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Defects Along the Th17 Differentiation Pathway Underlie Genetically Distinct Forms of the Hyper IgE Syndrome

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Abstract

Background—The hyper IgE syndrome (HIES) is characterized by abscesses, eczema, recurrent infections, skeletal and connective tissue abnormalities, elevated serum IgE and diminished inflammatory responses. It exists as autosomal dominant (AD) and recessive (AR) forms that manifest common and distinguishing clinical features. A majority of those with AD-HIES suffers from heterozygous mutations in Signal Transducer and Activator of Transcription 3 (STAT3) and impaired Th17 differentiation.

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Objective—To elucidate mechanisms underlying different forms of HIES.

Methods—A cohort of 25 Turkish children diagnosed with HIES were examined for *STAT3* mutations by DNA sequencing. Activation of STAT3 by IL-6 and IL-21 and STAT1 by interferon alpha (IFNα) was assessed by intracellular staining with anti-phospho (p)STAT3 and pSTAT1 antibodies. Th17 and Th1 cell differentiation was assessed by measuring the production of IL-17 and IFNγ, respectively.

Results—Six subjects had *STAT3* mutations affecting the DNA binding, SH2 and transactivation domains, including 3 novel ones. Mutation-positive but not mutation-negative HIES subjects exhibited reduced phosphorylation of STAT3 in response to cytokine stimulation, while pSTAT1 activation was unaffected. Both patient groups exhibited impaired Th17 responses, but whereas STAT3 mutations abrogated early steps in Th17 differentiation, the defect(s) in HIES patients with normal STAT3 affected more distal steps.

Conclusion—In this cohort of Turkish children with HIES, a majority had normal STAT3, implicating other targets in disease pathogenesis. Impaired Th17 responses were evident irrespective of the *STAT3* mutation status, indicating that different genetic forms of HIES share a common functional outcome.

Keywords

Hyper IgE syndrome; STAT3; Th17; IL-6; IL-21; RORγτ

Key Messages

- The hyper IgE syndrome (HIES) is caused by a heterogeneous set of genetic defects. *STAT3* mutations are associated with the autosomal dominant form of HIES, while other yet undefined genetic defect(s) underlie the autosomal recessive form(s).
- Different genetic forms of HIES act by distinct mechanisms to impair Th17 helper T cell differentiation.

Introduction

Hyper IgE syndrome (HIES) is an uncommon primary immune deficiency characterized clinically by recurrent infections, especially with *Staphylococcus aureus* and *Candida albicans*, leading to frequent skin and lung abscesses and pneumatocele formation. HIES is also characterized by dermatitis, eosinophilia and high serum levels of IgE. Inflammatory responses are characteristically aberrant in that infections cause tissue destruction but do not generate the expected warmth, redness and fever [1-3].

Insight into the molecular basis of HIES came with the discovery of a homozygous Tyrosine kinase 2 gene (*TYK2)* mutation in a patient with an HIES-like syndrome, with elevated serum IgE, T cell deficiency, and susceptibility to mycobacterial infections [4]. The subsequent investigation of Janus kinase (JAK)- Signal Transducer and Activator of Transcription (STAT) signaling pathways led to the identification of heterozygous mutations in STAT3 as the genetic cause of sporadic, autosomal dominant (AD) form of HIES [5, 6]. The mutations, which cluster in the DNA and SH2 binding domains, are either missense or inframe deletions that leave an expressed protein in place. They result in defective functional responses, including upregulation of immunoglobulin production in B cells and chemokine induction in mononuclear cells by IL-6, and impaired suppression of inflammatory cytokine production by IL-10 [5, 6].

STAT3 regulates multiple cytokine signaling pathways involved in the innate and adaptive immune responses, including the IL-6, IL-21, 23, 27 and IL-10 families, as well as granulocyte-colony stimulating factor and leptin [7, 8]. Many of these cytokines are critical to the differentiation of Th17 $CD4^+$ T cells, which consequently is impaired in AD-HIES [9-12]. Th17 cells are important for mounting inflammatory responses to bacterial and fungal pathogens, a function that is reflected in the spectrum of infections associated with HIES [13-17].

While AD-HIES associated with STAT3 mutations accounts for a substantial subset of HIES, at least two other subsets can be differentiated based on molecular and/or clinical basis. First, there are those sporadic patients with a clinical presentation similar to those of AD-HIES who are nevertheless negative for STAT3 mutations. Yet another subset of HIES has been described as AR-HIES with a clinically overlapping but distinct phenotype [18]. The patients suffer from recurrent infections with pathogens associated with AD-HIES, including *Staphylococcus aureus* and *Candida albicans*, but also from viral infections atypical for AD-HIES such as herpes simplex, herpes zoster and molluscum contagiosum. They do not manifest the skeletal or dental abnormalities of AD-HIES [18]. They have high serum IgE levels similar to those with AD-HIES, but their eosinophil count is typically higher. Many subjects suffer from central nervous system abnormalities, including cerebral aneurysms, strokes and infections, leading to fatal outcome. Autoimmune phenomena may also occur, including hemolytic anemia, thrombocytopenia and vasculitis. The molecular basis of AR-HIES and the role of STAT3 mutations in this HIES subset remain unknown.

In this study, we aimed to elucidate mechanisms underlying different forms of HIES and their functional outcome by investigating the incidence of STAT3 mutations and the competency of Th17 cell differentiation in a cohort of affected Turkish children.

