Transplantation of ACTH-Secreting Pituitary Tumor Cells in Athymic Nude Mice *

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Summary. Chronic excess of glucocorticoids results in Cushing's syndrome in humans. A common cause of excess cortisol secretion is the presence of an adrenocorticotropin secreting pituitary tumor which stimulates the adrenal cortex to produce excess glucocorticoids. ACTH-secreting AtT-20 mouse pituitary cells transplanted subcutaneously in oestrogenized athymic nude mice form tumors rapidly. Six weeks after receiving the tumor transplants, the mice weighed 45% more than normal mice due to the increase in body fat. The tumor-bearing mice exhibit the familiar "buffalo hump" appearance due to the abnormal distribution of body fat. The adrenal glands of the tumor-bearing animals are enlarged due to hypertrophy of the zona fasciculata. The foamy looking fasciculata cells in normal mice were converted to dense, eosinophilic cells in the tumor-bearing mice. Transplantation of normal pituitary glands to athymic nude mice with or without oestrogen treatment did not produce these morphological changes. The experimental model described here may be useful for future studies of Cushing's syndrome.

Key words: Athymic nude mice – ACTH pituitary tumor – Cushing’s syndrome – Adrenal – Glucocorticoids

The clinical and metabolic abnormalities associated with elevated cortisol secretion were described by Cushing 50 years ago (Cushing 1932). A common cause of excess cortisol secretion is the presence of an adrenocorticotropin (ACTH) secreting pituitary tumor which stimulates the adrenal cortex to produce an excess of glucocorticoids (Liddle 1980).

Cushing's syndrome in the dog (Capen and Martin 1975) and horse (Moore et al. 1979) has been reported. A model for Cushing's syndrome in mice was described by Furth (1955), who

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transplanted ACTH secreting pituitary tumors from mice of the strain LAF1. The tumor was grafted into the thigh muscle of the animal and palpable tumors developed after a long period of latency (varying from 110 to 207 days). An ACTH-secreting tumor cell line (AtT-20) was subsequently established in vitro from this transplantable pituitary tumor (Buonassisi et al. 1962). The AtT-20 cells were shown to produce biologically active ACTH (Eipper and Mains 1980; Gumbiner and Kelly 1981; Orth et al. 1973). These cells were also widely used to study the biosynthesis of ACTH (Mains and Eipper 1976).

Athymic nude mice, because of their deficiency in immune response, have been commonly used as graft recipients. Many tumor cells have been successfully transplanted into the nude mice (Fogh et al. 1977; Giovanella et al. 1974).

We describe here the morphological changes in athymic mice which had received a subcutaneous transplant of ACTH-secreting AtT-20 cells and the usefulness of these mice as an animal model of Cushing's syndrome.

Materials and Methods

Cell Culture

The AtT-20 mouse pituitary tumor cell line was obtained from the American Type Culture Collection, Rockville, MD., USA. Stock cultures of AtT-20 cells were maintained in T-75 flasks with Dulbecco's modified Eagle's medium supplemented with L-glutamine (4 mM), glucose (4.5 g/l), penicillin (100 IU/ml), streptomycin (100 μg/ml), bovine insulin (10 μg/ml) and 10% (v/v) fetal bovine serum. Cells were kept in a humidified atmosphere of 95% air – 5% CO2 at 37°C.

Animals

Four to five week old female Balb/c athymic nude mice were obtained from ARS/Sprague Dawley Division, Madison, WI, USA. Animals were kept under standard conditions for a 5 to 7 day period prior to use. The animals were kept inside a laminar-flow air filtration system, and food and water were supplied ad libitum.

Inoculation of Cells in Nude Mice

Cells were detached with Trypsin-EDTA in Hank's balanced salt solution and resuspended in a small volume of medium. AtT-20 cells (10⁶) were injected subcutaneously in the flanks of the animals. Some animals also received 500 μg oestradiol valerate once every 2 weeks. Oestrogen was injected subcutaneously in the dorsal midline, caudal to the neck. Pituitaries from normal female Sprague Dawley rats were transplanted subcutaneously into some of the athymic nude mice. Two pituitaries were transplanted into each nude mouse.

Four Groups of Mice Were Studied. Group 1 animals were injected with oestrogen and AtT-20 cells; group 2 animals received oestrogen only; group 3 animals received both oestrogen and pituitary transplants; group 4 animals received pituitary transplants only.

Histology of the Adrenals of the Nude Mice

At the completion of the experiments, the adrenals and whole pituitary glands from the various groups of animals were dissected and weighed. The adrenals were fixed in 3% paraformaldehyde and prepared for histological examination. Sections were stained by haematoxylin and eosin.

Results

The AtT-20 cells form tumors rapidly after transplantation into athymic nude mice. Palpable tumors can be observed 2 to 3 weeks after transplantation. The histology of the AtT-20 tumor is shown in Fig. 6.

The appearance of an oestrogenized normal athymic nude mouse and that