

# Defects in Jak–STAT-mediated cytokine signals cause hyper-IgE syndrome: lessons from a primary immunodeficiency

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## Abstract

**Hyper-IgE syndrome (HIES) is a primary immunodeficiency characterized by atopic manifestations and susceptibility to infections with extracellular bacteria and fungi, which frequently occur in the skin and lung. Atopic manifestations in HIES include extremely high serum IgE levels, eczema and eosinophilia. Most of the extracellular bacterial infections are associated with disproportionately milder inflammation than normal, which was originally described as having a ‘cold abscess’. Non-immunological abnormalities are also observed in most patients with HIES, including a distinctive facial appearance, scoliosis, hyper-extensive joints and retained primary teeth. Recent studies have demonstrated that hypomorphic mutations in signal transducer and activator of transcription 3 result in the classical multisystem form of HIES, whereas a null mutation in tyrosine kinase 2 causes the autosomal recessive form of HIES that is associated with viral and mycobacterial infections. Analyses of cytokine responses in both types of HIES have revealed defects in signal transduction for multiple cytokines including IL-6 and IL-23, leading to impaired T<sub>H</sub>17 function. These results suggest that the defect in multiple cytokine signals is the molecular basis of the immunological and non-immunological abnormalities in HIES and that the susceptibility to infections with extracellular bacteria and fungi in HIES might be associated with the defect in T<sub>H</sub>17 cell differentiation.**

## Introduction

Primary immunodeficiencies are genetically determined immune disorders. Research into these disorders, in conjunction with the study of ‘knockout’ mice, has led to remarkable progress in our understanding of the *in vivo* function of certain genes expressed in the immune system. Identification of the molecular origin of primary immunodeficiencies also provides benefit for the patients, resulting in earlier diagnosis and better treatment. The number of genes identified as responsible for primary immunodeficiencies has recently grown tremendously, reflecting the explosive expansion of knowledge in this field (Table 1). In this article, we will first briefly review the contribution of one primary immunodeficiency (agammaglobulinemia) to our understanding of the human immune system and later focus on another primary immunodeficiency, hyper-IgE syndrome (HIES).

### Agammaglobulinemia

The first primary immunodeficiency disorder was reported in 1952, when Ogden Bruton described an 8-year-old boy who

experienced recurrent, life-threatening pneumococcal pneumonia and septicemia (1). The patient was able to survive, thanks to the discovery and clinical use of antibiotics at that time, which enabled the identification of the disorder. Using a newly developed serum electrophoresis technique, Bruton identified that the boy was deficient in gammaglobulin. With the treatment using intramuscularly injected gammaglobulin isolated from healthy individuals, the patient’s susceptibility to bacterial infections was dramatically decreased. The disorder described in the report is now called X-linked agammaglobulinemia (XLA). Patients with XLA can remain healthy during the first few months of life, thanks to maternally transmitted IgG antibodies. Thereafter, the patients repeatedly suffer from infections with extracellular bacteria such as pneumococci and haemophilus unless prophylactic antibiotics or gammaglobulin therapy is initiated. Concentrations of Igs of all the isotypes are very low in the patients, and circulating B cells are almost absent. In the bone marrow, the number of pro-B cells is normal, but that of pre-B cell is

**Table 1.** Classification of primary immunodeficiencies

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|--|
| A. Combined T and B cell immunodeficiency  |
| 1. T-B- SCID (RAG1/2, Artemis, ADA)  |
| 2. T-B+ SCID ( $\gamma$ c, Jak3, IL-7R $\alpha$ , CD45, CD3 $\delta$ / $\epsilon$ / $\xi$ )    |
| 3. Omenn syndrome (hypomorphic mutation in Rag1/2, Artemis, IL-7R $\alpha$ )                   |
| B. Predominant antibody deficiencies   |
| 1. Agammaglobulinemia (Btk, $\mu$ heavy chain, $\lambda$ 5, Ig $\alpha$ , Ig $\beta$ , BLNK)   |
| 2. Common variable immunodeficiency (ICOS, CD19)   |
| 3. Hyper-IgM syndrome (CD40L, CD40, AID, UNG)  |
| C. Other well-defined immunodeficiency syndromes   |
| 1. HIES (STAT3, TYK2)  |
| 2. Wiskott-Aldrich syndrome (WASP)   |
| 3. DNA repair defects (ATM, MRE11, NBS1, BLM)  |
| D. Diseases of immune dysregulation  |
| 1. Familial hemophagocytic lymphohistiocytosis (PRF1, MUNC13D, STX11)                          |
| 2. X-linked lymphoproliferative syndrome (SH2D1A, XIAP)  |
| 3. Syndrome with autoimmunity (Fas, FasL, CASP10, CASP8, AIRE, FOXP3)                          |
| E. Congenital defects of phagocytes  |
| 1. Severe congenital neutropenia (ELA2, GFI1, G-CSFR)  |
| 2. Kostman disease (HAX1)  |
| 3. Leukocyte adhesion deficiency (ITGB2, FUCT1)  |
| F. Defects in innate immunity  |
| 1. Ectodermal dysplasia with immunodeficiency (NEMO)   |
| 2. IRAK4 deficiency (IRAK4)  |
| 3. Herpes virus encephalitis (UNC93B1, TLR3)   |
| G. Autoinflammatory disorders  |
| 1. Familial Mediterranean fever (MEFV)   |
| 2. Hyper-IgD syndrome (MVK)  |
| 3. Muckle-Wells syndrome (CIAS1/NALP3)   |
| H. Complement deficiencies (C1q, C1r, C1s, C4, C2, C3, C5, C6, C7, C8a, C8b, C9, factor I/H/D) |

