Eur J Cancer Clin Oncol, Vol. 19, No. 9, pp. 1255–1263, 1983.
Printed in Great Britain.

0277-5379/83$3.00+0.00

Sera from Irradiated Rats Contain Antibodies to a Ubiquitous Tumour-associated Antigen*

P. BENTVELZEN,† M. A. DUBBELD,† W. H. KOORNSTRA,† J. J. BROERSE† and M. J. VAN ZWIETEN‡
†*Radiobiological Institute TNO and* ‡*Institute for Experimental Gerontology TNO, Rijswijk, The Netherlands*

Abstract—*Female rats of the inbred strains BN/BiRij and WAG/Rij were irradiated with* 300 kV *X-rays, or* 15 MeV *or* 0.5 MeV *fast neutrons. Sera were collected several months after irradiation and found to be negative for antibodies reacting with the murine mammary tumour virus as tested by a solid-phase radioimmunoassay and an immunofluorescence absorption test. We found, however, that in an immunofluorescence assay several sera from irradiated rats reacted with a cytoplasmic antigen in rat mammary, ureter and skin carcinoma cell lines as well as a mouse mammary tumour and a transformed BALB/3T3 mouse fibroblast line. No reaction was found with normal fibroblast cell lines of rat or murine origin. Endpoint titres of the sera on tumour cells ranged from* 1:20 *to* 1:160. *Of* 48 *sera from unirradiated rats* 18 *also stained tumour cells, but usually at the low dilution of* 1:10. *Irradiation seems to enhance antibody activity to a ubiquitous tumour-associated antigen.*

INTRODUCTION

RATS HAVE been found to contain sequences in their normal cellular DNA which are partially homologous to the genome of the murine mammary tumour virus (MuMTV) [1, 2]. Since endogenous viral sequences may play a role in mammary carcinogenesis in mice [3–5], it is worthwhile investigating the possible role of the rat endogenous sequences in the development of mammary tumours.

We have reported earlier that rat mammary tumours of different aetiologies and histopathological appearance did not contain antigens which were related to MuMTV polypeptides [6]. This negative finding does not exclude the possibility, however, that transient expression of endogenous virus initiates the oncogenic process in the mammary gland. Imai *et al.* [7] reported that sera from neutron-irradiated rats have antibodies which react with MuMTV. They detected these antibodies with an immune adherence haemagglutination test and an immunofluorescence assay. In this study we could not detect antibodies to MuMTV in sera from irradiated rats by means of a highly sensitive and specific solid-phase radioimmunoassay. Using an immunofluorescence test, a reaction was found not only with mouse mammary tumour cells but also with other kinds of rodent tumour cell lines. No reaction was found with continuous normal cell lines.

Accepted 1 March 1983.
*This study was financially supported in part by the Koningin Wilhelmina Fonds, the Netherlands Cancer Organization, and Euratom.

MATERIALS AND METHODS

Animals

Female rats of the inbred strains WAG/Rij and BN/BiRij were used in this study. The irradiated animals were included in an extensive research program on radiation carcinogenesis of the mammary gland in rats [8]. These strains are bred in the barrier-maintained animal colony of our institutes. The background of the strains and the animal husbandry methods employed have been described before [9, 10]. The animals were irradiated at 8 weeks of age with 300 kV X-rays, or 15 MeV or 0.5 MeV fast neutrons.

Three-week-old female BN/BiRij rats were inoculated i.p. with 1 ml of a 10% cell-free extract from a virally induced BALB/cfC3H mouse mammary tumour. Twice a week thereafter the rats were injected with a similar freshly prepared tumour extract. After the 9th injection the animals were caged with (WAG × BN)F1 hybrid males. Milk samples were tested for the presence of the MuMTV envelope glycoprotein gp52 by

competitive radioimmunoassay [11]. Sera were collected from virus-positive animals 6 months after the last injection.

