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TRANSPLANTATION OF CULTURED JAAGSIEKTE (SHEEP PULMONARY ADENOMATOSIS) CELLS INTO ATHYMIC NUDE MICE

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ABSTRACT

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Epithelial cells of the 15.4 line, which were originally established from the adenomatous lung of a jaagsiekte case and which had been cultured *in vitro* for 22 generations, were injected subcutaneously into athymic nude mice. A slow-growing tumour which soon became cystic was established in each case. The cysts rapidly increased in size as a result of the accumulation of a slightly turbid secretion containing aggregates of tumour cells which rapidly refilled the cysts after the fluid had been withdrawn. Cultures were readily re-established from these cells and a chromosomal analysis proved that the tumour consisted of sheep cells. An epithelial cell lining, very similar to that found in the adenomatous lung alveoli of typical jaagsiekte, could be demonstrated histologically.

Résumé

TRANSPLANTATION SUR SOURIS GLABRES ATHYMIQUES DE CELLULES D'UNE CULTURE D'ADÉNOMATOSE PULMONAIRE OVINE (JAAGSIEKTE)

On a injecté sous la peau de souris glabres athymiques des cellules épithéliales de la lignée 15.4, *qui avait été établie à l'origine à partir du poumon adénomateux d'un cas de "jaagsiekte" et que l'on avait maintenue en culture* in vitro *pendant* 22 *générations. Dans chaque cas est apparue une tumeur à croissance lente, qui est bientôt devenue cystique. Les cystes ont grossi rapidement suite à l'accumulation d'une sécrétion légèrement trouble contenant des agrégats de cellules tumorales qui ont rapidement rempli à nouveau les cystes après évacuation du liquide. A partir de ces cellules de nouvelles cultures ont été établies sans difficulté et l'analyse chromosomique a montré qu'il s'agissait de cellules de brebis. Une étude histologique y a mis en évidence un revêtement de cellules épithéliales très semblable à celui que l'on observe dans les alvéoles pulmonaires adénomateux d'une "jaagsiekte" typique.*

INTRODUCTION

We recently reported the establishment of an epithelial cell line from lung lesions of sheep with jaagsiekte and the transmission of this disease to new-born lambs by means of intratracheal injections of the cells (Coetzee, Els & Verwoerd, 1976). The transplantation of jaagsiekte to lambs by means of a cell culture is at present the only way to prove the oncogenic or transformed character of the cells. However, as an experimental system, it is rather time-consuming owing to the very long incubation period (usually 6 months to one year or more) before clinical signs are seen. All attempts to transmit the disease to small laboratory animals having failed (unpublished results), the only possible alternative was to explore the use of congenitally athymic nude mice.

In the course of transplantation studies in lambs it emerged that transplantation of living cells can occur in nature and this at least partially explains the infectious nature of the disease (Coetzee *et al.*, 1976). This discovery suggested the possibility of using fixed, inactivated cells prophylactically. However, to produce the number of cells required for a cellular vaccine of this kind, the conventional cell culture techniques would be totally inadequate and new methods for the production of cells in bulk had to be explored.

Athymic nude mice have been used widely for growing a variety of cancer cells, mostly of human origin. The ability to grow these cells in nude mice has proved to be one of the most consistent criteria in demonstrating the transformed character of cultured cells (Freedman & Shin, 1974). The fast growth rate of many tumours in nude mice has also been exploited by various workers for the mass culture of certain cells (Freedman, Brown, Klinger & Shin, 1976). The use of nude mice therefore seemed to offer a possible solution to both above-mentioned problems.

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MATERIALS AND METHODS

Nude mice

A breeding nucleus of nude mice, obtained from the Laboratory Animals Centre, Carshalton, England, consisted of mice derived from pairs mated at random within the 8th back cross-generation of the nude gene onto a Balb/C inbred background.

Husbandry and breeding

The mice were kept in a Trexler type plastic isolator under controlled environmental conditions of temperature, light and humidity. Anal swabs were taken regularly for detecting the presence of pathogenic bacteria. Standard mouse pellets, sterilized by autoclaving, were supplemented by a vitamin concentrate which was sterilized by millipore filtration and added to the drinking water.

Homozygous nude males were randomly bred to heterozygous females but only the homozygous offspring used for our experiments.

