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Tissue Distribution of Mouse Mammary Tumor Virus (MMTV) Antigens and New Endogenous MMTV Loci in Japanese Laboratory Mouse Strains

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The distribution of mouse mammary tumor virus (MMTV) antigens was studied by the immunoperoxidase method in the II-TES and I-TES mouse strains as well as their progenitors, CS and DBA/2 strains. In the II-TES, I-TES and CS strains, and BALB/c mice foster-nursed with these strains, MMTV antigens were found not only in epithelial cells of the mammary glands but also in those of other tissues including the seminal vesicle, vas deferens, epididymis, prostate, parotid, submandibular, lacrimal, sebaceous, and urethral glands. In DBA/2 and BALB/cfDBA/2 mice, however, the MMTV antigens were found only in the mammary glands. Electron microscopic examination showed MMTV particles in these organs. When we examined the presence of *Mtv*-1 and 2 proviruses, which are known to be responsible for MMTV expression, in the genomes of the II-TES, I-TES, CS and DBA/2 strains by Southern blotting, *Mtv*-2 was not found in any of the mice and *Mtv*-1 was found in the II-TES and DBA/2 mice but not in the I-TES and CS mice. Instead, four new endogenous MMTV loci, which have never previously been reported in laboratory mouse strains, were detected in the genomes of the II-TES, I-TES and CS strains. One (designated *Mtv*-42) was common in the three strains and the other three (designated *Mtv*-43, 44 and 45) were common in the II-TES and I-TES strains or the II-TES and CS strains. These results thus suggest that new endogenous MMTV loci may be responsible for MMTV expression in a variety of tissues of these three strains.

Key words: Mouse mammary tumor virus — Endogenous *Mtv* loci — II-TES strain — I-TES strain — CS strain

The mouse mammary tumor virus (MMTV)[2] is a retrovirus which induces mammary tumors at high incidence in female mice of susceptible strains.[1-4] The genomes of all laboratory and wild mice examined contain one or more proviruses of MMTV.[5,6] The endogenous MMTVs are present in strains which do not produce infectious viruses and do not develop mammary tumors (e.g. BALB/c strain). Both genetic and biochemical studies have shown that three endogenous MMTV loci, *Mtv*-1 in the C3H and DBA/2 strains, *Mtv*-2 in the GR strain and *Mtv*-4 in the SHN strain, actively produce infectious MMTVs in milk and are responsible for mammary tumor development in susceptible strains,[5] although the *Mtv*-4 locus has not been identified in the genome of the SHN strain. In exogenous MMTV-free but endogenous MMTV-carrying mice, MMTV antigens and virus particles were detected in non-mammary tissues as well as in the mammary glands, suggesting that endogenous MMTVs are responsible for MMTV expression in a variety of organs.[7] On the other hand, in BALB/cfC3H mice carrying only exogenous MMTV, the expression of MMTV antigens was detected exclusively in the mammary glands by the immunoperoxidase method.[8,9] However, Bentvelzen and Brinkhof[10] reported that MMTV activity was exhibited by cell-free extracts from the salivary gland, kidney, testis and epididymis of BALB/cfC3H mice.

The II-TES and I-TES mouse strains were established by cross-breeding of the DBA/2 strain with the CS and NBC strains and the CS and SII strains, respectively (Fig. 1).[11-13] The latter three strains were derived from Japanese pet mice. II-TES, I-TES and CS mice have low mammary tumor incidence in our laboratory, while there is no information on its incidence in NBC and SII mice. Recently, we demonstrated that II-TES mice released large amounts of virulent MMTVs in milk despite their low mammary tumor incidence.[11] Genetic analysis suggested that II-TES mice carry two infectious endogenous MMTVs and a recessive gene conferring resistance to mammary tumorigenesis but not to release of MMTV in milk.[12]

In the present study, we investigated endogenous MMTVs and the distribution of MMTV antigen expression in the II-TES, I-TES and CS strains as well as in BALB/c mice foster-nursed by these strains. Southern

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[2] The abbreviations used are: MMTV, mouse mammary tumor virus; PAP, peroxidase anti-peroxidase, PBS; phosphate-buffered saline.

blot analysis suggested the presence of new endogenous MMTV loci in the genome of each strain.

MATERIALS AND METHODS

Mice Mice of the II-TES, I-TES, CS, DBA/2, AKR and BALB/c strains were provided by the Institute for Laboratory Animal Research, Nagoya University School of Medicine. GR mice were obtained from the Institute of Medical Science, University of Tokyo. The II-TES and I-TES strains[13, 14] were established in 1961 by cross-breeding of the DBA/2 strain with strains of Japanese pet mouse origin: NBC, S-II and CS (Fig. 1). The CS strain was established by cross-breeding of the S-II strain with the NBC strain.[13, 14] For foster-nursing, babies were collected by cesarian section and kept with foster mothers until 30 days old.

