

# The Open Reading Frames in the 3' Long Terminal Repeats of Several Mouse Mammary Tumor Virus Integrants Encode V $\beta$ 3-specific Superantigens

By Ann M. Pullen, Yongwon Choi, Elenora Kushnir, John Kappler,\* and Philippa Marrack\*<sup>†</sup>

From the Howard Hughes Medical Institute at Denver, Division of Basic Immunology, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206; and the \*Departments of Microbiology and Immunology and Medicine, and the <sup>†</sup>Departments of Biochemistry, Biophysics, and Genetics, University of Colorado Health Sciences Center, Denver, Colorado 80206

## Summary

Mice expressing the minor lymphocyte stimulation antigens, Mls-1<sup>a</sup>, -2<sup>a</sup>, or -3<sup>a</sup>, singly on the B10.BR background have been generated. Mls phenotypes correlate with the integration of mouse mammary tumor viruses (MTV) in the mouse genome. The open reading frames within the 3' long terminal repeats of the integrated MTVs 1, 3, 6, and 13 encode V $\beta$ 3-specific superantigens. Sequence data for these viral superantigens is presented, indicating that it is the COOH-terminal portion of the viral superantigen that interacts with the T cell receptor V $\beta$  element.

A number of murine alloantigens have been described that, in combination with MHC class II products, stimulate T cells bearing particular V $\beta$  elements (1-8). These alloantigens have been termed superantigens (9). Mice expressing such superantigens delete thymocytes bearing reactive V $\beta$  elements during development in the thymus (10). This clonal elimination of thymocytes maintains self-tolerance by preventing mature self-reactive T cells from reaching the periphery.

The analysis of peripheral expression of T cells bearing particular V $\beta$  elements has recently facilitated the mapping of several of these superantigens to particular chromosomal locations. Woodland et al. (11, 12) found no recombinants between the gene encoding a superantigen (*Etc-1*), which causes deletion of V $\beta$ 11- and V $\beta$ 5.2-bearing T cells, and a mouse mammary tumor virus (MTV)<sup>1</sup> integrant (*Mtv-9*) on chromosome 12. In addition, the genes for several other endogenous mouse superantigens map to the same chromosomal regions as MTV integrants (4, 13, 14). In the past, however, some recombinants between the genetic locations of mouse endogenous superantigens and *Mtvs* had been reported in the AKXD and BXD recombinant inbred mice. Suspecting that these reported recombinants may have been mistyped, Frankel et al. (15) retyped the collection of AKXD and BXD mice for MTV integrants and, after correcting previous typings,

found perfect correlation between the expression of several superantigens and presence of particular MTV integrants in these mice. Thus, these investigators suggested that MTV integrants themselves code for the endogenous mouse superantigens. Independently, Dyson et al. (16) arrived at the same conclusion after mapping several ligands that delete V $\beta$ 11<sup>+</sup> T cells.

Recently, we have shown that the infectious milk-borne MTV carried by C3H/HeJ mice codes for a superantigen that interacts with T cells bearing V $\beta$ 14 and -15 (17, 18). Furthermore, transfection experiments have shown that it is the product of the open reading frame (ORF) within the 3' long terminal repeat (LTR) of the C3H MTV that confers the ability to interact with TCR in a V $\beta$ -specific manner (18). Acha-Orbea et al. (19) have shown, using a transgenic model, that the ORF of the infectious MTV transmitted in the milk of GR mice also causes deletion of V $\beta$ 14<sup>+</sup> thymocytes. We have suggested the name viral superantigen (vSAG) for these ORF genes.

In this study, we have used mice expressing Mls-1<sup>a</sup> -2<sup>a</sup>, or -3<sup>a</sup> singly on the B10.BR background to demonstrate conclusively the correlation between the expression of these Mls phenotypes and the presence of *Mtv-7*, -1, and -6 (vSAG-7, -1, and -6), respectively. Transfection experiments showed that *Mtv-1* and *Mtv-3* vSAGs encode V $\beta$ 3-specific superantigens. The sequences presented here for the MTV vSAGs with V $\beta$ 3 specificity (*Mtv-1*, -3, -6, and -13) strengthen the hypothesis that it is the COOH-terminal region of the vSAG that interacts with the TCR V $\beta$ .

