ORIGINAL ARTICLE

STAT3 Mutations in the Hyper-IgE Syndrome

Steven M. Holland, M.D., Frank R. DeLeo, Ph.D., Houda Z. Elloumi, Ph.D.,
Amy P. Hsu, B.A., Gulbu Uzel, M.D., Nina Brodsky, B.S.,
Alexandra F. Freeman, M.D., Andrew Demidowich, B.A., Joie Davis, A.P.R.N.,
Maria L. Turner, M.D., Victoria L. Anderson, C.R.N.P., Dirk N. Darnell, M.A.,
Pamela A. Welch, B.S.N., Douglas B. Kuhns, Ph.D., David M. Frucht, M.D.,
Harry L. Malech, M.D., John I. Gallin, M.D., Scott D. Kobayashi, Ph.D.,
Adeline R. Whitney, B.A., Jovanka M. Voyich, Ph.D., James M. Musser, M.D., Ph.D.,
Cristina Woellner, M.Sc., Alejandro A. Schäffer, Ph.D., Jennifer M. Puck, M.D.,
and Bodo Grimbacher, M.D.

ABSTRACT

BACKGROUND

The hyper-IgE syndrome (or Job's syndrome) is a rare disorder of immunity and connective tissue characterized by dermatitis, boils, cyst-forming pneumonias, elevated serum IgE levels, retained primary dentition, and bone abnormalities. Inheritance is autosomal dominant; sporadic cases are also found.

METHODS

We collected longitudinal clinical data on patients with the hyper-IgE syndrome and their families and assayed the levels of cytokines secreted by stimulated leukocytes and the gene expression in resting and stimulated cells. These data implicated the signal transducer and activator of transcription 3 gene (*STAT3*) as a candidate gene, which we then sequenced.

RESULTS

We found increased levels of proinflammatory gene transcripts in unstimulated peripheral-blood neutrophils and mononuclear cells from patients with the hyper-IgE syndrome, as compared with levels in control cells. In vitro cultures of mononuclear cells from patients that were stimulated with lipopolysaccharide, with or without interferon-γ, had higher tumor necrosis factor α levels than did identically treated cells from unaffected persons (P=0.003). In contrast, the cells from patients with the hyper-IgE syndrome generated lower levels of monocyte chemoattractant protein 1 in response to the presence of interleukin-6 (P=0.03), suggesting a defect in interleukin-6 signaling through its downstream mediators, one of which is STAT3. We identified missense mutations and single-codon in-frame deletions in *STAT3* in 50 familial and sporadic cases of the hyper-IgE syndrome. Eighteen discrete mutations, five of which were hot spots, were predicted to directly affect the DNA-binding and SRC homology 2 (SH2) domains.

CONCLUSIONS

Mutations in *STAT3* underlie sporadic and dominant forms of the hyper-IgE syndrome, an immunodeficiency syndrome involving increased innate immune response, recurrent infections, and complex somatic features.

THE SYNDROME DESCRIBED AS JOB'S SYN-drome by Davis et al. in 1966[1] and as hy-perimmunoglobulinemia E by Buckley et al. in 1972[2] was originally characterized by recur-rent cold staphylococcal abscesses, pneumonia, eczema, hyperextensibility, and extreme elevation of IgE levels. Since then, additional features of the hyper-IgE syndrome have been recognized, includ-ing scoliosis, pathologic fractures, pneumatoceles, delayed dental deciduation,[3,4] coronary-artery an-eurysms,[5] brain lesions, and Chiari's malforma-tions.[6] Pneumonia in patients with the hyper-IgE syndrome is typically caused by infections with *Staphylococcus aureus*, *Haemophilus influenzae*, or *Strep-tococcus pneumoniae* and leads to pneumatoceles,[3] providing portals for fatal infections with bacte-ria and filamentous fungi.[7]

Eosinophilia, eczema, and elevated IgE levels focused attention on the T helper cell Th1/Th2 profile,[8] but no primary defect was uncovered. Cytogenetic and linkage data suggested the pres-ence of a locus on chromosome 4q, but no disease gene was found there.[9] Microarray-based approach-es have yielded inconsistent results.[10,11] We there-fore aimed to comprehensively describe the phe-notypes of affected persons in a large cohort and to assay gene expression and in vitro cytokine production to identify relevant pathways.

METHODS

PATIENTS AND CONTROLS

Patients suspected to have the hyper-IgE syndrome, or their parents, and their relatives gave written informed consent. These patients and relatives were enrolled in studies approved by the institu-tional review board of the National Institute of Allergy and Infectious Diseases. We collected blood samples while patients were clinically well and were not receiving corticosteroids or nonsteroidal antiinflammatory drugs. An empirical scoring sys-tem was used to calculate the risk of carrying the hyper-IgE syndrome trait on the basis of an ag-gregation of diagnostic features.[9] We arbitrarily categorized hyper-IgE syndrome scores of 0 to 15 as unaffected, 16 to 39 as possibly affected, 40 to 59 as probably affected, and 60 or more as defi-nitely affected.[9] We analyzed by microarray blood samples obtained from probands who were defi-nitely affected (had scores of 60 or more) and were over the age of 16 years. We also performed cyto-

kine phenotyping on these samples, in addition to those from persons with familial and sporadic hyper-IgE syndrome (which included possibly and probably affected persons). Samples from 50 pa-tients and 48 family members were available for sequence analysis. The race or ethnic group of pa-tients was determined by a physician author and by self report (for details, see the Supplementary Appendix, available with the full text of this ar-ticle at www.nejm.org). Control DNA was col-lected according to several approved protocols from unaffected persons: 125 whites, 15 Hispan-ics, 4 blacks, 2 Asians, 1 white Hispanic, 1 black Hispanic, and 10 persons of unknown ancestry. Race or ethnic group of these control subjects was self-reported.