Methods

Subjects

A total of 25 unrelated Turkish children with a diagnosis of HIES were enrolled. Diagnosis of HIES was given on the basis of elevated serum IgE levels, eczematoid rashes and unusual, severe, recurrent infections including recurrent pneumonias, skin and deep seated Staphylococcal abscesses, candidiasis and other fungal infections. HIES scores were calculated as described by Grimbacher et al [3]. The study was approved by the local Institutional Review Boards, and written informed consent was obtained from participating families. Control subjects included healthy parents of HIES subjects with no detectable STAT3 mutations (parent controls) and unrelated healthy subjects (unrelated controls).

STAT3 sequencing

Genomic DNA was prepared from peripheral blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA). Individual STAT3 exons and bordering intronic sequences were amplified using specific oligonucleotide primers and PCR, and the amplified fragments were sequenced using the ABI Big Dye Terminator mix (Applied Biosystems, Foster City, CA), and analyzed with a 3730xl DNA Analyzer (Applied Biosystems). Parents of subjects with identified *STAT3* mutations were screened for presence of those mutations. STAT3 cDNA of 12 of the 19 HIES subjects found not to harbor STAT3 mutations by genomic analysis were sequenced in their entirety and confirmed to be free of mutations.

Intracellular staining

Intracellular staining of phospho Y701-STAT1 (pSTAT1) and phospho Y705 STAT3 (pSTAT3) was carried out as described [19]. PBMC were expanded for 4 days with PHA 10 μg/ml + IL-2 100 units/ml. 1×10^6 cells were stimulated for 15min with IFNα, IL-6 or IL-21

(Peprotech, Rocky Hill, NJ) at a final concentration of 100ng/ml. The cells were then fixed with 1.6% paraformaldehyde, and permeabilized for 10 minutes with methanol. After washing, the cells were then stained with conjugated pSTAT1 or pSTAT3 (Alexa fluor 647; BD Biosciences, San Jose, CA) and analyzed by flow cytometry.

Th17 and Th1 differentiation

Peripheral blood T cell blasts were expanded by stimulation of peripheral blood mononuclear cells (PBMC) for 3 days with phytohemagluttinin (PHA) and IL-2. They were then differentiated for 4 days by stimulation with plate-bound anti-CD3 and soluble CD28 monoclonal antibodies (mAb) in the presence of irradiated feeder PBMC and Th17 polarizing cytokines (IL-1beta, IL-6, IL-21 and IL-23), or the Th1 polarizing cytokine IL-12 (all cytokines at 20 ng/ml) [9-11, 15]. Naïve T cells were isolated by negative selection using magnetic bead sorting (Miltenyi). They were stimulated with using magnetic beads bound with anti-CD2, CD3 and CD28 mAb (Miltenyi) and IL-2 at 100 units/ml (Th0) or Th17 polarizing cytokines, as above. Polarized T cells were resuspended at 2×10^6 cell/ml and stimulated with 20 ng/mL phorbol 12-myristate 13-acetate and 1 μg/mL ionomycin (Sigma-Aldrich, St Louis, Mo) for 48 hours, and the culture supernatant were assayed for IL-17 and IFNγ By ELISA.

Real Time PCR analysis

RNA was isolated from peripheral T cell blasts derived by stimulation of PBMC for 3 days with PHA and IL-2 or from Th0 and Th17 cells polarized from naïve T cells using the RNeasy kit (Qiagen). Reverse transcription was performed using Superscript III and oligo dT (Invitrogen Carlsbad, CA). Retinoic orphan receptor gamma t (RORγt) Taqman assay mixes were used with Taqman Universal Fast Master Mix (Applied Biosystems) and then ran on a Step-One-Plus machine (Applied Biosystems). Cytokine mRNA expression levels were analyzed using GAPDH as an endogenous control (Applied Biosystems). The relative expression levels were calculated using the delta-delta CT analysis, and the results were normalized to the average levels of RORγt in HIES subjects with STAT3 mutations.

Statistical analysis

Significance was calculated using Student's two-tailed t test or the Fisher's exact test. Multiple comparisons were carried out using one-way ANOVA with Newman-Keuls post-test analysis.

Results

Patient characteristics

A total of 25 unrelated children with a diagnosis of HIES were enrolled (Figure 1; Table 1 in the Repository). The mean age was 9.1 years with a range of 1.4 to 18.8. The mean HIES score for the entire cohort was 50.56 + 2.56 and the mean IgE level was 11,987 + 2508 IU/ml. Fifteen subjects were the product of consanguineous marriages (60%). Three children came from families with deceased affected siblings.

Analysis of clinical attributes revealed that all subjects suffered from one or more infectious complications associated with HIES. Eighteen subjects had a history of *Staphylococcus aureus* skin and/or deep abscesses, 22 had history of pneumonia, and eight developed pneumatoceles. Eighteen subjects had history of candidiasis. Nineteen subjects exhibited characteristic, HIES-associated facial features, while skeletal and connective tissue features associated with AD-HIES with STAT3 mutations, such as retained primary teeth, hyperextensible joints, and pathologic fractures were less common (Table I). Eight subjects had features associated with AR-HIES, including recurrent viral infections with papilloma virus, herpes family viruses, and/or molluscum contagiosum, autoimmunity, exaggerated

eosinophilia and/or affected (deceased) siblings with HIES to otherwise asymptomatic parents. The eight AR-HIES-like subjects lacked features associated with AD-HIES with STAT3 mutations: skeletal and connective tissue abnormalities and pneumatoceles [18]. Four subjects died in the course of the study, one with brain abscess, one with enhancing brain lesions, one with encephalitis, and the fourth with complications of lung disease. Additionally, three siblings of three subjects enrolled in this study have previously died of with the presumptive diagnosis of HIES.

