Pituitary-directed Leukemia Inhibitory Factor Transgene Forms Rathke's Cleft Cysts and Impairs Adult Pituitary Function

A Model for Human Pituitary Rathke's Cysts

Sadanori Akita,* Carol Readhead,* Lucia Stefaneanu,‡ Jeremy Fine,* Alina Tampanaru-Sarmesiu,‡ Kalman Kovacs,‡ and Shlomo Melmed*

**Department of Medicine, Cedars-Sinai Research Institute, University of California, Los Angeles School of Medicine, Los Angeles, California 90048; and ‡Department of Pathology, St. Michael's Hospital, University of Toronto, Toronto, Ontario, Canada M5B 1W8*

Abstract

Leukemia inhibitory factor (LIF) and LIF receptors are expressed in adenohypophyseal cells and LIF regulates pituitary hormone transcription and cell replication in vitro. Therefore, transgenic mice expressing pituitary-directed LIF driven by the rat growth hormone (GH) promoter were generated to evaluate the impact of LIF on pituitary development. Three founders were established with diminished linear growth and body weight (57–65% of wild type [WT]), and intense anterior pituitary LIF immunoreactivity. Cystic cavities observed in pituitary anterior lobes were lined by cuboidal, ciliated epithelial cells, focally immunopositive for cytokeratin and S-100 protein and immunonegative for adenohypophyseal hormones. Transgenic pituitaries showed decreased GH (40%) and prolactin (PRL) (26%) cells, and decreased GH and PRL mRNAs by in situ hybridization. ACTH cells increased 2.2-fold, whereas gonadotrophs and thyrotrophs were unchanged. Serum GH was undetectable (< 0.78 ng/ml), PRL levels were one third of WT ($P < 0.05$), IGF-I levels were 30% of WT ($P < 0.001$), and T4 was normal. 10 human pituitary Rathke's cysts studied all showed conclusive LIF immunoreactivity in cyst-lining cells. Thus, intrapituitary murine LIF overexpression causes cystic invaginations from the anterior wall of Rathke's cleft, suggesting failed differentiation of Rathke's epithelium to hormone-secreting cells. Arrested murine pituitary maturation with formation of pituitary Rathke's cleft cysts, GH deficiency, and short stature provide a model to study human Rathke's cyst pathogenesis. (*J. Clin. Invest.* 1997. 99:2462–2469.) Key words: Rathke's cyst • leukemia inhibitory factor • pituitary development • transgenic model

Introduction

Leukemia inhibitory factor (LIF),[1] a pleiotropic cytokine, is a determinant of neuronal, hematopoietic, uterine, and metabolic development and function (1, 2). LIF arrests embryonic stem cell differentiation and selectively inhibits primitive ectoderm formation in vitro (3). Disruption of LIF expression results in failure of uterine blastocyst implantation (4). Tissue-specific LIF overexpression in vivo leads to switching from noradrenergic sympathetic to cholinergic innervation in the pancreas (5) or to altered thymic epithelium and interconversion of thymic and lymph node morphologies (6). Injection of LIF-producing hematopoietic cells causes cachexia and lethality in mice (7), while LIF antiserum appears to protect animals from endotoxic shock (8). Pituitary LIF and its binding sites are expressed predominantly in human fetal corticotrophs and somatotrophs as early as 14 wk of gestation (9). LIF potently synergizes with corticotropin-releasing hormone to enhance propiomelanocortin (POMC) transcription and ACTH secretion in vitro (10). Conversely, LIF antagonizes corticotropin-releasing hormone–induced corticotroph proliferation by attenuating the cell cycle in S phase (11). Furthermore, injection of endotoxic lipopolysaccharide induces both LIF and LIF-receptor gene expression in the murine hypothalamus and pituitary concomitantly with ACTH induction (12). LIF chronically infused into mice bearing a disrupted LIF gene stimulates stress-induced circulating ACTH levels (13). These findings imply that LIF acts to regulate both fetal pituitary development and adult pituitary functional responses to stress. Therefore, we sought to determine the effects of pituitary-directed LIF overexpression in transgenic mice. The anterior pituitary arises from Rathke's pouch, which develops by invagination of oral ectoderm apposed to the diencephalon (14). As LIF arrests primitive ectoderm formation (3), we studied both the developmental and functional effects of LIF on the pituitary as an in vivo model of murine adenohypophyseal development. We demonstrate that pituitary-directed murine LIF expression results in persistent Rathke's cleft cysts and that the cells lining human Rathke's cysts strongly express immunoreactive LIF.

Methods

Microinjection and Southern screening. A plasmid containing the growth hormone (GH)–LIF fusion gene was constructed by ligation of a 320-bp (KpnI–XhoI) fragment of the rat GH promoter region (15) and a 670-bp (EcoRI–BamHI) fragment containing the full length murine LIF cDNA (2) into the polylinker region of pBluescript SK (Stratagene Inc., La Jolla, CA). The KpnI–BamHI sites encompassing the rGH-LIF fusion gene were ligated into pcDNA 3 vec-

Address correspondence to Shlomo Melmed, M.D., Endocrinology B-131, Cedars-Sinai Medical Center, 8700 Beverly Blvd., Los Angeles, CA 90048. Phone: 310-855-4691; FAX: 310-967-0119; E-mail: melmed@csmc.edu

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1. *Abbreviations used in this paper:* CREB, cAMP response element; CRH, corticotropin-releasing hormone; GH, growth hormone; HE, hematoxylin and eosin; LIF, leukemia inhibitory factor; POMC, propiomelanocortin; PRL, prolactin; WT, wild type.

tor (Invitrogen Corp., San Diego, CA), which contains bovine GH polyadenylation sites. The rGH-LIF fusion gene for microinjection was purified using agarose gel electrophoresis followed by electroelution and further purification with an Elutip (Schleicher & Schuell, Inc., Keene, NH). This fragment was microinjected into the pronuclei of $B6D2F_2$-fertilized mouse eggs, and the injected eggs transplanted to pseudopregnant foster mothers (16). Pups were screened by Southern blot analysis of tail DNA using a random primer-labeled 670-bp fragment containing the full length cDNA of mouse LIF. All mice were maintained in a specific pathogen-free environment and provided with food and water ad libitum.

RIAs. Mouse GH and prolactin (PRL) RIA kits (AFP10783B and AFP1077D, respectively) were provided by Dr. A.F. Parlow (Harbor-UCLA). The sensitivity of the GH and PRL RIAs was 0.78 and 0.4 ng/ml, respectively.

Light microscopy. Pituitaries were fixed in 10% buffered formalin and embedded in paraffin. 5-μm sections were stained with hematoxylin and eosin (HE) and the periodic acid-Schiff technique.

