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Characterization of Immunodeficiency in a Patient With Growth Hormone Insensitivity Secondary to a Novel *STAT5b* Gene Mutation

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ABSTRACT

STAT5 proteins are components of the common growth hormone and interleukin 2 family of cytokines' signaling pathway. Mutations in the *STAT5b* gene, described in 2 patients, lead to growth hormone insensitivity that resembles Laron syndrome. Clinical immunodeficiency was also present, although immunologic defects have not been well characterized thus far. Here we describe a 16-year-old girl who suffered generalized eczema and recurrent infections of the skin and respiratory tract since birth. She also suffered severe chronic lung disease and multiple episodes of herpetic keratitis. Clinical features of congenital growth hormone deficiency were observed, such as persistently low growth rate, severely delayed bone age, and postnatal growth failure resulting from growth hormone resistance. This combined phenotype of growth hormone insensitivity and immunodeficiency was attributable to a homozygous C→T transition that resulted in a nonsense mutation at codon 152 in exon 5 of the *STAT5b* gene. This novel mutation determined a complete absence of protein expression. The main immunologic findings were moderate T-cell lymphopenia (1274/mm^3), normal CD4/CD8 ratio, and very low numbers of natural killer (18/mm^3) and γδ T (5/mm^3) cells. T cells presented a chronically hyperactivated phenotype. In vitro T-cell proliferation and interleukin 2 signaling were impaired. $CD4^+$ and $CD25^+$ regulatory T cells were significantly diminished, and they probably contributed to the signs of homeostatic mechanism deregulation found in this patient. This new case, in accordance with 2 previously reported cases, definitely demonstrates the significant role of the STAT5b protein in mediating growth hormone actions. Furthermore, the main immunologic findings bring about an explanation for the clinical immunodeficiency features and reveal for the first time the relevant role of STAT5b as a key protein for T-cell functions in humans.

CYTOKINES SUCH AS interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, IL-21, and growth hormone (GH) mediate their responses through activation of the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway.[1]

Although the GH receptor (GHR) and the IL-2 receptor (IL-2R), in the presence of their specific ligands, recruit and activate different cytosolic JAK proteins (such as JAK2 for GHR and JAK1/3 for IL-2R), they share STAT5 proteins for their signaling pathways.[1]

The STAT5 proteins a and b show 96% homology[2] and are encoded by 2 distinct but closely related genes, *STAT5a* and *STAT5b*. They have been described as having both redundant and nonredundant roles in the response of cells to different growth factors.[3]

The exclusive role of STAT5 in mediating GH actions has been demonstrated in mice[3,4] and recently in humans[5,6] in 2 nonrelated patients with STAT5b mutations. These subjects presented GH insensitivity, resembling Laron syndrome, and also associated with recurrent se-

Drs Bernasconi and Marino contributed equally to this work.

Key Words: STAT5b, STAT5b mutation, immunodeficiency, growth hormone insensitivity

Abbreviations: IL, interleukin; GH, growth hormone; JAK, Janus kinase; STAT, signal transducer and activator of transcription; GHR, growth hormone receptor; IL-2R, interleukin 2 receptor; Treg, regulatory T cell; NK, natural killer; SDS, SD score; EBV, Epstein-Barr virus; Ig, immunoglobulin; IGF-I, insulin-like growth factor I; IGFBP-3, insulin-like growth factor–binding protein 3; FC, flow cytometry; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cell; rIL-2R, recombinant interleukin 2 receptor; MCFI, mean channel of fluorescence intensity; TCR, T-cell receptor

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vere infection diseases. However, it is well known that Laron syndrome is not associated with immunologic defects.[7]

In the immune system, the importance of STAT5 proteins as mediators of the IL-2 family of cytokines has been established on the basis of different mouse models, supporting their role in development, homeostasis, and proliferation of different lymphocyte populations.[8–11]

A severe immunologic phenotype was found in double–knock-out STAT5a/b-deficient mice.[11] Some of them exhibited autoimmune pathology,[9] similar to mice lacking IL-2 or its receptor components: IL-2Rα or IL-2Rβ.[12–14] Because the disease was associated with decreased numbers of $CD4^+CD25^+$ regulatory T cells (Tregs), which are required to suppress immune response and prevent autoimmunity,[15] it has been proposed that STAT5 might be critical for the maintenance of tolerance in vivo.[9] Particularly, the Stat5b-deficient mouse has less dramatic immunologic alterations than the double knock-out, characterized by decreased numbers of T cells, low proliferation of lymphocytes to mitogens, IL-2 or IL-15 activation, and very low natural killer (NK) cell number and function.[16]

We report here a novel *STAT5b* gene mutation that totally precluded protein expression in a patient with GH insensitivity and immunodeficiency. A thorough characterization of the immunodeficiency showed moderate lymphopenia, with very low numbers of NK and $\gamma\delta$ T cells (which contribute to mucosal immunity and host defense against intracellular pathogens), as well as functional defects of T cells. The finding of a reduced number of Tregs would suggest that in humans, as in mice,[17,18] Tregs require the activation of STAT5.

CASE REPORTS

The patient, a 16-year-old girl, was the first of 2 children of nonconsanguineous parents. She was born with a birth weight of 2500 g. She has had severe growth failure since the first year of life. She was first referred to another institution at the age of 6 years, at which time her height was 83.6 cm (SD score; [SDS]: −6.17), her weight was 9800 g (relative weight: 89%), and her bone age was 2.5 years (Fig 1).

Since her first months of life she presented generalized eczema and recurrent severe infections of the skin and respiratory tract that required multiple hospitalizations. She also presented recurrent episodes of pneumonia, for which she was hospitalized and treated with intravenous antibiotics. No microbiologic isolations were reported.

