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Tissue and Organ Distribution of Mammary Tumor Virus Antigens in Low and High Mammary Cancer Strain Mice

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Abstract—*Expression of mammary tumor virus (MTV) antigen was measured in a wide variety of organs and tissues of a series of high (GR, SHN, SHNf, DD, SLN, SLNf) and low (DDf, DDD, DDDf, KF, KFf, ddY, C57BL, BALB/c) mammary cancer strain mice. Tests were carried out by microimmunodiffusion (micro-ID) and immunoperoxidase tests on formalin-fixed tissues and radioimmunoassays in extracts for 2 viral proteins, MTVp27 from the viral core and MTVgp52 from the viral envelope. Organs with exocrine function, i.e. the mammary gland, salivary gland, coagulating gland and prostate, were mostly positive. The secretory epithelial cells of these organs showed viral antigen expression. Less positivity was encountered in brain, pancreas, stomach, urinary bladder, epididymis, uterus, thymus, spleen, lymph nodes and kidney, plasma and blood cell pools. Unexpectedly the uterus extracts showed MTV antigen expression, occasionally even by immunodiffusion, especially in mice of various DD stocks, but also in the GR strain. Another striking observation was the detection of MTV antigen expression in salivary glands in C57BL strain mice; most other organs of this strain (including the mammary glands) were negative. The implications of results of this extensive survey for MTV antigen expression are discussed for tumorigenesis by MTV of mammary gland and perhaps other tissues in the mouse.*

INTRODUCTION

THE MURINE mammary tumor virus is known to transform mammary gland cells and cause mammary cancer in mice. However, the virus is also expressed in organs other than the mammary gland, yet it does not seem to transform the cells of such other organs, perhaps with the exception of thymus cells in the GR and DBA strains, which give rise to thymic lymphomas (for a review see [1–3]).

Some of the central questions in MTV research still are why this virus is expressed in so many tissues, how expression is regulated and why only the mammary gland cells can be transformed with high efficiency.

Although an extensive amount of research has been carried out on the distribution of MTV in the mouse (for a review of older literature see [4]), there is still some confusion about expression of 'infectivity' [5], B and intracytoplasmic A particles [6] and MTV antigens [7–10].

We have carried out an extensive immunological study of the expression of MTV antigens in the various organs and tissues of both high and low mammary cancer strains maintained in a Japanese laboratory. This study is an extension of previous work on expression of this virus in mammary gland [11] and male genital organs [12].

While in most studies carried out to date only 1 or at most 2 immunological techniques were employed, we have used 3 assays simultaneously, notably the immunoperoxidase test to study expression at the cellular level and also radioimmunoassays based on pure proteins of both the core and the envelope of the B particle. The latter test permits the study of concordance and discordance of core and envelope protein antigens of the virus.

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Some surprising results were obtained. Salivary glands proved to be high in MTV expression, even in case of the C57BL strain. Another organ which was highly positive was the prostate. Lymphoid tissues show an erratic pattern of positivity. Exceptional positivity was also found in the uterus, pancreas, brain, bladder etc., organs not reported to be positive previously. In general positivity was more pronounced in high mammary cancer strains than in low cancer strains, but expression was found in all strains except the BALB/c.

MATERIALS AND METHODS

Mice

The dd mouse group is represented by (1) DD/Tbr, inbred by Dr. Y. Tsubura since 1957 having reached now the 70th inbred generation. The incidence of mammary tumor is 71% at an average 10 months of age; (2) the KF strain, which was inbred from the dd stock by Kitasato with simultaneous selection for susceptibility to *Salmonella* infection. In 1963 some of these mice were brought to Nara Medical College with a 22% mammary tumor incidence (13.7 months); (3) the DDD strain, a strain from the dd stock with simultaneous selection for a high mammary cancer incidence from 1962. The incidence of mammary cancer declined from the 20th inbred generation. The incidence of mammary tumor is 14% (13.7 months).

The Swiss mouse group is represented by (4) the SHN and SLN strains. They were inbred from the same stock of Swiss mice by Dr. H. Nagasawa with selection for a high and low cancer incidence respectively. The incidence of mammary tumor is 89% (7.2 months) and 69% (7.9 months) in SHN and SLN respectively.