Immunohistochemistry. The streptavidin–biotin peroxidase complex method was used. The primary antibodies included anti–mouse PRL, rat GH, rat β-TSH, rat β-FSH, rat β-LH and human ACTH (all kindly donated by National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland). The details of the procedure were described previously (9). For LIF demonstration, a rabbit anti–LIF antibody that binds to both human and murine LIF (BIODESIGN Intl., Kennebunk, ME) diluted 1:40 was used on sections microwaved in citrate buffer, pH 6 (three times, 5 min each) for antigen retrieval. Mouse uterus served as positive control tissue. The substitution of primary antibody with normal rabbit serum resulted in negative immunostaining. Monoclonal mouse anti–human epithelial membrane antigen (clone E29; Dako Corp., Carpinteria, CA) (diluted 1:50), rabbit anti–human factor VIII–related antigen (Dako Corp.) (diluted 1:1,000), monoclonal anti–human cytokeratin (CAM 5.2; Becton-Dickenson & Co., Mountain View, CA) (diluted 1:10), rabbit anti–cow glial fibrillary acidic protein (Dako Corp.) (diluted 1:1,000), rabbit anti–cow S-100 protein (Z311; Dako Corp.) (diluted 1:1,000), and anti–human neurofilament protein (2F11; Dako-NF) (diluted 1:100) were also applied. For the demonstration of phosphorylated cAMP response element (CREB) antibody 5322 (diluted 1:700) and unphosphorylated CREB antibody 244 (diluted 1:400) were used. These were kindly provided by Dr. M. Montminy (La Jolla, CA).

Pituitary cells immunoreactive for GH, PRL, ACTH, TSH, and LH were counted with a 40× objective lens within a 0.01–1-mm^2 ocular grid. The total number of positive cells was expressed as percentage of the total number of cells within the same area. The counting covered the entire horizontal section of a pituitary gland in every case.

10 human pituitaries collected at autopsy, fixed in buffered formalin, and embedded in paraffin were selected based on the presence of cysts at the border of anterior and posterior lobes. Immunocytochemistry for LIF was performed as described above using the same anti–LIF antibody.

In situ hybridization. 5-μm deparaffinized sections were used for in situ hybridization. GH, PRL, and POMC mRNAs were demonstrated by hybridization to oligodeoxynucleotide probes corresponding to amino acids 145–151 of mouse GH, 64–70 of mouse PRL, and 99–108 of rat POMC. The probes were 3′-end labeled with ^{35}S-dATP using a kit (NEP-100; DuPont Canada, Mississauga, Ontario, Canada). LIF and Pit-1 mRNAs were demonstrated using RNA probes. Pit-1 antisense and sense RNA probes were synthesized from a 672-bp cDNA template subcloned in pBluescript KS. After linearization with BamHI and HindIII, transcription was performed using T3 and T7 polymerase for antisense and sense probes, respectively. The oligoprobes and RNA probes were purified with NENSORB-TM cartridges (DuPont Canada). Details of hybridization conditions, autoradiography, and controls are described elsewhere (17). Silver grains signaling GH and PRL mRNAs were counted with a 100× oil objective over 50–80 adenohypophyseal cells. The mean number of silver grains representing nonspecific hybridization was obtained from the number of silver grains on intermediate and posterior lobes, and was subtracted from the mean number of silver grains on anterior lobe cells.

Results

Screening for transgene integration. After microinjection of an rGH mLIF fusion gene (Fig. 1 *a*) into fertilized mouse eggs, progeny of the resultant foster pregnancies were screened for

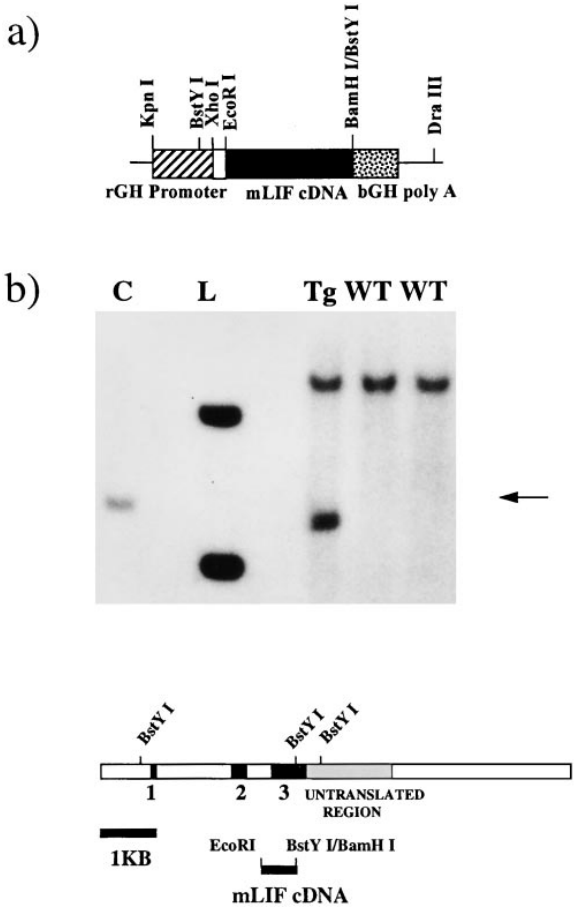

Figure 1. (*a*) The rGH-LIF construct used for microinjection. The rGH promoter region (320 bp, KpnI–XhoI) was cloned into pBluescript SK polylinker. A 670-bp EcoRI–BamHI fragment, containing the coding region of the mLIF cDNA was cloned 3′ to the promoter region. The rGH-mLIF cDNA hybrid fragment was excised from Bluescript using KpnI and BamHI restriction enzymes and cloned into the pcDNA 3 vector 5′ to the bovine GH polyadenylation sites. For microinjection, the 1.6-kb construct containing the rGH promoter region, the mLIF cDNA, and the bGH polyadenylation sites was excised from the vector sequences with KpnI and DraIII and the fragment was purified by gel electrophoresis. (*b*) Screening of pups for integration of the transgene using Southern blot analysis. Tail DNA from the pups was digested with BstYI and run on a 1% agarose gel. The DNA was transferred to nitrocellulose using an electroblotter. The blot was hybridized with a radiolabeled 670-bp EcoRI–BamHI mLIF cDNA fragment that contained exons 1, 2, and part of exon 3. This probe hybridizes to a 3.0-kb BstYI fragment from the endogenous LIF gene and a bridge 0.85-kb BstYI fragment unique to the transgene construct (*arrow*). The endogenous gene served as a copy number control (*C*). The 100-bp DNA ladder is also shown (*L*).

