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Transgenic Mice That Develop Pituitary Tumors

A Model For Cushing's Disease

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Transgenic mice that developed adrenocorticotropic hormone (ACTH)-producing pituitary tumors were generated with the polyoma early region promotor linked to a cDNA encoding polyoma large T antigen (PyLT). Light microscopic examination of the pituitaries showed normal morphology at 4 months of age, either unremarkable morphology or microadenoma formation at 9 months of age, and up to 5 mm large adenomas in clinically ill transgenic mice at 13–16 months of age. At age 9 months, transgenic mice weighed significantly more than corresponding control mice, but they began wasting at approximately 1 year of age. The adrenal glands of these older PyLT-1 mice showed a weight increase and exhibited a medullary hyperplasia. Subcutaneous transplants of transgenic pituitary tumors to nontransgenic, immunocompetent mice resulted in tumors with a morphology and ACTH immunoreactivity similar to the primary tumor. The effects of hypercorticotropism were more enhanced and occurred with a shorter latency in the mice carrying transgene pituitary transplants than in the PyLT-1 transgenic mice themselves. Moreover, these transplanted mice showed a weight increase with an axial deposition pattern and hypertrophy of the adrenal cortex that resembled the findings in human Cushing's disease. Plasma ACTH levels were significantly increased in clinically ill transgenic mice and even higher levels were found in the transplant mice. Thus, both murine models should be useful for studying Cushing's disease. (Am J Pathol 1992, 140:1071–1080)

The hypothalamus, pituitary gland and adrenal cortex constitute an endocrine axis. A hypothalamic peptide, corticotropin-releasing hormone (CRH),[1] stimulates the pituitary to secrete adrenocorticotropic hormone (ACTH); ACTH stimulates the adrenal cortex to produce and secrete glucocorticosteroids. Chronic ACTH overstimulation of the adrenal cortex induces the steroidogenic pathway by enhancing gene transcription of the involved enzymes.[2] Glucocorticoids are normally elevated in response to stress and fasting. In fasting, corticosteroids maintain blood glucose by stimulating gluconeogenesis. Feeding in the presence of high concentrations of glucocorticoids may cause hyperglycemia, leading to hyperinsulinism that in turn results in a central deposition of fat.[3]

Cushing's syndrome is the clinical result of chronic overexposure to endogenous cortisol (e.g., an adrenal cortical adenoma) or prolonged administration of synthetic glucocorticoids in humans. The latter is seen in large groups of patients treated for chronic autoimmune diseases, patients with serious allergies such as asthma, and certain groups of cancer patients. The side effects of glucocorticoid therapy often limit the therapeutic usefulness of the drugs. Excessive pituitary secretion of ACTH is called Cushing's disease and most often results from a pituitary tumor, usually designated as a microadenoma. The more common symptoms of the disease are truncal obesity, hypertension, hyperglycemia, muscular weakness, and dystrophic skin changes. Measurement of ACTH levels in plasma from the inferior petrosal sinuses can distinguish ACTH-secreting pituitary tumors from other causes of hyperadrenocorticotropism.[4] Microadenomas can be removed selectively through a transsphenoidal resection, leaving normal gland *in situ*. For the few patients with a vessel plexus surrounding the pituitary gland[5] and for patients with macroadenomas,[6] transsphenoidal surgery may be impossible or insufficient.

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Adjunctive therapies with radiation and drugs that decrease ACTH secretion or inhibit corticosteroid synthesis are available, but they have major shortcomings in terms of efficiency and side effects.[7]

Therefore, animal models for Cushing's syndrome and Cushing's disease are relevant for investigating in detail the systemic effects of ACTH and glucocorticoids and new treatment modalities. We have generated transgenic mice with a polyoma large T-antigen cDNA transgene that heritably form pituitary tumors with detectable symptoms at about 1 year of age. The pituitary tumor and the testes express the PyLT transgene at the mRNA level (VLB unpublished results). We have shown both at the light- and electron microscopic level that these tumors produce ACTH (R. Holm, unpublished results). We report that the PyLT-1 transgenic mice and immunocompetent mice carrying transplanted transgenic pituitary tumors show changes in total body weight, adrenal weight, and histology of several organs. In addition, the transplant mice have an axial fat deposition pattern and adrenal cortex hypertrophy. The circulating levels of ACTH are markedly increased in both transgenic and transplant mice. These mice may therefore be useful models for research related to Cushing's syndrome and Cushing's disease.

Material and Methods

Transgenic Mice

Transgenic mice were generated as described[8] by microinjection of the polyoma (Py) early region promotor linked to the cDNA encoding Py large T (PyLT) into the male pronuclei of fertilized eggs. The cDNA has a deletion in the late region and at the origin of replication that prevents autonomous replication. Transgenic pups in a litter were identified by the presence of PyLT in their genomic DNA. The DNA was isolated from tail biopsies taken at 2 weeks of age, digested with *Hind*III, transferred to nitrocellulose and hybridized with a ^{32}P-labelled probe against the Py sequences.[8] Nontransgenic mice were used as control animals in the study. The mice were kept in a room with humidified and filtered air together with other transgenic lineages. The facility is approved by the American Association for Accreditation for Laboratory Animal Care (AAALAC) and is under the care of Dr. J. Pick. The mice had food and water *ad libitum* and were inspected on a daily basis. Mice were anesthetized with 0.65 ml 2.5% avertin per 30 g animal weight (100% avertin is a weight/volume formulation composed of 5g 2,2,2 tribromoethanol (Aldrich Chemical Company Inc., WI) in 10 ml 2-methyl-2-butanol (Fluka Chemical Corp., NY)). The time course of weights and morphology were analyzed by killing transgenic mice together with age- and sex-matched controls at 4 and 9 months of age in addition to sick mice. Each group contained 4–5 experimental mice and 3–6 control mice.