She had been suffering from chronic diarrhea since she was 2 months old. Gastroenterological and laboratory evaluations ruled out cystic fibrosis and celiac disease. *Ascaris lumbricoides* and *Enterobius vermicularis* were found at different times; to our knowledge, no other organisms were isolated. At the age of 4 years she had

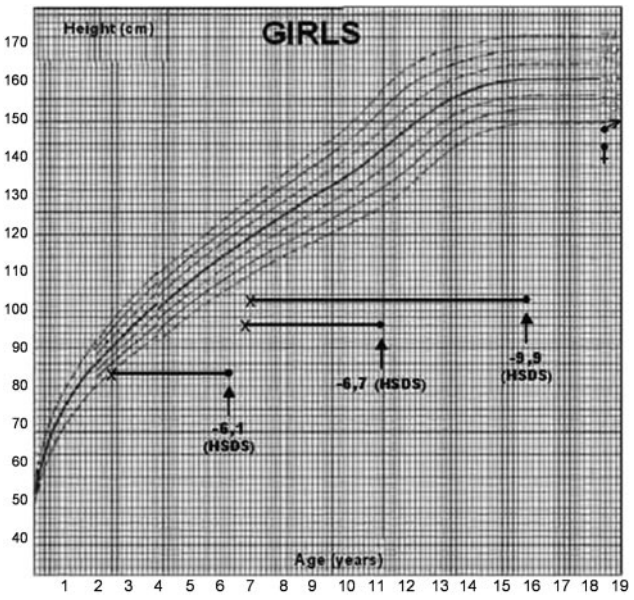

FIGURE 1
Our patient's growth chart. Height (in centimeters) and bone age (in years) are depicted by the ● and × symbols, respectively. Horizontal lines indicate the corresponding chronological and bone ages. Severe growth failure and delayed bone age were already present at 6 years of age.

prolonged varicella complicated with bacterial infection. Since the age of 10 years, she has had recurrent herpes zoster keratitis and uveitis of the left eye, with progressive loss of visual acuity.

No family history of immunodeficiency or growth failure was reported. However, short stature was observed in her mother. Paternal and maternal heights were 160 cm (SDS: −2.2) and 142.3 cm (SDS: −3.3), respectively. Her 10-year-old brother had normal height. At 16 years of age she was hospitalized because of respiratory distress and was referred to our immunology and endocrinology units. Clinical features of congenital GH deficiency were observed: prominent forehead, saddle nose, and high-pitched voice, with no signs of pubertal onset. She had a persistently low growth rate and severe growth failure (height: 102.7 cm [SDS: −9.91]; weight: 12 kg [SDS: −6.7]), and her bone age was severely delayed (7 years old). Basal serum triiodothyronine, thyroxine, thyrotropin, cortisol, luteinizing hormone, and follicle-stimulating hormone were normal for age and pubertal development. However, here serum prolactin level was persistently high in 3 different samples drawn with an interval time of 1 month (168, 102.3, and 143 ng/mL).

She had evidence of chronic lung disease with signs of chronic hypoxemia (clubbing). Active herpes keratitis and severe eczema were also present. Pharyngeal swabs and blood and sputum cultures were negative for virus, fungus, bacteria, and mycobacteria.

Serology analysis showed negative results for HIV, cytomegalovirus, and Epstein-Barr virus (EBV) immu-

noglobulin (Ig)M but positive results for cytomegalovirus and EBV IgG. The thorax computed tomography scan showed a ground-glass appearance with generalized micronodular images and bronchiectasis in the left lower lobe. Pulmonary-function tests showed mixed, restrictive and obstructive ventilative insufficiency of moderate degree. She was treated empirically with antibiotics without any response.

Lymphoid interstitial pneumonia was suspected; a lung biopsy was indicated, but the parents refused the test. Since then, the patient's pulmonary function has worsened, and permanent oxygen has been required. Because of the long history of recurrent infections, chronic lung disease, and severe growth retardation, immunodeficiency and GH deficiency or insensitivity was suspected.

The patient was lost to follow-up. Treatment with high doses of prednisone was started in another institution and, after 3 months, a partial improvement of the respiratory condition was observed (E.M. Rivas, verbal communication, 2005).

Our study was approved by the institutional review board of the Garrahan Pediatric Hospital. Informed consent, as well as assent, were obtained from the parents and patient, respectively.

METHODS

Serum Hormone Studies

Twenty-minute–interval basal serum GH was determined by a commercial chemiluminescent enzyme immunometric assay (Diagnostic Products Corporation, Los Angeles, CA).[19]

Insulin-like growth factor I (IGF-I) and IGF-binding protein 3 (IGFBP-3) levels were determined at basal conditions and during 5 consecutive days of treatment with 0.1 IU/kg per day of recombinant human GH. IGF-I was measured by radioimmunoassay after acid-ethanol extraction of serum,[20] and IGFBP-3 was measured by immunoradiometric assay with a diagnostic kit (Diagnostic System Laboratory, Webster, TX).

Serum prolactin was determined by microparticle enzyme immunoassay using a diagnostic kit (Axsym System; Abbott, Abbott Park, IL).

Flow-Cytometry Studies

Phenotypic analysis was performed after 3 color flow-cytometry (FC) standard methods[21] using monoclonal antibodies (mAbs) from Becton Dickinson (San Jose, CA) and a FACSort instrument (Becton Dickinson) equipped with CellQuest software (Becton Dickinson).

Perforin (PharMingen, San Diego, CA) in NK cells was determined on peripheral blood mononuclear cells (PBMCs) preincubated 24 hours with either medium alone or plus recombinant IL-2 (rIL-2) (50 IU) by standard intracellular staining methods.[21] Analysis was performed by gating the population-specific cell antigens, $CD3^{-}CD56^{+}$ and reporting the mean channel of fluorescence intensity (MCFI).

