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Screening for Autoantibodies in Children With Opsoclonus-Myoclonus-Ataxia

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Various paraneoplastic autoantibodies have been linked to discrete neurologic syndromes and tumors in adults, but little is known about their incidence in children. We report a cross-sectional study of known paraneoplastic antibodies in 59 children with opsoclonus-myoclonus-ataxia, 86% of whom were moderately or severely symptomatic, and 68% of whom had relapsed at the time of testing. This total number of patients includes 18 children with low-stage neuroblastoma (tested after tumor resection), six of whom had never been treated with immunosuppressants. All were seronegative for anti-Hu, anti-Ri, and anti-Yo, the three paraneoplastic antibodies most associated with opsoclonus-myoclonus or ataxia in adults. These data contrast with reports of anti-Hu-positive sera in children with high-stage tumors and suggest that anti-Hu, anti-Ri, and anti-Yo do not explain relapses in pediatric opsoclonus-myoclonus-ataxia. They underscore the need to search for unique autoantibodies, as well as cellular mechanisms of pediatric paraneoplastic disease.

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Introduction

Paraneoplastic neurologic syndromes affect cancer patients of all ages, but children differ from adults in the associated tumor types, as well as in clinical phenotypes [1]. Antineuronal autoantibodies have been the focus of considerable research on proposed autoimmune mechanisms underlying the remote effects of cancer, and many have been described in adults [2]. Only one of these, anti-Hu, has been reported in children with neuroblastoma [3], the most common extracranial malignant neoplasm of early life [4] and the tumor most often associated with opsoclonus-myoclonus-ataxia, also referred to as *opsoclonus-myoclonus syndrome*, *dancing eyes syndrome*, or *Kinsbourne syndrome* [5]. Despite positivity for Hu antigen in approximately 75% of neuroblastomas [6], serum anti-Hu antibodies are present in only approximately 4-15% of children with neuroblastoma [6,7], and there has been no large series of the paraneoplastic subgroup. The childhood incidence of other IgG autoantibodies associated either with opsoclonus-myoclonus-ataxia or ataxia in adults, such as anti-Ri [8] and anti-Yo [2], is unknown. Opsoclonus-myoclonus-ataxia, one of the few paraneoplastic syndromes shared by children and adults, is the focus of this study.

Because the diagnosis of neuroblastoma is made difficult by the tumor's high incidence of spontaneous regression [9], we included in our report children who never had a tumor. Some may have had neuroblastoma that involuted

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Table 1. Clinical information at time of testing for autoantibodies

Parameter	All Patients	Tumor Found	No Tumor Found
Number of cases	59	18 (30%)	41 (70%)
Sex			
Male	34 (58%)	11 (61%)	23 (56%)
Female	25 (42%)	7 (39%)	18 (44%)
Age at onset (yr)*	1.7 ± 0.1 (0.5–5.8)	1.5 ± 0.2 (0.8–2.8)	1.8 ± 0.2 (0.5–5.8)
Age at testing (yr)*	4.1 ± 0.3 (0.8–12)	4.4 ± 0.5 (1.6–8.7)	3.9 ± 0.4 (0.8–12.3)
Syndrome duration (yr)*	2.4 ± 0.3 (0.1–11.5)	2.9 ± 0.5 (0.7–7.9)	2.1 ± 0.4 (0.3–11.5)
Time between tumor resection and antibody testing (yr)	–	2.6 ± 0.4 (0.1–6.9)	–
Syndrome severity at testing			
Mild	8 (14%)	2 (11%)	6 (15%)
Moderate	24 (40%)	7 (39%)	17 (41%)
Severe	27 (46%)	9 (50%)	18 (44%)
Number relapsing	40 (68%)	13 (72%)	27 (66%)
Number previously untreated	20 (34%)	6 (33%)	14 (34%)
Number previously treated†	39 (66%)	12 (67%)	27 (66%)
ACTH	21	9	12
Prednisone	21	5	16
IVIG	15	6	9
Azathioprine	2	2	0
Chemotherapy	4	4	0
Plasmapheresis	1	1	0
Number currently treated at testing†	12 (20%)	4 (22%)	8 (20%)
ACTH	7	2	5
Prednisone	4	1	3
IVIG	1	1	0
Azathioprine	1	0	1

* Mean ± S.E.M. is given for age, duration, and interval time parameters. Ranges are given in parentheses.
† Some children received more than one type of therapy, therefore percentages of individual treatments do not tally to 100% and therefore are not given.

