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Leprechaunism: *In Vitro* Insulin Action Despite Genetic Insulin Resistance[1]

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ABSTRACT. We recently identified a female leprechaun infant with marked hyperinsulinemia [as high as 10,975 μU/ml (78,746 pmol/liter)], presumably secondary to insulin resistance. She had two physical findings suggestive of possible insulin action: cystic ovarian enlargement with gonadotropin-independent steroid secretion and persistent, severe myocardial hypertrophy. To examine the pathophysiology of this disorder we measured the *in vitro* sensitivity to insulin and other growth factors of erythroid progenitors and a T-lymphoblast cell line derived from her peripheral blood. Resistance to insulin was demonstrated by failure of her circulating erythroid progenitor cells to augment proliferation in response to physiologic concentrations of insulin (1–10 ng/ml). An immortalized T lymphoblast cell line was established by transforming the cells with the human retrovirus human T cell leukemia virus II. This cell line showed little or no response to physiologic concentrations of insulin contrary to consistently observed stimulation of colony formation by cell lines similarly derived from normals. The patient's T lymphoblasts, however, showed normal sensitivity to insulin-like growth factor I. In response to supraphysiologic insulin concentrations (25–1000 ng/ml), leprechaun T lymphoblasts showed significant augmentation of colony formation (peak 189% above baseline at 50 ng/ml); normal T lymphoblasts also showed responsiveness at these high insulin concentrations. Preincubation with a monoclonal antibody against the insulin-like growth factor I receptor (αIR-3 at 5000 ng/ml) blocked the *in vitro* effect of physiologic concentrations of insulin-like growth factor and supraphysiologic concentrations of insulin on leprechaun and control T lymphoblast colony formation, but had no clear effect upon the response to physiologic insulin concentrations. These findings document genetic insulin resistance in hematopoietic cells from a patient with leprechaunism. Response to supraphysiologic concentrations of insulin appears to be mediated via the insulin-like growth factor receptor mechanism which remains intact. Such action *in vivo* could account for the ovarian and cardiac findings in the patient. (*Pediatr Res* 22: 286–291, 1987)

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Abbreviations

BFU-E, erythroid progenitor cells or burst-forming units of the erythroid line
HTLV, human T-cell leukemia virus
T-LB, T lymphoblast
IGF-I, insulin-like growth factor I
LRF, luteinizing-hormone releasing factor
LH, luteinizing hormone
FSH, follicle-stimulating hormone
GH, growth hormone
GMP, guanosine monophosphate
AIB, aminoisobutyric acid
EB, Epstein-Barr
SI, Système International d'Unités

Leprechaunism (Donahue's syndrome) is a rare disorder characterized clinically by intrauterine and postnatal growth retardation, diminished fat and muscle tissue, characteristic facies, precocious puberty, and early death (1). The most common physiologic disturbance in this condition, however, is hyperinsulinism due to severe insulin resistance (2). While leprechaunism has been described in approximately 30 patients, only limited studies of the mechanism of the insulin resistance have been reported (3). Both insulin receptor and postreceptor defects have been proposed to account for the insulin resistance in different patients. Previous investigators have found persistence of the insulin resistance in cultured cells suggesting a primary genetic basis for the reduced insulin sensitivity.

We describe herein a patient with leprechaunism with marked hyperinsulinemia and many of the classical phenotypic features of the leprechaun state. There were two unusual physical findings: large, autonomously functioning, polycystic ovaries and persistent, diffuse, severe myocardial hypertrophy, abnormalities likely to be associated with insulin action. Using clonogenic assays employing both freshly isolated BFU-E (4–9) and HTLV-II transformed T-LB cells (10, 11), we demonstrated *in vitro* responses only at supraphysiologic concentrations of added insulin. This activity was completely blocked by pretreatment with antibody to the IGF-I receptor suggesting that the observed insulin action was mediated through the IGF-I receptor. Such a mechanism could account for the ovarian and cardiac findings in this and other leprechaun patients.

