ELSEVIER

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)-1β, 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 μg/ml (Molecular Probes), *S. aureus* peptidoglycan (PGN) at 30 μg/ml (Sigma Fluka), and *S. aureus* lipoteichoic acid (LTA) at 100 μg/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 μg/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 H_4Cl/0.1% $KHCO_3$/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× 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-1β 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