Cytokine 96 (2017) 49–53

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Short communication

Case-control, exploratory study of cerebrospinal fluid chemokines/cytokines and lymphocyte subsets in childhood Tourette syndrome with positive streptococcal markers

Michael R. Pranzatelli *, Elizabeth D. Tate, Tyler J. Allison [1]

National Pediatric Myoclonus Center and National Pediatric Neuroinflammation Organization, Inc., Orlando, FL, USA

ARTICLE INFO

Article history:
Received 19 December 2016
Received in revised form 28 February 2017
Accepted 2 March 2017

Keywords:
CSF B cells
CSF immunophenotyping
CSF chemokine profiling
GABHS
OCD
Pediatric neuroinflammation

ABSTRACT

A longstanding question is whether neuroinflammation is present in children symptomatic for Tourette syndrome (TS) with positive streptococcal serology and throat cultures. The objective was to directly test for it using modern hypothesis-driven approaches. Profiling studies for 14 immune cell types (flow cytometry), 7 chemokines/cytokines (ELISA), oligoclonal bands, and other immunoglobulins were performed in this IRB-approved study of 5 children with TS and streptococcal markers compared to data from 26 non-inflammatory pediatric neurological controls. Subjects were well-characterized clinically and with standardized scales for tics and obsessions/compulsions. Three subjects with TS (60%) had positive throat cultures for Group A beta-hemolytic strep, five had elevated anti-deoxyribonuclease-B titers (mean = 444), and 4 (80%) had elevated anti-streptolysin O titers (981). There were no significant differences between groups in the frequency of CSF B and T cell subsets or NK cells; the proportion of intracellularly-stained T helper type 1 (IFN-γ) or type 2 (IL-4) cells; the concentrations of B cell chemoattractants CXCL13, CXCL10; the B cell proliferation/survival cytokines BAFF and APRIL, or other chemokines (CCL19, CCL21, CCL22). None of the patients had positive CSF oligoclonal bands or an abnormal IgG index/synthesis rate. Parallel blood studies were negative. This novel study found no group CSF lymphocyte phenotypic abnormalities or elevated inflammatory mediators in childhood TS despite positive serology and throat cultures for Group A beta-hemolytic streptococci. It demonstrates feasibility of the methodology, and should serve as the basis for a larger study of putative streptococcal-associated neuroimmunological disorders.

1. Introduction

Group A beta-hemolytic streptococci (GABHS), or S. Pyogenes, is a pathogen of global import. Controversy exists as to whether it causes a diversity of purported autoimmune pediatric neuroinflammatory disorders beyond the well-substantiated immunogenicity of GABHS in Sydenham Chorea, as recently reviewed [1]. One such disorder caught in the crosshairs is Tourette syndrome (TS), a childhood-onset movement disorder diagnosed by the combination of motor and vocal tics for a minimum of one year, usually with neuropsychiatric comorbidities.

Tic exacerbations may or may not occur in the setting of positive serology for GABHS, noted to be more prevalent in TS than in pediatric controls [2]. Other than antibiotic treatment, a clinical management directive is lacking due to a gap in knowledge about whether the GABHS-associated subgroup has neuroinflammation. The focus of immunological studies has been on disputed antibasal ganglia antibodies [2–4], and most other markers have been studied in blood [5–8] rather than cerebrospinal fluid (CSF). However, CSF is in greater proximity to the central nervous system and more likely to be informative [9]. Also, routine CSF testing for inflammation has a much lower yield for diagnosing neuroinflammation than revealing the immunophenotype through flow cytometry [9,10] and measuring concentrations of inflammatory mediators [11–15].

Abbreviations: ASO, anti-streptolysin O; ADN-B, anti-deoxyribonuclease-B; CSF, cerebrospinal fluid; GABHS, Group A beta-hemolytic streptococci; OCD, obsessive-compulsive disorder; OCB, oligoclonal immunoglobulin G; TS, Tourette syndrome.

* Corresponding author at: National Pediatric Neuroinflammation Organization, Inc., 12001 Research Parkway, Suite 236, Orlando, FL 32826, USA.

E-mail addresses: mpranzatelli@omsusa.org (M.R. Pranzatelli), etate@omsusa.org (E.D. Tate), tallison@cmh.edu (T.J. Allison).

[1] Present address: Division of Child Neurology, Children's Mercy Hospital, 2401 Gillham Road, Kansas City, MO 64108, USA.

http://dx.doi.org/10.1016/j.cyto.2017.03.003
1043-4666/

Drawing from established neuroinflammatory disorders, we hypothesized that if neuroinflammation giving rise to autoantibodies was present, evidence of recruitment and proliferation of B cells in the CNS with possible dysregulation and skewing of T cell subsets would be found. This approach represents a paradigm shift in TS from the antibody to the B lymphocyte, which can act as an antigen-presenting cell, looking for direct evidence of B cell expansion. The aims of this study were to (1) determine the CSF and blood immunophenotype of well-characterized TS compared to non-inflammatory neurological disorders without TS [9,10]; (2) measure chemokines and other cytokines involved in recruiting B cells (CXCL13, CXCL10) [11,13] and maintaining an abnormal CNS niche (BAFF, APRIL) [16]; and (3) identify markers of intrathecal humoral involvement, such as oligoclonal immunoglobulin G (OCB) and IgG synthesis. Because some B cell mechanisms are T cell-dependent, we surveyed T cell subsets [9,10], including T helper type 1 (Th1) and Th2 cells as identified by intracellular staining of cytokines. Other chemokines included were CCL22, which preferentially attracts Th2 lymphocytes, and CCL19 and CCL21, both critically involved in antigen-engaged B cells, dendritic cells, and central memory T cells [14]. CSF B cell expansion and marked elevations of CSF BAFF, CXCL13, and CXCL10 have been found in anti-N-methyl-D-aspartate receptor encephalitis, multiple sclerosis, neuromyelitis optica, neuropsychiatric lupus, and paraneoplastic neurological disorders [9–15]. The present panel of markers has not been previously evaluated in TS.

