ANTIBODIES REACTING WITH CYTOPLASM OF SUBTHALAMIC AND CAUDATE NUCLEI NEURONS IN CHOREA AND ACUTE RHEUMATIC FEVER*

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Although a considerable body of evidence has implicated the Group A streptococcus in the pathogenesis of rheumatic fever, the exact role the organism plays in the initiation of the pathological process still remains unclear. Among various hypotheses entertained has been the concept that the Group A streptococcus, through its long association with its human host, has acquired antigens which cross-react with mammalian tissues. During the infection with the organism, the rheumatic susceptible individual may produce an unusual immune response to streptococcal antigens eliciting antibodies which bind to host tissues. This concept has been strengthened by the finding in many laboratories that Group A streptococci contain antigens which cross-react with a variety of mammalian tissues including cardiac and skeletal muscles (1–4), valvular tissue (5, 6), kidney (7), skin, and even certain neuronal tissues (8). Antibodies which bind to heart and skeletal tissue have also been found in the sera of acute rheumatic individuals, correlate well with clinical parameters of the disease, and are invariably seen with recurrences (9). The streptococcal nature of heart-reactive antibodies in acute rheumatic fever has been established by the absorption of these antibodies with streptococcal antigens (1, 9).

Despite these findings, it has been difficult to reconcile the presence of these tissue-reactive antibodies with one of the most striking clinical forms of acute rheumatic activity – namely, the occurrence of Sydenham's chorea. The clinical manifestations of this form of rheumatic fever are presumed to be associated with an inflammatory process or physiologic dysfunction of the brain (10, 11) and are often seen without any other evidence of rheumatic activity, i.e., carditis or arthritis.

Among the possibilities entertained to explain this unusual form of rheumatic activity is the concept that patients with chorea possess circulating antibodies directed against components of the central nervous system. The observation that a considerable proportion of children seen at the Free Rheumatic and Heart Center, Giza, Egypt, present primarily with recurrent bouts of chorea

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without the classical manifestations of arthritis and carditis offered us a unique opportunity to test this hypothesis. The present study provides evidence for the presence of antibodies to neuronal tissues. The streptococcal-induced nature of these antibodies was established by the fact that absorptions only with Group A streptococcal membranes abolish the immunofluorescent-staining pattern in neuronal cells. The tissue-specific staining pattern was evidenced by the fact that only isolated neurons abolished the immunofluorescent staining pattern. Moreover, serial studies of sera in a number of patients with chorea appeared to indicate that the presence of these antibodies correlates with the clinical course of disease. A preliminary report of these findings has recently been presented (12).

Materials and Methods

Clinical Material

RHEUMATIC FEVER PATIENTS. A group of 80 children with acute rheumatic fever including 77 patients seen at the Free Rheumatic and Heart Center were studied. In addition three patients from Port of Spain, Trinidad, with acute rheumatic activity were also included in the group. The diagnosis of acute rheumatic fever was made on the basis of the American Heart Association Modified Jones Criteria (13). 30 children (ages 6–14 yr) had rheumatic chorea and many of these patients had recurrent bouts of the disease. In addition a number of these patients had clear-cut physical findings of mitral stenosis and/or mitral regurgitation at the time of the study. An additional group of 50 other children, ages 6–13 yr had primarily active carditis and arthritis without choreiform manifestations. While the majority of these patients were receiving salicylates, three patients had received corticosteroids (5–15 mg of prednisone/day).

For the serial examination of the presence of neuronal antibodies, the sera from six patients with well-documented rheumatic chorea were selected from the serum banks of the Rheumatic Fever Service of The Rockefeller University Hospital, New York. In addition serial sera from four Egyptian patients and one patient from Trinidad were available for study.

CONTROLS. A wide variety of control sera were employed in these studies. They were selected from the following groups of individuals:

(*a*) 71 normal Egyptian and American subjects ranging in ages between 6 and 45 yr. (*b*) 19 acute poststreptococcal glomerulonephritic sera, kindly supplied by Dr. E. Potter, Northwestern University School of Medicine, Chicago, Ill. (*c*) Eight sera obtained from individuals who had uncomplicated streptococcal pharyngitis. The sera from groups 2 and 3 had anti-streptolysin (ASO)[1] titers greater than 250 Todd units. (*d*) 65 sera obtained from patients with systemic lupus erythematosus and rheumatoid arthritis. (*e*) 75 sera from a miscellaneous group of patients with neoplasms, bacterial infections, chronic obstructive lung disease, cirrhosis, and diverse neurological states (i.e. strokes, multiple sclerosis, Parkinson's disease, cerebral atrophy, paresis, peripheral neuropathy, and amyotrophic lateral sclerosis).

All sera to be studied were kept frozen at −20°C until use. Immediately before testing, the sera were heat inactivated at 56°C for 30 min. Sera from the serum bank at The Rockefeller University had never been frozen but had been stored sterilely at 4°C for periods of up to 30 yr.

