

Hyper-IgE Syndrome Is Not Associated With Defects in Several Candidate Toll-Like Receptor Pathway Genes

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ABSTRACT: The genetic basis of hyper-IgE syndrome (HIES), also known as Job syndrome, a primary immunodeficiency associated with recurrent skin and pulmonary infections, is unknown. We hypothesized that HIES is due to a defect in the Toll-like receptor signaling pathway. We used a whole blood cytokine assay to compare inflammatory responses to stimulation with specific Toll-like receptor (TLR) pathway agonists in four individuals with HIES and nine healthy controls. Production of tumor necrosis factor- α , interleukin (IL)-1 β , IL-6, and IL-12 was not impaired in response to stimulation with lipopolysaccharide, peptidoglycan, zymosan, lipoteichoic acid, *Staphylococcus aureus*, *Escherichia coli*, or *Streptococcus pneumoniae*. Interferon (IFN)- γ was reduced in HIES subjects in response to each of these stimuli. We sequenced several candidate genes from the TLR pathway in HIES individuals to determine whether any mutations were

associated with this syndrome. No novel mutations or polymorphisms were found in the coding regions of TLR1, TLR2, TLR6, MyD88, or TRAF6. In summary, although HIES individuals had an IFN- γ secretion defect, they also produced normal levels of several TLR-regulated proinflammatory cytokines. No unique mutations or polymorphisms were observed in several candidate genes from the TLR pathway. Our studies do not support a role for a defective TLR response in HIES individuals. *Human Immunology* 66, 842–847 (2005). © American Society for Histocompatibility and Immunogenetics, 2005. Published by Elsevier Inc.

KEYWORDS: inflammation; innate immunity; genetic predisposition to disease; hyper-IgE syndrome; Toll-like receptor

ABBREVIATIONS

HIES hyper-IgE syndrome
LPS lipopolysaccharide
LTA lipoteichoic acid

PGN peptidoglycan
TLR Toll-like receptor

INTRODUCTION

Hyper-IgE syndrome (HIES, also known as Job syndrome) is characterized by recurrent “cold” abscesses, pneumonia, and high levels of IgE. Additional features often include recurrent pneumonias complicated by

pneumatocele formation, bronchiectasis, recurrent candidiasis, distinctive facial features, bone fractures, eczema, delayed shedding of primary teeth, hyperextensible joints, scoliosis, and craniosynostosis [1]. The skin and pulmonary abscesses are most commonly caused by *S. aureus*, but other pathogens are also often involved, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Aspergillus* species. The genetic basis of HIES is unknown. Although many cases occur sporadically, autosomal-dominant and autosomal-recessive forms of this syndrome have been described [2, 3]. The autosomal-dominant form of the disease has been linked to chromosome 4q in a linkage analysis.

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Toll-like receptors (TLRs) are critical orchestrators of the innate immune response that recognize pathogens, regulate inflammatory signaling pathways, and influence formation of the adaptive immune response [4, 5]. We hypothesized that TLR pathway genes are associated with HIES because of the severe inflammatory defect in this syndrome [6], the central role of TLRs in recognizing *S. aureus* and other bacterial and fungal pathogens, the presence of several TLR genes on chromosome 4 (TLR1, TLR2, and TLR6), and the similarity of several human clinical features with phenotypes in mice with TLR pathway gene deletions (MyD88 and TRAF6) [7, 8]. Here, we use functional and genetic studies to examine the hypothesis that HIES is caused by a mutation in a TLR-pathway gene.

MATERIALS AND METHODS

Human Subjects and Data Collection

Approval for human study protocols was obtained from the human subjects review boards at the University of Washington Medical Center, the Western Institutional Review Board (for the Institute for Systems Biology), and the University of Utah Medical Center.