CASE REPORT

The patient was a 1480 g, 38 cm (50th centile for 30 wk gestation) female product of a term gestation born to a 19-yr-old

Filipine female (G2,PO,TAB1). The patient's father is black and unrelated to her mother.

At birth, the child was noted to have severe intrauterine growth retardation associated with depleted subcutaneous and muscle tissue. She also had peculiar facies characterized by a depressed nasal bridge, underdeveloped periorbital bony structures, posteriorly rotated and downwardly displaced pinnae with underdeveloped superior helices, a right preauricular skin tag, upturned nares, a very broad upper lip with a very thin vermilion border and underdeveloped philtrum, and a high-arched palate. A short, midsystolic murmur at the the lower left sternal border radiating to the back, protuberant nipples, diffuse hypertrichosis, and a prominent clitoris also were noted. A specific syndrome was not identified at this time. The karyotype was 46XX. Cardiologic evaluation, including echocardiogram, was consistent with diffuse myocardial hypertrophy.

At age 1.25 months, free peritoneal air was noted on a radiograph of the abdomen, but laparotomy failed to reveal any bowel perforation. However, the ovaries were noted to be quite enlarged (maximal diameter was 6–8 cm on the left and 5–6 cm on the right) and contained multiple follicular cysts, separated from each other by septa of normal stromal tissue.

At age 3.5 months, the patient first developed intermittent hyperglycemia and hypoglycemia and the diagnosis of leprechaunism was entertained. A serum insulin level was 2044 μU/ml (14,666 pmol/liter). A simultaneous C peptide level was 45 ng/ml (normal stimulated level = 1.5–9.0 ng/ml). Plasma glucose levels ranged between 38 and 144 ng/dl (2.09 and 7.93 mmol/liter). Somatomedin-C was <0.10 U/ml (normal for age 0.17–0.62 U/ml) and glycosylated hemoglobin was 3.5% (normal 4–7%). Pelvic ultrasound revealed persistence of bilateral ovarian enlargement (1.8 × 1 × 3 cm on the right and 2 cm in greatest diameter on the left) with both ovaries containing small cysts. Serum LH was 5 IU/liter (normal for age <2–20 IU/liter), FSH 2 IU/liter (normal for age <2–4 IU/L), and estradiol 7 pg/ml (25.7 pmol/liter; normal for age <30 pg/ml, <110 pmol/liter). Persistence of significant diffuse myocardial hypertrophy was noted.

At age 5.5 months bilateral breast development (Tanner III) was noted for the first time without other evidence of estrogen effect. A pelvic ultrasonogram again showed bilaterally enlarged ovaries. Basal LH was 2.5 IU/liter, FSH <1 IU/liter, estradiol 180 pg/ml (66 pmol/liter; normal for age <30 pg/ml, <110 pmol/liter), estrone 72 pg/ml (normal for age <10 pg/ml); and testosterone 0.76 ng/ml (2.64 nmol/liter; normal for age <0.10 ng/ml; <0.35 nmol/liter). Following a 100-μg intravenous bolus of LRF there was no significant increase of serum LH or FSH concentrations over basal levels. In an attempt to control the hypoglycemia which only responded to feeding every 2 h, diazoxide (5 mg/kg/day) was begun. The serum insulin level before diazoxide was 10,975 μU/ml (78,746 pmol/liter). Hyperglycemia ensued and responded to omission of diazoxide for 24 h. The diazoxide was resumed and ultimately increased to 10 mg/kg/day because of the recurrence of hypoglycemia; the serum insulin level 2 wk later was 117 μU/ml (840 pmol/liter). Throughout this time limited growth was noted. Subsequently, the family moved to another state where the baby died at 7 months of age. At autopsy, there was a normal-sized pancreas with marked islet-cell hyperplasia (immunohistochemical staining positive for insulin), nodular regeneration in the liver, a high-lying hemorrhagic left ovary, an enlarged right ovary, myocardial fibril hypertrophy, cortical brain atrophy, and a 2.0-cm pineal cyst. No specific cause of death was found.