Immunofluorescence Studies. Because of the probable localization of functional derangement in chorea to the areas which include the subthalamic and caudate nuclei (10, 11) particular care was taken in the selection of the tissues. Accordingly, fresh human brain was obtained from a 45-yr old previously healthy man within 4 h of death in a traffic accident. Specimens from caudate nucleus, subthalamic nucleus, cerebral cortex, and cross sections of medulla containing trigeminal nuclei, geniculate ganglion, and hypoglossal nuclei were dissected from fresh brain, cut into small pieces (3 x 3 x 5 mm), immediately embedded in OCT (Ames Co., Div. of Miles Lab. Inc., Elkhart, Ind.), and snap-frozen in dry ice/acetone. Unfixed frozen sections (4–6 μm) served as substrate for brain antigens. For screening, the sections were incubated with undiluted test sera for 30 min in a moist

[1] *Abbreviations used in this paper:* ANA, anti-nuclear antibodies; ASO, anti-streptolysin; DTT, dithiothreitol; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline.

chamber, rinsed twice in phosphate-buffered saline (PBS), pH 7.4, for 10 min and thereafter overlaid with fluorescein isothiocyanate (FITC)-conjugated pepsin-digested rabbit IgG antibodies to $F(ab')_2$ fragments of human IgG (14), washed, and mounted in PBS/glycerol. Stained sections were then examined by immunofluorescence microscopy, using a Zeiss fluorescence microscope with a mercury HBO 200 W lamp with BG12 primary filter, 53 Barrier filter and the Zeiss F.I.T.C. filter (Carl Zeiss, Inc., New York). Twofold titrations of positive sera were also performed.

To determine the immunoglobulin (Ig) class of the serum antibodies, FITC-conjugated antisera specific for α-, γ-, or μ-chains were used as the second layer in the indirect immunofluorescence test. Specificity of fluorescence was checked by using absorption of specific antisera with respective antigens, as well as by preincubation of the sections with unconjugated antisera.

All sera were also tested for anti-nuclear antibodies (ANA) by indirect immunofluorescence using unfixed frozen sections from human kidney and mouse liver as substrate for antigen (15). The sera were tested both undiluted and at a dilution of 1:10.

Absorption Experiments. When positive staining was recorded, the following absorption experiments were carried out. 1 mg of the antigen to be tested was incubated with 1 ml of both positive and negative sera. Group A streptococcal antigens included Type 6 protoplast membranes, cell walls, and Group A polysaccharide. A further test of specificity included absorptions of rheumatic fever sera with cell walls and membranes from unrelated bacteria (Group D streptococcus) as well as separate absorptions with human Cohn Fraction IV (Pentex Biochemical, Kankakee, Ill.). Initially, absorptions employed 1 mg of absorbing antigen/ml of human serum. In several instances, parallel absorptions using membranes from Group A as well as Group D streptococci were performed using a range of 1.0–0.001 mg/ml of serum.

In order to identify what central nervous system structures were involved with positive immunofluorescent staining, parallel sections from the same tissue blocks were fixed in formalin and stained with hematoxylin and eosin. In many instances when positive neuronal immunofluorescence was recorded, localization to neurons could be confirmed directly under UV fluorescence by identification of yellow granules of autofluorescence representing lipofuchsin in the cytoplasm of positively staining nerve cells. In order to rule out possible specificity for antigens present in the first brain studied, similar sections from fresh brain of five other individuals were also examined.

Streptococcal Strains. The streptococcal strains used in these studies were all obtained from The Rockefeller University Rheumatic Fever Service collection. These included S 43/100/2 (M-type 6), T 12/126/2 (M-type 12), D 678 (M-type 11), and A 928 and A 995 (types 55 and 57, respectively). As a control, a Group D streptococcal strain, A 932, was also used.

Preparation of Streptococcal Antigens. All organisms were grown in filter-sterilized dialyzed Todd-Hewitt broth (Difco Laboratories, Detroit, Mich. prepared in 20-liter carboys as follows: Todd-Hewitt (600 g) and yeast extract (Difco Laboratories) (60 g) were resuspended in 500 ml distilled water using heat. Antifoam (Dow Chemical, Midland, Mich.) was added to prevent excessive foaming. The solubilized media was then placed in dialysis tubing (Fisher Scientific Co., Pittsburgh, Pa.) and dialyzed against two changes of 10 liters of distilled water. The dialysate was finally sterilely filtered into a carboy using a Coors no. 2 candle (Coors Porcelain Co., Golden, Colo.). The carboys were then inoculated with 250 ml of a culture in the logarithmic phase of growth. After incubation at 37°C for 18 h, the cultures were harvested using a Sharples high speed centrifuge (Sharples-Stokes Div., Pennwalt Corp., Warminster, Pa.) and frozen at −70°C until processed further.

For the preparation of protoplast membranes, phage-associated lysin was prepared and purified through cellulose phosphate chromatography according to the procedure of Fischetti et al. (16). Group A streptococcal membranes were prepared using the purified Group C phage-associated lysin as described by Zabriskie and Freimer (4) with slight modification as follows: After thawing, the organisms were washed with saline and then resuspended in 0.06 M sodium phosphate buffer (pH 6.1) containing 4% sodium chloride and 5×10^{-4} μl dithiothreitol (DTT) (10 volumes buffer per gram wet weight of organisms). To this was added DTT-reactivated lysin (10,000 U lysin per gram wet weight of cells) and 5 mg of DNase (Sigma Chemical Co., St. Louis, Mo.). The mixture was incubated at 37°C for 2 h and monitored for protoplast formation by dark-field microscopy and Gram stain. After what appeared to be 100% conversion of intact streptococci to protoplasts, the suspension was incubated for an additional 30 min. The remainder of the treatments and procedures followed those of Zabriskie and Freimer (4) using 10 volumes buffer per gram wet weight of cells for all volumes. The final protoplast membrane preparation was lyophilized.

Group A and Group D cell walls and Group D membranes were prepared as follows: the organisms were thawed and washed two times in 0.01 M PBS, pH 7.6, and finally resuspended in distilled water. Cells were then fragmented in a Vibrogen cell mill (Rho Scientific Co., Commack, N. Y.) and assayed for breakage using the Gram stain. The glass beads were removed by filtration and cell walls were collected by centrifugation at 12,000 *g* for 60 min. The supernate was centrifuged further at 100,000 *g* for 1 h in a Beckman Model L-1 ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.). The pellet contained the cell membrane fraction. Both the cell wall and membrane fractions were washed three times with PBS and finally treated with DNase and RNase (Sigma Chemical Co.) for 2 h at 37°C. After extensive washings with PBS and finally distilled water, the fractions were lyophilized. Each fraction served as a test material for the absorptions.