Abbreviations:
ACTH = Adrenocorticotrophic hormone
IVIG = Intravenous immunoglobulins

and escaped detection [10]. The classification of these children is crucial but problematic. Although the term *paraviral* has been applied because of flu-like prodromal symptoms, documentation of a specific opsoclonus-myoclonus-ataxia-associated neuropathic virus, such as Epstein-Barr [11], Coxsackie B [12] and other enteroviruses [13], or St. Louis encephalitis [14], is not routine. Also, a viral-like presentation does not preclude finding an occult tumor [5]. In the absence of a bona fide pathogen, "idiopathic" may be preferable [15]. Detection of "paraneoplastic" autoantibodies in adults in whom no tumor was ever identified [6,8] further justifies screening children without a demonstrated neoplasm.

Patients and Methods

Patients

Fifty-nine children with opsoclonus-myoclonus-ataxia were referred to the National Pediatric Myoclonus Center over a 5-year period for neurologic relapse or failure to respond to therapy (68%) or for initial treatment (34%). Each underwent diagnostic testing for neuroblastoma, clinical phenotyping, and blood drawing for autoantibody screening. Table 1 lists patient characteristics. Clinical severity was assessed as mild, moderate, or severe using a global measure of dysfunction in more than one category, such as motor, cognitive, or behavioral. Children rated as "severe" had a marked gait disturbance, daily behavioral problems, and pronounced developmental delay. Past treatments included tumor resection, corticotropin (adrenocorticotrophic hormone), intravenous immunoglobulins, corticosteroids, azathioprine, cancer chemotherapy, or plasmapheresis. Table 2 presents the tumor characteristics.

Table 2. Tumor information

Parameter	No. of Patients
Tumor type	
Neuroblastoma	13 (72%)
Ganglioneuroblastoma	5 (28%)
Tumor location	
Thoracic (posterior mediastinal)	6 (33%)
Abdominal (adrenal, paraspinal)	12 (67%)
Tumor N-*myc*	
Amplified	0
Unamplified	18 (100%)
Tumor staging*	
1	15
2	3
3	0
4	0

* Neuroblastomas were staged by the International Neuroblastoma Staging System.

Autoantibody Detection

Sera for anti-Hu, anti-Ri, and anti-Yo were screened by Athena Diagnostics (Worchester, MA; formerly, Genica Pharmaceuticals Co.). Before 1996, anti-Hu and anti-Yo were evaluated by Specialty Laboratories, Inc. (Onc Quest, Inc., Santa Monica, CA). Titers were reported as negative if less than 10 U/mL. Detection of anti-Hu autoantibodies was performed by enzyme-linked immunosorbent assay using purified human recombinant antigen with confirmation of positive Hu tests by Western blot analysis (40-kDa band). The detection of Yo (62-kDa band) and Ri (40-kDa band) was also performed by Western blot analysis using recombinant human antigen.

Statistical Analysis

Differences between paraneoplastic and idiopathic groups were analyzed statistically by Fisher's exact test. *P*-values less than 0.05 were considered statistically significant.

Results

All of the children were still symptomatic at the time blood was drawn, 47 (80%) were not on treatment at the time of antibody testing, and 20 (34%) had never received immunotherapy. Of those previously treated, 54% received adrenocorticotrophic hormone (not high-dose protocol), 54% prednisone, and 38% intravenous immunoglobulin, administered at different times either alone or in combination with chemotherapy, azathioprine, or plasmapheresis. The tumor and idiopathic groups were similar demographically. There was no statistically significant difference between them in patient age at time of blood testing, age at syndrome onset, neurologic severity, syndrome duration, frequency of relapse, or treatment profile. The ratio of males to females was 1.4:1 regardless of etiology.

Neuroblastoma was 2.6 times more common than ganglioneuroblastoma and twice as often found in the abdomen as in the chest. In all patients the N-*myc* tumor gene was not amplified. All children survived their tumor, even those who received no antineoplastic therapy.

Of 59 children with opsoclonus-myoclonus-ataxia screened for serum autoantibodies, none had anti-Hu, anti-Ri, or anti-Yo. These included 18 with resected neuroblastoma, six of whom had never been treated with immunosuppressants of any type and eight others who had been treated in the past, but not within months before the relapse and autoantibody testing. Fifty-one (86%) of the seronegative children manifested moderate-to-severe neurologic symptoms, and 40 (68%) were relapsing at the time of testing.

