



HOMOLOGOUS TRANSPLANTATION OF BOVINE ETHMOID CARCINOMA CELLS *

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Abstract

A study was undertaken to transplant bovine ethmoid tumour cells into calves immunosuppressed with hydrocortisone. Twelve calves were equally grouped into two groups of control and immunosuppressed group. Trypsinized single cell suspension of freshly taken bovine ethmoid tumour was inoculated subcutaneously into all the calves. The animals were observed for four months. At the end of the observation period the calves were sacrificed and site of inoculation was taken for histopathology. The tumour failed to grow in calves even after providing favourable conditions. Haematological studies were also done to evaluate the immuno-competency of the animals. The failure to transplant bovine ethmoid carcinoma cells subcutaneously in calves even though majority of the requirements were satisfied may be due to the absence of some unknown factors required for the growth of neoplastic cells.

Key words : *Bovine ethmoid carcinoma cells, homologous transplantation*

A transplanted tumour offers an excellent model system for studying the tumour-host relationship and clues which aid in the clinical management of the tumour in the primary host. Several attempts were made for transplantation of the ethmoturbinate

neoplasms of the domestic animals into experimental animals with or without immunosuppression without success (Duncan *et al.*, 1967; Rajan *et al.*, 1972; Nair, 1973; Jayaraman *et al.*, 1979; Sulochana, 1980; Pospischil *et al.*, 1982; Karki and Rajan, 1986 and Chaudhary, 1994). Ajith (1994) transplanted ethmoid tumour of cattle by inoculating single cell suspension of the tumour subcutaneously in mice immunosuppressed with cyclosporine-A. This paper describes an attempt to transplant ethmoid carcinoma cells in calves subcutaneously.

Materials and Methods

Cattle bearing tumour of the ethmoturbinate mucosa and twelve neonatal calves which were not fed colostrum were utilised for the study. Calves were grouped equally into two. Group A of six calves were administered hydrocortisone sodium succinate (Neon) subcutaneously at the rate of 3 mg/kg body weight one day prior to transplantation and was repeated for three days. Group B was kept as control without immunosuppression.

Hank's balanced salt solution (Hi-Media) (HBSS) and TC 199 media (Hi-Media) prepared as per the manufacturers' direction by dissolving in deionized double distilled water and filtered through 0.2 µ

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membrane filter (Sartorius) under positive pressure were used as maintenance and culture media. Culture media was supplemented with 10 per cent foetal calf serum (CSIR, New Delhi). The antibiotic mixture added contained penicillin G - 200 IU/ml, streptomycin - 150 ug/ml, gentamycin - 50 ug/ml and nystatin - 100 IU/ml.

A strength of 0.25 per cent trypsin (1 :250 Difco) in phosphate buffered saline (Ca and Mg free) (Hi-Media) (PBS) was prepared in deionized double distilled water and sterilized by filtering through 0.2 μ membrane filter.

Tumour bearing cows were euthanised by exsanguination after stunning with captive bolt pistol. Fresh soft healthy tumour tissue was dissected out under sterile condition from the deeper portion avoiding the necrotic area. The tumour tissue was collected in HBSS with antibiotics. A part of the tumour tissue was taken in 10% formalin for histopathology.

The tumour tissue was washed several times using PBS containing antibiotics, to remove the debris. It was transferred into a Petri dish containing PBS. The superficial fascia was removed from the tumour mass. The tissue was then cut into small cubes of one millimetre size and washed several times with PBS. A few cubes of the tumour tissue were transferred into a beaker containing 100 ml of 0.25 per cent trypsin solution in PBS. The beaker was placed on a magnetic stirrer and stirred for 10 min using a magnetic stirring paddle. The supernatant was decanted and replaced with fresh 100 ml of 0.25 per cent trypsin and again stirred using magnetic stirrer for another 10 min. Serum (3 ml) was added to the suspension to neutralise the trypsin. The suspension was sieved through a double layered sterile muslin cloth into a sterile flask. The suspension was transferred to a centrifuge tube and was centrifuged at 1000 rpm for 5

min. The supernatant was poured off and the cell pellet was suspended in media with antibiotic. The viable cell concentration was adjusted to 1×10^6 viable cells per 0.25 ml after estimating live cell concentration by trypan blue staining.

