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ORIGINAL RESEARCH

# Novel *STAT3* Mutation Causing Hyper-IgE Syndrome: Studies of the Clinical Course and Immunopathology

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**Abstract**
*Purpose* Reporting a clinical case with a novel mutation in the signal transducer and activator of transcription 3 (*STAT3*) gene resulting in autosomal dominant hyper-immunoglobulin E syndrome (AD-HIES). Here we also had the opportunity to perform in-depth immunologic investigations to further understand the immunopathology of this primary immunodeficiency.
*Methods* The patient, a baby boy, was clinically assessed according to the scoring system developed by Grimbacher et al. and *STAT3* was investigated by DNA sequencing. Immunologic work-up consisted of lymphocyte phenotyping and proliferation assays, measurement of soluble mediators and routine investigations.
*Results* According to the Grimbacher score the patient was likely to have AD-HIES and a novel heterozygous *STAT3* mutation (c.1110-3C>A), causing a splice error, was identified. Lymphocyte phenotyping revealed decreased numbers of interleukin (IL)-17 producing T-helper lymphocytes and aberrant B-lymphocyte subsets. Proliferative in vitro lymphocyte responses against *C. albicans*, staphylococcal enterotoxins and pokeweed mitogen were supernormal at presentation, whereas only the elevated response to pokeweed mitogen persisted. The soluble mediators IL−5, −10, −12, −13, −15 and granulocyte colony stimulatory factor were elevated in serum.
*Conclusion* A novel heterozygous *STAT3* mutation causing defective splicing of exon 12 was identified. Lymphocyte

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phenotyping revealed deranged subpopulations. Despite the clinical picture with severe *C. albicans* and staphylococcal infections, the patient's lymphocytes mounted responses to these pathogens. The hypereosinophilia and high immunoglobulin E levels might partly be explained by elevated IL−5 and −13 as a result of lack of negative feedback from defective STAT3 signaling.

**Keywords** Job's Syndrome · immunodeficiency · Th17

## Introduction

The autosomal dominant hyper-immunoglobulin (Ig) E syndrome (AD-HIES) was described in the late 1960's and early 1970's as characterized by eczematous dermatitis, recurrent skin and pulmonary infections with elevated IgE. Thereafter the multiorgan nature of the disease has been outlined. In 2007, dominant-negative mutations in the signal transducer and activator of transcription 3 (*STAT3*) gene were found to be the cause of connective tissue abnormalities and recurrent infections [1, 2]. STAT3 plays an important role in immunologic processes, transducing signals downstream of the interleukin (IL) −2, −6 and −10 receptor families, receptor-type tyrosine kinases, Flt3 ligand, macrophage colony stimulatory factor (M-CSF), granulocyte CSF (G-CSF), interferon (IFN) family (IFN-γ, IFN-α/β). In addition, STAT3 signals downstream of other factors such as ciliary neurotropic factor, oncostatin M, leukemia inhibitory factor, leptin and growth hormone [3–6]. The immune defect in AD-HIES is heterogeneous. Poor humoral responses to antigens as well as deteriorated Ig levels have been reported [7, 8]. Phenotyping of immune cells have displayed patterns shared by most patients, i.e. low levels of IL-17 producing T-helper (Th17) lymphocytes [9–11] and circulating memory B lymphocytes [12, 13]. Subnormal responses to mitogens and allogeneic cells have also been observed [7]. Phagocyte function is normal in AD-HIES, while the Th17 lymphocyte deficiency has been associated to a decreased production of antimicrobial peptides [2].

Thus, AD-HIES is a primary immunodeficiency affecting several branches of the immune system with subsequent risk of severe infectious diseases. No curative treatment exists as the role of hematopoietic stem cell transplantation is unclear. Anti-staphylococcal and -fungal maintenance therapies as well as symptomatic treatment of connective tissue-related disease are applied by most centers [1, 2]. A beneficial effect of IgG infusions was recently demonstrated by a French national survey describing the clinical course of 60 AD-HIES patients [8].

Here, we report a new heterozygous *STAT3* mutation associated with AD-HIES, in a boy with classical features of the syndrome presenting within the first months of life. A multimodal investigation of the patient's immune system disclosed aberrations that may offer new insights into the immunopathology underlying AD-HIES.

## Subjects, Material and Methods

The patient and parents were investigated according to institutional guidelines and the study was performed in accordance with the declaration of Helsinki. Written informed consent was given by the parents. Healthy blood donors served as control subjects and their samples were obtained from the Department of Transfusion Medicine, Karolinska University Laboratory, Karolinska University Hospital.