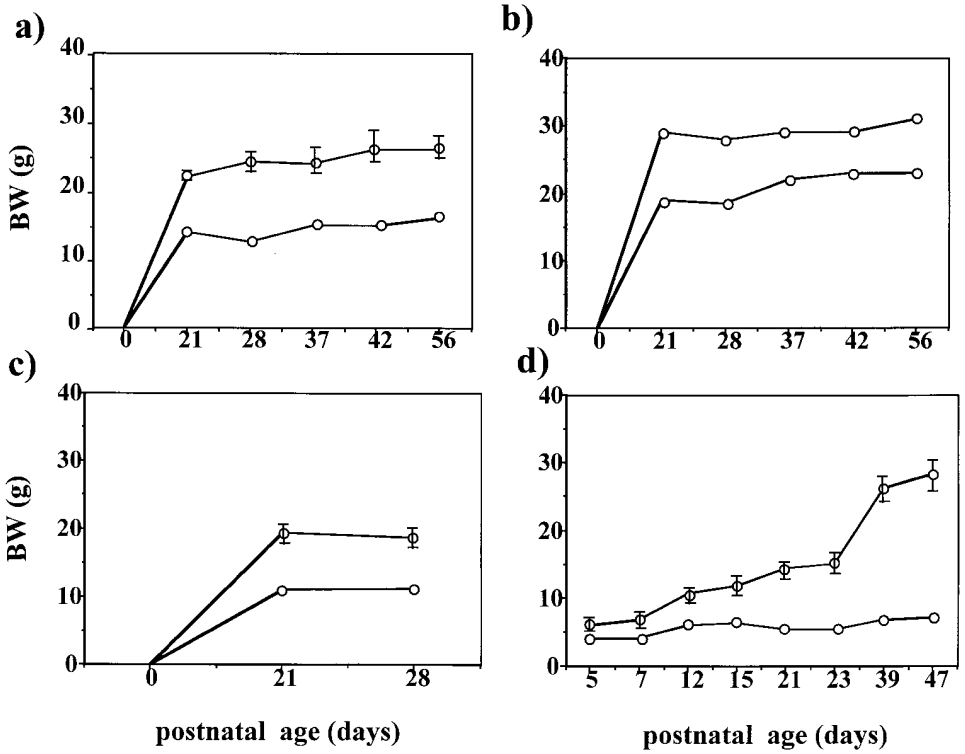

Figure 2. Body weights of three founders, F-0_1 (*a*), F-0_2 (*b*), and F-0_3 (*c*), and F-1_1(*d*). WT littermates and transgenic mice were weighed at 1000 hours on the indicated days. Each point represents mean±range of two, one, three, and two WT animals in *a–d*, respectively.

transgene integration. 250 pups were initially screened using an mLIF cDNA probe depicted in Fig. 1 *b* and integration of the rGH mLIF bGH polyA transgene was confirmed in three founder mice by Southern blot analysis. The LIF probe hybridized with a 0.85-kb BstYI bridging fragment unique to the transgene (Fig. 1 *b*). From 14 to 44 copies of the transgene were integrated into the founder mice. All three founders weighed less than wild-type (WT) littermates (Fig. 2) and as one (F-O_3) died at 4 wk, two were used for breeding. Since no pregnancies were achieved by 22 wk, their ovaries were transplanted to surrogate recipient B6D2F_1 female mice for subsequent establishment of transgenic progeny. Males were also infertile. Transgenic mice had a short life span of up to 5 mo, as compared with the expected 2 yr for WT littermates. At autopsy, they displayed diaphragmatic hypoplasia with chronic lung and liver congestion.

Fetal survival. The very low transgene integration rate (3:250) suggested that the transgene could be lethal in utero. Therefore, after microinjection, some timed pregnancies were terminated and fetal viability was assessed. After termination on embryonic day 19.5, 2/17 fetuses were dead at the time of isolation in utero and DNA analysis demonstrated transgene integration in the dead fetuses. 17 viable fetuses observed in five separate pregnancies did not exhibit transgene integration.

Hormone levels. Serum GH levels measured on three consecutive days were undetectable (< 0.78 ng/ml) in two transgenic mice (Fig. 3). IGF-I levels were also lower in transgenic animals (53–94 vs. 215–321 ng/ml, $P < 0.001$), and serum prolactin levels were decreased (5.4±0.43 vs. 14.4±2.19, $P < 0.05$). T_4 levels were not altered in transgenic animals.

GH gene analysis. To determine whether undetectable circulating GH was due to structural disruption of the GH gene by the transgene, the integrity of the murine GH gene was determined by restriction digestion and Southern analysis using a rat GH cDNA probe. DNA digestion with BstYI yielded a restriction map of GH that was unaltered compared with WT DNA (data not shown).

Pituitary morphology. On HE-stained sections, the architecture of the posterior and intermediate lobes showed no morphologic changes. The Rathke's cleft separated the anterior lobe from the intermediate lobe. In transgenic pituitary sections, the lining epithelium of Rathke's cleft formed deep invaginations into the anterior lobe and gave rise to cystic cav-

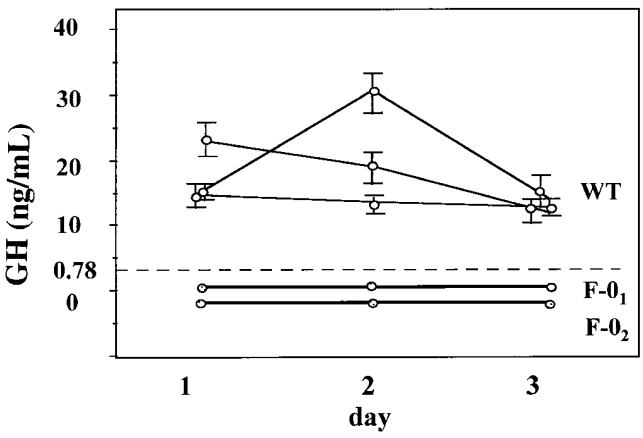

Figure 3. GH levels in WT and F-0_1, and F-0_2 mice. Retro-orbital blood was obtained from each animal under light anesthesia (methoxyfluorane, 10 s) at 1000 hours on three consecutive days. Each point represents mean±range of duplicate determinations in each animal.

