Very Late-Onset Group B Streptococcus Meningitis, Sepsis, and Systemic Shigellosis due to Interleukin-1 Receptor–Associated Kinase-4 Deficiency

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We describe a child with very late-onset group B Streptococcus sepsis and meningitis, systemic shigellosis, and chronic osteomyelitis. Peripheral blood cells obtained from the patient and her brother did not respond to stimulation with either interleukin-1β or lipopolysaccharide. Sequencing of the interleukin-1 receptor–associated kinase-4 gene revealed 2 novel mutations.

Interleukin (IL)–1 receptor associated–kinase-4 (IRAK-4) mediates signal transduction downstream of the toll-like receptor (TLR) and IL-1 receptor (IL-1R) superfamilies. Its deficiency leads to a defect in innate immunity that is primarily associated with infections of the gram-positive organisms Streptococcus pneumoniae and Staphylococcus aureus [1], although infections with the gram-negative organisms Pseudomonas aeruginosa, Neisseria meningitidis, Escherichia coli, Shigella sonnei, and Serratia marcescens have been reported [2–5]. Here, we describe 2 siblings with IRAK-4 deficiency due to novel mutations in the IRAK4 gene and an unusual clinical phenotype that included very late-onset Streptococcus agalactiae (group B Streptococcus [GBS]) sepsis and meningitis, systemic shigellosis, and chronic osteomyelitis.

Case report. The patient was born at term after an uncomplicated pregnancy and presented at 5.5 months of age with fever (temperature, 39.2°C), lethargy, and decreased oral intake and urinary output. She was somnolent but arousable, with a weak cry. Her anterior fontanel and her left tympanic membrane were bulging. She was tachycardic with brisk capillary refill. Neurologic examination was remarkable for right oculomotor nerve palsy.

The patient’s peripheral white blood cell count was 2900 cells/µL (71.9% neutrophils and 23.1% lymphocytes). Her spinal fluid was remarkable for a protein level of 165 mg/dL and a glucose level of 30 mg/dL; the peripheral glucose level was 101 mg/dL at the time. The spinal fluid was colorless and clear, with 255 nucleated cells/µL and without red blood cells (75% neutrophils, 10% lymphocytes, and 15% monocytes). Both blood and spinal fluid cultures grew GBS. A human immunodeficiency virus (HIV) antibody test was nonreactive.

At 32 months of age, the patient travelled to El Salvador and developed fever, headaches, abdominal pain, and bloody diarrhea. Upon her return to the United States, she was admitted to the hospital with severe hyponatremic dehydration. She was febrile (temperature, 39.6°C), tachycardic (heart rate, 172 beats/min), lethargic, and irritable and had a respiratory rate of 32 breaths/min. Her abdomen was soft, nondistended, and non-tender, without hepatosplenomegaly. Her white blood cell count at admission was 5900 cells/µL (52% neutrophils, 30% lymphocytes) and remarkable for a protein level of 32 mg/dL, a glucose level of 14 mg/dL, and a creatinine level of 0.49 mg/dL. Her erythrocyte sedimentation rate was 15 mm/h, with a C-reactive protein level of 140.6 mg/L. Spinal fluid was colorless and clear and remarkable for a protein level of 32 mg/dL, a glucose level of 82 mg/dL, a nucleated cell level of 1 cell/µL, and a red blood cell count of 45 cells/µL. Two blood cultures, two stool cultures, and her cerebrospinal fluid culture—in thioglycolate broth only—yielded S. sonnei (subgroup D). Flow cytometry and immunoglobulin findings were essentially normal. We treated the patient with parenteral ceftriaxone and gentamicin for a total of 3 weeks.

At 35 months of age, the patient was noted to be limping, which was not evaluated until five months later (at the age of 40 months) when she sustained a witnessed fall. Plain radiography findings were normal, and the patient was treated with nonsteroidal anti-inflammatory agents. During an additional evaluation 1 week later, she continued to have pain and was
bracing; her erythrocyte sedimentation rate was 60 mm/h. Computed tomography and magnetic resonance imaging of the pelvis were concerning for osteomyelitis of the right pubic ramus. The biopsy revealed bone, cartilage, and chronically inflamed fibrous tissue with focal granulation tissue formation. The results of blood, fungal wound, acid-fast bacilli wound, and bacterial wound cultures remained negative.

The 12-year-old brother of the index patient had episodes of bronchitis and pneumonia that required hospitalizations during his first 4 years of life. The parents were not consanguineous.

**Methods.** Informed consent was obtained. Whole blood was diluted to a ratio of 1:2 in Roswell Park Memorial Institute 1640 (GibcoBRL) and activated with either IL-1β (20 ng/mL; R&D Systems), lipopolysaccharide (LPS; 1 ng/mL of Salmonella minnesota R595; Sigma), phorbol 12-myristate 13-acetate plus ionomycin (PMA/Iono; 10^-7 M and 10^-5 M, respectively; Sigma), or tumor necrosis factor (TNF)-α (20 ng/mL; R&D Systems) for 48 h. Supernatants were collected after 48 h, and the secretion of IL-6 (in response to IL-1β, LPS, and PMA/Iono) and the secretion of IL-10 (in response to TNF-α and PMA/Iono) were measured using human IL-6 and IL-10 (CLB) enzyme-linked immunoassay kits.