Also represented are strains with a low mammary tumor incidence, C57BL (from the Aichi Cancer Center in 1974) and BALB/c (from Taisho Pharmaceutical Co. Ltd. in 1974).

Foster-nursing strains SHNfBALB/c, SLNfBALB/c, DD/TbrfC57BL, DDDfBALB/c and KFfBALB/c mice were produced to eliminate exogenous MTV in the milk in Nara Medical College in 1978.

The C3H/Hef, of American origin, was obtained from the Charles River Animal Farm in Japan in 1978.

Maintenance of mice

All strains were maintained by strict brother-sister mating. They were fed Oriental NMF pellets and received water *ad libitum*. Mice were kept in an animal room with a constant temperature of 22 ± 1°C and a humidity of 40%.

Examination of tumors

The mice were checked weekly for mammary tumors and mice with tumors were autopsied. Tumors were fixed in 10% formalin solution, embedded in paraffin and stained with hematoxylin and eosin for histological observation.

Antiserum to MTV

Antiserum was produced against ether-treated MTV virions (purified B particles) from a mammary tumor cell line originating in a DD/Tbr mammary tumor, which was obtained from Dr. H. Iwai of the Radiation Center of Osaka Prefecture. It was produced by immunizing rabbits with purified virions mixed with incomplete and complete Freund's adjuvant 2–3 times at 4-week intervals. Non-specific antibody in the antiserum was absorbed *in vitro* and *in vivo* as described previously [7].

Preparation of extracts from various organs of mice

Fresh organs were homogenized in 10 vol. of Ca^{2+}-and Mg^{2+}-free Earle's Balanced Salt Solution (EBSS, from Grand Island Biological Co., NY, U.S.A.). After addition of ether the homogenate was stirred vigorously for 3 min on a Vortex. After centrifugation at 850 ***g*** for 10 min the ether phase was discarded and the water phase was stored overnight at 4°C. The extract was centrifuged again and the supernatant was used in the micro-ID test. Whole blood was mixed with 0.5 ml 9% sodium citrate and was centrifuged at 850 ***g*** for 10 min. The supernatant (plasma) was used for radioimmunoassay. After addition of 1.5 times the volume of saline the homogenate was stirred on a Vortex and centrifuged again at 850 ***g*** for 10 min. The precipitation (RBC) was used for radioimmunoassay.

Immunoperoxidase test

Organs were fixed in 10% buffered formaline, dehydrated in alcohol, embedded in paraffin and sectioned at 3–5 μm. The paraffined sections were treated with 30% H_2O_2 (v/v) and ethanol for 30 min to reduce endogenous peroxidase. The sections were washed once with Tris-HCl buffer (0.05 M Tris-HCl buffer in saline, pH 7.6) followed by 5% (v/v) normal fetal calf serum, and then washed 3 times every 5 min with Tris-HCl buffer.

The rabbit anti-MTV serum was added and incubated for 60 min in a humidified box at room temperature. The sections were washed 3 times, each for 5 min with Tris-HCl buffer. Goat anti-rabbit gamma globlin diluted 1:20 was added and the sections were kept in a humidified box at room temperature for 30 min and then washed 3 times

with Tris-HCl buffer. The washed sections were incubated with rabbit antiperoxidase bound to horseradish peroxidase (PAP) (Miles-Yeda CTD), diluted 1:100 for 30 min and then washed 4 times for 5 min each with Tris-HCl buffer. After incubating with DAB-H_2O_2 solution [20 mg 3,3-diaminobenzidine: 4 HCl (Sigma) in 75 ml 0.05 M Tris-HCl buffer, pH 7.6, and 5% H_2O_2] for 5 min they were washed 3 times with Tris-HCl buffer and stained with hematoxylin as counterstaining.

Microimmunodiffusion test

Microimmunodiffusion (micro-ID) was performed at room temperature according to Crowl, with some modifications [12].

Radioimmunoassay

Purified MTVgp52 and MTVp27 was labeled with ^{125}I by the chloramine-T method, with some modifications described previously [11].

