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Absence of Antigens Related to Murine Mammary Tumour Virus Polypeptides in Rat Mammary Tumours*

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Abstract—*Fifty-two rat mammary tumours were tested by radioimmunoassay for the presence of antigens related to the envelope glycoprotein gp52 and core protein p28 of the murine mammary tumour virus and found to be negative. The tumours assayed were from WAG/Rij* (16 *cases*), *BN/BiRij* (16 *cases*) *and Sprague–Dawley* (20 *cases*) *rats. Thirty of the rats had been irradiated with fast neutrons*, 16 *with X-rays, one had been implanted with a* 17-β-*oestradiol pellet and* 5 *were untreated. The tumours studied included* 22 *fibroadenomas*, 2 *adenomas*, 19 *adenocarcinomas*, 4 *sarcomas*, 1 *carcinosarcoma, and* 4 *of undetermined type.*

INTRODUCTION

MAMMARY gland tumours develop in mice and rats under the influence of a complex of etiological, promoting and permissive factors. Oestrogenic hormones are of prime importance in the induction of these neoplasms [1, 2], although their carcinogenic action may be partly due to the release of the pituitary mammotropic hormone prolactin [3]. Exogenous carcinogens such as various chemicals [4, 5] or ionizing radiation [6, 7] are known to induce mammary tumours in either rodent species.

In the house mouse an RNA tumour virus plays a dominant role in the genesis of early mammary carcinomas [8]. Normal cellular DNA of every inbred mouse strain tested so far contains some proviral copies of this murine mammary tumour virus (MuMTV) [8, 9]. In a few strains the etiologic role of such endogenous MuMTV-s has been well documented [8]. Nonviral carcinogens as well as excessive hormonal stimulation induce the expression of endogenous MuMTV in the mouse mammary gland [10–12]. Mammary carcinomas arising in old, presumed virus-free, mice often contain MuMTV polypeptides [13]. There is no unequivocal evidence, however, for the etiological role of endogenous MuMTV in environmental or 'spontaneous' mammary carcinogenesis in mice from low-MuMTV-expressor strains.

In several other species of the rodent genus *Mus* a retrovirus distantly related to MuMTV has been detected [14]. Molecular hybridization studies revealed that under relaxed condition sequences could be detected in normal rat DNA which are homologous to MuMTV [5]. In view of a report that a retrovirus might be operational in radiation-induced rat mammary tumours [16], we have screened a number of rat mammary tumours for the presence of antigens related to MuMTV-polypeptides by means of radioimmunoassay.

MATERIALS AND METHODS

Tumours

The mammary tumours assayed were taken from female rats, which form a part of an extensive study in the effects of irradiation with X-rays and fast neutrons of different

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energies and oestrogenic hormone administration on mammary carcinogenesis in the rat [17, 18]. Three rat strains are studied, including the inbred WAG/Rij and BN/BiRij strains, and the partially-inbred Sprague–Dawley strain. The tumours were taken at necropsy and a 1–5 g portion was stored in liquid nitrogen until assayed. The remaining portions of the tumours were fixed in 10% neutral buffered formalin, embedded in paraffin, and 5 μm sections were stained with hematoxylin phtoxine–saffron.

Viral proteins

Murine mammary tumour virus produced in tissue culture by the Mm5mt/cl cell line [19] was provided through the Office Research Resources of the Biological Carcinogenesis Branch, National Cancer Institute, Bethesda, MD, U.S.A. From the virus preparations solubilized by nonionic detergents the glycoprotein with a molecular weight of 52,000 daltons (gp52) was isolated by means of Concanavalin A affinity chromatography followed by gel filtration on Sephadex G150. The major core protein with a molecular weight of 28,000 daltons (p28) was purified from the material unbound to the plant lectin by means of chromatography on phosphocellulose [20]. Antisera were raised against the purified polypeptides in rabbits and after absorption with fetal calf serum tested in several immunoassays and proven to be highly specific [21].

Radioimmunoassay

After thawing, tumour homogenates (10% w/v) were made with a Sorvall Omnimixer at 19,000 rev/min for 1 min in TEN-buffer (20 mM Tris–HCl, pH 7.6, 1 mM EDTA, 100 mM NaCl) containing 0.2% Triton X-100, 2 mg.ml^{-1} bovine serum albumin and 300 mg.ml^{-1} of protease inhibitor phenylmethylsufonylfluoride. The latter compound was added shortly before use from a stock solution containing 20 mg.ml^{-1} in isopropanol. The homogenates were centrifuged in a Sorvall RC-5 centrifuge for 30 min at 15,000 rev/min. Two-fold serial dilutions were made of the supernatant in the TEN-buffer + additions as described above. To 10 μl of test sample was added 40 μl of buffer and 10 μl of rabbit antiserum to gp52 or p28 of MuMTV at a dilution sufficient to precipitate 50% of the input radioactivity. After an incubation period of 2 hr at 37°C, 40 μl of iodinated gp52 or p28 in TEN buffer containing 6.5 mg.ml^{-1} 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 pig anti-rabbit immunoglobulin serum was added. Incubation was continued for 1 hr at 37°C and overnight at 4°C. Thereafter, 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.

