*Eur J Cancer Clin Oncol*, Vol. 21, No. 5, pp. 631-636, 1985.
Printed in Great Britain.

0277-5379/85$3.00+0.00


# Polyoma Virus-induced Hemangiomas in Grafts of Visceral Yolk Sac and of Embryos*

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**Abstract**—*The development of hemangiomas in grafts of visceral yolk sac and embryos after* in vitro *infection with polyoma virus is described. These hemangiomas were shown to be positive for the polyoma tumor-specific transplantation antigen(s) and to develop as well in recipient virgin as in pregnant rats. In grafts of fetal skin or gut infected with polyoma virus neoplastic transformation was never observed.*

## INTRODUCTION

THE ONCOGENIC activity of polyoma virus depends on the cell-mediated reactions of the host. Indeed, the virus only induces tumors in rats and mice inoculated within a few days after birth or in strongly immunosuppressed animals [1-3]. Normal adult rodents seem to be totally resistant to polyoma oncogenesis. Yet the injection of polyoma virus into the placenta of fetectomized rats induces benign hemangiomas and malignant hemangiosarcomas which derive from the exteriorized visceral yolk sac [4]. To explain the development of these vascular tumors in adult rats, three possibilities have been considered: (i) a particular sensitivity of the visceral yolk sac to neoplastic transformation that may eventually be linked to the growth and differentiation potential of the cells in this extra-embryonic membrane [5]; (ii) the non-immunogenicity of these vascular tumors; and (iii) the special immunological mother-fetus relationship that exists during pregnancy. In order to verify these different possibilities we studied the development of polyoma tumors after transplantation *in vivo* of virus-infected visceral yolk sac. It was shown that with this method vascular tumors are induced which are immunogenic and can develop in virgin as well as in pregnant rats.

Accepted 13 November 1984.
*This work was supported by the Belgian A.S.L.K. Cancer Fund and by the National Fund for Scientific Research (Belgium).
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## MATERIALS AND METHODS

Polyoma (Py) virus (Toronto strain, small plaque) was grown in mouse embryo fibroblast (MEF) cultures and prepared by the method of Crawford [6]. The virus was titrated by the plaque method of Dulbecco and Freeman [7] on mouse fibroblast cultures. The virus pool had a titer of $2 \times 10^9$ PFU/ml Eagle's minimum essential medium (MEM). One-milliliter aliquots were stored in sealed glass ampules at -90°C. The control medium was prepared from MEF cultures not infected with Py virus.

### *Surgical procedure*

Rats of the inbred R/A (Wistar albino) were used. Twelve days after mating (the day when the copulation plug was found was counted as day 0) the uteri with embryos were removed and put in Petri dishes with PBS. The visceral yolk sac (VYS) was dissected free from the Reichert membrane, amnion and placenta as previously described [8]. Pieces of VYS and embryo were incubated for 2 hr in 1 ml of Py virus or control medium. As control tissues, pieces of fetal (18-day-old) or adult (9-week-old) gut and abdominal skin were used. The adult tissues were rinsed five times in MEM and antibiotics prior to the incubation.

After *in vitro* incubation the tissues were implanted in the fat tissue of the supporting ligaments of the uterus of inbred 2-month-old R/A virgin or pregnant rats (day 12), fetectomized or not [5]. The recipients rats were killed 6 weeks after the syngeneic intraperitoneal grafting. The number and aspect of the nodules were verified and the implants with the surrounding tissue fixed for histological examination. The paraffin

blocks were cut serially and stained with erythrosin-hematoxylin, PAS and Tibor-Pap for reticulin.

## RESULTS

*Macroscopy and microscopy*

In the rats killed 6 weeks after grafting of pieces of VYS or embryo into the peritoneal cavity, small nodules displaying frequently a cystic appearance were found. Only in the animals implanted with Py virus-infected tissues, however, were hemorrhagic nodules observed (Fig. 1). They were mostly well encapsulated, their diameter varied from 2 to 12 mm and they were always found in connection with the cystic nodules. In the rats implanted with 18-day fetal skin or gut, infected or not with Py virus, small cystic nodules were found. The grafts of adult skin and gut were completely necrotized.

Histologically, the nodules derived from the VYS consisted mainly of small cysts lined by an endodermal or epidermal epithelium. The latter was frequently associated with skin appendages. The surrounding tissue consisted of mesenchymal tissue with cartilage, bone containing bone marrow cells and muscular tissue. Sometimes trophoblastic giant cells were also recorded.

All these tissues were found in the control as well as in Py virus-infected groups. Only in the latter group, however, were hemangiomas recorded, composed of cavernae filled with blood and lined by endothelial cells. The endothelial cells seemed to be well differentiated, mitoses were very rare (Figs 2-4). These hemangiomas developed as well in Py virus-infected VYS fragments implanted in virgin rats as in fetectomized or pregnant rats. They were also observed in the grafts of 12-day-old embryos infected with the virus (Table 1). Apart from hemangiomas, the implanted pieces of 12-day-old embryos gave rise to typical embryomas consisting of a mixture of proliferating mesenchymal tissue and well-differentiated structures such as gut, cartilage, nervous tissue and skin. In the group of rats implanted with pieces of embryo incubated with control fluid, the embryomas had the same histological appearance but hemangioma development was never observed.