STAT3 mutations

Six of the 25 patients were found to harbor heterozygous mutations in *STAT3* (Figure 1 in the Repository). Consistent with previous reports, all but one of the mutations affected the DNA binding and SH2 domains of STAT3. Three mutations affected the DNA binding domain. Of those, two missense mutations involved R382, a frequent target of mutations in AD-HIES (R382W and R382Q). The third was a novel mutation involving a deletion of the canonical G residue at position +1 of the splice donor site of intron 14 (IVS14 +1delG). This mutation is predicted to result in skipping of exon 14 during heteronuclear RNA editing, resulting in an in-frame 16 amino acid deletion in the DNA binding domain. The mutation was also found in the mother, who had history of eczema and skin abscesses in early childhood and eczema as an adult, and provides the only case of a transmitted mutation in this cohort. Two other mutations affected the SH2 domain of STAT3, including the previously described missense mutation Y657C, and a novel mutation at S668F. A third novel mutation involved the regulatory tyrosine residue at position 705 in the transactivation domain (Y705C). The latter is of particular interest as failure to phosphorylate the Y705 residue by cytokine receptor-coupled JAK kinases would abrogate the activation of STAT3 [20].

Genotype/Phenotype relationships

Comparison of patient characteristics between those with or without *STAT3* mutations revealed no statistically significant difference in age, serum IgE levels, HIES scores, or frequency and severity of infections. Two of the six subjects with and thirteen of the nineteen without STAT3 mutations came from consanguineous families. None of the eight subjects with AR-HIES like phenotype suffered from STAT3 mutations. Patients with *STAT3* mutations were more likely to have pneumatocele formation (5/6 versus 3/19, p=0.005), whereas patients without *STAT3* mutations had a significantly higher eosinophil count compared to those with mutations (7583 + 2514 vs 1154 +337, p=0.02 unpaired t test with Welch's correction) (Figure 1). The increased eosinophilia in the HIES group with no STAT3 mutations was in large measure related to the contribution of patient with AR-HIES-like phenotype (11980 + 5018 vs. 1154 +337, p=0.07 unpaired t test with Welch's correction). AR-HIES-like patients also suffered disproportionately from chronic viral infections as compared to those with AD-HIES-like patients (p=0.04 Fisher's exact test). Comparison of other clinical phenotypes between the AR-HIES and AD-HIES-like subgroups did not yield significant results, in part due to small sample size.

Cytokine-induced STAT3 and STAT1 phosphorylation

The series of steps leading to Th17 differentiation involve the sequential action of STAT3 activating cytokines, including IL-6 and IL-21 [21-24]. To evaluate the functional consequences of mutation positive and negative HIES status on the activation of STAT3 in response to Th17 polarizing cytokines, we examined STAT3 phosphorylation at the regulatory Y705 residue (pSTAT3) in T cells following IL-6 and IL-21 treatment. STAT3 mutations were associated with decreased pSTAT3 formation in response to both cytokines (Figures 2 and 3, respectively). In contrast to an earlier report that found impaired pSTAT3 formation in patients with mutations in the SH2 domain but not DNA binding domain [12], both the DNA-binding

and SH2 domain mutations were associated with decreased pSTAT3 formation. Analysis of the pSTAT3 response of patients with DNA binding versus SH2 domain mutations failed to reveal a statistically significant difference between the two subgroups, although the analysis is limited by the small sample sizes. On average, pSTAT3 induction was significantly lower in mutation-positive as compared to mutation negative HIES, while the latter group was not significantly different from normal controls (Figure 2, 3). Further breakdown of the mutation-negative HIES group into AR-like and AD-like individuals did not reveal any significant difference in IL-21-induced pSTAT3 formation between those two subgroups (data not shown).

TYK2 deficiency was found to cause of an HIES-like disorder in one individual [4]. To rule out TYK2 deficiency as an underlying cause of STAT3 mutation-negative HIES, the competency of TYK2 signaling was assessed by the induction of STAT1 phosphorylation at the regulatory Y701 residue (pSTAT1) in response to IFNα. This effect proceeds in a TYK2 (and JAK2)-dependent manner and is abrogated upon TYK2 deficiency. Results revealed that both STAT3 mutation-positive and -negative HIES individuals exhibited a similar range of pSTAT1 formation that, on average, was comparable to the response of control subjects. No patient failed to activate pSTAT1 formation, thus ruling out global TYK2 deficiency as an underlying diagnosis (Figure 2 in the Repository). These results indicated that the molecular defect(s) in HIES with normal STAT3 did not impair STAT3 activation by distinct receptor pathways.