### Clinical Investigation and HIES Scoring

To probe a tentative diagnosis of HIES, the scoring system by Grimbacher et al. was applied. This scoring system, accepted by the National Institutes of Health, is based on both clinical (e.g. eczema, skin abscesses, fractures, pneumonias, candidiasis etc.) and laboratory (i.e. serum IgE level and absolute eosinophil granulocyte count) criteria. Total-point score assignments at <10 points suggest that the subject is unlikely to carry an HIES genotype, whereas at the total score ≥15 points the subject is likely to carry an HIES genotype and ≥40 points are considered consistent with the diagnosis [14, 15].

### Genetic Investigations

Genomic DNA from the patient and the parents was prepared from peripheral blood mononuclear cells (PBMC). The coding region and splice sites of *STAT3* were bidirectionally sequenced. Putative mutations were confirmed in an independent sequencing reaction. Furthermore, genomic DNA was examined by array based comparative genomic hybridization using ExonArrayDx. The array contains DNA oligonucleotide probes in, or flanking, all exons and introns of the *DOCK8*. These analyses were performed by GeneDx, Inc., MD, USA.

The splicing mechanism at the site of the heterozygous *STAT3* mutation was assessed by RT-PCR using PBMC-derived RNA. The following *STAT3*-specific primers were used: 5′- CAGGTTGCTGGTCAAATTCC-3′ (forward primer) and 5′-CCGTTGTTGGATTCTTCCAT-3′ (reverse primer). Amplified products were gel-extracted after electrophoresis on agarose gel and cloned (TOPO-TA cloning kit; Invitrogen, CA, USA). Plasmid DNA was isolated and sequenced to detect aberrant splice products. The effect of the splice site heterozygous mutation was also assessed *in silico* using the programs NNSPLICE 0.9 version and Human Splicing Finder (HSF) version 2.4.1.

### Functional Testing of the STAT3 Signaling Pathway

The STAT3 signaling pathway was assessed by IL-10 inhibition of tumor necrosis factor (TNF) α release from lipopolysaccharide simulated monocyte-derived macrophages, as previously described by Woellner et al. [16]. Sampling occurred when the patient was in a stable clinical condition and healthy volunteer blood donors were used as control subjects.

### Flow Cytometry for Lymphocyte Phenotyping, Lymphocyte Cytotoxicity and Granulocyte Function

Lymphocyte subpopulations were quantified in fresh EDTA whole blood obtained from the patient and stained with the following mouse anti-human monoclonal antibodies: CD45-FITC, CD3-PC5, CD4-RD1, CD8-ECD, CD56-RD1, CD19-ECD (Cytostat 4-color mixes; Beckman Coulter, France), CD16-PE, HLA-DR-PE, CD38-FITC, CD21-PE (BD Biosciences, CA, USA), CD45RA-ECD (Beckman Coulter) CD27-FITC, CD45RO-RD1 (Dako, Denmark) and rabbit anti-human monoclonal antibodies IgD-PE (Southern Biotech, AL, USA) and IgM-APC (Biolegend, CA, USA). After incubation, the erythrocytes were lysed (IO Test Lysing Solution; Beckman Coulter) and cells were analyzed by flow cytometry (Navios; Beckman Coulter). Th17 lymphocytes were quantified according to the definition by Acosta-Rodriguez et al. [17], with staining using the following mouse anti-human monoclonal antibodies: CD45-PE, CXCR3-PC5, CCR4-PC7 (BD Biosciences), CCR6-FITC (Biolegend), CD8-Alexa F700, CD4-Krome Orange, CD3-Pacific blue (Beckman Coulter) and gating on $CD4^+CCR4^+CXCR3^-CCR6^+$ T lymphocytes. Incubation, lysing of erythrocytes and analysis were performed as described above. Th17 lymphocytes were reported as percentage of total CD4+ lymphocytes.

For assessment of natural cytotoxic (NK) cell-mediated cytotoxicity a standard 4-h $^{51}Cr$ assay was used [18]. A value <10 lytic units was considered pathological [19]. Cytotoxic lymphocyte exocytosis was assessed by flow cytometric quantification of CD107a surface expression on gated $CD3^-CD56^+$ NK cells and $CD3^+CD8^+CD57^+$ effector T-lymphocytes upon incubation of PBMC with different target cells, as previously described [20]. Samples were analyzed by flow cytometry (LSR Fortessa, BD Biosciences).