Antisera Goat antisera against the envelope (gp52) and core (p28) proteins of MMTV of RIII mice were provided by the Biological Carcinogenesis Branch, Division of Cancer Cause and Prevention, National Cancer Institute (Bethesda, USA). These polyclonal antibodies reacted with gp52 or p28 MMTV antigen expressed in the mammary gland of several mouse strains such as C3H, GR, DBA/2 and RIII strains. The secondary antibodies, swine anti-goat IgG, and rabbit peroxidase-anti-peroxidase complex were purchased from Toga, Inc. (Burlingame, USA) and Dakopatts (Copenhagen, Denmark), respectively. The optimum dilution of these antibodies was obtained with phosphate-buffered saline (PBS) containing 5% normal swine serum.

Immunohistochemical detection of MMTV antigens Tissues of mice were fixed in either 10% neutral buffered formalin or Carnoy's solution and embedded in paraffin. Paraffin blocks were cut and sections were mounted on glass slides treated with chrom alum gel. The MMTV antigens in paraffin-embedded tissues were demonstrated by a peroxidase anti-peroxidase (PAP) method. Paraffin sections were dewaxed and endogenous peroxidase activity was blocked by incubation for 5 min in 3% hydrogen peroxide aqueous solution. After washing with PBS, the sections were incubated in 5% normal swine serum at 37°C for 15 min to reduce nonspecific binding. After pouring off the excess normal swine serum, the slides were covered with the primary antibody for 30 min at 37°C, washed in PBS and incubated with the secondary antibody for 30 min at 37°C. After washing with PBS, the slides were covered with PAP solution for 30 min at 37°C, washed with PBS and stained with diaminobenzidine solution for 10–15 min at room temperature. Finally, after washing with PBS, the sections were counter-stained with hematoxylin, dehydrated and mounted.

The first appearance of gp52 MMTV antigen expression in virgin mice Virgin males and females of II-TES, II-TESfBALB/c and BALB/cfII-TES mice were killed every week from the age of 6 weeks. Tissues were kept for immunohistochemical examination of gp52 MMTV antigen expression.

Transfer of exogenous MMTV by spleen cells Spleen cells of 3-month-old BALB/c mice foster-nursed by II-TES, I-TES or CS mice were suspended in 3 ml of Hanks balanced solution. Two-month-old male and female BALB/c mice were injected intraperitoneally with 1 ml of the spleen cell suspension. The injected female mice were hormonally stimulated by mating with the injected male mice.

Electron microscopy Small tissue blocks were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.05 *M* phosphate buffer, post-fixed in 2% osmium tetroxide in 0.05 *M* phosphate buffer and embedded in Epon. Ultrathin sections were cut with an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a Hitachi 600 electron microscope at 100 kV.

Southern blotting DNAs were extracted from liver or spleen and completely digested with appropriate restriction endonucleases. The DNA samples were separated by electrophoresis in 0.5% agarose gels, transferred to a Gene Screen Plus filter (New England Nuclear Corp., Boston, USA) and hybridized with ^{32}P-labeled probe specific for a gag-pol (3.2 kb *Pst*I-*Eco*RI fragment), or an envelope (1.5 kb *Pst*I-*Bal*II fragment) or an LTR (1.5 kb *Bal*II-*Pvu*II fragment) sequence[15] (Fig. 4A), provided by Dr. A. Murakami (Institute for Virus Research, Kyoto University, Kyoto). Prehybridization, hybridization and washing were performed under the conditions recommended by New England Nuclear Corp.

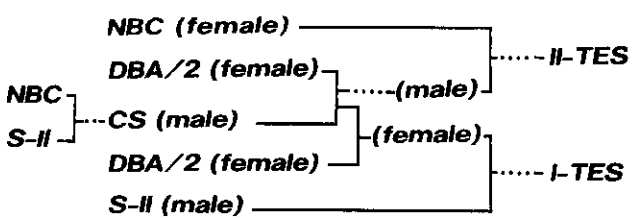

Fig. 1. History of the II-TES and I-TES strains.