Some examples of primary immunodeficiency and its molecular origins from the World Health Organization classification (29), the responsible molecules for each category are shown in parenthesis. ADA, adenosine deaminase; AID, activation-induced cytidine deaminase; AIRE, autoimmune regulator; ATM, ataxia telangiectasia mutated; BLM, Bloom's syndrome protein; BLNK, B cell linker protein; Btk, Bruton tyrosine kinase; CASP, caspase; CIAS1, cold-induced autoinflammatory syndrome; ELA, neutrophil elastase; FOXP3, forkhead box P3; FUCT1, GDP-fucose transporter 1; GFI1, growth factor independent 1; HAX1, HSL1-associated protein X1; ICOS, inducible costimulatory; ITGB2, integrin  $\beta$ -2; IRAK4, IL-1 receptor-associated kinase; MEFV, familial Mediterranean fever; MVK, mevalonate kinase; Mre11, meiotic recombination 11; NALP3, Nacht domain-, leucine-rich repeat and PYD-containing protein 3; NBS1, Nijmegen breakage syndrome 1; NEMO, NF- $\kappa$ B essential modulator; PRF1, perforin 1; RAG, recombinase-activating gene; SH2D1A, SH2 domain protein 1A; STX11, syntaxin 11; TLR, Toll-like receptor; UNC93B1, Unc-93 homolog B1; UNG, uracil-DNA glycosylase; WASP, Wiskott-Aldrich protein; XIAP, X-linked inhibitor of apoptosis protein.

severely decreased. Tonsils are usually very small, and lymph nodes are non-palpable because of the absence of germinal centers in the lymphoid tissues.

Patients with agammaglobulinemia are susceptible to encapsulated bacteria, including *Streptococcus pneumoniae* and *Haemophilus influenzae*. In contrast, they are not susceptible to infections by fungi, coliforms, intracellular bacterial and many kinds of viruses with the exceptions of hepatitis viruses and enteroviruses. This differential susceptibility of agammaglobulinemic patients to the microbial pathogens and the beneficial effects of therapeutic gammaglobulin highlighted a crucial role of antibodies in protection against

encapsulated bacteria. Furthermore, before the discovery of the primary immunodeficiencies, it was assumed that infections are attributable to excessive exposure to infectious agents or unusual properties of the infectious organisms involved. Thus, the recognition of the first human primary immunodeficiency disease has set the stage for an exponential increase in information about the functions of the various components of the human immune system and its defects as a result of 'experiments of nature' (2).

In 1993, two groups independently discovered a gene responsible for XLA, now called Bruton agammaglobulinemia tyrosine kinase. One group positionally cloned (see below) the responsible gene in human (3). The other group identified it as a new B cell-specific tyrosine kinase that is important in murine B cell signaling (4). The group next realized that the gene was located on the X chromosome. When the human gene counterpart was cloned, alterations in the nucleotide sequence in the tyrosine kinase were identified in patients with XLA.

Agammaglobulinemia is one of the prototypes of primary immunodeficiencies, and the process of identification of the molecular origin provides a typical example for many other primary immunodeficiencies (5–8). In most cases, a molecular origin is identified utilizing both genetic and immunological clues. Theoretically, there are two approaches for the identification of the genetic determinant for primary immunodeficiencies. One is a classical position-dependent approach (positional cloning) and the other is a position-independent approach. In positional cloning, the genes in the candidate region are selected for further evaluation on the basis of the expression pattern and/or function of the molecules in the region; here, phenotypic homology between humans and mice provides valuable clues toward identifying genes. Positional information reduces the number of possible candidate genes enormously. At present, this is very important because our ability to predict appropriate candidate genes is very limited.

### History of HIES

HIES (also called Job's syndrome; #147060 and #243700 in the Online Mendelian Inheritance in Man human genetic disease database) is another primary immunodeficiency syndrome, one of the members of the 'other well-defined immunodeficiency syndrome' category in the classification defined by the World Health Organization (Table 1). This disease was first described in 1966 by Davis and Wedgwood (9). Two patients reported by them had recurrent staphylococcal skin infections that lacked the features of typical inflammation including redness and warmth, and therefore Davis and Wedgwood coined the phrase 'cold abscess' for the phenomenon. The syndrome was further characterized by Buckley *et al.* (10), who noted that the recurrent staphylococcal skin infections and cold abscess formation in HIES were associated with severe dermatitis and highly elevated serum IgE levels. Later study further characterized the multi-system nature of HIES, in which the manifestations are not only restricted to the immune system but also extend to skeletal and dental abnormalities such as a unique facial appearance, scoliosis, osteoporosis, hyper-extensive joints

and retained primary teeth (11). In 2004, a novel form of HIES was identified in consanguineous families, suggesting the presence of autosomal recessive (AR) HIES (12).

### Clinical characteristics of HIES

As well as by high levels of serum IgE and recurrent bacterial infections, HIES is characterized by eczema similar to that found with atopic dermatitis (13, 14). The levels of IgE in the serum are extremely high—usually at least 100 times higher than those of normal individuals. The atopic dermatitis-like eczema usually starts in the neonatal period—much earlier than the onset of atopic dermatitis, which is a common skin disorder. In HIES, infections with extracellular bacteria generally commence in infancy and mainly involve the skin and lung. The most common bacterium involved in the infections is *Staphylococcus aureus*, although *S. pneumoniae*, *H. influenza* and enteric gram-negative bacteria are occasionally isolated in some infectious episodes of HIES. Fungal infections including mucocutaneous candidiasis and lung aspergillosis are also common in HIES.

