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Antineuronal Antibodies in Rheumatic Chorea

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The objectives of our study were to examine the sera of rheumatic chorea (RhCh) patients (those with acute or chronic RhCh or with a past history of RhCh) for the presence of antineuronal antibodies (ANeurA) and to correlate the results with disease activity, chronicity, and the number and durations of choreic attacks. Subjects were inpatients of the Pediatric Hospital, Ain Shams University, and outpatients of the Outpatient Pediatric Cardiology Clinic (both in Cairo, Egypt). Forty children with RhCh (mean age, 10.9 years) and 40 healthy controls were tested. An indirect-immunofluorescence technique was used for the detection of ANeurA. ANeurA were present in the sera of 100, 93, and 44% of the patients with acute, chronic, and past histories of RhCh, respectively. A definition of chronic chorea is presented for the first time. None of the control subjects had ANeurA in their sera. The presence of ANeurA correlated with disease activity. A statistically significant increase ($P < 0.01$) in the prevalence of ANeurA was found for patients with active chorea (acute and chronic) compared with the prevalence in patients with past histories of RhCh (controlled chorea). ANeurA were present in the sera of both patients with acute RhCh and patients with chronic RhCh, yet patients with acute RhCh showed more brightness and cell staining than chronic patients. The severity, number, and duration of each attack were not related to the presence of ANeurA. These results strengthen further the concept of autoimmunity being the basis for the pathogenesis of RhCh. The presence of ANeurA correlated with the activity of RhCh but not with the severity, number, or duration of attacks. Humoral immunity definitely plays a role in RhCh; thus, routine administration of corticosteroids to patients with acute RhCh is suggested to prevent neuron damage and chronicity. The chronicity of chorea is not due to a further increase in ANeurA but is probably due to sensitivity to these antibodies.

Rheumatic chorea (RhCh) or Sydenham's chorea, the most prevalently acquired childhood chorea (9) is characterized by adventitious choreic movements; muscle weakness; and disturbances of speech, voluntary movements, and gait (1, 15, 19). Patients have been described as anxious, inattentive, overtly sensitive, and distractible (5, 6, 21), and some exhibit obsessive-compulsive symptoms (17). RhCh's clinical manifestations have been attributed to an antibody-mediated immune response directed against a neural antigen, with stimulation of target cell activity in the corpus striatum (20). Exposure of a susceptible individual gives rise to exaggerated humoral and immune responses to those streptococcal antigens, which are cross-reactive with human brain tissue (23). The concept of antigenic mimicry explains the basis of this cross-reaction (2).

Although the incidence of RhCh has declined in the Western world, several cases of resurgence of rheumatic fever and hence RhCh have occurred in the past 10 years (8, 10). Chorea is still a common manifestation of rheumatic fever, particularly in developing countries. In addition, in many cases it tends to be recurrent and resistant to treatment.

Provoked by the occurrence of frequent, recurrent attacks of RhCh in the same patient, we carried out this work to answer several questions. Are there antineuronal antibodies (ANeurA) in the sera of patients with RhCh? Are these ANeurA related to the activity of the disease, the severity of a choreic attack, the number of attacks, or the duration of an attack? Do patients with chronic chorea have more ANeurA and does this excess lead to an increased number and brightness of stained brain neurons?

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MATERIALS AND METHODS

This study included 40 patients (30 females and 10 males) with RhCh (mean age ± standard deviation, 10.9 ± 2.3 years) and a control group of 40 healthy children (mean age ± standard deviation, 11 ± 2 years). The patients were divided into three groups: (i) children with acute RhCh ($n = 10$) experiencing their first attack of chorea; (ii) children with chronic RhCh ($n = 14$), i.e., with chorea that had recurred more than once after the patients were treated (with the patient remaining free of symptoms for 1 month or more between the attacks) or with chorea that persisted for more than 6 months (one patient); and (iii) children with past histories of RhCh ($n = 16$) (for whom >6 months had passed since the last attack and who did not have active chorea at the time of the study).

The control subjects were healthy children free of infection and who had received no medications for 2 weeks prior to sampling. A full history was taken from each patient, with stress being placed on determining the onset of the disease; the duration, distribution, and severity of choreic attacks; the number of attacks; and the time of the last attack. Complete clinical examinations were performed. The severity of the choreic movements was graded according to a standard neurological examination that included six tests for minimal brain dysfunction, namely, tests for adventitious movements, mirror movements, fine motor coordination, gross motor coordination, and unsteady gait. These parameters were graded as absent, mild, moderate, or severe (4).

