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Expression of Mammary Tumour Virus in Late-Appearing Mammary Carcinomas in Presumed Virus-Free Mice*

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Abstract—*Mammary tumours arising in mice which had been tested for the presence of mammary tumour virus (MuMTV) in the milk were tested for either the presence of infectious MuMTV by bioassay in female BALB/c mice or for the presence of the major MuMTV envelope glycoprotein gp52. In crosses involving the high cancer strain GRS, 16/17 tumours appearing before 1 yr of age contained a virulent virus. Only 2/19 of the late appearing tumours in virus positive mice, harboured a virus which induced tumours at an early age but 12 contained an infectious late-oncogenic MuMTV. In crosses involving the C3Hf mouse strain which develops tumours at a late age, only 2/28 tumours in virus positive mice contained a virulent MuMTV but 15 harboured a late-oncogenic strain. Of the 22 tumours which arose in so-called virus-negative mice in all crosses, 7 harboured an infectious MuMTV; it was highly virulent in 3 cases. In the immunoassay, all 41 tumours which developed in mice with virus positive milks were also positive for gp52. The majority (25/39) of tumours arising in virus negative mice were positive for the viral antigen, although the quantities were relatively low.*

INTRODUCTION

In an earlier study we have analyzed the genetical factors which controlled the release of endogenous murine mammary tumour virus (MuMTV) in some mouse strains [1]. It was found that virus release at a young age corresponded with an increased risk for mammary tumour development, but an appreciable number of so-called virus-free mice developed a mammary carcinoma at a late age.

The aim of the present investigation was to obtain evidence for switching on of MuMTV in these tumours. Initially, this was attempted by bioassay of cell-free extracts in female BALB/c mice. In a second phase, tumours were screened for the presence of the major viral envelope glycoprotein gp52.

MATERIALS AND METHODS

Mice

The strains BALB/c, C57BL, C3Hf and GRS were obtained from the breeding colony of the Radiobiological Institute TNO. For the crossing program, the maintenance of the animals and virus testing, see an earlier publication [1]. Tumours were taken at autopsy of the mice and immediately stored in liquid nitrogen.

Bioassay

After thawing, tumours were homogenized in phosphate buffered saline (5 ml/g of tumour) with a Sorvall ominimixer at 19,000 rev/min for 1 min. The suspension was spun at 20,000 rev/min in a Sorvall RC2-B superspeed centrifuge. All steps were carried out 4°C. The cell-free supernatant was collected and immediately injected i.p. into 4-week-old BALB/c mice (0.5 ml per animal). Twenty BALB/c mice were used for each tumour. Two weeks after the injection, the mice were subjected to forced breeding. The experiments were terminated when the females were 2 yr of age.

Immunoassay

A rabbit antiserum to purified gp52 absorbed with normal mouse serum and fetal calf serum and which has been shown to be highly

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specific for gp52 [2], was used in the Sepharose bead immunofluorescence assay.

The protein content of cell-free extracts of the mammary tumours was determined by the fluoram method [3] and subsequently equalized to 5 mg/ml using PBS with 2% Tween 80.

For comparison, a cell-free extract was prepared from a spontaneous rat mammary carcinoma to which was added purified MuMTV (0.07 mg/ml). Subsequently, 2-fold dilutions were made in PBS–Tween. The results obtained with this mixture in the Sepharose bead immunofluorescence assay functioned as reference for those data obtained with the murine tumours (Fig. 1).

In crosses involving the GRS strain which genetically transmits the virulent MuMTV-P [1], 16 out of 17 tumours which appeared before 1 year contained a virulent MuMTV. The incidences in the corresponding BALB/c groups at 1 year were at least 40%.

In the second category of tumours arising after the 1-year cut-off point in virus positive mice, only 2/19 contained a virulent virus. However, 12 other tumours induced an excess of tumours of over 60% at 2 yr, which might indicate the presence of a late-oncogenic MuMTV. Of the 8 tumours which arose, in animals which had virus negative milks, two were positive for an oncogenic MuMTV; one of these could be regarded as virulent.