Analysis of granulocyte function was performed by flow cytometric analysis of expression of CD11b after stimulation with N-formylmethionyl-leucyl-phenylalanine (fMLP), staining with mouse anti human monoclonal antibodies CD45-PC5 and CD11b-PC7 (Beckman Coulter). Furthermore, oxidative burst activity of granulocytes and monocytes was measured using the Phagoburst test (Orpegon Pharma, Germany). In short, the test measures the oxidation of the added fluorogenic substrate di-hydrorhodamine 123 to rhodamine123 per cell, after stimulation of heparinized whole blood with *E. coli*, fMLP and phorbol-12-myristate 13-acetate (PMA) measured in the FITC channel.

### FASCIA

The flow cytometric assay of specific cell-mediated immune responses in activated whole blood (FASCIA) was originally developed for vaccine studies at the Swedish Institute for Communicable Disease Control [21, 22]. In-house evaluation have proven FASCIA to be a robust assay comparable with standard mitogen proliferation assays and additionally it provides more information of the detected responses (Thunberg et al., manuscript in preparation). Briefly, heparinzed whole blood is diluted 1:9 in medium (RPMI supplemented with penicillin and streptomycin; Life Technologies, UK) and incubated with mitogens or antigens for 6–7 days. The following mitogens or antigens were used: *C. albicans* (20 μg/mL; Greer, NC, USA), concanavalin A (ConA, 10 μg/mL; Sigma-Aldrich, Sweden), influenza virus vaccine (influenza, 1:100; Fluarix from GlaxoSmithKline), pokeweed mitogen (PWM, 5 μg/mL; Sigma-Aldrich), staphylococcal enterotoxin A and B (SEA/B, 100 ng/mL of each; Sigma-Aldrich), tetanus toxoid (4 IU/mL; Statens Serum Institut, Denmark) and varicella zoster virus vaccine (VZV, 1:100; GlaxoSmithKline, Sweden). The incubated samples were thereafter stained with the following monoclonal antibodies: CD3-FITC/CD4-PE simultest mix (BD Biosciences) and CD19-PC7 (Beckman Coulter). Before analysis remaining erythrocytes were lysed (IO Test Lysing Solution; Beckman Coulter). Finally, samples were analyzed by flow cytometry (Navios; Beckman Coulter). Lymphoblasts, i.e. proliferating cells, were gated by scatter profile and counts during 80 s in the regions for $CD4^+$, $CD8^+$ ($CD3^+CD4^-$) and $CD19^+$ cells were recorded and adjusted to counts/μL blood.

### Measurement of Soluble Mediators

Soluble mediators in serum were measured with a 26-plex Milliplex human cytokine/chemokine kit. Sampling occurred when the patient was in a stable clinical condition several months after diagnosis. The multiplex procedure was performed according to the standardized protocol provided by the manufacturer (Millipore, MO, USA). Sample analysis was performed on a Luminex 200 (Luminex, TX, USA) using Milliplex Analyst Software (Millipore).

### Measurement of Pro-LL-37 (hCAP-18)

Analysis of plasma pro-LL-37 was performed as previously described [23]. In short, plasma was mixed with NuPAGE LDS Sample buffer under reducing conditions. Proteins were separated using 4–12 % NuPAGE Bis-Tris Gels in NuPAGE

MES SDS running buffer and blotted onto polyvinylidene difluoride filters (all products from Invitrogen). The pro-LL-37 was detected with specific polyclonal rabbit antibodies generated as previously described [24], followed by horseradish peroxidase (HRP)-conjugated antibobies (BioRad Laboratories, Munich, Germany). Bound antibody was detected using SuperSignal West Dura (Pierce Biotechnology) and evaluated by densitometry using Intelligent DarkBoxII (FujiFilm, Japan). Data analysis was performed using the NIH Image program ImageJ (http://rsb.info.nih.gov/nih-image).

## Results

### Case Report

An 8-month old boy was referred from the pediatric dermatology unit to the pediatric immunology clinic due to eczema, skin abscesses and elevated serum IgE. At 3–4 weeks of age, the boy presented with persistent eczema with growth of *S. aureus* in skin swabs. Within 4 weeks, several sparsely inflamed abscesses were formed (mainly on the patient's dorsal side) that did not respond to treatment with systemic antibiotics. Some improvement was seen after initiation of topical corticosteroid and antibiotic ointments. The parents recalled that the boy had manifested foot- and fingernail candidiasis as well as thrush. Moreover, when eating crackers the boy occasionally bleed from the gum. No severe infections requiring systemic antibiotic therapy were reported. The child welfare center record displayed no remarks, i.e. a normally developing child with good growth. There was no family history of eczema or infectious disease. At referral, the physical examination revealed 6 pale abscesses in the skin of the neck, back and extremities with a minor eczema in the forehead. The full blood count and chemistry revealed a microcytic anemia with low ferritin and a slight eosinophilia. Due to suspicion of HIES, an immunologic work-up was initiated (Table I). Serum IgE was markedly elevated. Immunologic investigations displayed aberrations consistent with HIES, prompting anti-fungal and -bacterial prophylactic treatment with fluconazole and cotrimoxazole. The AD-HIES diagnosis was later genetically confirmed, see below.