Cells

The following continuous cell lines were used: C3HMT/cl11, from a mouse mammary tumour [12]; C3H10T1/2, from normal mouse fibroblasts [13]; BALB/3T3, from normal mouse embryonic cells [14]; B3-SDT, a BALB/3T3 subline transformed by normal Sprague–Dawley rat DNA; WRE, from normal rat embryonic fibroblasts; R-1, from a rat rhabdomyosarcoma [15]; RUC-1, from a rat ureter carcinoma [16]; RSC-1, from a rat skin carcinoma [16]; SDRijMT/cl2-5, from a rat mammary carcinoma; and WRijMT/cl3, from a rat mammary carcinoma. The C3H10T1/2 line was grown in Eagle's basal medium, while all other cells were grown in Dulbecco's modification of that medium. Culture media were supplemented with 10% foetal calf serum. The cells were passaged by means of trypsinization when saturation density was reached, except for BALB/3T3 and C3H10T1/2, for which prescribed passage procedures were followed.

Antisera

Hyperimmune antiserum to the isolated viral core protein p28 of MuMTV was raised in a rabbit, absorbed with normal mouse serum and foetal calf serum and tested in various immunologic assays as previously described [12]. For the production of rat antisera to MuMTV, tissue-culture-derived virus (provided by the Biological Carcinogenesis Branch, National Cancer Institute, Bethesda, MD, U.S.A.) was first solubilized by incubation with 1% of the non-ionic detergent NNP 10 (Serva, Delden, The Netherlands) in PBS for 15 min at room temperature. The preparation was then diluted in PBS to a concentration of 200 μg viral protein/ml. Six-month-old female WAG/Rij rats were injected intramuscularly with 50 μg disrupted MuMTV emulsified in 0.25 ml complete Freund's adjuvant. This procedure was repeated 3 weeks later; 2 weeks thereafter the animals were injected with MuMTV emulsified in incomplete Freund's adjuvant. The animals were bled 3 weeks later.

Immunofluorescence

Microscope slides with wells (Toxoplasmosis slides, Bellco, Vineland, NJ, U.S.A.) were washed for 1 hr with 5% Decon (Decon Labs, Hove, U.K.) in distilled water. The slides were then rinsed with distilled water, dried with a cotton tip and sterilized overnight at 100°C. Freshly trypsinized cells were deposited in the wells of these slides: $1–5 \times 10^4$ cells per 50-μl droplet of growth medium. The slides were incubated for 24 hr at 37°C with 5% CO_2. In the case of the cell line C3HMT/cl11, 10^{-5} M dexamethasone was added. They were then washed ×3 with PBS at 4°C for 10 min. The cells were fixed with acetone at −20°C for 10 min and then washed as above. The slides were stored at −20°C. The cells were incubated with 2-fold dilutions of sera, beginning at 1:10, for 1 hr at 37°C and then washed as above, while the wash fluid was gently stirred. The slides were dried in air and the cells were then incubated for 1 hr at 37°C with a goat anti-rat immunoglobulin serum conjugated with fluorescein-isothiocyanate (Nordic, Tilburg, The Netherlands) diluted ×20. After washing the slides were covered with 50% glycerol in PBS and a cover slip and then sealed with nail polish. The slides were examined with a Leitz Orthoplan microscope using an FL40×/1.30 objective and GW ×4 oculars. Epi-illumination was accomplished with a mercury arc HBO 100W/2. The filtres used were 2 × KP490 + 1 mm GG 455 and K515.

Virus

One litre of cell-free tissue culture supernatant of C3HMT/cl11 cells was concentrated 10-fold with an Amicon CH3 apparatus using HIP100 hollow fibres. The concentrate was layered on top of a discontinuous sucrose gradient (5 ml 50% and 10 ml 20%) and centrifuged in a SW27 rotor for 3 hr at 140,000 *g* in a Beckman ultracentrifuge. The sharp light-scattering band above the 50% sucrose fraction was collected, resuspended in PBS, layered on top of a continuous sucrose gradient (20–50%) and again subjected to ultracentrifugation for 3 hr at 140,000 *g*. The fraction at a density of 1.16–1.18 was collected and stored at −20°C.

