JOURNAL OF VIROLOGY, May 1994, p. 3437–3442
0022-538X/94/$04.00+0

Vol. 68, No. 5

Distribution of Mouse Mammary Tumor Virus in Asian Wild Mice

SHUNSUKE IMAI,[1] MASAAKI OKUMOTO,[2] MINEKO IWAI,[2] SATOMI HAGA,[3] NOBUKO MORI,[2] NOBUMOTO MIYASHITA,[4] KAZUO MORIWAKI,[4] JO HILGERS,[5] AND NURUL H. SARKAR[6]*

Department of Pathology,[1] and Department of Anatomy,[3] Nara Medical University, Kashihara, Nara-ken, Department of Technology and Applied Science, Osaka Prefecture University, Sakai-shi, Osaka,[2] and National Institute of Genetics, Mishima-shi, Shizuoka-ken,[4] Japan; Research Laboratory for Oncological Gynecology, Department of Obstetrics and Gynecology, Academic Hospital, Free University, Amsterdam, The Netherlands[5]; and Department of Immunology and Microbiology, Medical College of Georgia, Augusta, Georgia[6]

Received 30 December 1992/Accepted 25 January 1994

Several groups of wild mice (*Mus musculus*) were captured from eight different locations in Asia and bred for several generations in a facility free of any laboratory strains of mice carrying mouse mammary tumor virus (MMTV). The distribution of endogenous MMTV proviral sequences in the liver tissues of these mice was investigated by using Southern blot hybridizations. Four categories of mice were identified. Mice originating from Bogor, Indonesia (Cas-Bgr); He-mei, Taiwan (Cas-Hmi/1); and Malaysia (Cas-Mal) were found to carry an endogenous MMTV provirus consisting of the *env*, *gag-pol*, and long terminal repeat sequences. Mice captured from Kojuri, Republic of Korea (Sub-Kjr); Nagoya, Japan (Mol-nag); and three Chinese provinces, Shanghai (Sub-Shh), Beijing (Sub-Bjn), and Jiayuguang (Sub-Jyg/1), appeared to carry defective proviruses. Some mice originating from He-mei (Cas-Hmi/2) and Jiayuguang (Sub-Jyg/2) were found to be completely free of endogenous MMTV. Interestingly, however, the Sub-Jyg/2 mice, after several generations of inbreeding, were found, unlike all of the other subspecies that we examined in the present study, to develop mammary tumors at a high incidence (80 to 90%) with a short period of latency. Electron microscopic examination of the mammary glands and mammary tumors of these mice revealed the presence of numerous intracytoplasmic A, immature, budding, and mature B particles. Furthermore, the mammary tumors were found to contain MMTV proviral sequences. It seems, therefore, that Sub-Jyg/2 mice carry an exogenous MMTV which contributes to their developing mammary tumors.

The use of laboratory mice has contributed to remarkable advances in understanding the molecular biology of retrovirus-induced carcinogenesis for the last decade. Several mouse strains, both classical and newly developed from wild mice, develop spontaneous mammary tumors. The underlying mechanism of mammary tumorigenesis in mice seems to involve the transcriptional activation by mouse mammary tumor virus (MMTV) of a family of oncogenes called *int* oncogenes, especially *int-1* (recently designated *Wnt-1* [21]) and *int-2* (7, 8, 20–22).

In most laboratory strains of mice, MMTV is transmitted exogenously via milk or saliva from mothers to their offspring (3, 17). In a few strains of mice, infectious MMTV has been found to be transmitted genetically; this type of virus is referred to as endogenous MMTV (3, 6, 10, 13). However, every laboratory mouse strain tested thus far has been shown to contain multiple copies of the MMTV genome in its normal cellular DNA (14). It is uncertain at present whether these endogenous MMTVs, although appearing not to be transcriptionally active, affect the responsiveness of mammary cells to infection by exogenous MMTV and thus affect mammary tumor development. To address this question properly and to study further the molecular basis of the variability in the frequency with which *int* oncogenes are activated in different strains of mice (15, 23), model experiments must be done with endogenous MMTV-free mice.

Some recent studies have examined the distribution of endogenous MMTV in several populations of wild mice from different geographical regions, namely, California, Morocco, Czechoslovakia, Denmark, and Spain. It has been found that whereas most wild mice contain endogenous MMTV, albeit in different copy numbers, some mice lack endogenous MMTV (5, 6, 9). To develop a mouse strain(s) free of endogenous MMTV, we analyzed 10 groups of mice originating from the wild in Japan, Indonesia, Taiwan, Malaysia, the Republic of Korea, and the People's Republic of China for the presence or absence of MMTV sequences and mammary tumor development. This work led us to the identification of two groups of mice, one from Taiwan (Cas-Hmi/2) and the other from the People's Republic of China (Sub-Jyg/2), that are free of endogenous MMTV. Interestingly, our results show that the Sub-Jyg/2 mice develop mammary tumors and carry exogenous MMTV.