Tissue Antigens.

SARCOLEMMA. Fresh human heart tissue (10 g) was homogenized in a Sorvall Omnimixer (DuPont Instruments, Sorvall Operations, Newtown, Conn.) in the presence of distilled water for 5 min at 4°C. The thick slurry was centrifuged at 14,000 *g* for 30 min, and the pellet which contained the sarcolemmal sheaths was then washed three times in saline. Next, sarcolemma sheaths were allowed to autolyze in a 100-fold (wt/vol) excess of distilled water for 24 h at 4°C. The sheaths were then centrifuged at 14,000 *g* for 30 min. This process was repeated three times. The sheaths were then treated with DNase and RNase at 37°C for 3 h and subsequently washed three times with distilled water and lyophilized. Under light microscopy, a negligible number of Z bands remained, indicating a loss of intracellular contents. This sarcolemmal sheath preparation served as a source of the insoluble antigenic matrix.

BRAIN. Finely chopped caudate nucleus from fresh normal human brain was utilized to prepare nerve cell bodies using the method described by Sellinger et al. (17), which employs differential centrifugation through sucrose–bovine serum albumin gradients. Fractions containing neuron cell bodies showed 85–90% neuronal perikarya and were used to absorb sera positive for anti-neuronal antibody.

LIVER. Human and mouse liver tissue extracts were prepared by freezing and thawing tissues five times followed by homogenization at high speed in a Sorvall Omnimixer (DuPont Instruments, Sorvall Operations) with an equal volume of PBS. The liver suspensions were then sonicated six times for 30 s in a Bronson sonifier (Bronson Instruments, Inc., Stamford, Conn.) and after centrifugation at 18,000 *g* for 30 min, the supernates as well as tissue pellets were used in absorption studies at a concentration of 1 mg/ml.

Results

Immunofluorescent Staining. When sera from a number of patients with chronic recurrent chorea were layered over sections of brain tissue and counterstained with fluoresceinated anti-human gamma globulin, a striking pattern of immunofluorescence was seen. The type of fluorescence is illustrated in Figs. 1 A and 1 B where large, bright areas of immunofluorescence are seen throughout the section. Comparison with companion hematoxylin eosin sections of the same area (Fig. 1 C) revealed that the staining pattern was localized in the cytoplasm of the neuronal cells of the caudate and subthalamic nuclei. In all cases the positive-staining patterns were localized to the cytoplasm of moderately large neurons. No nuclear membrane or nuclear staining was noted in these preparations. Fig. 1 D demonstrates that when normal sera were applied to the section, no specific fluorescence was seen. The smaller dots of fluorescence (see arrows) were lipofuchsin granules which exhibited yellow or orange autofluorescence present in normal brain sections.

In all instances, immunofluorescent staining was identified as the IgG class of immunoglobulin using H-chain-specific antisera. No staining for IgA or IgM was recorded. In order to rule out possible nonspecific IgG binding of chorea serum antibody to neurons through Fc structures and Fc receptors in neuronal