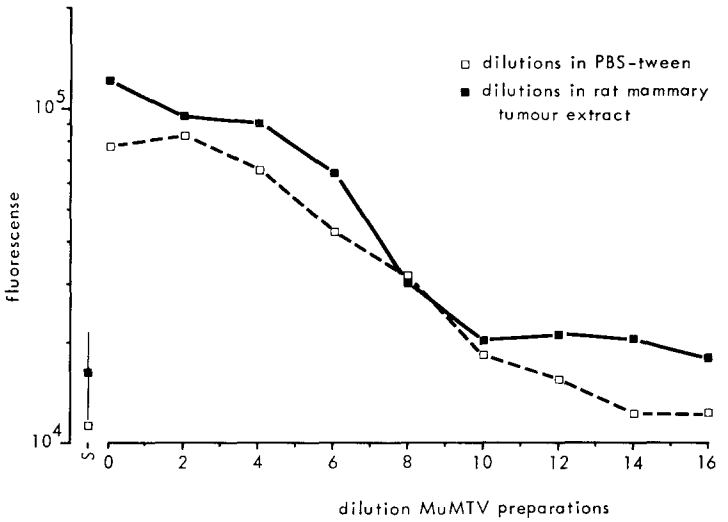

Fig. 1. Influence of an extract from a rat mammary tumour on titration of purified murine mammary tumour virus in the Sepharose bead immunofluorescence assay.

RESULTS

Bioassays

The tumour-inducing capacity of cell-free extracts from several tumours in BALB/c mice is presented in Table 1. The extracts were regarded as containing an infectious MuMTV when at least 60% of the injected groups of BALB/c mice developed mammary tumours. During this 4-year study, 8 separated groups of at least 25 untreated BALB/c mice were subjected to forced breeding. Tumour incidences ranged from 12 to 36%.

A virulent MuMTV strain was defined as one causing more than 20% mammary tumours in mice before 1 yr of age. Of the 337 control BALB/c mice only 3 developed a mammary carcinoma before that age.

In the series of crosses involving the C3Hf mouse strain (and excluding the GRS strain) several tumours appeared before 1 yr of age. However, from none of the 5 early tumours tested was a virulent virus retrieved. In 2 of the 23 tumours which appeared at a relatively late age in virus positive mice, a virulent MuMTV was shown to be present, while 13 others contained a virus that induced tumours at a late age. An infectious MuMTV was detected in 5/14 tumours which developed in so-called virus negative mice; two of these viruses proved to be virulent.

The results of the immunoassay for gp52 of another set of tumours are presented in Table 2. Those tumours which were scored as positive in this immunoassay had values con-

Table 1. Bioassay of mammary tumors for the presence of an infectious MuMTV

♀	Cross	♂	Virus status*	Tumor appearance†	No. of tumors tested	No. positive MuMTV‡	No. with virulent MuMTV§
1. C57BL	C57BL × GRS		+	early	6	5	5
			+	late	4	2	0
			−	late	2	0	0
2. C57BL	BALB/c × GRS		+	early	2	2	2
			+	late	3	3	1
3. BALB/c	BALB/c × GRS		+	early	3	3	3
			+	late	7	5	1
			−	late	3	1	1
4. BALB/c	C57BL × GRS		+	early	1	1	1
			+	late	2	1	0
			−	late	1	0	0
5. BALB/c	C3Hf × GRS		+	early	5	5	5
			+	late	3	3	0
			−	late	2	1	0
6. BALB/c	BALB/c × C3Hf		+	early	2	2	0
			+	late	6	4	1
			−	late	3	1	1
7. C3Hf	BALB/c × C3Hf		+	early	1	0	0
			+	late	7	4	0
			−	late	2	2	0
8. C3Hf	C57BL × C3Hf		+	late	3	2	0
			−	late	2	1	0
9. C57BL × C3Hf	C57BL × C3Hf		+	late	1	0	0
			−	late	4	0	0
10. BALB/c	C57BL × C3Hf		+	early	2	2	0
			+	late	6	5	1
			−	late	3	1	1

*Viral antigens in milk at 5–6 months of age.
†Early is tumor before 1 yr of age.
‡Positive is at least 60% tumors after 2 yr in recipients.
§Virulent is more than 20% tumors before 1 yr in recipients.