Origin of feral mice. Mice have been classified as *Mus musculus molossinus*, *M. m. castaneus*, *M. musculus* (undetermined subspecies), and *M. m. domesticus* (following the taxonomic classification proposed by Yonekawa et al. [28]) on the basis of their genetic constitution. The origins of the 10 groups of Asian mice that we analyzed in the present study are summarized in Table 1. It should be emphasized that each group of mice was derived from individual pairs of founder animals. Initially, six to eight mice captured from each location were randomly paired, and each pair was kept in a separate cage for breeding. The litters obtained from each of the successful breeding pairs were maintained separately and used for subsequent breeding by brother-sister mating. All of the different groups of mice were inbred by Kazuo Moriwaki at the National Institute of Genetics (Mishima, Japan). The designations of these groups of mice include abbreviations for their place of origin and their genetic lineage, as established on the

* Corresponding author. Mailing address: Department of Immunology & Microbiology, Medical College of Georgia, CB-2208, 1120 15th St., Augusta, GA 30912-2400. Phone: (706) 721-7657. Fax: (706) 721-7507.

TABLE 1. Distribution of endogenous MMTV sequences in mouse strains descended from Asian wild mice

Subspecies	Strain[a]	Trapping location[b]	No. of mice examined[c]	Size(s) (kb) of *Eco*RI fragment(s) hybridized with indicated MMTV probe		
				env	LTR	*gag-pol*
M. m. molossinus	Mol-Nag	Nagoya, Japan	5 (F_{32})	1.0	None	None
M. m. castaneus	Cas-Bgr	Bogor, Indonesia	2 (F_{12})	7.9	7.9	7.9
			3 F_{12}	7.9, 28	7.9, 28	7.9, 28
			1 F_{12}	28	28	28
	Cas-Hmi/1	He-mei, Taiwan	2 (F_6)	7.9	7.9	7.9
			1 F_6	7.9	7.9, 28	7.9, 28
	Cas-Hmi/2	He-mei, Taiwan	2 (F_6)	None	None	None
	Cas-Mal	Malaysia	2 (F_4)	10.3	9.3, 10.3	9.3
			4 F_4	7.9, 10.3	7.9, 9.3, 10.3	7.9, 9.3
Unclassified	Sub-Kjr	Kojuri, Republic of Korea	5 (F_{13})	1.0	None	None
	Sub-Jyg/1	Jiayuguang, People's Republic of China	3 (F_{20})	None	None	4.1
	Sub-Jyg/2	Jiayuguang, People's Republic of China	11 (F_{20})	None	None	None
	Sub-Shh	Shanghai, People's Republic of China	5 (F_{11})	None	6.8, 9.3	None
	Sub-Bjn	Beijing, People's Republic of China	7 (F_{16})	1.0	6.8	None

[a] Mol, Cas, and Sub indicate *M. m. molossinus*, *M. m. castaneus*, and unidentified subspecies, respectively. The three letters after the hyphen abbreviate the names of the localities from which the mice were initially trapped.

[b] The years when the mice were trapped are as follows: Mol-Nag, 1972; Cas-Bgr, 1984; Cas-Hmi/1 and Cas-Hmi/2, 1986; Cas-Mal, 1987; sub-Kjr, 1984; Sub-Jyg/1 and Sub-Jyg/2, 1981; Sub-Shh, 1981; and Sub-Bjn, 1980.

[c] The generation of the mice used is indicated in parentheses.

basis of genetic constitution and biochemical marker profiles such as mitochondrial DNA (28). Thus, those mice that were captured from Japan, Indonesia, Taiwan, and Malaysia have been characterized as subspecies *M. m. molossinus* (Mol) and *M. m. castaneus* (Cas), whereas those mice that were captured in the Republic of Korea and the People's Republic of China have not yet been typed genetically and are designated Sub, to indicate simply that they are subspecies of *M. musculus*.

MMTV proviral genomes in the DNAs of feral mice. The distribution of MMTV proviral genomes in liver tissues obtained from 10 groups of mice, belonging primarily to the generations indicated in Table 1, was examined by Southern blot hybridization of *Eco*RI-digested genomic DNAs. Representative results of this analysis are shown in Fig. 1 and are summarized in Table 1. *Eco*RI digestion of the genomic DNAs of Cas-Bgr, Cas-Hmi/1, and Cas-Mal mice produced, in general, a 7.9-kb fragment that hybridized with MMTV long terminal repeat (LTR) (Fig. 1A), *env* (Fig. 1B), and *gag-pol* (Fig. 1C) probes. Digestion of the DNAs of Cas-Bgr and Cas-Hmi/1 mice indicated the presence of an additional larger fragment (28 kb in size; hereafter referred to as the 28-kb fragment). Interestingly, however, some Cas-Bgr mice lacked the 7.9-kb *Eco*RI fragment but had the 28-kb fragment (Table 1). Thus, the 7.9-kb band most likely represents one endogenous provirus, whereas the 28-kb band represents another provirus that most likely does not have an *Eco*RI site in its genome. This conclusion is consistent with the observation that both *gag-pol* and LTR probes hybridized with the 28-kb fragment. The heterogeneity in the pattern of MMTVs that we observed in the DNA of Cas-Bgr mice (Fig. 1 and Table 1) may be due to either of the following causes: (i) the founder pair of mice was heterozygous with respect to at least two proviruses located in two different chromosomes or (ii) the six animals examined were not littermates, although they were from the same generation. It should also be mentioned that the probability of residual heterozygosity in the 12th-generation animals is significant (7% [11]).

