Eur J Cancer Clin Oncol, Vol. 17, No. 8. pp. 883–888, 1981.
Printed in Great Britain.

0014-2964/81/080883-06$02.00/0

Expression of the Murine Mammary Tumor Virus in the Milk of F1 Hybrids of Virus Negative Mouse Strains*

P. BENTVELZEN
Radiobiological Institute TNO, Rijswijk, The Netherlands

Abstract—*Milk samples of multiparous females from several mouse strains and their F1 hybrids were tested for the presence of the envelope gylcoprotein gp52 of the murine mammary tumour virous. F1 hybrids resulting from matings of the virus negative strain BALB/c with the virus negative strains AKR, ND2 or RFM were positive for the viral antigen. The progeny of backcrosses to either parent had considerable lower incidences of viral antigen-positive milks. It was concluded that different recessive genes in the BALB/c strain on the one hand and in the AKR, ND2 and RFM strain on the other cause inhibition of endogenous virus expression. In F1 hybrids the two sets of genes complement each other, resulting in virus release.*

INTRODUCTION

THE ROLE of a virus in early occurring mammary tumours in mice is well established. This murine mammary tumour virus (MuMTV) can be transmitted either via the mother's milk or genetically [1]. Every inbred mouse strain tested so far contains a few DNA copies of MuMTV-RNA in its normal cellular DNA [2]. Expression of such germinal proviruses is associated with an increased risk of mammary carcinoma [3, 4]. However, expression of endogenous viruses in the form of viral proteins synthesized in the mammary gland is inhibited in several mouse strains [3–5]. In the present study on the genetic regulation of expression of endogenous MuMTV, complementation resulting in the synthesis of MuMTV proteins was found in several F1 hybrids of virus negative strains.

MATERIALS AND METHODS

Mice

The following inbred mouse strains maintained at the Radiobiological Institute TNO were used: AKR, BALB/c, CBA, C3Hf, C57BL, GRS, ND2, RFM. Virological data and mammary tumour incidences have been obtained in earlier investigations of my laboratory [4, 6].

Milk samples were collected in the third lactation period of the 12th day postpartum.

Virus

Tissue culture derived MuMTV originating from the virus producing C3H mammary tumour cell line, Mm5mt/cl, was obtained through the Office of Resources and Logistics, Biological Carcinogenesis Branch, National Cancer Institute, Bethesda, Md., U.S.A.

Radioimmunoassay

The envelope glycoprotein gp52 was isolated from solubilized MuMTV by means of Concanavalin A affinity chromatography followed by gel filtration on Sephadex G150 according to Westenbrink *et al.* [7]. An antiserum has been raised in a rabbit against this viral antigen and proved to be highly specific in a variety of immunoassays [8]. Routine radioimmunoassay for gp52 as developed in my laboratory [8–11] were performed as follows: two-fold serial dilutions of skimmed milk were made in 20 mM Tris–HCl, pH 7.6, 1 mM EDTA, 100 mM NaCl (TEN buffer) containing 0.2% Triton X–100,

Accepted 25 February 1981.
*This work was supported in part by contract NO1 CP4–3328 with the Biological Carcinogenesis Branch, National Cancer Institute, NIH, Bethesda, Md., U.S.A.

2 mg/ml BSA and 300 μg/ml^{-1} of protease inhibitor phenylmethylsulfonyl-fluoride (PMSF). The latter compound was added shortly before use from a stock solution containing 20 mg/ml PMSF in isopropanol. To 10 μl of test antigen was added 40 μl of buffer and 10 μl of rabbit anti-gp52 serum, absorbed with foetal calf serum, at a dilution sufficient to precipitate 50% of the input radioactivity. After an incubation period of 2 hr at 37°C, 40 μl of gp52 iodinated according to the chloramine-T method [12], in TEN-buffer containing 6.5 mg/ml bovine serum albumin and 7.5% normal rabbit serum was added. After an additional incubation period of 2 hr at 37°C, 30 μl of swine anti-rabbit-immunoglobulin serum (Nordic, Tilburg, The Netherlands) was added. Incubation was continued for 1 hr at 37°C and overnight at 4°C.