<sup>1</sup> Abbreviations used in this paper: LTR, long terminal repeat; MTV, mammary tumor virus; ORF, open reading frame; vSAG, viral superantigens.

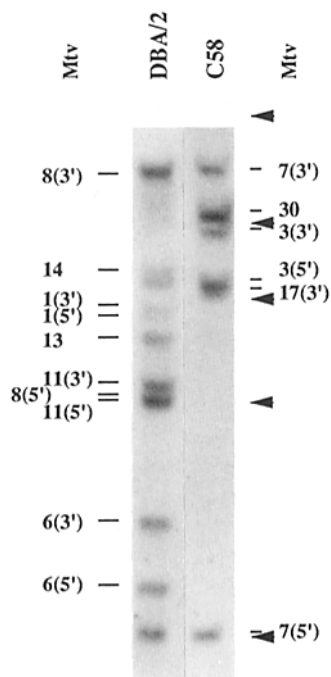
## Materials and Methods

**Mice.** Mice were generally purchased from The Jackson Laboratory (Bar Harbor, ME). Mice carrying *Mls-1<sup>a</sup>*, *Mls-2<sup>a</sup>*, or *Mls-3<sup>a</sup>* on the B10.BR background were bred in our own facility.

**Flow Cytometric Analyses.** Nylon wool-purified, peripheral blood and lymph node T cells were analyzed for expression of *Vβ3* and *Vβ6* as outlined previously (9). T cells were stained with biotinylated KJ25 (anti-*Vβ3*; reference 4) or RR4-7 (anti-*Vβ6*; reference 20) followed by PE-streptavidin (Tago Inc., Burlingame, CA). Two-color analyses of TCR *Vβ* expression and CD4 and/or CD8 used directly fluoresceinated GK1.5 (21) and 53.6.71 (22), respectively.

**Southern Analyses.** Liver or tail DNA was digested with *PvuII*, subjected to electrophoresis on 0.7% agarose gels, and transferred to nitrocellulose as described by Maniatis et al. (23). Filters were hybridized with a MTV LTR probe. The probe was an *EcoRI*-*BamHI* fragment of pTZ18R-ORF (18), derived from the milk-borne C3H MTV (kindly provided by Dr. John Majors, Washington University School of Medicine, St. Louis, MO). The probe was labeled by random priming (24). Filters were washed with two final washes with 0.1% SSC, 0.1% SDS at 58°C for 30 min.

**PCR Amplification, Cloning and Sequencing of MTV vSAGs.** Oligonucleotide primers specific for C3H MTV vSAG (5' sense, GGGAAATCTCGAGATGCCGCGCCTGCAG; and 3' antisense, GGGGATCCAACCTAACTTAAACCTTGG) were used to amplify the vSAGs from *PvuII*-digested genomic DNA from DBA/2 and C58 mice. The DNA was separated on a 0.7% agarose gel and gel pieces corresponding to the 3' LTRs of *Mtv-1*, -6, and -13 from DBA/2 and *Mtv-3* from C58 (Fig. 1) were excised. DNA purified using GeneClean (Bio 101 Inc., La Jolla, CA) was subjected to PCR amplification using a thermocycler (Cetus Corp., Norwalk, CT) and the following amplification conditions: 95°C melting, 50°C, annealing, and 72°C extension, each for 2 min. The amplified products were restricted with *BamHI* and *EcoRI* and cloned into pTZ18R. Multiple clones were sequenced by the chain termination method (25) using the Sequenase kit (United States Biochemical Corp., Cleveland, OH).



**Figure 1.** Southern blot of *PvuII*-digested DNA from DBA/2 and C58 mice. The filter was hybridized with a probe derived from C3H exogenous MTV LTR. Arrow heads designate molecular weight markers of 23.5, 9.4, 6.6, 4.3, and 2.3 kb.