Cell cultures

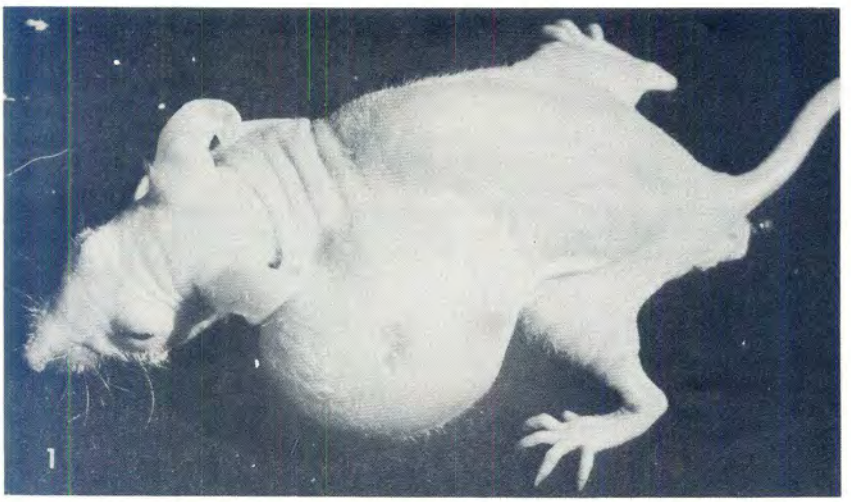
Cultures of the 15.4 cell line were established from stocks frozen away at generation 20, and used within 5 further passages. Standard cell culture techniques and media were used as previously described (Coetzee *et al.*, 1976).

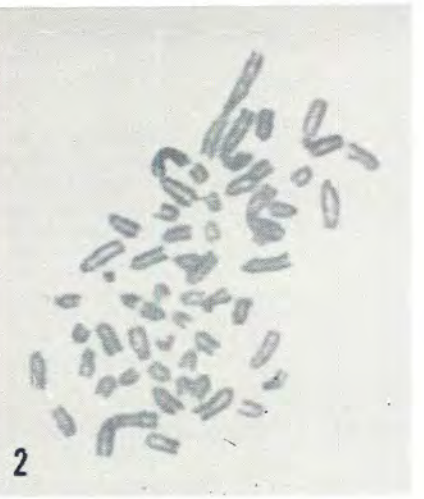
Transplantation

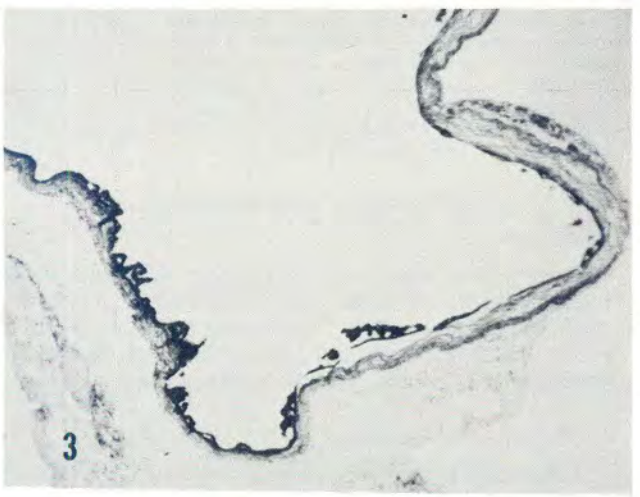
Two mice were used for the initial transplantation attempts. One was a 5-week-old male and the other a 7-week-old female. Both were injected subcutaneously, the former at one site with 1×10^7 cells, the other at each of 2 sites with 1×10^7 cells.

Control

One nude mouse was injected subcutaneously at 2 sites with 1×10^7 normal sheep lung epithelial cells grown in culture.







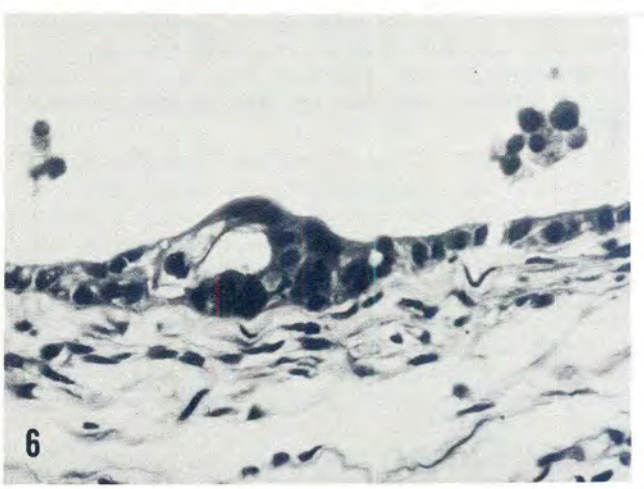


FIG. 1 Nude mouse with subcutaneous cystic tumour
FIG. 2 Karyotype of tumour cell in culture showing the 3 pairs of large metacentric chromosomes characteristic of sheep cells
FIG. 3–6 Photomicrographs of histological sections of tumour stained with H & E
FIG. 3 Low magnification of tumour cyst wall lined with epithelium. ×30
FIG. 4 Cyst wall lined with cuboidal epithelium, subepithelial layer consisting of histiocytes, fibroblasts and leucocytes and areolar connective tissue between cyst wall and subcutis. ×75
FIG. 5 Papilliform growth of tumour epithelium. ×200
FIG. 6 Desquamated epithelial cells undergoing necrosis. ×500

Re-establishment in cell culture

The fluid content of the cystic tumour was removed aseptically with a syringe, seeded directly into a plastic tissue culture flask containing medium, and incubated at 37 °C.