MATERIALS AND METHODS

Patients. Hematopoietic cells were obtained from the peripheral blood of the leprechaun when she was 4 months old. Control data for the BFU-E and T lymphoblast cell line experiments were derived from pools of normal adults, including seven males, 12 females, mean age (±SE) = 30.4 ± 1.69 yr, and normal children including three males, four females, including three infants ≤4 months of age (mean age = 6.50 ± 2.38 yr). All studies were performed with the approval of the UCLA Human Subject Protection Committee and with the informed consent of the parents of the infant with leprechaunism and of all control subjects and/or their parents.

BFU-E. Assays for the stimulatory effects of purified hormones on BFU-E were performed as previously described (4–9). Two to 10 ml of venous blood was collected in preservative-free sterile heparin. Buffy coat cells were separated by centrifugation and treated with ammonium chloride-Tris buffer at 37°C to lyse erythrocytes. The cell pellets were washed once with complete medium. Nucleated cells were adjusted to a final concentration of 3×10^6/ml, and plated at a concentration of 3×10^5/ml in Falcon flat-bottomed microtiter plates (5). Ten μl of either biosynthetic, recombinant human insulin in concentrations of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ng/ml, synthetic IGF-I (10, 11) in concentrations of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 ng/ml or highly purified human GH in concentrations of 10, 25, 50, 100, 200, 500 ng/ml (4), were added with phosphate-buffered saline (pH 7.4). The cells were cultured in methylcellulose at a final concentration of 0.8% (Methocel E4M premium, Dow Chemical Co., Midland, MI), Iscove's modification of Dulbecco's medium (Irvine Scientific, Santa Ana, CA), 30% fetal calf serum (selected lot), 10^{-4} M α-thioglycerol (Calbiochem, La Jolla, CA), penicillin-streptomycin, and 0.5 U/ml human urinary erythropoietin (SA, 44 U/mg protein; provided by National Heart, Lung, and Blood Institute), as previously described (6, 8). The plates were incubated at 37°C in high humidity at 8% CO_2 in air. After 10–14 days, large hemoglobinized colonies of erythroid cells (BFU-E) containing at least 50 cells were enumerated using an inverted microscope.

T-LB cell line studies. The methodology for peripheral blood T lymphocyte transformation by the human retrovirus HTLV II has been described previously (12, 13). Low-density peripheral blood mononuclear cells (5×10^5), obtained by Ficoll-Hypaque density-gradient separation, were cocultivated with an equal number of lethally irradiated (10,000 rad) late-passage Mo cells (11) in Iscove's medium supplemented with 20% fetal bovine serum. The Mo-T cell line was derived from the spleen of a patient with a T-cell variant of hairy-cell leukemia (14). A virus-infected immortalized T-cell line is produced in about 4 wk. Three thousand transformed T-cells/ml were cultured in microtiter plates and hormones were added as above; insulin was added at concentrations of 1–10 ng/ml as well as 25, 50, 100, 250, 500, or 1000 ng/ml. Clonogenic assays of HTLV-transformed cells were carried out in methylcellulose. All experiments were performed in duplicate or triplicate, with a maximal replicate variability of <5%. The unstimulated number of colonies (without added hormone) or baseline level is defined as 100%.

Anti-IGF-I receptor antibody. A highly specific monoclonal antibody to the IGF-I receptor (αIR-3) was kindly provided by Dr. Steven Jacobs (15, 16). Dose-response curves using insulin or IGF-I and the clonogenic bioassay system outlined above, with and without pretreatment with αIR-3, were generated. Based on previously published data (17), initial concentrations of αIR-3 tested were 50, 250, 500, and 5000 ng/ml. When using αIR-3, all cells were exposed for 1 h prior to adding insulin or IGF-I.