Th17 differentiation

To determine whether impaired Th17 differentiation is a common attribute of all HIES patients or only those with STAT3 mutations, we examined the expression in peripheral blood T cells of RORγτ, a STAT3-regulated transcription factor that is essential for induction of Th17 differentiation, as a measure of circulating Th17 cells [9, 25]. Results revealed that the levels of RORγτ mRNA were severely depressed in HIES subjects with or without STAT3 mutations as compared to parent control subjects (Figure 4A). Next, we examined the in vitro induction of Th17 differentiation in peripheral blood T cells of HIES subjects and their parent controls in response to mitogenic stimulation in the presence of polarizing Th17 cytokines (IL-1, IL-6 and IL-23). Results revealed that mutation positive and negative HIES subjects exhibited a profound deficit in Th17 differentiation with nearly absent IL-17 production as compared to controls (Figure 4B). In contrast, T cells of both groups exhibited comparable IFNγ production upon differentiation into Th1 cells in the presence of the Th1 polarizing cytokine IL-12 (Figure 4C). These results established that defective Th17 responses is a common attribute of HIES subjects both with and without STAT3 mutations.

The Th17 response in the peripheral blood, represented in above results, is dominated by pre-committed Th17 memory T cells that express high levels of RORγτ and secrete large amounts of IL-17 as compared to freshly differentiated Th17 cells [26]. To establish whether the defect in peripheral blood Th17 cells in STAT3 mutation-negative individuals results from the failed differentiation of naïve T cells into Th17 cells or from the failed maintenance of differentiated Th17 memory cells, we examined the induction of Th17 differentiation in naïve T cells of mutation positive and negative individuals and their parent controls. Results revealed that differentiating Th17 cells of mutation-negative individuals express RORγτ at levels equivalent to those of parent controls. In contrast, naïve T cells of STAT3 mutation-positive individuals failed to express RORγτ (Figure 4D). Differentiating Th17 cells of mutation-negative individuals expressed about half as much IL-17 as their parent control counterparts, whereas those of mutation positive individuals completely failed to secrete IL-17 (Figure 4E). These results suggest that unlike STAT3 mutations, the defect(s) in mutation negative HIES does not impair the initial steps in Th17 differentiation, but may impact later steps necessary for the terminal differentiation of Th17 cells and/or their long term persistence.

Discussion

Several findings were noted in this study of a Turkish pediatric cohort of HIES. First, the majority of subjects did not have detectable STAT3 mutations (19/25 or 76%). This is in contrast with other cohorts where the subjects are mostly adults with sporadic AD-HIES, in whom the rate of STAT3 mutations is high [5, 6, 12]. One difference is the large number of subjects that came from consanguineous families (15/25 or 60%), a reflection of the high consanguinity rate in the Turkish population [27]. This attribute allows for a higher representation of autosomal recessive forms of HIES in the patient population under study. However, a history of consanguinity was insufficient on its own to rule out sporadic heterozygous *STAT3* mutations, evidenced by the fact that 2 out of the six subjects with *STAT3* mutations came from consanguineous families. A second difference may be the markedly younger age group of the present cohort, allowing the catchment of more severe cases that may otherwise attrition with age (as evidenced by the high mortality in the patient group with normal STAT3).

The lack of detectable STAT3 mutations in a majority of HIES subjects of this cohort, confirmed by both genomic and cDNA sequencing, was corroborated by other studies. These included the failure to identify *STAT3* as a candidate locus by single nucleotide polymorphisms (SNP) array mapping (data not shown), and the normal IL-6 and IL-21-induced STAT3 phosphorylation at Y705 in mutation-negative as compared to mutation-positive group (Figures 2 and 3). Together these studies argue that STAT3 is not the direct genetic target in the STAT3 mutation-negative HIES group.

An important finding of this study is that the STAT3 mutation negative group shared with its mutation positive counterpart defective Th17 cell differentiation, consistent with shared pathogenic features underlying both disease types. Nevertheless, the underlying mechanisms for the impaired Th17 response appeared distinct. Whereas STAT3 deficiency abrogated the induction in naïve T cells of RORγτ expression, a requisite early step in their differentiation into Th17 cells, induction of RORγτ expression in naïve T cells of STAT3 mutation-negative HIES individuals progressed normally. Furthermore, whereas the production of IL-17 by freshly differentiated Th17 cells was virtually abolished, it was detectable in similarly treated T cells of mutation-negative HIES subjects, albeit at reduced levels compared to control cells. In contrast, the expression in unfractionated peripheral blood T cells of RORγτ and IL-17, a process dominated by memory Th17 T cells [26], was profoundly impaired in both patient populations. These results indicated that the genetic lesion(s) in mutation negative HIES acted at a locus along the Th17 differentiation pathway further downstream from that of STAT3, resulting in the impairment of distal steps in Th17 cell differentiation and long-term persistence.