Genomic DNA from whole blood cells or Epstein Barr virus–transformed B cells was isolated. The IRAK4 gene was sequenced from the amplified products as described elsewhere [4]. Total RNA was extracted from Epstein Barr virus–transformed B cells using TRIzol (Invitrogen); cDNA was prepared using SuperScript II reverse transcriptase (Invitrogen) in accordance with the instructions of the manufacturer. Full-length IRAK4 cDNA was ligated into TOPO-TA vectors (Invitrogen) and transfected into chemically competent E. coli (Invitrogen). We sequenced the products using a Big Dye Terminator (Amer sham). The primers and their conditions are available on request. For western blotting, total lysates were extracted from Epstein Barr virus–transformed B cells. The proteins were probed with rabbit anti-human IRAK-4 antibody (Turlarik) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (Santa Cruz Biotechnology).

**Results.** IL-6 secretion was undetectable in the index patient and her 12-year-old brother after stimulation with IL-1β or LPS, but secretion in response to PMA/Iono was comparable to that of control cells (Figure 1). Both parents and the un-
affected sister had normal responses to IL-1β and LPS stimulation (data not shown). The patient and her brother responded normally to TNF-α stimulation with IL-10 secretion.

We found 2 IRAK4 mutations that, to our knowledge, have not been reported previously. The first one, 1175G→T (located in the splicing site of exons 9–10), leads to a 10-base pair deletion at the beginning of exon 10 in the cDNA of the patient. The second mutation, Y430X, is a nonsense mutation in exon 11. As a result, the 2 patients are compound heterozygous (Figure 2). The consequence of the first mutation in cDNA and confirmation of the second one were performed by cloning and sequencing of the full-length IRAK4 cDNA. Western blot analysis was performed for the patient, a healthy control subject, and an identified patient with complete IRAK-4 deficiency. No IRAK-4 protein was detected in the patients (data not shown).

Discussion. Impaired TLR signaling is associated with very late onset GBS infection, systemic shigellosis, and osteomyelitis in this family. The review of a case report of Shigella meningitis associated with IRAK-4 deficiency [5] led to testing for impaired TLR signaling and the correct diagnosis in our patient. Systemic shigellosis is very unusual in the United States, where most cases of Shigella infection are due to S. sonnei, but bacteremia has been reported to occur in 12%–27% of patients aged <15 years with shigellosis in Bangladesh [6], where most isolates were identified as either Shigella flexneri or Shigella dysenteriae type 1. Seymour et al [7] suggested that S. sonnei bacteremia may reflect compromised host defenses, not bacterial virulence. Of note, the sedimentation rate in our patient was normal during the episode of shigellosis, despite the presence of septicemia due to gram-negative organisms; a poor inflammatory response is characteristic of IRAK-4 deficiency. Unlike the IRAK-4–deficient patient described by Chapel et al [5], Shigella infection did result in severe mucosal inflammation with hematocoezia in our patient. In any event, these 2 patients clearly indicate that the association of IRAK-4 deficiency and systemic shigellosis, 2 rare conditions, is not coincidental but causal.

Myeloid differentiation primary response gene 88 (MyD88) deficiency has to be considered in the differential diagnosis of IRAK-4 deficiency, because patients with MyD88 deficiency can have infections with a similar spectrum of organisms [8]. MyD88 is a cytoplasmic adaptor protein involved in coupling TLRs (with the exception of TLR3) and IL-1 receptor family members—specifically, IRAK-1 and IRAK-4. Although the clinical differentiation of these molecular defects remains difficult, sequencing of IRAK-4, as was done in our patients, establishes the diagnosis once impaired TLR signaling has been confirmed.

IRAK-4 deficiency is associated with invasive S. pneumoniae infection and Streptococcus milleri meningitis [4]. To our knowledge, this is the first association of IRAK-4 deficiency with very late-onset GBS sepsis and meningitis. GBS is a leading cause of neonatal morbidity and mortality in the United States [9] but a rare cause of systemic infection beyond the first 3 months of life. GBS colonization from the peripartum period has been reported to last beyond 9 months of age without causing clinical symptoms [10]. In the context of an immunodeficiency such as IRAK-4 deficiency, it may cause systemic disease. Very late-onset GBS disease was described in 3 infants who were infected with HIV [11]. We conclude that GBS should be added to the list of pathogens that are associated with IRAK-4 deficiency. Additional studies are warranted to determine the frequency of impaired TLR signaling in patients with very late-onset GBS infection.

The optimal treatment of such patients has not been established. Upon confirmation of the functional defect, we initiated treatment with trimethoprim-sulfamethoxazole and monthly intravenous immunoglobulin prophylaxis in the patient. Although the mortality rate has been reported to be as high as 43% in young children with IRAK-4 deficiency, the immune system eventually seems to compensate for this defect, because no deaths and no invasive infection have been reported in patients older than 8 and 14 years, respectively [4]. The brother of the patient illustrates this. Despite the favorable outcome in the brother, children in early childhood are at risk for significant invasive infections, as our patient experienced, and additional work is needed to define appropriate strategies for prophylaxis.

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References