During the first year since diagnosis, the patient's psychomotor development and growth have been normal. At follow-up 6 months after diagnosis, a nasopharyngeal swab revealed *A. fumigatus* colonization and the patient thus received itraconazole for 4 weeks. Nasopharyngeal swabs have thereafter been negative. Chest X-rays performed twice were normal. The patient has now, at an age of 20 months, developed a more apparent coarse face with a prominent forehead. This was not attributed to any form of craniosynostosis. No signs of hyperextensibility have been recorded.

### HIES Score

The patient's total Grimbacher score [14], 32 points, was well above the cut-off predictive of the HIES genotype (≥15 points) but not reaching the cut-off considered consistent with

**Table I** Clinical findings and basic laboratory work-up in a boy with AD-HIES due to c.1110-3C>A mutation of the *STAT3* gene

| | Diagnosis | 1 year follow-up | Reference interval | Unit |
|---|---|---|---|---|
| Clinical findings | | | | |
| Skin abscesses | 6 | 0 | n/a | number |
| Eczema | + | −/+ | n/a | n/a |
| Characteristic face | −/+ | + | n/a | n/a |
| Immunology | | | | |
| B-eosinophil granulocytes | 1.3 ↑ | 0.6 | 0.0–0.6 | $\times 10^9$/L |
| B-neutrophil granulocytes | 3.5 | 4.1 | 1.2–7.2 | $\times 10^9$/L |
| S-IgA | 1.6 | 0.24 | 0.07–0.55 | g/L |
| S-IgE | 980 ↑ | 890 ↑ | <22.3 | kU/L |
| S-IgG | 6.4 | 9.4 | 3.5–10.5 | g/L |
| S-IgM | 1.1 | 0.67 | 0.27–1.20 | g/L |
| Granulocyte function* | normal | n/t | normal | n/a |
| Microbiology | | | | |
| NPH-bacteria | neg | neg | neg | n/a |
| NPH-fungi | neg | neg | neg | n/a |
| S-anti *P. aeruginosa* exotoxin ab | 30 (neg) | 30 (neg) | <110 (neg) | U/mL |
| S-anti *S. aureus* alpha toxin ab | 240 (neg) | 90 (neg) | <700 (neg) | U/mL |
| S-anti *S. aureus* teichoic acid ab | 300 (neg) | 200 (neg) | <1,000 (neg) | U/mL |
| S-*C. albicans* IgE | 29 | 5.4 | <0.35 | kU/L |

*Abbreviations*: *B* blood; *S* serum; *Ig* immunoglobulin; * surface antigen expression and leukocyte oxidative burst; *NPH* nasopharyngeal swab; *ab* antibodies; ↑ elevated compared to control subjects; *neg* negative; *n/t* not tested; *n/a* not applicable

the diagnosis (≥40 points). According to the scoring system, the patient qualified for the following points: highest serum IgE 501–1,000 kU/L (4 points), >4 skin abscesses (8 points), highest absolute eosinophil granulocyte count >$0.8 \times 10^9$/L (6 points), severe worst stage eczema (4 points), fingernail candidiasis (2 points), 3 upper respiratory tract infections (1 point) and young-age correction ≤1 year (7 points). At diagnosis, patient's facies was not yet characteristic of HIES, thus no points (2 or 4) were given at the time. However, at follow-up the patient would have received 4 additional points.

## Novel *STAT3* Mutation Identified

Sequencing of *STAT3* revealed a heterozygous c.1110-3C>A mutation in intron 11 of the gene, located in proximity to the acceptor splice site of exon 12. Both parents were negative for the identified *STAT3* mutation. *STAT3* mutations affecting this splice site have previously been reported, i.e. c.1110-1G>T, c.1110-2A>G and c.1110-2A>C, and result in a p.D371_G380del in the DNA binding domain of STAT3 [16]. Both NNSPLICE and HSF confirmed a possible effect of the heterozygous c.1110-3C>A mutation on the acceptor splice site of exon 12, albeit to a lower extent than the previously described mutations affecting the same splice site. Nonetheless, RT-PCR revealed that the de novo mutation gave rise to an additional *STAT3* transcript in the patient relative to a healthy control (Fig. 1a). The cloning of RT-PCR products from the patient confirmed that the additional product lacked exon 12, confirming aberrant splicing of *STAT3* in the patient (Fig. 1b). The aberrant splicing product resulted in the loss of 10 amino acids compared to a normal STAT3 protein (p.D371_G380del). Functional assessment of the IL-10-dependent STAT3-mediated inhibition of LPS induced TNF release by macrophages revealed that patient cells displayed 77 % of maximum TNF release, relative to 7–17 % in healthy controls (Fig. 1c). These data are consistent with a disruption of STAT3 signaling in patient cells.