The STAT3 mutation-negative subjects were similar to their mutation positive peers in many aspects, including age, IgE levels, and HIES scores, and susceptibility to recurrent infections with *Staphylococcus aureus* and *Candida Albicans*. However, subjects with STAT3 mutations were particularly susceptible to pneumatocele formation, a reflection of the critical role played by STAT3-regulated pathways of resident airway tissues in mitigating acute injury. This is supported by the findings that conditional deletion of Stat3 in type II respiratory epithelial cells of mice results in exaggerated hyperoxia-induced lung injury and epithelial cell damage [28], whereas expression in the airway epithelium of a constitutively active STAT3 mutant is protective [29]. In contrast, the mutation negative group had subjects with a phenotype resembling HIES with STAT3 mutations (pneumatoceles, presence of skeletal and connective tissue manifestation) and others with an AR-HIES-like phenotype (lack of skeletal and connective tissue manifestation and pneumatoceles) and the presence of recurrent viral infections, exaggerated eosinophilia and/or autoimmunity) [18]. The latter group contributed in large measure to the higher levels of eosinophilia seen in the HIES group with no STAT3 mutations. The mechanism for the heightened susceptibility to viral infections in AR-like HIES

is not clear, and it suggests that the failure of Th17 differentiation in this subgroup is only one component of a more complex immunological deficit. These findings revealed that the STAT3 mutation-negative group is comprised of children with a heterogeneous set of defects rather than suffering a single underlying causative agency.

Examination of identified *STAT3* mutations revealed 3 novel one, including one affecting the DNA binding domain, another the SH2 domain and a third targeting the invariant regulatory tyrosine residue at position 705, distal to the SH2 binding domain. The latter mutant can still dimerize with both WT and mutant STAT3 [20]. Nevertheless, previous studies have indicated that STAT dimers containing an invariant tyrosine mutant maybe able to effect transcriptional activation of some genes in collaboration with other transcriptional activators [30, 31]. It is thus possible that STAT3 mutations associated with HIES, while acting in a dominant negative fashion, may also be permissive to the activation of a subset of STAT3-responsive genes.

A unique aspect of this cohort is the young age of its subjects, 9 years on average. The relatively high average HIES score (around 50) points to the severity of HIES in this cohort. Given that many features of HIES are age dependent, it is likely that additional, milder cases are missed until disease complications emerge later in life. Particularly notable are the fatalities in this cohort, involving four subjects (and three deceased siblings), all in the group with normal STAT3, which serves to emphasize the potentially lethal outcome of HIES in this patient population. Two of the 4 deceased subjects had features of AR-HIES (Table 1 in the Repository), in agreement with the previous observation of Renner et al that AR-HIES is associated with high mortality [18]. Further studies are needed to elucidate determinants of disease severity and outcome and therapeutic interventions aimed at forestalling development of serious disease complications such as pneumatocele and central nervous system manifestations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Davis SD, Schaller J, Wedgwood RJ. Job's Syndrome. Recurrent, “cold”, staphylococcal abscesses. Lancet 1966;1:1013–5. [PubMed: 4161105]
2. Buckley RH, Wray BB, Belmaker EZ. Extreme hyperimmunoglobulinemia E and undue susceptibility to infection. Pediatrics 1972;49:59–70. [PubMed: 5059313]
3. Grimbacher B, Holland SM, Gallin JI, Greenberg F, Hill SC, Malech HL, et al. Hyper-IgE syndrome with recurrent infections--an autosomal dominant multisystem disorder. N Engl J Med 1999;340:692–702. [PubMed: 10053178]
4. Minegishi Y, Saito M, Morio T, Watanabe K, Agematsu K, Tsuchiya S, et al. Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. Immunity 2006;25:745–55. [PubMed: 17088085]
5. Minegishi Y, Saito M, Tsuchiya S, Tsuge I, Takada H, Hara T, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature 2007;448:1058–62. [PubMed: 17676033]
6. Holland SM, DeLeo FR, Elloumi HZ, Hsu AP, Uzel G, Brodsky N, et al. STAT3 mutations in the hyper-IgE syndrome. N Engl J Med 2007;357:1608–19. [PubMed: 17881745]
7. Levy DE, Lee CK. What does Stat3 do? J Clin Invest 2002;109:1143–8. [PubMed: 11994402]