RESULTS

Distribution of MMTV antigens Expression of MMTV antigens (gp52 and p28) was examined in adult mice of the II-TES, I-TES and CS strains by the immunoperoxidase method. As shown in Table I, both antigens were detected not only in the mammary glands but also in various exocrine glands and tissues of these strains. In the salivary glands, the MMTV antigens were detected

Table I. Distribution of MMTV Antigens (gp52 and p28) Detected by the PAP Method in 8- to 12-Month-old Mice

Organ	Mouse strain						
	I-TES	II-TES	CS	BALB/cf I-TES	BALB/cf II-TES	BALB/cf CS	DBA/2
Mammary gland	+	+	+	+	+	+	+
Lacrimal gland	+	+	+	+	+	+	−
Parotid gland	+	+	+	+	+	+	−
Submandibular gland	+	+	+	+	+	+	−
Prostate gland	+	+	+	+	+	+	−
Seminal vesicle	+	+	+	+	+	+	−
Epididymis	+	+	+	+	+	+	−
Vas deferens	+	+	+	+	+	+	−
Urethral gland	+	+	+	+	+	+	−
Sebaceous gland	+	+	+	+	+	+	−

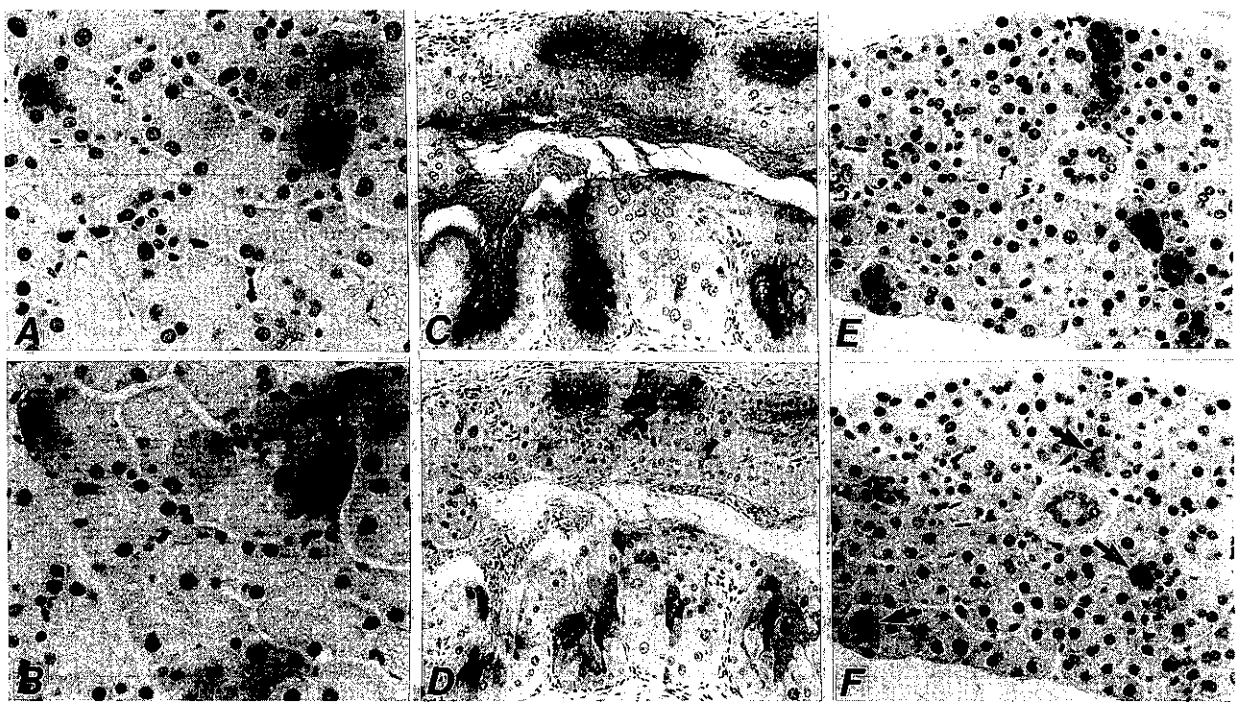

Fig. 2. Expression of MMTV antigens in II-TES mice. The lacrimal gland (A and B), vas deferens (C and D) and parotid gland (E and F) were stained with anti gp52 (A, C and E) or anti p28 (B, D and F) antibody by the PAP method. Both antigens were detected on the apical surface of some ductal or acinar cells and also in their cytoplasms. Arrows in F indicate positive staining.

only in serous cells of the parotid and submandibular glands (Fig. 2E and 2F). Among excretory glands of the eyes, the antigens were found in the lacrimal glands (Fig. 2A and 2B) but not in the Harderian and Meibomian glands. The antigens were also detected in the sebaceous glands but not in the sweat glands of the skin. Furthermore, the vas deferens (Fig. 2C and 2D), epididymis, ampulla, ampulla gland, seminal vesicles, coagulation, prostate and uretheral glands were antigen-positive tissues. The positive reaction for gp52 and p28 antigens was found on the apical surface of acinar or ductal cells of these organs and in their cytoplasms (Fig. 2) except the sebaceous glands, in which both antigens were seen only in the cytoplasm (data not shown). On the other hand, no antigen was found in the pancreas, ovary, testis, tongue, bulbourethra, prepuce, vulval and uterine glands.

