Supplementary Materials

Impaired T cell receptor activation in Interleukin-1 Receptor-associated Kinase-4-deficient patients

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Case Reports:

Patient 1

The patient, a 3 year old female, was born at term without complications to unrelated parents. Family history is negative for immune deficiency. Infectious history is remarkable for recurrent skin abscesses and impetigo, two urinary tract infections, recurrent otitis media, *S. pneumoniae* sepsis, *P. aeruginosa* brain abscess, *S. pneumoniae* cervical abscess. Maximum recorded temperatures with her brain abscess was 37.3^{o}C. Physical examination is unremarkable. Initial immunologic evaluations revealed white blood cell counts from 3,300 to 17,000 per microliter and neutrophil counts from 560 to 10,000 per microliter, and lymphocyte counts from 1,463 to 12,075 per microliter. Immunoglobulin levels were normal, except for low IgA levels (<5 to 13 mg/dl). Antibody titers to diphtheria and tetanus antigens, *H. influenzae*, and rubella were protective. Post immunization antibody titers following immunization with Pneumovax (Merck Pharmaceuticals, Whitehouse Station, NJ), which contains 23 pneumococcal polysaccharide antigens, were protective for 9/14 serotypes measured. T and B cell subsets were normal and T cell mitogen and antigen proliferation was normal. NK cell function was normal. CH50 was normal. HIV testing was negative. The patient has compound heterozygous mutations in IRAK-4 consisting of a nonsense mutation C877T (Q293X) on one allele and a 17 base pair deletion (870-887del) that results in a premature stop codon (I291X).

Patient 2

The patient, a 22 month old male, was born at term to unrelated parents. Family history is significant for an older sister with IRAK-4 deficiency. The patient was diagnosed in the first month of life based on absence of neutrophils to shed CD62L (L-selectin) in response to LPS, poly-IC, R848 and FSL lipopeptide. IL-6, TNFα, and IL-8 production by whole blood cells in response to IL-1, lipopeptide, LPS, poly-IC and R848 were absent. Immunoglobulin levels were normal at 11 months of age (IgG 386, IgA 29 and IgM 59 mg/dl) and at 22 months with IgG 651, IgA 31 and IgM 64 mg/dl, though IgE was elevated at 330U/ml (<110U/ml). Response to specific immunizations showed protective titers to hepatitis B vaccine, but failure to develop protective titers to varicella after two vaccinations with Varivax (Merck, Whitehouse Station, NJ). He did respond to MMR with mumps IgG, measles IgG and rubella IgG with protective titers. The patient did not develop protective titers to pneumococcus following vaccination with the polysaccharide antigens contained in Prevnar (diphtheria - pneumococcal conjugate vaccine; Pfizer, Kirkland Que.). The patient had a normal complete blood count, including normal lymphocyte populations. The patient has remained healthy since he was put on antibiotic prophylaxis shortly after birth. He has not had any invasive bacterial or fungal infections and has only had one episode of a mild bronchiolitis with parainfluenza 3. The patient has compound heterozygous mutations in IRAK-4 consisting of a deletion (631del_G) on one allele resulting in a premature stop codon at amino acid 212 (V212X). The other allele contains the point mutation C144G, resulting in a tyrosine to stop codon (Y48X) substitution at position 48.

Patient 3

The patient, a 4 year old female, was born at term to unrelated parents. Family history is negative for immune deficiency. Infectious history is remarkable for pneumococcal meningitis. The patient was discharged to home after therapy but was subsequently readmitted with proptosis and complete opthalmoplegia of left eye. CT scan/ MRI showed a retro-orbital mass. Biopsy showed skeletal muscle infiltrated with chronic inflammatory cells. Complete resolution of retro-orbital mass occurred following treatment with antibiotics and steroids, though she was left with residual left optic atrophy. She later developed an episode of pneumococcal arthritis of the left ankle. She subsequently developed fever and meningismus but no organisms were isolated from the CSF. She has had recurrent impetigo and cellulitis of foot, no organisms isolated. Immunologic evaluations revealed normal complete blood counts, normal T and B cell subsets, normal immunoglobulin levels, protective immune responses to the polysaccharide antigens contained in Pneumovax, normal classical and alternative complement function, and a heterozygous mutation in MBL. The patient has compound heterozygous mutations in IRAK-4 consisting of C877T (Q293X) and G893A (G298D).

Patient 4:

The patient is an 11 year male born at term to unrelated parents. Family history is negative for immune deficiency. Infectious history is significant for two episodes of pneumococcal meningitis, one year apart. Sequelae of his episodes of meningitis included stroke, seizures, mild hearing loss, and, possibly, mild learning impairment. The patient had multiple episodes of otitis media, requiring placement of myringotomy tubes at age 2 years. At eleven months of age he developed the intestinal intussuception,

complicated by perforation of the intestine and abscess formation in the peritoneum. He had several episodes of furuncles of the scalp and several skin infections, all of which responded well to treatment with antibiotics. He was also noted to have a weak febrile response, usually developing only low-grade fevers late in the course of an illness. Physical examination is unremarkable. Immunologic evaluation revealed a normal complete blood count and normal T and B cell subsets. Immunoglobulins were all within the normal range. Specific antibody titers demonstrated protective immune responses to protein antigens, such as tetanus toxoid, HiB conjugate vaccine, and the pneumococcal polysaccharide conjugate vaccine, Prevnar. However, immunization with pure polysaccharide antigens, contained in Pneumovax, did not result in increased titers. Complement function was normal. The patient is homozygous for a nonsense mutation in IRAK-4 consisting of C877T (Q293X).

Supplementary Table 1
T and B lymphocyte populations in IRAK-4 deficient patients

Patient#	1	2	3	4
CD3	2.7(2.3-6.5)	4.6(2.3-6.5)	3.0(2.4-6.9)	1.8(1.6-6.7)
CD3/4	1.6(1.5-5)	3(1.5-5)	1.9(1.4-5.1)	1.02(1-4.6)
CD3/8	0.9(0.5-1.6)	1.4(0.5-1.6)	1.04(0.6-2.2)	0.7(0.4-2.1)
CD19	1.3(0.6-3)	0.6(0.6-3)	1.7(0.7-2.5)	1.2(0.6-2.7)
CD56	0.05(0.1-1.3)	0.3(0.1-1.3)	0.36(0.1-1.3)	0.95(0.2-1.2)

Values are x 1000. Normal range in parentheses.