**Transfection.** The vSAGs of *Mtv-1* and *Mtv-3* were subsequently cloned into the eukaryotic expression vector pBDWMCβ2 (26). In case other retroviral products were necessary to ensure high expression of the vSAG products, the vSAG constructs were cotransfected with pUVH, a plasmid containing the 5' sequences of the endogenous C3H provirus *Mtv-1* and the 3' sequences of the milk-borne exogenous C3H MTV (27). These plasmids were linearized with *XbaI* and *Sall* respectively, and were transfected into the B cell line CH12.1 (28) by electroporation. Transfectants were selected with G418.

**Stimulation Assays.** Transfectants were screened for their ability to stimulate T cell hybrids expressing *Vβ3* (K25-49.16 and K25-59.6; reference 4) and *Vβ7* (KOX7-6.6; reference 18). 10<sup>5</sup> B cell transfectants were cultured with 10<sup>5</sup> T cell hybrids. Lymphokine production was assayed after 24 h using the indicator cell line HT-2 (29).

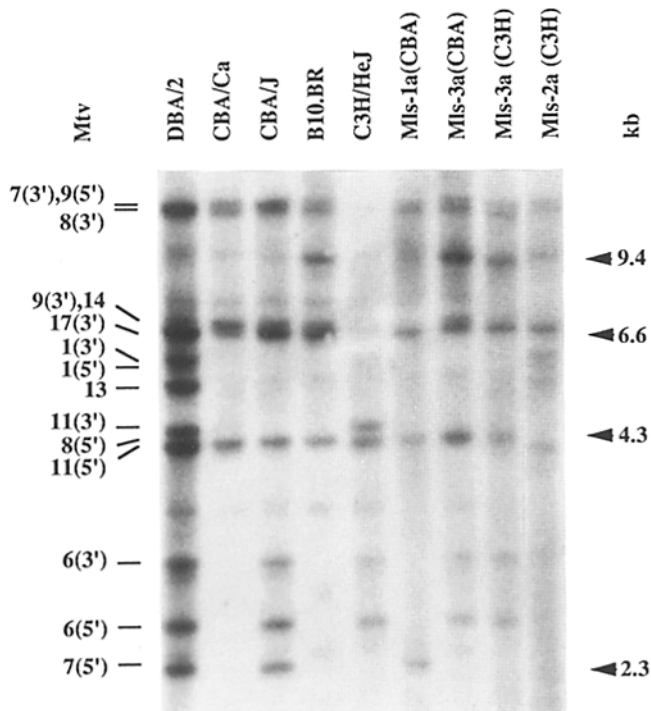
## Results and Discussion

**Endogenous Mouse Superantigens Map to MTV Integration Sites.** Previously published analysis of the progeny of a (C3H/HeJ × B10.BR)<sub>F1</sub> × B10.BR backcross has shown that C3H/HeJ mice express two *Vβ3*-specific superantigens, *Mls-2<sup>a</sup>* and *Mls-3<sup>a</sup>* (30). The progeny that expressed *Mls-2<sup>a</sup>* or *Mls-3<sup>a</sup>* were further backcrossed on to the B10.BR background. For each generation, the offspring were screened for *Vβ3* expression and the mice expressing low levels of *Vβ3*<sup>+</sup> peripheral blood T cells were used as breeders. Expression of *Mls-2<sup>a</sup>* causes partial deletion of *Vβ3*<sup>+</sup> T cells, while *Mls-3<sup>a</sup>* causes complete deletion of these cells (Table 1; and reference 30). Fig. 2 shows a Southern blot of genomic DNA from the backcrossed mice hybridized with a probe derived from C3H exogenous MTV LTR. Mice expressing the *Mls-2<sup>a</sup>* phenotype carried *Mtv-1*, while the *Mls-3<sup>a</sup>* mice carried *Mtv-6*. This is a formal demonstration that the *Mls-2<sup>a</sup>* phenotype correlates with the presence of *Mtv-1*, a possibility suggested by Frankel et al. (15).