Transplant Mice

Pituitary tumors were carefully excised from the transgenic animal (PyLT-1), minced with fine scissors and rinsed in sterile saline. Tumor pieces were further minced with sterile scalpel blades and forced through a 19G hypodermic needle before subcutaneous injection of 0.1 ml tumor slurry into 6–10 week B6D2F1 nontransgenic female mice (first passage). Tumors were passaged four times using this technique, using 3–4 week old B6D2F1 mice in passages 3 and 4. Between 4 and 15 test animals were transplanted in each experiment and accompanied by 3–5 controls. All transplant mice were females except for the third passage in which both genders were used. The transplant mice were weighed every 2–4 weeks, and the implant site was both inspected and palpated. Five months after transplantation all the third passage animals were anesthetized, and tumor diameter was measured. A group of six randomly chosen female transplant mice were autopsied at this point. The remaining nine females and eight males were followed for an additional 3 months.

Pathology

After lethal anesthesia, blood samples were obtained and the mice were subjected to complete autopsy. The organs were quickly removed, the weights were recorded after excess fat was trimmed, the specimens were fixed in 10% buffered formalin (Tissue-Fixx, Lerner Laboratories, PA), and they were processed for paraffin embedding or fixed and processed for ultrastructural analyses (R. Holm, unpublished results). The paraffin sections were stained in batches with H&E. Tumor specimens from five representative cases (two PyLT-1 mice and three mice receiving subcutaneous transplants) were processed for immunohistochemical demonstration of the anterior pituitary hormones using standardized techniques as previously described.[9,10] The antibodies were used in the commercially available prediluted forms: ACTH and PRL (both from Lipshaw, Detroit, MI), TSH, FSH, LH, and GH (all from Biomedica, Foster City, CA).

ACTH Measurement

Blood was obtained by heart puncture, transferred to chilled microfuge tubes, centrifuged and heparin plasma was stored at −70 C until assayed. ACTH was measured using a double-antibody radioimmunoassay (RIA) as de-

scribed by Nicholson et al.[11] The immunoreagents (human ACTH antiserum #AFP 6328031, human ACTH #AFP 2938C for iodination and rat reference preparation #rACTH-RP-1) were supplied by the National Hormone and Pituitary Program (NHPP) at the University of Maryland School of Medicine and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

Statistical Analysis

The relationship between the mean weights of the experimental and the control animal groups were evaluated nonparametrically using the Mann-Whitney two sample test in the NCSS 5.1 Graphics programme (Dr. J. L. Hintze, Kaysville, Utah 84037). A value of $P < 0.05$ was considered significant.

Results

Of the two transgenic mouse lineages generated with PyLT cDNA microinjection,[8,12] mice of one lineage (PyLT-1) developed pituitary tumors with a complete penetrance and a long latency (lifespan approximately 14 months). Mice of the second lineage (PyLT-2) developed a similar pituitary microadenoma but with a much lower penetrance of the phenotype (VLB, unpublished results). The PyLT-1 line is investigated here.

Weight Changes of Transgenic PyLT-1 Mice

To study the time course of the histomorphology in the pituitary gland and in organs affected by ACTH overproduction, symptom-free transgenic mice at 4 and 9 months of age were studied in addition to sick transgenic mice and compared with nontransgenic matched controls. The early signs of the PyLT-1 phenotype were an enlarged penis and enlarged scrotal pads in males, whereas female mice did not show specific early signs (VLB, unpublished results). When the mice became clinically ill, they huddled together, they developed both a spinal hump in the thoracic region and an unsteady gait, they showed fur changes (loss of hair and shine), and they had difficulty in easily reaching food and water. Sick mice deteriorated rapidly and were killed quickly after serious symptoms developed. The mean age (and standard deviation) at the time of autopsy for these animals was 15.5 (0.9) months for males and 13.1 (2.4) months for females. The age difference between the genders approached statistical significance ($P = 0.0679$).

Table 1 gives the weights of transgenic and control mice according to age and sex. Both male and female PyLT-1 mice showed a peak in total body weight at 9 months with subsequent weight loss. The difference between body weight of the experimental and the control group was statistically significant for females (weight loss) at 12 months ($P = 0.0143$) and for males (weight increase) at 9 months ($P = 0.0339$). Furthermore, at 9 months the increased body weight for female PyLT-1 mice approached statistical significance ($P = 0.083$). The mean weight of the adrenal glands increased with age in PyLT-1 female mice contrary to the stable trend in the control mice, and the difference was statistically significant at both 9 ($P = 0.0209$) and 12 months of age ($P = 0.0209$). For males, only the sick PyLT-1 mice differed from their control group ($P = 0.0106$) in adrenal weight. These findings are illustrated in Figure 1 where the total and adrenal weights are shown as the percentage of the corresponding control values. None of the groups dif-