Absorption

Ten microlitres of solubilized MuMTV (10 μg protein) were added to 0.1 ml serum. The mixture was gently stirred at room temperature for 2 hr. Precipitates were removed by centrifugation at 12,000 *g* for 15 min. Adjustment of serum dilutions for immunofluorescence was made in such a way that they corresponded to the dilutions of unabsorbed sera.

Solid-phase radioimmunoassay

A modification of the technique of Colombatti and Hilgers [17] was used. Wells of Falcon microtest II tissue culture plates containing 2 μg solubilized MuMTV in 50 μl PBS + 0.1% NNP10 were incubated at 37°C for 16 hr; 125 μl of a 1% bovine serum albumin (BSA) solution in PBS was then added and incubation continued at 37°C for 2 hr. Thereafter the wells were washed ×3 for 10

min in cold PBS with 1% BSA and 0.1% NNP10. The wells were then incubated with 50 μl rat serum diluted in PBS and 0.1% NNP10 at 37°C for 45 min. They were then washed ×3 with PBS + 0.1% NNP10. Subsequently the wells were incubated for 45 min at 37°C with a rabbit antiserum to rat immunoglobulins (provided by Dr. R. L. Coffman, DNAX, Palo Alto, CA, U.S.A.) and conjugated with ^{125}I according to the iodogen method [18]. If normal rabbit serum or rabbit antiserum to MuMTV-p28 were used, the wells were later incubated with purified protein A from *Staphylococcus aureus* (Pharmacia, Uppsala, Sweden) conjugated with ^{125}I.

The quantity of radioactivity added was about 17,500 pCi per well. After incubation, the wells were washed ×3 with PBS and 0.1% NNP10 and then 100 μl 4N NaOH was added. On the next day the radioactivity of the contents of the wells was determined in a gamma counter. As a control, a rabbit antiserum to the internal core protein p28 of MuMTV was used. Another control involved the testing of the rat sera on wells coated with BSA.

RESULTS

In the indirect immunofluorescence tests on acetone-fixed C3HMT/cl11 cells derived from a mouse mammary tumour a rabbit antiserum to the core protein p28 of MuMTV still stained the cells at a 1:12,500 dilution, while the syngeneic fibroblast line C3H10T1/2 was stained only at dilutions lower than 1:40. The serum from an infected rat still reacted with the mammary tumour cells at dilution 1:640, while the fibroblasts were negative at a serum dilution of 1:20.

As can be concluded from Table 1, 33 of 67 sera from irradiated rats reacted with C3HMT/cl11. None of the sera reacted with the syngeneic

Table 1. Reactivity of sera from irradiated rats with mouse mammary tumour cells in an immunofluorescence test

	Group	Radiation treatment	No. of animals	Time serum taken after irradiation (months)	No. positive on C3HMT/cl11	Endpoint titres
BN	I	0.4 Gy X-rays	7	4	4	1:20 (×2), 1:80; 1:160
	II	0.4 Gy X-rays	4	12	1	1:80
	III	1 Gy X-rays	3	23	1	1:160
	IV	1.6 Gy X-rays	5	3	2	1:40, 1:80
	V	4 Gy X-rays	2	24	1	1:20
	VI	0.15 Gy 15 MeV neutrons	4	10	1	1:80
	VII	0.5 Gy 15 MeV neutrons	3	25	1	1:160
	VIII	5 × 0.02 Gy 0.5 MeV neutrons	4	10	2	1:40, 1:160
			32			
WAG	I	0.08 Gy X-rays	4	9	2	1:10, 1:20
	II	0.4 Gy X-rays	4	8	1	1:40
	III	1.6 Gy X-rays	8	8	4	1:20 (×2), 1:40, 1:80
	IV	10 × 0.04 Gy X-rays	4	8	3	1:40 (×2), 1:160
	V	20 × 0.02 Gy X-rays	4	7	3	1:20, 1:40 (×2)
	VI	09.1 Gy 0.5 MeV neutrons	4	7	3	1:20 (×2), 1:40
	VII	0.15 Gy 15 MeV neutrons	3	25	1	1:40
	VIII	10 × 0.01 Gy 0.5 MeV neutrons	4	7	3	1:20 (×2). 1:160
			35			

