X-linked chronic granulomatous disease (CGD) caused by an intra-exonic splice mutation (CYBB exon 3, c.262G->A) is mimicking juvenile sarcoidosis

J. Brunner1,3, G. Dockter2, A. Rösen-Wolff1, J. Roesler2

1 Department of Pediatrics, Medical University of Innsbruck, 6020 Innsbruck, Austria; 2 University Hospital Carl Gustav Carus, 01307 Dresden, Germany; 3 Department of Pediatrics, University of Saarland, 66421 Homburg/Saar, Germany.

Please address correspondence to: Dr.med. Dipl.oec.med. Jürgen K.H. Brunner, Department of Pediatrics, Innsbruck Medical School, Anichstrasse 35, A-6020 Innsbruck, Austria. E-mail: juergen.brunner@uklibk.ac.at

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ABSTRACT

Background. Chronic granulomatous disease (CGD) is caused by mutations in genes encoding nicotinamide dinucleotide phosphate (NADPH) oxidase subunits.

Case report. A boy was diagnosed as having juvenile sarcoidosis because he presented with cervical and pulmonary lymphadenopathy with epitheloid cells and granuloma formation and high angiotensin converting enzyme. Later, a liver abscess was diagnosed. CGD was established by a dihydrorhodamine 123 (DHR) assay and genetic analysis revealed an unusual intra-exonic splice mutation in the CYBB gene encoding gp91-phox. It did not change the amino acid sequence and allowed for residual NADPH oxidase activity explaining the late onset of the disease.

Conclusion. CGD is an important differential diagnosis of juvenile sarcoidosis.

Introduction

Chronic granulomatous disease (CGD) is a rare inherited disorder of the innate immune system. The disease is characterized by severe infections due to distinct susceptibility to certain opportunistic bacteria such as Staphylococcus aureus, Burkholderia cepacia and Serratia marcescens, and fungi, especially Aspergillus species. Lymph nodes, lungs, liver, bones and skin are frequently involved in the infections, but any organ can be affected. In many cases such infections already occur in the first or second year of life, but later presentations, even in adults, have been described (1, 2).

CGD is also characterized by poor wound healing and dehiscence, and by uncontrolled inflammation often accompanied by the formation of granulomas that can have any size including microscopically small and huge like a fist. Such manifestations often occur in the lungs, the gut (Crohn-like disease), the uro-genital tract, and lymph nodes, but again any organ can be affected (3).

CGD is caused by the failure of leukocytes (mainly neutrophils, eosinophils, macrophages and monocytes, but also B and T-cells may be involved) to produce micbicidial reactive oxygen metabolites (ROM). Superoxide (O2-) is normally generated by the multicomponent enzyme complex nicotinamide dinucleotide phosphate (NADPH) oxidase (4-6). All other ROM are derived from O2-. Defects in any of four components of the NADPH oxidase (p47phox, p67phox, p22phox or gp91phox encoded by the NCF1, NCF2, CYBA and CYBB, respectively) can lead to CGD. The most common form is X-linked and caused by mutations in the CYBB gene that encodes the large subunit of flavocytochrome b558, gp91phox, the heme and flavin-containing core of NADPH oxidase. The 30 kb CYBB gene consists of 13 exons and is located on Xp21.1 (3, 7, 8).

The chronic granulomatous inflammation in CGD may resemble granulomatosis without underlying immunodeficiency. Therefore, CGD can mimic sarcoidosis, tuberculosis, hypersensitivity pneumonitis and Crohn’s disease (3). The case reported herein presented with mild manifestations mimicking sarcoidosis. His genetic defect involved an unusual intra-exonic splice mutation.

Case report

A ten-year-old boy had a history of a coccyx fistula and a small subcutaneous neck-abscess in his first year of life. At the age of seven years he suffered from a small subcutaneous cervical abscess (staphylococcus isolated) and cervical and pulmonary lymphadenopathy. High-resolution chest computed tomography scan revealed multiple nodes. A more comprehensive diagnostic workup was initiated. Erythema nodosum and uveitis did not occur. A Mendel-Mantoux test was negative. A biopsy of a cervical submandibular lymph node showed circumscribed, non-caseating granulomas with radially arranged epitheloid cells with pale nuclei. Ziehl-Nielsen and Wade-Fite stainings, and microbiological cultures failed to show acid-fast bacilli or mycobacterial growth. Subsequently, an increase in the serum level of angiotensin converting enzyme (ACE) was found (144 U/l, normal range 0-55 U/l). For all these reasons, the diagnosis...
of sarcoidosis was accepted. No specific treatment was started. At the age of ten years, the patient presented with fever and abdominal pain for 2 days. Abdominal ultrasound revealed a mass in the liver. Surgical evaluation and treatment by removal showed a large abscess (culture positive for Staphylococcus aureus) in the right lobe.