RESULTS

BFU-E proliferation. Leprechaun BFU-E colony augmentation was tested on one occasion in response to insulin, IGF-I, and growth hormone. Leprechaun BFU-E showed no colony augmentation at any added insulin concentration between 1–10 ng/ml (Fig. 1, *left*), whereas in 42 experiments with adult BFU-E, mean peak augmentation was 187 ± 3.4% above baseline occurring at 8 ng/ml of added insulin. In 16 experiments with BFU-E from infants and children, mean peak stimulation was

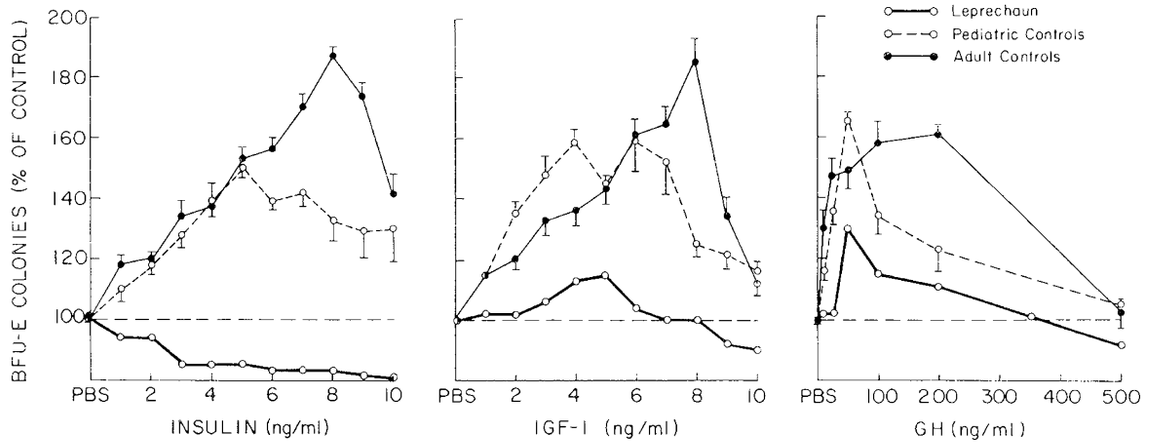

Fig. 1. BFU-E colony formation in response to insulin (*left*), IGF-1 (center), and GH (*right*). The y-axis represents colony formation as a percentage of control. The x-axis gives the concentration of added growth factor. To convert insulin (ng/ml) to SI units (pmol/liter), multiply by 179. To convert GH (ng/ml) to SI units (μg/liter), multiply by 1.00.

