Mls-1-like Superantigen in the MA/MyJ Mouse Is Encoded by a New Mammary Tumor Provirus That Is Distinct from *Mtv-7*

By Christine K. Rudy,* Eliyahu Kraus,* Ed Palmer,‡ and Brigitte T. Huber*

*From the *Department of Pathology, Tufts University School of Medicine, Boston, Massachusetts 02111; and the ‡National Jewish Center for Immunology and Respiratory Medicine, Division of Basic Sciences, Department of Pediatrics, University of Colorado Health Sciences, Denver, Colorado 80206*

Summary

Mls-1 is an endogenous superantigen that leads to in vivo deletion and in vitro stimulation of T cell receptor (TCR) Vβ6-, 7-, 8.1-, and 9-expressing cells. The MA/MyJ mouse deletes the identical set of TCR from its mature T cell repertoire; however, it does not contain *Mtv-7*, the murine mammary tumor provirus (MMTV), whose *sag* gene encodes Mls-1. Interestingly, the superantigen activity of this mouse strain segregates with a new mammary tumor provirus, *Mtv-43*, not seen in other inbred strains. The predicted amino acid sequence of the *sag* gene of *Mtv-43* was compared with that of *Mtv-7*. Strikingly, the COOH terminus of the two molecules is very similar, while all other MMTV-encoded superantigens differ 100% in this segment.

The endogenous superantigens (SAG)[1] comprise a family of molecules that exert a strong influence on the expressed TCR repertoire. Unlike conventional peptide antigens, which contact the third hypervariable region of both the α and the β chains of the TCR, SAG are recognized by the TCR Vβ gene product only (1). The prototype of an endogenous SAG is Mls-1, which was discovered some 20 yr ago by Festenstein (2). The biochemical nature of this ligand, however, remained an enigma until recently. The first breakthrough came from two independent groups who established that Mls-1-expressing mice delete TCR Vβ6$^+$ and Vβ8.1$^+$ T cells from their mature repertoire. These investigators also observed that Vβ6 and Vβ8.1 T cells respond to Mls-1 stimulation in vitro (3, 4). Similar results were obtained with other endogenous SAG (5–9). The genetic mapping of a Vβ5-tolerizing element close to the murine mammary tumor provirus (MMTV) *Mtv-9* provided a hint of the molecular nature of the endogenous SAG (10). This led us and others to map a series of endogenous SAG to various MMTV loci and infectious MMTV (11–14). Furthermore, the open reading frame in the U3 region of the MMTV LTR has been identified as the gene encoding the SAG (15–18a). Thus, the new name MMTV *sag* has been proposed for this retroviral gene (18a).

In our initial survey of the correlation between the presence of *Mtv-7* and the expression of an Mls-1 phenotype in inbred mice, we noticed one discrepancy, namely, the MA/MyJ strain that had been typed as Mls-1 positive (19, 20), but lacks *Mtv-7* (11). Intrigued by this observation, we analyzed the SAG activity of this mouse strain in detail. We described in this report striking similarities, as well as some differences, between the Mls-1 and the MA/MyJ-specific T cell stimulatory/tolerizing phenotypes. Using backcross segregation analyses, we were able to map the SAG activity of this mouse strain to a new MMTV, now called *Mtv-43*, which is not present in other inbred strains tested to date.

We have determined the nucleotide sequence of *Mtv-43 sag* and compared its predicted amino acid sequence with that of other MMTV *sag* genes. The *Mtv-7* and the *Mtv-43 sag*–encoded proteins are very similar. Most strikingly, their COOH terminus is very similar, while other MMTV *sag*–encoded proteins differ 100% in this segment. Thus, we conclude that this region is responsible for the TCR Vβ specificity that is shared between the *Mtv-7*- and the *Mtv-43*-encoded SAG. Interestingly, the predicted amino acid sequence of the *Mtv-43 sag* is closely related to that of an exogenous MMTV, which functionally resembles Mls-1 (20a).

Materials and Methods

Mice. MA/MyJ, B10.BR, CBA/J, AKR, and C3H/HeJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME).

[1] *Abbreviations used in this paper:* MMTV, murine mammary tumor provirus; SAG, superantigens.

(MA/MyJ × B10.BR)F_1, (MA/MyJ × B10.BR)F_1 × B10.BR, and B10.BR × (MA/MyJ × B10.BR)F_1 were bred at the animal facilities at Tufts University School of Medicine.

Antibodies. The following cytotoxic mAbs for C killing were obtained from American Type Culture Collection (Rockville, MD) anti-CD4 (RL 172), anti-CD8 (3.155), anti-HSA (J11D), anti-I-A^k (10.2-16), and anti-I-E^k (M5-114.25.2). Anti-Vβ mAbs were obtained from the following sources: Vβ2 (B20.6), B. Mallissen (Marseilles, France); Vβ3 (KJ25) and Vβ8.1 + Vβ8.2 (KJ16), P. Marrack and J. Kappler (Denver, CO); Vβ4 (KT4.10), K. Tomonari (London, UK); Vβ6 (44.22.1), H. Hengartner (Zurich, Switzerland); Vβ7 (TR130), I. Weissman (Stanford, CA); Vβ8.2 (F23.2); M. Bevan (Seattle, WA); and Vβ9 (MR10.2), O. Kanagawa (St. Louis, MO). They were all used in biotinylated form. Anti-CD4 (GK1.5) and anti-CD8 (53-6.72) were obtained from American Type Culture Collection and used in FITC-conjugated form.