Following this, 0.5 ml of cold TEN-buffer was added and precipitates were collected by centrifugation for 30 min at 3000 *g* at 4°C. Pelleted material was washed and radioactivity determined. To exclude the possibility of false positive results, positive samples were subjected to immunoprecipitation with anti-gp52 serum. The precipitates were then analyzed by SDS-polyacrylamide gelelectrophoresis [13]. The amount of protein in the milk samples was determined according to Lowry [14].

RESULTS

The sensitivity of the radioimmunoassay used in this study can be concluded from Fig. 1: the lowest detectable amount is 0.1 ng of purified gp52. In milk samples the lowest detectable amount of gp62 is 0.3 ng, corresponding with approximately 1 ng of MuMTV. The radioimmunoassay of gp 52 as practiced in my laboratory proved to be highly specific [8–11]. No cross-reaction was found with any of the other MuMTV structural proteins nor with murine leukaemia virus polypeptides. Organ or tumour extracts or milk had no significant effect upon the sensitivity and specificity of the assay.

A very high content of gp52 was found in the milk of exogenously infected BALB/c mice. In this case the value was 543 ng of gp52 per mg milk protein. Considerably less was found in the milk of a (BALB/c × ND2) F1 mouse producing endogenous MuMTV: 23 ng/mg protein. The BALB/c milk sample was completely negative, as has been repeatedly found in my laboratory. In comparison, in the supernatant of a mammary tumour cell line producing exogenous MuMTV's, the yield was 0.1 mg gp52 per liter tissue culture fluid, which is below average.

The expression of MuMTV gp52 at the third lactation period is given in Table 1 for the various inbred mouse strains. Strains AKR, BALB/c, CBA, C57BL, ND2 and RFM are consistently negative, while milk samples of C3Hf, DBAf and GRS are invariably positive. These results are in complete agreement with those obtained with the Sepharose bead immunofluorescence assay [4, 6]. Milk samples from strains BALB/c, C57BL, and ND2 which were taken at later lactation periods than the third also proved to be negative.

The occurrence of gp52 in milk samples of several F1 hybrids of these strains is shown in Table 2. The hybrids (BALB/c × C3Hf)F1, (BALB/c × GRS)F1, (C3Hf × GRS)F1, (CS57BL × BALB/c)F1, (C57BL × C3Hf)F1 and (C57BL × GRS)F1 have been tested before by means of the Sepharose bead immunofluorescence assay. Comparable results had been obtained [4]. No appreciable differences are found between the two methods. The hybrids

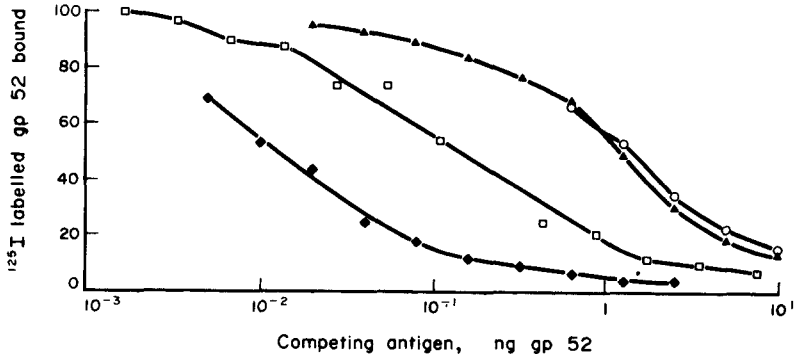

Fig. 1. Radioimmunoassay for MuMTV gp52 in mouse milk samples. ■: *purified go52*; □: *milk of BLAB/c mouse infected with MuMTV-S*; ▲: *supernatant mouse mammary tumour cell line C3HMT/c111*; ○: *milk of (BALB/c × ND2)F1 mouse*; *: *milk of BALB/c mouse.*

Table 1. Detection of MuMTV gp52 in milk of inbred mouse strains

Mouse strain	Number of mice	Number positive
AKR	15	0
BALB/c	35	0
CBA	8	0
C3Hf	6	6
C57BL	12	0
DBAf	7	7
GRS	5	5
ND2	13	0
RFM	7	0

MuMTV in their milk: (BALB/c × AKR)F1, (BALB/c × ND2)F1 and (BALB/c × RFM)F1. The BALB/c strain is one of the parents of each of these positive hybrids. The concentration of viral protein varies between 20–150 ng/mg milk protein in the samples of these hybrids. For comparison, in samples of BALB/c mice exogenously infected with MuMTV-S, it varies between 100 and 1200 ng/mg protein. Most F1 hybrids of virus negative strains do not contain MuMTV gp52 in their milk: (BALB/c × CBA)F1, (ND2 × AKR)F1, (ND2 × RFM)F1 and (RFM × AKR)F1.