Most cases of HIES are sporadic, but some familial cases of HIES have been reported, either with an autosomal dominant (AD) (11) or with an AR (15) mode of inheritance. Skeletal and dental abnormalities are observed in sporadic cases and familial cases with the AD form, but not those with the AR form that is characterized by severe, recurrent viral infections, extreme eosinophilia and neurological complications. From both clinical and etiological points of view, we classify HIES into two groups, type 1 and type 2, irrespective of modes of inheritance. Type 1 HIES displays abnormalities in multiple systems of the body including the skeletal and dental systems, whereas type 2 HIES shows abnormalities confined to the immune system (Table 2).

#### Type 1 HIES

This group of HIES represents the most common form of HIES, including both sporadic and familial AD inheritance (11). In addition to the recurrent staphylococcal skin and pul-

monary infections, atopic dermatitis and elevated serum IgE found in almost all the patients with HIES, patients with type 1 HIES display skeletal, dental and connective tissue manifestations. By the age of 16 years, all the patients show a distinctive facial appearance: coarse texture of facial skin, asymmetric facial appearance, prominent forehead, deep-set eyes, broad nasal bridge and bulky nasal tip. In this type of HIES, pneumonia is frequently followed by the formation of pulmonary cysts, most likely due to the impaired remodeling capability of the lung. Pulmonary cysts are frequently complicated by the superinfection of *Aspergillus* and multi-drug-resistant *Pseudomonas aeruginosa*, which is a serious problem in the morbidity and the mortality of patients with HIES (16).

#### Type 2 HIES

This group of HIES was reported relatively recently; six consanguineous families from Turkey and Mexico were suggested to carry the AR form of HIES (12). More than two patients were present in five out of the six pedigrees. The patients in these families did not show any apparent abnormalities in their skeletal and dental systems but suffered from recurrent and severe infections with *S. aureus*, *S. pneumoniae* or *H. influenzae*, as observed in type 1 HIES. Notably, most of the patients with type 2 HIES also suffered from recurrent viral infections such as chronic refractory molluscum contagiosum and herpes simplex virus infections, which were not identified in type 1 HIES. Furthermore, no pulmonary cyst was found in any of the patients with type 2 HIES, unlike in the patients with type 1 HIES. Seven out of 13 patients with type 2 HIES had neurological complications, and four of them died of the neurological symptoms. The origin of the neurological complications might be due to either primary to the type 2 HIES or secondary to latent infections in the central nervous system.

### Diagnosis of HIES

Clinical diagnosis of HIES is based on the criteria established by Grimbacher *et al.*, which scores clinical findings such as the number of skin abscesses (more than four times in a lifetime is 8 points) and pneumonia (more than three episodes in a lifetime is 8 points), parenchymal lung abnormalities (pneumatocele is 8 points), characteristic facial appearance (if typical, 5 points), newborn rash (if present, 4 points), hyper-extensibility (if present, 4 points) as well as laboratory findings such as high serum IgE concentrations ( $>2000$  IU ml<sup>-1</sup>, 10 points) and eosinophilia ( $>800$  mm<sup>-3</sup>, 6 points) (17). We clinically diagnose HIES if the score of the patient is above 40 points. Although not included in this scoring system, one of the unique characteristics of the HIES is the apparent lack of classical inflammatory responses, which might be included in the scoring system for the future.

### Defining the etiology of HIES

#### Early findings

The origin and the molecular pathology of HIES remained an enigma in spite of >40 years of extensive research, until the recent discovery of causative genes. Previous study by

**Table 2.** A classification of HIES

| HIES type            | Inheritance                         | Distinguishing clinical findings  |
|----------------------|-------------------------------------|---|
| Type 1 (multisystem) | Sporadic (>90% of cases)            | Skeletal and dental abnormalities (characteristic face, fracture with minor trauma, retained primary teeth, scoliosis and hyper-extensibility)  |
|                      | Familial with AD inheritance (rare) | Pulmonary cyst (pneumatocele)   |
| Type 2               | Familial with AR inheritance        | Severe viral infections (herpes simplex virus, molluscum contagiosum)<br>Central nervous system involvement (?)<br>Mycobacterial infections (some)<br>No pulmonary cyst<br>No skeletal manifestations |

Hill *et al.* (18) suggested that impaired neutrophil chemotaxis caused the susceptibility to extracellular bacterial infection; however, it was later realized that the defect was not consistently present in patients with HIES. After the identification of  $T_H1$  and  $T_H2$  cells in mice, many researchers investigated  $T_H1$  and  $T_H2$  cell differentiation in patients with HIES. Although some studies suggested a decreased  $T_H1$  cytokine production and a skewing toward  $T_H2$  cytokine production (19–21), the results were inconsistent from study to study and did not provide sufficient clues to identify the molecular origin of HIES. It should be noted that the patients with defects in IL-12 signaling manifest none of the common features of HIES, suggesting that the simple reduction of IFN- $\gamma$  in response to IL-12 is unlikely to cause HIES (22).