Table 1. *Weight Data: Differences Between Transgenic and Control Mice**

	Sex	Mice	Weight (SD)		
			4 months	9 months	12 months
Body weight (g)	F	PyLT-1	25.91 (3.09)	36.58 (6.96)	19.65 (2.29)†
		Control	25.45 (0.47)	29.42 (3.53)	28.51 (2.53)
	M	PyLT-1	32.41 (1.44)	45.78 (2.17)†	38.04 (2.63)
		Control	34.99 (1.27)	36.59 (2.14)	43.38 (6.45)
Adrenals (mg)	F	PyLT-1	6.72 (0.84)	7.80 (0.79)†	10.43 (1.36)†
		Control	6.30 (0.26)	5.98 (0.63)	5.85 (0.97)
	M	PyLT-1	5.14 (1.10)	4.75 (0.35)	7.80 (1.85)†
		Control	5.00 (0.87)	4.60 (0.95)	4.80 (1.01)
Ovaries (mg)	F	PyLT-1	10.92 (1.89)	14.93 (2.91)	8.50 (2.94)
		Control	12.77 (0.68)	13.08 (2.89)	12.00 (3.79)
Testes (g)	M	PyLT-1	0.22 (0.02)	0.21 (0.01)	0.18 (0.04)
		Control	0.23 (0.03)	0.22 (0.01)	0.24 (0.05)
Liver (g)	F	PyLT-1	1.16 (0.15)	1.49 (0.22)	1.01 (0.28)
		Control	1.09 (0.07)	1.28 (0.14)	1.21 (0.12)
	M	PyLT-1	1.58 (0.08)†	1.94 (0.32)	1.94 (0.24)
		Control	1.93 (0.15)	1.59 (0.05)	1.92 (0.28)

* The data were analyzed between the transgenic and the control mice for each sex.
† $P < 0.05$.

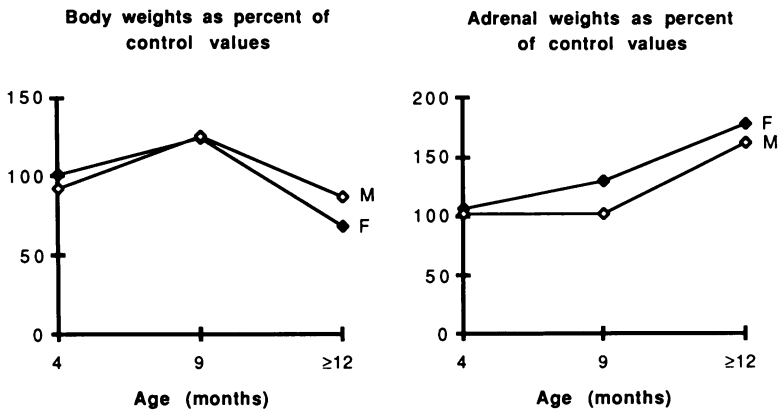

Figure 1. *The body weights (left) and adrenal weights (right) of male (M) and female (F) transgenic mice expressed as percentage of corresponding control values.*

fered statistically from control mice in weights of ovaries and testes (Table 1). The liver weights revealed a significantly higher weight for control males at the age of 4 months ($P = 0.0253$) (Table 1). Taken together, these data show that a peak in total body weight precedes the onset of serious symptoms and that the adrenal gland weights increase as the total body weights decrease in the oldest age group of PyLT-1 mice.

Pathology of Transgenic Mice

Pituitary and adrenal glands were examined by light microscopy both in the mice with macroscopic pituitary tumors and in the 4- and 9-month old asymptomatic mice. Due to the known importance of the pituitary-adrenal axis in the regulation and synthesis of sex hormones, histopathologic evaluation of the ovaries and testis was also performed. Furthermore, since the liver is central to gluconeogenesis and the kidney is the target organ for mineralcorticosteroids, these organs were also examined.

Age 4 Months

The pituitary glands of the transgenic mice (Figure 2A) had an architectural arrangement and cell distribution similar to that of the control mice. Specifically, no tumors or hyperplastic foci were observed. The adrenal glands did not show morphologic changes specific for the experimental group (Figure 2E). Both groups of mice included adrenal glands with subcapsular infiltration of small, mononuclear cells and minor degenerative changes in the reticular layer of the cortex. Sections from testis, ovary, liver, and kidney showed normal morphology, except for the finding of a unilateral cystic kidney in one transgenic animal (data not shown).

Age 9 Months

Of the four pituitary samples processed for light microscopic examination in this group, two were without morphologic changes, one showed a small nodule of hyperplastic cells, and one sample was classified as a microadenoma (Figure 2B). Both the nodule and the microadenoma were not obviously encapsulated. The adenoma cells varied in size and shape and were mainly organized in groups. Some mitotic figures and small necrotic areas were seen within this lesion.

Sections from male adrenal glands showed some increase in vacuolization of the cortical cell cytoplasm relative to the control adrenals. This vacuolization was more apparent in female adrenals, and in one out of three organs a minimal apparent medullary hyperplasia was also found (Figure 2F). The medullary changes consisted of nodules of large cells with darker cytoplasm than the ordinary medullary cells. Some mitotic figures were seen in the nodules. Sections from testis, ovary, liver, and kidney showed unremarkable morphology.