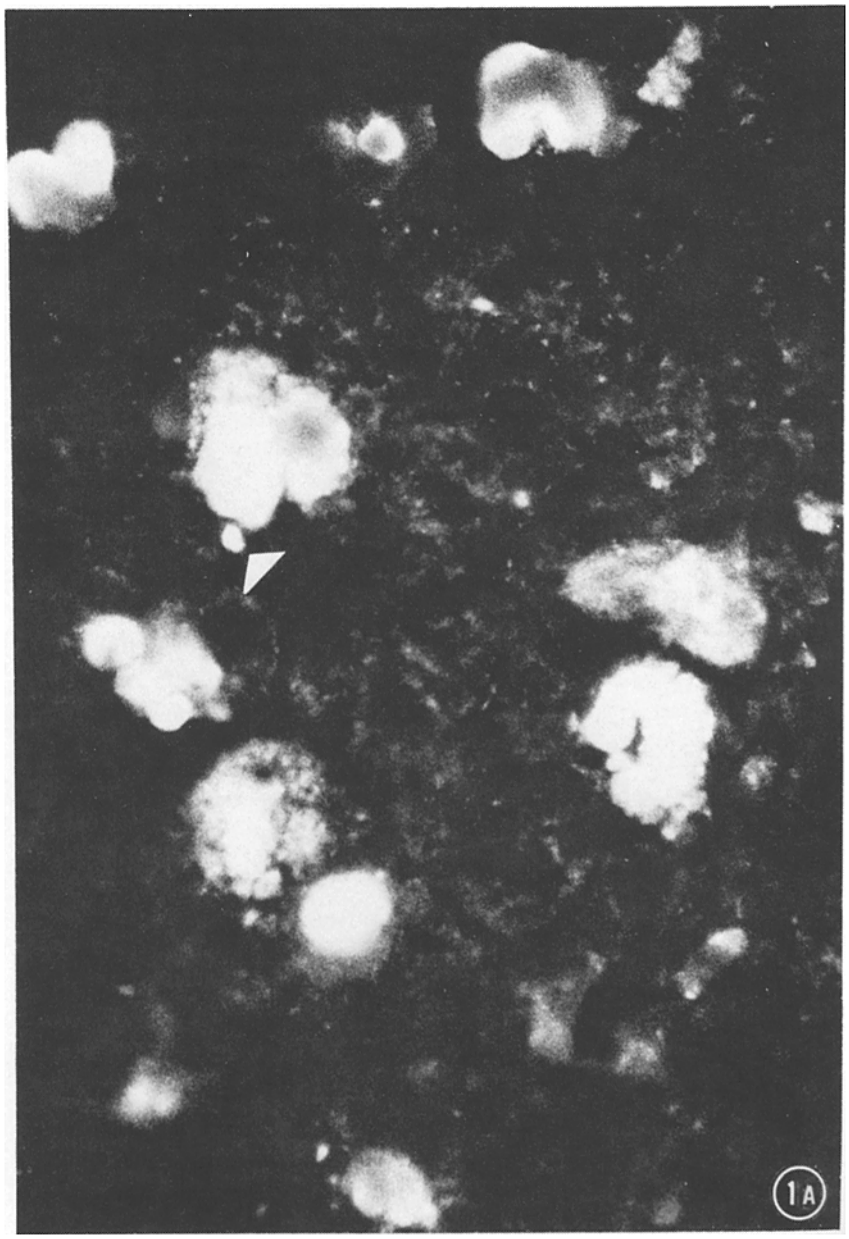
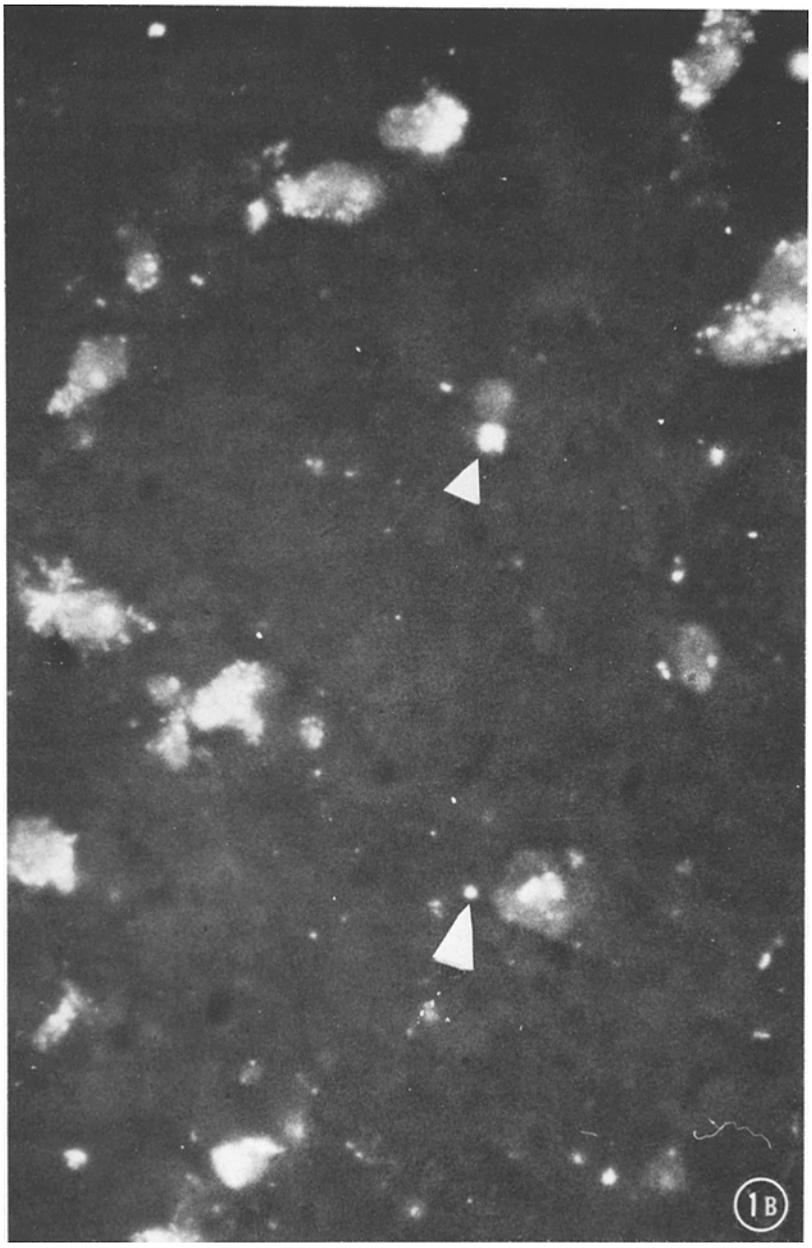


