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Brief Communication

A novel truncating mutation in MYD88 in a patient with BCG adenitis, neutropenia and delayed umbilical cord separation

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ABSTRACT

Mutations in *MYD88* cause susceptibility to invasive bacterial infections through impaired signaling downstream of toll-like receptors (TLRs) and IL-1 receptors. We studied a patient presenting with neutropenia, delayed umbilical cord separation, BCG adenitis, and*P. aeruginosa*pneumonia. Next-generation DNA sequencing identified a novel homozygous truncation mutation in *MYD88* that abolishes MyD88 expression. The patient's dermal fibroblasts had severely impaired IL-6 production after stimulation with ligands for the MyD88-dependent receptors TLR2, TLR4 and IL-1R, while responses to ligands for the MyD88-independent receptors TLR3 and TNF-α were preserved. Notably, secretion of TNF-α, which is essential for BCG control, was also impaired after LPS stimulation. In this first report of BCG infection in MyD88 deficiency, data suggest that MyD88-dependent TNF-α production contributes to control of mycobacterial disease.

To the editor:

Myeloid differentiation primary response 88 (MyD88) is a cytosolic adapter molecule that interacts with toll-like receptors (TLRs) and receptors of the interleukin-1-family of cytokines (IL-1Rs) via the Toll and IL-1R (TIR) domain [1]. MyD88 signaling induces the expression of inflammatory cytokines essential for effective innate and adaptive immune function [1]. Human mutations in MyD88 or the downstream signaling molecule Interleukin-1 Receptor Associated Kinase 4 (IRAK4) result in susceptibility to invasive bacterial infections, particularly *S. aureus, S. pneumoniae,* and *P. aeruginosa* [2–6]. MyD88 and IRAK4 deficiency patients fail to mount an inflammatory response to infections [4]. While T cell-dependent responses are intact, variable defects in humoral immunity, such as reduced IgG, IgA, and IgM levels, have been described. We report a novel *MYD88* C-terminal truncation mutation in a patient with neutropenia, adenitis after Bacillus Calmette-Guérin (BCG) vaccination, and *P. aeruginosa* pneumonia.

The patient is a son of two consanguineous Omani parents (Fig. 1A). The family history was notable for an older son who had delayed separation of the umbilical cord at 3 weeks of age and died at 7 months of age due to *Pseudomonas* sepsis notable for the absence of fever. The proband had delayed separation of the umbilical cord at 4 weeks of age. Vaccinated with BCG at birth, he subsequently developed culture-positive BCG adenitis at age of 3 months, successfully treated with isoniazid, rifampicin & ethambutol for 2 months followed by isoniazid and rifampicin for 4 months. At 5 months of age, he developed pneumonia due to *P. aeruginosa* and a supraclavicular lymph node abscess positive for methicillin resistant *S. aureus*. Immune evaluation at that time was notable for severe neutropenia, reduced numbers of B cells and reduced IgM (Table 1). His pneumonia and abscess were treated successfully with piperacillin-tazobactam and vancomycin and the patient is currently on prophylactic penicillin.

Targeted next-generation sequencing of a panel of 264 genes associated with primary immunodeficiency [7] identified a homozygous nonsense variant within TIR domain of *MyD88* (c.814C > T;p.Arg272Ter) which was verified by Sanger sequencing (Fig. 1B). The variant is present in the gnomAD database in heterozygous form in 3 alleles (MAF 1.19×10^{-5}), but has not been previously reported in the homozygous state. The mutation occurs prior to the sequence encoding 24 amino acids at the protein's C-terminus (Fig. 1C). If expressed, this truncation mutant's expected molecular mass is 30 kDa.