MICROARRAY ANALYSIS

We isolated polymorphonuclear leukocytes and peripheral-blood mononuclear cells from venous blood samples and carried out phagocytosis and microarray assays as previously described.[12,13] We labeled samples, allowed for hybridization of com-plementary RNA to microarrays (HU133 Plus 2.0, Affymetrix), and scanned the microarrays accord-ing to standard Affymetrix protocols. For each time point, we analyzed cells from 9 control donors of peripheral-blood mononuclear cells or 10 control donors of polymorphonuclear leukocytes and 7 pa-tients with the hyper-IgE syndrome, using a sepa-rate oligonucleotide array for each donor. One sample of stimulated control polymorphonuclear leukocytes for the 360-minute time point was lost owing to technical error.

We analyzed the gene-expression data by using Microarray Suite software, version 5.0 (Affymetrix), and GeneSpring software, version 5.0 (Silicon Ge-netics), as described previously,[12,13] but with modi-fications. We defined genes as being differentially expressed when all three of the following crite-ria were met: the gene was identified as "pres-ent" by the Microarray Suite software in 8 of the 9 samples of peripheral-blood mononuclear cells or 9 of the 10 samples of polymorphonuclear leukocytes from control subjects and in 6 of the 7 samples of cells from patients, the difference in gene expression between the samples from con-trol subjects and those from patients was twofold or greater, and the normalized signal intensity was at least 100. We carried out principal-component analysis using software from Partek and compared

the average difference intensity of transcript levels in cells from control subjects and patients with the use of Student's t-test. Complete microarray data are provided, with analysis, in Tables 1, 2, 3, 4, and 5 of the Supplementary Appendix. Raw data are available at the Gene Expression Omnibus Web site (www.ncbi.nlm.nih.gov/geo/) under accession number GSE8507. We visualized the implicated biochemical pathways using Ingenuity Pathways Analysis software (Ingenuity Systems).

CYTOKINES

Peripheral-blood mononuclear cells were prepared with the use of density centrifugation from heparinized whole blood, as previously described.[14] Cells were stimulated for 48 hours at 37°C in 5% carbon dioxide with phytohemagglutinin (1:100), phytohemagglutinin with interleukin-12 (10 ng per milliliter), lipopolysaccharide (200 ng per milliliter), lipopolysaccharide with interferon-γ (1000 IU per milliliter), or interleukin-6 (40 ng per milliliter). The supernatants were harvested and stored at –20°C until cytokine levels could be measured with the use of plate-based methods (Meso Scale Discovery) or bead-based methods (BioRad Luminex), according to the manufacturers' recommendations. The cytokine levels were compared with the use of a Mann–Whitney U test (GraphPad Software), with P values of less than 0.05 considered to indicate statistical significance.

SEQUENCING

Complementary DNA (cDNA) was prepared from peripheral-blood mononuclear cells and neutrophils of unrelated probands and control subjects and was sequenced for the signal transducer and activator of transcription 3 gene (*STAT3*) (for a list of primers, see the Methods in the Supplementary Appendix). Sequencing was performed on an Applied Biosystems 3100 sequencer and analyzed using Sequencher software (Gene Codes). We also sequenced cDNA from peripheral-blood leukocytes or transformed lymphoblastoid cell lines or genomic DNA of relatives. For sequencing of genomic DNA, polymerase-chain-reaction primers (available in the Supplementary Appendix) were chosen according to the proband's mutation. GenBank recognizes three human isoforms of *STAT3*: NP_644805.1, NP_003141.2, and NP_998827.1. The amino acid positions reported here are for the isoform NP_644805.1.

FLOW CYTOMETRY FOR DETECTION OF PHOSPHORYLATED STAT3

Peripheral-blood mononuclear cells from healthy adults or from patients with the hyper-IgE syndrome were isolated with the use of a Ficoll gradient. Peripheral-blood mononuclear cells (1×10^6) were incubated in 100 μl of phosphate-buffered saline with 2% fetal-calf serum, either without stimuli or with interleukin-6 (10 ng per milliliter) (R&D Systems), for 25 or 60 minutes. Cells were fixed, permeabilized, and stained, as previously described.[15] An Alexa Fluor 647 conjugated phospho-STAT3 antibody against phospho-S727 (BD Biosciences) was used to identify intracellular or intranuclear phospho-STAT3. Mouse IgG1 (BD Biosciences) was used as isotype control. Analysis was carried out on a FACSCalibur flow cytometer (BD Biosciences) with Cell Quest software (BD Biosciences).

BIOINFORMATICS ANALYSIS OF MISSENSE CHANGES IN STAT3

For the missense changes detected, we used bioinformatics methods encoded in the programs Sorting Intolerant from Tolerant (SIFT)[16] and PolyPhen.[17] SIFT reports the probability that the substitution is deleterious by predicting whether the substitution is or is not probably damaging. PolyPhen reports a score, with putatively benign changes scored from 0.00 to 1.50, possibly damaging changes scored from 1.51 to 2.00, and probably damaging changes scored as 2.01 or greater.