Ex Vivo Whole Blood Cytokine Assay

Whole blood cytokine assays were prepared by diluting venous blood 1:5 with RPMI (Life Technologies), plating in a 96-well dish, stimulating for 18 hours, and then harvesting supernatants, as previously described [9]. Stimuli included the following: ultrapure lipopolysaccharide (LPS) at 100 ng/ml, from *Salmonella* Minnesota R595 (List Biological Labs), zymosan at 125 μ g/ml (Molecular Probes), *S. aureus* peptidoglycan (PGN) at 30 μ g/ml (Sigma Fluka), and *S. aureus* lipoteichoic acid (LTA) at 100 μ g/ml (Sigma). Bacterial stimuli included *S. aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *S. pneumoniae* (ATCC 49619) and were used at 5×10^6 particles/ml. Bacteria were grown in tryptic soy broth (Remel) to stationary phase, washed in phosphate-buffered saline, and heat-killed at 65°C for 15 minutes. All stimuli except LPS and *E. coli* were incubated with polymyxin B at 10 μ g/ml for 30 minutes before addition to the assay. Cytokine levels were determined with a sandwich enzyme-linked immunosorbent assay (ELISA) technique (Duoset; R&D Systems). Statistical analysis was performed by comparing mean cytokine levels in the control and HIES groups with an unpaired Student's *t*-test.

Molecular Biology

Genomic DNA was purified from peripheral blood leukocytes. From 10 ml of heparinized blood, the red blood cells were lysed with 0.75% N H₄Cl/0.1% KHCO₃/1

mM EDTA and the leukocytes purified by centrifugation. The leukocytes were subsequently lysed in 0.2% SDS/200 mM NaCl/5 mM EDTA/100 mM Tris-HCl, pH 8.5. After brief centrifugation, the genomic DNA was precipitated with isopropanol, isolated by centrifugation, purified with a phenol/chloroform extraction and resuspended in 1 \times TE. Single nucleotide polymorphism (SNP) discovery and genotyping was performed by polymerase chain reaction (PCR) amplification of the genes of interest from genomic DNA, followed by sequencing. The forward and reverse primers for the indicated PCR products for each of the candidate genes are listed in Table 1. Sequencing reactions were performed with the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The sequencing was then determined with an Applied Biosystems 3730 DNA analyzer. Sequence was aligned and analyzed with the programs PHRED/PHRAP and CONSED [10].

RESULTS

We hypothesized that defects in TLRs that recognize gram-positive and fungal pathogens are associated with susceptibility to HIES. To screen for these defects, we used a whole blood cytokine assay from four individuals with HIES and nine controls to characterize the innate immune response to a panel of TLR ligands. The clinical features of the four HIES individuals are summarized in Table 2. These individuals had classic features of HIES consistent with the autosomal-dominant or sporadic forms of the syndrome, with IgE levels greater than 1000 IU/ml [1–3]. We stimulated whole blood for 18 hours and then measured cytokine levels by ELISA (Figure 1). Total leukocyte and lymphocyte counts were similar for all participants. The stimuli included several different TLR 1, 2, and 6 ligands: zymosan (model fungal stimulus), peptidoglycan from *S. aureus*, heat-killed *S. aureus*, and heat-killed *Streptococcus pneumoniae*. In addition, we used LPS and heat-killed *E. coli* as TLR4 stimuli. Whole blood from HIES and control individuals contained similar levels of interleukin (IL)-6 and IL-1 β in response to most of the stimuli tested.

For tumor necrosis factor (TNF)- α and IL-12, substantial cytokine levels were again detected for both the controls and HIES individuals. For some of the stimuli, there was a slight increase in TNF- α and IL-12 levels in the HIES subjects compared with controls (Figures 1A,D). The mean difference in TNF- α levels was significantly different for whole blood stimulated with PGN, LTA, LPS, *S. aureus*, and *E. coli* ($p < 0.05$, Student's *t*-test). For IL-12, the mean difference in cytokine levels was significantly different for PGN, LTA, LPS, and *E. coli* ($p < 0.05$). In contrast to TNF- α and IL-12, HIES individuals had decreased levels of interferon (IFN)- γ compared with