Single cell suspension obtained by trypsinization was injected subcutaneously in the flap of flank of all the calves, at the rate of 1×10^6 viable cells per inoculum. The experimental animals were observed for four months. The thickness of the skin at the site of inoculation was measured immediately and repeated monthly. All animals were sacrificed after the observation period. The site of inoculation, spleen, lungs, heart, liver and kidney were taken for histopathology. At the time of sacrifice blood samples were collected in EDTA for total leucocyte count and differential leucocyte count.

Results and Discussion

The gross and histopathological findings confirm that the tumour was a primary adenocarcinoma arising from the ethmoturbinate mucosa, which agreed with the observations made by Gangadharan, 1992. Thickness of the skin at the site of inoculation is shown in Table 1. Control animals revealed local oedema at the site of inoculation 24 h after transplantation which attained maximum size by first week and then gradually decreased in size. The animals of group B, showed congested blood vessels with infiltration of lymphocytes and macrophages at the inoculation site. Neoplastic cells were not detected at the site of inoculation. The absence of tumour growth in control animals could be due to the destruction of the neoplastic cells by the host immune cells as observed microscopically.

The animals of group A revealed initial swelling which subsided within 24 h. Histologically there was no evidence of tumour

Table 1: Skin thickness after transplantation

Months	Group A	Group B
0	0.410 \pm 0.010	0.419 \pm 0.006
1	0.313 \pm 0.004	0.410 \pm 0.075
2	0.320 \pm 0.004	0.368 \pm 0.093
3	0.331 \pm 0.003	0.417 \pm 0.005
4	0.392 \pm 0.003	0.420 \pm 0.006

Table 2: Total leucocyte count ($\times 10^3/\text{mm}^3$) and lymphocyte percentage

Parameter	Group A	Group B
Total leucocyte	4.90 \pm 0.15	9.25 \pm 0.21
Lymphocyte percentage	32.00 \pm 0.58	57.30 \pm 0.98

growth. The total leucocyte count and lymphocyte percentage are shown in Table 2. There was significant difference between the animals of group A and group B in these parameters. The total leucocyte counts in all experimental animals were very low when compared to the control. The differential leucocyte count of the immunosuppressed animals showed a very low lymphocyte percentage when compared to the control.

Jayaraman *et al.* (1979) suggested that the failure to transplant bovine ethmoid tumour cells may perhaps be due to the etiological agent not being present in these cells or remaining in an incomplete form which requires certain exciting condition for maturation and replication. Karki and Rajan (1986) attributed degraded condition of the tumour tissue and the absence of certain unknown factors required for the growth of the neoplastic cells in the recipient along with the role of infectious agent to the failure of tumour growth *in vivo*. Ebbers *et al.* (1986) suggested that nasopharyngeal carcinomas have difficulty in surviving in tissue culture system and even the transplantation of solid tumour mass into nude mouse was also not easy. There may also be various other factors like type of host, age of host, immune status of the recipient, type of tissue preparation, type of tumour, inoculation rate, viability of the cells and route of inoculation which would determine the transplantability of tumour.

Lin *et al.* (1990) transplanted nasopharyngeal carcinoma cells subcutaneously into the back of BALB/c nude mice. The tumour grew upto 2-3 months, and

attained a size of 2.2 cm. Ajith (1994) observed that ethmoid tumour could be successfully transplanted in mice treated with cyclosporine. A supports the assumption that immunosuppression is a prerequisite for the development of neoplasms. Lowering of the immunological barrier of the host therefore appears to be an important event in establishing neoplastic growth.

In the present study the experimental lot consisted of young animals which were naturally at a lower immunological competency and were immunosuppressed with hydrocortisone. Even after providing these conditions the tumour cells failed to grow which indicates the need of some unknown factors. Watanabe *et al.* (1980) suggested that subcutaneous route was more effective than I/P or I/V routes of inoculation probably due to better blood supply and presence of connective tissue framework for fixation and proliferation of the inoculum.

It has also been reported that the failure of some tumours to grow when inoculated subcutaneously (Al-Yamen and Willenborg, 1984) was probably due to factors such as lack of proper vascularisation or the lack of essential factors required for tumour growth, which is present in the original host but not in the recipient. In the tumour transplant obtained by Ajith (1994) moderate degree of vascularisation was present which might have facilitated the proliferation and growth of the tumour cells. The tumour cells transplanted in the present study did not initiate capillary proliferation which might have been a concurrent factor for the failure of transplant.

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