FIG. 1 A and B. Immunofluorescent photomicrographs of frozen sections of normal human brain 'caudate nucleus' showing positive staining reactions with cytoplasm of moderately large neurons using serum from a child with prolonged active chorea. Reference arrows indicate nonspecific yellow autofluorescence produced by lipofuchsin granules in neurons and nerve tissue. × 500.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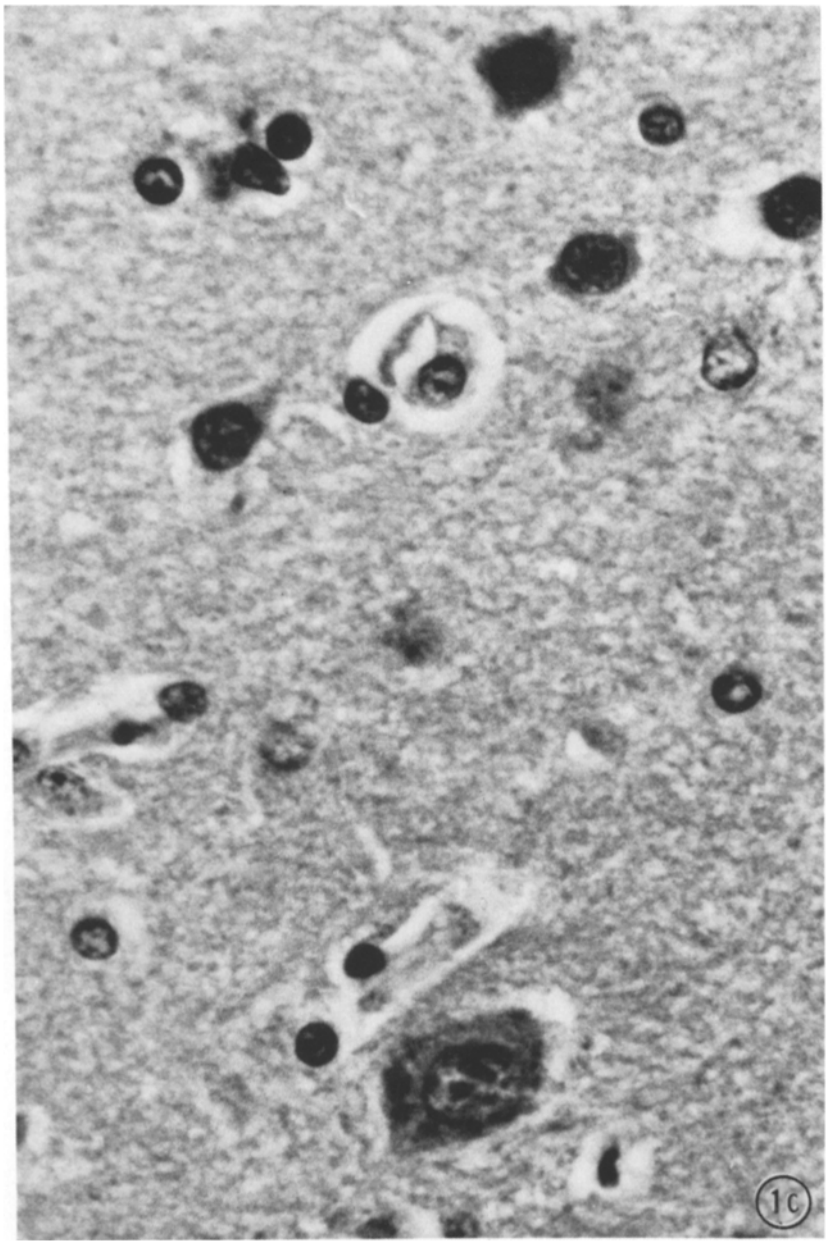

FIG. 1C. Hematoxylin and eosin section of normal caudate nucleus used as substrate for immunofluorescence testing showing relative size and density of neurons in this area. × 500.

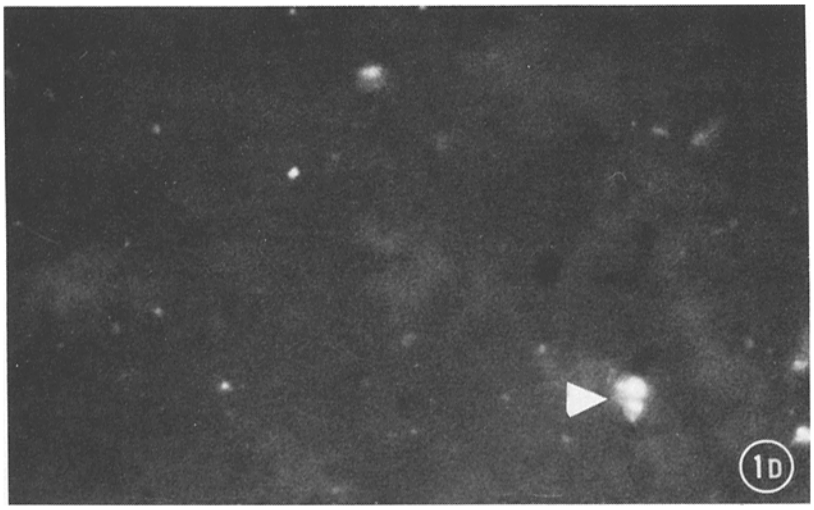

FIG. 1 D. Control section treated with normal serum showing background autofluorescence of lipofuchsin granules. × 500.

tissue, IgG was isolated from positive sera, pepsin digested, and $F(ab')_2$ fragments applied to test frozen sections. Application of fluorescein-labeled $F(ab')_2$ anti-$F(ab')_2$ confirmed specific binding of pepsin-digested antibody to neuronal cytoplasm.

Localization and Staining Patterns. Table I summarizes the results of nine chorea patients who exhibited positive immunofluorescent staining patterns. Using sections of caudate nucleus, subthalamic nucleus, cerebral cortex, and medullary nuclei, it can be seen that 9 of 22 sera from children with chorea showed concordant staining of neurons in both caudate and subthalamic nuclei. Two of the latter showed stronger staining (++) in caudate than in subthalamic (+) sections. Three sera also showed weak (±) staining of neuronal cells in cerebral cortex and five showed positive staining (± − +) of neurons in medullary nuclei. The staining intensity and numbers of cells staining were considerably greater in both caudate and subthalamic nuclei than in sections of cerebral cortex. Concomitant titrations for intensity of staining in caudate and medullary nuclei showed essentially similar titers in three sera, 286, 274, and 230; and lower titers using medullary nuclei in two, 8008 and 4162. No concomitant staining for nerve cell nuclei was recorded. By contrast, only 7 of 50 sera from children with rheumatic carditis showed caudate and subthalamic neuronal staining. Four of these seven showed concomitant medullary nuclei nerve cell staining. Higher titers of antibody reacting with caudate and subthalamic neurons (mean titer 5.7) were recorded in sera from children with chorea than were detected in the seven patients with carditis (mean titer 2.1).