Oligonucleotides. Six oligonucleotides were synthesized for this study. Two 23mers were used as primers for the PCR: a 5′ primer (position 343–357) 5′-AATTCGGACCACAGCCAACT-3′ includes an EcoRI restriction site, and a 3′ primer (position 1107–1121) 5′-GATCTCCAAGTCAGGAAACC-3′ includes a BglII restriction site. Two oligomers were used for sequencing: a 15mer (position 691–705) 5′-ATCTGTTGGTCT-3′, and a 21mer (position 925–945) 5′-TCCCTCTTCGGTGTACTC-3′. An *Mtv-7 sag*–specific 21mer (position 982–1003) 5′-GAAGCCAACGCGACCCCC-3′ and an *Mtv-43 sag*–specific 18mer (position 978–995) 5′-ATGCGACCGTTATGA-3′ were used for hybridizations.

MLR. Single cell spleen suspensions were prepared, and the red cells were lysed by hypotonic shock. B cell blast stimulators were obtained by treating spleen cells with anti-CD4 and anti-CD8 mAb plus guinea pig C (Organon Teknika-Cappel, West Chester, PA) for 30 min at 37°C, followed by a 18–24-h incubation in RPMI (Gibco Laboratories, Grand Island, NY), supplemented with Hepes, 2-ME, 2 mM L-glutamine (Gibco Laboratories), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco Laboratories), 10% FCS (Hazelton, Lenexa, KA), and 10 μg/ml LPS (Sigma Chemical Co., St. Louis, MO). B cell blasts were treated with mitomycin C (75 μg/ml) (Sigma Chemical Co.) before coculture with T cells. T cell responders were prepared by passage through nylon wool. MLR were carried out in 96-well plates by incubating 5×10^5 B cell blasts and 2×10^5 T cells in 200 μl Click's medium (Irvine Scientific, Neptune, NJ), supplemented with L-glutamine, penicillin, streptomycin, and 5% prescreened FCS (kindly provided by C. Janeway, New Haven, CT). The cells were incubated at 37°C in 7% CO_2 for 72–86 h, with 1 μC [^{3}H]TdR (New England Nuclear, Boston, MA) added for the last 12 h. The plates were harvested using a PHDcell harvester (Cambridge Technologies, Watertown, MA), and incorporation of [^{3}H]TdR into the DNA was counted on a β counter (Beckman Instruments, Inc., Fullerton, CA).

Flow Cytometry. Two-color fluorescence analyses were carried out on T cells that had been isolated from whole spleen cells by treatment with anti-I-A^k or anti-I-E^k and anti-HSA mAb plus a mixture of guinea pig C and rabbit C (Pel-Freez Clinical Systems, Brown Deer, WI) for 20 min at 37°C, followed by a second incubation with C for 20 min at 37°C. Cells were then incubated with the various biotinylated anti-Vβ mAbs for 20 min on ice, washed, and incubated with FITC-conjugated anti-CD4 or anti-CD8 mAb and PE streptavidin (Southern Biotechnology Associates, Inc., Birmingham, AL) for 20 min on ice. The cells were analyzed using the FACScan® (Becton Dickinson & Co., Mountain View, CA).

Southern Analyses. DNA was isolated from whole spleen cells, digested with PvuII (Gibco BRL, International Biotechnologies, Inc., New Haven, CT), and subjected to electrophoresis on an 0.8% Seakem (FMC BioProducts, Rockland, ME) agarose gel 21–23 h at 60 V, as previously described (11). The gel was denatured in 0.2 M NaOH, 0.75 M Tris, and neutralized in 1 M NaCl, 1.5 M Tris. For Southern analyses, the gel was blotted onto Zetabind (CUNO, Inc., Meriden, CT) or Biotrans (ICN Biomedicals, Inc., Irvine, CA) nylon over night in 20× SSC. The DNA was UV crosslinked to the nylon, and washed for 1 h at 65°C in 0.1× SSC, 0.5% SDS. The blots were prehybridized for 1 h at 65°C and then hybridized with a random-primed MMTV LTR probe (21) for 16–18 h at 65°C. The blots were washed and exposed for 1–3 d at −80°C on x-ray film. For oligonucleotide hybridizations, the agarose gel was dried, prehybridized for 1 h at 37°C, and then hybridized with an end-labeled oligomer for 16–18 h at 37°C, according to published procedures (22). The gel was washed and exposed for 4–7 d at −80°C on x-ray film.

PCR Amplification, Cloning, and Sequencing of Mtv-43 sag. The DNA for PCR was obtained by separating PvuII-digested DNA in low-melt agarose and isolating the band corresponding in size to the *Mtv-43* junction fragment (between 6.1 and 6.7 kb). The DNA was amplified by PCR (Perkin Elmer Cetus, Norwalk, CT) using the two primers described above. The PCR product was digested with EcoRI and BglII (Gibco BRL, International Biotechnologies, Inc.) and cloned into M13mp18 (New England Biolabs, Beverly, MA). Competent XL1-blue-*Escherichia coli* were transformed with the double-stranded phage. Phage plaques that hybridized to an MMTV LTR probe were picked for sequencing. Single-strand phage DNA was prepared by PEG precipitation from an over night culture. The DNA was sequenced using the Sequenase Kit (U.S. Biochemical Corp., Cleveland, OH) and both the M13-40 primer (U.S. Biochemical Corp.) and the two MMTV specific oligomers described above.