## Immunologic Findings

Phenotyping of lymphocytes at diagnosis and follow-up yielded normal numbers of T-, T-helper-, cytotoxic T- (CTL) and B-lymphocytes as well as NK-cells. The distribution of naïve, intermediate, memory and activated helper T-lymphocytes and CTLs was also stable over time and within normal range (Table II). In the B-lymphocyte subsets, deviations (compared to healthy older children/adults) were stable or more accentuated over time: low levels of marginal zone B-lymphocytes and switched memory B-lymphocytes, and relatively high levels of transitional B-lymphocytes. Remaining B-lymphocytes subsets were found at normal numbers. The patient had lower Th17-lymphocytes cells compared to healthy adult subjects (Table II).

Normal NK cell-mediated cytotoxicity (33 lytic units) and normal $CD3^-CD56^+$ NK cell degranulation in response to K562 cells (20.2 %), as well as normal $CD3^-CD56^+$ NK cell degranulation or $CD3^+CD56^-CD57^+$ cytotoxic T cell degranulation in response to P815 cells supplemented with anti-

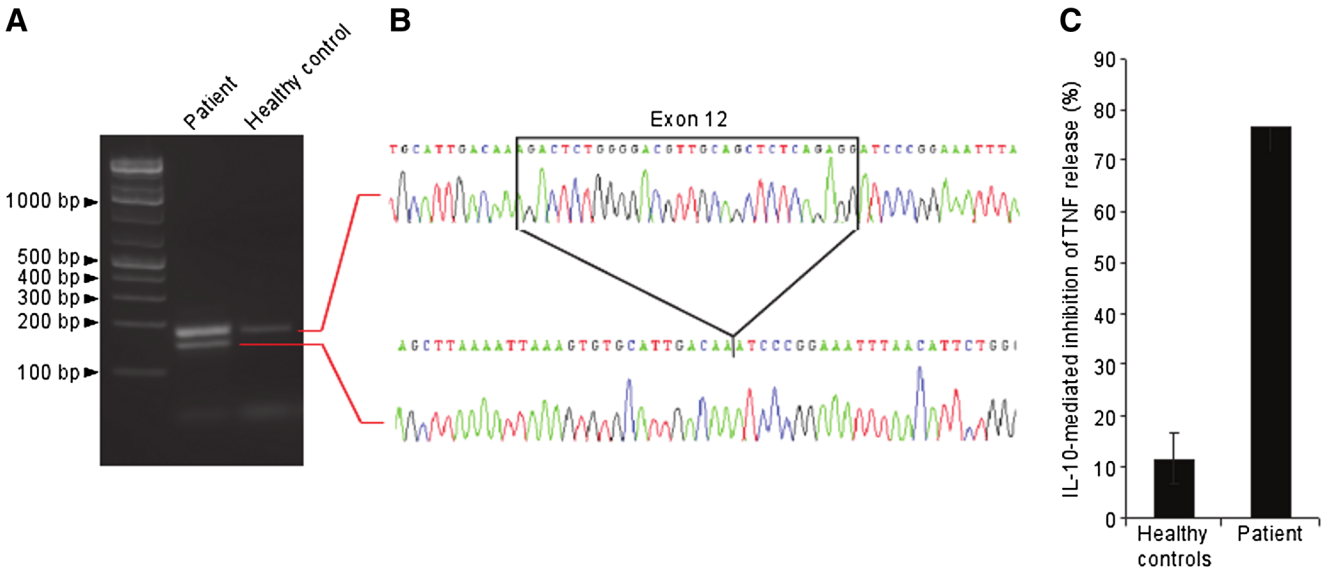


**Fig. 1** Transcriptional and functional analyses of the heterozygous *STAT3* c.1110-3C>A mutation. **a** A RT-PCR reaction produced a predicted 159 bp *STAT3* amplicon in a healthy control, whereas the patient additionally displayed an amplicon of 129 bp due to defective splicing of exon 12. **b** Sequencing of RT-PCR products from the patient revealed a transcript that lacked exon 12 of *STAT3*. **c** IL-10 mediated inhibition of TNF release in LPS-activated macrophages. Macrophages of five healthy controls as well as the the patient carrying the heterozygous *STAT3* mutation were pretreated with IL-10 and then stimulated with LPS. Supernatants were examined for the presence of TNF. The impact of IL-10 on LPS-induced TNF release is shown as percentage of maximum TNF release upon LPS stimulation. *Bars* indicate SD