8. Schindler C, Levy DE, Decker T. JAK-STAT signaling: from interferons to cytokines. J Biol Chem 2007;282:20059–63. [PubMed: 17502367]
9. Milner JD, Brenchley JM, Laurence A, Freeman AF, Hill BJ, Elias KM, et al. Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. Nature 2008;452:773–6. [PubMed: 18337720]
10. de Beaucoudrey L, Puel A, Filipe-Santos O, Cobat A, Ghandil P, Chrabieh M, et al. Mutations in STAT3 and IL12RB1 impair the development of human IL-17-producing T cells. J Exp Med 2008;205:1543–50. [PubMed: 18591412]
11. Ma CS, Chew GY, Simpson N, Priyadarshi A, Wong M, Grimbacher B, et al. Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3. J Exp Med 2008;205:1551–7. [PubMed: 18591410]
12. Renner ED, Rylaarsdam S, Anover-Sombke S, Rack AL, Reichenbach J, Carey JC, et al. Novel signal transducer and activator of transcription 3 (STAT3) mutations, reduced T(H)17 cell numbers, and variably defective STAT3 phosphorylation in hyper-IgE syndrome. J Allergy Clin Immunol 2008;122:181–7. [PubMed: 18602572]
13. Weaver CT, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu Rev Immunol 2007;25:821–52. [PubMed: 17201677]
14. Happel KI, Dubin PJ, Zheng M, Ghilardi N, Lockhart C, Quinton LJ, et al. Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae. J Exp Med 2005;202:761–9. [PubMed: 16157683]
15. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. Nat Immunol 2007;8:942–9. [PubMed: 17676045]
16. LeibundGut-Landmann S, Gross O, Robinson MJ, Osorio F, Slack EC, Tsoni SV, et al. Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. Nat Immunol 2007;8:630–8. [PubMed: 17450144]
17. Palm NW, Medzhitov R. Antifungal defense turns 17. Nat Immunol 2007;8:549–51. [PubMed: 17514204]
18. Renner ED, Puck JM, Holland SM, Schmitt M, Weiss M, Frosch M, et al. Autosomal recessive hyperimmunoglobulin E syndrome: a distinct disease entity. J Pediatr 2004;144:93–9. [PubMed: 14722525]
19. Krutzik PO, Irish JM, Nolan GP, Perez OD. Analysis of protein phosphorylation and cellular signaling events by flow cytometry: techniques and clinical applications. Clin Immunol 2004;110:206–21. [PubMed: 15047199]
20. Lim CP, Cao X. Structure, function, and regulation of STAT proteins. Mol Biosyst 2006;2:536–50. [PubMed: 17216035]
21. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006;441:235–8. [PubMed: 16648838]
22. Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, et al. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. Nature 2007;448:484–7. [PubMed: 17581588]
23. Ivanov, Zhou L., II; Spolski, R.; Min, R.; Shenderov, K.; Egawa, T., et al. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol 2007;8:967–74. [PubMed: 17581537]
24. Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature 2007;448:480–3. [PubMed: 17581589]
25. Ivanov II, Zhou L, Littman DR. Transcriptional regulation of Th17 cell differentiation. Semin Immunol. 2007
26. Chen Z, Tato CM, Muul L, Laurence A, O'Shea JJ. Distinct regulation of interleukin-17 in human T helper lymphocytes. Arthritis Rheum 2007;56:2936–46. [PubMed: 17763419]
27. Basaran N, Sayli BS, Basaran A, Solak M, Artan S, Stevenson JD. Consanguineous marriages in the Turkish population. Clin Genet 1988;34:339–41. [PubMed: 3229003]

28. Hokuto I, Ikegami M, Yoshida M, Takeda K, Akira S, Perl AK, et al. Stat-3 is required for pulmonary homeostasis during hyperoxia. J Clin Invest 2004;113:28–37. [PubMed: 14702106]
29. Lian X, Qin Y, Hossain SA, Yang L, White A, Xu H, et al. Overexpression of Stat3C in pulmonary epithelium protects against hyperoxic lung injury. J Immunol 2005;174:7250–6. [PubMed: 15905571]
30. Yang J, Chatterjee-Kishore M, Staugaitis SM, Nguyen H, Schlessinger K, Levy DE, et al. Novel roles of unphosphorylated STAT3 in oncogenesis and transcriptional regulation. Cancer Res 2005;65:939–47. [PubMed: 15705894]
31. Yang J, Liao X, Agarwal MK, Barnes L, Auron PE, Stark GR. Unphosphorylated STAT3 accumulates in response to IL-6 and activates transcription by binding to NFkappaB. Genes Dev 2007;21:1396–408. [PubMed: 17510282]

Abbreviations

AD-HIES	Autosomal Dominant hyper IgE syndrome
AR-HIES	Autosomal Recessive hyper IgE syndrome
HIES	hyper IgE syndrome
IFNα	interferon alpha
JAK	Janus kinase
mAb	monoclonal antibody
PBMC	peripheral blood mononuclear cells
PHA	phytohemagluttinin
pY	phospho-tyrosine
RORγτ	retinoic orphan receptor gamma t
SH2	Src homology 2
STAT	signal transducer and activator of transcription
Th	T helper
TYK2	tyrosine kinase 2