IL-2–Induced Phosphorylation of STAT5 Protein

On the basis of the method described by Fleisher et al,[22] PBMCs were used for the evaluation of STAT5 phosphorylation in basal conditions or under stimulation with 1000 IU of rIL-2. Permeabilized cells were incubated with anti-STAT5 (BD Transduction Lab, Lexington, KY), antiphospho-STAT5 (Zymed Laboratories, San Francisco, CA), and isotype control antibody (MOPC 31C Sigma, St Louis, MO). Data were reported as the percentage of lymphocytes staining positive for STAT5 or phospho-STAT5 versus cells stained with isotype control. The data of MCFI were analyzed also.

Lymphocytes Functional Assay

Lymphocyte proliferation was assessed as described previously[23] on PBMC culture using the [^{3}H]thymidine-incorporation method with optimal concentrations of mitogens (phytohemagglutinin, anti-CD3, *Staphylococcus enterotoxin* B, phorbol myristate acetate, and ionomycine) for 3 days and specific antigens (purified protein derivative, *Candida*, and tetanus toxoid) for 6 days. To asses IL-2 proliferative response under mitogenic stimulation, rIL-2 (50 μg/mL, 50 IU) was added.

Cytotoxic NK activity was measured on a ^{51}Cr-release assay as described previously[24] after 24 hours of preincubation of PBMCs either with medium alone or under rIL-2 (50 IU) stimulation. The percentage of cytotoxicity was determined according to the following formula: % cytotoxicity = (mean patient cpm − mean spontaneous cpm)/(mean cpm of maximum − mean spontaneous cpm) × 100.

Western Immunoblotting

Western immunoblotting was conducted by using mouse mAbs anti-human-STAT5 (BD Transduction Lab), anti-human-STAT5b (Santa Cruz Biotechnology, Santa Cruz, CA), and anti-human-actin (Santa Cruz Biotechnology) as an internal control.

EBV immortalized B-cell lines derived from the patient and a normal control were solubilized in radioimmunoprecipitation assay lysis buffer. Total protein concentration was determined by using a commercial protein assay (Bio-Rad, Hercules, CA). Samples were electrophoresed on 7% sodium dodecyl sulfate/polyacrylamide gels and electroblotted onto nitrocellulose membranes (Hybond ECL, Amersham, Piscataway, NJ). Proteins were detected with ECL chemiluminescence reagents (Amersham).

DNA Isolation, Amplification, and Sequencing

Each coding exon of the *STAT5b* gene and flanking intronic regions were amplified from the patient and rel-

ative genomic DNA using primers reported by Kofoed et al.[5] Each purified product (QiaQuick polymerase chain reaction purification kit, Qiagen, Valencia, CA) was used as a template for direct sequencing (automated ABI PRISM 310 capillary sequencer, Applied Biosystems, Foster City, CA). The nucleotide sequences obtained were compared with those for contig DNA containing the *STAT5b* gene (NT_010755 accession number gi 51474257).

Reference Values

All patient data were compared with age-related reference values of our immunology laboratory at the Hospital de Pediatría Garrahan.

Statistical Analysis

Differences between means were evaluated by using the Bonferroni test after 1-way analysis of variance.

RESULTS

Evaluation of the GH–IGF-I Axis

Basal GH was evaluated in 4 different serum samples at 0 (4.2 ng/mL), 20 (4.6 ng/mL), 40 (6.6 ng/mL), and 60 minutes (4.2 ng/mL). According to a previous report,[19] 6 ng/mL was the cutoff to define a normal serum GH response to pharmacological test. Thus, a basal GH value reaching 6.6 ng/mL was sufficient to discard the diagnosis of GH deficiency. In addition, the diagnosis of severe GH insensitivity was made, because no detectable IGF-I or IGFBP-3 was measured under basal conditions or after recombinant human GH treatment during the IGF-I-generation test.

Immunologic Studies

Moderate lymphopenia and hypergammaglobulinemia, mainly of IgG and IgE isotypes, was observed (Table 1). Serologic evaluations showed positive IgG antibodies for varicella herpesvirus, hepatitis A virus, EBV, cytomegalovirus, and herpes simplex virus I and II. Serum antibodies specific to recall antigens (tetanus toxoid and *Pneumococcus*) were within the reference range (Table 1), showing integrity of the antibody function. Complement proteins were below the reference levels, and a high titer (1/1000) of antinuclear antibody was detected.

The patient had moderate T-cell lymphopenia, with numbers of CD4 and CD8 T cells in the lower limit and

TABLE 1 **Immunological Parameters**

	Patient		Reference Values[a]	
	%	Per mm³	%	Per mm³
Lymphocytes		903–1820		2000–2700
T lymphocytes				
CD3	70	1274	65–72	1545–2489
CD4	35	783	32–38.5	771–1180
CD8	29	473	25–32.5	629–1128
TCR$\alpha\beta$	99	1261	54–66	1407–2187
TCR$\gamma\delta$	0.4	5	5–8	113–237
Activation T-cell profile				
HLA-DR	49		9.7–17.6	
CD95	97		10–15	
CD4/CD45RA	38		64.8–73.3	
CD4/CD45RO	92		39–57.1	
CD4/CD25	30		<10	
CD8/CD45RA	63		76.7–90.9	
CD8/CD45RO	81		25.0–44.4	
B lymphocytes, CD19	25	355	10–16	278–481
NK lymphocytes, CD56	1	18	10–19	113–237
Serum Ig[b]				
IgG, mg/dL	2530		940 ± 124	
IgM, mg/dL	121		59 ± 20	
IgA, mg/dL	157		148 ± 63	
IgE, IU/mL	631		≤200	
Antibody titers				
Tetanus toxoid, IU/mL	0.2		>0.1	
Streptococcus				
Pneumoniae, mg/liter[c]				
Basal	17		>113	
Poststimulus	250			

Percentage of TCR subset is expressed in 100% of T cells. The percentage of each activation antigen is expressed as 100% of T lymphocytes (HLA DR, CD95) and 100% of CD4 or CD8 T cells (CD25, CD45RA, CD45RO). Serum Igs were determined by nephelometry

[a] 25th and 75th percentiles of age-related normal values.