The pattern of MMTV proviral integration in Cas-Mal mice was found to be more complicated. For example, in this strain, a 10.3-kb band capable of hybridizing to LTR, *env*, and *gag-pol* probes was present in some mice, whereas in a set of samples from other Cas-Mal mice this band did not hybridize to the *gag-pol* probe (Fig. 1C and Table 1). The Asian mice were also found to fall into other categories in which endogenous MMTV proviruses were either completely absent or apparently defective. For example, only a 1.0-kb *env* fragment was found in the genomic DNA of Mol-Nag mice. Defective proviruses which hybridized only with the LTR probe, only with the *gag-pol* probe, or only with the *env* probe were found to be present in Sub-Shh, Sub-Kjr, and Mol-Nag mice, respectively. Sub-Bjn mice lacked endogenous *gag-pol* sequences. It should be noted that, by using the technique of liquid hybridization, one previous study detected the presence of MMTV-related sequences in two species of Asian mice, *Mus cervicolor* and *Mus careoli* (19), unrelated to those analyzed in the present study.

During the inbreeding of those mice that were captured from the Jiayuguang province of the People's Republic of China, two lines of mice (Sub-Jyg/1 and Sub Jyg/2, derived from a single breeding pair of founder mice) that either had or did not have endogenous MMTV, respectively, were found to be segregated after the eighth generation. This separation was demonstrated by using Southern blot hybridization to detect viral genomes in various organs of Sub-Jyg/1 and Sub-Jyg/2 mice. Some examples of our findings are presented in Fig. 2. Both male (lanes 1 to 3) and female (lanes 4 to 6) Sub-Jyg/1 mice (from the eighth generation) were found to carry part of an endogenous MMTV, namely, the 4.1-kb *gag-pol* fragment, in their livers (lanes 1 and 4), kidneys (lanes 3 and 6), and salivary glands (lanes 2 and 5), but were free of endogenous LTR or *env* sequences. DNA samples that were obtained from the offspring of these mice (up to the 20th generation) and were similarly analyzed also exhibited the presence of a 4.1-kb fragment (data not shown). By contrast, mice from several generations of the Sub-Jyg/2 subline that we examined were found to be completely devoid of MMTV *gag-pol*-hybridizing fragments in their livers (lanes 7 and 8), kidneys (lanes 9 and 11), salivary glands (lanes 12 and 13), and mammary glands (lane 14). Further, DNA samples from these tissues were also found not to contain MMTV LTR or *env* sequences. Our observations thus clearly indicate that, unlike Sub-Jyg/1 mice,