RESULTS

Table 1 shows the expression of MTV antigens in various organs as tested by a microimmunodiffusion test, the least sensitive of the 3 tests used. Positivity was most frequently scored in mammary glands and tumors, although in certain low mammary cancer strains (DDf, DDD, DDDf, ddY, BALB/c and C57BL) the normal glands are invariably negative. In male mice positivity is often found in the prostate, coagulating glands and seminal vesicle, while the testes were negative. The salivary glands are frequently positive in both male and female animals, even in certain low mammary cancer strains such as DDf, DDD, ddY and C57BL. While ovaries are invariably negative, uterus extracts occasionally reveal MTV positivity, e.g. in the DDD and SHN strains. The liver, thymus and spleen were never scored positive, but occasional positive results were obtained with kidneys (DD/Tbr and SHNf) and lymph nodes (DD/Tbr, SHN and SHNf).

Table 2 shows the organ distribution of MTV antigens at the cellular level using immunoperoxidase tests. Mammary glands and tumors were generally positive, except for the glands of the following strains: DDf, DDD, DDDf, BALB/c and C57BL. Also frequently positive was the prostate, with exceptions in the DDf, DDDf, BALB/c and C57BL strains. The uterus was found to be negative, except in the GR strain. Seminal vesicles and coagulating glands could be positive and negative, while the epididymis was only found positive in the GR strain. Testes and ovaries were invariably negative. Salivary glands were positive in the following strains: GR, DD/Tbr, DDD, KF and ddY. No positivity at all was found in liver, kidney, thymus, spleen and lymph nodes.

Positivity for MTV antigens in the epithelia of mammary glands, salivary glands, prostates and seminal vesicles was seen at the luminal or apical side of the glandular epithelia, not in myoepithelial elements or in stroma of these organs.

Table 3 depicts levels of two protein antigens of MTV: MTVgp52 (envelope) and MTVp27 (core). Measurements were carried out by a previously described radioimmunoassay in ng/mg of tissue extract (see Figs 1 and 2).

Levels of MTVgp52 could be very high, at about 7000 ng (about 7 μg/mg protein) in ddY mammary glands. However, they were also low, with levels less than 100 ng/mg in the same and other high mammary cancer strains. Apparently, the variation in levels is enormous. Low cancer strains, such as the DDf and the C57BL, did not show any positivity for MTV proteins in their mammary glands. Mammary tumors of the high mammary cancer strains were generally positive for MTV proteins. Of male animals, the prostate showed extremely high positivity, not only in the high mammary cancer strains (GR, SHN and DD/Tbr) but also in some of the low cancer strains (DDf, C3Hf and ddY), but the prostate of the C57BL strain was not found to be positive in 3 cases. Also frequently positive, generally with moderate levels of protein antigens, were the seminal vesicles of the high cancer strains, while 2 of the low cancer strains also showed some positive samples (C3Hf and ddY).

An unexpected high frequency of positivity was found in the salivary glands, although levels were never extremely high. All strains except the BALB/c, C57BL and DDf showed positivity.

While the thymus and spleen were always negative as tested by immunodiffusion and immunoperoxidase tests, the radioimmunoassay revealed occasional positive results, especially in the high cancer strains GR and SHN. In this case the levels of proteins were low.

There is not always a concordance in the expression of envelope and core proteins in the various tissues. In the C57BL strain, for example, positivity for MTVgp52 was found in the salivary glands, which showed negativity for MTVp27.

The two strains with the highest mammary tumor incidence, due to genetic transmission of MTV, GR and SHN, had at least 1 positive MTV test in every organ and tissue tested. These organs include the brain, thymus, lung, spleen, pancreas, stomach, uterus (except SHN), urinary bladder and red blood cell packs (in this case positivity is low and may be due to contaminating white blood cells).

In most strains, however, the above mentioned

Table 1. Organ distribution of MTV antigen in inbred strains of mice detected by the microimmunodiffusion test