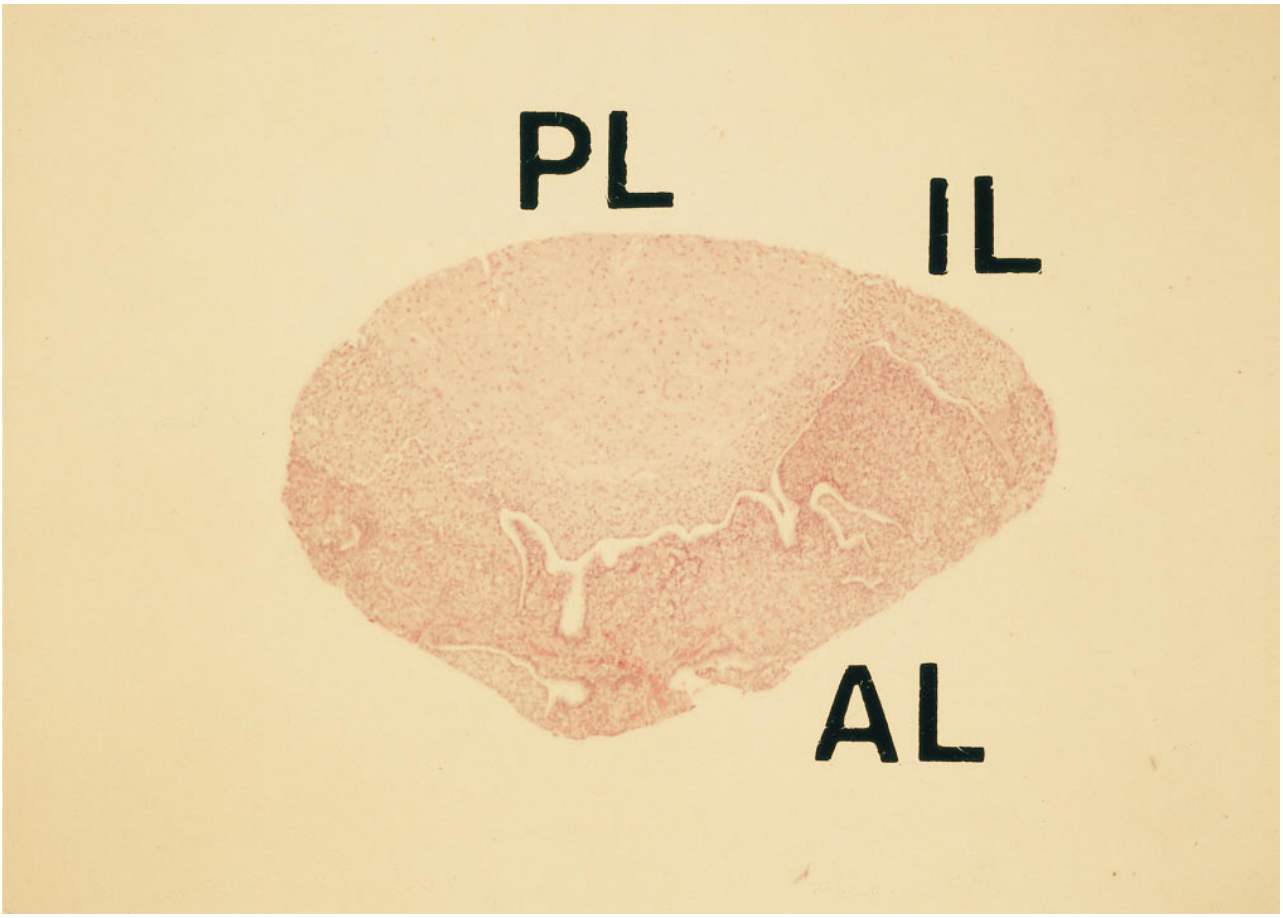

Figure 4. Horizontal section of transgenic pituitary. The anterior wall of Rathke's cleft proliferates and invaginates into anterior (*AL*), posterior (*PL*), and intermediate (*IL*) lobes. Invaginations into anterior lobe are filled with colloid. HE. Original magnification 50×.

ities filled with colloid (Fig. 4). In contrast with WT pituitaries, in transgenic mice, the cuboidal lining epithelium of the anterior wall of Rathke's cleft was ciliated (Fig. 5). The anterior lobes of transgenic mice contained a decreased number of acidophil cells and a variable number of cystic cavities filled with periodic acid-Schiff–positive colloid, occasional cell debris, and/or psammoma bodies. The pituitary cysts were evaluated for the presence of epithelial, endothelial, and neuronal immunomarkers, including epithelial membrane antigen, cytokeratin, factor VIII–related antigen, glial fibrillary acidic protein, S-100 protein, and anti–human neurofilament protein. Only cytokeratin and S-100 protein were present in epithelial cells lining the same cysts (Fig. 6), while others were immunonegative for all markers. In some cysts, one to two entrapped hormone-containing cells were found among epithelial cells.

In the anterior lobes of transgenic pituitaries, LIF immunoreactivity showed a diffuse pattern with moderate intensity in the majority of cells and intense LIF immunostaining in scattered foci of cells. In contrast, LIF immunoreactivity was diffuse in WT pituitaries (Fig. 7). Distinct cells immunostained for LIF were present in intermediate lobes of both transgenic and WT pituitaries. By in situ hybridization, the signal for LIF mRNA was weak and diffusely distributed over the anterior and intermediate lobe cells in both transgenic and WT pituitaries.

Immunocytochemistry for adenohypophysial hormones revealed the presence of all five types of hormone-containing cells (Table I). The cell counts revealed a 40% decrease of GH immunoreactive cells and a 26% decrease of PRL immunoreactive cells compared with the adenohypophyses of WT mice. Many GH immunostained cells were smaller, with an ovoid or angular contour in contrast with WT pituitaries, which had a round or polyhedral shape. In contrast, the absolute number of ACTH immunoreactive cells doubled in transgenic mice while

Table I. Quantification of Pituitary Trophic Hormone Cell Types

Cell type	WT	Transgenic
	%	%
GH	35	21
PRL	31	23
ACTH	15	33
TSH	9	10
LH	10	13

Immunopositive cells present in the horizontal section of each pituitary gland were determined within a 0.01-mm^2 ocular grid and expressed as a percentage of total cell number per pituitary.

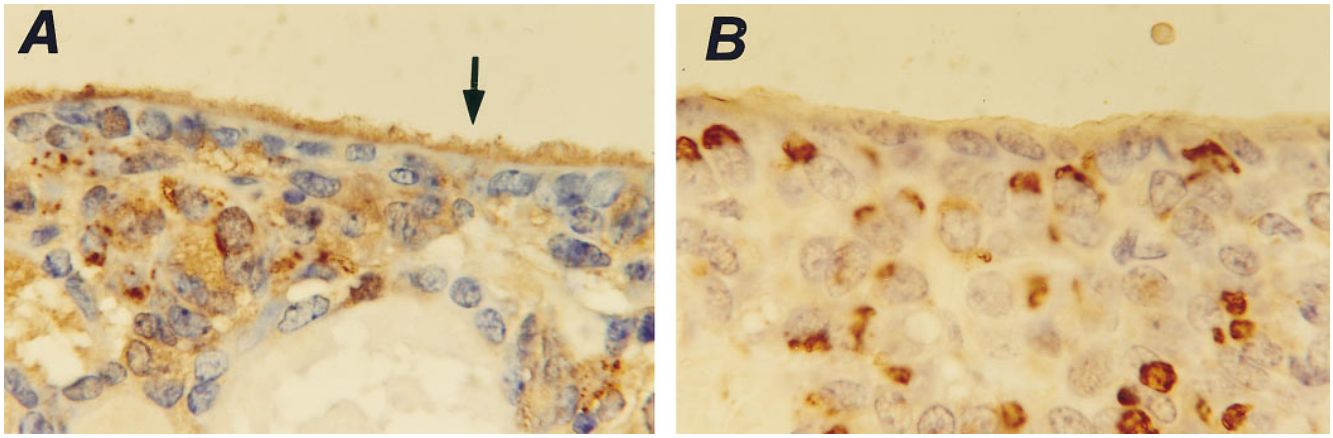

Figure 5. Epithelial lining of Rathke's cleft. (*A*) Epithelium lining the anterior wall of transgenic Rathke's cleft is ciliated (*arrow*) in contrast with a WT pituitary in which no cilia are seen (*B*). Immunostaining for PRL. Original magnification 1,250×.