More than 250 HIES patients have been reported in the literature. An HIES patient with mental retardation was identified to have a deletion of 15–20 cM on chromosome 4q. Linkage analysis of 19 families with 57 HIES patients demonstrated linkage to the proximal arm of chromosome 4q; however, 6 of the 19 families did not demonstrate linkage to this region of chromosome 4q. Another study suggested a polymorphism in the IL-4R (Q576R) linked to HIES; however, later study showed no correlation of the Q576R alleles with the HIES phenotype. Collectively, although many attempts were made to identify the molecular origin of HIES, they were not successful until we identified an AR-HIES patient associated with viral and intracellular bacterial infection 3 years ago.

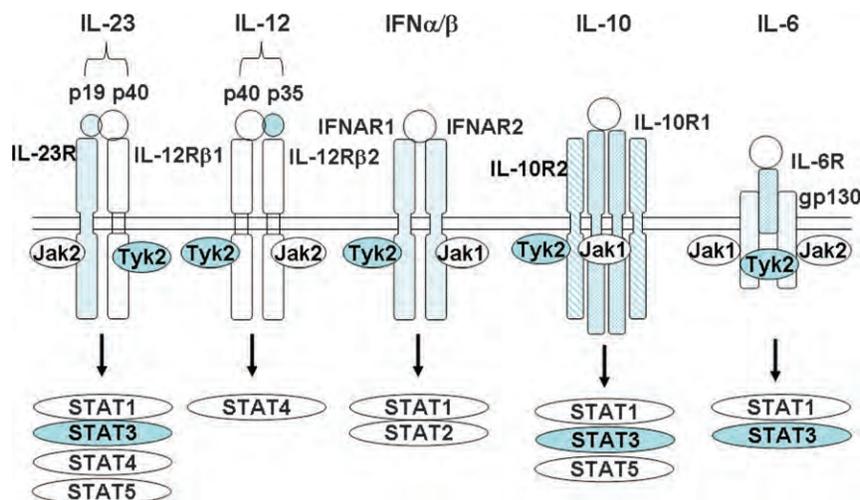
#### Tyrosine kinase 2 deficiency as the genetic origin for a sub-population of type 2 HIES

The first genetic origin identified in HIES was a null mutation of tyrosine kinase 2 (*TYK2*) (23). The identification of *TYK2* as the causative gene was suggested following the observations that a patient with HIES was susceptible to intracellular bacterial infections and had defective signaling by IL-12 and IFN- $\alpha$ . Tyk2 is one of the founding members of Janus kinase family (Jaks) (24, 25), which transduces signals initiated by most of cytokines (26–28). Jaks constitutively

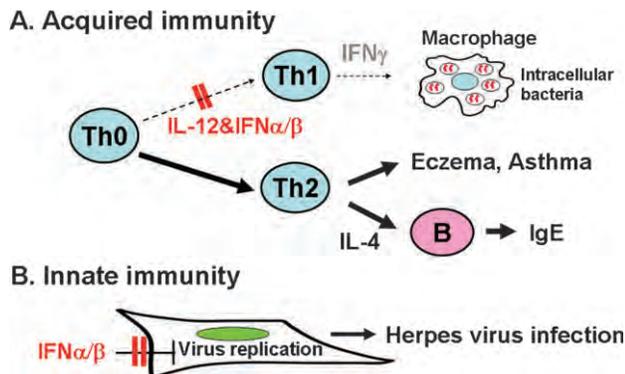
associate with the cytoplasmic domains of cytokine receptors, which lack intrinsic kinase activity, and upon ligand binding, phosphorylate receptor subunits. This recruits signal transducers and activators of transcription (STATs) and other adapters and signaling molecules. Jaks also phosphorylate and activate STATs, promoting nuclear translocation and transcription of STAT-responsive genes.

The patient had a homozygous 4-bp deletion in the coding region of *TYK2* gene, resulting in a premature stop codon and the absence of TYK2 protein. The parents of the patient were consanguineous, and both were heterozygous for the mutation, establishing the AR inheritance of the *TYK2* deficiency in the patient. The *TYK2*-deficient patient displayed typical phenotypes of type 2 HIES, including elevated serum IgE, atopic dermatitis, infections with extracellular bacteria and viral infections but no skeletal and dental abnormalities. Now this disorder is termed AR-HIES associated with susceptibility to virus and intracellular bacterial infections (29).

Blood cells from the patient showed severe defects in response to multiple cytokines including type I IFN (IFN- $\alpha$  and IFN- $\beta$ ), IL-6, IL-10, IL-12 and IL-23 (Fig. 1). Importantly, the cytokine signals were successfully restored by transducing the intact *TYK2* gene *in vitro*. Thus, the *TYK2* deficiency is likely to account for the clinical manifestations of the patient (Fig. 2). The susceptibility of the patient to viral infections could be explained by the defects in type I IFN signaling. IFN- $\alpha$ -induced tyrosine phosphorylation of Jak1, STAT1 and STAT2 was completely abrogated in the human *TYK2*-deficient cells. Furthermore, no inhibition in the replication of herpes simplex virus was observed in the patient's cells when pre-treated with IFN- $\alpha$ . One of the features characteristic of human *TYK2* deficiency is susceptibility to intracellular bacterial infection such as mycobacteria and salmonella. The absence of *TYK2* resulted in defects of both IL-12 and type I IFN signaling, which lead to impaired  $T_H1$  differentiation and IFN- $\gamma$  production. This defect likely accounts for the patient's susceptibility to intracellular bacterial infections as reported in other disorders with the defects in the IL-12 and IFN- $\gamma$  signaling circuit (30). When the patient's naive CD4