Abbreviations: MyD88, Myeloid differentiation primary response 88; TLR, toll-like receptor; IL-1R, interleukin-1 receptor; IRAK4, Interleukin 1 Receptor Associated Kinase 4; TIR, Toll and IL-1R; BCG, Bacillus Calmette-Guérin; LPS, Lipopolysaccharide; LAD, Leukocyte adhesion deficiency

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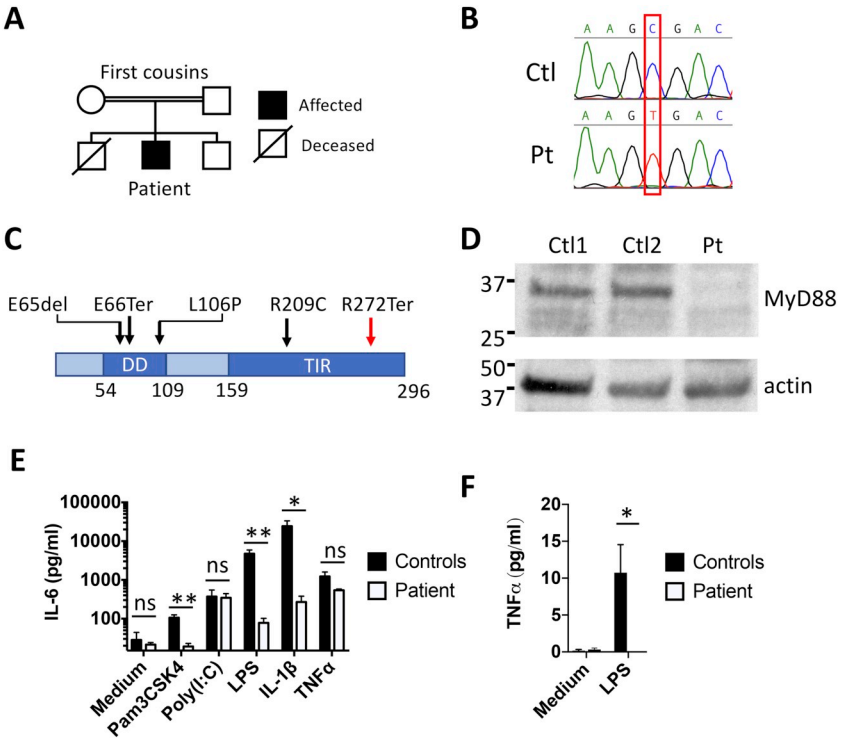

Fig. 1. A. Family pedigree. B. Sanger sequencing of the c.814C > T variant. C. MyD88 protein domains. Red arrow indicates position of the patient's mutation, black arrows indicate previously reported mutations. DD, Death domain; TIR, TIR domain. D. Immunoblot of dermal fibroblast lysates derived from patients and controls. Representative of 2 independent experiments. E, F. Toll-like receptor (TLR) signaling in patient fibroblasts. Dermal fibroblasts from patient and two healthy controls were stimulated as indicated and IL-6 (E) or TNF-α (F) production was assessed by ELISA. Pooled from 2 independent experiments. Columns and bars represent means and SEM. * $p < .05$, ** $p < .01$; Student's *t*-test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Immunological profile of the patient at eight months of age.

Clinical variable	Patient (reference range)
Hemogram	
Hemoglobin, g/dL	10.8 (10.4–12.5)
WBCs, 10^3 cells/μL	**6.4** (7.7–13.1)
Neutrophils, 10^3 cells/μL	**0.3** (2.5–6.4)
Lymphocytes, 10^3 cells/μL	**6.8** (2.3–5.5)
Monocytes, 10^3 cells/μL	0.6 (0.4–2.0)
Platelets, 10^3 cells/μL	**497** (185–399)
Lymphocyte subsets [12]	
$CD3^+$, 10^3 cells/μL	5.27 (1.90–5.90)
$CD3^+CD4^+$, cells/μL	3.88 (1.40–4.30)
$CD45RA^+CD31^+$, % $CD4^+$	**59.5** (65–90)
$CD45RA^+CCR7^+$ % $CD4^+$	**70.4** (76.7–91.4)
$CD45RA^+CCR7^-$, % $CD4^+$	**17.6** (0.1–1.9)
$CD45RA^-CCR7^+$, % $CD4^-$	**6.8** (6.7–15.6)
$CD45RA^-CCR7^-$, % $CD4^+$	5.23 (1.1–5.3)
$CD3^+CD8^+$, cells/μL	0.95 (0.50–1.70)
$CD45RA^+CCR7^+$, % $CD8^+$	**60.3** (62.1–94.0)
$CD45RA^+CCR7^-$, % $CD8^+$	10.7 (1.5–22.7)
$CD45RA^-CCR7^+$, % $CD8^-$	**6.7** (0.9–5.6)
$CD45RA^-CCR7^-$, % $CD8^+$	**22.3** (1.3–19.5)
$CD19^+$, 10^3 cells/μL	**0.23** (0.61–2.60)
Immunoglobulins [13]	
IgG, mg/dL	430 (217–904)
IgM, mg/dL	**19** (34–126)
IgA, mg/dL	81 (11–90)

Values in bold are outside of the reference range.