**Table II** Lymphocyte phenotyping and function in a boy with AD-HIES due to c.1110-3C>A mutation of the *STAT3* gene

| | Diagnosis | 1 year follow-up | Reference interval | Unit |
|---|---|---|---|---|
| Phenotyping | | | | |
| CD3+ (T) | 67 | 67 | 50–70 | % |
| CD3+CD4+ (Th) | 47 | 45 | 33–58 | % |
| CD3+CD8+ (Tc) | 19 | 20 | 13–26 | % |
| CD3+CD16/+CD56+ (NK-cells) | 3 | 3 | 2-13 | % |
| CD4+HLA-DR+ (activated Th) | 18 | n/t | 2–9 | % |
| CD8+HLA-DR+ (activated Tc) | 3 | n/t | 2–15 | % |
| CD4+CD45RA+RO- (naïve Th) | 53 | 60 | 57–100 | % |
| CD4+CD45RA+RO+ (intermediate Th) | 21 | 20 | – | % |
| CD4+CD45RA-RO+ (memory Th) | 27 | 19 | 0–36 | % |
| CD8+CD45RA+RO- (naïve Tc) | 49 | 69 | – | % |
| CD8+CD45RA+RO+ (intermediate Tc) | 22 | 21 | – | % |
| CD8+CD45RA-RO+ (memory Tc) | 30 ↑ | 10 | 1–17 | % |
| CXCR-CCR4+CCR6+ (Th17) | 1 ↓ | 0.5 ↓ | 3.8–5.6* | % |
| CD19+ (B) | 30 | 30 | 13–35 | % |
| CD19+IgD+CD27- (naïve B) | 88 | 93 | 90–95/84–93[#] | % |
| CD19+IgD+CD27+ (marginal zone B) | 2 ↓ | 2 ↓ | 3–8/3–10[#] | % |
| CD19+IgD-CD27+ (switched memory B) | 1 | 0.5 ↓ | 0,6–2,0[#] | % |
| CD19+IgM+CD38+ (transitional B) | 4 ↑ | 4 ↑ | <1[#] | % |
| CD19+IgM+CD21+ (relative mature B) | 74 | 96 | – | % |
| CD19+IgM+CD21- (activated/immature B) | 18 | 4 | – | % |
| CD19+IgM-CD38+ (plasmablast) | 1 | 4 | 0–3,2[#] | % |
| FASCIA | | | | |
| CD4+ candida | 3,010 ↑ | 51 | 51–1,014[#] | counts/μL |
| CD8+ candida | 160 ↑ | 0 | 0–49[#] | counts/μL |
| CD4+ conA | 848 | 3,497 | 620–3,800[#] | counts/μL |
| CD8+ conA | 379 | 1,479 | 180–1,757[#] | counts/μL |
| CD4+ influenza | 142 | 89 | 19–1,050[#] | counts/μL |
| CD8+ influenza | 21 | 31 | 0–202[#] | counts/μL |
| CD4+ pneumococci | 22 | 6 | 0–269[#] | counts/μL |
| CD8+ pneumococci | 3 | 0 | 0–13[#] | counts/μL |
| CD4+ PHA | 228 | 189 | 170–3,499[#] | counts/μL |
| CD8+ PHA | 29 ↓ | 27 ↓ | 76–3,640[#] | counts/μL |
| CD4+ PWM | 4,742 ↑ | 2,850 ↑ | 233–2,189[#] | counts/μL |
| CD8+ PWM | 822↑ | 909 ↑ | 50–549[#] | counts/μL |
| CD19+ PWM | 2,361↑ | 1,509 ↑ | 42–741[#] | counts/μL |
| CD4+ SEA/B | 10,252↑ | 6,781 | 553–7,743[#] | counts/μL |
| CD8+ SEA/B | 2,634↑ | 1,366 | 123–2,365[#] | counts/μL |
| CD4+ tetanus toxoid | 3 | 5 | 0–306[#] | counts/μL |
| CD8+ tetanus toxoid | 0 | 0 | 0–14[#] | counts/μL |
| CD4+ VZV | 0 | 6 | 0–154[#] | counts/μL |
| CD8+ VZV | 0 | 0 | 0–23[#] | counts/μL |