RESULTS

GENE-EXPRESSION PROFILING OF LEUKOCYTES

We found greater levels of expression of proinflammatory molecules in unstimulated and phagocytosis-stimulated leukocytes of patients with the hyper-IgE syndrome than in those of controls (Fig. 1 in the Supplementary Appendix and Tables 1, 2, 3, and 4 in the Supplementary Appendix). Thirty transcripts encoding proteins related to interferon signal transduction or typically induced by interferon showed elevated levels of expression in polymorphonuclear leukocytes from patients with the hyper-IgE syndrome (Table 1 in the Supplementary Appendix). Known or putative genes involved in host defense were up-regulated in polymorphonuclear leukocytes and peripheral-blood mononuclear cells from the patients (Fig. 1C in

Table 1. Stimulated Cytokine Production in Leukocytes from Patients with the Hyper-IgE Syndrome and in Those from Controls.*

Analyte and Stimulus	Patient	Control	P Value
		pg/ml	
TNF-α			
Unstimulated (LPS)	17.9±6.6 (N=38)	54.3±20.8 (N=32)	0.23
LPS	1368.0±192.3 (N=38)	597.3±86.8 (N=32)	0.003
LPS+interferon-γ	4244.0±382.5 (N=38)	3044.0±311.9 (N=32)	0.02
Unstimulated (SAC)	19.7±8.0 (N=31)	68.1±27.3 (N=24)	0.22
SAC	5651.0±519.9 (N=31)	2404.0±397.3 (N=24)	<0.001
HKLM	2807.0±364.8 (N=30)	862.4±101.7 (N=12)	<0.001
Interleukin-12p70			
Unstimulated	5.8±0.7 (N=31)	10.3±3.4 (N=24)	0.77
LPS	36.5±6.3 (N=30)	12.9±2.7 (N=23)	0.001
LPS+interferon-γ	837.1±132.2 (N=31)	385.3±93.0 (N=24)	0.003
Interferon-γ			
Unstimulated	36.4±4.5 (N=31)	43.7±7.4 (N=25)	0.75
PHA	127,610.0±17,956.0 (N=27)	71,238.0±11,382.0 (N=24)	0.03
PHA+interleukin-12	158,823.0±14,900.0 (N=29)	102,820.0±20,715.0 (N=23)	0.03
MCP-1			
Unstimulated	346.4±263.3 (N=10)	252.5±145.6 (N=6)	0.07
Interleukin-6	1761.0±878.7 (N=10)	4494.0±649.3 (N=6)	0.03

* Plus–minus values are means ±SD. TNF-α denotes tumor necrosis factor α, LPS lipopolysaccharide, SAC *Staphylococcus aureus* Cowan strain, HKLM heat-killed *Listeria monocytogenes,* and MCP-1 monocyte chemoattractant protein 1.

the Supplementary Appendix and Tables 1, 2, and 3 in the Supplementary Appendix). After phagocytosis, 15 genes encoding proteins related to interferon signal transduction or induced by interferon showed elevated levels of expression in polymorphonuclear leukocytes from patients, as compared with those from controls (Fig. 2 in the Supplementary Appendix and Table 4 in the Supplementary Appendix). Taken together, these data suggest that cytokine-related signal transduction,

Table 2. Results of STAT3 DNA Sequencing of Selected Probands and Family Members.*

Member	Race or Ethnic Group†	Hyper-IgE Syndrome Score‡	Mutation		
			Nucleotide and Amino Acid	Domain	Type
Family J001					
Proband	White	68	1144C→T, R382W	DNA binding	Sporadic
Daughter	White	55	1144C→T, R382W	DNA binding	Transmitted
Daughter	White	25	None		
Husband	White	1	None		
Family J002					
Proband	White	90	1865C→T, T622I	SH2	De novo
Daughter	White	80	1865C→T, T622I	SH2	Transmitted
Daughter	White	6	None		
Sister	White	16	None		
Sister	White	25	None		
Mother	White	4	None		
Father	White	33	None		
Wife	White	16	None		
Family J004					
Proband	White	87	1144C→T, R382W	DNA binding	Sporadic
Grandfather	White	Not scored	None		
Father	White	3	None		
Family J005					
Proband	White–Black	54	1144C→T, R382W	DNA binding	De novo
Father	Black	Not scored	None		
Mother	White	Not scored	None		
Family J006					
Proband	White	90	1151T→C, F384S	DNA binding	Sporadic
Family J007					
Proband	White	82	1268G→A, R423Q	DNA binding	Sporadic
Brother	White	4	None		
Sister	White	3	None		
Daughter	White	82	1268G→A, R423Q	DNA binding	Transmitted
Sister	White	24	None		
Niece	White	9	None		
Mother	White	6	None		
Family J008					
Proband	White	79	1145G→A, R382Q	DNA binding	De novo
Father	White	4	None		
Mother	White	3	None		
Family J009					
Proband	White	82	1832G→A, S611N	SH2	Sporadic

Table 2. (Continued.)

Member	Race or Ethnic Group†	Hyper-IgE Syndrome Score‡	Mutation		
			Nucleotide and Amino Acid	Domain	Type
Family J010					
Proband	White	85	1144C→T, R382W	DNA binding	De novo
Father	White	9	None		
Brother	White	1	None		
Sister	White	4	None		
Cousin	White	5	None		
Mother	White	0	None		
Family J011					
Proband	White	69	1909G→A, V637M	SH2	Transmitted
Sister	White	86	1909G→A, V637M	SH2	Transmitted
Mother	White	19	None		
Family J012					
Proband	White	84	1387delGTG, V463del	DNA binding	Sporadic
Family J013					
Proband	White	66	1150T→C, F384L	DNA binding	Sporadic
Family J014					
Proband	White	96	1909G→A, V637M	SH2	De novo
Father	White	14	None		
Mother	White	16	None		
Sister	White	38	None		
Sister	White	5	None		
Family J015					
Proband	White	96	1861T→G, F621V	SH2	Sporadic
Family J016					
Proband	Asian	65	1268G→A, R423Q	DNA binding	De novo
Son	Asian	34	1268G→A, R423Q	DNA binding	Transmitted
Daughter	Asian	10	None		
Daughter	Asian	34	1268G→A, R423Q	DNA binding	Transmitted
Wife	Asian	1	None		
Father	Asian	0	None		
Mother	Asian	1	None		
Family J017					
Proband	White	82	1145G→A, R382Q	DNA binding	Transmitted
Brother	White	3	None		
Father	White	48	1145A→G, R382Q mosaic	DNA binding	Sporadic
Mother	White	1	None		
Sister	White	6	None		
Brother	White	67	1145G→A, R382Q	DNA binding	Transmitted

Table 2. (Continued.)