Results

Mls Phenotype of the MA/MyJ Mouse. Although the MA/MyJ mouse has been described as having an Mls-1 phenotype (19), Ryan et al. (20) noted that T cells from this mouse strain are stimulated by H-2-matched spleen cells of an Mls-1 strain. Furthermore, we have established that MA/MyJ lacks *Mtv-7* (11), the endogenous MMTV that encodes Mls-1 (18a). These observations prompted us to further investigate the Mls phenotype of the MA-MyJ strain. Using H-2-matched stimulator and responder cells (see Table 1), we have confirmed

Table 1. *H-2 and Mls Phenotypes of Mouse Strains Used*

	H-2	Mls
MA/MyJ	k	?
B10.BR	k	–
AKR	k	1
CBA/J	k	1, 3
C3H	k	3, 4
BALB/c	d	3

Mls-1 (*Mtv-7*) is a Vβ6, Vβ7, Vβ8.1, and Vβ9 deleting phenotype; Mls-3 and Mls-4 (*Mtv-6* and *Mtv-1*, respectively) are Vβ3 deleting phenotypes.

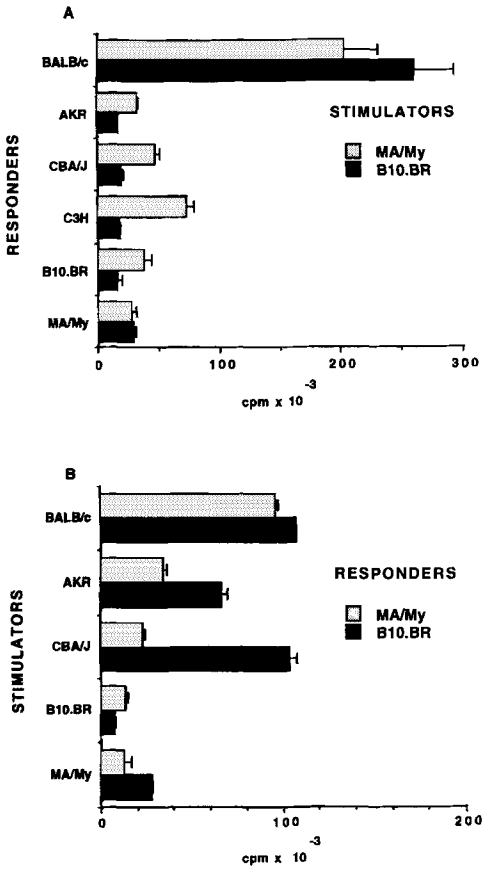

Figure 1. Primary MLR. (*A*) Response of T cells from four H-2-matched strains and one H-2-mismatched strain to LPS-activated B cells from B10.BR and MA/MyJ mice, respectively. (*B*) Response of B10.BR and MA/MyJ T cells to LPS-activated B cells from four H-2-matched strains and one H-2-mismatched strain. cpm is of [^{3}H]TdR incorporation.

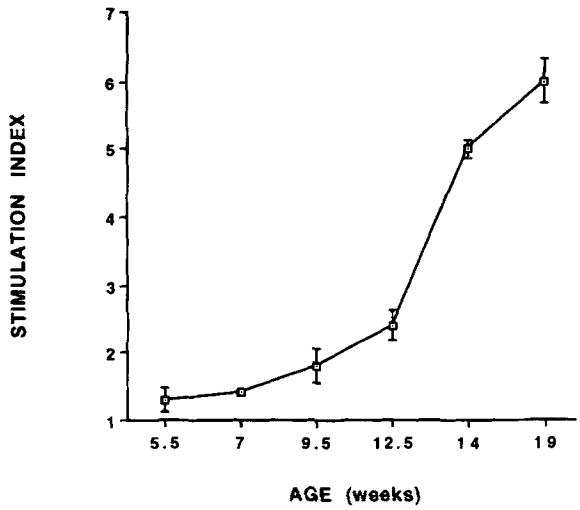

Figure 2. Age-related appearance of stimulatory activity. The stimulatory capacity of LPS-activated B cells from (MA/MyJ × B10.BR)F_1 mice in a primary MLR, measured on B10.BR T cells, is seen reliably only by 12–14 wk, and is further enhanced with increasing age.

and extended the finding of Ryan et al. (20). B cells from MA/MyJ mice induced a primary MLR in CBA/J-, AKR-, and C3H-derived T cells, as depicted in a representative experiment in Fig. 1 *A*. Stimulator cells were preactivated with LPS, because this treatment is known to increase the stimulatory capacity of endogenous SAG (10). Although the stimulatory activity of MA/MyJ B cells is relatively small in the strain combinations tested, it is very reproducible. In addition, MA/MyJ T cells responded to LPS-stimulated B cells from AKR and CBA/J mice (Fig. 1 *B*). From these results, we conclude that MA/MyJ mice do not express a strict Mls-1 phenotype. Interestingly, the stimulatory activity of MA/MyJ B cells is seen only by 12–14 wk of age (Fig. 2). This is significantly later in ontogeny than the manifestation of the Mls-1 phenotype (23), but resembles the appearance of SAG activity induced by infectious MMTV (15, 16).