[b] Reference values are expressed as median ± SD.

[c] INOVA kit for evaluation of all serotypes of the polyvalent vaccine.

below the reference range, respectively, but within the reference CD4/CD8 ratio. B cells were within the reference range. It is notable that an extreme reduction in percentages and in absolute counts of NK and γδ T cells was found (Table 1).

T lymphocytes showed a phenotype of chronically activated cells: skewed CD45RA/CD45RO ratio, high percentages of HLA-DR, CD95, and CD25 (Table 1 and Fig 2 A–F), and down-modulation of CD62L (Fig 2F).

Analysis of the $CD4^+CD25^+$ Treg population was based on the definition of Tregs by Baecher et al.[25] Despite the fact that T cells were hyperactivated, a minor population with high expression of CD25 could be detected that represented only 1.16% ± 0.36% ($n = 3$) of the total CD4 T cells (Fig 2C). According to our age-matched reference values of 4.04% ± 0.26% ($n = 7$), they were clearly diminished ($P < .01$). These cells constitutively expressed cytotoxic T-lymphocyte–associated protein 4 (CTLA4) and were $CD62L^{+high}$, indicating that, phenotypically, they were truly Tregs[25] (data not shown).

In vitro T-cell proliferation is shown in Table 2. Proliferation to mitogens (phytohemagglutinin, *S enterotoxin* B, phorbol myristate acetate/ionomycine, and anti-CD3) was consistently low and could not be restored to reference values despite rIL-2 stimulation. After natural exposure or vaccination, absence of proliferative response to recall antigens (tetanus toxoid, purified protein derivative, and *Candida*) was observed (Table 2). These results support the concept of a general functional T-cell defect besides the defect in the IL-2 signaling pathway.

TABLE 2 **T-Cell Proliferation in Vitro: Incorporation of $^3[H]$thymidine in cpm (Mean of Triplicates)**

Stimulus	Patient, No rIL-2	Patient, rIL-2	Reference Values, No rIL-2
Mitogens (3 d)			
Medium	2590	15 062	150–5851
Phytohemagglutinin M	53 137	54 001	93 235–186 129
S enterotoxin B	57 392	50 149	117 599[a]
Anti-CD3	42 565	48 843	85 038–176 294
PMA and ionomycine	71 691	ND	80 223–238 517
Antigens (6 d)			
Medium	2178	ND	Up to 5000
Candida	709	ND	14 850–61 814
Tetanus toxoid	554	ND	14 850–61 814
Purified protein derivative	397	ND	13 080–56 558

ND indicates not done.

[a] The reference value indicated was measured in the same experiment.

The presence of high T-cell activation, hypergammaglobulinemia, antinuclear antibodies, and diminution of $CD4^+CD25^+$ Tregs suggests a defect in the peripheral homeostasis mechanisms.

