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Characterization of B-Cells in tonsils of patients diagnosed with pediatric autoimmune neuropsychiatric disorder associated streptococcus☆

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ABSTRACT

Objective: To determine if Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus (PANDAS) patients demonstrate a significantly different number of B-Cells or markers of activity when compared to recurrent Group A Streptococcus or Obstructive Sleep Apnea patients.
Study design: Prospective Cohort Study.
Study setting: Academic University Hospital.
Methods: Tonsil tissue was collected from twenty-two patients in the operating room and organized into three groups. Ten clinically diagnosed PANDAS, six Group A Streptococcus and six Obstructive Sleep Apnea patients were included in this study. Each tissue sample was extracted with MSD Tris Lysis Buffer and protein lysates were analyzed for CD 19, B-Cell Activating Factor and B-Cell Activating Receptor by western blot methods.
Results: Based on ANOVA analysis, there was no significant difference in the expression of B-Cell Activating Factor, B-Cell Activating Receptor or CD 19 when comparing the three study groups by western blot analysis methods.
Conclusions: In this prospective cohort study, it appears that PANDAS patients do not demonstrate increased number of B-Cells, expression of B-Cell Activating Factor or B-Cell Activating Receptor when compared to Group A Streptococcus or Obstructive Sleep Apnea cohorts. As a result, further evaluation of the cell-mediated immune system is warranted to provide further insight into the pathophysiology of PANDAS. In addition, we must investigate if PANDAS patients only demonstrate increased B-Cell number or activity when undergoing an acute Tic/OCD exacerbation.

1. Introduction

Currently, Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcus (PANDAS) remains a highly controversial diagnosis due to the paucity of basic science available to the medical community in the literature. More specifically, our current understanding of the condition's pathophysiology remains unknown, which further intensifies the disagreement about its existence as a viable diagnosis [1,2]. Many investigators believe that the exacerbation of OCD and Tic symptoms are due to autoantibodies attacking the neurons of the basal ganglia similar to Syndenham's Chorea in patients suffering from Group A Strep pharyngitis [3–5]. Previous basic science attempts failed to identify unique antibodies which may cause the aforementioned symptom exacerbation [6]. As a result, the medical community lacks agreed upon serum markers or diagnostic tests to further identify PANDAS in the clinical setting.

Some investigators have hypothesized that tonsillectomy, if properly performed by an otolaryngologist, may provide therapeutic benefit due to the possible association of Group A Streptococcus (GAS) pharyngitis and the exacerbation of OCD and Tic occurrences [7,8]. After evaluating the literature, mixed results remain at this time, but this may be due to the fact that

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control groups were not properly implemented into these studies or that retrospective parental opinion was utilized to measure outcomes rather than psychiatric evaluation post-operatively [7,8]. As a result, much of the data regarding PANDAS remains retrospective, with very few prospective studies which aim to reduce bias. One method of improving our current understanding of PANDAS would be to further explore basic science methods such that the medical community may have better insight into the mechanism of this condition.

In this study, our group chose to evaluate the lymphatic tissue for immunological markers that could demonstrate the involvement of the humoral immune system as a means of PANDAS pathophysiology. B-Cell Activating Factor (BAFF) and B-Cell Activating Factor Receptor (BAFF-R) were utilized to help quantify the activity of B-Cells in the tonsil tissue of each study participant. Both BAFF and BAFF-R have been implicated in several autoimmune disorders and are well characterized as one of the most significant markers of B-Cell activity [11–13]. These two markers were chosen because in our most recent study, we evaluated several interleukins that could account for increased humoral activity such as IL1, IL2 and IL6 but, we identified no significant difference between affected and non-affected cohorts [14]. After review of the medical and basic science literature, we chose to evaluate BAFF and BAFF-R due to their significant involvement in autoimmune disorders [11–13]. Here, we utilized a separate biological assay to identify the above B-Cell markers and performed a more focused evaluation of the humoral immune system within affected patient's tonsil tissue.

If PANDAS is indeed an autoimmune disorder, patients would likely maintain either increased activity of their B-Cell population or significantly more B-Cells residing in the lymphatic tissue. In this manuscript, our group hypothesized that PANDAS patients may have significantly more B-Cells or markers of B-Cell activity, which could be identified in the lymphatic tissue. In order to evaluate this possibility, our group prospectively enrolled PANDAS, GAS and Obstructive Sleep Apnea (OSA) patients at our tertiary academic center to evaluate their tonsil tissue by western blot analysis. In order to characterize each patient's B-Cell number, we utilized CD 19, a B-Cell marker that is found on all stages of B-Cells, except the progenitor line [9,10].