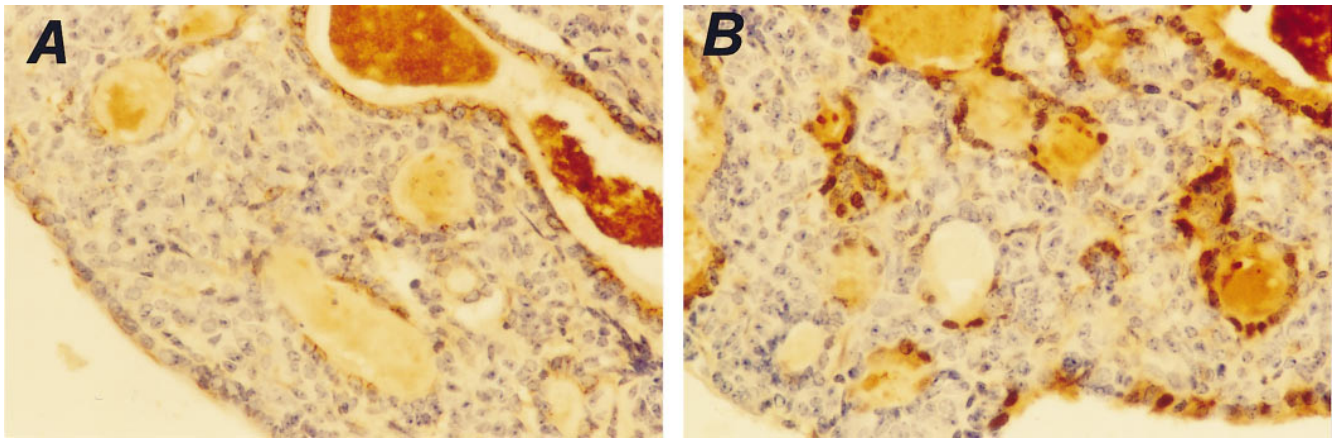

Figure 6. Epithelial cells lining the cysts are immunoreactive for cytokeratin (*A*) and for S-100 protein (*B*). Streptavidin-biotin peroxidase method. Original magnification 500×.

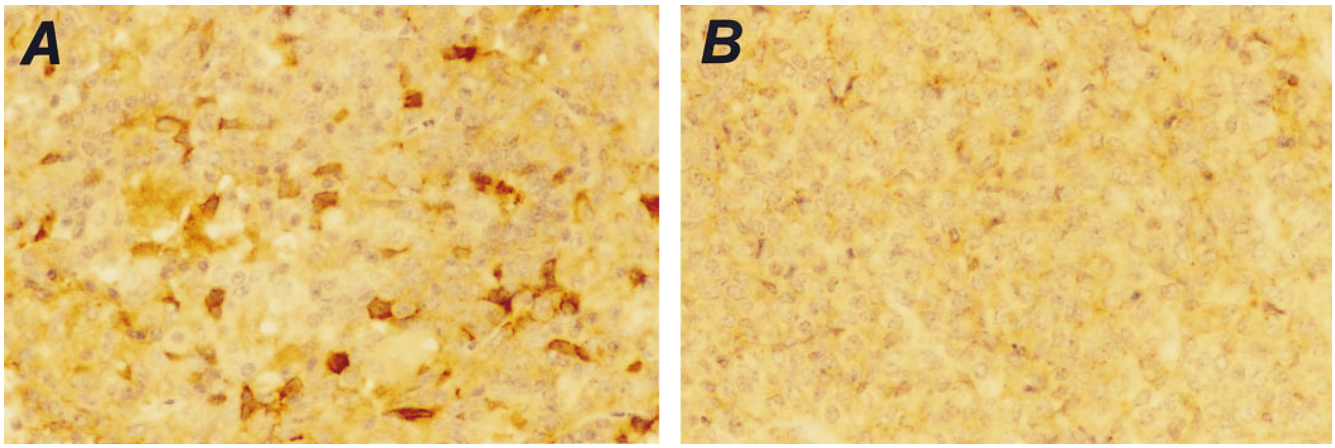

Figure 7. Pituitary LIF immunoreactivity. In the anterior lobe of a transgenic mouse, LIF immunoreactivity is intense in scattered cells (*A*), while in a WT pituitary, LIF has a diffuse, weak distribution (*B*). Negative controls yielded no signal when incubated with preimmune serum. Streptavidin-biotin peroxidase method. Original magnification 500×.