Strain	Sex	Age (months)	Liver	Kidney	Thymus	Spleen	Lymph node	Salivary gland	Testis/ ovary	Epididymis	Seminal vesicle	Coagul. gland	Prostate/ uterus	Mammary: gland	Mammary: tumor
GR	M	3–7	0/6	ND	ND	0/6	ND	2/4	0/6	ND	0/4	ND	2/4	–	–
		8–17	0/10	ND	ND	0/10	ND	4/4	0/10	ND	5/7	–	7/7	–	–
	F	7	0/3	0/3	0/3	0/3	ND	3/4	ND	–	–	–	0/3	3/3	3/3
DD/Tbr	M	2–7	0/3	0/3	0/3	0/3	1/3	2/2	0/18	0/18	0/18	0/18	0/18	–	–
		8–26	0/4	0/4	0/4	0/4	1/3	2/4	0/27	1/27	0/27	0/27	19/27	–	–
	F	8–15	0/6	1/4	0/3	0/4	0/4	2/5	0/6	–	–	–	0/6	2/5	4/4
DDf	M	1–18	0/15	0/14	0/15	0/15	0/13	1/16	0/23	0/23	1/23	0/23	1/23	–	–
	F	1–18	0/11	0/11	0/11	0/11	0/11	0/11	0/10	–	–	–	0/10	0/11	–
DDD	M	2–7	0/3	0/3	0/3	0/3	0/3	0/5	0/3	0/3	0/6	0/6	0/6	–	–
		8–27	0/3	0/4	0/2	0/2	0/4	5/13	ND	ND	1/9	1/9	1/13	–	–
	F	8–17	0/3	0/3	0/3	0/3	0/3	2/12	0/1	–	–	–	1/1	0/13	1/1
DDDf	M	5–10	0/2	0/2	0/2	0/2	0/2	0/2	0/6	0/6	0/6	0/6	0/6	–	–
	F	5–10	0/6	0/6	0/6	0/6	0/6	0/6	0/6	–	–	–	0/6	0/6	–
KF	M	2–7	0/3	0/3	0/3	0/3	0/3	1/8	0/9	0/9	1/9	1/9	1/9	–	–
		8–19	0/4	0/4	0/4	0/4	0/4	3/10	0/11	0/11	2/11	1/11	7/11	–	–
	F	7–18	0/4	0/4	0/4	0/3	0/4	4/10	0/3	–	–	–	0/4	3/10	2/2
KFf	M	7–8	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	–	–
	F	7–8	0/1	0/1	0/1	0/1	0/1	0/1	–	–	–	–	0/1	1/1	–
SHN	M	2–7	0/3	0/3	0/3	0/3	0/3	2/3	0/11	0/11	4/11	0/11	4/11	–	–
		8–18	0/3	0/3	0/2	0/2	1/2	5/9	0/11	0/10	10/11	0/10	6/10	–	–
	F	8–12	0/4	0/4	0/3	0/4	1/4	7/7	0/4	–	–	–	2/5	6/6	4/4
SHNf	M	8–12	0/1	ND	0/3	0/3	1/3	2/3	0/3	0/3	2/8	0/8	3/8	–	–
	F	8–12	0/3	1/3	0/3	0/3	1/2	3/1	0/2	–	–	–	0/2	3/3	2/2
SLN	M	2–7	0/2	0/2	0/2	0/2	0/2	1/2	0/6	0/6	3/9	1/9	2/9	–	–
		8–10	0/10	0/10	0/10	0/10	0/10	4/10	0/10	0/10	8/10	4/10	4/10	–	–
	F	12	ND	0/3	ND	ND	0/3	1/3	ND	–	–	–	0/3	2/2	2/2
SLNf	M	5–10	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	–	–
	F	5–10	0/3	0/3	0/3	0/3	0/3	0/3	0/3	–	–	–	0/3	1/3	–
ddY	M	5–13	0/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4	1/6	1/6	1/5	–	–
	F	8–13	0/3	0/3	0/3	0/3	0/3	0/3	0/3	–	–	–	0/3	0/3	–
BALB/c	M	7–22	0/12	0/12	0/12	0/12	0/12	0/12	0/13	0/13	0/13	0/13	0/13	–	–
	F	12–27	0/14	0/14	0/14	0/14	0/14	0/14	0/14	–	–	–	0/14	0/14	–
C57BL	M	13–23	0/7	0/7	0/7	0/7	0/7	2/14	0/7	0/7	0/14	0/7	0/14	–	–
	F	9–23	0/6	0/6	0/6	0/6	0/6	0/6	0/6	–	–	–	0/6	0/6	–

ND = Not done.

