NOD2-associated autoinflammatory disease: an exploratory study of its pathogenesis

Snr, We previously reported an autoinflammatory disease associated with nucleotide-binding oligomerization domain containing protein 2 (NOD2) gene mutations, designated as NOD2-associated autoinflammatory disease (NAID) [1, 2]. To date, none of the studies of NOD2 mutations have demonstrated spontaneous inflammation in NOD2-associated diseases [3]. Herein we report a patient with NAID to illustrate the pathogenic role of NOD2 mutations and explore the pathogenesis of the disease.

A 23-year-old white man presented with recurrent fever and rash. On physical examination, his blood pressure was 143/104 mmHg and his temperature was 36.8°C; he appeared well developed and nourished, with a body weight of 85 kg; the remainder of the physical examination was unremarkable except for multiple abdominal surgical scars.

The scars resulted from multiple surgical procedures due to a complex disease that began to manifest itself 4 h after a normal vaginal delivery. The patient began vomiting bilious material with massive abdominal distension. He underwent colostomy with stoma for suspected Hirschsprung’s disease. Soiling continued after surgery and re-anastomosis was performed at 10 months of age.

The patient was doing reasonably well until age 13 years, when he developed several episodes of abdominal pain. Oesophagogastroduodenal endoscopy and inflammatory bowel disease (IBD) markers were negative. Colonoscopy demonstrated multiple lymphoid nodules with rare aggregates of submucosal eosinophils. An exploratory laparotomy disclosed ascites and oedematous bowel, and a subtotal colectomy was performed. There was a marked expansion of submucosal lymphoid tissue of the ileum with activated T cells admixed with eosinophils, without characteristic features of IBD or vasculitis (Fig. 1A and B). Mesenteric lymph node contained interfolicular hyperplasia (Fig. 1C) and activated T cells. At age 15 years he underwent laparoscopic lysis of adhesions and construction of a continent ileostomy T-pouch. At post-surgical day 3 he developed abdominal pain and sepsis-like symptoms. Subsequent exploratory laparotomy with small bowel and stomach biopsies was not revealing, but partial colon excision showed diffuse active colitis without ulcers, granulomas or dysplasia. Examination of mesenteric lymph nodes demonstrated reactive lymphoid hyperplasia. Immunohistochemistry stains showed positive IL-17 cells in the colon and lymph node tissues, notably the colon mucosa (Fig. 1D and E).

The patient had had nearly 20 episodes of flu-like symptoms, high-grade fever and sunburn-like rash on the axilla, neck, trunk and extremities (Fig. 1F) over the past 10 years. A skin biopsy showed spongiform dermatitis. After invariable treatment of each flare with prednisone 50 mg/day with tapering over 6 days, on the second day the rash and fever faded and resolved completely within 3 days. There was an episode of conjunctivitis, but without uveitis. There was no arthralgia.

Laboratory work showed mild leucocytosis with eosinophilia, elevated IgE at 432.0 (normal <114) kU/L, elevated ESR and negative serology for systemic autoimmune diseases. At early active disease, plasma and intracellular [CD4, CD8, natural killer (NK) and natural killer T (NKT) cell] levels of cytokines, such as TNF-α, IFN-γ, IL-4, IL-6 and IL-10, were normal. There were increased proportions

References

of blood CD4+ cells expressing CD69+ and CD71+, but normal cytotoxic cells expressing perforin and granzyme B, and a normal T cell receptor (TCR) Vβ repertoire clonogram. Genetic testing revealed heterozygosity for the NOD2 variants IVS8 +158 and Leu1007fsinsC and a new variant, Arg709Gln.

This case is characterized by neonatal onset and recurrent, severe gastrointestinal (GI) manifestations with resolution following multiple bowel surgeries. Recurrent episodes of high fever and dermatitis continued. The genotypic feature was the detection of the triple NOD2 gene mutations, including the new variant, Arg709Gln. Hirschsprung’s disease was excluded due to the presence of ganglion cells. There was meagre histopathological evidence of Crohn’s disease. Additionally Th1 and Th2 cytokines are involved in Crohn’s disease and ulcerative colitis, respectively [4], but they were normal in this patient. The absence of granulomas and the presence of GI symptoms would argue against Blau’s syndrome [5].

NAID is a new entity characterized by recurrent fever, dermatitis and inflammatory arthritis [1, 2]. Patients with GI symptoms account for ~60% of cases. Distal extremity swelling occurs in approximately one-third of patients [6].

NAID is currently associated with the NOD2 variants IVS8 +158, R702W and R703C [7]. The new variant, Arg709Gln, is close to the aforementioned variants. The phenotype and genotype of this patient are congruent with NAID. The aetiopathogenesis of NAID is currently unclear, however, this case allowed us to explore the pathogenesis of the disease for the first time. It also demonstrates unprecedented evidence of the intrinsic role of the triple dose NOD2 mutations in NOD2-associated diseases, perhaps resulting from a gene dosage effect, as in this case. The interaction of these mutations and environment could contribute to the disease, possibly involving the loss/gain of function. Our data also suggest that Th1/Th2 cells and their cytokines may not contribute to NAID. Rather, some type of activated T cells, possibly Th17 cells and latency-associated peptide-positive T cells [8], may play a role.

**Rheumatology key message**

- This case provides unprecedented evidence of the intrinsic role of the triple dose nucleotide-binding oligomerization domain containing protein 2 (NOD2) mutations in NOD2-associated diseases.

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**References**


**Fig. 1** Haematoxylin and eosin stain showing submucosal lymphoid hyperplasia of the ileocecal valve with prominent reactive germinal centres present.
Successful treatment of calcinosis with infliximab in a patient with systemic sclerosis/myositis overlap syndrome

Sir, Calcinosis is a debilitating manifestation of many connective tissue diseases, particularly JDM and SSC. Little is known about the pathogenesis of this condition, and its treatment remains a challenge. Previously used measures of therapeutic response (clinical examination, X-ray and scintigraphy) have been inconsistent and insensitive.

We present a case of severe calcinosis where the response to an anti-TNF agent was assessed by serial pelvic CT imaging at baseline, 7 and 41 months post-therapy. The patient was diagnosed with limited SS/myositis overlap syndrome in 2007, based on clinical features and positive immunology (ANA 1:1600, PM-Scl positive). Creatinine kinase (CK) was significantly elevated and EMG demonstrated low-grade myositis (a muscle biopsy was not performed). Although affected by arthritis, myositis and moderate pulmonary fibrosis, the patient was principally debilitated by calcinosis. Severe lesions occurred across several sites, affecting particularly the fingers and pressure areas, including the buttocks and hips. The lesions were painful and prone to ulceration and infection, and many discharged continuously. Intratendon calcification was proved radiologically, and local shortening of the triceps tendon disabled the patient by preventing the hand from reaching the mouth.

Multiple therapeutic interventions failed to treat the calcinosis. CYC, NSAIDs and corticosteroids provided no benefit and the lesions continued to worsen. Minocycline and MTX improved finger lesions and rendered myositis subclinical, but calcific deposits remained severe. Surgical excision of the lesions resulted in wound dehiscence. In 2008, specialist consensus was to initiate infliximab, based on clinical experience and individual case reports. Infliximab 3 mg/kg was infused at 0, 2 and 6 weeks, and then every 8 weeks thereafter. Pelvic CT at 7 months demonstrated reduced calcification with no new lesions when compared

![Fig. 1](image-url)