The tissue distribution of the MMTV antigens in BALB/c mice foster-nursed by the three strains was almost the same as that in foster mothers (Table I). In contrast, in DBA/2 and BALB/cfDBA/2 mice the antigens were found only in the lactating mammary glands.

The first appearance of gp52 MMTV antigen expression in virgin mice In II-TES, II-TESfBALB/c and BALB/cfII-TES virgin male mice, appearance of MMTV expression was first detected in the vas deferens and lacrimal glands at the age of 7 weeks (data not shown). In virgin female mice, the lacrimal glands were the first organ with detectable MMTV expression, as early as at 7 weeks of age. Expression of MMTV antigens in the mammary glands was seen in 11-week-old females (data not shown).

Detection of virus particles by electron microscopy Antigen-positive organs in BALB/cfII-TES and BALB/cfCS mice were studied by electron microscopy. In the sebaceous glands, A particles were observed in the cytoplasm (Fig. 3A), but neither budding nor B particles could be detected. Clusters of A particles were seen in undifferentiated peripheral cells and partially differentiated sebaceous cells. In other virus antigen-positive organs, numerous virus particles were observed, including mature B particles, the budding form on the surface of the luminal border and intracytoplasmic A particles (Fig. 3B and 3C). In addition, A particles were sometimes found around the microvesicular bodies, especially in ductal cells of the vas deferens (Fig. 3C).

Transfer of exogenous MMTV by spleen cells To investigate whether spleen cells of II-TES mice can transfer infectious MMTV to mice of other strains, 2-month-old BALB/c mice were intraperitoneally injected with spleen cells from BALB/cfII-TES mice. The expression of MMTV antigens was examined at 6 months after inoculation by the immunoperoxidase method. The tissue distribution of MMTV antigens in these mice was similar to that in II-TES and BALB/cfII-TES mice (data not shown). Both gp52 and p28 antigens were found on the apical surface of acinar or ductal cells of antigen-positive organs. In the BALB/c mice inoculated with the spleen cell suspension from BALB/cfI-TES and BALB/cfCS mice, the gp52 MMTV antigen was detected in the lacrimal glands and vas deferens, although this examination was carried out only at 10 weeks after injection.

Endogenous MMTV loci in II-TES, I-TES and CS strains To compare endogenous MMTVs in the II-TES, I-TES and CS strains with those in the GR, DBA/2, AKR and BALB/c strains, DNA from each mouse was hybridized with 5′ *gag-pol* and 3′ *env* probes of MMTV (Fig. 4A). The nomenclature and numbering system for loci of endogenous MMTVs given here are based on the pro-