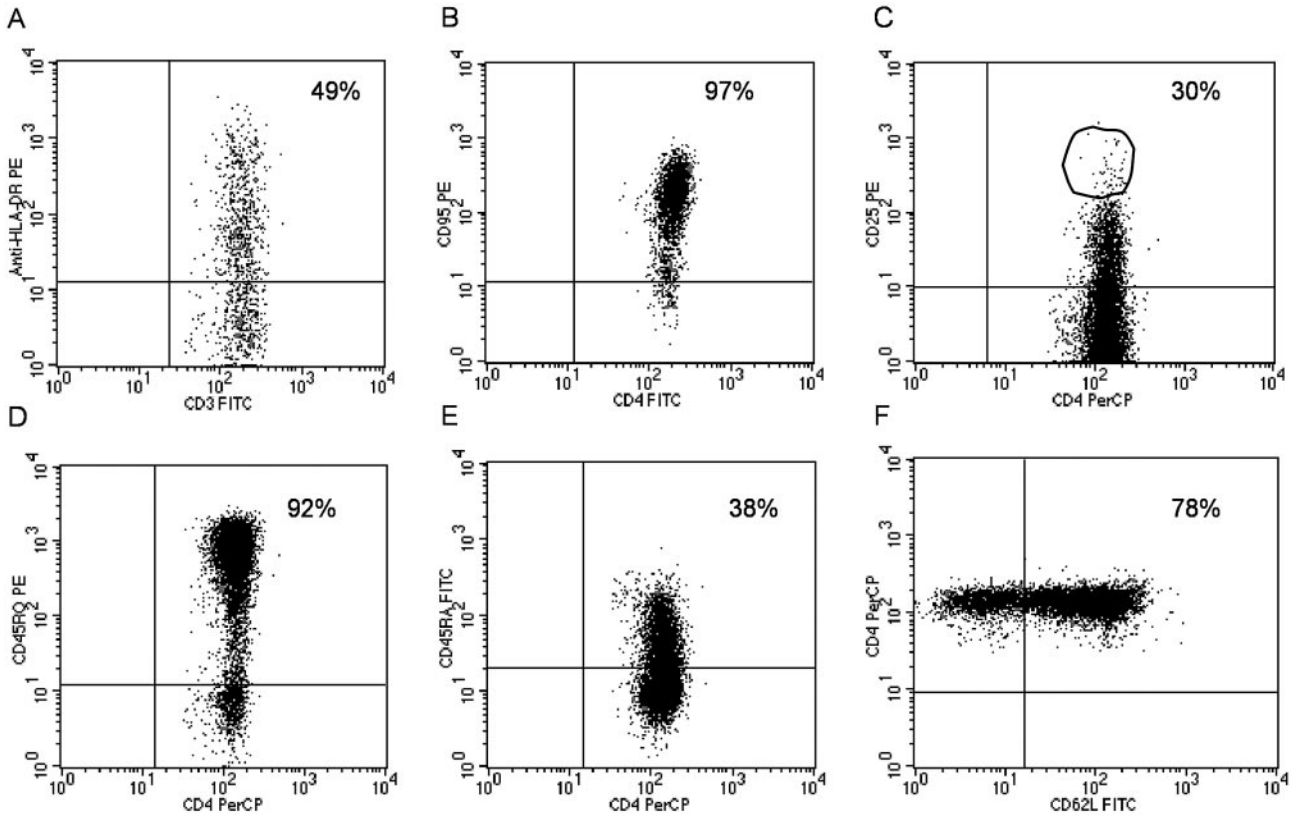

FIGURE 2

Our patient's T-cell–activation profile. A, Expressed in 100% of T cells; B–F, 100% of CD4 T cells. Analysis of Tregs was performed by selecting the $CD4^+CD25^{high}$ T-cell population indicated by a circle (in C). FITC indicates fluorescein isothiocyanate; PerCP, perydin clorophyl protein; PE, phycoerythrin.

To analyze the IL-2 signaling pathway, STAT5 phosphorylation under IL-2 stimulation was evaluated by FC.

In the patient's lymphocytes, as shown in Fig 3A, STAT5 protein phosphorylation in response to high concentrations of rIL-2 was reduced to less than half of the control MCFI value (206 vs 462 ± 51.8 [n = 3]).

The expression of STAT5 proteins was reduced compared with the normal control (MCFI value: 291 vs 464 ± 62.7 [n = 3]) (Fig 3B).

As a whole, these results suggest that the functional impairment in IL-2-mediated signaling might be the consequence of an alteration in either of the 2 STAT5 proteins.

Molecular Studies

To evaluate the expression of both STAT5a and STAT5b proteins, immunoblot analysis was performed by using an anti-STAT5 mAb and 7% sodium dodecyl sulfate/polyacrylamide gels that discriminate STAT5a (91 kilodaltons) from STAT5b (84 kilodaltons) proteins, as shown in Fig 4A. The STAT5b protein is not detected at all in the patient's sample, whereas the related STAT5a protein level is comparable to those found in the normal B-cell line. The absence of STAT5b was confirmed with an immunoblot analysis using a specific anti-human STAT5b mAb (Fig 4B).

To determine the genetic basis for the apparent lack of immunodetectable STAT5b, *STAT5b* coding exons were sequenced. The results revealed a novel single-nucleotide change, a homozygous C→T transition, that resulted in a nonsense mutation at codon 152 (R152X). The parents and brother were heterozygous for this mutation. The stop-codon mutation in the *STAT5b* gene is in exon 5, which encodes the coiled-coil domain, very close to the NH_2-terminal domain, predicting that the protein should not be immunodetectable.

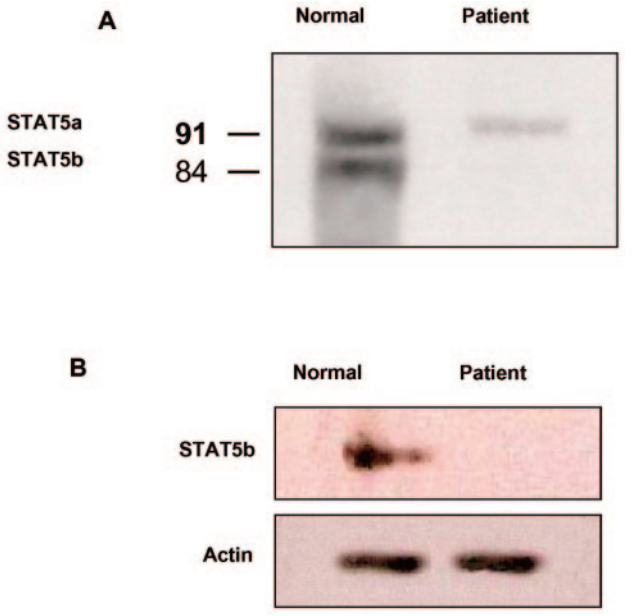

FIGURE 4
Immunoblot analysis of STAT5 expression in our patient's and a normal control's EBV immortalized B-cell lines using anti-human-STAT5-mouse mAb (A) and anti-human-STAT5b-mouse mAb (B) in 7% sodium dodecyl sulfate/polyacrylamide gels. The STAT5b protein is not detected at all in the patient's sample, whereas the related STAT5a protein is detected at levels comparable to those found in the normal B-cell line.

NK Activity and Perforin Expression Induced by IL-2

Because IL-2 is a potent in vitro stimulator of NK cytolytic activity, we evaluated if this function was affected in the absence of the STAT5b protein. A clear 2.5-fold (from 8% to 20%) and 10-fold (from 3% to 30%) in-