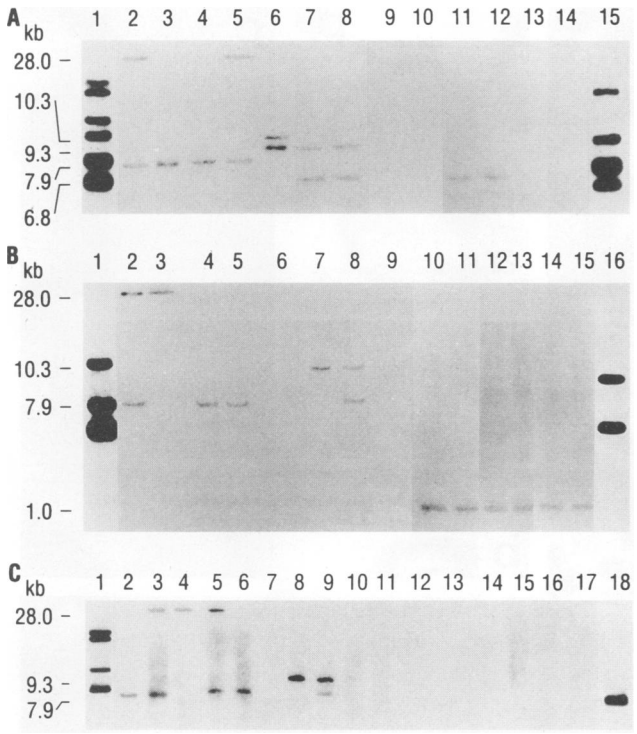

FIG. 1. Detection of MMTV sequences in the genomic DNAs of a number of mouse strains (Table 1) originating from Asian wild mice. Liver tissue samples were homogenized in TNE buffer (0.02 M Tris-HCl [pH 8.0], 0.1 M NaCl, 0.001 M EDTA), and incubated for 1 h at 37°C in the presence of 0.5% SDS and 100 μg of RNase A per ml. The extract was then incubated for 12 h or more after addition of proteinase K at a final concentration of 100 μg/ml, and DNA was extracted several times with a mixture of phenol and chloroform (10). Ten micrograms of DNA was digested with *Eco*RI, size separated by electrophoresis through a 0.7% agarose gel, and transferred to nitrocellulose filters by the procedure of Southern (26). The membranes were hybridized with MMTV LTR (A), *env* (B), and *gag-pol* (C) probes. The *gag-pol* (3.2-kb *Pst*I-*Bgl*II fragment), LTR (1.4-kb *Pst*I fragment), and *env* (1.6-kb *Pst*I-*Bgl*II fragment) DNAs were provided by C. Dickson, R. Nusse, and A. Murakami, respectively. All probes were labeled with [^{32}P]dCTP (3,000 Ci/mmol; Amersham) by nick translation (10). Hybridizations were done at 65°C for 18 h in a mixture of 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate), 20 mM Na_2PO_4 (pH 6.5), 0.01% bovine serum albumin, 0.1% Ficoll, 0.05% polyvinylpyrrolidone, 10% dextran sulfate, and 0.1 mg of calf thymus DNA per ml. After hybridization, the filters were washed sequentially in 2× SSC containing 0.1% sodium dodecyl sulfate (SDS) for 5 min at room temperature, twice in 0.1× SSC plus 0.1% SDS for 15 min at 65°C, and in 2× SSC for 5 min at room temperature (14) and then autoradiographed. (A) Lanes: 1, STS (control); 2 and 3, Cas-Bgr; 4 and 5, Cas-Hmi/1; 6, Cas-Mal; 7 and 8, Sub-Shh; 9 and 10, Sub-Kjr; 11 and 12, Sub-Bjn; 13 and 14, Mol-Nag; 15, BALB/c (control). (B) Lanes: 1, STS; 2 to 4, Cas-Bgr; 5, Cas-Hmi/1; 6, Cas-Hmi/2; 7 and 8, Cas-Mal; 9, Sub-Shh; 10 and 11, Sub-Kjr; 12 and 13, Sub-Bjn; 14 and 15, Mol-Nag; 16, BALB/c. (C) Lanes: 1, STS; 2 to 4, Cas-Bgr; 5, Cas-Hmi/1; 6 and 7, Cas-Hmi/2; 8 and 9, Cas-Mal; 10 and 11, Sub-Shh; 12 and 13, Sub-Kjr; 14 and 15, Sub-Bjn; 16 and 17, Mol-Nag; 18, BALB/c.

which carry endogenous MMTV, Sub-Jyg/2 mice completely lack endogenous MMTV (Table 1).

The clear segregation of Sub-Jyg/1 and Sub-Jyg/2 mice, with or without endogenous MMTV, respectively, during inbreeding is probably due to one of the following reasons. (i) Wild

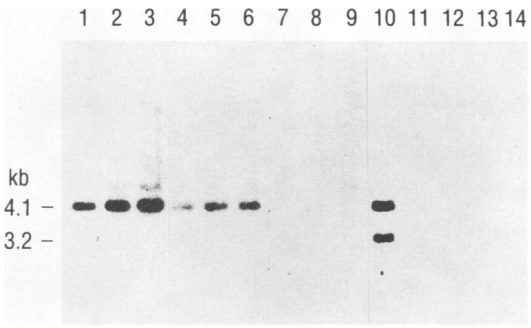

FIG. 2. Southern blot hybridization of *Eco*RI-digested cellular DNA of Sub-Jyg/1 (lanes 1 to 6) and Sub-Jyg/2 (lanes 7 to 14) mice with an MMTV *gag-pol* probe. The DNA was extracted from livers (lanes 1, 4, 7, and 8), salivary glands (lanes 2, 5, 12, and 13), kidneys (lanes 3, 6, 9, and 11), mammary gland (lane 14), and mammary carcinoma (lane 10). Tissue samples obtained from both female (lanes 1, 2, 3, 4, 7, 9, 10, 12, and 14) and male (lanes 5, 6, 8, 11, and 13) mice were analyzed. The methods used were the same as those described in the legend to Fig. 1.

mice that originally lacked endogenous MMTV may have acquired endogenous MMTV recently as a result of continuous exogenous MMTV infection, although evidence for such an event has not been previously described. (ii) It is possible that Chinese mice originally had an endogenous proviral genome but that during breeding the provirus was lost spontaneously because of a relatively high mutability of the proviral genome. (iii) Since we have shown that endogenous MMTV genomes segregate stably during breeding, it is reasonable to postulate that random segregation of a limited number of MMTV proviral loci has occurred within a given colony. Thus, Sub-Jyg/2 mice might have been heterozygous for MMTV genomes in the early generations, but the crossing of these mice might have produced MMTV provirus-negative and -positive mice by random assortment.