TCR Vβ Repertoire of the MA/MyJ Mouse. Since endogenous SAG lead to deletion of T cells expressing certain TCR Vβ genes (3, 4, 6, 24–26), we analyzed the TCR Vβ repertoire in the peripheral T cells of the MA/MyJ mouse. A comparison was carried out with T cells from H-2-matched AKR and B10.BR mice. Using two-color flow cytometry, the expression of eight Vβ chains was analyzed in CD4$^+$ as well as in CD8$^+$ T cells. While no significant differences were observed in the level of Vβ2, Vβ3, Vβ4 (results not shown), and Vβ8.2 T cells in the three strains, it is very striking that the MA/MyJ mouse showed reduced Vβ6, Vβ7, Vβ8.1, and Vβ9 T cells, compared with the B10.BR mouse (Fig. 3). This

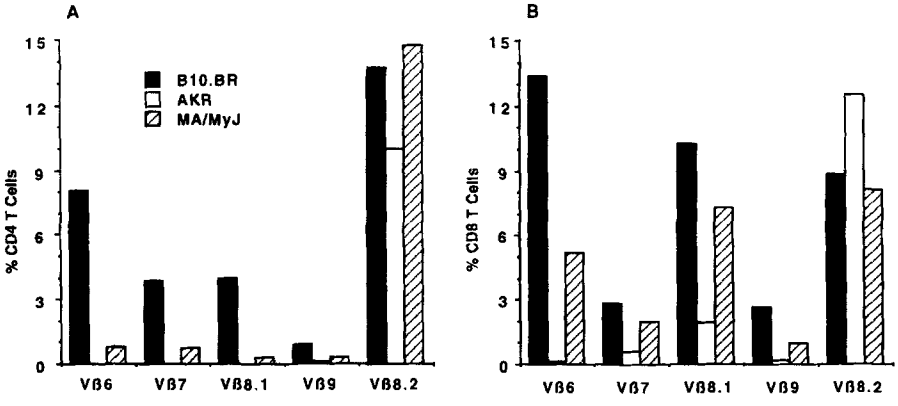

Figure 3. Selected TCR Vβ gene usage in B10.BR, AKR, and MA/MyJ strains. Vβ6, 7, 8.1, and 9 are absent in CD4$^+$ T cells (*A*) and reduced in CD8$^+$ T cells (*B*) of the Mls-1-positive strain AKR. The same Vβs are highly reduced in CD4$^+$ T cells of the MA/MyJ mouse (*A*), while their reduction is not as pronounced in CD8$^+$ T cells in this strain (*B*). The B10.BR strain is not depleted of these four TCR Vβs (negative control). Vβ8.2 is not deleted in either strain.

is precisely the TCR Vβ deletion phenotype of the Mls-1-positive AKR mouse. However, while these four Vβs were virtually absent form the CD4+ T cells in the AKR mouse, only a partial deletion was seen in the MA/MyJ mouse, despite the fact that mice >4 mo of age were tested. The difference in magnitude of deletion in AKR vs. MA/MyJ mice is even more noticeable in the CD8+ subset of T cells. The incomplete deletion of the set of Mls-1-associated Vβs in the MA/MyJ mouse is consistent with functional data obtained in in vitro stimulation assays; i.e., we have observed that several Vβ6 and Vβ8.1 T cell hybridomas are unable to respond to MA/MyJ-derived B cell blasts, while mounting a good response to Mls-1 (results not shown). The fact that MA/MyJ does not delete Vβ3 indicates that this mouse strain does not express an Mls-2, Mls-3, or Mls-4 phenotype.

MMTV Profile of the MA/MyJ Mouse. We had initially observed in Southern blot analysis that the genome of the MA/MyJ mouse does not contain *Mtv-7*, but has a new MMTV provirus (11), now designated *Mtv-43*. From the hybridization pattern seen in PvuII-digested chromosomal DNA, probed with an MMTV LTR fragment, we conclude that MA/MyJ mice have *Mtv-8*, *-9*, *-17*, and *29*, in addition to the unique *Mtv-43* (Fig. 4 *A*). By probing the Southern blot with a DNA fragment that was isolated from the 3' flanking region of *Mtv-7* (11), we noticed that the hybridization pattern of MA/MyJ is different from that of *Mtv-7*-positive strains (Fig. 4 *B*). Thus, it is unlikely that *Mtv-43* maps to the vicinity of *Mtv-7* on chromosome 1, or that this new provirus is a product of an *Mtv-7* duplication.

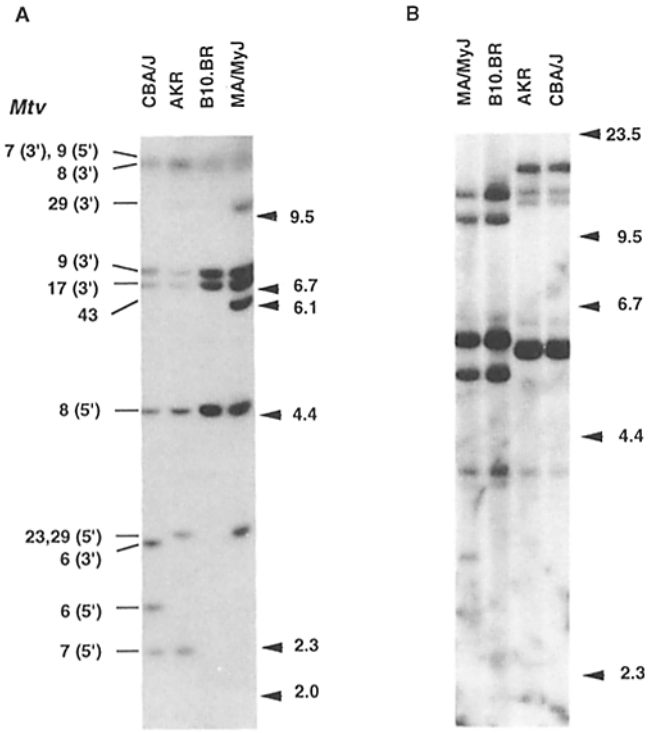

Figure 4. MMTV expression analysis. (*A*) Genomic DNA from CBA/J, AKR, B10.BR, and MA/MyJ mice was digested with PvuII, Southern blotted, and hybridized to a ^{32}P-labeled MMTV LTR probe. The junction fragments of the various MMTVs are marked. (*B*) Genomic DNA from MA/MyJ, B10.BR, AKR, and CBA/J mice was digested with PvuII, Southern blotted, and hybridized with a ^{32}P-labeled fragment, isolated from the *Mtv-7* 3' flanking region. Molecular mass markers are shown on the right.