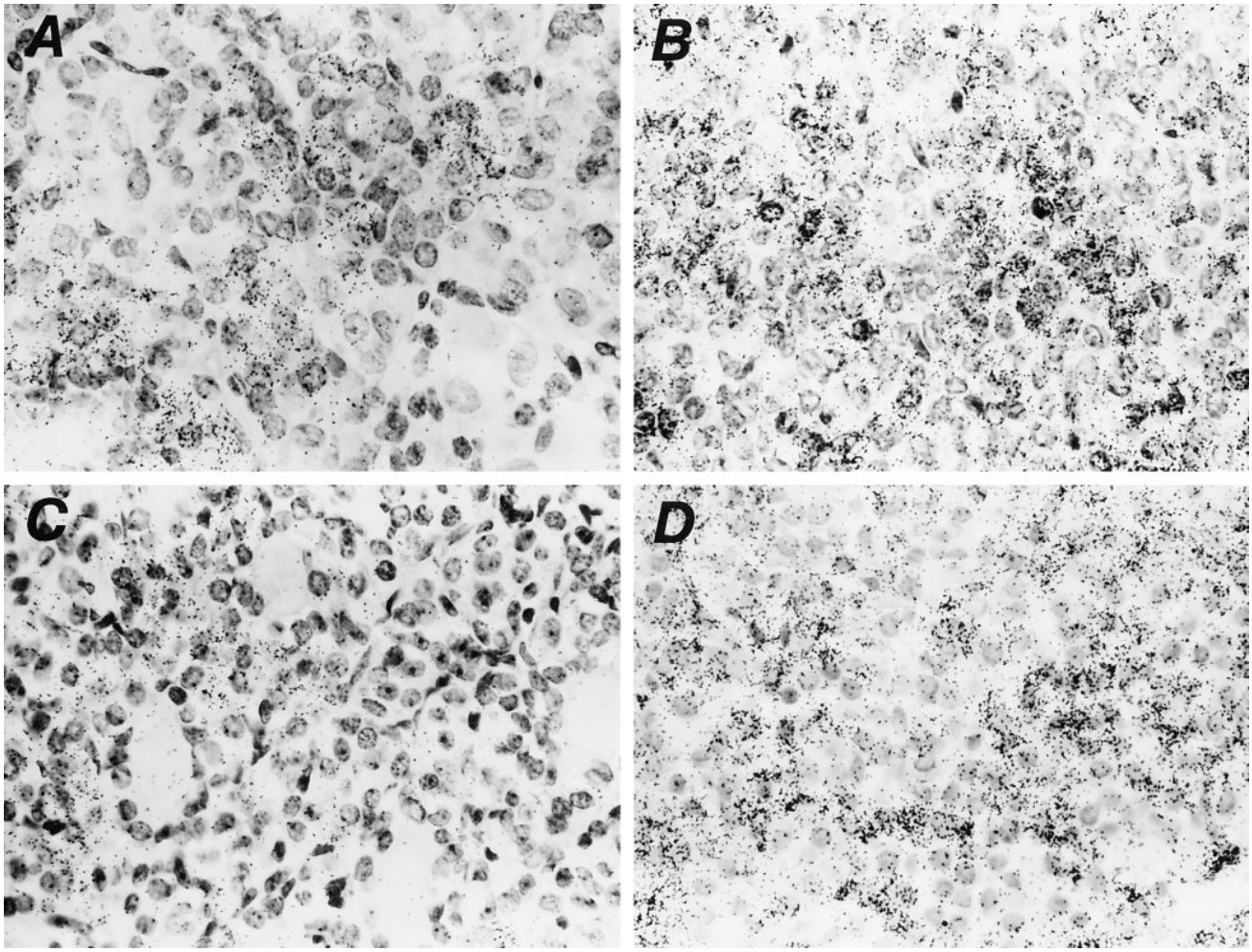

Figure 8. In situ hybridization for pituitary GH (*A* and *B*) and PRL (*C* and *D*) mRNA in transgenic (*A* and *C*) and WT (*B* and *D*) pituitary sections, respectively. Oligo-dT probes were 3′ labeled with ^{35}S-dATP and, after hybridization and autoradiography, silver grains were counted over 50–80 cells. Nonspecific hybridization signals (grains over intermediate and posterior lobes) were subtracted to determine specific binding to anterior pituitary cells. Original magnification 500×.

TSH and LH immunopositive cells were unchanged. After in situ hybridization, quantification of silver grains signaling GH and PRL mRNAs showed a 70 and 74% reduction, respectively, in transgenic mice (Fig. 8). No change in the intensity of POMC mRNA hybridization signal was found compared with WT pituitaries.

Immunocytochemistry for CREB and phosphorylated CREB revealed a marked decrease in the number of nuclei immunoreactive for phosphorylated CREB in the anterior lobes of transgenic pituitaries (Fig. 9). No change in the intensity of hybridization signal for Pit-1 mRNA was found in the adenohypophyses of transgenic animals in comparison with WT.

Human Rathke's cysts. The human pituitaries contained several different-sized cysts in the area representing the vestigial intermediate lobe. They were lined by one or multiple layers of epithelial cells and were filled with colloid and cellular debris (Fig. 10). Some cysts were lined by cuboidal or columnar ciliated cells, and some by flattened cells. Strong LIF immunoreactivity was present predominantly in the cysts with cuboidal-columnar epithelium. Cysts with flat cells were usually immunonegative for LIF. Some cysts contained both positive and negative LIF areas.

Discussion

The development of pituitary cysts occurs commonly in a wide variety of species, including humans. The murine Rathke's pouch is evident at gestational day 8.5 and by days 12–14, its lumen is compressed by the developing adenohypophysis and stalk. By 16 days, all three functional components of the pituitary, including the anterior, intermediate, and posterior lobes, are evident (14). Pituitary trophic hormone expression occurs from day 11.5, beginning with the α-subunit of glycoprotein hormones, and these are restricted to the definitive Rathke's pouch (18). GH expression is only evident at E 15.5–16.5. In rodents, Rathke's pouch cavity persists in the adult pituitary as Rathke's cleft (19). Mature hormone expression, however, is restricted to the differentiated trophic cell types of the anterior pituitary (20).

In humans, remnants of Rathke's pouch, an ectodermal di-

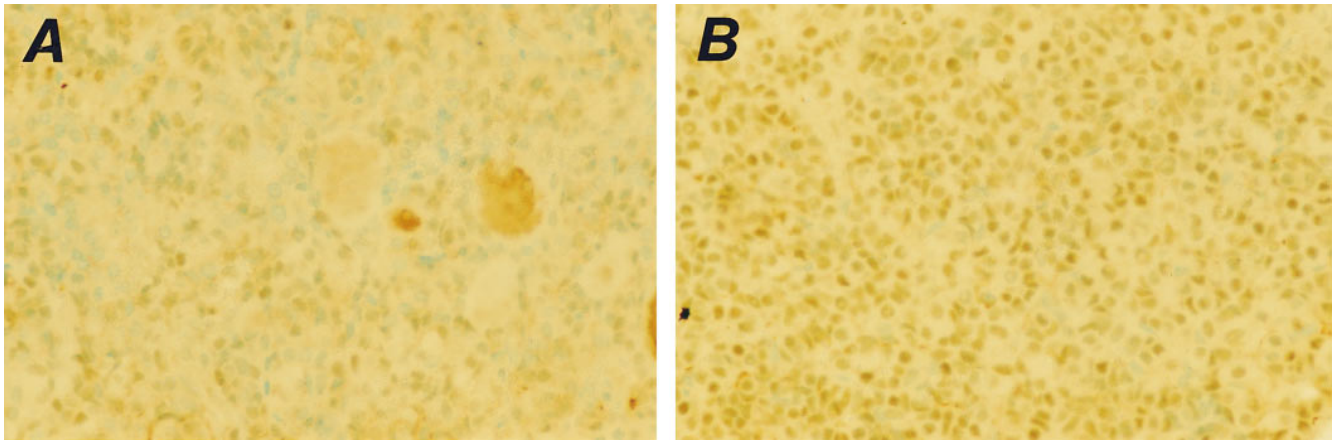

Figure 9. Immunoreactivity of phosphorylated CREB in pituitary sections. Using antiserum (5322) directed against phosphorylated CREB, immunoreactivity is present in a reduced number of nuclei in the anterior lobe cells of transgenic pituitary (*A*) compared with a WT pituitary (*B*). Original magnification 500×.

verticulum arising from the foregut, may give rise to Rathke's cysts (21). Histologically, these mucus-containing cysts are characteristically lined by cuboidal to columnar ciliated epithelial cells that do not express immunopositive staining for the pituitary trophic hormones. In contrast, craniopharyngiomas are thought to originate from squamous cell rests of the craniopharyngeal duct (20). The cellular lining of all 10 Rathke's cysts studied stained positively for LIF. At autopsy, Rathke's cleft cysts are found in up to one third of pituitary glands (22). Clinically, cysts may present as large (> 2 cm) masses, most of which are asymptomatic; but clinical features determined by the local effects of an expanding pituitary mass include headaches, visual field defects, cranial nerve dysfunction, and diabetes insipidus (22). Pituitary failure with trophic hormone deficiencies occurs as a result of impinging on surrounding normal pituitary cells leading to growth failure, hypogonadism and, rarely, thyroid or adrenal failure (23). Functional pituitary adenomas may also arise from within a cyst wall, and $\sim 2\%$ of resected pituitary adenomas contain Rathke's cleft epithelial tissue (22–24).

