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Brief communication

Gain-of-function STAT1 mutations are associated with intracranial aneurysms

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ABSTRACT

Chronic mucocutaneous candidiasis, characterized by persistent or recurrent fungal infections, represents the clinical hallmark in gain-of-function (GOF) signal transducer and activator of transcription 1 (STAT1) mutation carriers. Several cases of intracranial aneurysms have been reported in patients with GOF STAT1 mutation but the paucity of reported cases likely suggested this association still as serendipity. In order to endorse this association, we link the development of intracranial aneurysms with STAT1 GOF mutation by presenting the two different cases of a patient and her mother, and demonstrate upregulated phosphorylated STAT4 and IL-12 receptor β1 upon stimulation in patient's blood cells. We also detected increased transforming growth factor (TGF)-β type 2 receptor expression, particularly in $CD14^+$ cells, and a slightly higher phosphorylation rate of SMAD3. In addition, the mother of the patient developed disseminated bacille Calmette-Guérin disease after vaccination, speculating that GOF STAT1 mutations may confer a predisposition to weakly virulent mycobacteria.

1. Introduction

In 2003, Dupuis et al. have described and genetically elucidated a new immunodeficiency syndrome caused by biallelic loss-of-expression and loss-of-function mutations in signal transducer and activator of transcription 1 (STAT1) alleles, leading to complete absence of the wild type protein [1]. Heterozygous dominant gain-of-function (GOF) mutations in STAT1 gene were detected for the first time in 2011 by whole-exome sequencing and genome-wide linkage analysis in patients with chronic mucocutaneous candidiasis (CMC) [2,3]. CMC represents the main clinical disease phenotype in GOF STAT1 mutation carriers and is characterized by persistent or recurrent infections mostly by *Candida albicans* [3]. Four cases of intracranial aneurysms in CMC patients prompted the question whether aneurysms are probably associated with STAT1 GOF mutations [4–7]. In addition, five cases of intracranial aneurysms in GOF STAT1 mutation carriers with CMC have been mentioned so far [3,8,9]. In order to endorse this association, we herein present the two different cases of a patient and her mother with STAT1 GOF mutations having the occurrence of intracranial aneurysms in common.

2. Materials and methods

2.1. Analysis of phosphorylated STAT1, 3, and 4

For phosphorylated STAT1 (pSTAT1) measurements, EDTA blood was either stimulated with 1000 U/mL interferon-γ (IFNγ) or, for pSTAT3, with 100 ng/mL interleukin(IL)-6 or with 20 ng/mL phorbol 12-myristate 13-acetate (PMA) plus 1 μg/mL ionomycin for 15 min. Cells were permeabilized with Perm Buffer III (BD, USA) and stained for surface markers CD3-PC7, CD14-PC5 (both from Beckman Coulter, USA) and anti-pSTAT1 AlexaFluor 488 (pY701) or anti-pSTAT3-PE (pS727; both from BD). Data were collected by FACSCanto (BD) and analyzed with FCS4 Express™ (De Novo Software, USA). For pSTAT4 analysis, peripheral blood mononuclear cells (PBMC) were isolated by Ficoll

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separation and split into two tubes containing RPMI-1640 medium supplemented with 10% fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, and 1 mM sodium pyruvate (R10). 4.8 μg/mL phytohaemagglutinin (PHA) was added to each tube. Cells were cultured four days. After three days, medium was exchanged with R10 medium without PHA. IL-12 was added to one tube in a final concentration 5 ng/mL (PHA + IL-12 activated) and incubated for 10 min. Cells were permeabilized with Perm Buffer III and stained with CD3-PC7, CD4-PC5 (Beckman Coulter) and anti-pSTAT4 (pY693, BD).

2.2. Analysis of IFN-γ receptor 1 (IFNγR1) and IL-12 receptor β1 (IL-12Rβ1)

IFNγR1 (CD119): EDTA blood was co-stained with CD3-PC7, CD14-PC5, and with either CD119-PE (BD) or the respective isotype control (Mouse IgG1κ; BD). IL-12Rβ1 (CD212): PBMC were isolated and cultured as described above. Cells were stained with CD3-PC7, CD4-PC5, and with either CD212-PE (BD) or the respective isotype control (Mouse IgG1κ; BD).

2.3. Measurements of TGF-β 1, phospho-SMAD3, expression of TGF-β receptor 2, and anti-cardiolipin antibodies

Blood was obtained by venous puncture and drawn in serum separator tubes. Transforming growth factor (TGF)-β1 concentrations were measured using enzyme linked immunoassay (ELISA; eBioscience, USA). To analyze activated SMAD3, phospho-SMAD3 (ser423/425) was measured in cell lysates of PBMC (3×10^5 cells) using InstantOne™ ELISA (eBiosciences). All samples were ran in duplicates. Expression of TGF-β receptor 2 was analyzed by means of ChipCytometry, a technique that allows a stepwise quantitative analysis of biomarkers on cells using unlimited set of markers (http://www.chipcytometry.com) [10]. ChipCytometry-based analysis was carried out as previously described [11]. Briefly, cells were allowed to attach inside of cell-adhesive microfluidic chips for 30 min and subsequently subjected to ChipCytometry on a Zeiss AxioImager M2 automated microscope equipped with a Plan Apochromat ×20/0.8 objective and Zeiss AxioCam MRm camera. In a stain-bleach cycle, we documented fluorescence staining iteratively as a result of staining with CD14, CD3, CD4, CD8 and TGF-β receptor 2 in order to characterize various lymphocyte subpopulations. Analysis of image data was done using Zellkraftwerk.Explorer software (version 16.08.2016). Serum anti-cardiolipin IgG and IgM levels were determined using QUANTA Lite™ ACA ELISA kit according to the manufacturer's protocol (INOVA Diagnostics, USA).