Table 2. *Segregation Analysis of SAG Activity*

	No. of mice	Percent Vβ6*	Percent S.I.‡
B10.BR	12	8.4 (0.30)	0
(MA/MyJ × B10.BR)F_1	13	1.7 (0.09)	100
Backcross mice			
B10.BR-like	38	8.9 (0.19)	2 (1.14)
F_1-like	58	1.9 (0.08)	85 (4.96)
Discordant	2	8.4	47
	3	3.2	9

* Percentage of CD4+ T cells that express Vβ6, as determined by two-color flow cytometry. The numbers in parentheses are the SEM.
‡ Stimulation Index from MLR using B10.BR T cells. Results are expressed as a percentage of the stimulation index (cpm [experimental]/cpm[control]) obtained with (MA/MyJ × B10. BR)F_1 B cells. The numbers in parentheses are the SEM.

Segregation Analyses of the MA/MyJ SAG Activity and Mtv-43. We next examined whether the MA/MyJ SAG activity segregates with the newly identified MMTV provirus *Mtv-43* in backcross mice. For this purpose, we analyzed (MA/MyJ × B10.BR)F_1 × B10.BR, and B10.BR × (MA/MyJ × B10.BR)F_1 mice for three parameters: (*a*) In vitro MLR stimulatory activity, as measured by B10.BR T cells; (*b*) level of Vβ6 expression in the CD4+ T cell subset; and (*c*) presence or absence of *Mtv-43*. The two types of backcross mice were used to test for potential maternal transmis-

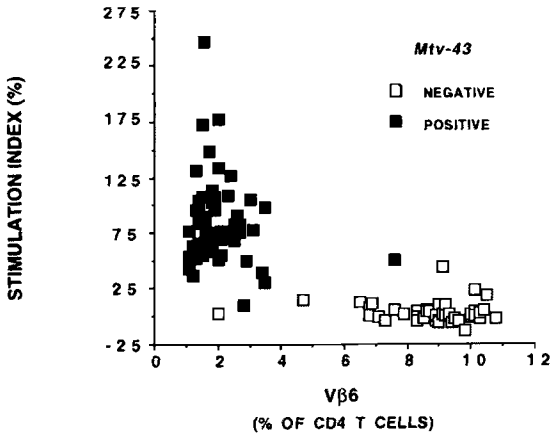

Figure 5. Segregation analyses of the MA/MyJ superantigen activity and *Mtv-43*. (MA/MyJ × B10.BR)F_1 × B10.BR backcross mice (101) were typed for Vβ6 level (*x*-axis), ability to stimulate B10.BR T cells (*y*-axis) and presence (*filled squares*) or absence (*open squares*) of *Mtv-43*. The stimulation index obtained with (MA/MyJ × B10.BR)F_1 B cells (100%).

sion of MA/MyJ-related SAG activity; however, no difference in transmission ratio was observed in the two groups. The results obtained with 101 backcross mice are shown in Table 2 and Fig. 5.

To normalize the MLR data, the stimulatory activity of each backcross mouse is expressed as percentage of the stimulatory activity of (MA/MyJ × B10.BR)F_1 B cell blasts, tested on B10.BR T cells in the same experiment (100% value). Despite variations seen in individual mice, there are clearly two groups of backcross mice that can be functionally distinguished: The F_1-like mice, which stimulate B10.BR T cells and have a reduced number of Vβ6 CD4$^+$ T cells (58 mice), and the B10.BR-like mice, which have neither stimulatory activity, nor delete Vβ6 T cells (38 mice). This distribution fits segregation of the SAG phenotype as a single genetic trait at a 95% confidence level by χ^2 analysis (27). Five mice were discordant; i.e., their stimulatory phenotype did not correlate completely with their level of Vβ6 cells. This may be a reflection of natural variations in the onset of phenotypic expression of the MA/MyJ-specific SAG during ontogeny (see Fig. 2) and/or the variability of the assays used. Alternatively, other undefined factors may also influence the phenotypic expression.

Typing for *Mtv-43* was done by Southern blotting of PvuII-digested genomic DNA, using an MMTV LTR probe (see Fig. 4 *A*). As is evident in Fig. 5, the presence of the *Mtv-43* provirus correlates well with the expression of the MA/MyJ-specific SAG.