Six weeks after implantation, the 18-day-old fetal skin and gut still displayed the histological characteristics of the tissue but hemangiomas or other neoplastic growth was never seen in the Py virus-infected group. The grafts of adult skin and gut were necrotized.

*Polyoma tumor specific transplantation antigens (TSTA)*

The presence of Py-TSTA on the hemangiomas was shown in recipient 9-week-old virgin rats after three weekly preimmunizations with $10^8$ PFU Py virus or with $5 \times 10^6$ allogeneic Py-induced kidney sarcoma cells (BN/PNS). In the Py virus pre-immunized animals none of the 12 virgin rats implanted with an *in vitro* infected VYS developed an hemangioma and only 1/12 pre-immunized with Py tumor cells (BN/PNS). In the control groups, however, pre-immunized with $5 \times 10^6$ allogeneic carcinogen-induced fibrosarcoma cells (BN/DMBA) or injected with PBS, 7/12 and 5/9 rats respectively developed hemangiomas ($P < 0.01$). The number of teratomatous nodules was nearly the same in all groups at the end of the experiment (6 weeks).

## DISCUSSION

These results support the hypothesis that the vascular tumors derived from the VYS after

*Table* 1. *Tissue sensitivity to Py virus as tested by grafting* in vivo

| Incubated with: | Tissue | Recipient rats (No.) | | No. and histology of nodules | | No. of rats with hemangioma |
|---|---|---|---|---|---|---|
| Py virus | 12-day VYS | pregnant | (4) | 5 | | 1 |
| | | fetectomized | (3) | 9 | teratoma | 2 |
| | | virgin | (15) | 26 | | 7 |
| | 12-day embryo | virgin | (2) | 6 | embryoma | 4 |
| | 18-day fetal gut | virgin | (2) | 7 | cyst (gut) | 0 |
| | 18-day fetal skin | virgin | (2) | 8 | cyst (skin) | 0 |
| Control fluid | 12-day VYS | pregnant | (2) | 5 | | 0 |
| | | fetectomized | (3) | 10 | teratoma | 0 |
| | | virgin | (6) | 12 | | 0 |
| | 12-day embryo | virgin | (2) | 4 | embryoma | 0 |
| | 18-day fetal gut | virgin | (1) | 4 | cyst (gut) | 0 |
| | 18-day fetal skin | virgin | (1) | 4 | cyst (skin) | 0 |

Each recipient R/A rat was implanted intraperitoneally with six tissue fragments.