Table 2. Organ distribution of MTV antigen in inbred strain of mice detected by immunoperoxidase

Strain	Age (months)	Sex	No. mice tested	Liver	Kidney	Thymus	Spleen	Lymph node	Salivary gland	Testis/ ovary	Epididymis	Seminal vesicle	Coagul. gland	Prostate/ uterus	Mammary: gland	Mammary: tumor
GR	7	M, F	4	-	-	-	-	-	+	-	+	+	+	+	+	+
DD/Tbr	8	M, F	4	-	-	-	-	-	+	-	-	+	+	+/-	+	+
DDf	16	M, F	5	-	-	-	-	-	-	-	-	-	-	-	-	NT
DDD	10	M, F	2	-	-	-	-	-	+	-	-	-	-	+/-	-	+
DDDf	10	M, F	2	-	-	-	-	-	-	-	-	-	-	-	-	NT
ddY	10	M, F	3	-	-	-	-	-	+	-	-	+	+	+/-	+	+
KF	8	M, F	4	-	-	-	-	-	+	-	-	+	+	+/-	+	+
KFf	8	M, F	3	-	-	-	-	-	-	-	-	-	-	+/-	+	NT
SHN	8	M, F	4	-	-	-	-	-	+	-	-	+	+	+/-	+	+
SHNf	8	M, F	3	-	-	-	-	-	+	-	-	+	+	+/-	+	+
SLN	8	M, F	3	-	-	-	-	-	+	-	-	+	+	+/-	+	+
SLNf	10	M, F	3	-	-	-	-	-	-	-	-	-	-	+/-	+	NT
BALB/c	20	M, F	5	-	-	-	-	-	-	-	-	-	-	-	-	NT
C57BL	18	M, F	5	-	-	-	-	-	-	-	-	-	-	-	-	NT

NT = No tumor is observed.

organs and tissues are rarely if at all positive, and if so only low levels of proteins are found. Note that the stomach of the ddY and also C57BL strains is somewhat more frequently positive with appreciable levels of MTV proteins. Plasmas of mammary tumor free mice are generally negative, with the exception of those of GR-strain male and female mice. This correlates well with positivity found in red blood cell packs.

DISCUSSION

The main organ systems for endogenous (GR, SHN) and exogenous (DD/Tbr) MTV expression are glands with exocrine secretory functions, i.e. mammary glands, salivary glands, coagulating glands (seminal vesicle) and the prostate. Since the DDf, a low cancer strain freed of the milk-factor [13], shows a very limited expression of endogenous MTV in these organs (except salivary glands and the prostate), it follows that expression in most organs of the DD/Tbr is of exogenous viral origin. This implies two important points: (1) that all these organs have receptors for MTV allowing exogenous virus to enter; and (2) that expression of endogenous and exogenous proviral sequences is governed by the same principle. Since exogenous MTV-DNA is known to enter the host genome at many different places, it is not the position in the genome which seems to allow for expression but, rather, the viral genome itself. Since it is known that the viral genome carries a sequence which is an acceptor site for the dexamethasone-receptor complex, it may be that glucocorticoid action in the organs with exocrine secretion is the main stimulus for its expression (for a review see [14]).

Expression of MTV in other organs and tissues seems to be much more erratic than in the above-mentioned exocrine organ systems. MTV has occasionally been found in lymphoid tissues. The same is true for EM studies: MTV particles have sometimes been found in such tissues, but most of the time were not demonstrable (for a recent review see [6]). Neoplastic tissue of lymphoid origin, however, has frequently been found to be positive for particles, especially intracytoplasmic A particles. Such positivity is confined to some high mammary cancer strains such as the GR [15], DBA/2 [16] and ICRC strains [17].

Since MTV antigens are found in all thymic lymphomas of the GR strain since MTV-DNA sequences are amplified in such lymphomas [18] and also because the GR-Mtv-2$^-$ strain [19] does not show these lymphomas, it seems likely that this virus is involved in the causation of the lymphomas of thymic origin in the GR strain. This may also be the case for certain lymphomas

Table 3. MTV gp52 and p27 antigen expression in various organs of mice by radioimmunoassay (ng/mg *protein*)