Table 2. Detection of muTV gp52 by means of radioimmunoassay in the milk of various F1 hybrids

Hybrid No.	Female*	Male*	Number of tested offspring mice	Number positive	%
1	BALB/c	AKR	8	3	38
2	BALB/c	CBA	12	0	0
3	BALB/c	C3Hf	6	6	100
4	BALB/c	DBAf	7	7	100
5	BALB/c	GRS	3	3	100
6	BALB/c	ND2	20	15	75
7	BALB/c	RFM	16	16	100
8	C3Hf	CBA	11	1	9
9	C3Hf	GRS	11	11	100
10	C57BL	BALB/c	5	1	20
11	C57BL	CBA	8	0	0
12	C57BL	C3Hf	9	0	0
13	C57BL	GRS	2	2	100
14	DBAf	C3Hf	8	8	100
15	ND2	AKR	9	0	0
16	ND2	RFM	7	0	0
17	RFM	AKR	6	0	0

*Virus-positive mouse strains are underlined.

which were sired by GRS males have very large quantities of gp52 in their milk, as was to be expected on the basis of biological studies [15]. Not every hybrid in which the C3Hf strain is involved is positive. As previously noted, the genome of the C57BL strain can inhibit the expression of endogenous MuMTV-L from C3Hf in their F1-hybrid; this is in contrast to genome of the BALB/c strain. The CBA strain seems to have similar inhibitory properties in its F1 hybrid with C3Hf as the C57BL strain.

The F1 hybrid of the virus releasing strains DBAf and C3Hf is also virus positive. Surprising was the high incidence of virus positive animals among some F1 hybrids of mouse strains which do not produce endogenous

The incidence of gp52 positive animals in a series of crosses is presented in Table 3. The 100% incidence in the test cross of virus negative BALB/c to the positive F1 hybrid (C3Hf × DBAf) indicates that the genes which allow release of endogenous MuMTV in the latter two strains [3, 4, 16] are located at approximately the same site in the same linkage group, if not allelic. Otherwise, several virus negative animals would be expected. If the genes were located in different linkage groups, approximately 25% of the animals produced in this test cross would have been negative for gp52.

The incidences of virus positive animals in back crosses of unexpected positive F1

Table 3. Detection of MuMTV gp52 by means of radioimmunoassay in the milk of progeny mice of several crosses

Female	Male	Number of tested offspring mice	Number positive	%
BALB/c	(C3Hf × DBAf)F1	42	42	100
BALB/c	(BALB/c × AKR)F1	61	12	20
AKR	(BALB/c × AKR)F1	25	8	32
BALB/c	(BALB/c × RFM)F1	22	10	46
RFM	(BALB/c × RFM)F1	31	10	32
BALB/c	(BALB/c × ND2)F1	33	11	33
ND2	(BALB/c × ND2)F1	27	12	44
RFM	(BALB/c × ND2)F1	44	15	34

sistently lower than in the F1 hybrids. These results can be explained by considering that different recessive genes in the BALB/c strain which inhibit endogenous virus release are complemented by other recessive genes from either AKR, ND2 or RFM strains, also not allowing virus production. In the back crosses to either parent, the inhibitory gene from one strain may become homozygous as in the parental strain, resulting in suppression of virus release into the milk.

DISCUSSION

Several publications have dealt with the detection of MuMTV polypeptides in mouse milk samples by means of radioimmunoassay [17–23]. In general, the results of the various studies agree quite well, particularly when high-cancer strains are concerned. Some authors report on low levels of viral proteins in the milk of presumably virus free low-cancer mouse strains [18, 22]. In their studies, the BALB/c strain is slightly positive, while in other [4, 17, 23] and our own investigations, this strain proves to be consistently negative for MuMTV in the milk. In our laboratory we have repeatedly tested BALB/c milk samples taken at various lactation periods and consistently found them to be negative. The quantity of MuMTV gp52 detectable with our method is so low that the discrepancy in results cannot be ascribed to the insensitivity of our technique. The difference in results must be due to either genetic differences among the various BALB/c sublines or to the measurement of nonviral artifacts. Some molecular-hybridization data obtained by Morris *et al.* [24] suggested the presence of an exogenous MuMTV in some BALB/c lines with a moderate mammary tumour incidence, including ours. Obviously, this hypothetical virus does not manifest itself during several lactation periods.