TABLE 1 Primers for PCR

PCR product	Name (Direction) ^a	Sequence (5'–3')
TLR1	TLR1-1 (F)	GTCAGCCATGACTAATTTTC
	TLR1-11 (R)	GCTGTAGAAATCTGCTATAC
TLR2-A (5')	TH31 (F)	GGT CCC AAA GCA TGC TAC TCC TGG
	TH28 (R)	CATAACCTGAAACAAACT TTCATC GG
TLR2-B (3')	TH20 (F)	TCCATTTTTTCAGAACTATCCACTGG
	TH24 (R)	TCCTCAAATGACGGTACATCCACG
TLR6-A (5')	T6-2 (F)	GTGGAGGTT TGAGAGTAA CCATCCG
	T6-18 (R)	GGCATATCC TTCGTCATG AGACC
TLR6-B (3')	T6-3 (F)	CACATGCTG TGTCTCATGCACCAAGC
	T6-16 (R)	GGCTAACCTCACCGCCTAGCTCAGTTCCCC
MyD88-A (5')	MyD88-15 (F)	GCAGATTCTACTTCTTACGCC
	MyD88-24 (R)	CCAGAGGTAGGACTATTATCTCC
MyD88-B (3')	MyD88-13 (F)	CTCATCTTTCCTCTCCTGGAAAGGGC
	MyD88-25 (R)	GGACATGAAATGTGATGTCCAGC
TRAF6-Exon2	2.5 (F)	GTG TGC TAA GTA CTG CGG
	2.3 (R)	AGT CAC TCC CCA GTC TGC
TRAF6-Exon3	3.5 (F)	GCT GGA ATC TAT CCC ACC AT
	3.3 (R)	AGG GTT GGC ATT AAT TCA GC
TRAF6-Exon4	4.5 (F)	GTT CTG TGG TGT GCA TTA GA
	4.3 (R)	TAG ACT CAG AGT CGG AGT CA
TRAF6-Exon5	5.5 (F)	ATA CAG GTG TAC CAC CTC AG
	5.3 (R)	CTG GAT GTT TTG TTC TCC CT
TRAF6-Exon6	6.5 (F)	CAC CTA TTC AAC TGC CAA TG
	6.3 (R)	GTA TGC CTT TGC TTC TGC TC
TRAF6-Exon7	7.5 (F)	CCC TGG ATT CTA CAC TGG
	7.3 (R)	CAA GGC GGT AGT GAT TTT CA

^a Direction is designated as forward (F) or reverse (R).

controls for all of the tested stimuli (Figure 1E). These differences were statistically significant for *S. aureus*, *E. coli*, and *S. pneumoniae* ($p < 0.05$). Overall, these results indicate that HIES subjects have a selective defect in IFN- γ production in whole blood that is stimulated with

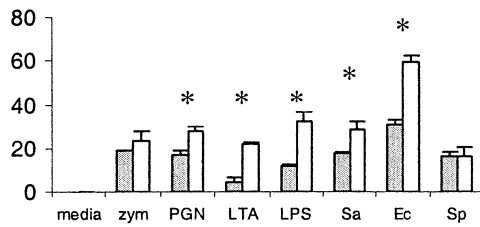
TABLE 2 Clinical features of four patients with hyper-IgE syndrome

Characteristic	Patient			
	1	2	3	4
Sex	M	F	F	M
Age (years)	24	45	30	45
Recurrent skin abscesses	Y	Y	Y	Y
Recurrent pneumonia	Y	Y	Y	Y
Pneumatoceles	Y	Y	Y	Y
Bronchiectasis	N	Y	Y	Y
Recurrent candidiasis	Y	Y	Y	Y
Recurrent fractures	N	Y	Y	Y
Coarse facies	Y	Y	Y	Y
Eczema	Y	Y	Y	Y
Elevated IgE ^a	Y	Y	Y	Y
Elevated eosinophils ^a	Y	Y	Y	Y
Delayed dental shedding	Y	Y	Y	Y
Scoliosis	Y	Y	Y	Y

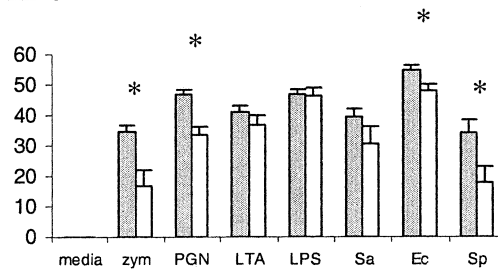
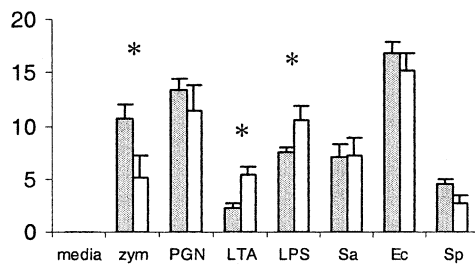
^a Values of IgE >1000 IU/ml and eosinophils >500 cells/mm³ are considered elevated.

a broad range of TLR ligands, including TLR1, 2, 4, and 6 agonists. However, this defect does not involve several other cytokines, including TNF- α and IL-12, that have higher levels in HIES subjects compared with controls.