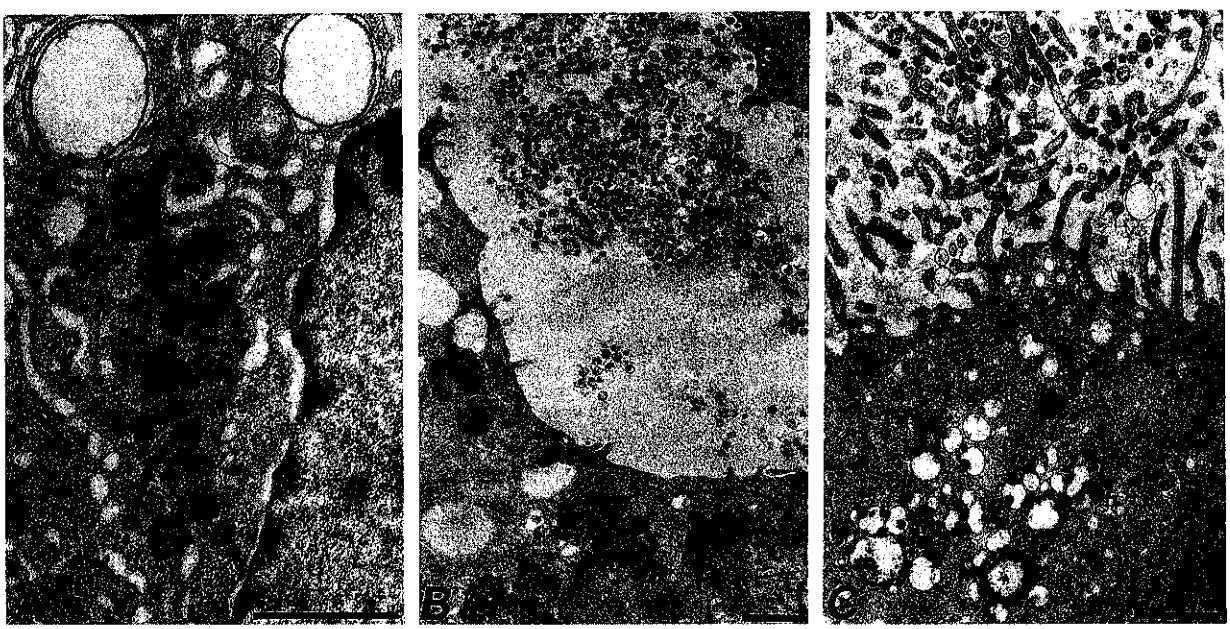

Fig. 3. Electron micrographs of MMTV antigen-positive organs in BALB/cfCS and BALB/cfII-TES mice. A, a cell of the sebaceous gland of a 6-month-old BALB/cfCS mouse. Clusters of A particles were observed in the cytoplasm. B, an acinar cell of the lacrimal gland of a 6-month-old BALB/cfII-TES mouse. There were many B particles in the lumen and clusters of A particles in the cytoplasm. C, a ductal cell of the vas deferens of a 6-month-old BALB/cfII-TES mouse. Mature B particles were seen in the lumen. A particles were found around multivesicular bodies and pinocytic vesicles of the cytoplasm. Bar, 1 μm.