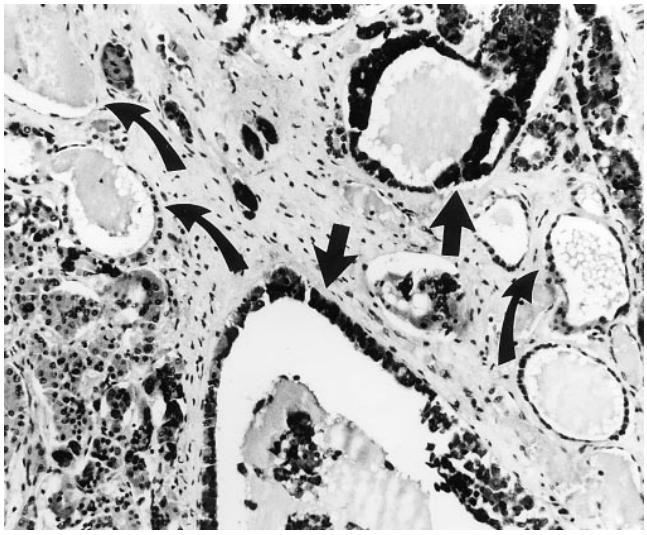

Figure 10. In a human pituitary, the cuboidal epithelium of Rathke's cysts (*arrows*) is immunoreactive for LIF, whereas cysts with flat epithelium show no LIF positivity (*curved arrows*). Original magnification 120×.

The inappropriate expression of LIF in the embryonic mouse pituitary resulted in abnormal development of the anterior wall of Rathke's cleft. In this transgenic model, pituitary cysts arise by proliferation of epithelial cells of the anterior wall of Rathke's cleft. The cysts appear embryonic in origin and are lined with epithelial cells immunopositive for cytokeratin and S-100 protein and immunonegative for trophic hormones. Expression of LIF in the embryonic pituitary also resulted in disturbed somatotroph development as evidenced by the decreased number of somatotrophs, reduced signal for GH mRNA, and undetectable serum GH.

The degree of growth retardation observed in these transgenic animals is greater than that seen in other models in which somatotroph lineage was ablated using either thymidine kinase (25) or diphtheria toxin (26), or when the GH gene was functionally inactivated using mutated CREB (27). Since the LIF transgene is directed by the GH promoter, it is expected to be expressed at the same time (embryonic day 15.5) and in the same cell type as endogenous GH. Therefore, dysregulation of the somatotrophs may be due to the high overproduction of a transgene product within the somatotroph and a generalized disruption of the cellular machinery; alternatively, it could be due to autocrine effects of LIF on the somatotroph. Disruption of the cellular machinery by the transgene is unlikely since other transgenes have been expressed using this promoter and endogenous somatotroph function was not disrupted (25–27). The low levels of GH mRNA and undetectable circulating GH imply defective GH biosynthesis. However, LIF does not suppress GH gene expression in vitro (manuscript in preparation). As the three separate founder lines all expressed a similar GH-deficient phenotype, disruption of the endogenous structural GH gene by the transgene is also unlikely to have caused GH deficiency. There was also no change in the GH structural gene restriction map in DNA derived from transgenic mice. In this regard, the GH promoter is only expected to be expressed in the acidophilic precursor stem cell common to both GH and PRL, and despite the pres-

ence of Pit-1, the pituitary expression of GH is decreased. Interestingly, the expression of phospho-CREB, which is associated with transactivation of GH transcription (28), was lower in the transgenic mice, supporting an indirect inhibition of GH manifest in this model. Earlier pituitary transgene expression may be achieved with the alpha-subunit promoter, and we have observed a similar dwarf phenotype using this promoter (Yano, Melmed, et al., unpublished observations).

Human fetal corticotrophs express abundant LIF binding sites and, in this model, pituitary LIF overexpression resulted in corticotroph hyperplasia. The relative abundance of corticotroph cells may reflect paracrine stimulation of this cell type (9). In vitro, it has been shown that LIF is a potent inducer of POMC transcription (10). In vivo, the diffusible alternatively spliced form of LIF mRNA transcript is expressed in the stressed pituitary (12), lending further credence to a paracrine role for LIF in POMC induction. The ability to regulate fetal somatotroph and corticotroph proliferation suggests a developmental intrapituitary function for LIF after embryonic day 15, when GH expression is initiated (29).

Expression of the transgene was lethal in utero in at least 12% of fetuses screened by embryonic day 19.5. Intrauterine lethality may occur because of ellicit extrapituitary expression of the GH promoter. Alternatively, LIF may be secreted in these animals and high circulating levels may have a deleterious systemic impact (7). Postnatal transgenic survivors may have had lower intrapituitary LIF expression and these viable mice may reflect a less favorable transgene integration site. Thus, overexpression of pituitary LIF during development selectively inhibits or stimulates adenohypophyseal cells. LIF causes formation of invaginated Rathke's cleft cysts and interferes with the normal development of somatotrophs and GH production. In contrast, ACTH-producing cells are abundantly present. This is in keeping with LIF's ability to markedly change the course of in vivo cellular differentiation patterns (5, 6). Recently, a novel homeobox gene, RPX, restricted to early Rathke's pouch development, was isolated (30). As RPX expression became extinct coincidentally with expression of Pit-1 and specific trophic hormone-secreting cells, it will be important to determine whether LIF impacts on embryonic RPX expression. In the pituitary-directed LIF transgenic mice, formation of cystic cavities associated with hypo-expression of GH and PRL thus serves as a model for human pituitary cysts. This embryologic disorder of cystic lesions derived from rests of Rathke's pouch cells provides a model for human pituitary cystic disorders, which may be associated with growth hormone deficiency (22).

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References

1. Patterson, P.H. 1994. Leukemia inhibitory factor, a cytokine at the interface between neurology and immunology. *Proc. Natl. Acad. Sci. USA.* 91:7833–7835.