Table 2. Reactivity of control rat sera with mouse mammary tumour cells in an immunofluorescence test

Strain	Age at blood sampling (months)	No. of animals	No. positive on C3HMT/cl11	Endpoint titres
BN/BiRij	6	7	2	1:10 (×2)
	12	4	3	1:10 (×3)
	24	3	1	1:40
WAG/Rij	4	25	5	1:10 (×5)
	12	4	4	1:10 (×3), 1:20
	24	5	3	1:80 (×3)

C3H10T1/2 fibroblast line. Titres on C3HMT/cl11 cells ranged from 1:20 to 1:160. In Table 2 the reactivity of sera from various groups of unirradiated animals is presented. The positive reactions of sera from animals younger than 1 yr of age were usually detectable only at the low dilution of 1:10. All of those 2-yr-old animals of which the sera reacted with C3HMT/cl11 cells at a relatively high dilution (1:40–1:80) had a mammary tumour at the time of blood sampling. None of the sera from unirradiated animals reacted with the normal C3H10T1/2 fibroblast line in the immunofluorescence test. In summary, a substantial number of irradiated rats had antibodies in their serum which reacted with a mouse mammary tumour line. The titre of these antibodies was substantially higher than in the positive sera from control animals.

The positive reaction with C3HMT/cl11 mammary tumour cells in contrast to the negative reaction with syngeneic C3H10T1/2 normal fibroblasts might indicate the presence of antibodies to the murine mammary tumour virus polypeptides. Incubation of the sera from four irradiated rats with disrupted virus did not affect the reactivity with C3HMT/cl11 cells, however. For instance, the serum from a BN/BiRij rat irradiated with 0.4 Gy of X-rays and bled 4 months after irradiation still had a titre of 1:80, while the titre in the serum of an infected BN/BiRij rat was reduced from 1:640 to 1:20 by this absorption procedure.

In the solid-phase radioimmunoassay a hyperimmune rabbit antiserum directed against the core protein p28 of MuMTV gave very strong reactions with the virus when compared with bovine serum albumin (BSA), as can be concluded from Fig. 1. The titre of this antiserum as expressed as that serum dilution which precipitates 50% of maximal detectable radioactivity is 1:16,400. The rat antiserum to MuMTV strongly reacted with MuMTV but also with BSA, although the difference in reactivity with the two antigens is still very great (Fig. 2). The serum from an infected BN/BiRij rat did not react so strongly with MuMTV but considerably more than with BSA (Fig. 3). The sera from an uninfected rat and a neutron-irradiated rat did not react with MuMTV (Fig. 4). None of the 67 sera from the irradiated animals reacted with MuMTV. Three control sera from 6-month-old BN/BiRij rats and three sera from unirradiated 6-month-old WAG/Rij rats also did not bind to MuMTV. Four sera from irradiated rats have been tested with this technique on the purified MuMTV polypeptides p28, p12, gp52 and gp36 and no reaction was found with either (results not shown).

All sera from irradiated rats which reacted with C3HMT/cl11 as well as some negative ones were tested by indirect immunofluorescence on several other cell lines such as the rat mammary tumour line SDRijMT/cl2-5 (see Fig. 5). As can be concluded from Table 3, the sera which stained the murine mammary tumour line also reacted