Member	Race or Ethnic Group†	Hyper-IgE Syndrome Score‡	Mutation		
			Nucleotide and Amino Acid	Domain	Type
Family J020					
Proband	Black	100	1144C→T, R382W	DNA binding	Sporadic
Mother	Black	Not scored	None		
Family J021					
Proband	Black	71	1145G→A, R382Q	DNA binding	Sporadic
Family J022					
Proband	White	93	1909G→A, V637M	SH2	Sporadic
Family J029					
Proband	White	62	1939A→G, N647D	SH2	Transmitted
Mother	White	4	None		
Sister	White	52	None		
Sister	White	69	1939A→G, N647D	SH2	Transmitted
Niece	White	29	None		
Niece	White	56	1939A→G, N647D	SH2	Transmitted
Family J030					
Proband	Black	65	1145G→A, R382Q	DNA binding	De novo
Father	Black	4	None		
Mother	Black	10	None		
Family J035					
Proband	White	77	1909G→A, V637M	SH2	Sporadic
Daughter	White	76	1909G→A, V637M	SH2	Transmitted
Daughter	White	27	None		
Wife	White	1	None		
Family J045					
Proband	Hispanic	64	1144C→T, R382W	DNA binding	Sporadic
Family J053					
Proband	White	87	1915C→G, P639A	SH2	Sporadic
Family J054					
Proband	White	78	1393T→G, S465A	DNA binding	Sporadic
Family J068					
Proband	White	73	1144C→T, R382W	DNA binding	Sporadic
Family J074					
Proband	White	69	1954G→A, E652K	SH2	Sporadic
Husband	White	1	None		
Daughter	White	64	1954G→A, E652K	SH2	Transmitted
Daughter	White	54	1954G→A, E652K	SH2	Transmitted
Family J083					
Proband	White	84	1909G→A, V637M	SH2	Sporadic
Sister	White	36	None		
Mother	White	4	None		

Table 2. (Continued.)

Member	Race or Ethnic Group†	Hyper-IgE Syndrome Score‡	Mutation		
			Nucleotide and Amino Acid	Domain	Type
Family J087					
Proband	White	69	1387delGTG; V463del	DNA binding	Sporadic
Daughter	White	82	1387delGTG; V463del	DNA binding	Transmitted
Family J088					
Proband	White–Hispanic	76	1145G→T, R382L	DNA binding	Sporadic
Family J098					
Proband	White	65	1909G→T, V637L	SH2	Sporadic
Family J100					
Proband	Hispanic	81	1909G→A, V637M	SH2	Sporadic
Family J112					
Proband	White	52	1970A→G, Y657C	SH2	Sporadic
Son	White	31	1970A→G, Y657C	SH2	Transmitted
Family J113					
Proband	White	58	1145G→A, R382Q	DNA binding	Sporadic
Mother	White	Not scored	None		
Family J121					
Proband	White	65	1930delCAG; Q644del	SH2	Sporadic

* Probands and family members are included if DNA samples were obtained for sequencing. SH2 denotes SRC homology 2.
† Race or ethnic group was determined by a physician author or was self-reported.
‡ The hyper-IgE syndrome scores were arbitrarily categorized as follows: 0 to 15, unaffected; 16 to 39, possibly affected; 40 to 59, probably affected; and 60 or more, definitely affected.

such as that involving the interferons and STATs, is altered in patients with the hyper-IgE syndrome (Fig. 1D in the Supplementary Appendix).

CYTOKINE EXPRESSION IN LEUKOCYTES

Consistent with the microarray data, cytokine protein levels were elevated in the supernatants of leukocytes from patients with the hyper-IgE syndrome as compared with those of controls, depending on the stimulus (Table 1). For example, tumor necrosis factor α (TNF-α) levels were higher in the supernatants of leukocytes from patients with the hyper-IgE syndrome than in those from controls, after the leukocytes were stimulated by the toll-like receptor 4 agonist lipopolysaccharide alone (P=0.003) or by lipopolysaccharide and interferon-γ (P=0.03). TNF-α levels were also elevated in the supernatants from leukocytes of patients after exposure to the toll-like receptor 2 agonist heat-killed *Listeria monocytogenes* (P<0.001)

or to *S. aureus* Cowan strain (P=0.001). Similarly, stimulation with lipopolysaccharide or lipopolysaccharide and interferon-γ resulted in elevated interleukin-12p70 production in patients with the hyper-IgE syndrome, as compared with controls (P=0.001 and P=0.003, respectively). In response to the nonspecific T-cell mitogen phytohemagglutinin or phytohemagglutinin and interleukin-12, interferon-γ levels were also elevated in the patients (P=0.03 for both comparisons). In contrast, we found diminished production of monocyte chemoattractant protein 1 in leukocytes from patients, as compared with control leukocytes, after exposure to interleukin-6 (P=0.03) (Table 1).

In aggregate, these data indicated a defect leading to elevated cytokine production in response to certain stimuli but impaired signaling through the interleukin-6 receptor. Since the production of monocyte chemoattractant protein 1 after stimulus with interleukin-6 was impaired in the leu-

kocytes of patients with the hyper-IgE syndrome, we decided to investigate components of the interleukin-6 signal-transduction pathway.