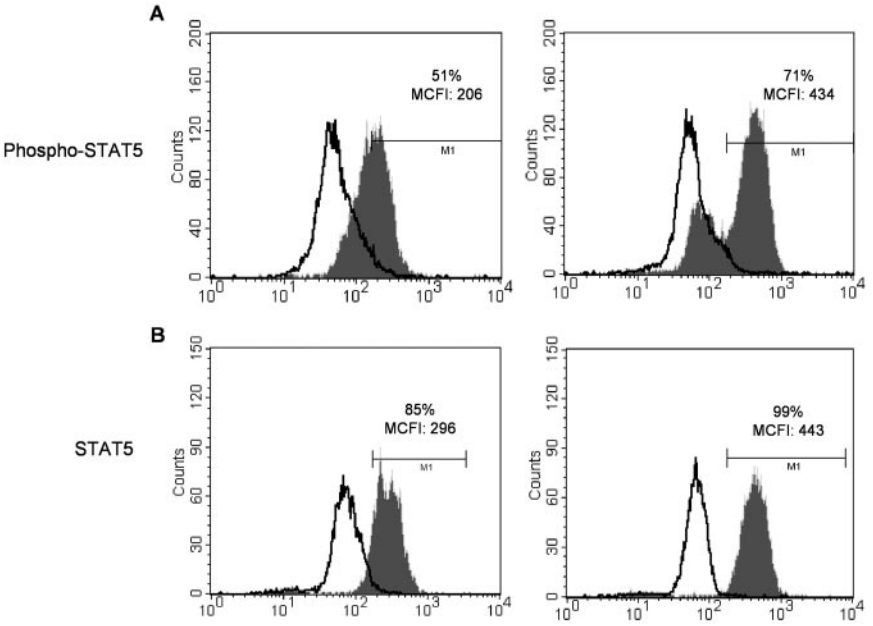

FIGURE 3
Reduced rIL-2-induced STAT5 phosphorylation and total STAT5 protein expression in patient's PBMCs. A, Phospho-STAT5 expression in IL-2-stimulated cells (shaded histogram) overlaid with histograms of phospho-STAT5 in unstimulated cells (line) of our patient (left) and a normal control (right). Quantification by FC was performed by setting the marker against isotype control to determine the percentage of cells staining positive for phospho-STAT5 and its intensity (MCFI). STAT5 phosphorylation in basal conditions is coincident with the isotype control in both the patient and the normal control. B, Total STAT5 protein expression in unstimulated cells (shaded histogram) versus isotype control (line) of our patient (left) and a normal control (right). The percentage of cells positive for STAT5 and MCFI are expressed.

crease in cell lysis in the presence of IL-2,was found in the patient when compared with basal conditions. In the control samples, an average of a twofold increase in NK activity was observed (from 29 ± 17.2 to 60 ± 22.9 [n = 5]; P = .042). Although the range of lysis enhancement in the patient was higher, it did not reach the normal control level of cytotoxic activity, possibly influenced by the low NK cell number.

Because perforin is one of the key molecules for NK-cell–mediated cytolysis, the upregulation of the protein expression by IL-2 was also analyzed by FC on NK cells. Corresponding to the NK cytotoxicity results, IL-2 increased the protein MCFI value from 136 ± 14.8 to 179.5 ± 10.6 (n = 3; P = .014) (controls: 147 ± 18.0 to 174.7 ± 23.3 [n = 4]). These data suggest that STAT5b is not a limiting factor for mediating IL-2 actions in NK cells.

DISCUSSION

The studies described for this patient lend support, for the first time, to the relevance of STAT5b not only in the human GH signaling cascade but also in the cytokine-mediated immune response. We describe here a novel mutation in the STAT5b gene in a patient with a combined phenotype of GH insensitivity and immunodeficiency secondary to lymphocyte functional alterations.

The nonsense mutation, located in exon 5 of the *STAT5b* gene, predicts a severely truncated protein that retains only the NH_2-terminal domain. Because it lacks the tyrosine-activation domain, it is expected to be devoid of any biological activity. Immunoblot analysis failed to detect the STAT5b protein, probably because the mutant *STAT5b* product suffers a rapid degradation or it is not expressed at all.

As in the 2 patients described previously,[5,6] basal normal-to-high concentration of serum GH and extremely low serum IGF-I and IGFBP-3 levels were found in this case. Moreover, exogenous GH treatment did not further increase serum IGF-I or IGFBP-3 levels, confirming that STAT5b abnormality affects GHR signaling cascade. Similarly to the other 2 cases with *STAT5b* gene mutations[5,6] and to those observed in GHR deficiency,[7] near-normal size at birth and severe postnatal growth failure were observed. Therefore, this postnatal growth pattern reinforces the relevance of the GH-GHR-STAT5b-IGF-I pathway in human postnatal growth. In this regard, the STAT5b protein in STAT5 knock-out models is also essential for GH biological response in terms of postnatal growth and IGF-I expression. However, the postnatal growth observed in the patients is in contrast to that of mice, in which only male animals are affected.[4]

The persistently high hyperprolactinemia suggests a state of prolactin resistance. In this regard, prolactin specifically stimulates phospho-STAT5b in cultured hypothalamic dopaminergic neurons.[26] In addition, a specific intracellular pathway of prolactin-negative feedback in the hypothalamus is mediated by STAT5b, as it has been demonstrated in STAT5b knock-out mice, showing grossly elevated serum prolactin concentrations.[27] Therefore, the patient's hyperprolactinemia suggests that STAT5b plays a role in mediating the negative feedback action of prolactin also in humans.

The relevant role of STAT5 proteins in the immune system thus far has been defined only in mouse models. Similarly to the 2 patients reported, in our case clinical signs of immunodeficiency were clearly found, because she suffered from recurrent pulmonary infections, persistent herpesvirus infection, chronic eczema, and diarrhea. However, the immune system abnormalities in the 2 patients with *STAT5b* gene mutations already reported have not been well characterized.

In our patient, similar to the STAT5b knock-out[16] mouse, phenotypic alterations, moderate T-cell lymphopenia, and highly reduced NK and γδ T cells were found. Moreover, the T-cell–hyperactivated status resembles the STAT5a/b double–knock-out mice[11] and, interestingly, also the knock-out mice for IL-2Rβ, the signaling-IL-2R chain that serves as a STAT5 docking site.[28]

T cells were functionally defective, which was clearly documented by the in vitro low proliferative response to mitogens and antigens. Even in the presence of rIL-2, no proliferation improvement was observed. These results reflect a generalized defect in T-cell functions that could be a consequence of both defective STAT5 signals through T-cell receptor (TCR)[29] and IL-2R. Moreover, resistance to IL-2 takes place even in the presence of normal amounts of STAT5a and one half of IL-2–STAT5 phosphorylation, which reinforces the concept that STAT5a and STAT5b are not completely redundant.[3] Thus, different from mice,[11] a full STAT5 function would be necessary in humans for a complete immune response.