Sequence Comparison of Mtv-43 and Mtv-7 sag. We have recently cloned and sequenced the *Mtv-7 sag* gene and shown in transfection studies that it encodes Mls-1 (18a). It was of interest, therefore, to compare the *Mtv-43 sag* sequence with that of *Mtv-7*. For this purpose, genomic DNA from the MA/MyJ strain was digested with PvuII, and DNA corresponding in size to the *Mtv-43* junction fragment was isolated from an agarose gel. Its specificity was tested in a Southern blot that was hybridized with an MMTV LTR probe (Fig. 6). The MMTV *sag* sequences were amplified by PCR from nucleotide 343 through the 3′ end, including 112 nucleotides of the 3′ untranslated region. The 5′ end was not obtained, because the comparison of other MMTV *sag* sequences indicates that they are all very homologous in this part of the gene (18a). The PCR products were cloned into M13mp18, and single-strand phage DNA was prepared from plaques that hybridized to an MMTV LTR probe. From the DNA shown in Fig. 6, lane *A*, which contained only the *Mtv-43* junction fragment, four independent full-length clones of identical nucleotide sequence were obtained. On the other hand, from the DNA shown in Fig. 6, lane *B*, which contained both the *Mtv-17* and the *Mtv-43* junction fragments, two independent full-length clones of different nucleotide sequence were obtained, one corresponding to the *Mtv-17 sag* sequence (Beutner, U., W. N. Frankel, M. S. Cole, J. M. Coffin, and B. T. Huber, unpublished data) (results not shown), and the other corresponding to the sequence of the former four clones. Thus, we conjectured that this latter DNA sequence is derived from the *Mtv-43 sag* gene. Fig. 7 shows its nucleotide

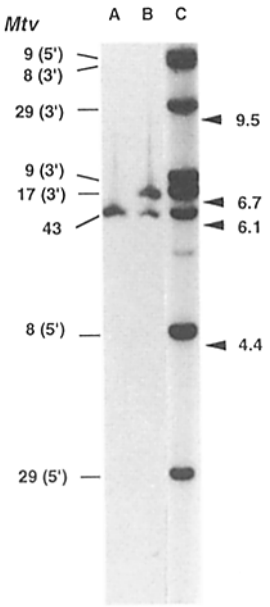

Figure 6. MMTV specificity of isolated DNA used as PCR template. PvuII-digested genomic DNA from MA/MyJ mice was run on a low melt agarose gel, the DNA corresponding in size to the *Mtv-43* junction fragment was isolated, and part of it was run again on an agarose gel (lanes *A* and *B*). PvuII-digested genomic DNA from MA/MyJ mice was run as a control (lane *C*). The gel was Southern blotted and hybridized with a ^{32}P-labeled MMTV LTR probe. The MMTV junction fragments are marked.

sequence, lined up with the corresponding *Mtv-7 sag* sequence (18a). The two genes are 97.8% homologous over a stretch of 688 nucleotides tested, indicating that they are closely related. To confirm the identity of the putative *Mtv-43 sag* sequence, two oligomers were synthesized spanning the nucleotide differences in the 3′ end of the *Mtv-7* and the *Mtv-43 sag* genes. Using them as probes revealed they that hybridized specifically to the respective junction fragments of *Mtv-7* and *Mtv-43* in AKR and MA/MyJ genomic DNA (Fig. 8).

The predicted amino acid sequence of the *Mtv-43 sag* gene was compared with that of other MMTV *sag* products (Fig. 9). Interestingly, the COOH terminus has a high degree of homology in the *Mtv-43 sag*– and the *Mtv-7 sag*–derived proteins. Over a stretch of 20 amino acids, there are only two substitutions that have a minor impact on the structure, as they represent conservative changes. This is very striking, because the COOH terminus of the *Mtv-7 sag*–encoded protein differs completely from that of all other MMTV *sag* gene products cloned and sequenced so far.

Discussion

The MA strain exhibited a high incidence of spontaneous mammary tumors (77% in breeding females and 64% in virgin females) and, thus, may have carried an infectious MMTV. The MA/MyJ strain, which is free of spontaneous carcinogenesis, was derived from a single MA female that did not develop mammary tumors (28). This is of special interest, because the MA/MyJ strain contains a new MMTV provirus in its genome, now designated *Mtv-43*, that is not present in other inbred strains (11). Most other endogenous MMTVs are shared between various inbred strains. The infectious MMTV of the MA strain could have integrated into the germline, resulting in transmission of a provirus in Mendelian