In light of this cytokine production defect in HIES, we selected several TLR pathway genes for genetic analysis. In addition to the four subjects summarized in Table 2, we also analyzed DNA from seven additional HIES subjects that have been previously described [11]. The ethnic background of these 11 HIES subjects was white. We PCR-amplified and sequenced the coding region of TLR1, TLR2, TLR6, MyD88, and TRAF6 because of their central role in detecting gram-positive and fungal pathogens and mediating the innate immune response. For all five genes, we sequenced the entire coding region for at least five individuals. In addition, we had more than 75% of the coding region sequenced for at least an additional three people. For TLR1 and MyD88, we had complete coverage of the coding region in all 11 subjects. We detected several synonymous and nonsynonymous polymorphisms (Table 3). None of the nonsynonymous polymorphisms caused a nonsense mutation. All of the detected polymorphisms have been described previously and are annotated in the dbSNP database at the National Center for Biotechnology Information (NCBI). These results indicate that HIES is not

A. TNF- α 

B. IL-6

C. IL-1 β 

D. IL-12

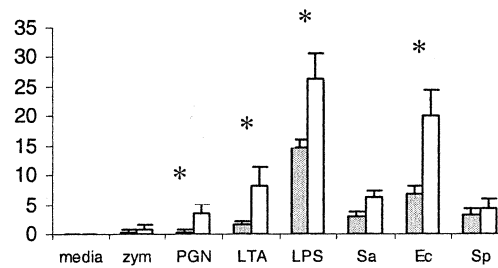
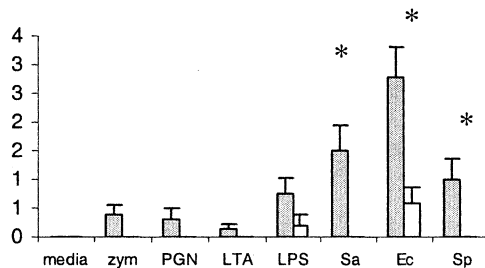
E. IFN- γ 

FIGURE 1 Whole blood cytokine response after TLR stimulation in HIES individuals. Whole blood was obtained from four HIES individuals (open boxes) and nine controls (gray boxes) and stimulated with different TLR agonists for 18 hours. Cytokine levels (ng/ml) were measured by ELISA. zym, zymosan; PGN, peptidoglycan; LTA, lipoteichoic acid; LPS, lipopolysaccharide; Sa, heat-killed *S. aureus*; Ec, heat-killed *E. coli*; Sp, heat-killed *S. pneumoniae*. Data represent mean cytokine values \pm SEM. * = $p < 0.05$ by Student's t-test.

associated with a unique or severe coding region mutation in any of these TLR pathway genes.

DISCUSSION

We examined the hypothesis that mutations in TLR pathway genes are associated with susceptibility to HIES. We did not observe any unique mutations associated with HIES in five critical TLR pathway genes. We selected these candidate genes for several reasons. First, staphylococcal abscesses and pneumatoceles in HIES are "cold," indicating a severe *in vivo* inflammatory and innate immune response defect. Mutations in TLRs

could profoundly impair inflammatory pathways as a result of their critical regulatory role of the innate immune response. Second, TLRs 1, 2, and 6 map to chromosome 4, where the autosomal-dominant form of HIES is genetically linked [2]. These TLRs also mediate recognition of a broad range of bacteria and fungi, including gram-positive organisms such as *S. aureus*. Third, mutations and polymorphisms in the TLR pathway have already been associated with susceptibility to different infections and inflammatory defects [12–14]. Finally, mice with gene deletions of TLR signaling pathway genes have some phenotypes that are similar to HIES. MyD88-deficient mice have decreased production of

TABLE 3 DNA polymorphisms in HIES subjects^a

Gene (chrom)	SNP BP	SNP AmAc	Genotype			dbSNP
			AA	Aa	aa	
TLR1 (4p14)	G239C	R80T	7	2	2	rs5743611
	A743G	N248S	9	2	0	rs4833095
	A1518G	S506S	0	2	9	rs5743614
	T1805G	I602S	1	1	9	rs5743618
TLR2 (4q32)	C597T	N199N	1	6	3	rs3804099
	T1350C	S450S	7	2	1	rs3804100
	G2258A	R753Q	9	1	0	rs5743708
	G2343A	A781A	9	1	0	rs5743709
TLR6 (4p14)	C745T	P249S	2	4	2	rs5743810
	C1083G	T361T	4	3	1	rs3821985
	A1263G	K421K	5	2	1	rs3775073
	T1932G	A644A	4	4	0	rs5743818
MyD88 (3p22)	None					
TRAF6 (11p13)	None					

^a The coding region of candidate genes was amplified by PCR from genomic DNA and sequenced. AmAc = amino acid; BP = base pair; chrom = chromosomal location. Genotypes: AA = homozygous wild type; Aa = heterozygote; aa = homozygous for uncommon allele. dbSNP rs number is from the single nucleotide polymorphism database at the National Center for Biotechnology Information.