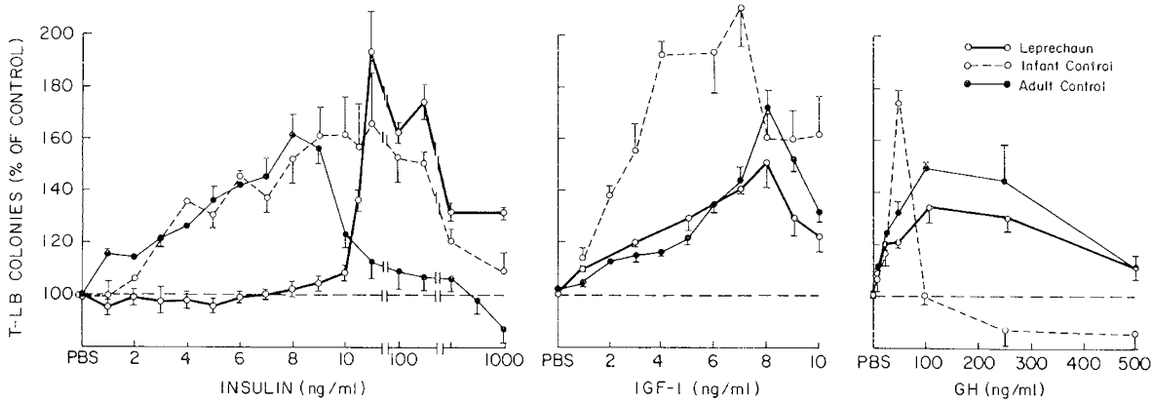

Fig. 2. Colony formation by HTLV-transformed T-cell-line in response to insulin (*left*), IGF-1 (*center*), and GH (*right*). Sensitivity to insulin was evaluated at both low (physiologic) concentrations [1–10 ng/ml and high (supraphysiologic) concentrations (25–1000 ng/ml)].

150 ± 2.6% above baseline occurring at 5 ng/ml of added insulin. The addition of any insulin caused less proliferation of leprechaun BFU-E than in plates without added insulin, a phenomenon previously observed in some obese subjects (8). Leprechaun BFU-E showed some augmentation of colony formation in response to IGF-I (115% above baseline) albeit less than either control group (Fig. 1, *center*); mean peak augmentation in 16 experiments with adult BFU-E was 185 ± 8.4% above baseline occurring at 8 ng/ml of added IGF-I and in 14 experiments with BFU-E from infants and children, mean peak stimulation was 159 ± 9.8% above baseline occurring at 6 ng/ml of added IGF-I. Leprechaun BFU-E also showed decreased GH responsiveness (130% above baseline) compared to both control groups (Fig. 1, *right*); in 15 experiments with adult BFU-E, mean peak augmentation was 161 ± 3.1% above baseline occurring at 200 ng/ml of added GH and in 15 experiments with BFU-E from infants and children, mean peak stimulation was 165 ± 3.3% above baseline occurring at 50 ng/ml of added GH.

Clonogenic stimulation of transformed T-LB. As with the BFU-E, leprechaun T-LB (mean of 15 experiments) showed no augmentation of colony formation in response to insulin concentrations up to 9 ng/ml (Fig. 2, *left*). Minimal responsiveness was observed at 10 ng/ml. Significant proliferation was consistently seen at concentrations of insulin above 10 ng/ml with a mean peak augmentation of 193 ± 15% above baseline occurring at 50 ng/ml. Control transformed infant T-LB (mean of eight experiments) also showed augmented proliferation of both physiologic and supraphysiologic insulin concentrations. Control adult T-LB showed stimulation as high as 139% above baseline at 250 ng/ml of added insulin (in five of 10 experiments). Peak responsiveness of leprechaun T-LB to IGF-I (150 ± 9.3%) (Fig. 2, *center*, mean of 10 experiments) was within two standard deviations of mean responses of control cells (five infant and 7 adult experiments for IGF-I). Peak responsiveness of leprechaun T-LB to GH (133 ± 6.7%) (Fig. 2, *right*, mean of eight experiments) was normal compared to adult controls (mean of six experiments) and slightly decreased compared to infant controls (mean of three experiments).

T-LB proliferation after αIR-3 pretreatment. No augmentation of either leprechaun or control T-LB colony proliferation was seen in response to αIR-3 alone (at concentrations between 50–5000 ng/ml, data not shown). Using IGF-I concentrations of 1–10 ng/ml, 500 ng/ml of αIR-3 partially blocked and 5000 ng/ml totally blocked augmentation of leprechaun (Fig. 3, *left*) and adult control (Fig. 3, *center*) T-LB colony formation. Infant control T-LB (Fig. 3, *right*) showed significantly but not completely blocked colony augmentation in response to 5000 ng/ml of αIR-3.