Concurrently, we have backcrossed the *Mls* genes of CBA/J on to the B10.BR background. Deletion of *Vβ6*<sup>+</sup> T cells was used as a marker for *Mls-1<sup>a</sup>* expression (3) and the absence of *Vβ3*-bearing cells was taken to indicate the presence of another *Mls* gene. *Mls-1<sup>a</sup>* expression correlates with the

**Table 1.** *Vβ3*-specific Deletion of T Cells by Endogenous Mouse Superantigens

	Source of <i>Mls</i> genes	Percent <i>Vβ3</i> <sup>+</sup> lymph node T cells
B10.BR		3.5
CBA/J		0.1
C3H/HeJ		0.1
B10.BR- <i>Mls-3<sup>a</sup></i> (F6)	CBA/J	0.1
B10.BR- <i>Mls-2<sup>a</sup></i> (F8)	C3H/HeJ	0.5
B10.BR- <i>Mls-3<sup>a</sup></i> (F7)	C3H/HeJ	0.1



**Figure 2.** Endogenous mouse superantigens map to MTV integration sites. A Southern blot of PvuII-digested DNA from inbred and recombinant mouse strains was hybridized with a probe derived from C3H/HeJ endogenous MTV LTR. The higher molecular weight bands for C3H/HeJ are faint due to partial degradation of the DNA sample. *Mls-1a(CBA)* designates B10.BR-*Mls-1<sup>a</sup>* (F6), *Mls-3a(CBA)* designates B10.BR-*Mls-3<sup>a</sup>*(F6), *Mls-3a(C3H)* designates B10.BR-*Mls-3<sup>a</sup>*(F6), and *Mls-2a(C3H)* designates B10.BR-*Mls-2<sup>a</sup>*(F7). Assignments are made for the MTV integrants that are known to be present in these mouse strains.

presence of *Mtv-7* on chromosome 1 (Fig. 2; and reference 15). It has previously been reported that, unlike C3H/HeJ and DBA/2, CBA/J carries only one V $\beta$ 3-specific superantigen (31–33). The expression of this superantigen correlates with the presence of *Mtv-6* (Table 1 and Fig. 2), and it is therefore termed *Mls-3<sup>a</sup>*.

**Four Different MTV Integrants Encode V $\beta$ 3-specific Superantigens.** Analyses of V $\beta$ 3 expression in RI mice of the AKXD and BXD series suggested that DBA/2 mice carry at least two genes encoding V $\beta$ 3-specific superantigens (4, 14); one mapping to chromosome 4 and a second to chromosome 16. Reanalysis of these data and the MTV integration sites for the RI strains suggested that DBA/2 mice actually carry three V $\beta$ 3-specific superantigens, *Mls-2<sup>a</sup>*, *Mls-3<sup>a</sup>*, and one other, mapping to the retroviral integrants *Mtv-1*, *Mtv-6*, and *Mtv-13* on chromosomes 7, 16, and 4, respectively (15). There is, however, evidence for yet another endogenous mouse superantigen that reacts with T cells bearing V $\beta$ 3. Several strains, including C58, NZB, NOD, and CE, have low levels of V $\beta$ 3 expression but do not carry *Mtvs* 1, 6, or 13 (4, 15, 34). Fairchild et al. (35) have recently demonstrated, by analyzing the progeny of a (B10.S(9R)  $\times$  NOD)F<sub>1</sub>  $\times$  B10.BR backcross, that the V $\beta$ 3 deletion phenotype of NOD mice correlates with the presence of *Mtv-3* on chromosome 11. To study the

relationship between these four V $\beta$ 3-specific MTV integrants, we cloned and sequenced the 3' LTV vSAGs of these viruses as described in Materials and Methods.

The *Mtv-1* vSAG sequence from DBA/2 (Fig. 3) differed from the published sequence for *Mtv-1* from C3H (36) at two positions; first by a C-G mutation in codon 55 resulting in a Pro-Ala substitution, and second, by the insertion of a single base at the third position of codon 315. The resulting protein encoded by this ORF is therefore three amino acids longer. Since *Mtv-1* in C3H mice interacts with V $\beta$ 3<sup>+</sup> T cells, it suggests that the terminal three amino acids of the *Mtv-1* vSAG in DBA/2 are not required for T cell stimulation or, alternatively, it is possible that the published sequence for C3H *Mtv-1* may be wrong.