Clinically Ill Mice (Mean Age: 13–16 Months)

All mice had pituitary tumors that were round, soft, and up to 5 mm in diameter. Grossly, the surface was dark red to brown, and sagittal sections through the tumor and cerebral hemispheres showed compression of neighboring structures. Microscopically, the tumor resembled an adenoma as characterized by groups and cords of tumor cells nested around centrally located blood vessels (Figure 2C, and Figure 4 for example of blood vessel). Mitotic figures were sparse. The tumor border showed compressed tissue but no genuine capsule. Many pituitary tumor cells showed a weak positive immunoreactivity for ACTH in the cytoplasm. In a minority of the cells, the reaction was intense (Figure 2D). No immunoreactivity was found in the cells of interest with antibodies against GH, PRL, LH, FSH, or TSH.

In five of the seven adrenal samples in this group, both the cortex and the medulla could be satisfactory evaluated. There was some nodular hyperplasia of the medulla in two samples and marked hyperplasia in another two samples. One of these samples also showed necrotic foci (Figure 2G) and numerous mitoses in the

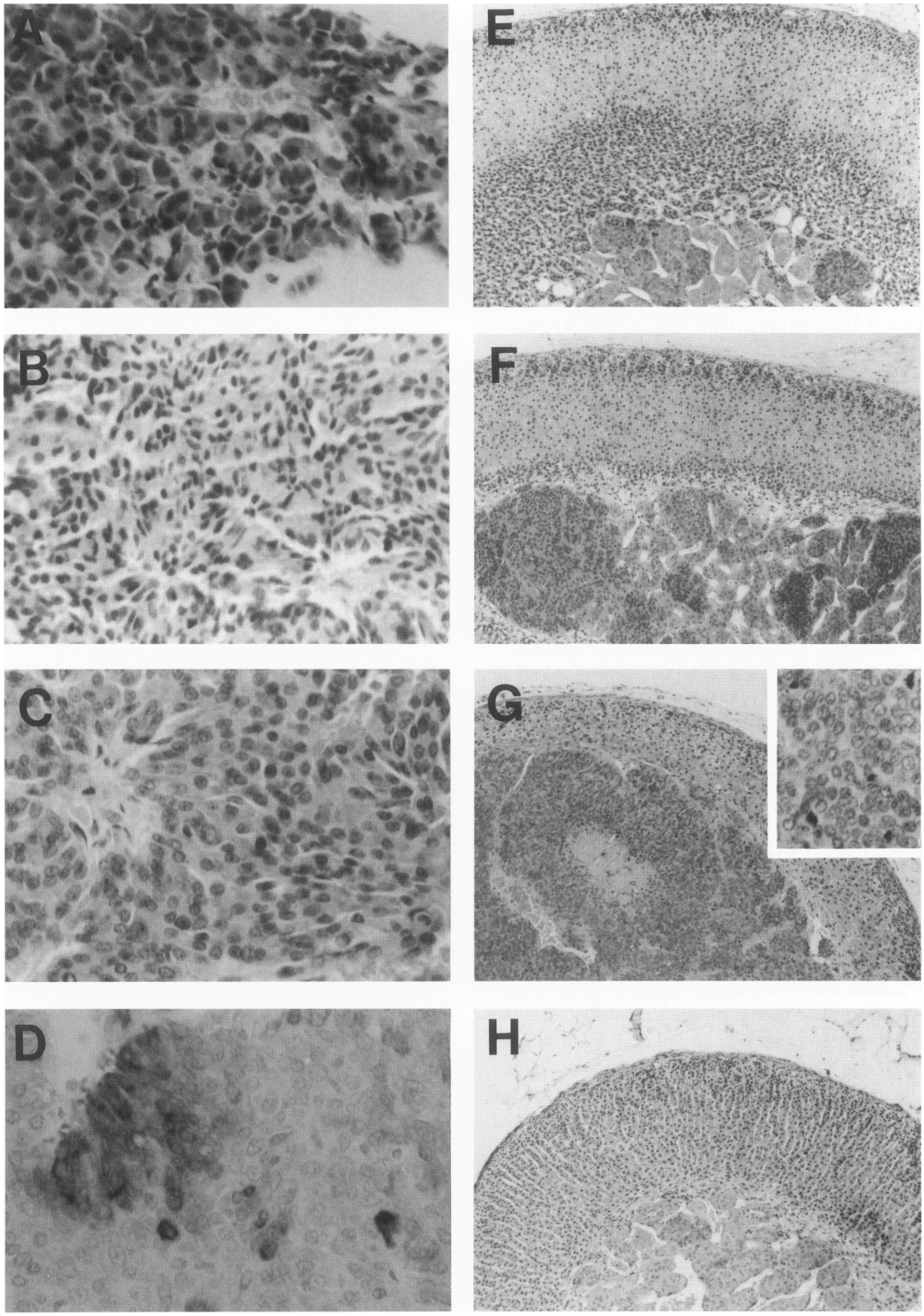

Figure 2. *Light microscopy of pituitary* (A–D) *and adrenal* (E–H) *glands. Representative sections of:* A: *Unremarkable pituitary in a 4-month-old PyLT-1,* B: *Microadenoma (whole photographic field) in a 9-month-old PyLT-1,* C: *Macroadenoma in an aged PyLT-1 transgenic mouse,* D: *ACTH-positive immunoreactivity in a PyLT-1 pituitary tumor.* E: *Histologically unremarkable adrenal in a 4-month-old PyLT-1 (note medulla in bottom center).* F: *Adrenal with nodular medullary hyperplasia in a 9-month-old female PyLT-1.* G: *Adrenal with nodular medullary hyperplasia and central necrosis showing stretched cortex overlying medullary zone (inset: numerous mitoses in medulla).* H: *Control: Adrenal in 12-month-old mouse (*A–D ×*40,* E–H ×*10, all sections H&E except* D *which was stained with AEC/hematoxylin counterstain).*