2. Materials and methods

2.1. PANDAS patient enrollment and tissue collection

The Georgetown University School of Medicine Institutional Review Board approved the following study and informed consent was obtained from the parents of each individual patient. The PANDAS cohort consisted of ten pediatric patients referred to our clinic by a pediatric neurologist associated with our academic center. Each patient was no older than eighteen years of age. Inclusion criteria by our pediatric neurologist included clinically diagnosed PANDAS patients based on the DSM IV criteria. This criteria includes: (1) Presence of Obsessive Compulsive Disorder and/or tic disorder, (2) Onset before puberty, (3) Episodic presentation symptoms, (4) Positive Group A Streptococcus culture with neurologic motor abnormalities. Exclusion criteria included history of rheumatic fever, autism spectrum disorders, psychotic disorders or other autoimmune disorders. The average time to tonsillectomy from last acute tonsillitis event was 2.3 months.

Each patient underwent tonsillectomy by our senior author and tissue was frozen at −80 °C until protein extraction with our associate at the National Institute of Health. All tonsil tissues were stored in the same location and extracted together to reduce treatment bias.

2.2. Group A streptococcus control group selection

The GAS Control Group consisted of six patients that underwent tonsillectomy for recurrent tonsillitis due to documented GAS by throat cultures. Patients were referred from primary care providers or self-referred to our outpatient clinic setting. Each patient was no more than eighteen years of age at the time of tonsillectomy. The average number of acute tonsillitis events included 3.5 per year with ranges of 2 to 5. In addition, the average time to tonsillectomy from last acute tonsillitis was 1.6 months. Exclusion criteria included: history of rheumatic fever, PANDAS diagnoses, autism spectrum disorders, psychotic disorders or other autoimmune disorders.

2.3. Obstructive sleep apnea control group selection

The OSA Control Group consisted of six patients who underwent tonsillectomy for moderate to severe OSA as determined by polysomnogram studies. Patients again were enrolled from the outpatient clinic setting and were no more than eighteen years of age at the time of tonsillectomy. Apnea Hypopnea Index values ranged from 6 to 13 with an average of 9.1. Exclusion criteria included: history of rheumatic fever, previous GAS infections, PANDAS diagnoses, autism spectrum disorder, psychotic disorders or other autoimmune disorders.

2.4. Western blot analysis

Tonsil tissue from 6 GAS, 6 OSA and 10 PANDAS patients were lysed in Tris lysis buffer as previously described [14]. Protein from the 22 samples were separated by molecular weight using SDS-PAGE before being transferred to PVDF membranes using the semi-dry Trans-Blot Turbo transfer system (Biorad). Membranes were then blocked using Odyssey Blocking Buffer (Li-Cor) for 1 h at room temperature and then probed overnight at 4 °C with primary antibodies against proteins of interest. These include BAFF (Abcam ab8396 1:1000), BAFF Receptor (Abcam ab112506 1:1000) and CD19 (Abcam ab 134114 1:1000). β-actin (Sigma A1978 1:15000) was used as the housekeeper protein. Following 3 washes with TBST (Tris-Buffered Saline and Tween), blots were incubated for 1 h at room temperature using the appropriate secondary antibody (Li-Cor 926-68020 and 926-32211 1:15000). All primary and secondary antibodies were diluted in 1:1 mixture of Odyssey Blocking Buffer and TBS + 0.1% Tween-20. Blots were then washed another 3 times with TBST and then imaged on a Li-Cor Odyssey CLx Infrared Imaging System. Protein band of interest were Quantified using Li-Cor Image Studio software and normalized to the corresponding β-actin value.

2.5. Statistical analysis

A one-way analysis of variance (ANOVA) was used to compare the means in expression of each of the cytokines and B-cell number between the 3 aforementioned study cohorts. A P-value <0.05 was considered statistical significant when comparing between the three groups. The size of the cohorts in the study were determined a priori such that there would be at least 90% power to detect a minimum effect size of 1, based on a one-way ANOVA with a significance level of 0.05. SAS software Version 9.3 (SAS Institute Inc, NC) was used for the analysis.

3. Results

3.1. Evaluation of CD19 marker in tonsillectomy samples

After western blot analysis, we identified no significant difference in the normalized expression of CD 19 when comparing