The *Mtv-6* and *Mtv-13* vSAGs were amplified from DNA from RI strains BXD-8 and BXD-1, respectively. Each of these RI strains carries only one MTV integrant that encodes a V $\beta$ 3-specific superantigen derived from DBA/2 (37). The *Mtv-1* and *Mtv-6* vSAG genes only differ by eight nucleotides (Fig. 3); however, it is striking that their amino acid sequences are identical. This finding is surprising, since it is known that the products of these genes differ in the efficiency with which they cause T cell deletion. Mice bearing *Mtv-6* completely delete V $\beta$ 3<sup>+</sup> T cells, while *Mtv-1* expression results in only partial deletion of these T cells (4, 14, 15, 30). Perhaps differences elsewhere in the MTV genomes, or the different chromosomal integration sites, cause expression of the various V $\beta$ 3-specific MTV superantigens in different tissues or at different levels.

The *Mtv-13* vSAG differs from that of *Mtv-1* by seven nucleotides; five of these differences are silent and the other two result in amino acid differences at positions 28 and 45 (Fig. 3). The similarity of the three MTV integrants encoding V $\beta$ 3-specific superantigens derived from DBA/2 mice may be due to the insertion of similar MTVs at distinct sites in the mouse genome, on chromosomes 16, 4, and 7, or alternatively, it may be due to the transfer of the provirus from one chromosomal location to another with subsequent point mutations.

The sequence of *Mtv-3* vSAG from C58 differs from the *Mtv-1* vSAG by 10 amino acids (Fig. 3). All these residues, except Thr at position 317, have been found in other MTV vSAGs. Similar relationships between other MTV vSAGs can be observed in other published sequences (38–41) (Fig. 4). On the whole, amino acids found substituted in one vSAG can also be found in other ORFs, suggesting the occurrence of multiple recombination events; possibly by recombination between milk-borne infectious viruses, or alternatively, by gene conversion between the integrated proviruses.

Comparisons of the published sequences of vSAGs from the exogenous C3H MTV (39) and integrated MTVs 1, 8, 9, and 11 (36, 40, 41), led us to speculate that the 30 or so amino acid residues at the COOH-terminal of the vSAGs were responsible for the V $\beta$  specificity (18). This was supported by the demonstration by Acha-Orbea et al. (19) that transgenic mice expressing a variant of the C3H MTV did not delete V $\beta$ 14<sup>+</sup> T cells, whereas the wild-type virus, which differs from the variant at amino acids 288–315 of the ORF, clearly caused the deletion of V $\beta$ 14-bearing T cells.

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1          10          20          30          40
MTV1 ATGCCCGCCCTGCACGAGAAATGGTTGAACCTCCCGAGAGTGTCTTACACTTAGGAGAGAAAGCCAAAGGGGTTGTTTCCCAAGGACGACCCGCTGCGTGCACGCGGATGAGCCCA
MTV3 M P R L Q Q K W L N S R E C P T L R R E A A K G L F P T K D D P S A C T R M S P
MTV6 . . . . .
MTV13 . . . . . T
I

50          60          70          80
MTV1 TCAGACAAAGACATACTCATTCTCTGCTGCAAACTTGGCATAAGCTCTGCTTTCCTGGGGCTATGGGGGAAAGTTCGGTTCGCTCGCAGGGCTCTCACCCCTTGACTCTTT--AAT
MTV3 S D K D I L I L C C K L G I A L L C L G L L G E V A V R A R R A L T L D S F - N
MTV6 . . . . . T
MTV13 . . . . . T
G
M

90          100         110         120
MTV1 AACTCTTCTGTGCAAGATTACAATCTAAACGATTCCGAGAACTCGACCTTCCCTCGGGGCAAGGACCACAGCCAACTCCTCTTACAAGCCACACCGACTTGTCTTCAGAAATGAAA
MTV3 N S S V Q D Y N L N D S E N S T F L L G Q G G P Q P T S S Y K P H R L C P S E I E
MTV6 . . . . . T
S . . . . . N . . . . . F
C