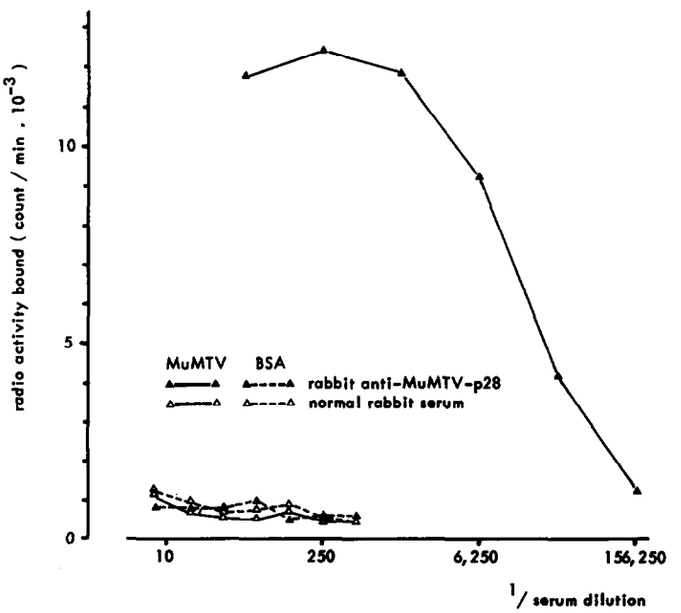

Fig. 1. Solid-phase radioimmunoassay with a rabbit antiserum to the core protein p28 of murine mammary tumour virus.

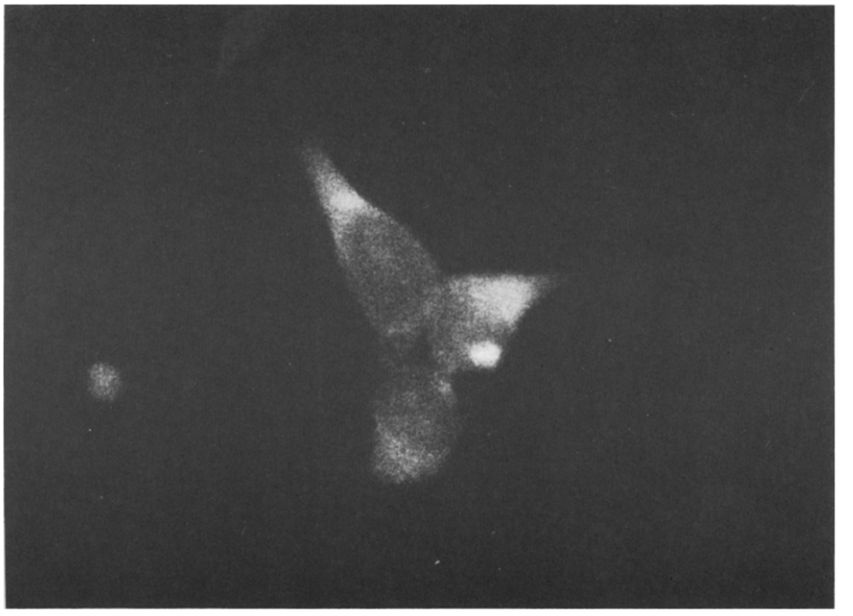

Fig. 5. Indirect immunofluorescence test on acetone-fixed SDRijMT/c12-5 rat mammary tumour cells with serum from a 15 MeV *neutron-irradiated BN/BiRij rat taken* 10 months *after irradiation.*