Mammary tumors in breeding mice. From each of the 10 mouse strains (Table 1), more than 100 breeding female mice belonging to different generations were kept for 15 to 20 months in order to determine whether or not they would develop mammary tumors. Nine of the 10 mouse strains, Mol-Nag, Cas-Bgr, Cas-Hmi/1, Cas-Hmi/2, Cas-Mol, Sub-Kjr, Sub-Jyg/1, Sub-Shh, and Sub-Bjn, were found to develop mammary tumors sporadically; taken together, these mice exhibited an extremely low incidence of mammary tumors (less than 1%) that developed late in life (later than 18 months of age). By contrast, approximately 10% of Sub-Jyg/2 mice had mammary tumors by the sixth generation. From the 12th generation, the frequency of tumor development began to increase; the tumors seemed to be independent of pregnancy. By the 18th generation, the incidence of mammary tumors became relatively high (89%) and tumor latency became shorter (less than 1 year of age). Histological examination of the tumors has indicated that they are type B adenocarcinomas (according to Dunn's classification [8a]).

Distribution of MMTV in mammary tumors and in normal tissues of Sub-Jyg/2 mice. In view of our finding that Sub-Jyg/2 mice do not carry endogenous MMTV but develop mammary tumors, we considered the possibility that the etiologic agent of the mammary tumors of these mice may be milk-borne MMTV. To examine this possibility, we initially analyzed a number of mammary tumors by Southern blot hybridizations for the presence of MMTV proviral sequences, by radioimmu-