Strain	Age (mo.)		Sex	Brain	Salivary gland	Thymus	Liver	Lung	Spleen	Pancreas
GR	8–10	gp52	M	1/3(12)	5/5(20–71)	1/5(6)	0/3	0/3	1/5(1)	2/3(10–12)
			F	0/3	3/3(22–70)	1/3(7)	0/3	1/2(15)	0/3	1/3(2)
		p27	M	0/3	2/3(25–134)	0/3	0/3	1/3(79)	0/4	0/3
			F	0/3	2/2(87–162)	1/3(25)	0/3	0/2	1/4(20)	0/3
SHN	7–12	gp52	M	1/3(9)	9/9(4–148)	2/9(7–189)	0/2	0/3	3/9(3–13)	0/3
			F	1/5(9)	3/4(8–11)	0/5	0/2	1/5(11)	2/5(7–18)	2/4(3)
		p27	M	0/3	4/4(9–325)	0/5	0/2	0/3	1/6(7)	1/3(95)
			F	0/5	3/4(2–5)	0/1	0/2	4/5(71–158)	0/2	1/4(20)
DD	10–13	gp52	M	0/3	7/7(27–237)	1/4(4)	0/3	0/3	0/4	0/3
			F	0/3	2/8(33–42)	2/6(22–41)	0/3	0/3	1/6(8)	1/2(24)
		p27	M	0/3	7/7(9–325)	0/5	0/3	0/3	1/6(7)	0/3
			F	0/3	4/4(2–11)	ND	0/3	1/3(11)	0/1	0/2
DDf	12–14	gp52	M	0/3	4/11(9–325)	0/9	0/3	0/2	0/9	0/3
			F	0/3	0/11	0/7	0/2	0/3	0/8	0/3
		p27	M	0/3	2/2(47)	0/2	0/2	0/2	0/6	0/3
			F	0/3	1/3(5)	0/4	0/2	0/3	0/3	0/3
C3Hf	12–15	gp52	M	ND	3/3(7–64)	0/3	0/1	ND	0/3	ND
			F	ND	1/3(5)	0/3	0/1	ND	0/3	ND
		p27	M	ND	1/6(9)	0/6	0/1	ND	2/7(5–6)	ND
			F	ND	0/3	1/3(28)	0/1	ND	0/3	ND
ddY	12–15	gp52	M	ND	7/7(3–55)	0/6	0/1	ND	0/7	ND
			F	1/3(26)	2/4(4–1482)	0/4*	0/1	2/4†(3–11)	0/4	1/2(7)
		p27	M	ND	3/3(6–406)	0/1	0/1	ND	0/2	ND
			F	0/3	0/1	0/2*	0/1	1/4†(12)	ND	0/2
C57BL	17–20	gp52	M	0/2	4/5(6–64)	0/3)	0/3	0/1	0/3	0/3
			F	0/2	1/6(5)	0/3	0/2	0/3	0/4‡	0/3
		p27	M	0/2	2/5(40–203)	0/1	0/3	0/1	0/3	0/3
			F	0/2	0/3	ND	0/2	0/3	0/1‡	0/3
BALB/c	13–22	gp52	M	0/1	0/3	ND	0/1	0/1	ND	0/2
			F	0/1	0/3	0/1	0/1	0/2	ND	0/1
		p27	M	0/1	0/3	ND	0/1	0/1	ND	0/2
			F	0/1	0/3	0/1	0/1	0/2	ND	0/1

of the DBA/2, ICRC and perhaps even low mammary cancer strains.

An extensive study using bioassays for MTV has been carried out by Bentvelzen and Brinkhof [20]. Infectivity was found in cell-free extracts from four organs: the salivary glands, kidney, testis and epididymis, from the BALB/cfC3H strain. This implies that infectious B particles should be present in those organs. This would fit with the findings of the present study of viral antigen expression, except for the testis. Perhaps the latter exception is due to a strain difference and the BALB/cfC3H is unique in its high expression of virions in the testis.

Bioassays by Bentvelzen and Brinkhof [20] were also carried out with intact cells of a variety of organs. This does not necessarily require the expression of fully mature, infectious B particles (for theoretical considerations see [2]). Bioactivity was found in the thymus, spleen, bone marrow, salivary glands, liver, kidney, testis and epididymis. This implies that the bioactivity test could well be of extremely high sensitivity, because radioimmunoassays of certain organs (the liver and testis) of many strains in this study did not reveal any positivity for antigens. Some discrepancies observed, especially for the thymus and spleen, may have to do with improper processing of viral antigens or the 'mottled' expression (see later) observed in many of these mouse strains.