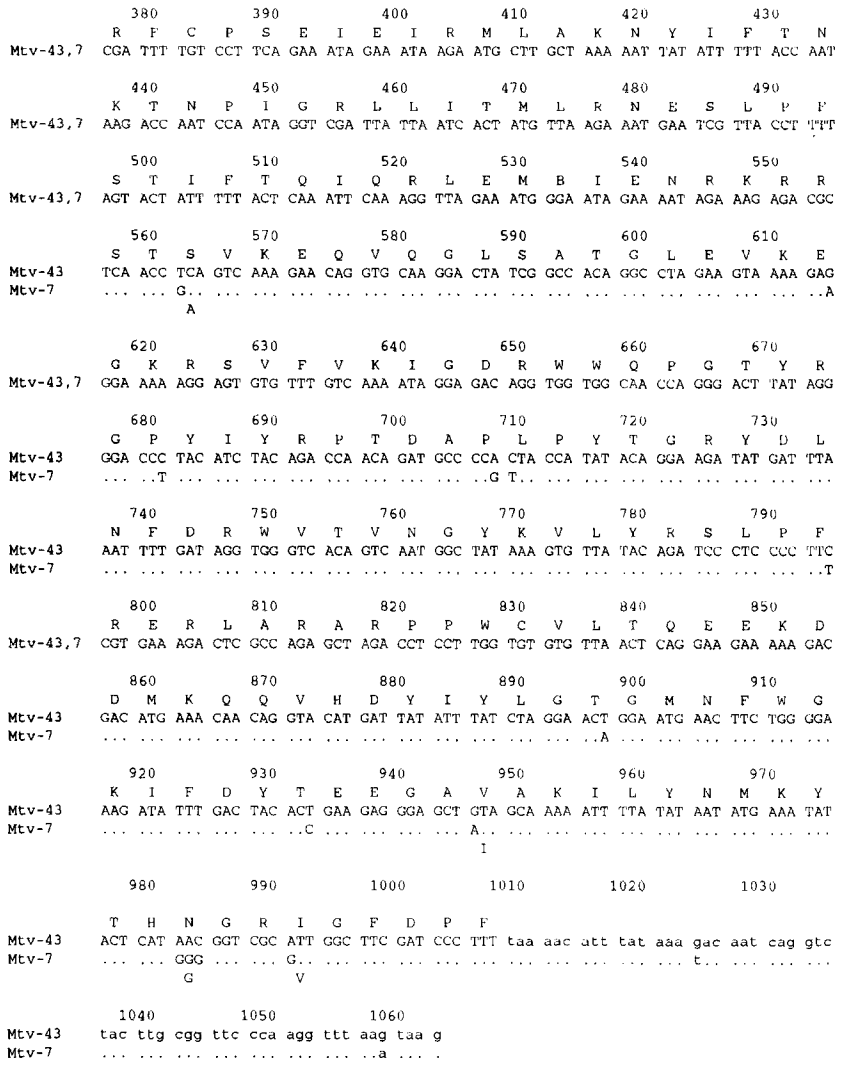

Figure 7. Nucleotide sequence of *Mtv-43 sag* (nucleotides 376–1063), compared with that of *Mtv-7 sag*. Identical nucleotides are represented by dots. The lowercase letters after position 1009 indicate the beginning of the 3′ untranslated region. The amino acids are shown above. These sequence data are available from EMBL/GenBank/DDBJ under accession number X64541.

fashion. Unfortunately, this hypothesis cannot be tested, because the original MA strain is no longer available. However, the fact that an infectious MMTV that has a very similar *sag* sequence to *Mtv-43 sag* was recently isolated supports this idea (20a). The implication of this finding is that the integrated provirus protects the animal from the infectious virus, probably by deleting the T cells able to respond to the virus (29). This is plausible because it has been shown that only T cells are able to transfer the milk-borne infectious virus from its primary residence in the gut to the mammary tissue (30).

The striking finding is that the TCR Vβ deletion pattern in the mature T cell repertoire of the MA/MyJ mouse is identical to that of Mls-1 strains; i.e., Vβ6-, 7-, 8.1-, and 9-expressing CD4$^+$ T cells are significantly reduced in MA/MyJ and virtually absent in Mls-1 mice. This deletion pattern is seen in CD8$^+$ T cells as well, but is less pronounced in the MA/MyJ mouse than in the Mls-1-positive AKR mouse. A difference is also noted in the time point when the stimulatory activity of these two SAG can be functionally detected during ontogeny. The MA/MyJ-derived SAG appears much later in life than Mls-1. The disparate penetration of the two SAG phenotypes could be due to differences in the level of expression, controlled partially by cellular flanking sequences. Alternatively, differences in the coding sequences of the two MMTV *sag* genes could lead to two products that differ slightly in their affinity for the TCR Vβ (see below). A similar delay in expression has been noted for the SAG encoded by infectious MMTV (14, 16) (20a), but this may be due to the time needed for the accumulation of infectious virus in the relevant lymphocyte populations.

The TCR Vβ deletion in vivo is a much more sensitive readout of SAG expression than in vitro stimulation of T cells expressing the particular Vβ chains. In fact, many SAG do not stimulate primary T cells at all, although they will stimulate selected T cell hybrids expressing the relevant TCR Vβ chains (31). We have made a similar observation with the *Mtv-43*-encoded SAG, which leads to efficient deletion of Vβ6 CD4$^+$ T cells in vivo, but has limited in vitro stimulatory capacity (Fig. 1). This latter function can only reliably be measured by using LPS-induced B cell blasts, a