On the other hand, we observed that the expression of IL-2Rα chain (CD25) could not be sustained in our patient's T cells in culture even in the presence of rIL-2 (data not shown). The fact that IL-2Rα is controlled by IL-2 through STAT5 proteins[16,30] suggests that the expression of a high-affinity receptor is also affected, and it might have an influence in the lack of response to IL-2.

Nevertheless, the possible impact of the absence of STAT5b on impaired thymic function should also be considered in the context of T-cell lymphopenia, skewed CD45RA/CD45RO ratio, proliferative defects, and chronic infection. Additional studies designed to evaluate thymic output could clarify this point.

Contrary to T cells, B lymphocytes were normal in number and function, suggesting that STAT5b protein is mainly relevant in mediating T-cell–lineage functions. However, the presence of hypergammaglobulinemia and anti-nuclear antibodies, even without clinical autoim-

mune disease, suggests a deregulation of B cells, probably as a result of an impaired control by defective T cells.

Several evidences show that IL-2 is not only an important growth factor for T cells through TCR stimulation but also exhibits a critical role in the maintenance of in vivo tolerance to self-antigens, because spontaneous accumulation of activated T cells and autoimmunity develop in IL-2[31] or IL-2Rβ[14] knock-out models or downstream signaling-component knock-out models such us STAT5a/b$^{-/-}$ mice.[9,11] However, the role of STAT5 proteins in the regulation of the immune response in humans has not been clearly established. One of the mechanisms for maintenance of peripheral tolerance by IL-2 is through the generation of $CD4^+CD25^+$ Tregs.[32] In this regard, it has been described in mice that STAT5 protein signaling is required for IL-2 Treg development and homeostasis.[17,18] In knock-out mouse models for STAT5a/b, the reduction of this T-cell subset was considered to contribute to the loss of tolerance detected in a group of them. The reduction in Tregs correlated with a high rate of cell death in this population.[9]

Our findings are compatible with a deregulation of the mechanisms of tolerance in our patient, such as the detection of high titers of antinuclear antibody, hypergammaglobulinemia, and T-cell activation. However, hypergammaglobulinemia and T-cell activation could also be secondary to infections. Moreover, on the basis of the characteristics of the pulmonary disease of the previously reported patients,[5,6] we suspected a similar chronic pulmonary illness as a consequence of lymphocytic infiltration. Because a significant decrease in Treg number that might contribute to this phenotype was found, we propose that in humans complete STAT5 function is required for establishing normal tolerance, although additional studies are necessary to confirm this proposal.

Similarly to STAT5 knock-out mice,[11,16] a severe reduction in our patient's NK-cell number was found, suggesting that either development or homeostasis of these cells in the periphery depend on STAT5b. For both mechanisms, dependence on the IL-15/STAT5 pathway seems to be crucial.[33] However, in contrast to the STAT5b knock-out model, the patient's NK cells showed a response to IL-2, increasing NK cytotoxic function, and upregulating perforin. Although this could be secondary to the activation of an alternative pathway,[34] the recurrent herpetic infections in our patient suggest an inadequate NK surveillance, considering the crucial role of these cells in innate immune defense against viruses.[35] Moreover, primary immunodeficiencies with selective deficiency in NK cells suffer mainly infections with herpes family viruses.[36,37]

CONCLUSIONS

We have demonstrated T-cell–function defects, a low number of NK and $\gamma\delta$ T cells, IL-2 signaling defects at the T-cell level but preserved in NK, and deregulation of homeostasis (probably related to a decreased number of Tregs) in a patient with GH insensitivity resulting from a novel mutation in the *STAT5b* gene.

This mutation results in alterations in the signaling pathways of GHR, cytokine receptors related to T lymphocytes such as IL-2 receptor, and probably prolactin receptor. This is explained biologically by the fact that STAT5b is a signal transduction protein shared by all these factor receptors.

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REFERENCES

1. Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW. Signaling through the JAK/STAT pathway recent advances and future challenges. *Gene.* 2002;285:1–24
2. Liu X, Robinson GW, Gouilleux F, Groner B, Hennighausen L. Cloning and expression of Stat5 and an additional homologue (Stat5b) involved in prolactin signal transduction in mouse mammary tissue. *Proc Natl Acad Sci U S A.* 1995;92:8831–8835
3. Teglund S, McKay C, Schuetz E, et al. Stat5a and Stat5b proteins have essential and nonessential or redundant roles in cytokine responses. *Cell.* 1998;93:841–850
4. Udy GB, Towers RP, Snell RG, et al. Requirement for Stat5b for sexual dimorphism of body growth rates and liver gene expression. *Proc Natl Acad Sci U S A.* 1997;94:7239–7244
5. Kofoed EM, Hwa V, Little B, et al. Growth hormone insensitivity associated with a STAT5b mutation. *N Engl J Med.* 2003; 349:1139–1147
6. Hwa V, Little B, Adiyaman P, et al. Severe growth hormone insensitivity resulting from total absence of signal transducer and activator of transcription 5b. *J Clin Endocrinol Metab.* 2005; 90:4260–4266
7. Laron Z. Laron syndrome (primary growth hormone resistance or insensitivity): the personal experience 1958–2003. *J Clin Endocrinol Metab.* 1996;89:1031–1044
8. Yao Z, Cui Y, Watford WT, et al. Stat5a/b are essential for normal lymphoid development and differentiation. *Proc Natl Acad Sci U S A.* 2006;103:1000–1005
9. Snow JW, Abraham N, Ma MC, Herndier BG, Pastuszak AW, Goldsmith MA. Loss of tolerance and autoimmunity affecting multiple organs in Stat5a/5b deficient mice. *J Immunol.* 2003; 171:5042–5050
10. Lord JD, McIntosh BC, Greenberg PD, Nelson BH. The IL-2 receptor promotes lymphocyte proliferation and induction of the c-*myc bcl-2* and *bcl-x* genes through the trans-activation domain of Stat5. *J Immunol.* 2000;164:2533–2554
11. Moriggl R, Topham DJ, Teglund S, et al. Stat5 is required for IL-2-induced cell cycle progression of peripheral T cells. *Immunity.* 1999;10:249–259
12. Horak I, Lohl J, Ma A, Smith KA. Interleukin-2 deficient mice: a new model for studying. *Immunol Rev.* 1995;148:35–44
13. Willeford DM, Chen J, Ferry JA, Davidson L, Ma A, Alt FW. Interleukin-2 receptor α chain regulates the size and content of the peripheral lymphoid compartment. *Immunity.* 1995;3: 521–530
14. Suzuki H, Kundig TM, Furlonger C, et al. Deregulated T cell