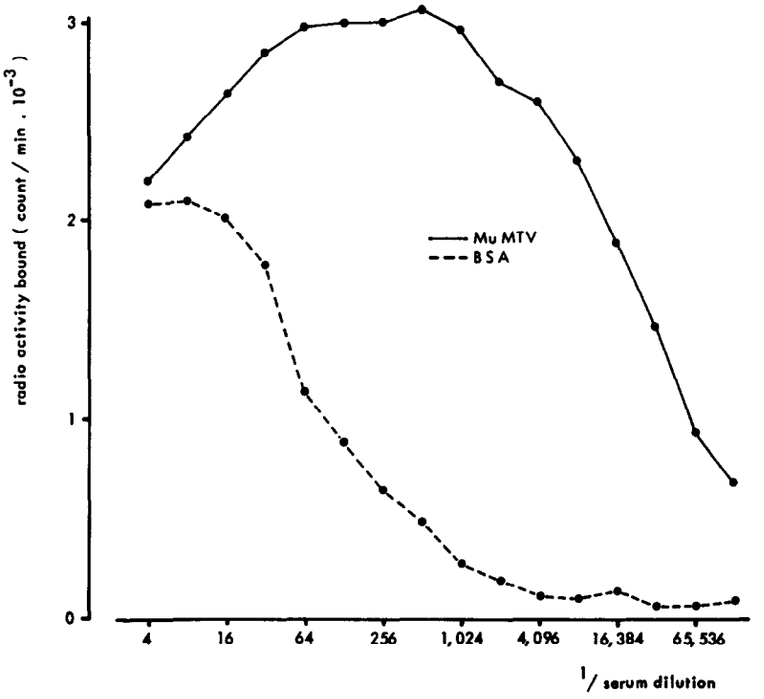

Fig. 2. Solid-phase radioimmunoassay with a rat antiserum to solubilized murine mammary tumour virus.

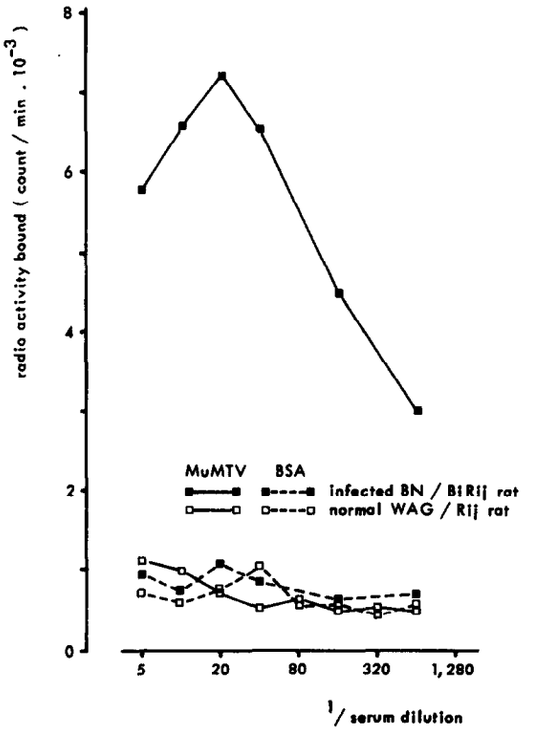

Fig. 3. Solid-phase radioimmunoassay for the detection of antibodies to the murine mammary tumour virus in the serum of an infected BN/BiRij rat.

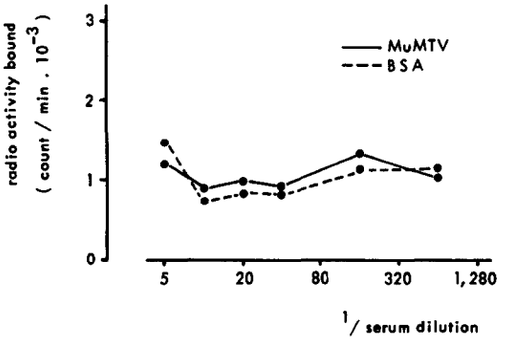

Fig. 4. Solid-phase radioimmunoassay for the detection of antibodies to the murine mammary tumour virus in the serum of a BN/BiRij rat, irradiated ×5 *with* 0.02 Gy 0.5 MeV *neutrons.*

with other tumour lines but not with normal mouse or rat fibroblast lines. The titres of the sera reacting with these other tumour lines also ranged between 1:20 and 1:160.

DISCUSSION

The finding of anti-MuMTV antibodies in sera from neutron-irradiated rats as reported by Imai *et al.* [7] was based on an immunofluorescence reaction with virus-producing mouse mammary tumour cells. The observations were made at the low dilution of 1:5 of the rat sera. Such a low dilution is not used in our laboratory because of anticipated non-specific reactions.