**Fig. 1.** Surface cytokine receptors and their associated intracellular Jaks (Jak1, Jak2 and Tyk2) and STATs.



**Fig. 2.** Pathogenesis of TYK2 deficiency. (A) In acquired immunity, the absence of TYK2 results in defects of both IL-12 and type I IFN signaling, which lead to impaired  $T_H1$  differentiation and IFN- $\gamma$  production. This defect likely accounts for the patient's susceptibility to intracellular bacterial infections. The patient's naive CD4 T cells showed enhanced  $T_H2$  differentiation and over-production of IL-4 in addition to impaired  $T_H1$  differentiation, reflecting the  $T_H2$ -dominant phenotypes of the patient, such as eczema, asthma and elevated serum IgE level. (B) In innate immunity, the absence of TYK2 results in defective type I IFN signaling, resulting in susceptibility to herpes virus infections.

T cells were cultured *in vitro*, enhanced  $T_H2$  differentiation was observed in addition to the impaired  $T_H1$  differentiation, reflecting the  $T_H2$ -dominant phenotypes of the patient, such as atopic dermatitis, asthma and elevated serum IgE levels. Since IL-10 plays a critical role in peripheral T cell tolerance to allergens, it might be reasonable to speculate that allergic symptoms in the TYK2 deficiency were associated, at least in part, with the defective IL-10 signaling.

The functional importance of the Jaks was demonstrated by studies in cell lines defective in cytokine signaling and confirmed by the establishment of knockout mice (31–36). Although TYK2 was originally described as essential for the type I IFN signaling in a human fibroblast cell line (24, 25), the establishment of Tyk2-deficient mice revealed that Tyk2 was not absolutely essential for responses to type I IFN in mice (37, 38). Tyk2 $^{-/-}$  mice displayed a lack of responsiveness to a small amount of IFN $\alpha$ , but a high concentration of IFN $\alpha$  could fully transduce its signals even in the absence of Tyk2. Thus, the function of Tyk2 in mice appears to be compensated for *in vivo*, partly by other kinases, most likely other Jaks, which is not the case in humans.

#### Hypomorphic mutations in STAT3 cause type 1 HIES

The identification of the TYK2 deficiency in a patient with type 2 HIES suggested to us that a similar defect in cytokine signaling might be observed in the type 1 HIES. To explore this possibility, we first examined the responses to IL-6, IL-10, IL-12 and IFN $\alpha$  of peripheral blood cells from typical type 1 HIES patients. IgM secretion from the patients' B cells after stimulation with EBV in the absence or the presence of IL-6 demonstrated that the signaling of IL-6 was impaired in type 1 HIES. In addition, the suppression of LPS-induced production of tumor necrosis factor (TNF) $\alpha$  by IL-10 was also impaired in the patients. Thus, both IL-6 and IL-10 signaling pathways were defective in type 1 HIES, as in TYK2 defi-

ciency. In contrast, neither IL-12 nor IFN $\alpha$  signaling was impaired in patients with type 1 HIES.

In a survey of candidate molecules involved in both IL-6 and IL-10 signaling, but not involved in the type I IFN and IL-12 signaling, we identified heterozygous, dominant negative, mutations in the DNA-binding domain of STAT3 in the patients (39). The DNA-binding domain of STAT3 is highly conserved among different species in its amino acid sequence, and the alterations were not found in 1000 unrelated healthy individuals analyzed. STAT3 is located on human chromosome 17q21 but not 4q that was reported to contain a disease locus for familial AD-HIES (17). None of the eight HIES patients in our study had a known family history of HIES, and no mutation was detected in the STAT3 cDNAs from all the parents and seven siblings of the patients. Therefore, the mutations are likely to have occurred *de novo* in the patients with this form of HIES.

Later study further identified mutations in the STAT3 gene in most of the patients with type 1 HIES, including the patients described in the first report of HIES (16, 40). The position of mutations was located in the DNA-binding domain of STAT3 in the first eight patients, which extended to the Src homology 2 domain and the transactivation domain (41, 42). The clinical characteristics of the patients have been indistinguishable between those with mutations in the DNA-binding domain and those with mutations in other domains of STAT3 molecule, and there seems no clear phenotype-genotype relationship in the HIES.

STAT3 is a transcription factor, which binds to the STAT3-responsive elements in the promoters of various genes including acute-phase proteins (43–46). STAT3 plays a critical role in responses to many cytokines, including IL-6, IL-10, IL-22, IL-23 and IL-27 (47–49). A null mutation of the STAT3 gene in mice demonstrated that STAT3 was essential for embryonic survival near the time of implantation (E6.5–E7.5) (50). It is reasonable to speculate that the difference between AD inheritance of hypomorphic mutations in human and early embryonic lethality of a null mutation in mice is due to the fact that the human mutations have residual STAT3 activity. It is, however, still possible that human and mouse STAT3 have distinct functions. Future study should clarify this issue. Mice with tissue-specific deletion of STAT3 were established, which demonstrated the critical role of STAT3 in cell migration, survival, proliferation, apoptosis and inflammation in each tissue, including skin, mammary glands, liver, thymic epithelium, respiratory epithelium, neurons, lymphocytes and macrophages (51). Thus, it is likely that wide range of manifestations in type 1 HIES patients corresponds to the diverse function of STAT3 *in vivo* in human.

