Vβ 8.2 polymorphism

Surprisingly, *Mtv-7*-expressing mice could be divided into three groups according to the percentage of TcR Vβ8.2$^+$ cells found in their CD4$^+$ and CD8$^+$ subsets. The majority of them were found to have fully deleted TcR Vβ8.2$^+$ cells while others showed only partial deletion. One mouse was shown with a percentage of TcR Vβ8.2$^+$ cells similar to what was seen in animals lacking *Mtv*. These observations led us to postulate that mice showing partial or complete deletion contained a TcR Vβ8.2 fragment reactive to *Mtv-7* products. As the BALB/c strain and its *Mtv-7*-congenic BALB.D2 are known to possess a non-reactive TcR Vβ8.2 fragment [37], it was assumed that reactivity to *Mtv-7* seen in our backcross mice was brought by the 38CH strain. 38CH TcR Vβ8.2 gene was amplified from tail DNA by PCR using specific primers. The resulting products were cloned and sequenced. Comparison of 38CH TcR Vβ8.2 sequence with BALB/c sequence [37] revealed a nucleotide substitution accounting for one amino acid change (Asn to Asp) in position 19 (numbering according to Happ et al. [38]) (Fig. 2). This TcR Vβ8.2 polymorphism has been previously reported to confer *Mtv-7* SAg reactivity in wild mice [39]. In a three-dimensional representation of the putative TcR structure, position 19 is located in a region of the Vβ8.2 domain well away from the peptide/MHC recognition site but in close proximity of the HV4 loop, known to be crucial in the interactions with endogenous superantigens [37, 40]. The gain of reactivity to *Mtv-7* was linked to the loss of glycosylation site on the TcR Vβ8.2 fragment resulting from the Asn to Asp mutation [41].

To analyze further SAg specificity of the 38CH TcR Vβ8.2, we tested its reactivity to MMTV(SW), a virus known to produce SAg with TcR Vβ specificity identical to *Mtv-7* (TcR Vβ6,Vβ7,Vβ8.1 and Vβ9 in common laboratory mice) [14], and to the SAg of MMTV(SHN), known to react with BALB/c TcR Vβ8.2 fragment [32]. The identification of *Mtv*$^-$ mice homozygote for the TcR Vβ8.2 mutant was carried out by screening the animals for the absence of BALB/c TcR genes in their genome as described in Sect. 2.7. BALB/c and *Mtv*$^-$ mice were injected in the left footpad with MMTV(SW) or MMTV(SHN). Four days later, the draining popliteal lymph nodes were taken and the percentages of CD4$^+$ TcR Vβ8.2$^+$ cells were determined by FACS analysis. As seen in Fig. 3, both types of TcR Vβ8.2$^+$ cells proliferated when stimulated by MMTV(SHN). On the contrary, MMTV(SW) did not induce proliferation among these cells (although, as expected, it stimulated TcR Vβ6$^+$ cells). These observations brought us to the conclusions that (1) MMTV(SHN) recognition by Vβ8.2$^+$ T cells was not dependent on the amino acid substitution found in position 19 on the TcR Vβ8.2 fragment. This opposes the conclusion made by others that predicts reactivity towards MMTV SAg as being dependent on the change in the glycosylation site within the

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                                                     1                                   10
                                 K  H  M  E  A  A  V  T  Q  S  P  R  N  K  V  A  V  T  G  G  K
Balb/c   ttatagaacacatggaggctgcaGTCACCATGGAGGCTGCAGTCACCCAAAGCCCAAGAAACAAGGTGGCAGTAACAGGAGGAAAG

38CH                             ------------------------------------------------------------

                   20                            30                            40
 V  T  L  S  C  N  Q  T  N  N  H  N  N  M  Y  W  Y  R  Q  D  T  G  H  G  L  R  L  I  H  Y  S  Y
GTGACATTGAGCTGTAATCAGACTAATAACCACAACAACATGTACTGGTATCGGCAGGACACGGGGCATGGCTGAGGCTGATCCATTATTCATAT
                D
---------------G--------------------------------------------------------------------------------

             50                            60                            70
 G  A  A  S  T  E  K  G  D  I  P  D  G  Y  K  A  S  R  P  S  Q  E  N  F  S  L  I  L  E  L  A  T
GGTGCTGGCAGCACTGAGAAAGGAGATATCCCTGATGGATACAAGGCCTCCAGACCAAGCCAAGAGAACTTCTCCCTCATTCTGGAGTTGGCTACC

--------------------------------------G---------------------------------------------------------

       80
 P  S  Q  T  S  V  Y  F  C  A  S  G  D
CCCTCTCAGACATCAGTGTACTTCTGTGCCAGCGGTGATgcacagtgatgtggggtttcctcc

-----------------------------------------
```

Figure 2. Comparison of nucleotide sequences of BALB/c and 38CH TcR Vβ8.2 genes. The BALB/c sequence is from [37].

Figure 3. Frequency of lymph node TcR Vβ8.2$^+$ CD4$^+$ cells in BALB/c mice and *Mtv*$^-$ mice homozygous for 38CH TcR Vβ8.2 genes after injection with MMTV(SW)- or MMTV(SHN)-infected milk. Each group contained three animals. The results shown are representative of two experiments.

TcR HV4 domain that would bring an Asn to Asp substitution in position 19 [39]; (2) the failure of MMTV(SW) to stimulate TcR Vβ8.2 mutant$^+$ cells may result from a lack of recognition between MMTV(SW) SAg and the TcR Vβ8.2 mutant. This seems unlikely as MMTV(SW) shares near complete homology and identical TcR Vβ specificity with *Mtv-7* [14], from which SAg is known to interact with the TcR Vβ8.2 mutant ([37, 39] and this report). Another possibility would be that the interaction between the MMTV(SW) SAg and the TcR Vβ8.2 mutant is too weak to stimulate *in vivo* clonal expansion of TcR Vβ8.2$^+$ cells after injection of the virus. The fact that this is known to occur for TcR Vβ8.1$^+$ T cells supports this idea [14]. Also in tune with this hypothesis is the previous observation of the weak interaction between *Mtv-7* and the TcR Vβ8.2 mutant in *in vitro* experiments involving the stimulation of TcR transfectants by *Mtv-7*-expressing cells [44].

In this report we have described a useful animal model for the study of MMTV biology. As MMTV is known to have considerably modified the repertoire of the mouse immune system, raising animals lacking endogenous MMTV or containing single *Mtv* locus was required for the comparison of immune responses generated after infection in the presence/absence of different MMTV gene products. Such observations may bring valuable information about strategies adopted by retroviruses to manipulate the host's immune system and to establish successful infection.

Received November 7, 1994; in final revised form December 15, 1994; accepted December 15, 1994.

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