Discussion

This study represents the largest reported paraneoplastic autoantibody screening in childhood opsoclonus-myoclonus-ataxia. The data reveal that, in the experience of the National Pediatric Myoclonus Center, three serum autoantibodies associated either with paraneoplastic opsoclonus-myoclonus-ataxia or ataxia in adults were not found in children with moderate-to-severe opsoclonus-myoclonus-ataxia and relapses. In a recent screening of sera from the Children's Cancer Group Serum Bank, 13 of 16 children with neuroblastoma-associated opsoclonus-myoclonus-ataxia had IgG antineuronal antibodies, but only four had anti-Hu and the rest did not have a previously identified autoantibody [7]. Although we did not find anti-Hu antibodies in our patients, both studies agree that anti-Ri and anti-Yo were not encountered.

On the issue of anti-Hu, the studies are complementary rather than contradictory. In the CCG study, 85% of patients with neuroblastoma had blood drawn less than 6 months after onset and before treatment, whereas the majority of our patients were evaluated later, on average 2 years after tumor resection and other therapies. Prior treatment may have eradicated autoantibodies. However, persistently positive anti-Hu serologies have been reported in children with neuroblastoma and a paraneoplastic syndrome despite treatment [3,6]. If anti-Hu antibodies were present initially, they did not persist, even in the presence of neurologic relapses or refractory symptoms. Such was the case in 10 other children with neuroblastoma who were evaluated several years after initial presentation, four of whom had never been treated [16].

It also may be significant that three of four children with opsoclonus-myoclonus-ataxia in the CCG questionnaire study who were positive for anti-Hu had stage 3 neuroblastoma, whereas the tumors in our patients were stages 1 or 2. In a previous report, children who were seropositive for anti-Hu all had stage 4 neuroblastoma [6]. Low-stage neuroblastoma with absence of N-*myc* amplification is more typical of pediatric opsoclonus-myoclonus-ataxia [16,17].

Another difference between studies is that we made comparisons between opsoclonus-myoclonus-ataxia of paraneoplastic and idiopathic etiologies, whereas the other study compared neuroblastoma groups with and without opsoclonus-myoclonus-ataxia, discovering the same autoantibodies in the nonparaneoplastic group. Because of the rarity of opsoclonus-myoclonus-ataxia, it is useful to combine the data of various studies that used the same methodology for antibody detection. Such an approach yields an apparent incidence of anti-Hu seropositivity in children with both neuroblastoma and opsoclonus-myoclonus-ataxia of approximately 10% or five of 49 patients: zero of 18 (our study), zero of 10 [16], one of five [6], and four of 16 [7]. This finding differs little from the 4-15% of anti-Hu-positive sera in children with neuroblastoma who do not have a paraneoplastic syndrome [6,7]. Our sample of 18 paraneoplastic patients lacks the evidence to discount the belief that anti-Hu has some role for some opsoclonus-myoclonus-ataxia in neuroblastoma, but clearly anti-Hu is not a frequent culprit in this disorder, its relapses, or persistence.

We also think it is likely, given the lack of demographic differences between the two groups in our study, that

many of the "idiopathic" group at one time did have neuroblastoma. If that is the case, then the sample size and statistical power increases substantially, with 14 additional patients not previously treated. More direct methods of testing that hypothesis are underway at our center.

Of both studies, it may be said that not sampling cerebrospinal fluid may preclude finding autoantibodies, because titers can be higher in cerebrospinal fluid than in serum. However, when cerebrospinal fluid is positive for anti-Hu or anti-Ri, the patient usually is also seropositive [3,18,19], and titers may be several-fold higher in serum than in cerebrospinal fluid [3].

Our study illustrates the high incidence of relapses in childhood opsoclonus-myoclonus-ataxia, their occurrence despite tumor resection, and persistence despite immunotherapy. Relapse after treatment is one of the most difficult challenges to the successful therapy of pediatric opsoclonus-myoclonus-ataxia. It is likely that our center attracts more refractory patients, in which case the incidence of relapse may be inflated by referral bias. Also, these figures reflect relapse rate before initiation of our combination treatment protocols.

Finally, in not finding the adult type of paraneoplastic autoantibodies, our study underscores the need to view childhood opsoclonus-myoclonus-ataxia as an entity distinct from opsoclonus-myoclonus-ataxia in adults. Although routine screening for these autoantibodies is not cost-effective, the data stress the importance of looking for new autoantibodies, as well as cellular mechanisms of autoimmune disease to explain relapses in children with opsoclonus-myoclonus-ataxia. Such studies are in progress at our center.

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