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Letter to the Editor

Constant B cell lymphocytosis since early age in a patient with *CARD11* mutation: A 20-year follow-up

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Dear Sir,

Recently a rare primary immunodeficiency, gain of function mutation (GOF) in the caspase recruitment domain family member 11 (*CARD11*) with a hallmark of constant B cell lymphocytosis was described [1]. *CARD11* is a scaffold protein that is essential in the activation of the canonical nuclear factor κB (NF-κB) pathway in lymphocytes. Both loss and GOF mutation of *CARD11* have been described. Inactivation of *CARD11* causes severe combined immunodeficiency, whereas GOF mutation leads to constitutive activation of NFκB [1–5]. The activation seems to be stimulatory in B cell explaining the B cell lymphocytosis, but on the T cell side renders T cell anergy and causes mild T cell immunodeficiency. Since the first publication in 2012 of *CARD11* GOF mutation, altogether four different mutations have been described in seven patients from five families [1–3]. The clinical picture of these patients is reminiscent of immunodeficiency with frequent upper respiratory tract infections (RTI) and poor antipolysaccharide antibody response. Some patients have chronic Epstein Barr virus (EBV) infection. The disorder is also referred as B cell expansion with NF-κB and T cell anergy (BENTA). We present 20-year follow-up of a patient with GOF mutation of *CARD11*.

The patient was born after full-term pregnancy to non-consanguineous parents. At the age of two weeks, she was hospitalized for upper RTI and two weeks later for pneumonia. At four months she had respiratory syncytial virus pneumonia and otitis caused by *Escherichia coli*. Sulfacotrimoxazole was started as prophylaxis. The antibiotic had to be discontinued ten days later because of fever, urticaria followed by septic infection and agranulocytosis. Bone marrow (BM) examination revealed hypoplasia of all cell lines. The BM recovered during the following months but had excess amount of lymphocytes consisting mainly of polyclonal B cells. At one year of age she was diagnosed with splenomegaly and at two years with intra-abdominal and mediastinal lymphadenopathy in CT. Lymph node (LN) and spleen biopsy PAD showed atypical lymphoid hyperplasia with disturbed structure in some of the LN follicles.

During childhood, she has continuous upper and lower RTIs, lymphadenopathy and enlarged spleen. At two years she converted EBV antibody positive. Plasma EBV nucleic acid detection was found positive at age seven and has since fluctuated up to 86,000 copies/ml. At four she had varicella and at eleven shingles. At eight years, intravenous immunoglobulin (ivig) was started. At this point she had IgG of 5.55 g/l, IgA 0.27 g/l, IgM 0.62 g/l, tetanus antibodies slightly above the level of protection (0.12 IU/ml) and low/absent serospecific polysaccharide pneumococcal antibodies with no response to polysaccharide vaccine. Despite ivig and prophylactic antibiotics, RTIs have continued. Consequently, she has developed bronchiectasis, tympanic perforations, hearing defect and has constant nasal congestion with occasional sinus infections.

Numerous examinations have been made to reveal the diagnosis. Several CT, MRI, and also PET—CT scans, as well as bone marrow examinations have been performed because of suspected lymphoproliferative disease and unknown diagnosis. Lymphocyte proliferation stimulation indexes (SI) were normal at eight months, low when she continuously had infections and recovered again when infections diminished (Fig. 1). Total lymphocyte values were within normal limits at one month of age but increased with infections and comprise up to 90% of B-lymphocytes, mostly naïve transitional cells (Fig. 1). CD27 positive memory cells are low and switched memory cells (IgM^+/IgD^+) are nonexistent (Supplementary Table). Complete CD27 deficiency, CATCH22 microdeletion and IL-2 inducible T-cell kinase (ITK) mutations were excluded. $TCR\alpha\beta^+CD4^-CD8^-$ double negative T cells were elevated (up to 8.0%) causing suspicion of autoimmune lymphoproliferative syndrome (ALPS) which could not be verified. Finally in 2015, whole exome sequencing (WES) revealed a *de novo CARD11* mutation (chr7:2984163, C > T, CARD11:NM_032415: exon5:c.G367A:p.G123S) explaining the patient's clinical picture.