To examine whether the augmenting effect of supraphysiologic insulin concentrations upon leprechaun and control T-LB growth might be due to stimulation through the IGF-I receptor, the responsiveness of T-LB was evaluated after pretreatment with αIR-3 (Fig. 4). Since the early peak augmentation of proliferation of T-LB generally occurs at added insulin concentrations of 7–9 ng/ml and the late peak between 100–500 ng/ml, the effect of αIR-3 pretreatment was tested at these concentrations of added insulin. Although no concentration of αIR-3 completely blocked the augmenting effect of insulin at 7 ng/ml of insulin, increasing

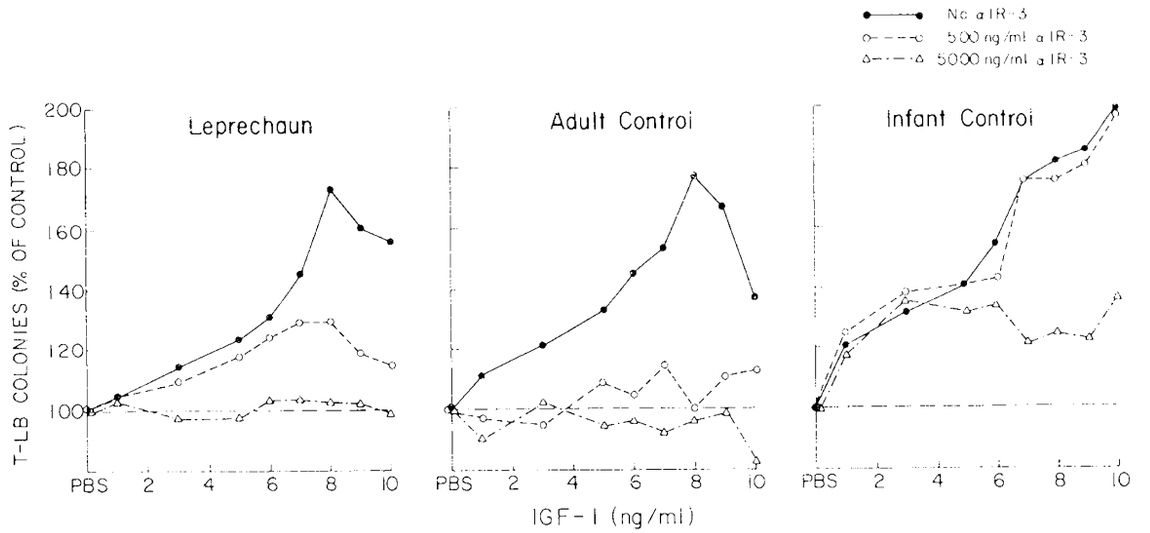

Fig. 3. Effect of preincubation with αIR-3 on T-LB colony augmentation in response to IGF-1. The responses of the leprechaun T-LB are shown on the *left*, the adult control T-LB in the center, and the infant control T-LB on the *right*.