medulla (Figure 2G, inset). The cells of the cortex were vacuolated to varying degrees. The overall size of the adrenal gland was increased and the cortex thickness was reduced compared with age-matched control adrenals (Figure 2H). Sections from testis, ovary, liver, and kidney showed normal morphology, except for the finding of a hemangioma in the liver of one transgenic animal (data not shown).

Taken together, the morphologic data indicate that neoplastic changes in the pituitary of the PyLT-1 transgenic mice are usually not apparent until after the age of 9 months and that the most pronounced morphologic change in the adrenals of older PyLT-1 mice is a medullary hyperplasia. The cells of the adrenal cortex show an increased vacuolization, which is indicative of increased steroid synthesis. Although the adrenal cortical observations fit a model of hypercorticotropism, the adrenal medullary findings were unexpected.

Transplanted Tumors

To further characterize the biological properties of the tumor while searching for an animal model of Cushing's disease that exhibited shorter latency, the transgenic pituitary tumor was serially transplanted in immunocompetent mice. The transplanted tumors grew slowly and in the second passage it took about 9 months for the tumor mass to reach approximately 1 cm in diameter. In third and fourth passages, the tumors took only 5 months to reach a comparable size. The male mice with transplanted pituitary tumors had penis and scrotal pad enlargement similar to PyLT-1 transgenic mice and mice of both genders showed a neck hump of increased fat deposition. The latter sign was more apparent in female mice.

The mice with third passage transplant tumors were anesthetized at 6 months of age to allow for accurate measurement of tumor size. In Figure 3 the total body weight of the animal is plotted against the tumor diameter. A positive, but weak relationship between the two parameters was noted for males (correlation coefficient: 0.60). The tumor size ranged up to 10 mm in diameter, and the coefficient for the regression line was 0.276. The regression line crossed the y-axis at 38.4 g and the mean weight of male controls was 29.6 g (SD 1.67). A stronger correlation was found for female mice (correlation coefficient: 0.78) and the tumors were larger, up to 16 mm. The regression coefficient was 0.7236, and the regression line crossed the y-axis at 27.3 g, close to the mean weight of female controls at 24.4 g (SD 1.87). Thus, female mice that received transplants differed from their male counterparts in having tumor burdens that reached a larger

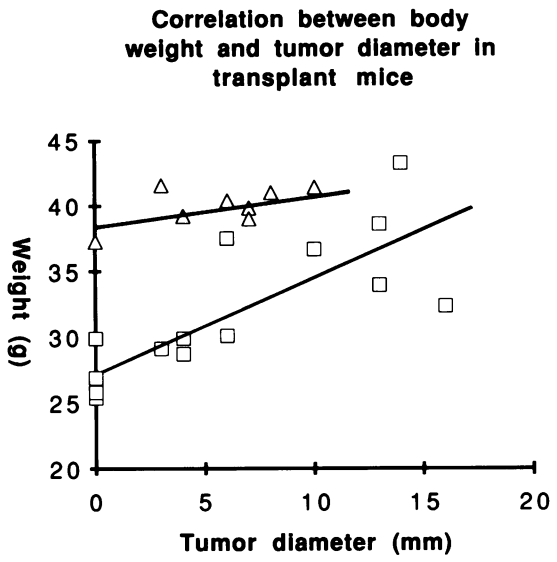

Figure 3. *Scatter grams with regression lines for body weight versus tumor diameter in 6-month-old male (△) and female (□) mice carrying 3rd-passage transgenic pituitary tumors (see text for details).*

size and they had a higher correlation between tumor size and body weight.

Table 2 gives the weights of 6- and 9-month-old female mice that received transplants and of 9-month-old female PyLT-1 transgenic mice, the latter from Table 1. Comparison is made between the two age groups of mice with transplanted pituitary tumors and between transplanted and PyLT-1 mice of the same age. Total body weight as well as adrenal and liver weights all show a statistically significant increase from 6 to 9 months in mice with transplanted tumors. A similar difference is seen between the PyLT-1 and transplanted mice of the same age, although the difference did not reach statistical significance for liver ($P = 0.0771$). For the ovaries, no significant weight differences were found. Comparison of the adrenal weights between Tables 1 and 2 suggest that the transplant mice are clinically in a state of hypercorticotropism at 6 months of age, and that this state is significantly enhanced at 9 months of age (Table 2).

