

Cellular Immunity Before and After Leptin Replacement Therapy

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ABSTRACT

Background: The few identified leptin-deficient children have immune deficiency.

Aims: To evaluate whether a newly-identified leptin-deficient boy has immune defects; to assess the immune changes during leptin replacement.

Methods: A 5 year-old boy with congenital leptin deficiency was evaluated before, 2 weeks and 6 weeks after the initiation of recombinant methionyl human leptin. Thymic volume was measured by computed tomography. Humoral immunity was assessed by measuring levels of several immunoglobulins. Cellular immunity was evaluated by the analysis of lymphocyte proliferation in response to mitogens. Lymphocyte subsets were quantified by flow cytometry.

Results: At baseline, thymic volume was increased. The lymphocyte subsets count and humoral/cellular immunities were normal. After treatment, proliferative response to mitogens increased by 1.5- to 3-fold, and lymphocyte count decreased by 17%.

Conclusions: Immune defects are not an obligatory feature of congenital leptin deficiency. Even in the absence of significant immune defects, leptin replacement therapy enhanced T-cell responsiveness.

KEY WORDS

deficiency, immunity, leptin, obesity, T cell

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INTRODUCTION

Leptin is one of several cytokine-like hormones produced by adipocytes, also known as adipokines, whose multiple functions go beyond that of regulating food intake. We have previously described the endocrine, metabolic, anthropometric, and neurological effects of leptin¹⁻¹², by assessing its effects on the only adults to date identified as leptin-deficient. More recently, we have reported the effects of leptin replacement on the neurocognitive development of a leptin-deficient boy, from the same family as the adult patients¹³. We have observed that leptin replacement led to (i) substantial weight loss, (ii) resolution of hypertension and hyperinsulinemia, (iii) improvements of lipid profile, and (iv) enhancement of neurocognitive development of that child¹³. However, the effects of leptin replacement on immunity in that patient have not yet been characterized.

As an adipokine, the hallmark of leptin biology is pleiotropy, characterized by multiple and marked effects on metabolic and immune functions¹⁴⁻¹⁶. Its immune effects include the stimulation of hematopoiesis and lymphopoiesis, the activation of monocytes, dendritic cells (DC) and macrophages (leading to the production of Th1 type cytokines)¹⁷, the maturation and survival of DC¹⁸, the activation of neutrophils and natural killer (NK) cells (and the subsequent stimulation of their gene expression, of the production of reactive oxygen species and of chemotaxis)¹⁹⁻²², and the modulation of the adaptive immunity (by enhancing T cell survival and stimulating the production of pro-inflammatory cytokines such as IFN- γ and IL-2)²³. Leptin also regulates thymic homeostasis and prevents apoptosis.

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In humans, leptin deficiency is associated with substantially increased mortality, due to increased risk of infection at early ages⁸. Leptin-deficient children have a marked reduction in the CD4+ T-cell count and reduced T-cell proliferation²⁴. Patients with leptin receptor mutations have a modest reduction in the absolute CD4+ T-cell counts, but with similar CD4+:CD8+ ratios. Also, those patients have a compensatory increase in CD19+ cells, and reduced proliferative responses to polyclonal stimuli specific to T cells²⁵. Cytokine expression impairment is more pronounced in leptin-deficient patients, as compared to patients with leptin receptor mutations. Treatment of a child with congenital leptin deficiency with r-metHuLeptin led to a substantial increase in white blood cell count, and to the marked improvement of asthmatic crisis²⁶. Conversely, in humans without congenital leptin deficiency, it is speculated that hypoleptinemia associated with malnutrition and nutritional deficiency might protect individuals from autoimmune diseases, but predispose to infections²⁷.

Therefore, immune dysfunction is thought to be common in patients with disorders of leptin function. Mortality is increased in both situations: leptin deficiency and mutations of the leptin receptor. The aim of this study was to describe immune findings in a leptin-deficient boy, before and during the course of treatment with recombinant methionyl human leptin (r-metHuLeptin).

PATIENT AND METHODS

The patient is a 5 year-1 month-old boy born to a highly consanguineous Turkish family. Three of his relatives are homozygous for the nonconservative missense leptin gene mutation (Cys-to-Thr in codon 105). The mutation renders them leptin deficient, and the endocrine and metabolic findings have been described elsewhere^{3,13}. Apart from being obese, the child had no medical problems, and no important history of infectious diseases was noted.