The solid-phase radioimmunoassay as used in our study proved to be as sensitive as immunofluorescence, if not superior. This technique is based on the same immunological principle as the indirect cytoplasmic immunofluorescence assay. Both tests involve the binding of a rat antibody to

*Table 3. Reactivity of rat sera in immunofluorescence tests on normal and tumour cell lines**

Source of sera	No. of sera	Cell lines BALB/3T3	B3-SDT	WRE	R-1	RUC-1	RSC-1	SDRijMT	WRijMT
Irradiated; reacting with C3HMT/cl11	33	—	1:59	—	1:61	1:57	1:64	1:82	1:82
Irradiated; not reacting with C3HMT/cl11	10	—	—	—	—	—	—	—	—
Controls; reacting with C3HMT/cl11	5	—	1:15	—	1:10	1:15	1:23	1:26	1:15
Controls; not reacting with C3HMT/cl11	5	—	—	—	—	—	—	—	—

*The geometric means of endpoint titres are presented.

an antigen fixed onto a solid substrate and its detection by labelled antibodies to rat immunoglobulins. The rabbit antiserum to rat immunoglobulins used for the radioimmunoassay reacts with IgM and all IgG subclasses and proved to be highly useful for the detection of natural antibodies to various antigens. It seems highly unlikely that an antibody which was detected by means of immunofluorescence could not be found in the solid-phase radioimmunoassay. The negative findings with the latter technique strongly indicated to us that antibodies to MuMTV were absent in sera from irradiated rats in our study.

It is possible that the W/Fu rats used by Imai *et al.* [7] more easily express their endogenous MuMTV-related sequences than our rat strains.

The positive reactions of several sera in immunofluorescence tests with mouse mammary tumour cells and the negative reactions with a syngeneic fibroblast line initially suggested to us that the sera contained antiviral antibodies. Incubation of four rat sera with solubilized MuMTV polypeptides did not affect immunofluorescence of C3HM/cl11 cells in our study. However, serum from a rat infected with MuMTV lost its reactivity in this test after the absorption procedure. The sera from the four irradiated rats reacted with an antigen present in C3HMT/cl11 cells which is not a MuMTV polypeptide.

The sera which stained the murine C3HMT/cl11 line also reacted with several rat cell lines developed from carcinomas originating in different organs. No normal epithelial cell line was available to exclude the possibility that the antibody was directed against an epithelium-specific differentiation antigen. However, these rat sera also reacted with a rat rhabdomyosarcoma line. They also stained a transformed BALB/3T3 fibroblast line but not the parental line or other normal fibroblast lines. The reaction seems to be due to a common antigen of tumour cell lines. The production of this antigen, which need not be exclusively associated with malignant tumours, might also have been induced by regeneration of radiation-damaged tissues.

An example of ubiquitous tumour-associated antigen is a polypeptide with a molecular weight of 53,000 dalton which has been found in a great variety of cell lines derived from malignancies having different aetiologies [19, 20] in several vertebrate species [21]. Since this antigen is located mainly in the nucleus [20], it is probably not identical with the one detected by sera from irradiated rats.

Another example is a murine antibody, Ca 1, which detects a tumour-specific antigen in sections of different kinds of human malignant tumours on using the immunoperoxidase technique [22]. It is possible that the tumour-associated antigen detected in rodent tumour cell lines in our study is comparable to this human tumour antigen.

We are attempting to increase the titre of antibodies to the ubiquitous tumour-associated antigen in sera from irradiated rats in order to enable its immunoprecipitation and further characterization.

Acknowledgements—Ms. Beleke van Ham and Mr. C. A. J. Poot are thanked for excellent biotechnical assistance, Dr. J. J. Haaijman for valuable suggestions and Dr. A. C. Ford for correcting the manuscript.

REFERENCES

1. DROHAN W, SCHLOM J. Differential distribution of mouse mammary tumor virus-related sequences in the DNA's of rats. *JNCI* 1979, **62**, 1279–1286.
2. CALLAHAN R, DROHAN W, TRONICK S, SCHLOM J. Detection and cloning of human DNA sequences related to the mouse mammary tumor virus genome. *Proc Natl Acad Sci USA* 1982, **72**, 5503–5507.