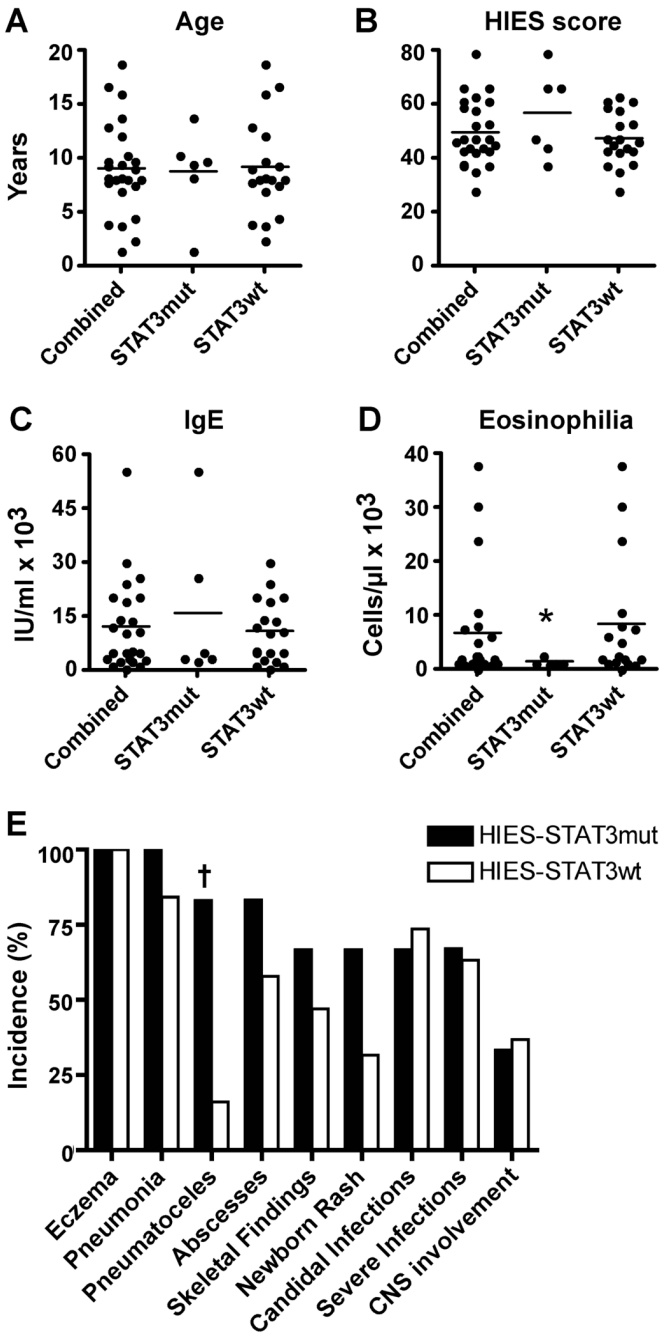

Figure 1.

Characteristics of the HIES cohort for Age (A), HIES score (B), serum IgE levels (C) and blood eosinophilia (D). Disease complications in HIES-STAT3wt versus HIES-STAT3mut group. Abscesses include those in the skin and viscera; skeletal involvement includes fractures, retained primary teeth and hyper-extensibility; severe infections are those requiring hospitalization; CNS involvement includes infections, infarcts, vessel occlusion and ischemic injury. *p=0.02 for eosinophil counts (Student's two tailed t test) and †p=0.005 for pneumatocele formation (Fisher exact test) in HIES-STAT3wt versus HIES-STAT3mut group.

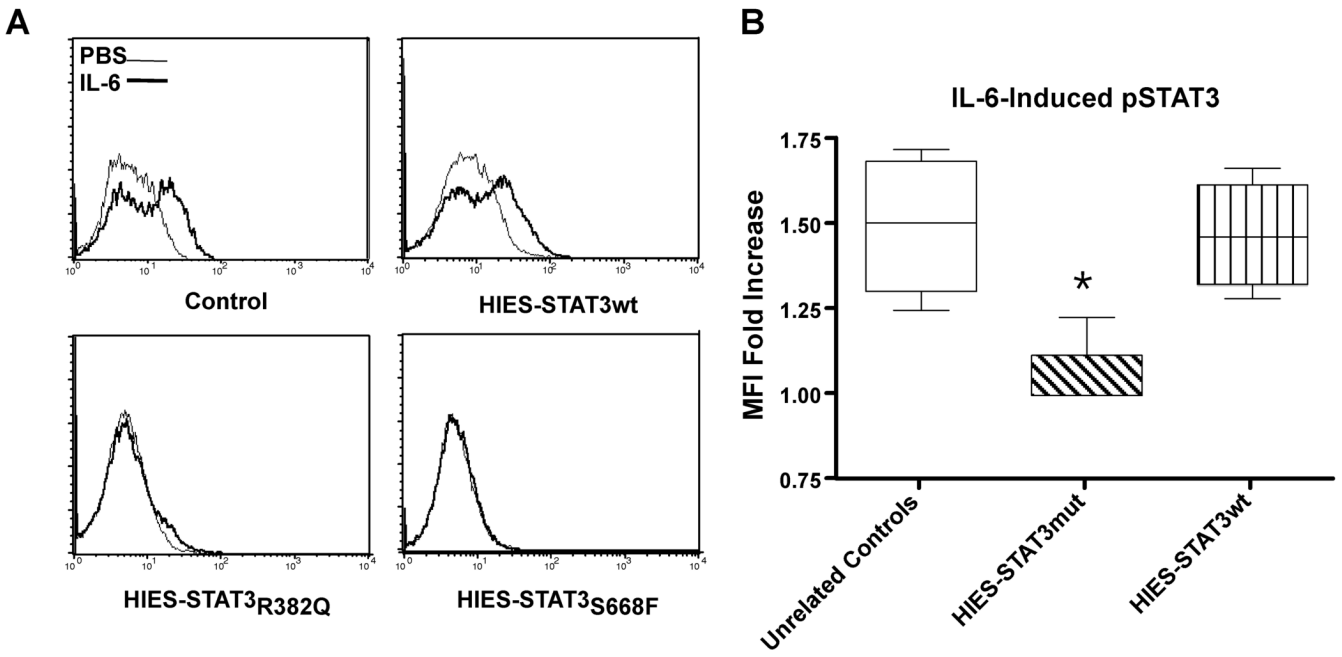

Figure 2.

Defective IL-6-induced STAT3 phosphorylation in T cells of HIES subjects with, but not without, STAT3 mutations. **A**. Representative intracellular staining profile of pY705STAT3 in T cells of a control subject, an HIES subject with normal STAT3 sequence (HIES-STAT3wt) and HIES subjects with DNA binding and SH2 domain mutations (HIES-STAT3R382Q and HIES-STAT3Y668F, respectively), following stimulation with IL-6 for 15 min. B. *$p<0.01$ for HIES patients with STAT3 mutations (HIES-STAT3mut) versus HIES-STAT3wt or unrelated controls (n=4 for each group).