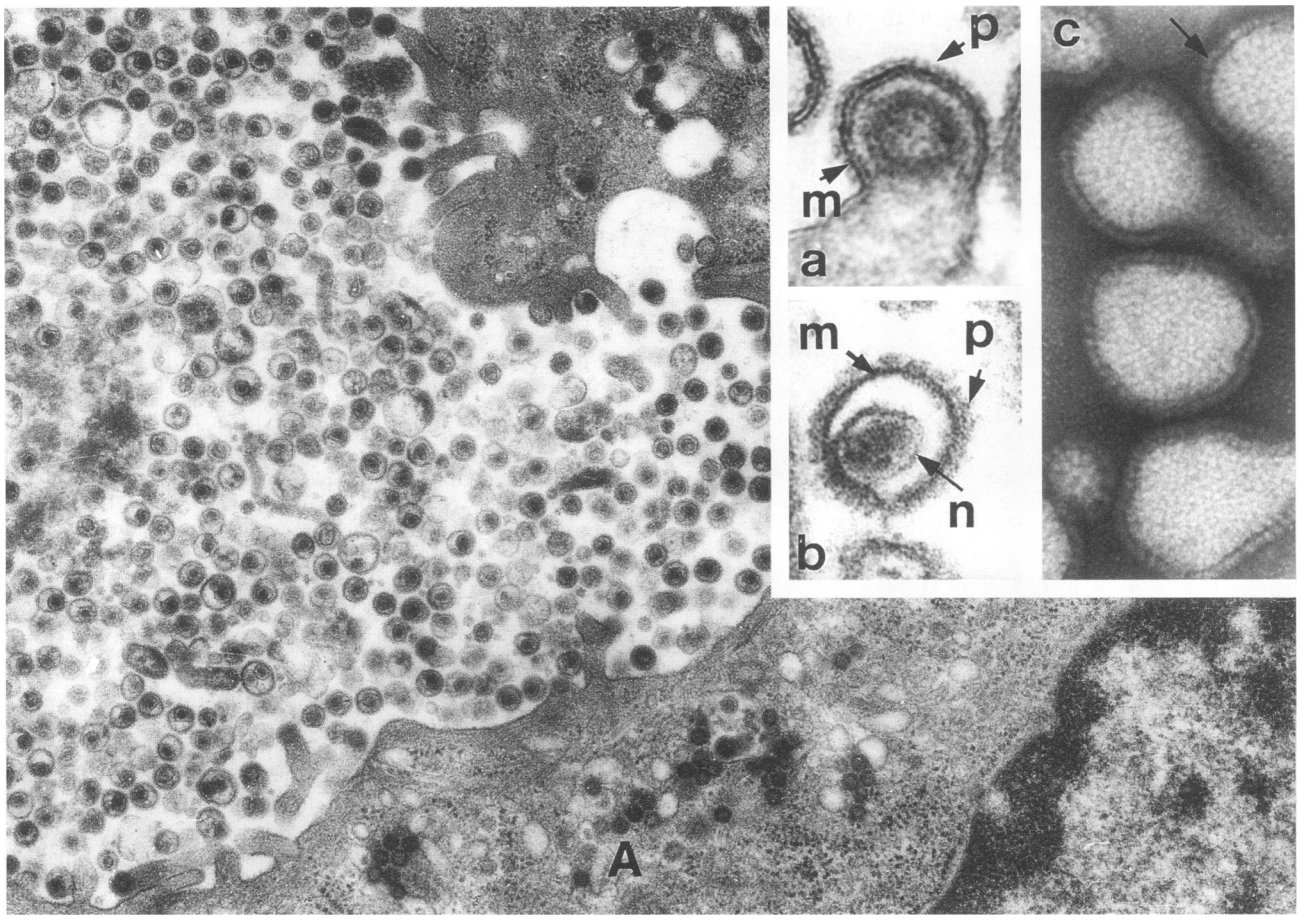

FIG. 3. Electron microscopic detection of particles resembling intracytoplasmic A, budding, immature, and mature MMTV particles in Sub-Jyg/2 mouse mammary tumors and in the culture medium of Sub-Jyg/2 mammary tumor cells. Tumor tissue was minced into small pieces and fixed in Karnovsky's solution. After being rinsed overnight in 0.1 M cacodylate buffer, the tissues were fixed in 1% osmium tetroxide for 2 h at 4°C, dehydrated with ethanol, and embedded by conventional procedures (25). Thin sections of the specimens were prepared with an ultramicrotome, stained with uranyl acetate and lead acetate, and examined in an electron microscope. The culture medium was harvested and centrifuged at 10,000 $\times$ *g* for 20 min to remove the cell debris. The supernatant was collected and recentrifuged at 100,000 $\times$ *g* for 1 h, and the pellet was dissolved in a small volume of phosphate-buffered saline, fixed with 1% glutaraldehyde, and examined in the electron microscope after being negatively stained with phosphotungstic acid (pH 7.0). The high-resolution micrographs of the budding and mature particles shown in insets a and b (thin sections of tumor cells) and c (negative staining of particles obtained from the culture medium) clearly demonstrate the presence of surface projections (p and arrows). A indicates the location where many intracytoplasmic A particles can be seen. m, viral membrane; n, viral nucleoid. Magnifications: main panel, $\times$40,000; inset a, $\times$150,000; inset b, $\times$170,000; inset c, $\times$180,000.

noassays (RIA) for the expression of viral antigens, and by electron microscopy for the production of putative MMTV particles. An example of the presence of MMTV sequences in mammary tumors of Sub-Jyg/2 mice as detected by a *gag-pol* probe is shown in Fig. 2 (lane 10). Further screening by LTR, *env*, and *gag-pol* probes of 15 mammary tumors that developed in Sub-Jyg/2 mice from different generations showed the presence of multiple MMTV proviral integrations (data not shown).

To determine whether the integrated proviruses that we detected in tumor tissues were expressed, we initially prepared soluble antigens from tumor tissues as well as a variety of normal tissues from Sub-Jyg/2 mice and examined the preparations by RIA for the presence of the major envelope glycoprotein of MMTV (gp52). The reason for investigating the expression of MMTV gp52 in these normal tissues was to ascertain if the distribution of the viral antigen in Sub-Jyg/2 mice resembles the distribution that has been found in other strains of mice carrying both endogenous and exogenous MMTVs.

The MMTV gp52 antigen that was used in this study was prepared from purified MMTV obtained from the milk of the highly inbred mammary tumor-producing mouse strain DD/Tbr. The methods for the purification of gp52, the production of anti-MMTV gp52 monoclonal antibody, and the RIA have been described previously (12, 16, 18). The monoclonal antibody was found to react with the MMTVs from other strains of laboratory mice. Our studies of the distribution of MMTV gp52 antigen in various tissues of Sub-Jyg/2 mice revealed that, in addition to mammary tumors and normal mammary glands, salivary glands, prostates, seminal vesicles, spleens, lymph nodes, and kidneys also expressed variable amounts of MMTV gp52. The level of expression was found to be highest in mammary tumors and mammary glands, followed by lymphoid tissues.

The results of our electron microscopic examination of all of

the tissues mentioned above correlated very well with those obtained by RIA, with some exceptions. In general, electron microscopy (Fig. 3) detected intracytoplasmic A particles as well as budding, immature, and mature B particles in the mammary glands (Fig. 3), mammary tumors, salivary glands, prostates, and seminal vesicles. However, no A, mature, or immature MMTV particles were found to be present in spleens, lymph nodes, or kidneys of Sub-Jyg/2 mice, although the RIA detected the expression of MMTV gp52 in these organs. Although virus particles resembling MMTV (designated M432) have been isolated from Asian mice (*M. cervicolor*) (4), the particles that we found in Sub-Jyg/2 mice (designated Jyg-MMTV) appear to be morphologically distinct from the M432 virus. While the envelope of Jyg-MMTV exhibits distinct surface projections (Fig. 3), a characteristic feature of classical MMTV particles (24), the surfaces of the M432 particles do not seem to contain projections (4).