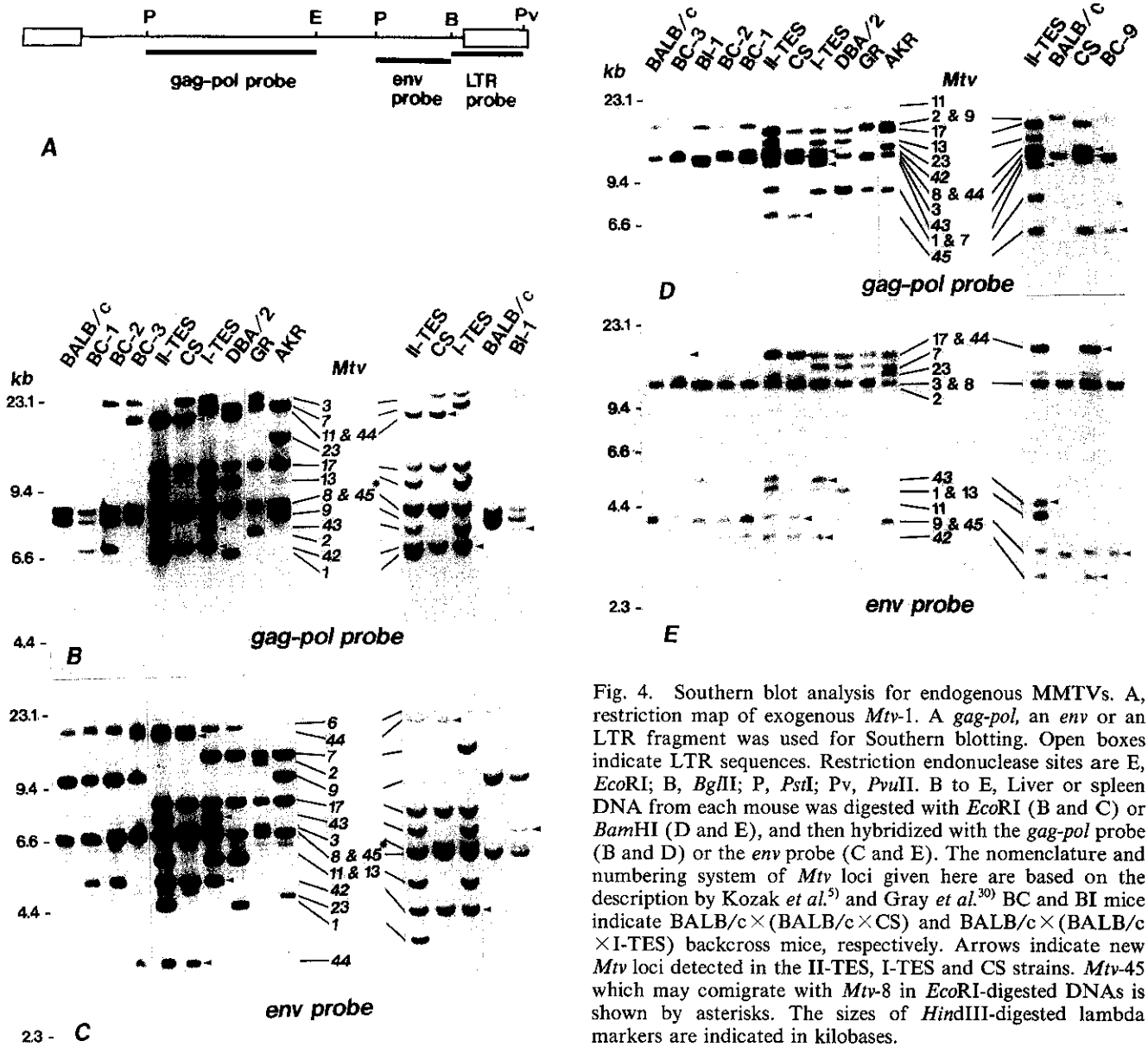

Fig. 4. Southern blot analysis for endogenous MMTVs. A, restriction map of exogenous *Mtv*-1. A *gag-pol*, an *env* or an LTR fragment was used for Southern blotting. Open boxes indicate LTR sequences. Restriction endonuclease sites are E, *Eco*RI; B, *Bgl*II; P, *Pst*I; Pv, *Pvu*II. B to E, Liver or spleen DNA from each mouse was digested with *Eco*RI (B and C) or *Bam*HI (D and E), and then hybridized with the *gag-pol* probe (B and D) or the *env* probe (C and E). The nomenclature and numbering system of *Mtv* loci given here are based on the description by Kozak *et al.*[5] and Gray *et al.*[30] BC and BI mice indicate BALB/c×(BALB/c×CS) and BALB/c×(BALB/c×I-TES) backcross mice, respectively. Arrows indicate new *Mtv* loci detected in the II-TES, I-TES and CS strains. *Mtv*-45 which may comigrate with *Mtv*-8 in *Eco*RI-digested DNAs is shown by asterisks. The sizes of *Hin*dIII-digested lambda markers are indicated in kilobases.

posal by Kozak *et al.*[5] The results are summarized in Tables II and III. When DNAs were digested with *Eco*RI, both probes detected the same sizes of bands in the II-TES mouse DNA as those corresponding to *Mtv*-1, 8, 13 and 17 proviruses (Fig. 4B and 4C, Table II). A fragment corresponding to *Mtv*-6 was detected only by *env* probe (Fig. 4C). Similarly, DNAs from I-TES and CS mice contained the bands corresponding to *Mtv*-3, 6, 7, 8, 13, and 17, and *Mtv*-3, 6, 8 and 17, respectively. These proviruses were also detected in *Bam*HI-digested DNAs (Fig. 4D and 4E), although we do not know which *Bam*HI fragment includes *Mtv*-6. In addition to these bands, a 6.7 kb 5′ *Eco*RI fragment and a 5.2 kb 3′ *Eco*RI fragment (designated *Mtv*-42) were observed in II-TES, and I-TES and CS mice, and a 6.9 kb 5′ *Eco*RI fragment and a 7.2 kb 3′ *Eco*RI fragment (designated *Mtv*-43) were seen in II-TES and I-TES mice (Fig. 4B and 4C, Table III). These fragments were not correlated to the bands determined by the standardized and provisional nomenclature for endogenous MMTV loci.[5] Furthermore, in II-TES and CS mice the *gag-pol* probe hybridized to a 15.0 kb *Eco*RI fragment which comigrated

Table II. Endogenous *Mtv* Loci Present in Mouse Strains

Strain	*Mtv* locus
AKR	7,[a] 8, 9, 17, 23
GR	2, 3, 7, 8, 14,[b] 17
DBA/2	1, 6, 7, 8, 11, 13, 14,[b] 17
BALB/c	6, 8, 9
II-TES	1, 6, 8, 13, 14,[b] 17, 42, 43, 44, 45
I-TES	3, 6, 7, 8, 13, 14,[b] 17, 42, 43
CS	3, 6, 8, 17, 42, 44, 45
BC-1[c]	6, 8, 9, 42
BC-2[c]	3, 6, 8, 9, 42
BC-3[c]	3, 6, 8, 9, 44
BC-9[c]	3, 6, 8, 9, 45
BI-1[d]	3, 6, 8, 9, 14,[b] 43

a) *Mtv*-7 of AKR mice was previously designated as *Mtv*-22.[17]
b) *Mtv*-14 was detected only by an LTR probe.
c) BC, BALB/c×(BALB/c×CS) backcross mouse.
d) BI, BALB/c×(BALB/c×I-TES) backcross mouse.