#### Molecular pathogenesis and $T_H17$ defect in HIES

Although the molecular origin of the HIES has been identified, we still know very little about the molecular pathogenesis of the HIES. There are at least four important questions: what is the molecular mechanism of skeletal and dental manifestations; why do patients with HIES display little or no inflammatory response in spite of severe infections; why do patients with HIES have atopic manifestations and why do

patients with HIES suffer from extracellular bacterial infections confined to the skin and lung?

STAT3 plays important roles in the differentiation of both osteoblasts and osteoclasts *in vitro*, and mice deficient for STAT3 in osteoblasts show an osteoporotic phenotype (52). When osteoclasts were generated from peripheral blood monocytes in culture with M-colony-stimulating factor 1 and receptor activator of nuclear factor  $\kappa$ B ligand (also known as TNF superfamily, member 11), those from HIES patients with STAT3 mutations showed higher bone resorption activity compared with those from control subjects (39). This may reflect the skeletal and dental abnormalities observed in patients with HIES.

Another remarkable clinical feature of HIES is that patients are often afebrile and doing well, despite serious pneumonia or dermal pathology (referred as cold abscess) (9). Moreover, the acute-phase responses, such as increased serum C-reactive protein levels during infections, were diminished in the patients. STAT3 was originally identified as a protein binding to the IL-6-responsive element in the genes encoding hepatic acute-phase proteins (43), and the liver-specific inactivation of STAT3 leads to an impaired acute-phase response in mice. Thus, the apparent lack of classical inflammatory responses in HIES patients could be attributed to defective signaling of IL-6.

We have no clear explanation for the mechanism underlying the high levels of serum IgE in patients with HIES. We and others considered the possibility that signaling a defect of IL-21 might be the origin of high levels of IgE in the serum. Surprisingly, a recent report indicated that although IL-21 in mice suppresses IL-4-induced IgE production, IL-21 in human induces IgE production by CD40 ligand-stimulated naive B cells (53). Future study should clarify these unanswered questions.

STAT3 plays a critical role in  $T_H17$  development (54), and IL-17 produced by  $T_H17$  cells is protective in host defense against extracellular bacteria (55–57). IL-22 stimulates cells in the skin and respiratory systems to produce  $\beta$ -defensins through STAT3 activation (58, 59). Thus, the susceptibility to extracellular bacterial infection could be attributed, at least in part, to defects in  $T_H17$  development and IL-22 signaling. Indeed, defective  $T_H17$  development and function in patients with HIES due to mutations in STAT3 was recently demonstrated (60–62). Future studies should address why and how defective  $T_H17$  cells specifically cause the susceptibility to staphylococcal infections in the skin and lung.

### Conclusion and perspective

The identification of human TYK2 deficiency revealed the critical functions of TYK2 in the transduction of multiple cytokine signals involved in the innate and acquired immunity and demonstrated that TYK2 has broader and more profound functions in humans compared with mice. The establishment of TYK2 deficiency as an etiology for a rare form of AR-HIES associated with susceptibility to viral and intracellular bacterial infections suggested us that the classic type 1 HIES is also associated with the deficiency in multiple cytokine signals, which led to the identification of dominant-negative STAT3 as a major molecular origin of type 1 HIES. These studies highlight the multiple and critical roles played

by STAT3 in humans *in vivo* and provide important information for the physician taking care of patients with HIES.

The identification of STAT3 as a major causative gene for type 1 HIES enables us to definitively diagnose the HIES patients very early in life. The key feature for early diagnosis used to be newborn rash, which is frequently associated with the susceptibility to staphylococcal infection and high serum IgE levels. Earlier definitive diagnosis at the DNA level facilitates the early start of prophylactic antibiotics, which is likely to prevent the pneumatocele formation. Preventive action should improve the quality of life of the HIES patients. Although our current attempts to improve treatment for HIES mainly focuses on early diagnosis and the prevention of disease progression, future treatment options should include stem cell transplantations and gene-targeted therapies.

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### Abbreviations

|      |  |
|------|--|
| AD   | autosomal dominant                               |
| AR   | autosomal recessive                              |
| HIES | hyper-IgE syndrome                               |
| Jaks | Janus kinase family                              |
| STAT | signal transducer and activator of transcription |
| TNF  | tumor necrosis factor                            |
| TYK2 | tyrosine kinase 2                                |
| XLA  | X-linked agammaglobulinemia                      |

### References

- 1 Bruton, O. C. 1952. Agammaglobulinemia. *Pediatrics* 9:722.
- 2 Conley, M. E., Rohrer, J., Rapalus, L., Boylin, E. C. and Minegishi, Y. 2000. Defects in early B cell development: comparing the consequences of abnormalities in pre-BCR signaling in the human and the mouse. *Immunol. Rev.* 178:75.
- 3 Vetrie, D., Vorechovsky, I., Sideras, P. *et al.* 1993. The gene involved in X-linked agammaglobulinemia is a member of the src family of protein-tyrosine kinases. *Nature* 361:226.
- 4 Tsukada, S., Saffran, D., Rawlings, D. *et al.* 1993. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* 72:279.
- 5 Yel, L., Minegishi, Y., Coustan-Smith, E. *et al.* 1996. Mutations in the mu heavychain gene in patients with agammaglobulinemia. *N. Engl. J. Med.* 335:1486.
- 6 Minegishi, Y., Coustan-Smith, E., Wang, Y. H., Cooper, M. D., Campana, D. and Conley, M. E. 1998. Mutations in the human lambda5/14.1 gene result in B cell deficiency and agammaglobulinemia. *J. Exp. Med.* 187:71.
- 7 Minegishi, Y., Rohrer, J., Coustan-Smith, E. *et al.* 1999. An essential role for BLNK in human B cell development. *Science* 286:1954.
- 8 Minegishi, Y., Coustan-Smith, E., Rapalus, L., Ersoy, F., Campana, D. and Conley, M. E. 1999. Mutations in Igalpha (CD79a) result in a complete block in B-cell development. *J. Clin. Invest.* 104:1115.
- 9 Davis, S. D., Schaller, J. and Wedgwood, R. J. 1966. Job's syndrome. Recurrent, "cold", staphylococcal abscesses. *Lancet* 1:1013.
- 10 Buckley, R. H., Wray, B. B. and Belmaker, E. Z. 1972. Extreme hyperimmunoglobulinemia E and undue susceptibility to infection. *Pediatrics* 49:59.