STAT3 MUTATIONS IN THE HYPER-IgE SYNDROME

Expression levels of the interleukin-6 receptor (IL6R or CD126) and the interleukin-6 signal transducer (IL6ST, CD130, or gp130) were normal on cells from patients with the hyper-IgE syndrome (data not shown). We inferred that Janus kinase 1 was intact in cells from patients, because it mediates TNF-α production in response to interferon-γ. STAT3 is physically associated with gp130, and its deletion in animal models and inhibition in tumor-cell lines is associated with increased production of interferon-γ and TNF-α.[18] We therefore sequenced *STAT3* in DNA samples obtained from 50 persons affected with the hyper-IgE syndrome and 48 unaffected relatives from 35 independent and unrelated white, Asian, Hispanic, or black families (Table 2). Seven patients had de novo *STAT3* mutations, 17 had familial transmission of *STAT3* mutations, and 26 had sporadic *STAT3* mutations (defined as those for which the parents were reported to be clinically unaffected and, in cases in which DNA was available for analysis, did not carry a *STAT3* mutation), 1 of whom had chimerism for the *STAT3* mutation. All *STAT3* mutations localized to regions encoding the DNA-binding or SH2 domains; several mutational "hot spots" were apparent (Fig. 1). For example, 13 mutations from unrelated patients affected the arginine (R) residue at position 382 in the DNA-binding domain. Several other residues in the DNA-binding domain — phenylalanine (F) at position 384, arginine (R) at position 423, and valine (V) at position 463 — were each affected by at least two de novo mutations. The SH2 domain has a hot spot at position 637; we found seven mutations from unrelated patients that involved the valine (V) residue normally found at this position. All mutations are missense or in-frame deletions, consistent with protein expression, and affect residues included in all splice variants of *STAT3*.[19] We did not find any of the mutations in the 158 unaffected control subjects of various ancestral origins.

The hyper-IgE syndrome scores[9] generally correlated well with the *STAT3* genotypes. Parents of probands with sporadic mutations from whom DNA samples could be obtained did not have the *STAT3* mutation, 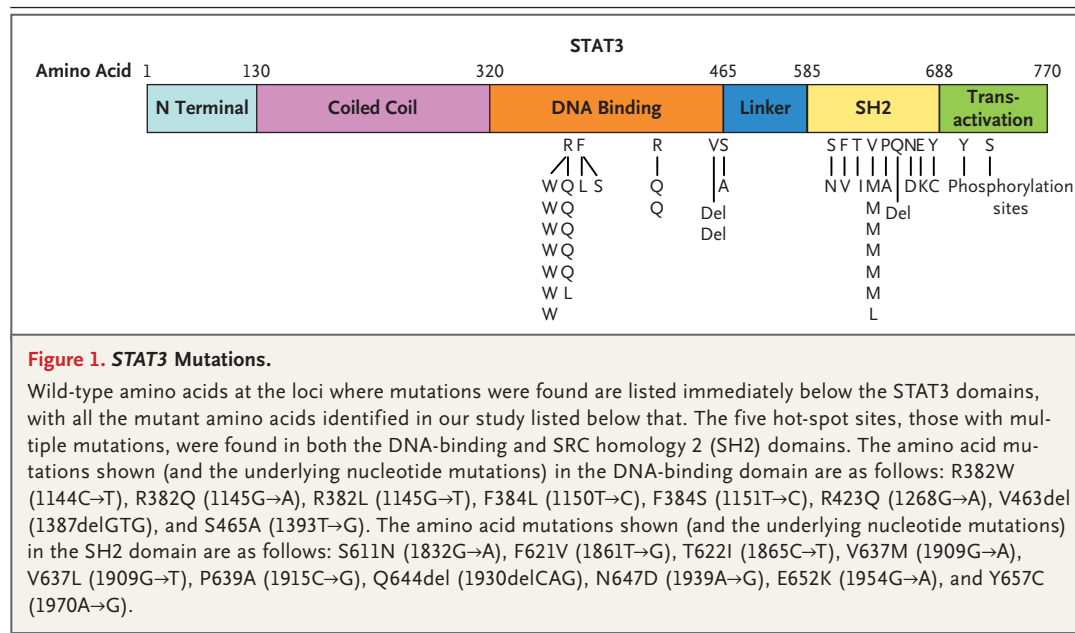indicating a substantial incidence of new mutations (Fig. 2). In Families J002, J014, and J016, the mutation in the proband was de novo and was associated with a high hyper-IgE syndrome score in the proband and offspring (if present), as well as with clinical disease. We could not collect DNA from both parents of every proband, but even in families in which DNA from one parent was available (such as Family J007), if a *STAT3* mutation occurred in a definitely affected individual, it was transmitted with disease as a stable autosomal dominant trait. Relatives of the proband who were possibly or probably affected (hyper-IgE syndrome scores of 16 to 59) did not necessarily have mutations, such as the father of the proband in Family J002 who had a hyper-IgE syndrome score of 33 and the sister in Family J014 who had a score of 31. Because the scoring system is tied to medical complications that accumulate over time, the scores of 34 in the children with a *STAT3* mutation in Family J016 are consistent with being affected with the hyper-IgE syndrome, since they were only 11 and 14 years old at the time of testing.

BIOINFORMATICS ANALYSIS WITH SIFT AND POLYPHEN

Thirteen of the 16 missense mutations identified are predicted to be possibly or probably damaging to STAT3 function according to at least one of the two methods (Table 6 in the Supplementary Appendix). R423Q, a recurrent mutation, is not predicted to be damaging but is clearly associated with disease in Families J007 and J016.