Table III. Restriction Fragments of New *Mtv* Loci Detected in II-TES, I-TES and CS Strains

Mtv locus	*Eco*RI fragment (kb)		*Bam*HI fragment (kb)	
	5′	3′	5′	3′
42	6.7	5.2	13.7	3.5
43	6.9	7.2	12.0	5.2
44	15.0	3.4&15.0	13.2	16.0
45	ND	ND	7.0	3.8

ND, Not determined. kb, kilobases.

with *Mtv*-11 found in DNA of DBA/2 mice (Fig. 4B). However, when DNAs were digested with *Bam*HI, no *Mtv*-11 band was observed in these two strains (Fig. 4D and 4E), suggesting that the 15.0 kb 5′ *Eco*RI fragment could represent a new endogenous MMTV locus. In agreement with this result, the *env* probe detected unique 3.4 and 15.0 kb *Eco*RI fragments in the two strains (designated *Mtv*-44 in Fig. 4C). The three new *Mtv* loci detected in the II-TES, I-TES and/or CS strains were also different from *Mtv*-27~38 which have recently been described.[16-19]

BALB/c×(BALB/c×CS) backcross mice (BC) were used to study the cosegregation of these newly identified endogenous MMTV loci. BC-1 and BC-2 mouse DNAs contained *Mtv*-6, 8, 9 and 42 and *Mtv*-3, 6, 8, 9 and 42, respectively (Fig. 4B and 4C, Table II). *Mtv*-42 locus was found concordantly with 13.7 kb 5′ and 3.5 kb 3′ *Bam*HI fragments (Fig. 4D and 4E). BC-3 mouse carried a 15.0 kb 5′ *Eco*RI fragment and 3.4 kb and 15.0 kb 3′ *Eco*RI fragments besides *Mtv*-3, 6, 8 and 9 (Fig. 4B and 4C). The three new *Eco*RI fragments detected in the BC-3 mouse also cosegregated in all of 15 other backcross mice examined (data not shown), indicating that these fragments represent the same endogenous MMTV locus (*Mtv*-44). This locus appears to correspond to 13.2 kb 5′ and 16.0 kb 3′ *Bam*HI fragments (Fig. 4D and 4E). In addition, the fact that the *env* probe detected the 3.4 and 15.0 kb fragments indicated that a single *Eco*RI site is present in a portion of the *env* region of *Mtv*-44. We further examined a BALB/c × (BALB/c × I-TES) mouse (designated BI-1). DNA from this mouse contained *Mtv*-3, 6, 8, 9 and 43 (Fig. 4B and 4C). As shown in Fig. 4D and 4E, *Mtv*-43 produced 12.0 kb 5′ and 5.2 kb 3′ *Bam*HI fragments in DNAs from II-TES, I-TES and BI-1 mice.

When DNAs were digested with *Bam*HI, a new 7.0 kb 5′ band (designated *Mtv*-45 in Fig. 4D) was found in DNAs from II-TES and CS mice. This locus is different from *Mtv*-44 because the 7.0 kb 5′ *Bam*HI fragment was absent in the BC-3 mouse. Instead, BC-9 backcross mouse DNA was found to contain this *Bam*HI fragment (Fig. 4D). In addition, the *env* probe detected a common 3.8 kb *Bam*HI fragment which comigrated with *Mtv*-9 in II-TES, CS and BC-9 mice (Fig. 4E). Since *Mtv*-9 is absent in II-TES and CS mice (Fig. 4B and 4C), these *Bam*HI fragments might represent another new endogenous MMTV locus (*Mtv*-45). In *Eco*RI-digested DNAs, the intensity of the bands coresponding to *Mtv*-8 in II-TES and CS mice appears to be stronger than that of the bands of other endogenous MMTV loci. Thus these *Eco*RI bands may contain *Mtv*-45 besides *Mtv*-8 (Fig. 4B and 4C).

To investigate the presence of LTR sequences in endogenous MMTVs, the filters were rehybridized with an LTR probe (Fig. 4A). This probe detected all bands which were hybridized with the *gag-pol* and *env* probes (data not shown), suggesting that the four new endogenous MMTVs identified here contain full viral genomes. In addition, *Mtv*-14 was found in GR, DBA/2, II-TES, I-TES and BI-1 mice (Table II).