Chromosomal analysis

Chromosome spreads were prepared using the technique previously described for the karyotypic analysis of the 15.4 cell line (Coetzee *et al.*, 1976).

Histology

The tumours were removed *in toto* and fixed in 10% buffered formalin for paraffin embedding. Sections were cut and stained with H and E in the conventional manner.

Results

Tumour growth

A small hard nodule was first observed at the site of injection of the first recipient 7 days after transplantation. The tumour slowly increased in size for about 2 weeks, when growth seemed to stop. Two weeks later the tumour suddenly started to increase rapidly in size and reached a diameter of about 3 cm (Fig. 1). At this stage it ruptured and was found to consist of a large single cyst. The rupture healed and the cyst started filling up again until it reached its original size in about a week's time. At this stage the fluid content was aspirated aseptically with a syringe and used to establish a cell culture. The contents of the cyst was withdrawn 3 times more, so that 4 cell cultures were established. This animal, as well as the 2nd one which had developed 2 smaller tumours, was then sacrificed for histopathological examination.

Cell cultures

Cells from the tumour content adhered and formed confluent cultures within 2 weeks. All 4 cell cultures formed monolayers indistinguishable in appearance from the original 15.4 cultures.

Chromosome preparations from these cultures clearly showed the presence of the 3 pairs of large, metacentric chromosomes characteristic of sheep cells, leaving no doubt that the tumours consisted of ovine cells and not of transformed mouse cells (Fig. 2).

Control

There was no evidence of tumour growth in the mouse injected subcutaneously with normal sheep lung epithelial cells.

Macro- and microscopic pathology

The tumours were clearly defined, soft, and easily separable from the skin and subcutaneous tissue.

A section through the cystic tumour showed a cavity lined with an epithelial layer (Fig. 3). In some places, the epithelium consisted of a single layer of cells (Fig. 3 and 4), whereas in others it consisted of several layers. In these areas it was papilliform and projected into the cavity (Fig. 5). In parts the epithelium appeared to rest on a basal layer which, however, was not continuous.

The epithelial cells were cuboidal in shape, had microvilli and, in general appearance, closely resembled the epithelial cells encountered in natural cases of jaagsiekte.

The epithelial and basal layers rested on a subepithelial layer consisting of fibroblasts, histiocytes and a few leucocytes, particularly neutrophils. Numerous capillaries and some lymphatics were present in this layer (Fig. 6). On its outer circumference, clearly differentiated connective tissue with collagen was in evidence, being separated from the subcutaneous tissue by loose, areolar connective tissue.

Some of the desquamated epithelial cells present in small groups or in larger sheets (Fig. 5) had undergone necrosis and become mineralized.

Discussion

The successful transplantation of the 15.4 jaagsiekte cells into nude mice constitutes the first successful adaptation of this tumour to an experimental animal other than sheep. A control nude mouse, injected with epithelial cells from a lung of a normal sheep, did not develop any tumours, confirming that the ability of the jaagsiekte cells to form a subcutaneous tumour in the nude mouse depends on their being transformed, that is, being oncogenic.

Transplantation into nude mice instead of into lambs can therefore be used to ascertain the tumorigenicity of cell lines established from jaagsiekte lungs. This constitutes a saving both in cost and time, as it took only 2 weeks to produce a tumour in the mice against 4–6 months in lambs.

In view of the epithelial nature of the tumour cells, the cystic nature of the tumour in the mouse was not entirely unexpected and may be regarded as an indication that these cells have retained some of their specialized functions. It is disappointing, though, from the point of view of mass cell production. In spite of their size, the tumours contained only a relatively small number of cells and could not be used for large-scale cell production.

The ability of the epithelial cells to line the cyst cavity and the distinct tendency of the epithelial lining to become papilliform in places are responsible for the close histological resemblance between the cystic tumour and the lung lesion in a sheep with jaagsiekte.

Since the completion of this work, we have learned that Drs W. B. Martin and J. B. Sharp of the Moredun Institute, Edinburgh, Scotland obtained results similar to ours by the subcutaneous implantation of fragments of lung tumour tissue (personal communication).

References

COETZEE, SUZANNE, ELS, H. J. & VERWOERD, D. W., 1976. Transmission of jaagsiekte (ovine pulmonary adenomatosis) by means of a permanent epithelial cell line established from affected lungs. *Onderstepoort Journal of Veterinary Research*, 43, 133–142.

FREEDMAN, VICTORIA H., BROWN, A. L., KLINGER, H. P. & SHIN, S.-I., 1976. Mass production of animal cells in *nude* mice with retention of cell specific markers. *Experimental Cell Research*, 98, 143–151.

FREEDMAN, VICTORIA H. & SHIN, S.-I., 1974. Cellular tumorigenicity in *nude* mice: Correlation with cell growth in semi-solid medium. *Cell*, 3, 355–359.