activation and autoimmunity in mice lacking interleukin-2 receptor β. *Science.* 1995;268:1472–1476
15. Shevach EM. Certified professionals: $CD4^+CD25^+$ suppressor T cells. *J Exp Med.* 2001;193:F41–F45
16. Imada K, Bloom ET, Nakajima H, et al. Stat5b is essential for natural killer cell-mediated proliferation and cytolytic activity. *J Exp Med.* 1998;188:2067–2074
17. Antov A, Yang L, Vig M, Baltimore D, Van Parijs L. Essential role for Stat5 signaling in $CD25^+CD4^+$ regulatory T cell homeostasis and the maintenance of self-tolerance. *J Immunol.* 2003;171:3435–3441
18. Burchill MA, Goetz CA, Prlic MO, et al. Distinct effect of Stat5 activation on $CD4^+$ and $CD8^+$ T cell homeostasis: development of $CD4^+CD25^+$ regulatory T cells versus $CD8^+$ memory T cells. *J Immunol.* 2003;171:5853–5864
19. Chaler E, Belgorosky A, Maceiras M, Mendioroz M, Rivarola MA. Between-assay differences in serum growth hormone (GH) measurements: importance in the diagnosis of GH deficiency in childhood. *Clin Chem.* 2001;47:1735–1738
20. Belgorosky A, Rivarola MA. Insulin-like growth factor binding protein (IGFBP)-3-bound IGF-I and IGFBP-3-bound IGF-II in growth hormone deficiency. *Horm Res.* 1999;52:60–65
21. Robinson P. *Handbook of Flow Cytometry Methods*. New York, NY: Wiley-Liss, Inc; 1993
22. Fleisher TA, Dorman SE, Anderson JA, Vail M, Brown MR, Holland SM. Detection of intracellular phosphorylated STAT-1 by flow cytometry. *Clin Immunol.* 1999;90:425–430
23. Fischer MB, Hauber I, Eggenbauer H, et al. A defect in the early phase of T-cell receptor-mediated T-cell activation in patients with common variable immunodeficiency. *Blood.* 1994;84:4234–4241
24. Baker KS, DeLaat CA, Steinbuch M, et al. Successful correction of hemophagocytic lymphohistiocytosis with related or unrelated bone marrow transplantation. *Blood.* 1997;89:3857–3863
25. Baecher AC, Brown JA, Freeman GJ, Hafler DA. $CD4^+CD25^{high}$ regulatory cells in human peripheral blood. *J Immunol.* 2001;167:1245–1253
26. Ma FY, Anderson GM, Gunn TD, Goffin V, Grattan DR, Bunn SJ. Prolactin specifically activates signal transducer and activator of transcription 5b in neuroendocrine dopaminergic neurons. *Endocrinology.* 2005;146:5112–5119
27. Grattan DR, Xu J, McLachlan MJ, et al. Feedback regulation of PRL secretion is mediated by the transcription factor, signal transducer, and activator of transcription 5b. *Endocrinology.* 2001;142:3935–3940
28. Suzuki H, Duncan GS, Takimoto H, Mark TW. Abnormal development of intestinal intraepithelial lymphocytes and peripheral natural killer cells in mice lacking the IL2 receptor β chain. *J Exp Med.* 1997;185:499–505
29. Welte T, Leitenberg D, Dittel BN, et al. STAT5 interaction with the T cell receptor complex and stimulation of T cell proliferation. *Science.* 1999;283:222–225
30. John S, Robbins CM, Leonard WJ. An IL-2 response element in the human IL-2 receptor alpha chain promoter is a composite element that binds Stat5 Elf-1 HMG-I(Y) and a GATA family protein. *EMBO J.* 1996;15:5627–5635
31. Sadlack B, Merz H, Schorle H, Schimpl A, Feller AC, Horak I. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell.* 1993;75:253–261
32. Malek TR. The main function of IL-2 to promote the development of T regulatory cells. *J Leukoc Biol.* 2003;74:961–965
33. Cooper MA, Bush JE, Fehniger TA, et al. In vivo evidence for a dependence on interleukin 15 for survival of natural killer cells. *Blood.* 2002;100:3633–3638
34. Zhou J, Zhang J, Lichtenheld MG, Meadows GG. A role for NF-KB activation in perforin expression of NK cells upon IL-2 receptor signaling. *J Immunol.* 2002;169:1319–1325
35. Brown MG, Dokun AO, Heusel JW, et al. Vital involvement of a natural killer cell activation receptor in resistance to viral infection. *Science.* 2001;292:934–937
36. Biron CA, Byron KS, Sullivan JL. Severe herpes virus infections in an adolescent without natural killer cells. *N Engl J Med.* 1989;320:1731–1735
37. Etzioni A, Eidenschen C, Katzk R, BeckR, Casanova JL, Pollac S. Fatal Varicella associated with selective Natural Killer cell deficiency. *J Pediatr.* 2005;146:423–425

Characterization of Immunodeficiency in a Patient With Growth Hormone Insensitivity Secondary to a Novel STAT5b Gene Mutation

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