Taken together, our observations clearly indicate that (i) Sub-Jyg/2 mice are infected with exogenous milk-borne MMTV, (ii) the pattern of viral expression in various tissues of these mice is similar to that observed in most laboratory strains of mice with a high incidence of mammary tumors (10), and (iii) Jyg-MMTV is most likely the causative agent of the mammary tumors in Sub-Jyg/2 mice. In addition, our results show that the major glycoprotein of Jyg-MMTV, a component of the viral surface projections, is antigenically related to the major glycoproteins of MMTVs of other inbred strains of *M. m. domesticus*. Since Sub-Jyg/2 mice develop mammary tumors at an incidence of 80 to 90% by 8 to 10 months of age, it is likely that Jyg-MMTV is highly infectious and/or virulent. Thus, Sub-Jyg/2 mice are unlike the other groups of mice similarly derived from wild mice in that the incidence of mammary tumors in the other groups of mice has been found to be very low (<5%) and the tumors developed late in life (>18 months) (1, 2, 8). It is possible that feral MMTV, in general, is not very infectious, unlike that carried by Sub-Jyg/2 mice, or alternatively that most feral mice are not highly susceptible to virus infection and/or virus replication. The infectivity of Jyg-MMTV, which can be obtained from the milk or tumors of Sub-Jyg/2 mice, in other inbred groups of wild mice and/or in laboratory strains of mice must be determined in order to ascertain the virus-host relationships between Jyg-MMTV and the genetic background of mice.

Our finding of the expression of MMTV gp52 at high levels in lymphoid tissues is interesting in view of the fact that previous studies have detected the presence of MMTV antigens, but not budding or mature MMTV particles, in lymphoid tissues of a number of strains of laboratory mice, and that a role for T cells in MMTV pathogenesis has been suggested (27). Although it is not yet clear how T cells participate in the process of mammary cell infection by exogenous MMTV or why these cells do not produce MMTV particles, we speculate that T cells may, as with other strains of laboratory mice, also be involved in MMTV pathogenesis in Sub-Jyg/2 mice.

In summary, our screening of the 10 groups of breeding mice that were initially captured in the wild from several places in Asia resulted in the identification of two groups of mice (Cas-Hmi/2 and Sub-Jyg/2) which are completely free of endogenous MMTV. Further, our results show that one of these groups of mice (Sub-Jyg/2) carries exogenous MMTV that causes mammary tumors at a high incidence. Sub-Jyg/2 mice provide an opportunity to generate an MMTV-free subline by using exogenous MMTV-free mice, such as BALB/c and C57BL mice (17), as foster nurses for the offspring of Sub-Jyg/2 mice. The availability of Cas-Hmi/2 and Sub-Jyg/2 mice thus constitutes a valuable addition to the several strains of mice that are currently used for studies of the molecular biology of MMTV-induced mammary tumorigenesis.

This research was supported in part by Public Health Service grants CA-45123 and CA-45127 awarded to N. H. Sarkar by the National Institutes of Health.

REFERENCES

1. **Andervont, H. B.** 1952. Biological studies on the mammary tumor inciter in mice. Ann. N.Y. Acad. Sci. **54:**1004–1011.
2. **Andervont, H. B., and T. B. Dunn.** 1962. Occurrence of tumors in wild house mice. J. Natl. Cancer Inst. **28:**1153–1163.
3. **Bentvelzen, P., and J. Hilgers.** 1980. Murine mammary tumor virus, p. 311–355. *In* G. Klein (ed.), Viral oncology. Raven Press, New York.
4. **Callahan, R., R. E. Benveniste, C. J. Sherr, G. Schidlovsky, and G. J. Todaro.** 1976. A new class of genetically transmitted retrovirus isolated from *Mus Cervicolor*. Proc. Natl. Acad. Sci. USA **73:**3579–3583.
5. **Callahan, R., W. Drohan, D. Gallahan, L. D'Hoostelaere, and M. Potter.** 1982. Novel class of mouse mammary tumor virus-related DNA sequences found in all species of Mus, including mice lacking the virus proviral genome. Proc. Natl. Acad. Sci. USA **79:**4113–4117.
6. **Cohen, J. C., and H. E. Varmus.** 1974. Endogenous mammary tumor virus DNA varies among wild mice and segregates during inbreeding. Nature (London) **278:**483–496.
7. **Dickson, C.** 1987. Molecular aspects of mouse mammary tumor virus biology. Int. Rev. Cytol. **108:**119–147.
8. **Dickson, C., R. Smith, S. Brookes, and G. Peters.** 1984. Tumorigenesis by mouse mammary tumor virus: proviral activation of a cellular gene in the common integration region *int*-2. Cell **37:**529–536.

8a. **Dunn, T. B.** 1959. Morphology of mammary tumors in mice, p. 38–84. *In* F. Homburger (ed.), Physiopathology of cancer, 2nd ed. Hoeber, New York.