We examined all subjects to determine their erythrocyte sedimentation rate (ESR) and antistreptolysin-O (ASO) titers and the presence of C-reactive protein (CRP). We also performed an X ray of the chest and heart, an electrocardiogram, and an echodoppler exam on each subject.

Diagnoses were made according to the revised Jones criteria (16) and after exclusion of other causes of chorea.

Patients with acute chorea associated with rheumatic carditis received corticosteroides and haloperidol. Patients with pure chorea received haloperidol only.

Immunofluorescent technique. The sera of the patients and controls were examined for the presence of ANeurA with fluorescein isothiocyanate (FITC)-labeled antiserum of human immunoglobulin G (IgG; Behring Werke Laboratories) in an indirect-immunofluorescence test based on that described by Wilson et al. (22). Frozen sections from the caudate nucleus were used for slide antigens and were prepared as follows. Unfixed tissue from the caudate nucleus was dissected from a fresh human brain which had been obtained (after parental consent) from an autopsied stillborn cadaver, aged 34 weeks, within 5 h after death. The sections were thawed and allowed to dry thoroughly at room temperature and then fixed in acetone for 10 min and dried. They were then incubated with undiluted test sera for 45 min at 37°C in a moist chamber, rinsed three times in phosphate-buffered saline (PBS; pH 7.4) for 30 min, and thereafter overlaid with FITC-labeled antihuman IgG (diluted 1:10 in PBS [pH 7.4]) for 45

TABLE 1. Number and severity of RhCh attacks and rheumatic manifestations of the studied groups[a]

RhCh (no. of patients)	No. of patients with:										
	Previous attacks numbering:					Mild RhCh	Moderate RhCh	Severe RhCh	P.H. Rh arthritis	Rh. MR	Pure chorea
	0	1	2	3	>3						
Acute (10)	10						3	7	4	3	3
Chronic (14)		1[b]	4	2	7	2	5	7	3	4	7
P.H. (16)		9	5	2		NA	NA	NA	5	5[c]	8

[a] P.H., past history; P.H. Rh arthritis, past history of rheumatic arthritis; Rh. MR, rheumatic mitral regurge; NA, no active chorea at the time of the study.
[b] The attack lasted more than 8 months.
[c] Two patients had past histories of arthritis and mitral regurge.

min at 37°C in a moist chamber. Finally, the sections were rinsed three times in PBS and mounted in buffered glycerol, pH 9. Stained sections were then examined in a UV microscope. FITC-derived positive immunofluorescence appeared apple green. In contrast, lipofuchsin granule-derived autofluorescence appeared yellow or orange. Specificity of positive immunofluorescence was checked by including negative controls in which only the FITC-labeled secondary-antibody layer was used.

Positive results were analyzed according to the following grading systems. (i) The numbers of stained neurons were graded as few (less than 5 cells per high-power field [HPF]), moderate (5 to 10 cells/HPF), and many (>10 cells/HPF). (HPF = 40 × 10.) (ii) The brightness of fluorescent staining was graded as faintly bright, moderately bright, and very bright fluorescence.

Statistical analysis. The results were analyzed by using analysis of variance and the chi-square test in a computer database program.

RESULTS

The numbers of choreic attacks, severity of the attacks, and rheumatic manifestations of the studied groups are shown in Table 1. All the patients in the acute RhCh group were experiencing their first choreic attack. Its duration ranged from 2 to 12 weeks (mean, 4.6 weeks). The onset of the disease in the chronic RhCh group ranged from 8 months to 9 years (mean ± standard deviation, 3.39 ± 2.73 years) prior to sampling. In the group of patients with past histories of RhCh, the choreic attacks occurred 8 months to 8 years (mean, 2.73 years) prior to sampling. At the time of the study, all patients in this group were free of chorea.

Patients with pure chorea or those with past histories of rheumatic arthritis were negative for CRP and their ESR and ASO titers were not raised. Only patients with concomitant chorea and carditis had raised ESR (mean, 80 mm Hg in the first hour) and raised ASO titers (mean, 600 Todd units) and were positive for CRP.