Pathology of Transplant Mice

In one of the six transplanted mice autopsied at 6 months of age, no palpable tumor was evident, but a 1–2 mm thick nodule of suspected tumor tissue was found by dissection. Microscopic examination confirmed the adenomatous pituitary nature of this tissue. The five other transplanted tumors taken at the same time showed similar histomorphology to that seen in the primary tumor but with a higher mitotic activity (Figure 4). Immunocytochemically, the transplanted tumor tissue showed the same pattern of immunoreactivity as the primary pituitary

Table 2. *Weight Data Comparison Between Female Transplant Mice and Transgenic Mice (PyLT-1)*

Parameter	Group	Age*	Mean weight (SD)	*P*-value
Body weight (g)	Transplant	6	30.26 (3.34)	0.0201†
	Transplant	9	42.33 (3.53)	0.0339‡
	PyLT-1	9	36.58 (6.96)	
Adrenals (mg)	Transplant	6	8.97 (2.09)	0.0201†
	Transplant	9	19.97 (6.71)	0.0339‡
	PyLT-1	9	7.80 (0.79)	
Ovaries (mg)	Transplant	6	11.47 (2.26)	NS
	Transplant	9	13.53 (2.56)	NS
	PyLT-1	9	14.93 (2.91)	
Liver (g)	Transplant	6	1.43 (0.18)	0.0389†
	Transplant	9	2.00 (0.30)	NS
	PyLT-1	9	1.49 (0.22)	

* No. of mice in each group: transplant 6-month n = 6, transplant 9-month n = 3, PyLT-1 9-month n = 4.
† Significant difference when comparing transplant mice 6- and 9-months old.
‡ Significant difference when comparing 9-month-old transplant mice to PyLT-1.
NS = not significant.

tumor tissue. It was immunoreactive for ACTH (Figure 4) and negative for GH, PRL, LH, FSH, and TSH. Figure 5 illustrates the light microscopic findings for adrenals, ovaries, and liver in 9-month-old transplant mice compared with control mice of the same age. The adrenal gland from the animal with transplanted tumor is larger than the control (Figure 5A–B) due to cortical hypertrophy. Pathologic abnormalities of the adrenal medulla were not revealed. The ovaries showed follicular cysts in various stages of maturation in both groups of mice, but only ovaries in the control animals contained corpus lutea (Figure 5C–D). The liver sections showed hydropic degeneration in the transplanted mice and two of three liver specimens also showed macrovesicular fatty change. Liver sections from control mice showed no pathologic abnormality.

Thus, the transplanted tumors shared the morphologic and immunohistochemical characteristics of the primary tumors. The experimental animals showed an adrenal gland hypertrophy confined exclusively to the cortex. The lack of corpora lutea would indicate ovulatory disturbances that are possibly due to androgen overproduction as a secondary effect of ACTH stimulation. The reversible injury (fatty change) seen in the liver could result from increased gluconeogenesis by chronic ACTH stimulation.

ACTH Plasma Levels

The weight and histologic changes seen in both the transgenic mice and the transplanted tumors, and the selective ACTH immunoreactivity of the tumors strongly indicated elevated systemic levels of ACTH in both types of mice. Corticotropic cell adenomas can, however, occur in the absence of high blood levels of ACTH.[13] We therefore established a radioimmunoassay for measuring blood levels of rodent ACTH. Data for clinically ill female PyLT-1 transgenic mice are presented in Figure 6 and compared with control mice and mice with transplanted tumors (2nd passage) of similar age. The median value in the control group (n = 3) was <10 μg/l compared with 685 μg/l in the transgenic (n = 4) and 1750 μg/l in the transplant mice (n = 5). The median value for the transgenic mice was about 70-fold that of the control value whereas the transplant group had a median value about twofold that of the transgenic group. Both differences were statistically significant. These data conclusively show that the ACTH immunoreactive pituitary adenomas in the PyLT-1 transgenic mice are functioning and associated with elevated blood levels of ACTH. Moreover, maximum circulating levels of ACTH were found in immunocompetent mice with subcutaneous transplants of the pituitary tumor.

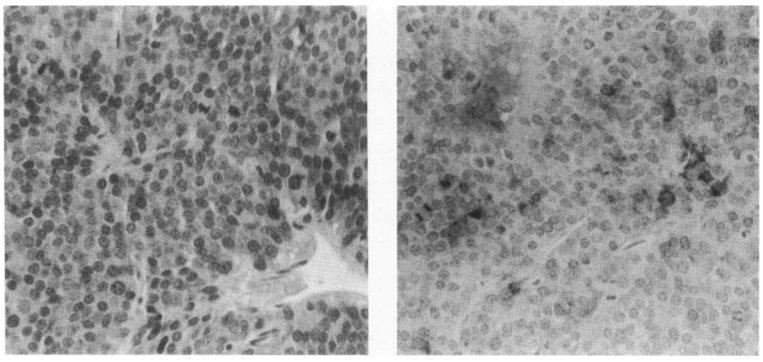

Figure 4. *Photomicrograph of transplanted pituitary tumor. Left: H&E section showing an adenomatous tumor with numerous mitoses (40×). Right: Same tumor showing ACTH-positive immunoreactivity (AEC/hematoxylin ×40).*