FUNCTIONAL STUDIES

Phosphorylation and expression of STAT3, as determined with the use of flow cytometry, was equivalent in the leukocytes from patients and those from controls (Fig. 3 in the Supplementary Appendix). The trafficking of STAT3 from the cytoplasm into the nucleus of leukocytes from patients was grossly normal (data not shown). Unlike in mouse models of *STAT3* mutation,[20] superoxide production by neutrophils from patients in response to opsonized zymosan and N-formyl-methionyl-leucyl-phenylalanine and phorbol myristate acetate was normal, as was killing of staphylococci by neutrophils in vitro (data not shown). Although interferon-γ and related proinflammatory cytokines prolong neutrophil survival, and our microarray suggested that this should be the case,[21] the rates of spontaneous and phagocytosis-induced apoptosis did not differ between poly-

Figure 1. *STAT3* Mutations.

Wild-type amino acids at the loci where mutations were found are listed immediately below the STAT3 domains, with all the mutant amino acids identified in our study listed below that. The five hot-spot sites, those with multiple mutations, were found in both the DNA-binding and SRC homology 2 (SH2) domains. The amino acid mutations shown (and the underlying nucleotide mutations) in the DNA-binding domain are as follows: R382W (1144C→T), R382Q (1145G→A), R382L (1145G→T), F384L (1150T→C), F384S (1151T→C), R423Q (1268G→A), V463del (1387delGTG), and S465A (1393T→G). The amino acid mutations shown (and the underlying nucleotide mutations) in the SH2 domain are as follows: S611N (1832G→A), F621V (1861T→G), T622I (1865C→T), V637M (1909G→A), V637L (1909G→T), P639A (1915C→G), Q644del (1930delCAG), N647D (1939A→G), E652K (1954G→A), and Y657C (1970A→G).

morphonuclear leukocytes from patients with the hyper-IgE syndrome and those from controls (data not shown). This is consistent with an inability of mutant STAT3 to promote cytokine-mediated delay of neutrophil apoptosis.[22]
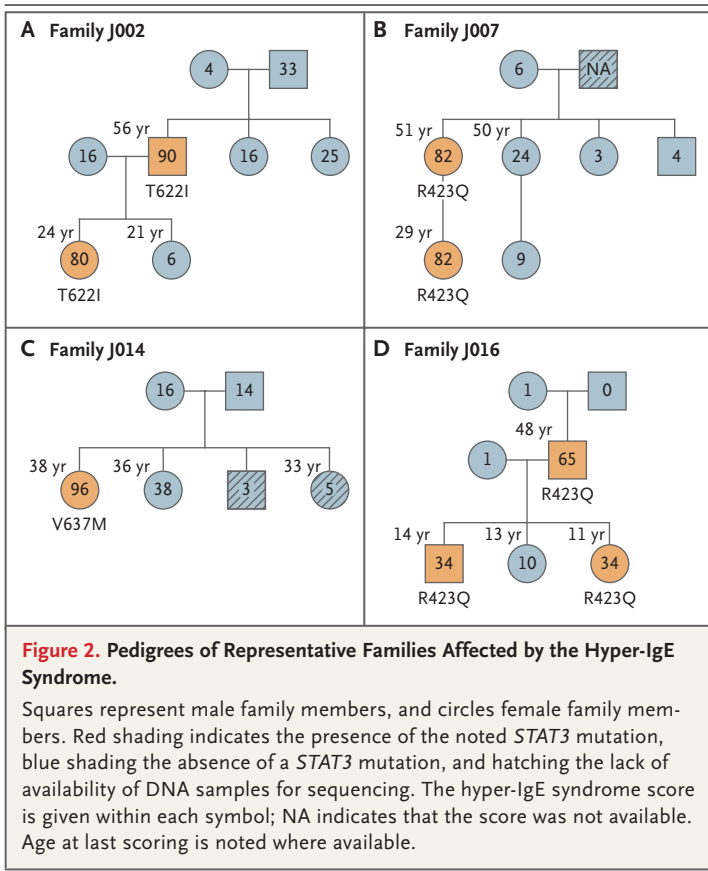
DISCUSSION

STAT3 mutation is the predominant cause of sporadic and familial hyper-IgE syndrome, although other genomic loci may also be involved. Within the gene encoding STAT3, we found mutational hot spots, indicated by independent mutations that affect the same codons, in the evolutionarily conserved SH2 and DNA-binding domains of STAT3. These mutations are predicted not to affect protein levels of STAT3 and are consistent with the survival of those carrying the mutations. In support of STAT3 as the cause of the hyper-IgE syndrome, Minegishi et al. found *STAT3* mutations affecting the DNA-binding domain in 8 of 15 patients examined.[23] Therefore, all mutations associated with the hyper-IgE syndrome identified to date are restricted to the DNA-binding or SH2 regions of *STAT3*. These mutations are likely to preserve protein levels, phosphorylation, and nuclear localization. Mice with half the normal dose of Stat3 seem unaffected, suggesting that haploinsufficiency is an unlikely pathogenic mechanism.[24]

A patient with a newly discovered primary immunodeficiency with mild IgE elevation due to homozygous recessive tyrosine kinase 2 deficiency has also been reported,[25] but this patient differs from our patients with classic hyper-IgE syndrome in the pattern of inheritance, susceptibility to viral infection, the degree of IgE elevation, and the absence of nonimmune features. Patients with the syndrome reported as autosomal recessive hyper-IgE syndrome also differ from those with the classic hyper-IgE syndrome described here in that they have fewer pulmonary infections and pneumatoceles, more viral infections, and vasculitis.[26] In keeping with these clinical differences, an index patient with autosomal recessive hyper-IgE syndrome from our original report[26] had no *STAT3* mutations (data not shown).