Immunoblotting of patient-derived fibroblast lysates using an anti-MyD88 antibody specific to the protein's N-terminus revealed no full-length protein or truncation mutant (Fig. 1D).

Analysis of the responses of the patient fibroblasts to MyD88-dependent and independent agonists was performed to verify the functional impact of the mutation. IL-6 secretion in response to the MyD88-dependent receptor agonists IL-1β, TLR1/2 agonist PAM3Csk4, and TLR4 agonist LPS were reduced in patient-derived dermal fibroblasts compared to controls (Fig. 1E). In contrast, IL-6 secretion in response to the TLR3 ligand poly (I:C) and TNF-α, both MyD88-independent agonists, were similar to healthy controls (Fig. 1E). Importantly, TNF-α, a cytokine critical for control of mycobacterium [8], was also reduced after LPS stimulation (Fig. 1F).

An early sign of immunodeficiency in the patient was delayed umbilical cord separation, which has previously been reported in at least 10 patients with MyD88 or IRAK4 deficiency [4]. Delayed separation is also seen in a number of other disorders impacting neutrophil number and/or function including leukocyte adhesion deficiency, Rac2 deficiency, and congenital or alloimmune neutropenia [4,9]. In cases of MyD88 and IRAK4 deficiency, defective neutrophil mobilization and/or neutropenia has been attributed to impaired IL-8 production in response to TLR ligands [4]. Thus, IRAK4 deficiency and MyD88 deficiency should be considered as a potential etiology in patients with delayed umbilical cord separation and neutropenia.

Adenitis is a known complication of the BCG vaccine even in healthy individuals [10]. BCG induces signaling vial TLR2, TLR 4, and TLR9 [11]. As TNF-α is known to be essential for immunity against mycobacterium [8], the patient's impaired secretion of TNF-α in response to LPS stimulation identifies a potential mechanism for his susceptibility to mycobacterial disease. MyD88-independent pathways, such as T cell-driven production of IFN-γ, likely prevented disseminated mycobacterial disease in our patient. Additional studies are needed to quantify the risk of BCG vaccine for use in this population.

This report adds a novel mutation to the four previously reported to cause MyD88 deficiency. The E65del, E66Ter and L106P mutations lead to dramatically reduced or undetectable protein expression, while the R209C mutation abolishes protein function without affecting protein expression [4,6]. The novel MyD88 mutation we report abrogates protein expression, resulting in neutropenia, *P. aeruginosa* infection, and delayed umbilical cord separation, and was associated with BCG adenitis, likely due in part to defective MyD88- dependent TNF-α production. Our findings demonstrate the pleiotropic effects of MyD88 on immune function and expand the clinical spectrum of MyD88 deficiency.

1. Material and methods

1.1. Immunoblotting

Cell lysates were immunoblotted using a polyclonal Ab raised against the N-terminus of MyD88 (ThermoFisher cat# 38-5800) or anti-actin (clone ACTN05 (C4), Abcam) followed by HRP-conjugated goat anti–rabbit antibody or goat anti–mouse antibody conjugated to horseradish peroxidase.

1.2. MyD88 signaling assays

Control or patient-derived dermal fibroblasts were stimulated for 24 h in the presence of medium, IL-1β (Peprotech), Pam3CSK4 (Invivogen), LPS (Invivogen), poly (I:C) (Invivogen) or TNF-α (Peprotech). After 24 h, supernatants were harvested and IL-6 or TNF-α was assessed by ELISA.

Declaration of Competing Interest

The authors have declared that no conflict of interest exists.

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