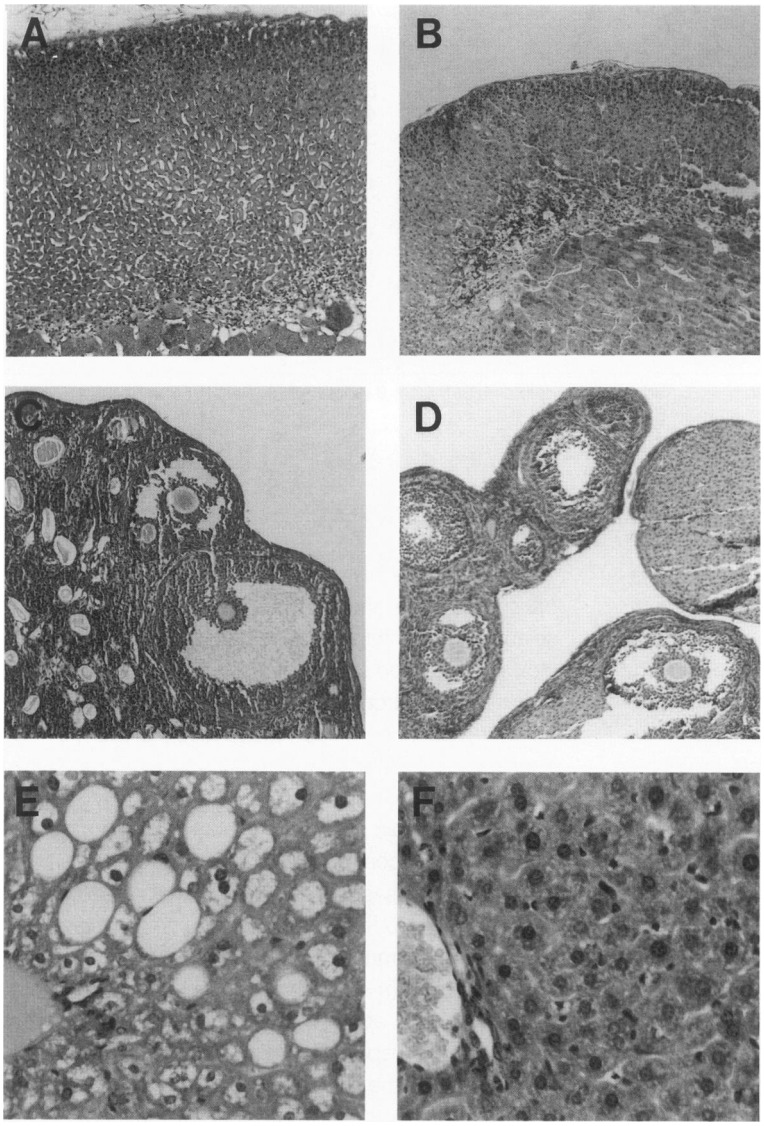

Figure 5. *Photomicrographs of an adrenal gland, ovary, and liver in a 9-month-old transplant mouse (left row) and in a control mouse (right row). The adrenal cortex is thicker in the transplanted animal* **(A)** *than the control* **(B)**. *Sections of ovaries from the transplanted mouse show follicles at different stages of maturation* **(C)** *but no corpus luteum are seen. This is in contradiction to the control mouse* **(D)**. *A section of liver from transplanted mouse shows fatty changes in the hepatocytes* **(E)** *as opposed to the control mouse* **(F)**. *All sections were stained with H&E.* **A–D** *×10,* **E–F** *×40.*

Discussion

The introduction of oncogenes into the mammalian germline and their expression has resulted in the development of tumors in a wide range of tissues in transgenic mice.[14,15] Transgenic mice carrying a hybrid gene consisting of the polyoma early region promotor linked to the polyoma large T antigen cDNA heritably develop pituitary tumors that are positive for transgene expression (VLB, unpublished results).[8,12] We describe the pathology of these mice and we show that they provide the first murine model for Cushing's disease. The model adds the advantages of an *in vivo* system to work related to Cushing's disease, and supplements *in vitro* studies with the AtT-20 cell line[16,17] that has been the main experimental model in the field.

The PyLT-1 transgenic mice and especially immunocompetent mice with transplanted PyLT-1 pituitary tumors shared physical signs, histopathologic characteristics and metabolic changes with Cushing's disease patients. All affected mice had elevated blood ACTH levels. The transplant mice showed weight gains with an axial deposition pattern that resembled the buffalo hump of patients with Cushing's disease. The transgenic mice and the transplant mice showed fur changes including dull hair and loss of hair that probably correlate with the dystrophic skin changes seen in patients. Although the transgenic mice showed an unexpected adrenal medullary hyperplasia, the transplant mice showed only a marked hyperplasia of the adrenal cortex. Thus, the histopathology of the adrenal glands of the transplant mice resembles that of Cushing's disease patients. Livers of transplant mice showed fatty degeneration that indicated distortions in carbohydrate metabolism. This interpreta-

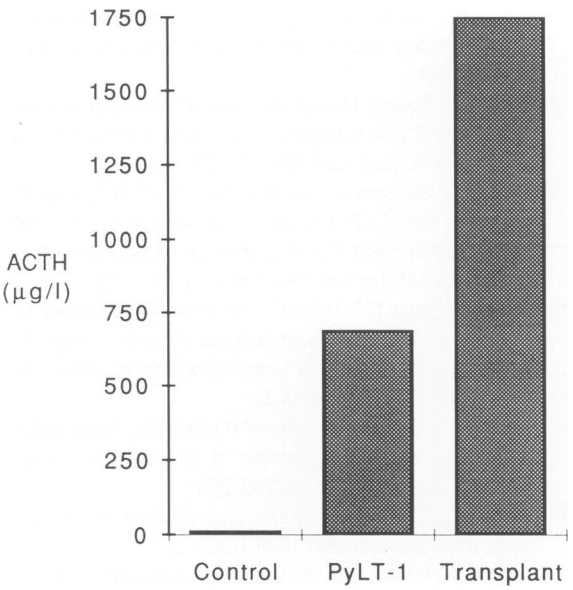

Figure 6. *Median plasma ACTH in 1-year-old female mice.*

tion is further supported by the demonstration of hyperglycemia in transplant mice (AH and VLB, unpublished data), and this condition is seen in patients with Cushing's disease.