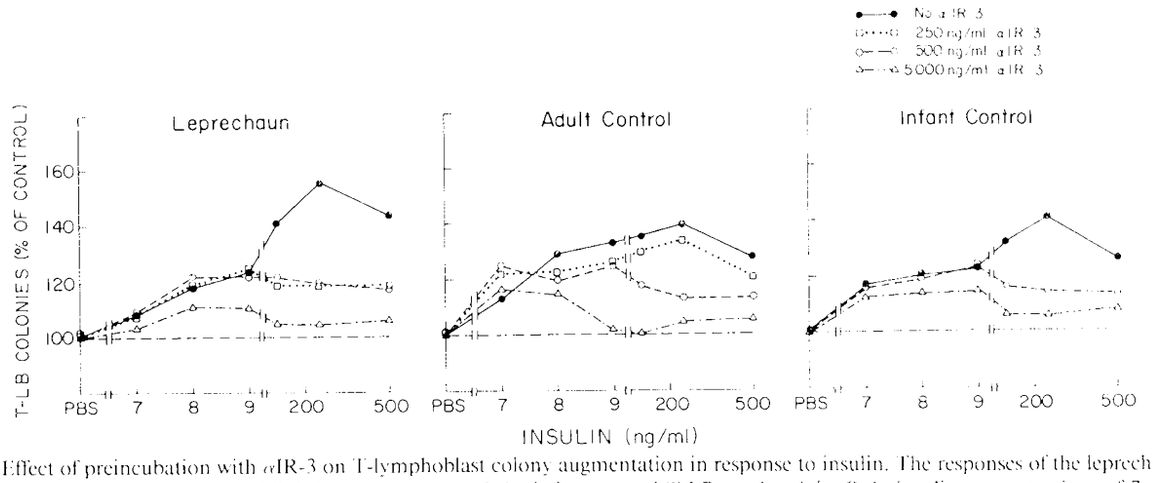

Fig. 4. Effect of preincubation with αIR-3 on T-lymphoblast colony augmentation in response to insulin. The responses of the leprechaun T-LB are shown on the *left*, the adult control T-LB in the *center*, and the infant control T-LB on the *right*. Only insulin concentrations of 7, 8, 9, 100, 250, and 500 ng/ml were used (note breaks in x-axis).

concentrations of αIR-3 began to decrease the proliferative capacity of leprechaun (Fig. 4, *left*), adult control (Fig. 4, *center*), and infant control (Fig. 4, *right*) T-LB to all higher added insulin concentrations, with almost complete blockade achieved at an αIR-3 concentration of 5000 ng/ml.

DISCUSSION

Using *in vitro* clonogenic bioassays, we have documented near-complete resistance to physiologic concentrations of insulin in a newly described leprechaun infant. Absent augmentation of BFU-E colony formation by insulin could still reflect *in vivo* environmental influences since the BFU-E represent a primary tissue explant. On the other hand, because the virally transformed T-lymphoblast cell line is many generations removed from the *in vivo* environment, the significantly blunted response of these cells to physiologic insulin concentrations must reflect genetically programed insulin resistance. The specificity of the insulin resistance is suggested by the presence of normal responsiveness to IGF-I.

Detailed evaluation of the mechanism of the insulin resistance in leprechaunism has been attempted in only four previous patients. The most extensively studied patient, Arkansas I (Ark-1), had variable findings, including normal insulin binding to monocytes and cultured fibroblasts (18), decreased insulin binding to monocytes (19), erythrocytes (20), and cultured fibroblasts (21), and increased binding affinity with decreased sensitivity of binding to alterations in pH and temperature using EB-virus-transformed B-lymphocytes (22, 23). More recently, Elsas *et al.* (2) reexamined cultured fibroblasts from this patient and found no high-affinity insulin binding and normal low-affinity binding (2).

In contrast, another leprechaun patient, Winnipeg/NIH, was reported to have a >80% reduction in insulin binding to cultured fibroblasts (3, 24). Insulin-stimulated glucose incorporation was markedly blunted, but insulin-mediated methyl-AIB uptake was normal in this patient's fibroblasts. These results were nearly identical when the fibroblasts were exposed to IGF-I, suggesting that this leprechaun had a receptor defect common to the homologous insulin and IGF-I receptors involving coupling of ligand signal for glucose incorporation, but not for methyl-AIB uptake. In a third leprechaun patient, Chapel Hill, there was decreased insulin binding to liver, normal insulin binding to fibroblasts, and blunted fibroblast uptake of both ^{3}H-glucose and ^{3}H-AIB in response to both insulin and IGF-I (25, 26).

A fourth leprechaun, Minnesota/NIH, had more than a 90% reduction in insulin binding to EB-virus-transformed B-lymphocytes, presumably on the basis of decreased receptor number (27). These findings suggest that the leprechaun state may result from heterogeneous biochemical/molecular defects. While we

have not yet defined the molecular basis of the insulin resistance in our patient, *e.g.* binding, transmembrane-signaling, postreceptor defects, etc., there remains near-total insulin resistance at physiologic concentrations implying that the insulin receptor/effector mechanism is nonfunctional. Unlike the Winnipeg/NIH and Chapel Hill patients, our leprechaun had normal activity of the IGF-I receptor/effector mechanism.