Patients with both RhCh and rheumatic carditis developed rheumatic mitral regurge (Table 1). The results of the electrocardiogram, heart X ray, and echodoppler exam were used to confirm cardiac involvement.

ANeurA status and the numbers and brightness of stained neurons of the chorea patients are shown in Table 2. Control subjects showed a complete absence of ANeurA.

Patients with active, uncontrolled chorea (acute- and chronic-RhCh groups) showed a statistically significant increase in the presence of ANeurA compared to prevalence in patients with controlled chorea, i.e., patients with past histories of RhCh (Table 3). Moreover, the chronic-RhCh patients showed a statistically significant increase in the presence of ANeurA compared to the prevalence in patients with past histories of RhCh (Table 3).

The prevalence of ANeurA was significantly increased when the onset of the rheumatic attack was less than 6 months prior to sampling. Nevertheless, the presence of ANeurA was not related to either the number of choreic attacks or the duration of an attack (Table 4).

The numbers of stained neurons and their brightness in specimens from patients with mild, moderate, and severe chorea are shown in Tables 5 and 6.

DISCUSSION

It was stated that Sydenham's chorea is a central nervous system disease, often of insidious onset and finite duration (3). In Egypt, where approximately 50% of patients with RhCh develop recurrent chronic attacks of chorea, it is difficult to tell exactly when these attacks will end. Although the disease is of finite duration, the patient suffers from unpredictable periods of abnormal movements and behavior disorders, failing scholastic performance, and missed days at school and this situation causes psychic stress to the whole family.

In this work, the patients' sera were tested against an antigen prepared from the caudate nucleus of a stillborn fetus. This particular site was chosen based on the findings of Kienzle et al. (13), who found by magnetic resonance imaging that the site of pathology for RhCh was located in this area. This location correlated with anatomical areas thought to be susceptible to cross-reaction with IgG antibodies that form in response to streptococcal infection.

When the three groups of patients positive for ANeurA were compared, we found that the patients with acute RhCh had the

TABLE 2. ANeurA status and brightness and number of stained neurons by group

RhCh (no. of patients)	No. of patients							
	Positive for ANeurA	Negative for ANeurA	With neurons that stained with brightness[a]:			With indicated no. of neurons		
			+	++	+++	Few	Moderate	Many
Acute (10)	10	0		6	4	1	2	7
Chronic (14)	13	1	4	9		3	10	
P.H.[b] (16)	7	9	3	4		7		

[a] +, faintly bright; ++, moderately bright; +++, very bright.
[b] P.H., patients with past histories of RhCh.

TABLE 3. ANeurA in the three groups of RhCh patients

Group[a] (no. of sera tested)	No. (%) of sera		*P* value[b]
	Positive for ANeurA	Negative for ANeurA	
Acute+ChRhCh (24)	23 (96)	1 (4)	<0.001
P.H. RhCh (16)	7 (44)	9 (56)	
ChRhCh (14)	13 (93)	1 (7)	<0.001
P.H. RhCh (16)	7 (44)	9 (56)	

[a] Acute+ChRhCh, groups of patients with acute and chronic RhCh ChRhCh) were pooled (patients with active chorea); P.H. RhCh, group of patients with past histories of RhCh (patients with controlled chorea).
[b] A *P* of <0.001 is highly significant.

TABLE 5. Relationship between the number of stained neurons and the severity of chorea

Severity of chorea[a]	No. (%) of patient specimens with:			Total no. of patients
	Few neurons	Moderate no. of neurons	Many neurons	
Mild	1 (100)	0	0	1
Moderate	1 (12.5)	5 (62.5)	2 (25.5)	8
Severe	2 (14)	7 (50)	5 (36)	14
Total	4 (17.5)	12 (52.5)	7 (30)	23

[a] Severity of chorea was assessed for uncontrolled cases, i.e., for patients in the acute-RhCh group ($n = 10$) and chronic-RhCh group ($n = 14$), excluding the patient who did not have ANeurA.

highest percentage of positivity for ANeurA in serum (100%). Patients with chronic RhCh also had a high percentage of positivity (93%), while patients with past histories of RhCh showed the least positivity (44%).