- 11 Grimbacher, B., Holland, S. M., Gallin, J. I. *et al.* 1999. Hyper-IgE syndrome with recurrent infections—an autosomal dominant multisystem disorder. *N. Engl. J. Med.* 340:692.
- 12 Renner, E. D., Puck, J. M., Holland, S. M. *et al.* 2004. Autosomal recessive hyperimmunoglobulin E syndrome: a distinct disease entity. *J. Pediatr.* 144:93.
- 13 Minegishi, Y. and Karasuyama, H. 2007. Hyperimmunoglobulin E syndrome and tyrosine kinase 2 deficiency. *Curr. Opin. Allergy Clin. Immunol.* 7:506.
- 14 Grimbacher, B., Holland, S. M. and Puck, J. M. 2005. Hyper-IgE syndromes. *Immunol. Rev.* 203:244.
- 15 Renner, E. D., Pawlita, I., Hoffmann, F. *et al.* 2005. No indication for a defect in toll-like receptor signaling in patients with hyper-IgE syndrome. *J. Clin. Immunol.* 25:321.
- 16 Freeman, A. F., Kleiner, D. E., Nadiminti, H. *et al.* 2007. Causes of death in hyper-IgE syndrome. *J. Allergy Clin. Immunol.* 119:1234.
- 17 Grimbacher, B., Schaffer, A. A., Holland, S. M. *et al.* 1999. Genetic linkage of hyper-IgE syndrome to chromosome 4. *Am. J. Hum. Genet.* 65:735.
- 18 Hill, H. R., Ochs, H. D., Quie, P. G. *et al.* 1974. Defect in neutrophil granulocyte chemotaxis in Job's syndrome of recurrent "cold" staphylococcal abscesses. *Lancet* 2:617.
- 19 Del Prete, G., Tiri, A., Maggi, E. *et al.* 1989. Defective *in vitro* production of gamma- interferon and tumor necrosis factor-alpha by circulating T cells from patients with the hyperimmunoglobulin E syndrome. *J. Clin. Invest.* 84:1830.
- 20 Borges, W. G., Augustine, N. H. and Hill, H. R. 2000. Defective interleukin-12/interferon-gamma pathway in patients with hyperimmunoglobulinemia E syndrome. *J. Pediatr.* 136:176.
- 21 Chehimi, J., Elder, M., Greene, J. *et al.* 2001. Cytokine and chemokine dysregulation in hyper-IgE syndrome. *Clin. Immunol.* 100:49.
- 22 Wood, P. M., Fieschi, C., Picard, C., Ottenhoff, T. H., Casanova, J. L. and Kumararatne, D. S. 2005. Inherited defects in the interferon-gamma receptor or interleukin-12 signalling pathways are not sufficient to cause allergic disease in children. *Eur. J. Pediatr.* 164:741.
- 23 Minegishi, Y., Saito, M., Morio, T. *et al.* 2006. Human Tyk2 deficiency reveals requisite roles of Tyk2 in multiple cytokine signals involved in innate and acquired immunity. *Immunity* 25:745.
- 24 Firnbach-Kraft, I., Byers, M., Shows, T., Dalla-Favera, R. and Krolewski, J. J. 1990. tyk2, Prototype of a novel class of non-receptor tyrosine kinase genes. *Oncogene* 5:1329.
- 25 Velazquez, L., Fellous, M., Stark, G. R. and Pellegrini, S. 1992. A protein tyrosine kinase in the interferon alpha/beta signaling pathway. *Cell* 70:313.
- 26 Ihle, J. N. 1995. Cytokine receptor signalling. *Nature* 377:591.
- 27 Liu, K. D., Gaffen, S. L. and Goldsmith, M. A. 1998. JAK/STAT signaling by cytokine receptors. *Curr. Opin. Immunol.* 10:271.
- 28 O'Shea, J. J., Gadina, M. and Schreiber, R. D. 2002. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. *Cell* 109 (Suppl):S121.
- 29 Geha, R. S., Notarangelo, L. D., Casanova, J. L. *et al.* 2007. Primary immunodeficiency diseases: an update from the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee. *J. Allergy Clin. Immunol.* 120:776.
- 30 Casanova, J. L. and Abel, L. 2002. Genetic dissection of immunity to mycobacteria: the human model. *Annu. Rev. Immunol.* 20:581.
- 31 Muller, M., Briscoe, J., Laxton, C. *et al.* 1993. The protein tyrosine kinase JAK1 complements defects in interferon-alpha/beta and -gamma signal transduction. *Nature* 366:129.
- 32 Neubauer, H., Cumano, A., Muller, M., Wu, H., Huffstadt, U. and Pfeffer, K. 1998. Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. *Cell* 93:397.
- 33 Nosaka, T., van Deursen, J. M., Tripp, R. A. *et al.* 1995. Defective lymphoid development in mice lacking Jak3. *Science* 270:800.
- 34 Parganas, E., Wang, D., Stravopodis, D. *et al.* 1998. Jak2 is essential for signaling through a variety of cytokine receptors. *Cell* 93:385.
- 35 Park, S. Y., Saijo, K., Takahashi, T. *et al.* 1995. Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. *Immunity* 3:771.
- 36 Rodig, S. J., Meraz, M. A., White, J. M. *et al.* 1998. Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. *Cell* 93:373.