130         140         150         160
MTV1 ATAAGAAATGCTGCTAAAACATATATTTTACCAATGAGACCAATCCAATAGGTCGATTATTAATCATGATGTTAAGAAATGAATCTTTGCTTTTAGCACTATATTTACTCAAATCAA
MTV3 I R M L A K N Y I F T N E T N P I G R L L I M M L R N E S L S F S T I F T Q I Q
MTV6 . . . . . T . . . . . A . . . . . CT . . . . . A . . . . . AC . . . . . T . . . . . T
K . . . . . T . . . . . P
T
T

170         180         190         200
MTV1 AGGTTAGAAATGGAATAGAAAATAGAAAGAGACGCTCAACCTCAGTTGAAGAACAGGTGCAAGGACTAAGGGCCTCAGGCCTAGAAGTAAAAGGGGAAAGAGGAGTCCGCTTGTCAA
MTV3 R L E M G I E N R K R R S T S V E E Q V Q G L R A S G L E V K R G K R S A L V K
MTV6 . . . . . A . . . . . GTT . . . . . L . . . . . T
A

210         220         230         240
MTV1 ATAGGAGACAGGTGGTGGCAACCAGGACTTATAGGGACCTTACATTTACAGACCAACAGACGCCCCCGTTACCATATACAGGAAGATATGATTTAAATTTTGATAGGTGGTGCACAGTC
MTV3 I G D R W W Q P G T Y R G P Y I Y R P T D A P L P Y T G R Y D L N F D R W V T V
MTV6 . . . . . A . . . . . C . . . . . T . . . . .
C . . . . . C . . . . . C
C

250         260         270         280
MTV1 AACGGCTATAAAGTGTATACAGATCCCTCCCCTTTCGTGAAAGGCTCGCCAGAGCTAGACCTCCTTGGTGTGTGTGCTCAAGAAGAAAAGACGACATGAAACAACAGGTACATGAT
MTV3 N G Y K V L Y R S L P F R E R L A R A R P P W C V L S Q E E K D D M K Q Q V H D
MTV6 . . . . . A . . . . . AA . . . . . G . . . . . T
A

290         300         310
MTV1 TATATTTACTAGGAACAGGAATGATA---CATGG---AAAGTATTTTATAACAGTAGAGAGGAGGCCAAAAGACATATAATAGAACATATTAAGGCTTTCCTTACGCTTTCTAA
MTV3 Y I Y L G T G M I - H W - K V F Y N S R E E A K R H I I E H I K A L P L A F *
MTV6 . . . . . G . . . . . G . . . . . . . . . . A . . . . . A . . . . . T
G . . . . . G . . . . . . . . . . A . . . . . T
A

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Figure 3. Sequences of Vβ3-specific MTV viral superantigens. Dashes designate spaces to allow alignment with the sequences of the other MTV vSAGs as shown in Fig. 4. These sequence data have been included in the EMBL Data Libraries under accession numbers X63024 (MTV1), X63025 (MTV3), X63026 (MTV6), and X63027 (MTV13).