These types of 'discrepancies' for positivity and negativity in lymphoid tissues can also be found by comparing results with immunological assays. Some authors have reported the presence of MTV antigens in lymphoid tissues, mainly spleens of various strains [7, 8], while other authors [10] did not get any positive results with spleens and lymph nodes. Earlier positive results were obtained by the Belgian group using the more sensitive radioimmunoassay [9]. It may indeed be that individual mice of some of these inbred mouse strains, especially those of the high cancer strains, are indeed very different in their expression of endogenous and exogenous MTV.

Table 3. Continued

Stomach	Testis/ ovary	Seminal vesicle	Prostate/ uterus	Urinary bladder	Mammary gland	Mammary tumor	Plasma	RBC
0/3	0/3	5/5(11-132)	8/8(147-15111)	3/3(33-347)	-	-	1/3(18)	1/3(1)
1/3(14)	ND	-	2/3(125-202)	2/3(5-57)	6/6(199-8924)	6/6(36-952)	2/2(3-21)	2/2(6-7)
1/3(69)	0/3	3/3(13-27)	5/5(161-42667)	2/3(19-170)	-	-	1/3(2)	1/3(7)
0/3	ND	-	3/3(22-9892)	1/3(363)	3/3(1826-2270)	3/3(418-5225)	2/2(7-10)	1/2(4)
1/3(7)	0/5	9/9(13-3157)	11/11(93-5333)	0/4	-	-	0/2	0/2
1/4(11)	0/4	-	0/10	1/5(36)	4/8(57-1131)	2/2(2286)	1/5(1)	0/5
0/3	0/3	5/5(15-216)	5/5(15-216)	0/4	-	-	0/2	0/2
0/4	0/3	-	0/6	0/5	0/4	ND	0/5	1/5(6)
0/3	0/6	6/7(6-160)	7/7(98-1971)	0/3	-	-	1/3(0.4)	0/3
0/2	0/6	-	6/9(3-9216)	0/3	8/9(43-2916)	5/6(457-5225)	0/1	0/1
0/3	0/6	7/8(15-216)	4/5(192-2845)	1/3(197)	-	-	0/3	0/3
0/2	0/6	-	1/3(33)	0/3	3/3(34-1527)	4/4(37-2612)	0/1	0/1
0/3	0/5	0/9	2/11(13-17)	0/3	-	-	0/3	0/3
0/3	0/4	-	0/10	0/3	0/5	NT	0/3	0/3
0/3	0/5	0/7	0/5	0/3	-	-	0/3	0/3
1/3(25)	0/4	-	0/3	0/3	0/5	NT	0/3	0/3
ND	ND	0/3	0/3	ND	-	ND	ND	ND
ND	ND	-	0/3	ND	0/3	ND	ND	ND
ND	ND	1/5(5)	1/3(16)	ND	-	ND	ND	ND
ND	ND	-	0/3	ND	1/3(13)	ND	ND	ND
ND	ND	5/7(16-226)	5/5(13/622)	ND	-	-	ND	ND
1/3(30)	ND	-	0/3	0/3	5/5(5-7349)	3/3(21-5225)	2/3(9-89)	1/3(1)
ND	ND	4/5(12-32)	1/1(40)	ND	-	-	ND	ND
2/3(39-165)	ND	-	0/1	2/3(1-513)	1/1(1787)	2/3(2776)	0/3	2/3(4-12)
1/3(7)	0/7	0/5	0/6	0/3	-	-	1/3(1)	0/2
0/3	0/6	-	0/3	0/3	0/6	NT	0/3	0/2
1/3(82)	0/7	0/5	0/6	0/3	-	-	1/3(6)	0/2
0/3	0/6	-	0/3	0/3	0/3	NT	0/3	0/2
0/1	0/1	0/3	0/3	0/1	-	-	0/1	0/1
0/2	0/2	-	ND	0/1	0/2	NT	0/1	0/1
0/1	0/1	0/3	0/3	0/1	-	-	0/1	0/1
0/2	0/1	-	ND	0/1	0/2	NT	0/1	0/1

NT = No tumor observed; ND = not done.
*Thymoma. †Lung adenoma.‡Sarcoma.

In some individuals expression in one or very few cells may lead to rapid spread of the virus and measurable amounts of antigens (and sometimes high levels), while in other individuals of the same strain the initial expression in one or few cells does not take place and the organ stays negative throughout life. Such a 'mottled' type of expression has also been demonstrated in cases of retroviruses of the C type in mice.