*Abbreviations*: [#] based on older children and/or healthy adult controls; *CD* cluster of differentiation; *T* T-lymphocytes; *Th* T-helper lymphocytes; *Tc* cytotoxic T-lymphocytes; *NK* natural cytotoxic; *Th17* interleukin 17 producing helper T-lymphocytes; *B* B-lymphoctes; *FASCIA* flow cytometric assay of specific cell-mediated immune responses in activated whole blood; *ConA* concanavalin A; *PHA* phytohemagglutinin; *PWM* pokeweed mitogen; *SEA/B*, staphylococcal enterotoxoin A and B, *VZV* varicella zoster virus; ↑ elevated compared to control subjects; ↓ lower compared to control subjects; *n/t* not tested; * compared to 3 healthy adults

CD16 or anti-CD3 monoclonal antibodies (42.4 % or 53.7 %, respectively) were observed. Granulocyte function was found normal by means of CD11b expression as well as metabolic activation (leukocyte oxidative burst).

The FASCIAs (using healthy older children and/or adults as controls) performed at diagnosis indicated normal or near normal CD4+ (T-helper lymphocyte) and CD8+ (CTL) responses to the mitogens PHA and ConA as well as to the antigens influenza, tetanus toxoid, pneumococci and VZV. Conversely, increased responses were seen after stimulation with *C. albicans*, PWM and SEA/B both in the CD4+ and CD8+ fractions, as well as in the CD19+ fraction in the case of PWM. At follow-up, after initiation of prophylactic measures, the responses against *C. albicans* and SEA/B were found normalized, while supernormal responses were seen against PWM. Detailed results are shown in Table II.

Analysis of cytokines and chemokines in the patient's serum displayed some deviations relative to healthy older children/adult subjects. Decreased to normal levels were seen for GM-CSF, IFN-γ, IL-1α, IL-17 and macrophage inflammatory protein-1β. Increased levels were found for eotaxin, G-CSF, IL-5, IL-8, IL-10, IL-12(p70), IL-13, IL-15 and TNF-α. Normal levels were observed for IFN-α, IL-2, IL-3, IL-4, IL-6, IL-7, IL-12(p40), IL-16, IFN-γ inducible protein 10, monocyte chemotactic protein-1, macrophage inflammatory protein-1α and TNF-β (Table III).

The plasma concentration of pro-LL-37 was 86 % of reference plasma, which is within the range of normal variation among healthy pediatric subjects (40–120 %) [23], indicating that this pro-peptide is transcribed and translated into protein in the neutrophil bone-marrow precursors.

## Discussion

Dominant-negative *STAT3* mutations are the genetic cause of AD-HIES. The immunopathology is partly understood, but remains the focus of extensive research [7, 1, 2]. Here we describe a patient with a novel heterozygous *STAT3* mutation (c.1110-3C>A) resulting in defective splicing with subsequent interference of the STAT3 signaling pathway. Other mutations

**Table III** Cytokine/chemokine analysis in a boy with AD-HIES due to c.1110-3C>A mutation of the *STAT3* gene

| Cytokine/chemokine | Patient level | Mean, 5th–95th percentile of controls[#] | Unit |
|---|---|---|---|
| Eotaxin | 173 ↑ | 23, 0–97 | pg/mL |
| Granulocyte colony stimulatory factor | 46.9 ↑ | 9, 12–27 | pg/mL |
| Granulocyte/macrophage colony stimulatory factor | 7.1 ↓ | 23, 0–97 | pg/mL |
| Interferon-α | 14.4 | 15, 0–54 | pg/mL |
| Interferon-γ | 6.7 ↓ | 33, 0–100 | pg/mL |
| Interferon-γ inducible protein 10 | 1,047 | 359, 184–1,244 | pg/mL |
| Interleukin-1α | 7.2 ↓ | 43, 0–213 | pg/mL |
| Interleukin-1β | 3.85 | 1, 0–11 | |
| Interleukin-2 | 3.2 | 2, 0–7 | pg/mL |
| Interleukin-3 | <8.5 | 1, 0–3 | pg/mL |
| Interleukin-4 | <8 | 2, 0–7 | pg/mL |
| Interleukin-5 | 5.5 ↑ | 0, 0–0 | pg/mL |
| Interleukin-6 | 10.5 | 9, 0–36 | pg/mL |
| Interleukin-7 | 8.1 | 3, 0–19 | pg/mL |
| Interleukin-8 | 55.6 ↑ | 9, 0–22 | pg/mL |
| Interleukin-10 | 70.4 ↑ | 9, 0–31 | pg/mL |
| Interleukin-12 (p40) | 109 | 27, 0–126 | pg/mL |
| Interleukin-12 (p70) | 16.7 ↑ | 3, 0–7 | pg/mL |
| Interleukin-13 | 7.8 ↑ | 1, 0–4 | pg/mL |
| Interleukin-15 | 7.5 ↑ | 1, 0–6 | pg/mL |
| Interleukin-17 | <2.8 ↓ | 8, 0–23 | pg/mL |
| Macrophage inflammatory protein-1α | <9 ↓ | 36, 0–79 | pg/mL |
| Macrophage inflammatory protein-1β | <9 ↓ | 31, 12–51 | pg/mL |
| Monocyte chemotactic protein-1 | 454 | 259, 101–642 | pg/mL |
| Tumor necrosis factor-α | 30.4 ↑ | 4, 0–14 | pg/mL |
| Tumor necrosis factor-β | 11.1 | 3, 0–18 | pg/mL |