3. Results

3.1. Case reports

We report about a 27-year-old patient who was diagnosed CMC at the age of 20. Already in infancy, she was treated almost regularly with nystatin and miconazole due to recurrent *Candida* infections in the oral cavity and vagina. At the age of three years, she suffered from left-sided hemiparesis after transient herald symptoms (weakness of the left leg associated with headache). Magnetic resonance imaging (MRI) revealed ischemic punctate lesions in the area of the right putamen and right globus pallidus. CSF analyses showed lymphocytic pleocytosis with 16 leukocytes per μL. In the following years, antifungal medications were administered at different time intervals and different doses to treat tenacious oral, esophageal, vaginal, and cutaneous candidiasis. The second cranial MRI, performed at the age of 14, revealed an elongated aneurysmal enlargement from the right carotid till middle cerebral artery. The subsequently performed intracranial arteriography (Fig. 1A, B) as well as computer tomography (CT) of chest and abdomen did not show any further aneurysms (not shown). No changes were detected in a control MRI, performed at the age of 26 (Fig. 1C–F, H–J), and prompted genetic testing for STAT1 GOF mutation which resulted in the identification of heterozygous STAT1 mutation in exon 10 (c.800C>T p.Ala267Val) affecting the coiled-coil (CC) domain. Besides *Candida* infections, the disease course was complicated by bacterial infections resulting in otitis media, bilateral bronchopneumonia, and sinusitis. Cone beam computed tomography of the paranasal sinus was performed due to increasing rhinorrhea and difficulty in breathing. Images revealed polypoid cystic changes in the right ethmoid cells, right sphenoid sinus, and right maxillary sinus (Fig. 1G). Sinus surgery including septoplasty and conchotomia was performed twice at the age of 25 and 26. Pathology showed chronic sinusitis with polypoid hyperplasia, squamous metaplasia, and inflamed sinus mucosa with erosions. Moreover, hypothyroidism has been diagnosed in childhood but so far no thyroid antibodies were found. Table 1 summarizes the most recent immunological parameters of this patient.

The mother of the patient received bacille Calmette-Guérin (BCG) vaccination a few days after her birth (no longer recommended in Germany since 1998). At the age of one, she developed acute lymphadenopathy in the left inguinal region which increased in size over a period of one year with reddening of the skin and, later on, resulting in an abscess in this region. Histopathology examination of the lymph nodes revealed the presence of epitheloid cells covered with tubercle bacilli but no granulomas. At the age of two, a resistance in the left lower abdomen and acid resistant bacteria in large quantities in feces and gastric juice were detected. In the following years, she had almost constantly oral, nail, and vaginal thrush. At the age of 18, surgical treatment due to left-sided psoas muscle abscess containing *E. coli* and streptococci bacteria was performed. Four years later, she suffered from left-sided brachiofacial sensorimotor stroke. The corresponding CT did not show any signs of infarction and ultrasound examination of carotid and vertebral arteries was unremarkable. CSF analyses revealed a scanty pleocytosis with 6 leukocytes per μL and distinctly elevated total protein (3390 mg/L). She recovered from stroke except some deficits in fine motor skills affecting the left hand. She started suffering from daily relapsing fever up to 39 °C, generalized weakness, inappetence, and difficulty swallowing. Because of these symptoms and significant weight loss (total body weight of 48 kg), she was admitted to hospital in the 34th week of gestation. As the vaginal swab test was positive for Group B streptococcus, chorioamnionitis was speculated. Vaginal birth was initiated in the 35th week of gestation. After delivery, she developed recurrent diarrhea and reported about pain in the lower abdomen. Abdominal ultrasonography showed enlarged spleen and target-like changes in some bowel loops. Leukocyte scintigraphy revealed acute inflammation of the intestine which aroused suspicion of Crohn's disease. A few months later, she sustained a perforation of the jejunum compounded by a subsequent severe peritonitis. The whole peritoneum including the resected part of the jejunum was infected by myriads of acid resistant bacteria but without the development of granulation tissue, suggesting non-reactive tuberculosis. Large liquefied lesions containing macrophages were found almost in the entire peritoneum as well as in the mesentery. Histopathology examination of mesenteric lymph nodes revealed caseous lesions surrounded by clusters of epithelioid cells and calcification. Treatment with antituberculostatic drugs (rifampicin, streptomycin, and isoniazid) was commenced. Several months later, small bowel resection and peritoneal lavage and, only few weeks later, resection of the ileum and ileostomy were performed due to necrotic lesions. She developed several enterocutaneous fistula whose secret was shown to contain acid-resistant bacteria and yeast. Treatment with somatostatin in order to decrease fistula output and to accelerate their closure remained ineffective. She continued losing weight up to a total body weight of 41 kg. At the age of 26, she suffered from left-sided oculomotor nerve palsy with rigid pupil. The CT scan revealed localized calcified lesions in basal ganglia, in the right temporal lobe, and one lesion in the left occipital lobe. Antituberculostatic drugs were stopped because of adverse events (not specified in the medical records). Despite committed treatment efforts, she died of pulmonary