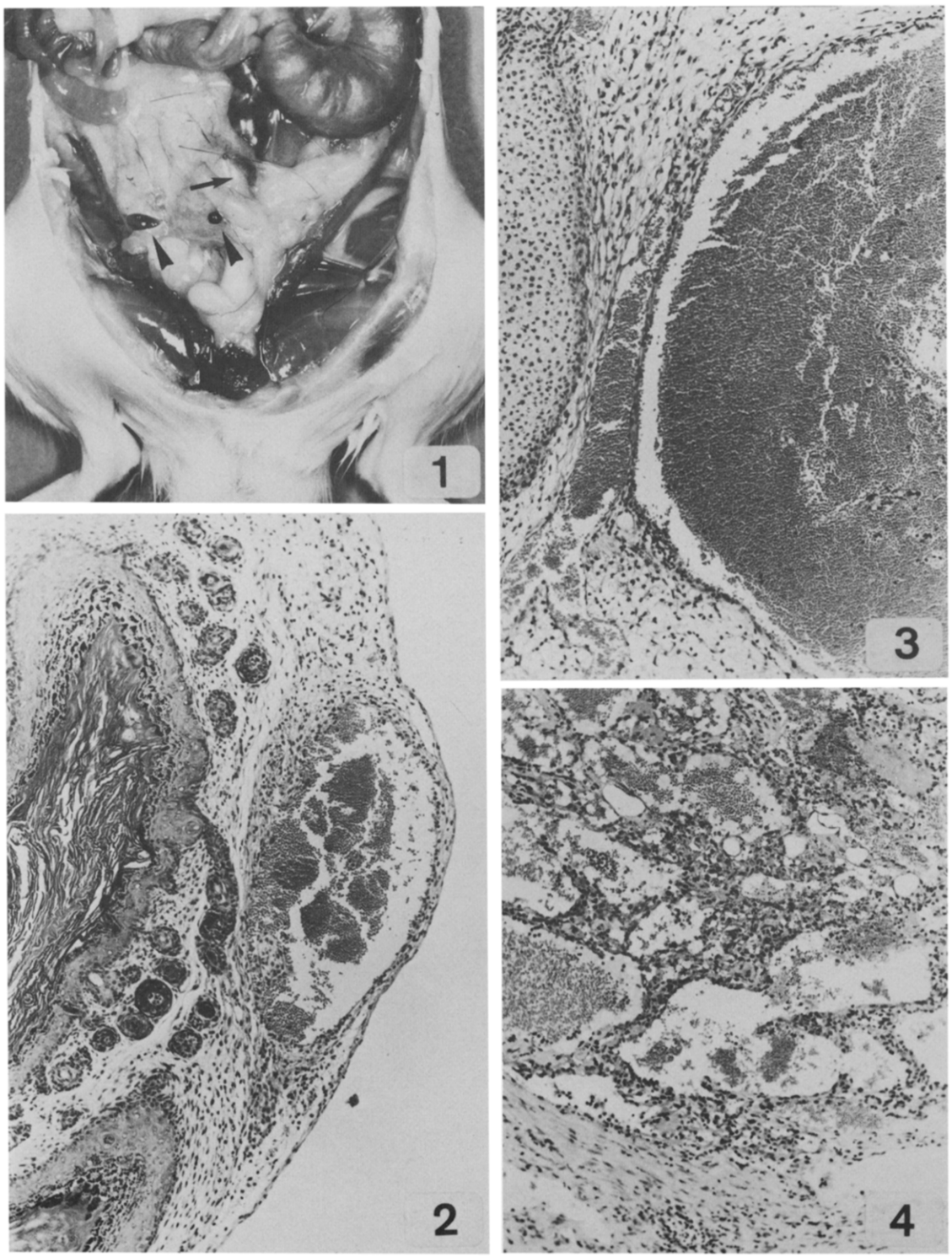


*Fig. 1. Macroscopical appearance of hemangiomas (head arrow) 6 weeks after transplantation of visceral yolk sac infected* in vitro *with polyoma virus. A teratomatous nodule is also seen (arrow).*

*Fig. 2. Microscopical appearance of small hemangioma in teratomatous tissue (epidermal cysts with skin appendages). H&E,* ×160.

*Fig. 3. Hemangioma surrounded by mesenchymal tissue and cartilage. H&E,* ×160.

*Fig. 4. Hemangioma composed of multiple small cavernae. H&E,* ×160.

injection of Py virus in the placenta of fetectomized rats [4] is due to the particular sensitivity of this extra-embryonic fetal membrane for neoplastic transformation. Indeed, the explanations based upon the role of the immunologic privileged mother-fetus relationship that exists during pregnancy or on the possibility that the Py-induced vascular tumors are not immunogenic are not compatible with the results reported. By the immunization experiments we could demonstrate the presence of Py-TSTA on the benign hemangiomas. The antigenicity of the malignant Py-induced hemangiosarcomas had already been shown [4]. From the present experiments one can conclude that, like their malignant counterpart, the benign vascular tumors also possess these antigens and are immunogenic. If during pregnancy the exteriorized VYS was somehow protected against an immune reaction, one would expect the VYS implants infected with Py virus to give rise to hemangiomas only when implanted in pregnant or in fetectomized rats. Since in the virgin rats the implanted Py-infected VYS developed hemangiomas, the presence of the particular hormonal and immunologic environment which exists during pregnancy is not required. We can, however, not exclude the possibility that the surgical procedure (laparatomy) required for the implantation of the VYS Py-infected fragments allows the development of antigenic tumors by the inhibition of the cellular immune response in the peritoneal cavity [9].

Hence one has to conclude that the 12-day-old displaced VYS and embryonic tissue is particularly sensitive to neoplastic transformation. This sensitivity may be linked to the embryonic stage of these tissues, to their capacity to proliferate or to both [10–12]. The capacity of the displaced VYS to proliferate and to differentiate has been well documented [5, 13]. Also, after implantation in the peritoneal cavity this extra-embryonic membrane seems to display, albeit to a lesser extent, this property since it frequently gives rise to teratomatous nodules containing various well-differentiated tissues [14, present experiments].

Fetal skin and gut which show proliferation in normal conditions did not, however, display neoplastic transformation. This may be due to the tropism of Py virus in the rat and hamster for mesenchymal tissue which proliferates in displaced VYS and embryonic tissue, while in the fetal skin and gut the cell proliferation is mainly observed in the epithelium. This oncongenic activity displayed by the Py virus seems to be particulary pronounced for endothelial cells since no other mesodermal tumors, apart from hemangiomas, were observed in these experiments. Interestingly enough, the same selectivity for vascular tissue was observed in fetectomized rats injected into the placenta with the virus and in immunosuppressed rats [4, 15]. In both cases the only Py tumors recorded were of vascular origin. This particular selectivity of the Py virus for vascular endothelial cells deserves further *in vitro* investigations.

Since the rat VYS cultured in organ culture displays the potentiality to proliferate and to differentiate [8], it may represent an interesting alternative model for the mouse salivary glands [16, 17] and kidney [18] extensively studied for their sensitivity *in vitro* to the transforming capacity of Py virus.

**Acknowledgements**—We are indebted to Miss Ria Van Laer, Mr J. Goebels, Mr C. Seghers and Mr G. Hermans for skilful technical assistance. The editorial help of Mrs Jane Putzeys is appreciated.

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