Although Swedo et al (17) similarly demonstrated the presence of ANeurA in 91% of their patients with acute RhCh, they did not categorize their patients into groups with acute and chronic disease. Another study (18) failed to detect ANeurA in patients with past histories of RhCh. This finding was explained by the extended period between the onset of the patient's illness and the time of sample collection, which was more than 1 year. These results are different from ours, since our patients with past histories of RhCh still demonstrated the presence of ANeurA even years after their attacks. The lower prevalence of ANeurA in our group of patients with past histories of RhCh is probably related to the fact that they did not exhibit choreic movements at the time of the study. Therefore, the prevalence of ANeurA increased significantly during disease activity (i.e., in patients with acute and chronic active chorea).

The clinical categorization of active chorea, according to its course, into acute and chronic is put forward in this work for the first time. We use the term chronic chorea to describe (i) chorea that recurred after being treated and of which the patient remained free for 1 month or more between the attacks or (ii) chorea that persisted for more than 6 months in spite of treatment and tended to increase with tapering of treatment. Levels of prevalence of ANeurA were not statistically different in the groups of patients with acute and chronic chorea, indicating that chronicity was not associated with a further increase in positivity for ANeurA. Since damage of some basal ganglion neurons was observed in brain magnetic resonance images of RhCh patients (12), it is suggested that the chronicity of RhCh is not due to an increase in the level of ANeurA but rather to a sensitivity of the viable basal ganglion neurons to any increase in the level of ANeurA.

Surprisingly, an increase in the number of choreic attacks was associated with an insignificant change in the prevalence of ANeurA. Many cells with the brightest staining were seen in specimens from patients with acute chorea; in contrast, specimens from patients with past histories of RhCh showed the lowest numbers of stained cells.

The ESR, ASO titers, and levels of CRP were normal in patients with pure chorea because of the long latent period between streptococcal infection and chorea (11). Frequently, chorea is the sole manifestation of rheumatic fever.

The results of this work strengthen further the immunologic basis of the pathogenesis of rheumatic fever, since they demonstrate that an antigen-antibody reaction occurs in patients with RhCh. This reaction can explain the previously reported (7) effectiveness of corticosteroids in controlling RhCh. Moreover, of our patients with active chorea, those who received corticosteroids for associated carditis showed a more rapid improvement of their choreic movements than those who received haloperidol alone. Hence, administration of corticosteroids to patients with acute RhCh is suggested to prevent the antigen-antibody reaction, which might damage brain nuclei. Although humoral immunity is definitely activated in patients with RhCh, the lack of a specific relation between the level of ANeurA and the severity, number, and durations of attacks suggests the presence of an additional mechanism that aids in the damage of these neurons. The role of cell-mediated immunity in RhCh previously suggested by other studies (14) remains to be verified.

Thus, it is concluded from this work that ANeurA are

TABLE 4. Relation between the presence of ANeurA and the onset, number, and durations of attacks in RhCh patients

Characteristic of choreic attack(s) in patients	No. (%) of patients		*P* value[a]
	Positive for ANeurA	Negative for ANeurA	
Onset			
<6 mo from sampling	23 (96)	1 (4)	<0.001
>6 mo from sampling	7 (31)	9 (69)	
No.			
1	15 (75)	5 (25)	>0.05
>1	15 (75)	5 (25)	
Duration			
<6 wk	26 (74)	9 (26)	>0.05
>6 wk	4 (80)	1 (20)	

[a] $P < 0.001$, highly significant; $P > 0.05$, insignificant.

TABLE 6. Relationship between the brightness of stained neurons and the severity of chorea

Severity of chorea	No. (%) of specimens with indicated brightness of staining[a]			Total no. of cases
	+	++	+++	
Mild	1 (100)	0	0	1
Moderate	1 (12.5)	6 (75)	1 (12.5)	8
Severe	2 (14)	9 (64)	3 (21)	14
Total	4 (17.5)	15 (65)	4 (17.5)	23

[a] +, faintly bright; ++, moderately bright; +++, very bright.

present in the sera of RhCh patients. These ANeurA are related to disease activity but not to the severity, number, or durations of the choreic attacks. Chronicity of RhCh is not due to an increase in levels of ANeurA above the levels seen in patients with acute RhCh but is probably due to an increased sensitivity of the remaining viable neurons to any rise in ANeurA.

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