3. BENTVELZEN P, BRINKHOF J, HAAIJMAN JJ. Genetic control of endogenous murine mammary tumour viruses reinvestigated. *Eur J Cancer* 1978, **14**, 1137–1147.
4. BENTVELZEN P, HILGERS J. Murine mammary tumor virus. In: KLEIN G. *Viral Oncology*. New York, Raven Press 1980, 311–355.
5. BREZNIK T, COHEN JC. Altered methylation of endogenous viral promotor sequences during mammary carcinogenesis. *Nature* 1982, **295**, 255–257.
6. BENTVELZEN P, WESTENBRINK F, BROERSE JJ, VAN ZWIETEN MJ. Absence of antigens related to murine mammary tumour virus polypeptides in rat mammary tumours. *Eur J Cancer* 1981, **17**, 407–410.
7. IMAI M, SUMI C, NAGAYOSHI S, HOSHINO M, YOKORO K. Immunological reaction between sera from neutron-irradiated W/Fu rats and mouse mammary tumor virus. *Gann* 1981, **72**, 132–140.
8. BROERSE JJ, KNAAN S, VAN BEKKUM DW, HOLLANDER CF, NOOTEBOOM AL, VAN ZWIETEN MJ. Mammary carcinogenesis in rats after X- and neutron irradiation and hormone administration. In: *Late Biological Effects of Ionizing Radiation*. Vienna, Int. Atomic Energy Ag., 1978, Vol. II, 13–27.
9. HOLLANDER CF. Current experience using the rat in aging studies. *Lab Anim Sci* 1976, **26**, 320–328.
10. BUREK JD. *Pathology of Aging Rats. A Morphological and Experimental Study on the Age-associated Lesions in Aging BN/Bi, WAG/Rij and (WAG×BN)F1 Rats*. West Palm Beach, FL, CRC Press 1978.
11. WESTENBRINK F, KOORNSTRA W. Expression of MuMTV antigens in rabbits and rats infected with MuMTV. *Virology* 1979, **98**, 493–496.
12. 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.
13. REZNIKOFF CA, BRANKOW DW, HEIDELBERGER C. Establishment and characterization of a cloned line of C3H mouse embryo cells sensitive to postconfluence inhibition of division. *Cancer Res* 1973, **33**, 3231–3238.
14. AARONSON SA, TODARO GJ. Development of 3T3-like lines from BALB/c mouse embryo cultures: transformation susceptibility to SV40. *J Cell Physiol* 1968, **72**, 141–148.
15. BARENDSEN GW, BROERSE JJ. Experimental radiotherapy of a rat rhabdomyosarcoma with 15 MeV neutrons and 300 kV X-rays. I. Effects of single exposure. *Eur J Cancer* 1969, **5**, 373–391.
16. BARENDSEN GW, JANSE HC, DEYS BF, HOLLANDER CF. Comparison of growth characteristics of experimental tumours and derived cell cultures. *Cell Tissue Kinet* 1977, **10**, 469–475.
17. COLOMBATTI A, HILGERS J. A radioimmunoassay for virus antibody using binding of ^{125}I-labeled protein. *J Gen Virol* 1979, **43**, 395–401.
18. FRAKER PJ, SPECK JC JR. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3a,6a-diphenyleglycoluril. *Biochem Biophys Res Commun* 1978, **80**, 849–857.
19. DELEO AB, JAY G, APELLA E, DUBOIS GC, LAW LW, OLD LJ. Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. *Proc Natl Acad Sci USA* 1979, **76**, 2420–2424.
20. DIPPOLD WG, JAY G, DELEO AB, KHOURY G, OLD LJ. P53 transformation-related protein: detection by monoclonal antibody in mouse and human cells. *Proc Natl Acad Sci USA* 1981, **78**, 1695–1699.
21. SIMMONS DT, MARTIN MA, MORA PT, CHANG C. Relationship among Tau antigens isolated from various lines of simian virus 40-transformed cells. *J Virol* 1980, **34**, 650–657.
22. MCGEE JO'D, WOODS JL, ASHALL F, BRAMWELL ME, HARRIS M. A new marker for human cancer cells. 2. Immunohistochemical detection of the Ca antigen in human tissues with the Ca 1 antibody. *Lancet* 1982, **ii**, 7–10.