The CBA strain produces MuMTV in several laboratories, presumably due to the sudden activation of endogenous MuMTV [1]. Our subline, which is closely related to the one of he Netherlands Cancer Institute, is genetically quite distinct from most CBA sublines. It does not produce MuMTV. The AKR and RFM strains are not known to be MuMTV producers. The ND2 strain, which has been developed from an outbred germfree colony in Notre Dame University, Indiana, U.S.A., has never been shown to produce MuMTV in our laboratory.

Most mouse strains do not spontaneously release an endogenous MuMTV. However, in several so-called nonexpressor strains, significant quantities of MuMTV related RNA can be found in lactating mammary glands [25–28]. The absence of viral structural proteins in these glands indicates a genetically controlled inhibition at the translational level.

Except for one study [29], most laboratories report low numbers of MuMTV RNA molecules to be present in lactating mammary glands of BALB/c mice [25–28, 30]. The absence of viral polypeptides in this strain seems to be due to inhibition at the transcriptional level. The BALB/c strain is quite permissive to the expression of endogenous mammary tumour viruses of those mouse strains which release such viruses spontaneously [31]. It seems that, in F1 hybrids of BALB/c with some other virus negative strains, the genome of the BALB/c mouse allows the translation of the MuMTV-RNA molecules and subsequent processing of the precursor proteins to take place.

In genetic terms, dominant BALB/c genes complement the inhibition of virus expression caused by recessive genes of the three other mouse strains. An alternative explanation is that dominant genes from either AKR, RFM or DN2 allow the manifestation of the latent, exogenous MuMTV suspected to be present in our BALB/c colony [24].

Acknowledgements—Mr. D. S. Luyt is thanked for excellent technical assistance, Dr. F. Westenbrink and Mr. W. Koornstra for the development of the radioimmunoassay technique used in this laboratory.