Replacement treatment with r-metHuLeptin (initially supplied by Amgen, Inc. Thousand Oaks, CA, USA and subsequently by Amylin, San Diego, CA, USA) was started at age 5 years 1 month. The

drug was administered once a day in the evening (18.00-20.00 h), with a starting dose of 1.36 mg/day SQ. Details regarding treatment procedures and outcomes are described elsewhere¹³.

This n-of-1 trial was approved by the University of California, Los Angeles Institutional Review Board (UCLA IRB), and informed parental consent and patient's assent was obtained for all studies. The patient was evaluated at the UCLA General Clinical Research Center (GCRC) as an in-patient.

Thymus computed tomography was performed before treatment, by obtaining axial scans of the chest with a slice thickness of 3 mm from the thoracic inlet to the lower chest without the administration of an oral contrast. Thymic volume was calculated after 3D reconstruction using the Vitrea software, and compared to the normal children's reference range.

Humoral immunity was assessed at baseline and six weeks after r-metHuLeptin was initiated, by measuring the levels of IgG (including subclasses), IgA, IgM and IgE. Titers of antibodies against *Haemophilus influenza* B, *Tetanus* toxoid, and pneumococcal antibody IgG were also evaluated before and after treatment.

Flow cytometry was undertaken before and six weeks after treatment initiation. The percent and absolute count of the lymphocyte subsets (CD3, CD4, CD8, CD19 and CD16/CD56) were calculated by using four-color flow cytometry analysis, based on the selection of CD45+ non-granular cells.

Analysis of lymphocyte proliferation in response to mitogens was performed before, two weeks and six weeks after treatment initiation. Lymphocyte proliferation in response to the mitogens phytohemagglutinin (PHA), concanavalin A (Con-A) and pokeweed mitogen (PWM) was determined 4 days after their administration. The response to *Tetanus* and *Candida* antigens was assessed on day 7. Briefly, the patient's purified peripheral blood mononuclear cells (lymphocytes and monocytes) were incubated with the test substances and, during the last 24 hours of the culture, tritiated thymidine was added to the medium. The extent of proliferation was determined by measuring the radioactivity taken up by the dividing cells and was reported

as a stimulation index (SI). This is the ratio of radioactivity (as counts per minute [CPM]) with stimulation to the CPM, or without stimulation (background).

RESULTS

Before treatment, thymic soft tissue was well visualized in the retrosternal location, without evidence of fatty infiltration. The thymic volume was type 4 (defined as moderate amount, of greater extent, as compared to the volumes seen in normal children)²⁸.

At baseline, humoral immunity was normal, as levels of IgG (including subclasses), IgA, and IgM were within the normal range. Levels of IgE were above normal (368 IU/ml, normal <20 IU/ml), indicating the likelihood of atopic allergy. There were no changes in the levels of immunoglobulins six weeks after leptin replacement was initiated. Titers of *Haemophilus influenza* B antibodies were undetectable, and titers of *Tetanus* toxoid antibody were >7.0 IU/ml, indicating previous vaccination against *Tetanus*, but not against *Haemophilus influenza* B. Titers of pneumococcal antibody IgG were below 2 µg/ml for types 1, 4, 6B, 9N, 12F, 14, 23F and 18C, and above 2 µg/ml for types 3, 8, 19F and 7F. Vaccination with Pneumovax 23 (Merck & Co, Inc, Rahway, NJ, USA) was administered, and pneumococcal antibody IgG titers were reassessed after 6 weeks (under leptin replacement). These titers increased at least 2-fold (except for antibodies type 19F, 23F, 7F and 18C).

Before treatment, all cell populations were within normal ranges (by complete blood count with differential and blood smear), including lymphocytes ($3.5 \times 10^3/\mu\text{l}$). Six weeks after leptin replacement was initiated, the absolute lymphocyte count was still normal ($2.9 \times 10^3/\mu\text{l}$) but lower than baseline. When analyzed by flow cytometry, the absolute (cells/cmm) and the percent counts of lymphocyte subsets were all normal at baseline. Concomitantly with the decrease in the absolute lymphocyte count, the absolute counts of CD3, CD4 and CD19 cells decreased six weeks after leptin was initiated (Table 1).

Cell-mediated immunity was assessed by lymphocyte proliferate response to mitogens PHA, Con-

A, PWM, *Tetanus* and *Candida*. There was a strong proliferative response to mitogens and to *Candida*, and no proliferative response to *Tetanus*, before and 6 weeks after leptin was initiated (Table 2).

