Increased Serum Levels of Interleukin-12 and Tumor Necrosis Factor-Alpha in Tourette's Syndrome

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Background: *The hypothesis that common infections can modulate the onset and course of tic disorders and early-onset obsessive-compulsive disorder (OCD) in pediatric populations is longstanding. To date, most investigations have focused on the hypothesis of molecular mimicry and humoral immune responses. This study was carried out to investigate whether cytokines associated with the innate immune response or T cell activation were altered under baseline conditions and during periods of symptom exacerbation.*

Methods: *Forty-six patients with Tourette's syndrome and/or early-onset OCD, aged 7–17 years, and 31 age-matched control subjects participated in a prospective longitudinal study. Ratings of clinical severity and serum were collected at regular intervals, and serum concentrations of 10 cytokines were measured repeatedly.*

Results: *Interleukin-12 and tumor necrosis factor α concentrations at baseline were elevated in patients compared with control subjects. Both of these markers were further increased during periods of symptom exacerbation.*

Conclusions: *These findings suggest that symptom exacerbations are associated with an inflammatory process propagated by systemic and local cytokine synthesis that might involve the central nervous system. We conclude that, in the future, longitudinal studies of children with neuropsychiatric disorders should examine the involvement of innate and T cell immunity.*

Key Words: Tourette syndrome, early-onset obsessive-compulsive disorder, PANDAS, interleukin-12, tumor necrosis factor α

Tic disorders, obsessive-compulsive disorder (OCD), and related conditions are prevalent disorders, affecting as many as .25%–3% of the pediatric population (Heyman et al 2003; Robertson 2003). They are chronic, relapsing disorders that can be associated with marked impairment and disability. Although clinical care has improved over the past decade, a significant number of patients fail to respond adequately or experience intolerable side effects to available treatments.

The etiologies of these disorders are unknown. The hypothesis that infections can modulate the clinical appearance of tic disorders dates from the 1800s. The past decade has seen the re-emergence of the hypothesis that postinfectious immune mechanisms account for at least some cases of Tourette's syndrome (TS) and OCD. It is well known that group A β-hemolytic streptococci (GABHS) can trigger immune-mediated disease (Bisno 2000; Stollerman 1997). Rheumatic fever, one of the most commonly recognized examples of a delayed nonsuppurative complication of GABHS infection, usually occurs a few weeks to several months after streptococcal infection in susceptible persons. Rheumatic fever typically involves the joints, heart, and the central nervous system (CNS). The CNS manifestations usually take the form of Sydenham's chorea; however, some patients with Sydenham's chorea also display motor or phonic tics, obsessive-compulsive (OC) symptoms, or features suggesting attention-deficit/hyperactivity disorder (Swedo et al 1989). On the basis of these associations, Swedo et al (1998) proposed that pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection (PANDAS) represents a distinct clinical entity that includes many cases of TS and OCD. The PANDAS hypothesis has stimulated clinical and basic research and has resulted in clinical and scientific controversy (Kurlan and Kaplan 2004; Singer and Loiselle 2003; Swedo et al 2004).

To date most of the immunologic studies of early-onset OCD and tic disorders have focused on the link between newly acquired GABHS infections and symptom exacerbations (Luo et al 2004; Murphy et al 2004; Swedo et al 1998), the presence of potentially pathogenic antineural antibodies (Kiessling et al 1993; Kirvan et al 2003; Morshed et al 2001; Singer et al 1998), and the presence of the D8/17 surface antigen on lymphocytes (Luo et al 2004; Murphy et al 1997; Swedo et al 1997), but these results have not been definitive. In contrast, relatively few studies have examined the possible role of cytokines and T cell function in these disorders, and most of these have been limited to studies of adult OCD patients (Brambilla et al 1997; Denys et al 2004; Mittleman et al 1997; Monteleone et al 1998).

This prospective longitudinal study was carried out to investigate whether early-onset OCD and tic disorders are associated with altered serum concentrations of cytokines that are characteristically released during either T cell activation, including interferon (INF)-γ, interleukin (IL)-2, IL-4, IL-5, IL-6, and IL-10; or during local inflammation IL-12, INF-α, tumor necrosis factor (TNF)-α, and brain-derived neurotrophic factor (BDNF). The serum concentrations of these cytokines were determined in patients with TS and/or early-onset OCD at multiple time points, including study entry (baseline), the regular 4-month visit that occurred before the symptom exacerbation, acute symptom exacerbation, and at a point 2 months after the onset of an acute symptom exacerbation. A set of exploratory analyses were also planned to determine whether the PANDAS subgroup had a distinctive cytokine profile and whether the subject's gender, age, and/or medication status were related to cytokine levels.

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Methods and Materials

Subjects

Forty-six children, aged 7–17 years, with a chronic tic disorder, OCD, or both and 31 healthy children without these disorders were assessed at baseline and followed prospectively for periods ranging from 4 to 24 months.

All patients were followed at the Yale Child Study Center Tic Disorder–Obsessive-Compulsive Disorder Specialty Clinic. Expert clinicians using DSM-IV criteria made all psychiatric diagnoses based on all available information. Exclusion criteria included an intelligence quotient of <75; serious medical illness; major sensory handicaps (e.g., blindness, deafness); major neurologic disease (including a seizure disorder); head trauma resulting in loss of consciousness; current (past 6 months) psychiatric disorder that could interfere with participation, such as major depression; psychosis; and autism or another pervasive developmental disorder. All parents provided written informed consent after the study was described to them in detail. A separate assent form was used to ensure the informed participation of the child and adolescent subjects.