- 37 Shimoda, K., Kato, K., Aoki, K. *et al.* 2000. Tyk2 plays a restricted role in IFN alpha signaling, although it is required for IL-12-mediated T cell function. *Immunity* 13:561.
- 38 Karaghiosoff, M., Neubauer, H., Lassnig, C. *et al.* 2000. Partial impairment of cytokine responses in Tyk2-deficient mice. *Immunity* 13:549.
- 39 Minegishi, Y., Saito, M., Tsuchiya, S. *et al.* 2007. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature* 448:1058.
- 40 Renner, E. D., Torgerson, T. R., Rylaarsdam, S. *et al.* 2007. STAT3 mutation in the original patient with Job's syndrome. *N. Engl. J. Med.* 357:1667.
- 41 Holland, S. M., DeLeo, F. R., Elloumi, H. Z. *et al.* 2007. STAT3 mutations in the hyper-IgE syndrome. *N. Engl. J. Med.* 357:1608.
- 42 Renner, E. D., Rylaarsdam, S., Anover-Sombke, S. *et al.* 2008. 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.* 122:181.
- 43 Akira, S., Nishio, Y., Inoue, M. *et al.* 1994. Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. *Cell* 77:63.
- 44 Zhong, Z., Wen, Z. and Darnell, J. E. Jr 1994. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. *Science* 264:95.
- 45 Wegenka, U. M., Buschmann, J., Luttkicken, C., Heinrich, P. C. and Horn, F. 1993. Acute-phase response factor, a nuclear factor binding to acute-phase response elements, is rapidly activated by interleukin-6 at the posttranslational level. *Mol. Cell. Biol.* 13:276.
- 46 Luttkicken, C., Wegenka, U. M., Yuan, J. *et al.* 1994. Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. *Science* 263:89.
- 47 Nakajima, K., Yamanaka, Y., Nakae, K. *et al.* 1996. A central role for Stat3 in IL-6-induced regulation of growth and differentiation in M1 leukemia cells. *EMBO J.* 15:3651.
- 48 Williams, L., Bradley, L., Smith, A. and Foxwell, B. 2004. Signal transducer and activator of transcription 3 is the dominant mediator of the anti-inflammatory effects of IL-10 in human macrophages. *J. Immunol.* 172:567.
- 49 Minami, M., Inoue, M., Wei, S. *et al.* 1996. STAT3 activation is a critical step in gp130-mediated terminal differentiation and growth arrest of a myeloid cell line. *Proc. Natl Acad. Sci. USA* 93:3963.
- 50 Takeda, K., Noguchi, K., Shi, W. *et al.* 1997. Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality. *Proc. Natl Acad. Sci. USA* 94:3801.
- 51 Akira, S. 2000. Roles of STAT3 defined by tissue-specific gene targeting. *Oncogene* 19:2607.
- 52 Itoh, S., Udagawa, N., Takahashi, N. *et al.* 2006. A critical role for interleukin-6 family-mediated Stat3 activation in osteoblast differentiation and bone formation. *Bone* 39:505.
- 53 Avery, D. T., Ma, C. S., Bryant, V. L. *et al.* 2008. STAT3 is required for IL-21-induced secretion of IgE from human naive B cells. *Blood* 112:1784.
- 54 Yang, X. O., Panopoulos, A. D., Nurieva, R. *et al.* 2007. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. *J. Biol. Chem.* 282:9358.
- 55 Toy, D., Kugler, D., Wolfson, M. *et al.* 2006. Cutting edge: interleukin 17 signals through a heteromeric receptor complex. *J. Immunol.* 177:36.
- 56 Aujla, S. J., Chan, Y. R., Zheng, M. *et al.* 2008. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. *Nat. Med.* 14:275.
- 57 Zheng, Y., Valdez, P. A., Danilenko, D. M. *et al.* 2008. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat. Med.* 14:282.
- 58 Kreyenborg, K., Etzensperger, R., Dumoutier, L. *et al.* 2007. IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. *J. Immunol.* 179:8098.

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- 59 Wolk, K., Kunz, S., Witte, E., Friedrich, M., Asadullah, K. and Sabat, R. 2004. IL-22 increases the innate immunity of tissues. *Immunity* 21:241.
- 60 Milner, J. D., Brenchley, J. M., Laurence, A. *et al.* 2008. Impaired T(H)17 cell differentiation in subjects with autosomal dominant hyper-IgE syndrome. *Nature* 452:773.
- 61 Ma, C. S., Chew, G. Y. J., Simpson, N. *et al.* 2008. Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3. *J. Exp. Med.* 205:1551.
- 62 de Beaucoudrey, L., Puel, A., Filipe-Santos, O., Cobat, A., Ghandil, P. *et al.* 2008. Mutations in STAT3 and IL12RB1 impair the development of human IL-17-producing T cells. *J. Exp. Med.* 205:1543.