STAT3 mutations in the hyper-IgE syndrome clarify many disparate aspects of the syndrome that have puzzled investigators. Targeted mutations in mice show specific roles of Stat3 in organogenesis, organ preservation, and organ-specific inflammation, distinct from hematopoiesis. *Stat3* deficiency specific to the pulmonary epithelium in mice exposed to hyperoxia causes excessive lung inflammation and airspace enlargement,[27] and lung expression of *Stat3* is critical for repression of the inflammatory response to lipopolysaccharide[20]; these observations are consistent with the development of pneumatoceles after infection in patients with the hyper-IgE syndrome. Myeloid-specific *Stat3* deficiency causes increased myelopoiesis and eosinophilia in mice,[28]

A Family J002

4 — 33

56 yr

16 90 16 25
T622I

24 yr 21 yr

80 6
T622I

B Family J007

6 — NA

51 yr 50 yr

82 24 3 4
R423Q

29 yr

82 9
R423Q

C Family J014

16 — 14

38 yr 36 yr 33 yr

96 38 3 5
V637M

D Family J016

1 — 0

48 yr

1 65
R423Q

14 yr 13 yr 11 yr

34 10 34
R423Q   R423Q

Figure 2. Pedigrees of Representative Families Affected by the Hyper-IgE Syndrome.

Squares represent male family members, and circles female family members. Red shading indicates the presence of the noted *STAT3* mutation, blue shading the absence of a *STAT3* mutation, and hatching the lack of availability of DNA samples for sequencing. The hyper-IgE syndrome score is given within each symbol; NA indicates that the score was not available. Age at last scoring is noted where available.

and eosinophilia is a consistent feature of the hyper-IgE syndrome.[3] Hematopoiesis-specific *Stat3* deletion in the mouse is associated with osteoclast generation and osteopenia,[29] phenotypes also found in persons with the hyper-IgE syndrome.[30] Ligand binding of the receptor for interleukin-22 (composed of IL-22R1 and IL-10R2), expressed predominantly on epithelial cells (including those making up the skin and lung),[31] invokes STAT3-mediated expression of β-defensins.[32] Impaired defensin production in the skin and lung may explain the predilection for skin and lung abscesses in patients with the hyper-IgE syndrome. The depletion of Stat3 in mouse cardiac myocytes is associated with increased production of TNF-α by these cells and with cardiac inflammation and dysfunction,[33] consistent with coronary-artery aneurysms recently described in adult patients with the hyper-IgE syndrome.[5] Similarly, a deficiency of Stat3 in the mouse brain is associated with

increased inflammation, demyelination, and astrocytosis in response to nerve injury,[34] consistent with the parenchymal brain lesions found in persons with the hyper-IgE syndrome.[6]

STAT3 up-regulates myeloid adhesion,[35] expression of PU.1,[36] expression of secondary granule proteins in neutrophils,[37] the interleukin-23 receptor, the generation of interleukin-17–producing CD4+ T lymphocytes,[38] and the antiinflammatory effects of interleukin-10.[18] In contrast, *STAT3* down-regulates expression of *T-bet*, *GATA3*, *IL12Rb2*, and *IFNγ*,[38] as well as the formation of osteoclasts.[29] Therefore, *STAT3* deficiency leads to the up-regulation of many cytokines secreted by type 1 helper T cells (e.g., interferon-γ or TNF-α), and down-regulation of the inflammatory and antiinflammatory responses governed by interleukin-6 and interleukin-10.[20] Patients with the hyper-IgE syndrome have retarded and inadequate inflammatory responses in the skin, leading to cold abscesses, and destructive inflammation in the lung, leading to pneumatoceles. The extraordinary elevation of the IgE level in persons with the disorder, seen from birth through adulthood and uncorrelated with eosinophilia,[3] may reflect the known role of STAT3 in mediating interleukin-21 receptor signaling, since interleukin-21 receptor α–knockout mice have elevated IgE levels.[39]

In conclusion, the newly recognized genetic cause of the hyper-IgE syndrome — *STAT3* mutation — affects complex, compartmentalized somatic and immune regulation. The discovery of this genetic cause opens new doors to understanding organ-specific infection, inflammation, and therapy.