RESULTS AND DISCUSSION

The good sensitivity of the radioimmunoassay employed in this study can be concluded from Fig. 1. When purified MuMTV, disrupted by the non-ionic detergent NNP10, is added as competing antigen 50% displacement is found at 0.63 ng gp52 in the virus preparation. When lactating mammary glands of the GRS mouse strain, which produces large quantities of endogenous MuMTV, are tested in this assay 1.2 μg gp52 is found per mg tissue protein. When extra MuMTV is added to the GRS mammary gland extract a shift of the competition curve is found to the position expected on the basis of adding the values of isolated MuMTV to that of the mammary gland. Competition with WAG/Rij rat mammary gland extract showed a slight reduction of the percentage ^{125}I-labelled gp52 precipitated at excess protein. This effect is considered to be nonspecific [21]. The mixing of the rat tissue extract and MuMTV did not result in a decrease of the quantity of gp52. This also did not occur when MuMTV was mixed with rat liver or a mammary tumour which was negative for MuMTV-gp52 (data not shown). Similar results were obtained for MuMTV-p28.

The rat mammary tumours studied included 22 fibroadenomas, 2 adenomas, 19 adenocarcinomas, 1 carcinosarcoma and 4 sarcomas. In 4 additional cases, multiple mammary tumours were present (e.g. fibroadenoma and adenocarcinoma) and the tumour type assayed could no longer be determined. Thirty of the 52 tumours were from neutron-irradiated rats, 16 from X-irradiated rats, 1 from a rat which had been implanted with a 17-β-oestradiol pellet, and 5 were from untreated control rats. Sixteen of the tumours were from WAG/Rij rats, 16 from BN/BiRij rats and 20 from Sprague–Dawley rats. None of these tumours gave a positive result in the radioimmunoassay for either viral polypeptide.

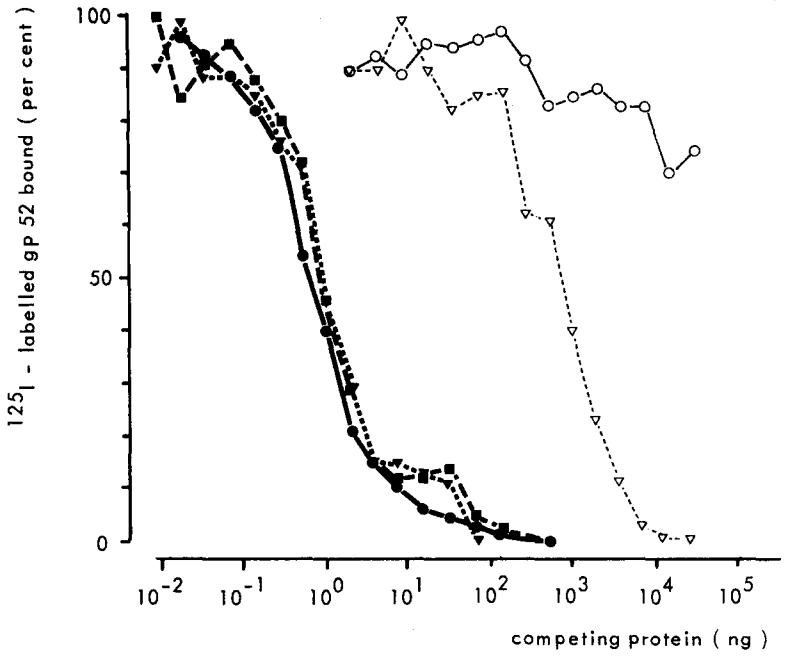

Fig. 1. Influence of organ extracts on competition radioimmunoassay for the murine mammary tumour virus polypeptide gp52. (■) *Disrupted MuMTV only,* (▽) *GRS mouse mammary gland extract,* (●) *GRS extract+MuMTV,* (○) *WAG/Rij rat mammary gland extract,* (▼) *WAG/Rij extract + MuMTV.*

These negative findings indicate that if the observed partial homology between MuMTV and normal rat DNA [15] concerns a somewhat related endogenous rat virus, either the *gag* and *env* genes of the two viruses are not homologous or these genes are not expressed in rat mammary tumours. It is possible that homology is found at the 3′-end of the viral genome which harbours the *mam*-gene, presumably active in the neoplastic conversion of mammary cells [8]. The most likely conclusion, however, is that no virus related to the murine mammary tumour virus is active in rat mammary carcinogenesis.

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