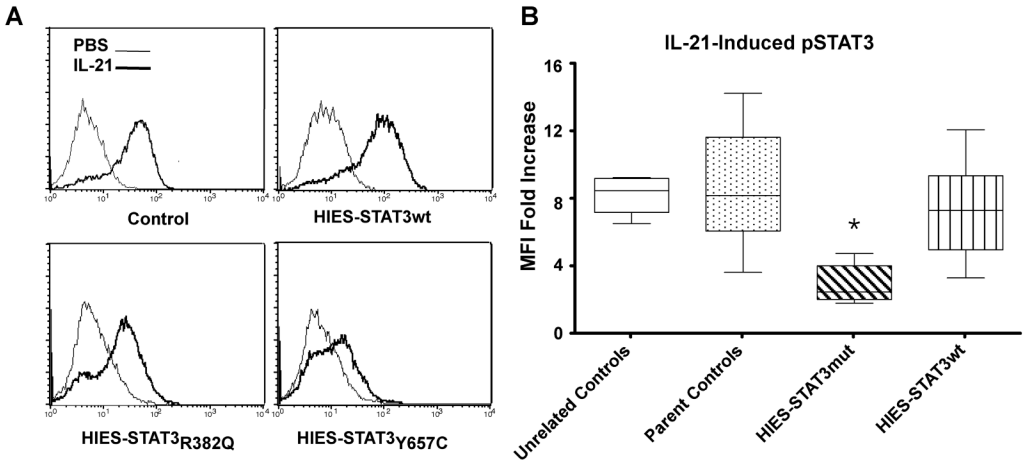

Figure 3.

Defective IL-21-induced STAT3 phosphorylation in T cells of HIES patients with STAT3 mutations. **A**. Representative intracellular staining profile of pY705STAT3 in T cells of a control subject, an HIES subject with normal STAT3 sequence (HIES-STAT3wt), and HIES subjects with DNA binding and SH2 domain mutations (HIES-STAT3R382Q and HIES-STAT3Y657C, respectively), following stimulation with IL-21 for 15 min. **B**. *$p<0.001$ for unrelated and parent controls (n=6 and 17, respectively) versus HIES patients with STAT3 mutations (HIES-STAT3mut; n=5), and for HIES-STAT3wt (n=15) versus HIES-STAT3mut.

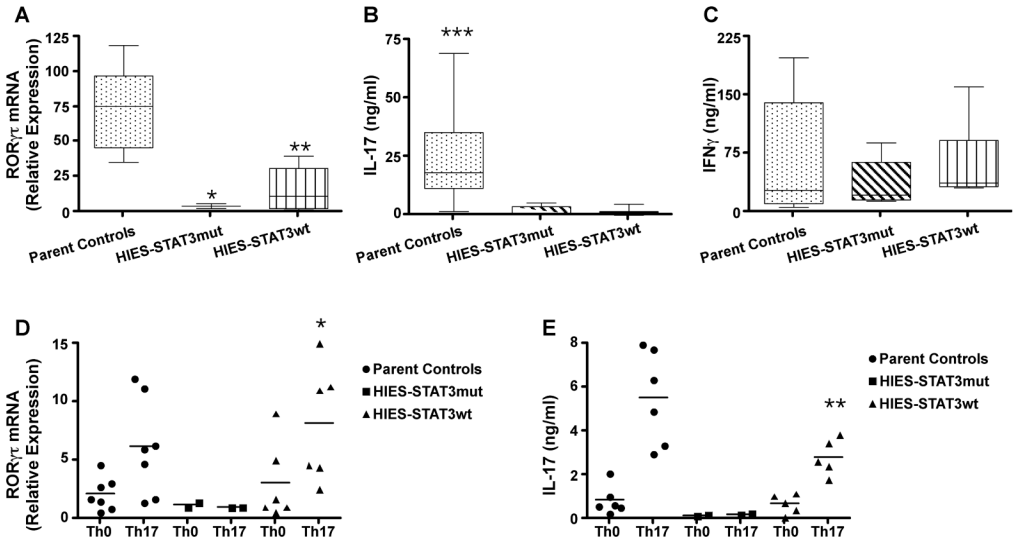

Figure 4.

Impaired Th17 differentiation is a common attribute of different forms of HIES. **A**. Real time PCR analysis of RORγτ mRNA levels in peripheral blood T cell blasts of Parent control subjects (n= 7), HIES without STAT3 mutations (HIES-STAT3wt, n=7) and HIES subjects with STAT3 mutations (HIES-STAT3mut; n=3); p= *0.01 and **0.001 for parent controls versus HIES-STAT3mut and HIES-STAT3wt subjects, respectively. **B**. IL-17 production by peripheral blood T cells of HIES and control subjects following Th17 differentiation; p=***0.001 for controls (n=17) versus HIES-STAT3mut (n=6) and HIES-STAT3wt (n=12), respectively. **C**. IFNγ production following Th1 differentiation is not impaired in HIES groups. **D, E**. RORγτ mRNA expression (D) and IL-17 production (E) in Th0 and Th17 cells derived from naïve T cells of control, HIES-STAT3mut and HIES-STAT3wt subjects; *p<0.05 for Th17 HIES-STAT3wt versus Th17 HIES-STAT3mut (D), and **p<0.01 for Th17 HIES-STAT3wt versus Th17 control or Th17 HIES-STAT3mut (E).