Our findings of significant clonogenic augmentation of T-LB at supraphysiologic concentrations of added insulin, with a dose-dependent competitive inhibition of this stimulation by preincubation with the anti-IGF-I-receptor antibody, suggest that insulin action can occur via the IGF-I receptor. The measured serum insulin concentrations in our patient, as high as 459 ng/ml (78,746 pmol/liter), were in the range in which insulin significantly augmented *in vitro* T-LB colony formation (25–1000 ng/ml; 4484–179,375 pmol/L). Similarly, Flier *et al.* (17), using human skin fibroblasts, found that αIR-3 inhibited [^{3}H]thymidine incorporation at insulin concentrations >1000 ng/ml, but not below. These conclusions are consistent with the generally held view that insulin at low (physiologic) concentrations acts *in vivo* through the insulin receptor predominantly promoting glucose uptake and other metabolic functions, whereas at high concentrations, insulin, acting through the IGF-I receptor, serves as a mitogen, stimulating [^{3}H]thymidine uptake, DNA synthesis, and cell replication (15, 17).

The unusual clinical findings of persistent, diffuse myocardial hypertrophy and cystic ovaries may be clinical markers of insulin action through the IGF-I receptor. The presence of massive cystic ovarian enlargement and autonomous steroidogenesis (as judged from the flat serum gonadotropin responses on the LRF test) could result from direct insulin action upon the ovary. Fifty percent of previously reported leprechaun infants have had ovarian enlargement, although previously noted precocious puberty in these patients has been attributed to hypothalamic-pituitary hyperfunction despite normal gonadotropin measurements in several patients (1). IGF-I receptors have been described in normal human ovarian tissue (28). In the swine granulosa cell model (29), stimulation with IGF-I results in production of pregnenolone and progesterone. Adashi *et al.* (30) also have reported that highly purified IGF-I (50 ng/ml) induced a 93% increase in [^{35}S]sulfate incorporation into extracellular proteoglycans in a primary culture of rat granulosa cells (30). The constellation of ovarian enlargement, cyst formation, and abnormal steroidogenesis has been noted in other patients with resistance to insulin action upon glucose disposal, *e.g.* thin women with polycystic ovarian disease and no acanthosis nigricans (9), patients with type A insulin resistance (31), and in uremic individuals (32). The ovarian tissue of one obese woman with polycystic ovarian disease, acanthosis, and marked hyperinsulinemia responded *in vitro* to stimulation by insulin (500 ng/ml) with production of androstenedione and testosterone (33).

While insulin is known to stimulate myocardial hypertrophy in the infant of the diabetic mother and in the infant with nesidioblastosis (34), the mechanism by which this occurs is unknown. Both insulin and IGF-I receptors have been found in heart and other vascular endothelium (35, 36). It is possible that the significant myocardial hypertrophy observed in our patient and in other leprechauns (1, 25, 27) could result from persistent hyperinsulinemia acting through the IGF-I receptor.

Selective insulin action on some tissues, but not others, in the face of resistance to insulin action on carbohydrate disposal, could be related to the density (and/or affinity) of IGF-I receptors in a given tissue. This concept is supported by the work of D'Ercole *et al.* (37) who have shown the highest specific binding of ^{125}I-somatomedin-C (12.5%) to membranes derived from adult pig ovarian tissue compared to all other pig tissues examined. Lack of response by muscle and fat tissue of the leprechaun could reflect limited IGF-I binding to these tissues. Using rat tissue membranes, D'Ercole et al. (37) found low specific binding (4.7%) to muscle tissue and no specific binding to adipocytes. Skottner *et al.* (38) have also noted that human fat cells do not possess IGF-I receptors. Thus, the presence or absence of IGF-I receptors may ultimately determine whether and where the growth-promoting actions of insulin can occur in insulin-resistant states.

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