A general summary of results recorded with the entire group of 80 sera from patients with acute rheumatic fever, as well as the various control groups is shown in Table II. The differences between the prevalence of positive reactions in chorea and carditis sera and normal controls was significant ($P < 0.005$ and $P < 0.05$). In general, control sera showed negative reactions. 1 of 31 normal adult sera showed a weak (±) reaction and none of 24 normal children's sera were

TABLE I

Relative Immunofluorescence Reactions of Sera from Patients with Rheumatic Chorea and Rheumatic Carditis

Patient no.	Age	Sex	Caudate nucleus (titer)	Sub-thalamic nucleus	Medullary nuclei (titer)	Cerebral cortex	ANA
	yr						
Chorea							
286	11	F	+ (8)	+	+ (4)	±	0
303	10	F	+ (8)	+	0 –	0	0
229	12	F	+ (2)	+	0 –	0	0
284	9	F	+ (2)	+	0 –	0	0
8088	11	M	+ (8)	+	± (undiluted)	±	0
274	9	F	++ (16)	+	+ (16)	±	0
272	8	M	+ (2)	+	0 –	0	0
230	9	M	++ (4)	+	+ (4)	0	0
4162	9	F	+ (2)	+	+ (undiluted)	0	0
			5.7 mean titer				
Carditis							
4460	13	M	+ (2)	+	+	0	0
228	13	F	+ (4)	+	0	0	0
245	13	F	+ (4)	+	0	0	0
5116	10	M	+ (undiluted)	+	0	0	0
4154	11	F	+ (undiluted)	+	+ (undiluted)	0	0
4145	5	M	+ (undiluted)	+	± (undiluted)	0	0
4006	13	F	+ (2)	+	+ (undiluted)	0	0
			2.1 mean titer				

positive. None of eight sera obtained from patients with uncomplicated streptococcal pharyngitis were positive, and 1 of 19 sera obtained from patients with acute poststreptococcal glomerulonephritis was positive. The small proportion of positive sera observed in patients with systemic lupus erythematosus and juvenile rheumatoid arthritis were weakly positive only in undiluted serum. Patients with other diverse destructive or degenerative neurological conditions including Parkinson's disease, cerebrovascular accidents, and amyotrophic lateral sclerosis were uniformly negative.

Further analysis of clinical records among chorea patients revealed that patients with the most severe and prolonged bouts of chorea were most likely to show positive neuronal immunofluorescence. Thus patients 274 and 230 in Table I had shown multiple severe attacks lasting 6–12 mo in both instances. In addition the appearance of the antibody appeared to be correlated with the duration of chorea, thus only one of eight patients with chorea of 2 mo or less had detectable antibodies while three of three patients who had chorea continuously for 6 mo or longer had positive tests for anti-neuronal staining. Finally, it was also noted that there appeared to be a relationship between the general clinical severity of chorea and the presence of positive staining as well as the titer of anti-neuronal antibodies (Table III).

Sequential analysis of sera from six patients which had been stored in The

TABLE II

Prevalence of Antibodies Reacting with Caudate and Subthalamic Nuclei in Comparative Groups of Normals and Patients Studied

Group studied	No. positive/no. studied	%	
Normal controls			
Children	0/24*	(0)	
Adults	1/31	(3.2)	
Streptococcal patients			
Rheumatic carditis	7/50	(14.0)	[$P < 0.005$]‡
Rheumatic chorea	14/30	(46.6)	[$P < 0.05$]‡
Poststreptococcal glomerulonephritis	1/19*	(5.2)	
Streptococcal pharyngitis	0/8	(0)	
Nonstreptococcal disease			
Connective tissue disease			
Systemic lupus erythematosus	2/27*	(7.4)	
Adult rheumatoid arthritis	0/10	(0)	
Juvenile rheumatoid arthritis	2/28*	(7.1)	
Neurological disease			
Multiple sclerosis	0/21	(0)	
Strokes, Parkinson's disease, amyotrophic lateral sclerosis, peripheral neuropathy	0/15	(0)	
Miscellaneous diseases			
Pneumonia, Hodgkin's disease, cirrhosis, myocardial infarctions	1/20*	(5.0)	

* Mean titer of all positive reactions in miscellaneous controls tested was 1 or present in undiluted serum only.

‡ P values refer to difference from normal controls.

TABLE III

Relationship of General Clinical Severity of Chorea to Result of Fluorescent Antibody Tests

	No. (+)/no. tested	Titers
Mild	2/13	Undiluted; 1:2
Moderate	7/12	1:2; 1:2; 1:2; 1:2; 1:4; 1:8; 1:8
Severe	5/5	1:2; 1:2; 1:4; 1:8; 1:16

Rockefeller University Serum Bank were also tested in conjunction with sera from five patients serially followed in Egypt or Trinidad. Evidence for temporal relationship between appearance of antibody and clinical onset of chorea was present in many patients. It appeared that detectable anti-neuronal antibody was associated with onset of chorea in many patients but often persisted for considerable periods of time (1–4 mo) after the subsidence of choreiform activity. A representative series of serial studies are shown in Fig. 2. On the other hand several instances were recorded in which chorea occurred in the absence of detectable antibody. In many of these latter cases the clinical episode of chorea represented a mild attack or the first recorded in the patient.

All patients with positive neuronal cytoplasmic fluorescence and carditis

showed severe active carditis. No correlation was found between age or sex and positive reactions. In addition, no correlation was recorded with ASO titers.