MTV	VR	1	10	20	30	40	50	60	70	80	90	100	110
Consensus		MPRLQQKWLNSRECFPLRRRAAKGLFPTKDDPSACTRMSFSDKDIILICCKLQIALCLLQGLQEVAVRARRALDST-NMSSVQDYMLNNSHSTLLOQQPQFTSSTK											
MTV-C3H	14,15	.....P.G.....EK.....F.....S.....R.....											
MTV-GR	14	.....G.....EK.....H.....D.....											
MTV-1 (C3H)	3	.....P.....D.....											
MTV-1, MTV-6	3	.....D.....											
MTV-3	3	.....S.....											
MTV-13	3	.....I.....M.....D.....											
MTV-8	11	.....											
MTV-9	5,11	.....A.....S.....											
		120	130	140	150	160	170	180	190	200	210	220	
Consensus		PHRFPSBIRIEMLAKWYIFTNKTWPIGRLLITMLRNESLSFSTIIFTQIQRLMGIENRKRSTSVREQVQGLASGLEVKRGRSVVVKIGDRNWQPGFTYRPT											
MTV-C3H	14,15	.....V.....K.....I.....TT.....K.....											
MTV-GR	14	..I..L.....K.....V.....K..I.....K.....R.....											
MTV-1 (C3H)	3	..L.....E.....M.....R.....R...AL.....											
MTV-1, MTV-6	3	..L.....E.....M.....R.....R...AL.....											
MTV-3	3	..L.....P.....T.....R...AL.....											
MTV-13	3	..L.....E.....M.....R.....R...AL.....											
MTV-8	11	..L.....M.....R.....R...TL.....											
MTV-9	5,11	..Y.....I.....P.....A..K...S..T.....											
		230	240	250	260	270	280	290	300	310	320		
Consensus		DAPLPYTGRIYDLNFRWVTVNGYKVLVRSLLPFRRLARARPPWCVLQREKDDMKQVVDYIYLOTGM...WGKIFH-TKEGAVA-LIHSISA-T-QMSYNG*											
MTV-C3H	14,15	.....W.....M..S.....HF.....T..G...Y..K..Y...YE..											
MTV-GR	14	.....W.....I.....M..EK.....HF...V.....G...Y..K..Y...YD..											
MTV-1 (C3H)	3	.....S.....IH...V..YNSR..E..KRHI...K..LP*											
MTV-1, MTV-6	3	.....S.....IH...V..YNSR..E..KRHI...K..LPLAF*											
MTV-3	3	.....S.....IH...V..YNSR..E..KRHI...K..LPLTF*											
MTV-13	3	.....S.....IH...V..YNSR..E..KRHI...K..LPLAF*											
MTV-8	11	.....I.....NV.....Y.....RQL.....D..F.....											
MTV-9	5,11	.....M.....NV.....Y.....R..L.....D..F.....											
MTV-11	11	.....FS.....NV.....Y.....RQL.....D..F.....											

**Figure 4.** Comparison of amino acid sequences for MTV viral superantigens. The consensus sequence was derived from the published MTV vSAG sequences (36, 38–41).

The finding that all the MTV vSAGs that stimulate  $V\beta 3^+$  cells have similar sequences, which differ significantly from the other MTV vSAG sequences at the 3' end, strengthens the hypothesis that the 3' end of the MTV ORF encodes the  $V\beta$ -binding portion of the vSAGs (Fig. 4). This hypothesis is currently being further tested by analyzing T cell responses to chimeric vSAGs.

B cell transfectants expressing the *Mtv-1* and *Mtv-3* vSAGs were generated to test whether the products of these genes stimulate  $V\beta 3^+$  T cells. CH12.1, a B cell lymphoma derived from B10.A (28), was used as the recipient cell line.

Since B10 congenic mice have high levels of  $V\beta 3$  expression (4, 34), we inferred that this B cell would not carry any  $V\beta 3$ -reactive MTV integrants that might confound the results. Transfectants expressing *Mtv-1* vSAG or *Mtv-3* vSAG stimulated  $V\beta 3^+$  T cell hybrids, K25-49.16 (Table 2) and K25-59.6, while transfectants expressing the hybrid vSAG C3H construct (pUVH) alone did not stimulate these T cells (data not shown). Thus, in conclusion, these experiments confirm that the vSAGs of these endogenous MTV integrants are superantigens, as has been previously demonstrated for the infectious MTVs.

**Table 2.** Stimulation of  $V\beta^+$  T Cell Hybrids by Transfectants Expressing *Mtv-1* ORF or *Mtv-3* ORF

CH12.1 transfectants	IL-2 production by T cell hybrids	
	K25-49.16 ( $V\beta 3$ )	KOX7-6.6 ( $V\beta 7$ )
	<i>U/ml</i>	
CMTV1-25	80	—*
CMTV1-35	160	—
CMTV1-40	320	—
CMTV1-43	160	—
CMTV3-4	640	—
CMTV3-8	320	—
CMTV3-27	320	—
CMTV3-4.4†	160	—
CMTV3-4.5†	640	—
CMTV3-4.7†	640	—

\*  $\leq 10$  U of IL-2/ml.

† These transfectants are clones of CMTV3-4.

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Address correspondence to Ann Pullen, Department of Immunology, University of Washington, I 264 Health Services Building, SL-05, Seattle, WA 98195.

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