When a family entered the study, information concerning the patient was collected in a two-stage process, as previously described (Findley et al 2003; Lin et al 2002; Luo et al 2004). The first stage consisted of the collection of information concerning symptoms associated with TS and OCD according to a self-and-family report (Tourette Syndrome International Consortium for Genetics 1999) based on the tic inventory, ordinal severity scales of the Yale Global Tic Severity Scale (YGTSS) (Leckman et al 1989), and the symptom checklist and ordinal scales of the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) (Goodman et al 1989; Scahill et al 1997). The semi-structured interview used to assess and diagnose PANDAS cases was based on those used by the Pediatrics and Developmental Neuropsychiatry Branch of the National Institute of Mental Health Intramural Program (Swedo et al 1989). In a second stage of assessment, an experienced clinician reviewed these symptom ratings with the child and the parent to ensure their accuracy and validity. Comorbid psychiatric diagnoses were made with all available information, including data collected with the Schedule for Affective Disorders and Schizophrenia for School-Age Children (Kaufman et al 1997). The Human Investigation Committee at Yale University School of Medicine approved each of these studies, and all parents provided informed consent.

Ratings of Symptom Severity

Tic symptom severity was rated with the tic portion of the YGTSS ($YGTSS_{TIC}$). Obsessive-compulsive symptom severity was rated with the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS). The $YGTSS_{TIC}$ and the CY-BOCS are widely used scales with excellent psychometric properties. In addition, symptoms of depression and anxiety were assessed with the Children's Depression Rating Scale-Revised (CDRS-R) (Poznanski et al 1984) and the Revised Children's Manifest Anxiety Scale (RCMAS) (Reynolds and Paget 1985), respectively. Tic and OC symptom exacerbations were identified according to previously described two-stage algorithms (Lin et al 2002).

In the case of tic symptoms, exacerbations were identified when the current monthly YGTSS rating exceeded the previous monthly rating by 9 points (Δ $YGTSS_{TIC}$ score > 9) and the current $YGTSS_{TIC}$ score exceeded 19. For OC symptoms, exacerbations were identified when the current monthly CY-BOCS rating exceeded the previous monthly rating by 7 points (Δ CY-BOCS score > 7) and the current CY-BOCS score exceeded 16. Finally, when considering the summed total of tic and OC symptoms, we identified as exacerbation points where the Δ score ($YGTSS_{TIC}$ + CY-BOCS) was greater than 14 and the current score ($YGTSS_{TIC}$ + CY-BOCS) exceeded 33.

Procedures

This study was part of a larger, ongoing, 24-month prospective longitudinal study. In addition to the monthly clinical assessments, serum specimens were collected at regular 4-month intervals. If an exacerbation of tic or OC symptoms was detected, two additional assessments were performed (one as soon as possible after the detection of the exacerbation and another 2 months later). Each of these assessments also included a collection of serum.

Determination of Cytokine and BDNF Content in Serum by Enzyme-Linked Immunosorbent Assay

The serum specimens were aliquoted and placed in a −20° freezer for 24 hours and then transferred to a −80° freezer for long-term storage before assay. The serum concentrations of IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, INF-α, INF-γ, and TNF-α were measured in duplicate by multiplex enzyme-linked immunosorbent assay (ELISA) with the Pierce SearchLight technology (Pierce Boston Technology, Woburn, Massachusetts) (http://www.searchlightonline.com) (Moody et al 2001). The concentration of serum BDNF was measured in duplicate with the capture ELISA kit (BDNF E_{max} ImmunoAssay System; Promega, Madison, Wisconsin) (http://www.promega.com/techserv/tools/elisasst/BDNFEmax.htm).

Data Analysis

The results are reported as mean ± 1 SD. SAS standard mixed and general linear model procedures (SAS Institute, Cary, North Carolina) were used to compare serum cytokines and BDNF levels across diagnostic groups. Pearson correlation coefficients were calculated to test for possible associations among variables. To evaluate the relationship between the cytokine levels and tic, OC, and depressive symptom severity measures across time, we conducted a series of repeated-measures analyses with mixed linear model procedure in SAS. Statistical significance for all analyses was set at the level of .05 for a two-tailed test.

Results

Description of Study Cohort

The age at baseline, the gender distribution, age of symptom onset, and symptom severity scores at baseline are presented in Table 1 and indicate that the sample is a relatively typical group of clinically referred pediatric patients with TS and early-onset OCD. On average, the patient group endorsed more symptoms of anxiety and depression than did the control subjects at baseline (Table 1). A majority of the patients (37 of 46, 80%) were receiving medications at baseline to control their tic and/or OCD symptoms; 18 patients (39%) were receiving α agonists; 20 (43%) patients were receiving selective serotonin reuptake inhibitors (SSRIs); and 14 (30%) patients were receiving neuroleptics. Among the 9 patients studied at exacerbation, 6 were receiving α agonists, 6 were receiving SSRIs, and 3 were receiving neuroleptics.

Baseline Comparisons

At baseline, serum IL-12 and TNF-α levels were elevated in the patients compared with control subjects (Table 2). On