2. Gearing, D.P., J.A. King, and N.M. Gough. 1988. Complete sequence of murine myeloid leukemia inhibitory factor (LIF). *Nucleic Acids Res.* 16:9857.

3. Shen, M.M., and P. Leder. 1992. Leukemia inhibitory factor is expressed by the preimplantation uterus and selectively blocks primitive ectoderm formation in vitro. *Proc. Natl. Acad. Sci. USA.* 89:8240–8244.

4. Stewart, C.L., P. Kasper, L.J. Brunet, H. Bhatt, F. Konkgen, and S. J. Abbandanzo. 1992. Blastocyst implantation depends on maternal expression of leukemia inhibitory factor. *Nature (Lond.).* 359:76–79.

5. Bamber, B.A., B.A. Maters, G.W. Hoyle, R.L. Brinster, and R.D. Palmiter. 1994. Leukemia inhibitory factor induces neurotransmitter switching in transgenic mice. *Proc. Natl. Acad. Sci. USA.* 91:7839–7843.

6. Shen, M.M., R.C. Skoda, R.D. Cardiff, J. Campos-Torres, P. Leder, and D.M. Ornitz. 1994. Expression of LIF in transgenic mice results in altered thymic epithelium and apparent interconversion of thymic and lymph node morphologies. *EMBO (Eur. Mol. Biol. Organ.) J.* 13:1375–1385.

7. Metcalf, D., and D.P. Gearing. 1989. Fetal syndrome in mice engrafted with cells producing high levels of the leukemia inhibitory factor. *Proc. Natl. Acad. Sci. USA.* 86:5948–5952.

8. Block, M.I., D.L. Fraker, G. Strassmann, K.G. Billingsley, W.S. Arnold, C. Perlis, and H.R. Alexander. 1993. Passive immunization of mice against D factor blocks lethality and cytokine release during endotoxemia. *J. Exp. Med.* 178:1085–1090.

9. Akita, S., J. Webster, S.G. Ren, H. Takino, J. Said, O. Zand, and S. Melmed. 1995. Human and murine pituitary expression of leukemia inhibitory factor-novel intrapituitary regulation of adrenocorticotropin hormone synthesis and secretion. *J. Clin. Invest.* 95:1288–1298.

10. Ray, D.W., S.G. Ren. and S. Melmed. 1996. Leukemia inhibitory factor (LIF) stimulates proopiomelanocortin (POMC) expression in a corticotroph cell line: role of stat pathway. *J. Clin. Invest.* 97:1852–1859.

11. Stefana, B., D.W. Ray, and S. Melmed. 1996. Leukemia inhibitory factor (LIF) induces differentiation of pituitary corticotroph function: a neuro-endocrine phenotypic switch. *Proc. Natl. Acad. Sci. USA.* 93:12502–12506.

12. Wang, Z., S.G. Ren, and S. Melmed. 1996. Hypothalamic and pituitary leukemia inhibitory factor gene expression in vivo: a novel endotoxin-inducible neuro-endocrine interface. *Endocrinology.* 137:2947–2953.

13. Akita, S., J. Malkin, and S. Melmed. 1996. Disrupted murine leukemia inhibitory factor (LIF) gene attenuates adrenocorticotropic hormone (ACTH) secretion. *Endocrinology.* 137:3140–3143.

14. Rugh, R. 1990. Organogeny in the mouse: its reproduction and development. R. Rugh, ed. Oxford Univ. Press, New York. 259–261.

15. Miller, W., and N.L. Eberhardt. 1983. Structure and evolution of the growth hormone gene family. *Endocr. Rev.* 4:97–130.

16. Hogan, B., R. Beddington, F. Costantini, and E. Lacy. 1994. Manipulating the mouse embryo. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 219–252.

17. Lloyd, R.V., L. Lin, W.F. Chandler, E. Hovath, L. Stefaneanu, and K. Kovacs. 1993. Pituitary specific transcription factor messenger ribonucleic expression in adenomatous and nontumorous human pituitary tissues. *Lab. Invest.* 69:570–575.

18. Simmons, D.M., J.W. Voss, H.A. Ingraham, J.M. Holloway, R.S. Broide, M.G. Rosenfeld, and L.W. Swanson. 1990. Pituitary cell phenotypes involve cell-specific Pit-1 mRNA translation and synergic interactions with other classes of transcription factors. *Genes Dev.* 4:695–711.

19. Ikeda, H., and T. Yoshimoto. 1991. Developmental changes in proliferative activity of cells of the murine Rathke's pouch. *Cell Tissue Res.* 263:41–47.

20. Scheithauer, B.W. 1985. Pathology of the pituitary and sellar region: exclusive of pituitary adenoma. *Ann. Pathol.* 20:67–154.

21. Ikeda, H., J. Suzuki, N. Sasano, and H. Niizuma. 1988. The development and morphogenesis of the human pituitary gland. *Anat. Embryol.* 178:327–336.

22. Ross, D.A., D. Norman, and C.B. Wilson. 1992. Radiologic characteristics and results of surgical management of Rathke's cysts in 43 patients. *Neurosurgery.* 30:173–179.

23. Mindermann, T., and C.B. Wilson. 1995. Pediatric pituitary adenomas. *Neurosurgery.* 36:259–269.

24. Mukherjee, J.J., N. Islam, G. Kaltsas, D. Lowe, M. Charlesworth, F. Afshar, P.J. Trainer, J.P. Munson, G. Besser, and A.B. Grossman. 1996. Rathke's cleft cyst: not such a benign lesion. *J. Endocrinol.* 151(Suppl. 014).

25. Borelli, E., R.A. Heyman, C. Arias, P.E. Sawchenko, and R.M. Evans. 1989. Transgenic mice with inducible dwarfism. *Nature (Lond.).* 339:538–541.

26. Behringer, R.R., L.S. Mathews, R.D. Palmiter, and R.L. Brinster. 1988. Dwarf mice produced by genetic ablation of growth hormone-expressing cells. *Genes Dev.* 1:453–461.

27. Struthers, R.S., W.W. Vale, C. Arias, P.E. Sawchenko, and M.R. Montminy. 1991. Somatotroph hypoplasia and dwarfism in transgenic mice expressing a non-phosphorylatable CREB mutant. *Nature (Lond.).* 350:622–624.

28. Bertherat, J., P. Chanson, and M. Montminy. 1995. The cyclic adenosine 3′, 5′-monophosphate-responsive factor CREB is constitutively activated in human somatotroph adenomas. *Mol. Endocrinol.* 9:777–783.

29. Voss, J.W., and M.G. Rosenfeld. 1992. Anterior pituitary development: short tales from dwarf mice. *Cell* 70:527–530.

30. Hermesz, E., S. Mackem, and K.A. Mahon. 1996. Rpx: a novel anterior-restricted homeobox gene progressively activated in the prechordal plate, anterior neural plate and Rathke's pouch of the mouse embryo. *Development (Camb.).* 122:41–52.