Studies of specificity. Because of the previous studies indicating a relationship between heart-reactive antibody and Group A streptococcal antigens, various absorption studies were carried out. Table IV indicates results of representative data obtained after absorptions. Complete absorption of positive staining of neuronal cytoplasm was recorded in six of seven sera tested using 1 mg/ml of Group A Type 6 membranes. By contrast, no diminution in staining or absorption was recorded after absorption of positive serum or serum dilutions with Group D streptococcal membranes or cell walls. In Table IV it can be seen that no absorption was noted using purified Group A Type 6 carbohydrate but moderate diminution of staining was present after absorption with Group A Type 6 cell walls. These latter preparations, however, are known to contain significant amounts of materials derived from Group A membranes (4). No diminution in positive staining was recorded after absorption with Cohn Fraction IV. In addition control absorptions using both Group A and Group D streptococcal antigens did not abolish reactivity of control-positive ANA. Absorption of positive sera from patients with chorea using isolated neurons from human caudate nucleus produced complete disappearance of positive immunofluroescence, whereas absorptions using human or mouse liver did not result in detectable diminution of staining (Table IV). Sonicated preparations of human cerebral cortex and mouse liver, as well as isolated caudate neurons did produce some diminution in control ANA reactivity. In Table V are shown relative absorptive capacities of various streptococcal membrane and cell wall preparations when used with four sera containing positive neuronal cytoplasmic antibody reactivity. All sera tested were adjusted by dilution to give equivalent degrees of fluorescent staining of brain sections. Progressive loss of absorptive capacity was noted as lower concentrations of Group A membranes were employed. Apparent equivalent reactivity in absorption was recorded using serum 4154 from a patient with clinical carditis but without detectable evidence of choreiform activity.

An attempt was next made to test relative absorptive capacities of Group A streptococcal membranes derived from a pharyngeal (T-12) strain and those from a higher serotype (T-57) isolated from skin sores of a patient with acute glomerulonephritis. Representative results of such an experiment shown in Table VI indicated that Type 12 membranes were more effective in absorbing out anti-neuronal antibody reactivity than those from a Type 57 strain. Similar greater absorptive capacity was documented in some other positive sera when Types 11 and 12 were compared with Types 55 and 57, whereas in other individual sera equal absorptive capacity was found when membranes from Types 6, 11, and 12 were compared with those from Types 55 and 57.

In view of the demonstrated absorption of neuron-reactive antibodies by various streptococcal membrane preparations, it was of interest to determine whether rabbit antisera made against purified Group A streptococcal membranes exhibited the same pattern of neuronal staining. No positive reactions were noted when three rabbit antisera to Group A Types 6 and 12 membranes were applied to fresh human brain caudate nucleus sections. Positive reactions were, however, recorded concurrently with these same rabbit antisera using

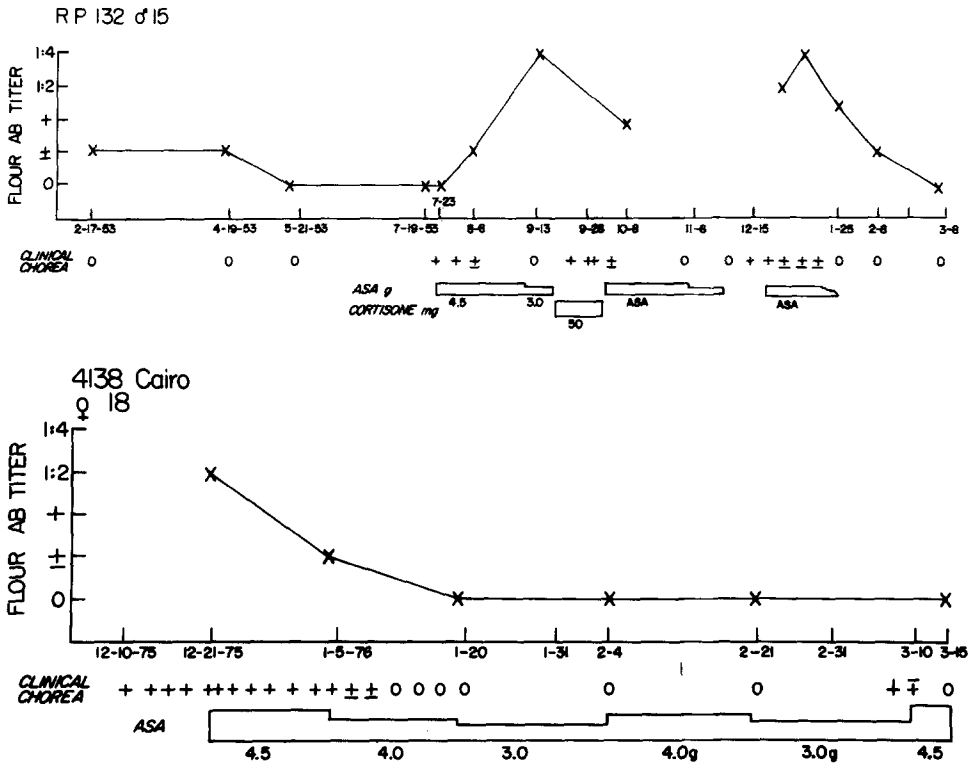

FIG. 2. (A) Serial studies of serum samples of a Rockefeller University patient before and after several clinical episodes of chorea. It can be seen that although weak anti-neuronal immunofluorescence was present without clinically manifest chorea on 2-17-53 and 4-19-53, the subsequent clinical episodes of chorea were associated with a rise in titer of anti-neuronal antibody staining. Antibody was no longer evident 7 wk after subsidence of clinical chorea. (B) Serial studies of Cairo patient 4138 show presence of anti-neuronal antibody detected on admission, 12-21-75, coincident with moderately severe chorea. Besides aspirin therapy the patient also received phenobarbital and reserpine. Subsequent subsidence of clinical chorea was associated with disappearance of detectable serum anti-neuronal antibody. A transient mild recurrence of chorea on 3-8-75 was not accompanied by the presence of detectable serum anti-neuronal antibody.

human sarcolemmal preparations. In addition, sera strongly positive for human neuronal cytoplasmic fluorescence were not absorbed using preparations of human sarcolemma at 1.0–0.01 mg/ml. These data indicated that antigens in human sarcolemma and neuronal structures although both cross-reactive with streptococcal antigens were not identical.