9. **Escot, C., E. Hogg, and R. Callahan.** 1986. Mammary tumorigenesis in feral *Mus cervicolor popaeus*. J. Virol. **58:**619–625.
10. **Etkind, P. R., and N. H. Sarkar.** 1983. Integration of new endogenous mouse mammary tumor virus proviral DNA at common sites in the DNA of mammary tumors of C3Hf mice and hypomethylation of the endogenous mouse mammary tumor virus proviral DNA in C3Hf mammary tumors and spleens. J. Virol. **45:**114–123.
11. **Green, E. L.** 1975. Breeding systems, p. 11–22. *In* The Staff of the Jackson Laboratory (ed.), Biology of the laboratory mouse, 2nd ed. Dover Publications, Inc., New York.
12. **Imai, S., J. Morimoto, Y. Tsubura, Y. Iwai, M. Okumoto, T. Takamori, and J. Hilgers.** 1983. Tissue and organ distribution of mammary tumor virus antigens in low and high mammary cancer strain mice. Eur. J. Cancer Clin. Oncol. **19:**1011–1019.
13. **Imai, S., Y. Tsubura, J. Hilgers, and R. Michalides.** 1983. A new locus (*Mtv*-4) for endogenous mammary tumor virus expression and early mammary tumor development in the SHN mouse strain. JNCI **71:**517–521.
14. **Kozak, C., G. Peters, R. Pauley, V. Morris, R. Michalides, J. Dudley, M. Green, M. Davisson, O. Prakash, A. Vaidya, J. Hilgers, A. Verstraeten, N. Hynes, H. Diggelmann, D. Peterson, J. C. Cohen, C. Dickson, N. Sarkar, R. Nusse, H. Varmus, and R. Callahan.** 1987. A standardized nomenclature for endogenous mouse mammary tumor viruses. J. Virol. **61:**1651–1654.
15. **Marchetti, A., J. Robbins, G. Campbell, F. Buttitta, F. Squartini, M. Bistocchi, and R. Callahan.** 1991. Host genetic background effect on the frequency of mouse mammary tumor virus-induced rearrangements of the *int-1* and *int-2* loci in mouse mammary tumors. J. Virol. **65:**4550–4554.
16. **Massey, R. J., L. O. Arthur, R. C. Nowinski, and G. Schochetman.** 1980. Monoclonal antibodies identify individual determinants on mouse mammary tumor virus glycoprotein gp52 with group, class, or type specificity. J. Virol. **34:**635–643.
17. **Moore, D. H., C. A. Long, A. B. Vaidya, J. B. Sheffield, A. S. Dion, and E. Y. Lasfargues.** 1979. Mammary tumor virus. Adv. Cancer Res. **29:**347–418.

18. **Morimoto, J.** 1987. Production and properties of monoclonal antibodies against mouse mammary tumor virus (DD-MMTV)—with special reference to horizontal transmission of MMTV. J. Nara Med. Assoc. **38:**501–516.
19. **Morris, V. L., E. Madeiras, G. M. Reingold, J. M. Bishop, and H. E. Varmus.** 1977. Comparison of mouse mammary tumor virus-specific DNA in inbred, wild and Asian mice and tumors and normal organs from inbred mice. J. Mol. Biol. **114:**73–91.
20. **Nusse, R.** The *int* genes in mammary tumorigenesis and in normal development. 1988. Trends Genet. **4:**291–295.
21. **Nusse, R., A. Brown, J. Papkoff, P. Scambler, G. Shackleford, A. McMahan, R. Moon, and H. Varmus.** 1991. A new nomenclature for *int*-1 and related genes: the *wnt* gene family. Cell **64:**231–232.
22. **Nusse, R., and H. E. Varmus.** 1982. Mammary tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell **31:**99–109.
23. **Peters, G.** 1990. Oncogenes at viral integration sites. Cell Growth Differ. **1:**503–510.
24. **Sarkar, N. H.** 1987. Oncovirinae: type B oncovirus, p. 257–272. *In* M. V. Nermut and A. C. Steven (ed.), Perspectives in medical virology (animal virus structure), vol. 3. Elsevier, New York.
25. **Sarkar, N. H., and D. H. Moore.** 1972. Electron microscopy in mammary cancer research. J. Natl. Cancer Inst. **28:**1051–1058.
26. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
27. **Tsubura, A., M. Inada, S. Imai, A. Murakami, N. Oyaizu, R. Yasumizu, Y. Ohnishi, H. Tanaka, S. Morii, and S. Ikehara.** 1988. Intervention of T-cells in transportation of mouse mammary tumor virus (milk factor) to mammary gland cells *in vitro*. Cancer Res. **48:**6555–6559.
28. **Yonekawa, H., O. Gotoh, Y. Tagashiro, Y. Matsushima, L. I. Shi, W. S. Cho, N. Miyashita, and K. Moriwaki.** 1986. A hybrid origin of Japanese mice "Mus musculus molossinus", p. 62–67. *In* M. Potter, J. H. Nadeau, and M. P. Cancro (ed.), The wild mouse in immunology. Springer-Verlag, Berlin.