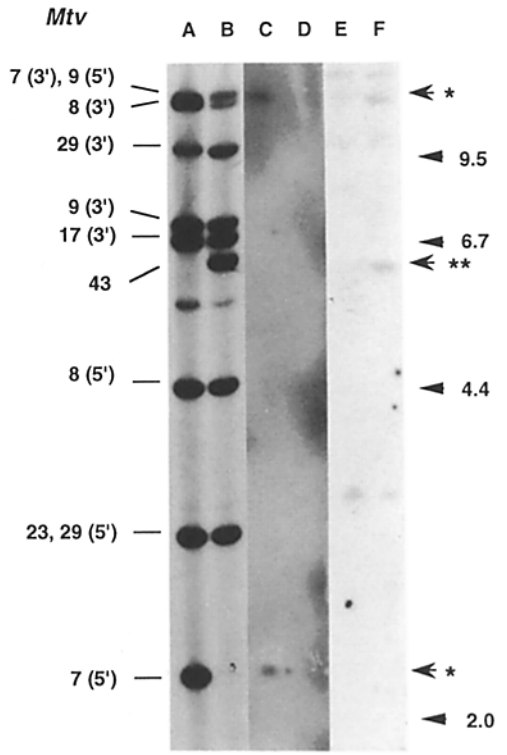

Figure 8. Combination of *Mtv-43 sag* 3′ nucleotide sequence. PvuII-digested DNA from AKR (lanes *A*, *C*, and *E*) and MA/MyJ (lanes *B*, *D*, and *F*) was subjected to electrophoresis and either blotted and hybridized to a ^{32}P-labeled MMTV LTR probe (lanes *A* and *B*), or dried and hybridized to a ^{32}P-labeled *Mtv-7*-specific oligomer (lanes *C* and *D*) or an *Mtv-43*-specific oligomer (lanes *E* and *F*). The junction fragments of the various MMTVs are marked, and molecular mass standards are shown on the right. The relevant bands in lanes *C* and *F*, respectively, are marked (**Mtv-7* junction fragments in *C*; ***Mtv-43* junction fragment in *F*).

```
          101                                                 150
    Mtv 7  GPQPTSSYKP HRFCPSEIEI RMLAKNYIFT NKTNPIGRLL ITMLRNESLP
    Mtv 43 .......... .......... .......... .......... ..........
    Mtv 1  .......... ..L....... .......... .E........ .M.......S
    Mtv 8  .......... ..L....... .......... .......... .M.......S
    Mtv 9  .......... ...Y...... .......... .......... .I........
MMTV C3H   .......... .......... .......... .......... V.........S
MMTV BR 6  .......... .QP....... .......... .......... V.........
MMTV GR    .......... ..I..L.... .....K.... .......... V.........S

          151                                                 200
    Mtv 7  FSTIFTQIQR LEMGIENRKR RSTAVKEQVQ GLSATGLEVK EGKRSVFVKI
    Mtv 43 .......... .......... ...S...... .......... ..........
    Mtv 1  .......... .......... ...S.E.... ..R.S..... R....AL...
    Mtv 8  .......... .......... ...S.E.... ..R.S..... R....TL...
    Mtv 9  .......... .......... .......... .......... K.........
MMTV C3H   .........K .......... ...SIE.... ..LT...... K..K......
MMTV BR 6  .......... .......... H..S.E.... ..R.S..... R....AL...
MMTV GR    .........K .......... ..KSIE.... ..L.S..... K..K......

          201                                                 250
    Mtv 7  GDRWWQPGTY RGPYIYRPTD APLPYTGRYD LNFDRWVTVN GYKVLYRSLP
    Mtv 43 .......... .......... .......... .......... ..........
    Mtv 1  .......... .......... .......... .......... ..........
    Mtv 8  .......... .......... .......... .......... ..........
    Mtv 9  .......... .......... .......... ...N...... ..........
MMTV C3H   ......L... .......... .......... ..W....... ..........
MMTV BR 6  .......... .......... .......... .......... .........S
MMTV GR    .......R.. .......... .......... ..W.....I. ..........

          251                                                 299
    Mtv 7  FRERLARARP PWCVLTQEEK DDMKQQVHDY IYLGTGM-NF WGKIFDYTEE
    Mtv 43 .......... .......... .......... .......-.. ..........
    Mtv 1  .......... .....S.... .......... ........IH .-.V.YNSR.
    Mtv 8  .......... .......... ..I....... ........V. .....H..K.
    Mtv 9  .......... .......... .......... ........V. .....H..K.
MMTV C3H   .......... ...M.S.... .......... ........H. .....H-.K.
MMTV BR 6  .......... ...M...... N......... .......SSI .....H-.K.
MMTV GR    .......... ...M..EK.. .......... ........H. ...V.H-.K.

          300                  323
    Mtv 7  GAIAKILYNM KYTHGGRVGF DPF*
    Mtv 43 ..V....... ....N..I.. ...*
    Mtv 1  E.KRHIIEHI .ALP*
    Mtv 8  ..V.RQ.EHI SADTF.MSYN G*
    Mtv 9  ..V.RL.EHI SADTF.MSYN G*
MMTV C3H   .TV.GLIEHY SPKTY.MSYY E*
MMTV BR 6  RTV.ALIEHY SAKTY.MSYY D*
MMTV GR    ....GLIEHY SAKTY.MSYY D*
```

Figure 9. Comparison of the predicted amino acid sequence *Mtv-43 sag* (residues 112–323) with that of nine other MMTV *sag* products. Identical amino acids are represented by dots, and gaps are represented by dashes.

treatment that has been demonstrated to increase the T cell stimulatory capacity of endogenous SAG (10). We have screened a panel of Vβ6 and Vβ8.1 T cell hybrids, most of which were not stimulated by MA/MyJ-derived B cells, although they all reacted to Mls-1 (results not shown). This is consistent with the partial deletion of Vβ6 and Vβ8.1 T cells seen in MA/MyJ mice in vivo.

Since the TCR Vβ deletion pattern of Mls-1 and the *Mtv-43*-encoded SAG is remarkably similar, it was of interest to determine the nucleotide sequence of *Mtv-43 sag* and compare its predicted amino acid sequence with that of *Mtv-7 sag*. The finding that the two genes are virtually identical offers an explanation for their functional similarity. At the COOH terminus, only 2 of 20 residues are different, representing conservative changes and, thus, probably have a limited influence on the secondary structure. However, as mentioned above, the two SAG, although very similar, differ slightly in their antigenic strength. It is possible that this is caused by these conservative amino acid changes.

As can be seen in Fig. 9, most of the heterogeneity in the predicted MMTV *sag* sequences occurs at the COOH terminus. The COOH-terminal sequence shared by *Mtv-7* and *Mtv-43 sag* products is completely different from other infectious and endogenous MMTV *sag* sequences published. The shared Vβ deletion pattern and 3′ sequences provide a strong indication that the Vβ specificity of SAG molecules is determined by the COOH terminus. The fact that an infectious MMTV has been isolated that is very similar to the two MMTV proviruses *Mtv-7* and *Mtv-43* indicates a common origin of these three viruses (20a).

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Address correspondence to Brigitte T. Huber, Department of Pathology, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111.

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Note added in proof: The 3′ and 5′ designation for the *Mtv-9* junction fragments has been reversed in Figs. 4, 6, and 8.

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