It has been reported that the C57BL strain is completely negative for MTV antigen expression, notably by Hendrick *et al.* [9]. These authors tested the mammary glands, seminal vesicles, brain, spleen, kidneys, heart and liver. In our study also the C57BL was negative in these organs, but positive in the salivary glands. This makes the C57BL quite a unique strain, apparently carrying an endogenous MTV which shows a very restricted pattern of expression, since the mammary glands and the prostate (the other organs with the highest expression in all other strains tested so far) are completely negative. It would be of interest to isolate this virus from salivary glands and to study its biological characteristics.

So far we have not found a report in the literature claiming that the uterus, pancreas, stomach and urinary bladder of mice could express MTV. We have obtained quite a number of extremely high positive results with uteri from the DD/Tbr, DDD, ddY, GR and SHN strains. It may be that the exogenous MTV of the DD stocks, notably the one in the DD/Tbr strain, can readily infect the uterus and become expressed in that organ. This may have implications for the transmission of the virus in this strain, and unborn mice could become infected *in utero*, as shown by Andervont [21], rather than infected after birth via the mother's milk. This would have to be a relatively rare event, however, since it is

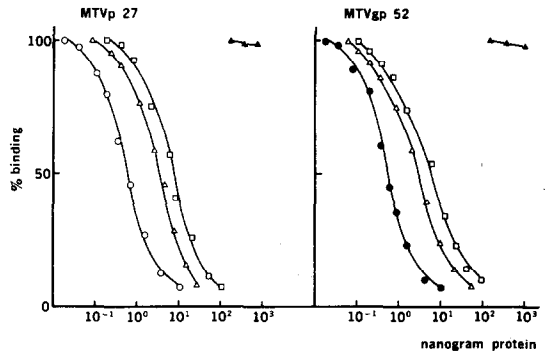

Fig. 1. Standard competition radioimmunoassays for MTVp27 and MTVgp52. O——O Purified MTVp27; ●——● purified MTVgp52; □——□ extract of DD mammary tumor; △——△ extract of GR mammary tumor; ▲——▲ extract of DD liver.

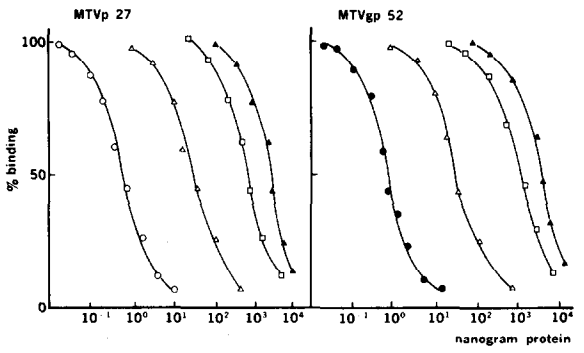

Fig. 2. Standard competition radioimmunoassays for MTVp27 and MTVgp52. O——O Purified MTVp27; ●——● purified MTVgp52; □——□ extract of DD prostate; △——△ extract of DDf salivary gland; ▲——▲ extract of C57BL salivary gland.

easy to establish a DDf strain by foster-nursing DD/Tbr babies on a low cancer strain. It could nevertheless be worthwhile to try to determine this mode of transmission using the DD/Tbr in view of the never-confirmed report by Andervont [21].

The question why MTV can become expressed in organs other than the mammary gland and still not cause cancer (prostate and salivary gland cancers have never been thought to be caused by MTV in mice [22]) still remains central to the understanding of MTV as a mammary cancer-causing agent in mice. If there is a 'mam' gene [1, 23], such a gene may indeed only cause transformation of the mammary gland. If amplification of proviral sequences are necessary to cause mammary cancer by MTV, 'activating' a normal cellular gene that causes transformation, it seems harder to understand why this event could not take place in organs other than the mammary gland. In fact this may occur in the occasional thymocyte in strains like GR, DBA/2 and ICRC, all showing low incidences of MTV expressing lymphomas. The question is why it does not occur in the salivary glands, prostate, seminal vesicles, epididymis, uterus etc. Perhaps it does occur in a certain number of these cases, and are more neoplasms of the mouse the result of direct MTV action?

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