Several other transgenic mice with pituitary neoplasia have been analyzed in addition to those reported here. Murphy et al[18] found anterior pituitary tumors in addition to pancreatic β-cell tumors in mice carrying the upstream sequences of the bovine vasopressin gene linked to SV40 T antigen. Windle et al[19] used the same oncogene linked to the promotor/enhancer region of the human glycoprotein hormone alpha-subunit, and the resulting transgenic mice develop anterior pituitary tumors. A clonal cell line from these tumors synthesizes and secretes alpha-subunit protein. In a third system, pituitary hyperplasia was found in mice carrying a rat growth hormone promotor-cholera toxin hybrid transgene.[20] In these models, however, there was no evidence of ACTH-producing tumors. Thus, transgenic mouse models presently available have features that mimic various diseases involving the anterior pituitary: multiple endocrine neoplasia type 1,[18] gigantism,[20] nonfunctional pituitary adenoma,[19] and Cushing's disease (present report).

Both transgenic mice and mice with transplanted tumors were analyzed in this study. The primary PyLT-1 pituitary tumors were adenomas based on classic morphologic features such as moderate cellular atypia and lack of metastatic capacity. The long latency of both the primary and the transplanted tumors is in accordance with the growth rates of pituitary adenomas in human patients.[21] Transgenic mice showed a weight peak at 9 months of age followed by wasting. The initial weight increase is most likely related to ACTH secretion from the mircoadenoma, whereas the weight decrease seen in older animals with a generally poor physical condition is likely to be caused by the mass effect of the macroadenoma. The tumor generally reached 5 mm in diameter and compressed the midbrain and the cerebral hemispheres. The mice carrying pituitary tumor transplants, however, increased their weight far beyond the peak values of the transgenic mice and developed the axial fat deposition pattern that mimics Cushing's disease.

The adrenal weights increased with age both in transgenic and in transplanted mice, but two important differences were found: the weight increase was significantly higher in the transplanted mice, and the adrenals in these mice showed a marked thickening of the fasciculate and reticulate layers of the cortex, as would be expected after ACTH overstimulation. The sick PyLT-1 mice, in contrast, usually showed a nodular hyperplasia of the adrenal medulla. The surrounding cortex seemed attenuated rather than hypertrophic, but it should be emphasized that morphometric analyses have not been performed. The increased vacuolization of the adrenal cortex in these old transgenic mice indicates, however, that the cortical cells were exposed to elevated ACTH levels.

There are several possible explanations for the nodular hyperplasia of the adrenal medulla in the old transgenic mice. These include: 1) a direct stimulus on the medulla by the glucocorticoids, 2) compression by the pituitary macroadenoma on adjacent midbrain with altered output to the adrenal medulla through the sympathetic nervous system, 3) expression of the transgene in hypothalamic cells secreting corticotropin-releasing hormone (CRH), and 4) expression of the transgene in the adrenal medulla. Although we have no direct evidence in support of these models, we favor the hypothesis that the transgene is expressed in the adrenal medulla for several reasons. First, the transplant mice showed no adrenal medullary hyperplasia, suggesting that a systemic effect is not involved. Second, the histology of the adrenal medullary hyperplasia, with numerous mitotic figures and areas of necrosis, suggests that the cells are not dependent on external growth stimulation. Finally, oncogene expression in more than one neuroendocrine cell type in transgenic mice has been previously reported.[18,22] In the present study, we have analyzed the pituitary, the adrenals, the ovaries, and the testes. In forthcoming studies, the thyroid, parathyroid, and the pancreas will be evaluated for pathology.

The aged female PyLT-1 mice had to be killed at a mean age of 13.1 (SD 2.4) months (n = 6) compared with 15.1 (SD 0.9) months for males (n = 5). Although the *P* value for the difference between the groups was statistically not significant at 6.79%, the observation suggests that a sex-related growth stimulus such as estrogen may be involved in tumor growth. The observed

higher maximal tumor size and higher correlation between tumor diameter and body weight in female transplant mice supports this hypothesis.

Both primary pituitary tumors and the tumors transplanted to immunocompetent mice showed a similar histomorphology, and both showed positive immunoreactivity only for ACTH and pathologically elevated levels of ACTH in the blood. However, the transplant mice differed in several respects from the PyLT-1 transgenic mice. First, the latency of the transplanted tumors was shorter and this is an advantage for experimental work. Second, transplant mice had a marked adrenal cortical hypertrophy that was less complex than the adrenal pathology of transgenic mice with hypertrophy of the adrenal medulla. Finally, transplant mice had a consistent weight gain with axial deposition that is characteristic of Cushing's disease rather than the wasting seen in old transgenic mice. The mice with transplanted PyLT-1 pituitary tumors may, therefore, be the most useful of the two murine models for the study of Cushing's disease.

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