REFERENCES

1. Davis SD, Schaller J, Wedgwood RJ. Job's syndrome: recurrent, "cold," staphylococcal abscesses. Lancet 1966;1:1013-5.
2. Buckley RH, Wray BB, Belmaker EZ. Extreme hyperimmunoglobulinemia E and undue susceptibility to infection. Pediatrics 1972;49:59-70.
3. Grimbacher B, Holland SM, Gallin JI, et al. Hyper-IgE syndrome with recurrent infections — an autosomal dominant multisystem disorder. N Engl J Med 1999; 340:692-702.
4. Buckley RH. The hyper-IgE syndrome. Clin Rev Allergy Immunol 2001;20:139-54.
5. Ling JC, Freeman AF, Gharib AM, et al. Coronary artery aneurysms in patients with hyper IgE recurrent infection syndrome. Clin Immunol 2007;122:255-8.
6. Freeman AF, Collura-Burke CJ, Patronas NJ, et al. Brain abnormalities in patients with hyperimmunoglobulin E syndrome. Pediatrics 2007;119:e1121-e1125.
7. Freeman AF, Kleiner DE, Nadiminti H, et al. Causes of death in hyper-IgE syndrome. J Allergy Clin Immunol 2007;119: 1234-40.
8. Del Prete G, Tiri A, Maggi E, et al. Defective in vitro production of γ-interferon and tumor necrosis factor-α by circulating T cells from patients with the hyperimmunoglobulin E syndrome. J Clin Invest 1989;84:1830-5.
9. Grimbacher B, Schäffer AA, Holland SM, et al. Genetic linkage of hyper-IgE syndrome to chromosome 4. Am J Hum Genet 1999;65:735-44.
10. Chehimi J, Elder M, Greene J, et al. Cytokine and chemokine dysregulation in hyper-IgE syndrome. Clin Immunol 2001; 100:49-56.
11. Tanaka T, Takada H, Nomura A, Ohga S, Shibata R, Hara T. Distinct gene expression patterns of peripheral blood cells in hyper-IgE syndrome. Clin Exp Immunol 2005;140:524-31.
12. Kobayashi SD, Voyich JM, Buhl CL, Stahl RM, DeLeo FR. Global changes in gene expression by human polymorphonuclear leukocytes during receptor-mediated phagocytosis: cell fate is regulated at the level of gene expression. Proc Natl Acad Sci U S A 2002;99:6901-6.
13. Kobayashi SD, Voyich JM, Braughton KR, et al. Gene expression profiling provides insight into the pathophysiology of chronic granulomatous disease. J Immunol 2004;172:636-43.
14. Holland SM, Dorman SE, Kwon A, et al. Abnormal regulation of interferon-γ, interleukin-12, and tumor necrosis factor-α in human interferon-γ receptor 1 deficiency. J Infect Dis 1998;178:1095-104.
15. Fleisher TA, Dorman SE, Anderson JA, Vail M, Brown MR, Holland SM. Detection of intracellular phosphorylated STAT-1 by flow cytometry. Clin Immunol 1999;90:425-30.
16. Ng PC, Henikoff S. Accounting for human polymorphisms predicted to affect function. Genome Res 2002;12:436-46.
17. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res 2002;30:3894-900.
18. Murray PJ. The JAK-STAT signaling pathway: input and output integration. J Immunol 2007;178:2623-9.
19. Maritano D, Sugrue ML, Tininini S, et al. The STAT3 isoforms alpha and beta have unique and specific functions. Nat Immunol 2004;5:401-9.
20. Welte T, Zhang SSM, Wang T, et al. STAT3 deletion during hematopoiesis causes Crohn's disease-like pathogenesis and lethality: a critical role of STAT3 in innate immunity. Proc Natl Acad Sci U S A 2003;100:1879-84.
21. Colotta F, Re F, Polentarutti N, Sozzani S, Mantovani A. Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. Blood 1992;80:2012-20.
22. Sakamoto E, Hato F, Kato T, et al. Type I and type II interferons delay human neutrophil apoptosis via activation of STAT3 and up-regulation of cellular inhibitor of apoptosis 2. J Leukoc Biol 2005;78:301-9.
23. Minegishi Y, Saito M, Tsuchiya S, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature 2007;448:1058-62.
24. Takeda K, Noguchi K, Shi W, et al. Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality. Proc Natl Acad Sci U S A 1997;94:3801-4.
25. Minegishi Y, Saito M, Morio T, 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.
26. Renner ED, Puck JM, Holland SM, et al. Autosomal recessive hyperimmunoglobulin E syndrome: a distinct disease entity. J Pediatr 2004;144:93-9.
27. Hokuto I, Ikegami M, Yoshida M, et al. Stat-3 is required for pulmonary homeostasis during hyperoxia. J Clin Invest 2004;113:28-37.
28. Panopoulos AD, Zhang L, Snow JW, et al. STAT3 governs distinct pathways in emergency granulopoiesis and mature neutrophils. Blood 2006;108:3682-90.
29. Zhang Z, Welte T, Troiano N, Maher SE, Fu X-F, Bothwell ALM. Osteoporosis with increased osteoclastogenesis in hematopoietic cell-specific STAT3-deficient mice. Biochem Biophys Res Commun 2005;328:800-7.
30. Freeman AF, Ling J, Collins M, et al. Minimal trauma fractures and reduced bone mineral density in hyper IgE syndrome. Presented at the Pediatric Academic Societies' Annual Meeting, Toronto, May 5–8, 2007. abstract.
31. Wolk K, Sabat R. Interleukin-22: a novel T- and NK-cell derived cytokine that regulates the biology of tissue cells. Cytokine Growth Factor Rev 2006;17:367-80.
32. Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R. IL-22 increases the innate immunity of tissues. Immunity 2004;21:241-54.
33. Jacoby JJ, Kalinowski A, Liu M-G, et al. Cardiomyocyte-restricted knockout of STAT3 results in higher sensitivity to inflammation, cardiac fibrosis, and heart failure with advanced age. Proc Natl Acad Sci U S A 2003;100:12929-34.
34. Okada S, Nakamura M, Katoh H, et al. Conditional ablation of Stat3 or Socs3 discloses a dual role for reactive astrocytes after spinal cord injury. Nat Med 2006;12:829-34.
35. Wooten DK, Xie X, Bartos D, et al. Cytokine signaling through Stat3 activates integrins, promotes adhesion, and induces growth arrest in the myeloid cell line 32D. J Biol Chem 2000;275:26566-75.
36. Panopoulos AD, Bartos D, Zhang L, Watowich SS. Control of myeloid-specific integrin alpha$_M$beta$_2$ (CD11b/CD18) expression by cytokines is regulated by Stat3-dependent activation of PU.1. J Biol Chem 2002;277:19001-7.
37. Wang L, Arcasoy MO, Watowich SS, Forget BG. Cytokine signals through STAT3 promote expression of granulocyte secondary granule proteins in 32D cells. Exp Hematol 2005;33:308-17.
38. Yang XO, Panopoulos AD, Nurieva R, et al. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. J Biol Chem 2007;282:9358-63.
39. Ozaki K, Spolski R, Feng CG, et al. A critical role for IL-21 in regulating immunoglobulin production. Science 2002;298: 1630-4.

Copyright © 2007 Massachusetts Medical Society.