DISCUSSION

In II-TES, I-TES and CS mice, MMTV antigens were detected in epithelial cells of various excretory glandular organs of both sexes and of male accessory sex organs as found in mice of other strains carrying active endogenous MMTVs.[5,7] In contrast, the MMTV antigens were observed only in the mammary glands of DBA/2 mice. Our study on MMTV proviruses showed that *Mtv*-1, one of the active endogenous MMTVs, is present in the II-TES and DBA/2 strains but not in the I-TES and CS strains. *Mtv*-2, another active provirus, was not detected in any of them. It seems unlikely that *Mtv*-1 is responsible for MMTV expression in various tissues of II-TES mice,

because the distribution of MMTV antigens was different between II-TES and DBA/2 mice. Instead, II-TES, I-TES and CS mice contained one common endogenous MMTV (*Mtv*-42) which has not previously been reported in the standardized nomenclature for endogenous MMTVs.[5] It is conceivable that the MMTV provirus expressed in II-TES and I-TES mice is derived from one of the active endogenous MMTVs in CS mice, a progenitor of II-TES and I-TES mice. Thus, *Mtv*-42 could be a good candidate for an active provirus in the three strains. In addition, we identified three other new endogenous MMTV loci (*Mtv*-43, 44 and 45) which are present in two of the three strains and may be expressed as infectious viruses.

II-TES, I-TES and CS mice and BALB/c mice foster-nursed by these mice expressed the MMTV antigens (gp52 and p28) in the sebaceous glands but not in the sweat glands (Table I). Conversely, both antigens were positive in the sweat glands of the GR and SHN strains carrying *Mtv*-2 and *Mtv*-4, respectively.[8] In the sebaceous glands of these two strains, only p28 antigen was found. In addition, the antigens were not detected in either of these glands of BALB/cfC3H, BALB/cfGR and BALB/cfSHN mice.[8] These results also suggested that the active endogenous MMTV in II-TES, I-TES and CS mice represents a new locus which is not related to *Mtv*-1, *Mtv*-2 or *Mtv*-4.

Tsubura *et al.*[20] demonstrated that *Mtv*-2 controlled the first appearance of endogenous MMTV expression in the mammary glands of virgin female GR mice in which the MMTV antigen (gp52) appeared very early in life (14 days). On the other hand, *Mtv*-1 gene was related to the antigen expression in the late period of life (200–240 days). However, the exogenous MMTVs of C3H mice took 65–80 days and the exogenous MMTVs of GR mice took 140 days for the first expression of the antigens in the mammary glands.[20] The endogenous and exogenous MMTVs of II-TES mice expressed their antigens in the mammary glands early in life (11 weeks), as did the endogenous and exogenous MMTVs of SHN mice (65 days).[20] Our results confirm the previous finding[20] that the variation in the time of the first appearance of MMTV expression depends on active endogenous MMTVs. It has been widely accepted that MMTVs are variable in respect of biological activity, immunological properties and morphology.[21–23]

In BALB/cfC3H mice, exogenous MMTVs from *Mtv*-1 can infect lymphoid cells, both T and B cells, and be integrated into their DNAs. Subsequently, viruses are transferred to the mammary glands by the T cells.[24, 25] BALB/c mice inoculated with spleen cells from BALB/cfII-Tes mice showed the same tissue distribution of MMTV antigens as that of the donors, indicating that lymphoid cells are able to transfer the exogenous MMTVs from II-TES mice to both the mammary and extramammary glands of BALB/c mice.

The proviral MMTVs found in II-Tes and I-Tes mice were inherited from DBA/2 and CS mice. However, *Mtv*-43 in II-Tes and I-Tes mice was not observed in the progenitors. These unique fragments are not inherited from S-II and NBC mice because these mice are also the progenitors of CS mice (Fig. 1). This endogenous MMTV might be a result of infection in the germ line during an early stage of the cross-breeding program. It was reported that *Mtv*-26 (formerly *Mtv*-22) in B61. C-KH-84 mice is also an integrated MMTV in the germ line because neither of the parent strains (BALB/c and C57BL/6) carries this provirus.[26] This finding supports the idea that endogenous MMTV sequences were acquired by multiple, infrequent, and independent infection of the germ line.[27] The germ-line acquisition of ecotropic murine leukemia proviruses was also shown to occur at low frequency.[28, 29]

Endogenous MMTVs which are actively expressed in the mammary glands are believed to be responsible for mammary tumorigenesis.[5, 7] We previously reported that II-TES mice carry a recessive gene restricting mammary tumorigenesis but not inhibiting MMTV release in milk.[11, 12] MMTVs appearing in II-TES milk was found to be oncogenic when introduced into BALB/c mice by foster nursing.[12] However, since there is no record of mammary tumor in the II-TES, I-TES and CS strains in our breeding data, these results suggest that these mouse strains might also share a gene conferring resistance to mammary tumor development.[11, 12]

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