IFN- γ by antigen-stimulated T cells as well as elevated levels of serum IgE [7]. TRAF6 knockout mice have defects in bone metabolism (including tooth eruption defects) and cytokine signaling [8]. Together, these studies suggest a potential role for the TLR pathway in HIES pathogenesis. However, we were unable to detect an obvious mutation after screening five TLR pathway genes. Although we had small numbers of subjects and cannot definitively rule out a primary role for these five genes in HIES pathogenesis, the data from our combined genetic and functional studies do not support this hypothesis.

The functional cytokine studies did not reveal an HIES defect in secretion of several proinflammatory cytokines that are regulated by TLR pathways, including TNF α , IL-1 β , IL-6, and IL-12. In contrast, we did find a pronounced defect in IFN- γ production in HIES subjects, an observation that has been described previously [11, 15–17]. Individuals with IL-12 and IL-12R β 1 deficiency also have impaired IFN- γ production in response to stimuli such as LPS [18–21]. Despite a similar cytokine defect, HIES individuals have a markedly different clinical phenotype compared with patients with IL-12/IL-12R β 1 mutations. IFN- γ is predominantly produced by T cells and NKT cells that are influenced by TLR-dependent IL-12 secretion from monocytes, macrophages, and dendritic cells. The absence of a functional defect in secretion of IL-12 or several other proinflammatory cytokines suggests that the IFN- γ defect in HIES individuals is not directly regulated by TLR pathways. Interestingly, HIES subjects had higher levels of TNF- α

and IL-12 for several of the stimuli compared with controls. These higher levels might be generated by influences from accessory pathways that are unrelated to TLR stimulation. For example, the decreased levels of IFN- γ in HIES subjects may affect the activation state of monocytes and macrophages and subsequently influence cytokine production. Speculation about such mechanisms is difficult without an understanding of the specific genetic defect in HIES. Genes from T-cell or NKT-cell signaling pathways may be more promising candidates for HIES pathogenesis. Taken together, our studies do not support a role for mutations in TLR pathway genes and HIES pathogenesis.

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REFERENCES

- Grimbacher B, Holland SM, Gallin JI, Greenberg F, Hill SC, Malech HL, Miller JA, O'Connell AC, Puck JM: Hyper-IgE syndrome with recurrent infections—an autosomal dominant multisystem disorder. *N Engl J Med* 340:692, 1999.
- Grimbacher B, Schaffer AA, Holland SM, Davis J, Gallin JI, Malech HL, Atkinson TP, Belohradsky BH, Buckley RH, Cossu F, Espanol T, Garty BZ, Matamoros N, Myers LA, Nelson RP, Ochs HD, Renner ED, Wellinghausen N, Puck JM: Genetic linkage of hyper-IgE syndrome to chromosome 4. *Am J Hum Genet* 65:735, 1999.
- Renner ED, Puck JM, Holland SM, Schmitt M, Weiss M, Frosch M, Bergmann M, Davis J, Belohradsky BH, Grimbacher B: Autosomal recessive hyperimmunoglobulin E syndrome: a distinct disease entity. *J Pediatr* 144:93, 2004.
- Aderem A, Ulevitch RJ: Toll-like receptors in the induction of the innate immune response. *Nature* 406(6797):782, 2000.
- Akira S, Takeda K: Toll-like receptor signalling. *Nat Rev Immunol* 4:499, 2004.
- Hill HR, Ochs HD, Quie PG, Clark RA, Pabst HF, Klebanoff SJ, Wedgwood RJ: Defect in neutrophil granulocyte chemotaxis in Job's syndrome of recurrent "cold" staphylococcal abscesses. *Lancet* 2(7881):617, 1974.
- Schnare M, Barton GM, Holt AC, Takeda K, Akira S, Medzhitov R: Toll-like receptors control activation of adaptive immune responses. *Nat Immunol* 2:947, 2001.
- Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, Morony S, Capparelli C, Van G, Kaufman S, van der Heiden A, Itie A, Wakeham A, Khoo W, Sasaki T, Cao Z, Penninger JM, Paige CJ, Lacey DL, Dunstan CR, Boyle WJ, Goeddel DV, Mak TW: TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev* 13:1015, 1999.