The mutation of our patient resides within the LATCH domain of *CARD11* and has been described previously [1]. Immunologic phenotyping of B cells was compatible with previous descriptions and showed high numbers of naïve cells and has a significant increase in transitional B cells (Supplementary Table) [1–3]. T cells and $CD4^+$ T cells were moderately low in numbers, especially in early childhood, when compared to age matched control values and also to values reported in other published cases. The high proportion of $TCR\alpha\beta^+CD4^-CD8^-$ double negative T cells has been shown in three previously reported cases [1]. Elevated double negative T cells is not diagnostic for ALPS, as has been shown in recent reports [6].

This report shows for the first time the evolution of lymphocytosis during 20 years in a single patient. The follow-up of previously reported patients has been shorter, patients were younger or they were treated with immunomodulatory agents. The only immunomodulatory treatment our patient received was ivig. In early infancy before the recurrent infections, the absolute number and percentage of total lymphocytes were within normal limits (6,1 × 109/l/62%), increased with infections to decline again towards adulthood when the infections diminished (Fig. 1). Brohl et al. have speculated that EBV could be a contributing factor to persistent lymphocytosis and high B cell numbers [2]. However, in our patient absolute B cell values were high at least a year before EBV seroconversion at two years and declined during the follow-up although EBV viremia persisted. Natural killer T (NKT) cells have been shown to be diminished/absent in immunodeficiencies like *ITK*

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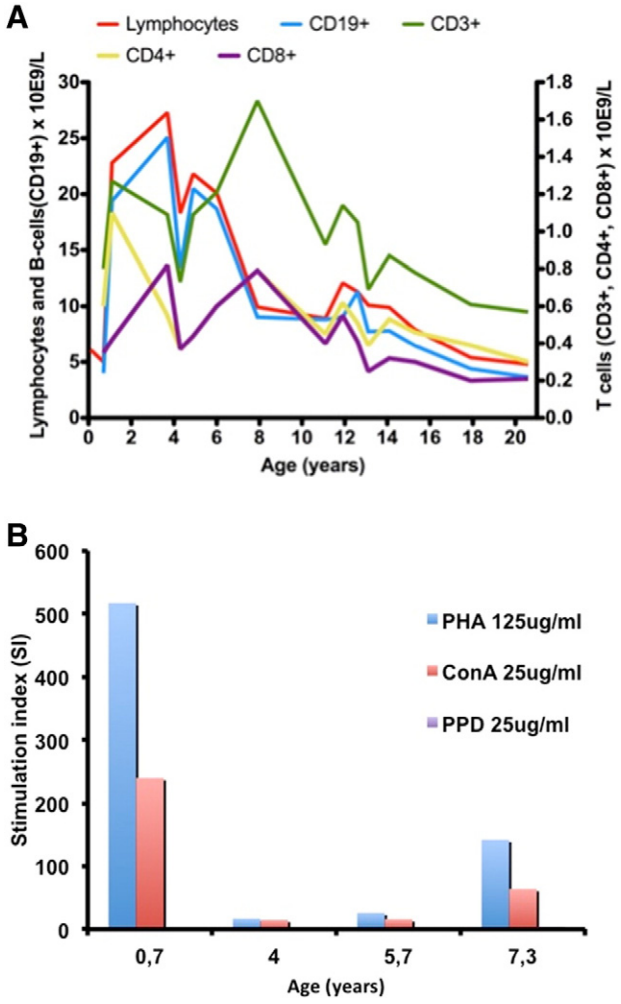

Fig. 1. Patient's peripheral blood B- and T-lymphocyte values (A) and lymphocyte proliferation stimulation indexes (B) during the 20-year follow-up. Stimulation indexes (SI) were calculated as follows: lymphocyte radiolabeled thymidine incorporated response in counts per minute (cpm) to phytohemagglutinin (PHA) and concanavalin A (ConA) divided with background incorporation (cpm).

mutation, X-linked lymphoproliferative disease (XLP) and X-linked inhibitor of apoptosis (XIAP) all of which may present with EBV viremia [7]. The (NK) T cell $CD3^+CD16^+CD56^+$ numbers and percentages of our patient were under detection limit. The number of NKT cells was not reported in connection with the other patients with *CARD11* mutation so their impact remains speculative [1,2].

Prognosis of patients with BENTA is unknown but one case of B cell chronic lymphocytic leukemia has been described [1]. Treatment of BENTA is supportive but close follow-up is needed because of possible predisposition to malignancy. Since survival to adulthood seems common and lymphocyte differential is needed to detect B cell lymphocytosis and B cell lymphocytosis gets milder with aging, patients with mutation could be missed. This report also shows that WES is a cost effective alternative in selected cases.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.clim.2016.02.002.

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