2. Materials and methods

2.1. Study design

This was a cross-sectional, case-control study in a convenient sample. Five school-aged children with TS and waxing and waning of tics and neuropsychiatric symptoms in the context of GABHS were recruited through Dr. Pranzatelli's Pediatric Movement Disorders Clinic at SIU School of Medicine (Springfield, IL). None had explosive onset of obsessive-compulsive disorder. Exclusion criteria were prior immunotherapy, other autoimmune disorders, non-streptococcal febrile illness or immunization within 1 month, contraindications to lumbar puncture, and other symptomatic causes of TS. Parents of subjects meeting inclusion/exclusion criteria signed informed consent and children also gave assent for this Institutional Review Board-approved protocol entitled "Immunophenotyping of Cerebrospinal Fluid Lymphocytes in Tourette Syndrome." The clinical aspects of the study were conducted from 2004 to 2008, but chemokine/cytokine assays, included to strengthen the study, were performed on the banked samples from 2008 to 2013 as they became available in the laboratory of the principal investigator.

2.2. Screening

Reference range values for serum streptococcal markers were used: anti-streptolysin O (ASO) < 200 and anti-deoxyribonuclease-B (ADN-B) < 1:170. Only throat cultures positive specifically for GABHS were included. Subjects were required to have at least 2 positive streptococcal markers. Patient 1 had pharyngitis with 2 negative throat cultures despite tic re-emergence and labile mood. Patient 2 had a prior GABHS infection, elevated C-reactive protein of 6.4, ASO of 929, and required antibiotic retreatment before slow, partial, self-reported resolution of tics. Patient 3 had a previous ADN-B of 680, but normal ASO. Screening of 16 patients with TS revealed 44% positive for ASO, 71% for ADN-B, 15% for GABHS throat culture, and 21% reporting tic exacerbation with pharyngitis (see Supplementary table). ASO is elevated in 15% of pediatric controls [2].

2.3. Clinical assessments

The principal investigator conducted a diagnostic interview in the company of parents (as informants) or separately (when teenagers) concerning tic disorder symptoms, associated symptoms, and psychosocial issues. Patients were examined off all medications for TS for one week: clonidine (patients 1–3), olanzapine (patient 3), methylphenidate and sertraline (patient 4), and risperidone (patient 5). The Tic Inventory of the Yale Global Tic Severity Scale (YGTSS) was administered by the co-investigator (E.D.T) [16]. Additional information concerning each of the YGTSS anchor points was obtained. The Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS) was used to rate obsessions and compulsions [17].

2.4. Controls

Controls were age- and gender-matched from a cohort of 26 children with a variety of non-inflammatory neurological disorders, comprising ataxia, developmental delay, headaches, seizures, and non-TS movement disorders, who were already immunophenotyped in the same manner as part of diagnostic evaluations [9,15]. Control samples were collected on a regular basis throughout the study period.

2.5. Sample collection (lumbar puncture and blood drawing)

Lumbar puncture usually was performed the day following clinical assessments. Extra precautions to prevent traumatic lumbar puncture were taken [9]. The first 3 ml of CSF went for quantitative immunoglobulins, IgG synthesis rate, oligoclonal bands, cell count, and chemistries. An additional 8–10 ml was collected on ice for lymphocyte subset analysis and cytokine measurements. Blood for parallel studies also was drawn. TS and non-TS samples were handled in the same manner.

2.6. Flow cytometry/immunophenotyping

Fresh CSF and blood were brought promptly to the flow cytometry lab, and lymphocyte subset analysis, using anti-CD45 (pan leukocyte) and anti-CD-14 (monocyte) for gating, was performed according to published methods [9]. To determine if the Th1 subset predominated and cytokine production was increased, we also intracellularly stained CSF CD4(+) T cells for IFN-γ and IL-4 as prototypic Th1 and Th2 cytokines, respectively. Th1 cells were CD4(+) IFN-γ(+); Th2 cells, CD4(+)IL-4(+).

2.7. Chemokine/cytokine assays and other tests

All cytokine assays were performed in duplicate on batched CSF/serum samples stored at −80 °C [15], with controls and TS on the same assay plates. BAFF, CXCL10, CXCL13, CCL19, CCL21, and CCL22 were measured using Quantikine human-specific enzyme-linked immunosorbent assay kits (R & D Systems, Inc., Minneapolis, MN) as per the manufacturer. The assay sensitivity was 3–4 pg/ml for BAFF, 0.41–4.46 pg/ml for CXCL10, 2 pg/ml for CXCL13, 15.6 pg/ml for CCL19, 9.9 pg/ml for CCL21, and <62.5 pg/ml for CCL22. The kit for APRIL, purchased from Bender MedSystems, Inc. (Burlingame, CA), had an assay sensitivity of 0.4 ng/ml. For CSF studies, the intra-assay CV ranged from 3 to 7%. The inter-assay CV values were also acceptable (3–8.4%). Tests for OCB, which were measured by isoelectric focusing and immunofixation, and CSF and serum immunoglobulins were performed in the clinical lab.