Table 2. Presence of gp52 in mammary tumors obtained in several crosses

Cross No.*	Virus status†	No. of tumors tested	No. negative	No. with less than 30 ng gp52‡	No. with 30–100 ng gp52‡	No. with > 100 ng gp52‡
1	+	6			1	5
	−	1	1			
2	+	6				6
3	+	3				3
	−	2	1		1	
4	+	3				3
	−	4	2		2	
5	+	4			1	3
	−	3	2	1		
6	+	5				5
	−	5	2	1	2	
7	+	6				6
	−	5	1	1	2	1
8	+	3				3
	−	4	2	1	1	
9	+	3			1	2
	−	2	1	1		
10	+	2				2
	−	13	2	10	1	

*For nature of crosses, see Table 1.
†MuMTV antigens in milk at 5–6 months of age.
‡per mg/ml protein.

siderably higher than background levels (more than 5 S.D.).

All 41 tumours which arose in mice which were positive for MuMTV in their milk at earlier testing [1] were positive for the MuMTV glycoprotein gp52. The tumours contained more than 30 ng of gp52 per mg tissue protein. Of the 39 tumours which arose in so-called virus-negative mice, 25 were positive for gp52, although the values were generally lower (less than 30 ng/mg tissue protein).

DISCUSSION

Molecular hybridization studies revealed that every mouse strain tested so far contains multiple proviral copies of MuMTV in its normal cellular DNA [4, 5]. A number of host genes which control the release of endogenous MuMTV have been identified [1, 6, 7].

Germinal mutations in such controlling genes, leading to spontaneous release of MuMTV, are associated with an increased risk for mammary tumour development [1]. In the GRS strain, one may even regard mammary carcinoma as being an hereditary disease, due to the action of the *Mtv-2* gene which is responsible for the release of large amounts of MuMTV-P [1, 7].

In the previous study a substantial number of mice which were negative for MuMTV antigens in their milk nevertheless developed a mammary neoplasm [1]. This finding prompted the present investigation on whether these tumours would result from the switching-on of endogenous MuMTV at a late age, as has been occasionally observed in the BALB/c mouse strain [8, 9].

As was to be expected, the majority of early tumours in crosses involving the GRS strain contained a potent MuMTV, while only a few of the late tumours harboured such a virus. Most of the tumours which arose at a late age in mice with relatively small amounts of MuMTV antigens in the milk contained an infectious MuMTV but usually of the late-oncogenic type. The later appearance of mammary neoplasms in recipient BALB/c mice under influence of the extracts from late tumours cannot be due to the small quantities of virus in the inoculum, since concentrated samples of MuMTV-L also induce a high incidence of tumours in BALB/c mice but at a late age [1, 8].

In the crosses involving the C3Hf mouse strain (except for the one in which the GR strain was also involved), a somewhat different pattern is found. None of the tumours arising before 1 yr of age contains an agent which can induce early mammary neoplasms on inoculation into BALB/c mice. It seems that the release of MuMTV-L by the C3Hf mouse genome in combination with the extreme susceptibility of the BALB/c mouse genome leads to the early appearance of many tumours, while exogenous infection of BALB/c with MuMTV-L only evokes the late appearance of many tumours.

The major finding is that a significant minority of tumours which developed in 'virus-free' mice contained an infectious MuMTV. It is an attractive assumption that these tumours resulted from activation of endogenous MuMTV at a late age.

In the immunoassay, all tumours which arose in virus positive animals were positive for the MuMTV glycoprotein. More interestingly, the majority of tumours (25/39) which arose in so-called virus negative animals were also positive for gp52 of MuMTV. Again, it is attractive to assume that the latter group of tumours result from switching-on of virus in ageing mice.

Still, a substantial number of tumours did not contain MuMTV specific proteins. Partial switching-on of endogenous MuMTV, viz. of the presumed *mam*-gene [10], might possibly be responsible for the genesis of these tumours. It must be kept in mind, however, that cellular genes other than those from endogenous MuMTV can be involved in the maintenance of the transformed state of mammary cells.

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