9. Hallstrand TS, Ochs HD, Zhu Q, Liles WC: Inhaled IFN-gamma for persistent nontuberculous mycobacterial pulmonary disease due to functional IFN-gamma deficiency. *Eur Respir J* 24:367, 2004.
10. Gordon D, Abajian C, Green P: Consed: a graphical tool for sequence finishing. *Genome Res* 8:195, 1998.
11. Borges WG, Augustine NH, Hill HR: Defective interleukin-12/interferon-gamma pathway in patients with hyper-immunoglobulinemia E syndrome. *J Pediatr* 136:176, 2000.
12. Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL, Schwartz DA: TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 25:187, 2000.
13. Hawn TR, Verbon A, Lettinga KD, Zhao LP, Li SS, Laws RJ, Skerrett SJ, Beutler B, Schroeder L, Nachman A, Ozinsky A, Smith KD, Aderem A: A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to Legionnaires' disease. *J Exp Med* 198:1563, 2003.
14. Picard C, Puel A, Bonnet M, Ku CL, Bustamante J, Yang K, Soudais C, Dupuis S, Feinberg J, Fieschi C, Elbim C, Hitchcock R, Lammas D, Davies G, Al-Ghonaïm A, Al-Rayes H, Al-Jumaah S, Al-Hajjar S, Al-Mohsen IZ, Frayha HH, Rucker R, Hawn TR, Aderem A, Tufenkeji H, Haraguchi S, Day NK, Good RA, Gougerot-Pocidallo MA, Ozinsky A, Casanova JL: Pyogenic bacterial infections in humans with IRAK-4 deficiency. *Science* 299(5615):2076, 2003.
15. Del Prete G, Tiri A, Maggi E, De Carli M, Macchia D, Parronchi P, Rossi ME, Pietrogrande MC, Ricci M, Romagnani S: Defective *in vitro* production of gamma-interferon and tumor necrosis factor- α by circulating T cells from patients with the hyper-immunoglobulin E syndrome. *J Clin Invest* 84:1830, 1989.
16. Ito R, Mori M, Katakura S, Kobayashi N, Naruto T, Osamura Y, Aihara Y, Yokota S: Selective insufficiency of IFN-gamma secretion in patients with hyper-IgE syndrome. *Allergy* 58:329, 2003.
17. Ohga S, Nomura A, Ihara K, Takahata Y, Suga N, Akeda H, Shibata R, Okamura J, Kinukawa N, Hara T: Cytokine imbalance in hyper-IgE syndrome: reduced expression of transforming growth factor beta and interferon gamma genes in circulating activated T cells. *Br J Haematol* 121:324, 2003.
18. Altare F, Durandy A, Lammas D, Emile JF, Lamhamedi S, Le Deist F, Drysdale P, Jouanguy E, Doffinger R, Bernardin F, Jeppsson O, Gollob JA, Meinel E, Segal AW, Fischer A, Kumararatne D, Casanova JL: Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. *Science* 280(5368):1432, 1998.
19. Altare F, Lammas D, Revy P, Jouanguy E, Doffinger R, Lamhamedi S, Drysdale P, Scheel-Toellner D, Girdlestone J, Darbyshire P, Wadhwa M, Dockrell H, Salmon M, Fischer A, Durandy A, Casanova JL, Kumararatne DS: Inherited interleukin 12 deficiency in a child with bacille Calmette-Guérin and *Salmonella enteritidis* disseminated infection. *J Clin Invest* 102:2035, 1998.
20. Casanova JL, Abel L: Genetic dissection of immunity to mycobacteria: the human model. *Annu Rev Immunol* 20:581, 2002.
21. de Jong R, Altare F, Haagen IA, Elferink DG, Boer T, van Breda Vriesman PJ, Kabel PJ, Draaisma JM, van Dissel JT, Kroon FP, Casanova JL, Ottenhoff TH: Severe mycobacterial and *Salmonella* infections in interleukin-12 receptor-deficient patients. *Science* 280(5368):1435, 1998.