REFERENCES

1. HILGERS J, BENTVELZEN P. Interaction between viral and genetic factors in murine mammary cancer. *Adv Cancer Res* 1978; **26**:143–195.
2. COHEN JC, VARMUS HE. Endogenous mammary tumour virus DNA varies among wild mice and segregates during inbreeding. *Nature* 1979; **278**:418–423.
3. VAN NIE R, VERSTRAETEN AA. Studies of genetic transmission of mammary tumor virus by C4Hf mice. *Int J Cancer* 1975; **16**:922–932.
4. BENTVELZEN P, BRINKHOF J, HAAIJMAN JJ. Genetic control of endogenous murine mammary tumour viruses reinvestigated. *Eur J Cancer* 1978; **14**:1137–1147.
5. NUSSE R, MICHALIDES R, BOOT LM, RÖPCKE G. Quantification of mouse mammary tumor virus structure proteins in hormone-induced mammary tumors of low mammary tumor mouse strains. *Int J Cancer* 1980; **25**:377–383.
6. BENTVELZEN P, BRINKHOF J. Ubiquity of natural antibodies to the mammary tumour virus in mice. *Arch Geschwulstf* 1980; **50**:193–203.
7. WESTENBRINK F, KOORNSTRA W, BENTVELZEN P. The major polypeptides of the murine-mammary tumor virus isolated by plant-lectin affinity chromatography. *Eur J Bioch* 1977; **76**: 85–90.
8. WESTENBRINK F, KOORNSTRA W, CREEMERS P, BRINKHOF J, BENTVELZEN P. Localization of murine mammary tumor virus polypeptides on the surface of tumor cells. *Eur J Cancer* 1979; **15**:109–121.
9. WESTENBRINK F, KOORNSTRA W. The purification and characterization of a major glycoprotein of the murine mammary tumor virus. *Anal Biochem* 1979; **94**:40–47.
10. WESTENBRINK F, KOORNSTRA W. Expression of MuMTV antigens in rabbits and rats infected with MuMTV. *Virology* 1979; **98**:493–496.
11. BENTVELZEN P, WESTENBRINK F, BROERSE JJ, VAN ZWIETEN MJ. Absence of proteins related to murine mammary tumour virus polypeptides in rat mammary tumours. *Eur J Cancer* 1981; **17**: 407–410.
12. GREENWOOD FC, HUNTER WM, GLOVER JS. The preparation of I-labelled human growth hormone of high specific radioactivity. *Biochem J* 1963; **89**:114–123.
13. WEBER K, OSBORN M. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. *J Biol Chem* 1969; **244**:4406–4412.
14. LOWRY OH, ROSEBROUGH NJ, FARR AL, RANDALL RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**:265–275.
15. MÜHBOCK O, BENTVELZEN P. The transmission of the mammary tumor virus. *Perspect Virol* 1968; **6**:75–87.
16. VERSTRAETEN AA, VAN NIE R. Genetic transmission of mammary tumour virus in the DBAf mouse strain. *Int J Cancer* 1978; **21**:473–475.
17. VERSTRAETEN AA, HAGEMAN PC, KWA HG. A radioimmunoassay for MTV-antigens. *Eur J Cancer* 1973; **9**: 155–157.
18. PARKS WP, HOWK RS, SCOLNICK EM, OROSZLAN S, GELDEN RV. Immunochemical characterization of two major polypeptides from murine mammary tumor virus. *J Virol* 1974; **13**:1200–1210.
19. NOON MC, WOLFORD RG, PARKS WP. Expression of mouse mammary tumor viral polypeptides in milks and tissues. *J Immunol* 1975; **115**:653–658.
20. RITZI E, MARTIN DS, STOLFI RL, SPIEGELMAN S. Plasma levels of a viral protein as a diagnostic signal for the presence of tumor: The murine mammary tumor model. *Proc Nat Acad Sci U.S.A.* 1976; **73**:4190–4194.
21. SHEFFIELD JB, DALY T, DION AS, TARASCHI M. Procedures for radioimmunoassay of the mouse mammary tumor virus. *Cancer Res* 1977; **37**:1480–1485.
22. IMAI S, HILGERS J. Levels of mammary tumor virus proteins (MTV p27 and MTV gp52) in the milk of low and high mammary cancer mouse strains of Japanese origin compared with European and American Strains. *Int J Cancer* 1979; **24**:359–364.
23. ALTROCK BW, CARDIFF RD. Mouse mammary tumor virus infections: viral expression and tumor risk. *J Nat Cancer Inst* 1979; **63**: 1075–1080.
24. MORRIS VL, VLASSCHAERT JE, BEARD CL, MILAZZO MF, BRADBURY WC. Mammary tumors from BALB/c mice with a reported high mammary tumor incidence have acquired new mammary tumor virus DNA sequences. *Virology* 1980; **100**:101–109.

25. VARMUS HE, QUINTRELL N, MEDEIROS E, NOWINSKI RC, SARKAR NH. Transcription of mouse MTV genes in tissue from high and low tumor incidence mouse strains. *J Mol Biol* 1973; **79**:663–679.
26. VAIDYA AB, LASFARGUES EY, SHEFFIELD JB, COUTINHO WG. Murine mammary tumor virus (MuMTV) infection of an epithelial cell line established from C57BL/6 mouse mammary glands. *Virology* 1978; **90**:12–22.
27. MICHALIDES R, VAN DEEMTER L, NUSSE R, VAN NIE R. Identification of the MTV-2 gene responsible for the early appearance of mammary tumors in the GR mouse by nucleic acid hybridization. *Proc Nat Acad Sci U.S.A.* 1978; **75**:2368–2372.
28. MICHALIDES R, VAN DEEMTER L, NUSSE R, RÖPCKE G, BOOT L. Involvement of mouse mammary tumor virus in spontaneous and hormone-induced mammary tumors in low-mammary-tumor mouse strains. *J Virol* 1978; **27**:551–559.
29. MCGRATH CM, MARINEAU EJ, VOYLES BA. Changes in MuMTV DNA and RNA levels in BALB/c mammary epithelial cells during malignant transformation by exogenous MuMTV and by hormones. *Virology* 1978; **87**:339–353.
30. PAULEY RJ, ROSEN JM, SOCHER S. Mammary tumour virus and casein gene transcription during mouse mammary development. *Nature* 1978; **275**:455–457.
31. BENTVELZEN P. Host-virus interactions in murine mammary carcinogenesis. *Bio. chem Biophys Acta* 1974; **355**:236–259.