DISCUSSION

In this study, we showed that, in our patient, congenital leptin deficiency is associated with normal humoral and cellular immunities, except for a very low proliferative response to *Tetanus*. In the short-term, r-metHuLeptin did not increase the proliferative response to *Tetanus*, whereas responses to other mitogens increased by up to 3-fold. Concomitantly, leptin replacement decreased the absolute lymphocyte count (though still within normal range). Finally, thymic volume was increased at baseline, as compared to the children's reference volume.

Previous studies report that leptin-deficient children have higher susceptibility to infections due to T cell hyporesponsiveness^{24,26}. In discordance with those findings, our patient did not have a history of recurrent infections, cell lymphopenia or impairment of lymphocyte function. On the contrary, after r-metHuLeptin was initiated, his absolute lymphocyte counts decreased. However, his proliferative responses to mitogens (other than *Tetanus* toxoid) did increase in response to leptin.

The absence of recurrent infections is strong evidence that our patient, besides having a different genotype (missense, instead of frameshift mutation), also has a different phenotype in regard to immunity, which did not change significantly after r-metHuLeptin was initiated. This may be the reason as to why he survived leptin deficiency. However, other obese children in his family, who were presumably leptin deficient, died early in life due to febrile illnesses (presumably infections). It is unclear why our patient has a different immune phenotype from the other leptin-deficient children identified so far. Possibly, the different types of mutations leading to leptin deficiency can determine diverse phenotypes. In addition, we hypothesize that the absence of infections in our patient, as opposed to the occurrence of fatal infections among relatives in the same family, might be explained by the 'variable expressivity' phenomenon. Although more frequently seen in auto-

TABLE 1

Lymphocyte subsets before and 6 weeks after initiation of leptin, measured by flow cytometry

	Before leptin	After 6 weeks on leptin	Children's reference range
CD3	63% (2261)	64% (1580)	62-80% (1610-4230)
CD4	34% (1144)	33% (766)	35-51% (900-2860)
CD8	27% (916)	27% (627)	22-38% (630-1910)
CD4/CD8 ratio	1.25	1.22	1.0-2.1
CD19	27% (1006)	22% (574/)	21-28% (700-1300)
CD16+CD56	9% (341)	11% (273)	

Values expressed as percentages (cells/cmm).

TABLE 2Proliferative response (SI) to phytohemagglutinin (PHA), concanavalin A (Con-A), pokeweed mitogen (PWM), *Tetanus* and *Candida*, before and after initiation of leptin

	Baseline	2 weeks	6 weeks
PHA 5 µg	184 (177)	290 (83)	373 (117)
PHA 1 µg	25 (21)	36 (7)	44 (19)
Con-A 5 µg	40 (32)	109 (34)	86 (47)
Con-A 1 µg	17 (17)	33 (13)	40 (19)
PWM 5 µg	28 (25)	49 (21)	43 (28)
PWM 1 µg	34 (28)	71 (22)	46 (30)
<i>Tetanus</i> 2 µg	2 (7)	27 (63)	5 (49)
<i>Tetanus</i> 1 µg	1 (6)	19 (54)	2 (33)
<i>Candida</i> 10 µg	45 (5)	211 (27)	99 (28)
<i>Candida</i> 5 µg	32 (2)	184 (20)	75 (32)

SI = stimulation index.

Controls' SIs are shown in parentheses.

somal dominant diseases, variable expressivity can occur in autosomal recessive traits, leading to different degrees of expression of the disease²⁹.

This study has some limitations. First, although we compared data with age-matched reference values, we did not include normal controls. Second, due to the rarity of the disease, only one leptin-

deficient child was included in the study. Finally, we did not repeat the studies due to restrictions regarding the volume of blood that was allowed to be drawn.

In conclusion, our data demonstrate that immune defects are not a constant feature of genetically-based leptin deficiency. The immune

phenotype associated with leptin deficiency appears to be determined not only by the absence of leptin, but also by the genotype and by variable expressivity. We also show here that even in the absence of significant immune defects, leptin replacement therapy enhances T cell responsiveness. Further studies need to evaluate the outcomes of leptin replacement therapy in hypo- or aleptinemic patients with normal immune responses (such as patients with lipodystrophy or anorexia nervosa). The potential role of leptin treatment for disorders of immunity may be either beneficial (by increasing immune response against infections) or negative (by facilitating autoimmune diseases), depending on the clinical context.

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Conflict of interest: none.

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