*Abbreviations*: [#] based on older children and/or healthy adult controls;↑ and ↓,elevated and lower compared to control subjects, respectively

of the same splice-site have been previously described [16]. All heterozygous *STAT3* mutations so far associated with AD-HIES are single nucleotide changes or short in-frame deletions or insertions. The clinical phenotype of our patient, classically presenting with both mucocutaneous candidiasis and staphylococcal skin infections, obtained a high score (despite the young age) according to classification scheme by Grimbacher and colleagues [14, 15], and is therefore consistent with genetic/functional findings and the diagnosis of AD-HIES. Scoring suspected cases of HIES is helpful as it can guide the clinician regarding which patients that should undergo genetic testing.

The immunopathology underlying AD-HIES is complex, as STAT3 is involved in numerous immunologic processes [7, 4]. Lymphocyte phenotyping displayed a possible excess of transitional B-lymphocytes and a diminished percentage of marginal zone B-lymphocytes at both first visit and follow-up as well as diminished percentages of switched memory B-lymphocytes on follow-up. This pattern is suggested to be characteristic for AD-HIES patients and has also been proposed as a routine analysis in the diagnostic work-up prior to genetic investigations [8]. However, the B-lymphocyte phenotyping should be interpreted with caution, especially in small children, due to scarce and varying reference values between laboratories. Generally, small children are expected to have higher numbers of immature transitional B-lymphocytes than adults [25]. As expected, the Th17 lymphocyte population was reduced, but no other deviations in T-lymphocyte subpopulations were observed. This contrasts data from Siegel et al., who elegantly showed that AD-HIES T-lymphocytes have an intrinsic defect in memory differentiation resulting in a diminished central memory T-lymphocyte population [26]. However, our patient is still young and it is possible that this aberration will become more pronounced with time. In contrast to a report by Buckley [7], our patient was able to mount cellular responses to *C. albicans*. Before initiation of antimicrobial prophylaxis the patient cells displayed high responses to *C. albicans*, SEA/B and PWM. Only the response to PWM has persisted. Hence, the AD-HIES cells show no defect in response to mitogens and infectious antigens as assessed by modern methodology (FASCIA). These results are in line with the early studies [7]. The patient also displayed normal cytotoxic lymphocyte function. Contrary to what has been reported for HIES-patients with other types of heterozygous *STAT3* mutations, we could not find any deficiency in the neutrophil production of the antimicrobial peptide pro-protein of LL-37 (hCAP18). This is in line with the findings that the ability of HIES-patients to respond to different microbial challenges may vary and expression might be more appropriately assessed locally at mucous membranes [2]. Additionally, it should also be noted that a partial deficiency in splicing, possibly differentially affecting distinct cell types, might explain the lack of expected immune cell abnormalities in our patient.

We also performed cytokine/chemokine profiling. Interestingly, the cytokines G-CSF, IL-10, IL-12, IL-15 and IL-5, that all have signaling pathways involving STAT3 [4], were elevated. Moreover, IL-13 was found at high levels and IL-17 at low levels. These findings seem to be explained by compensatory mechanisms caused by defective STAT3 signaling and inadequate responses to the cytokines, giving rise to reduced negative feedback. The cytokine profile may partly explain the eosinophilia and elevated IgE in AD-HIES, as IL-13 and -5 are associated with IgE production and eosinophilia [27, 28]. This is supported by Garraud et al., who showed that blockage of IL-13 significantly reduced the IgE production in an experimental setting [29]. To our knowledge this kind of wide cytokine profiling has not been reported earlier, why it merits studies in a larger patient cohort.

To summarize, we report an AD-HIES patient with a novel heterozygous *STAT3* mutation. Our patient displays classical clinical features and deviations in lymphocyte subsets consistent with the diagnosis. Remarkably, to previous studies, the AD-HIES cells were highly responsive to *C. albicans* and the patient's cytokine profile displayed increased IL-5 and -13 levels concordant with the high IgE level and eosinophilia, which are characteristic in AD-HIES.

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