Diagnostics and genetic evaluation
A dihydrorhodamine 123 (DHR) assay (9, 10) revealed a strongly decreased H2O2 production by the patient’s neutrophils after stimulation with phorbol-myristat-acetat (PMA, Fig. 1). The mother of the patient had two neutrophil populations, one DHR positive showing a functionally normal subset and one DHR weakly positive cell subset, as seen in the patient, indicative for the x-linked form of CGD. The diagnosis of CGD was confirmed by lucigenin enhanced chemiluminescence. Parental informed consent to genetic testing was given. A c.262G->A mutation (numbering 1A2T3G...; 298G->A according to ENSEMBL) was found in the last bp position of exon 3 of the CYBB gene in genomic DNA (Table I). The mother of the patient was heterozygous for this mutation. The putative amino acid sequence remained unchanged. As shown in Table 1 the wildtype splice sequence at the exon 3 - intron 3 - junction deviates already from the extended splice consensus sequence in positions -4, -3, -2, and +6 (11). Thus, the additional deviation in position -1 (77% conserved), caused by the mutation found, easily weakens the splice site. Accordingly, only a minor fraction of the mRNA showed normal exon 3 splicing. This finding explains the residual NADPH oxidase activity found in functional tests (Fig. 1). After establishing the diagnosis a prophylactic medication with trimetoprim and itraconazole was started promptly. The patient is without any clinical complaints.

Discussion
The presentation of the patient was remarkable because for the first 9 years of his life no pathognomonic symptoms of CGD occurred and only minor infections developed. Instead, bilateral lymphadenopathy, high serum level of ACE, and non-caseating granulomas with epitheloid cells as revealed by a lymph node biopsy pointed to the diagnosis of juvenile sarcoidosis. ACE is a serologic marker for sarcoidosis and is produced by epitheloid cells and macrophages (12). Elevated ACE levels in CGD are reported (13). The clinical spectrum of this form of sarcoidosis is heterogeneous, ranging from asymptomatic patients with an abnormal chest radiograph to widespread organ involvement. In early childhood sarcoidosis (EOS), the triad of rash, arthritis and uveitis is typical. In adolescence, the diagnostic findings resemble
those in adults with primary pulmonary manifestation (14, 15). A possible relationship between different granulomatous disorders such as sarcoidosis, Crohn’s disease, and CGD has been discussed (13, 16, 17). The sarcoïd-like granulomatous inflammation in CGD and other immunodeficiencies are similar to granulomatosis without underlying immunodeficiency (18-20). CGD may be a risk factor for developing chronic inflammatory disorders with formation of non-casating granulomas, such as sarcoidosis (13). However, CGD can mimic sarcoidosis. In industrialized countries, abscesses in the liver may be indicative of CGD as an underlying disease, especially when caused by *Staph. Aureus* (3, 21). Therefore, the big liver abscess in our patient prompted us to perform a DHR assay which revealed X-linked CGD with residual NADPH oxidase activity (22). The mutational analysis revealed an unusual intra-exonic splice mutation in the *CYBB* gene (see above “genetic evaluation”). The wildtype deviates already in four and the mutated sequence in five positions from the extended splice consensus sequence (Table I). Therefore, it can be expected to be mostly “ignored” by the spliceosome. The residual NADPH oxidase activity can be best explained by the finding that few mRNA molecules are still spliced correctly. However, the skipped exon 3 is an in-phase-zero exon. Therefore, even though unlikely, it is not completely excluded that the truncated gp91-phox protein is still functional to some minor degree. In any case, the residual NADPH oxidase activity can explain the late onset of CGD in our patient even though there is wide variability in CGD with and without such activity. *CYBB* mutations in gp91-phox deficiency are family-specific and comprise a wide range of different genomic aberrations (22, 23). Fewer than 10% of X-CGD cases have a reduced gp91phox protein level with measurable amounts of O2-. These patients, as ours, may have variable clinical symptoms and may also present at an older age more often than classical X-CGD patients (3). The correct diagnosis of CGD is important because it dictates appropriate prophylaxis, treatment, and genetic counselling. Regular follow-up visits of CGD patients can prevent or mitigate complications, improve life quality, and facilitate decisions for risky treatment such as bone marrow transplantation. Our case and others demonstrate that patients suspicious for certain forms of sarcoidosis should also be evaluated for CGD (13, 24, 25).

**Conclusion**

CGD can present with clinical symptoms indicative of other diseases and is an important differential diagnosis of certain types of sarcoidosis. Residual NADPH oxidase activity can result in late onset forms of CGD with unusual manifestations and longer intervals between disease episodes. However, because such episodes can still be life threatening, “variant” patients need the same care as “classical” CGD patients.

**References**