Discussion

The present study demonstrates the presence of IgG antibodies in sera from children with rheumatic fever reacting with neuronal cytoplasmic antigens which appear to be preferentially increased in caudate and subthalamic nuclei of human brain. A higher proportion of sera (46.6%) were positive in children with chorea than were recorded in those with active carditis (14.0%). In many instances there was a direct correlation of positive fluorescent antibody reac-

TABLE IV

Results of Absorption of Serum Showing Positive Immunofluorescent Reactions with Neuronal Cytoplasm in Caudate Nuclei using Streptococcal Antigens, Tissue Homogenates, and Cohn Fraction IV

	Reaction tested	Unabsorbed	Group A T6 membrane (D471)	Group A T6 cell wall (D471)	Group A T6 carbohydrate (D471)	Group D membrane (A932)	Group D cell wall (A932)	Isol. neurons caudate nucleus	Lyophil cerebral cortex	Lyophil mouse liver	Cohn Fraction IV
Serum 298 (chorea) undiluted	Caudate nucleus neuron staining	+	0	±	+	+	+	0	+	+	+
Serum W.W. (SLE) 1/50	Antinuclear antibody	++	++	++	++	++	++	+	+	+	++

TABLE V

Absorption of Four Sera from Children with Acute Rheumatic Fever using Membranes from Group A and Group D Streptococci and Subsequent Immunofluorescence of Neuronal Cytoplasm of Human Caudate Nuclei Neurons

Concentration of antigen used for absorption	Serum 286 (chorea)		Serum 274 (chorea)		Serum 230 (chorea)		Serum 4154 (carditis)	
	Group A T6 membrane	Group D membrane	Group A T6 membrane	Group D membrane	Group A T6 membrane	Group D T6 membrane	Group A T6 membrane	Group D membrane
mg/ml								
1	0*	+*	0	+	±	+	0	+
0.1	±	+	±	+	+	+	+	+
0.05	+	+	+	+	+	+	+	+

* Refers to presence of positive staining of neurons in frozen sections of human caudate nucleus by serum after absorption. Staining with unabsorbed serum dilution positive (+) in all instances.

tions with the length and severity of previous chorea attacks. Reactivity of antibodies for neuronal cytoplasmic constituents appeared to be higher in caudate and subthalamic structures than in other brain tissue (cerebral cortex or medullary nuclei) containing similar high proportions of neurons. However, presence of moderate to weak staining in neuronal cytoplasm of cells in cerebral cortex or medullary nuclei indicated that the reactive structures were not localized only to the corpus striatum.

Of particular importance were the absorption experiments which appeared to support the concept that staining of caudate and subthalamic neurons represented cross-reactions between antigens present in Group A streptococcal membranes and neuronal cytoplasm. Clear-cut absorption using Group A membranes and to a lesser extent cell walls in parallel with the absence of absorption with Group D streptococcal membranes or cell walls demonstrated specificity of Group A membrane-related antigens. In addition, positive immunofluorescence was completely removed using absorption with preparations of neurons from human caudate nucleus. There was no correlation between positive neuronal

TABLE VI
Comparative Absorptive Capacity of T12 and T57 Group A Streptococcal Membranes for Absorption of Anti-Neuronal Antibody

Serum tested	Dilution	Group A streptococcus type 12*		Group A streptococcus type 57‡	
		Membranes (1 mg/cc)	Cell walls (1 mg/cc)	Membranes (1 mg/cc)	Cell walls (1 mg/cc)
8088 (acute chorea)	Undiluted	+	+	+	+
	1:2	0	+	+	+
	1:4	0	±	0	+

* Strain 12 represents original isolate from pharyax.
‡ Strain 57 isolated from skin lesions of Trinidad patient with streptococcal pyoderma and acute glomerulonephritis.

staining and ANA. Furthermore, under immunofluroescence microscopy it was possible to differentiate peripheral neuronal cytoplasmic fluorescence from antinuclear reactions. The latter fluorescence was seen in virtually all sera from patients with systemic lupus erythematosus and in many sera from patients with rheumatoid arthritis. This pattern was clearly differentiated from the globular homogeneous fluorescence seen with cytoplasmic neuronal staining (Table IV). As a further test for specificity, it was noted that control sera obtained from normal Egyptian children and adults living in the same environment as well as a separate group of normal adults showed a low incidence (1/55) of positive reactions. Similar results were obtained in a large number of miscellaneous sera obtained from a wide variety of disease controls including subjects with widespread heterogeneous neurological lesions as in multiple sclerosis, Parkinson's disease, or cerebral infarctions. It was difficult to establish a direct or complete correlation between cytoplasmic neuron staining and chorea since 14.0% of children with active carditis alone, who had never had detectable chorea, also showed weak but definite staining reactions. It seems likely that neuronal cytoplasmic staining in this group of patients represents the same general specificity of antibody since abolition of staining was also accomplished by absorption with Group A Type 6 streptococcal membranes (Table V). Serial studies of sera from children during and before onset of chorea using the streptococcal bank sera stored in some instances for 30 yr provided evidence for temporal relationship of antibody activity to chorea attacks. More recent serial sera obtained from patients studied both in Egypt and Trinidad also showed disappearance of antibody after subsidence of chorea. There were, however, several instances of clear-cut acute chorea in which serial sera were studied where no anti-neuronal antibody was detected. In all of these individuals the clinical chorea episode recorded was either mild or the first clinically detected in that particular patient. It seems possible that studies of the anti-neuronal antibody in serial cerebrospinal fluid samples might provide insight into situations where chorea is present but serum antibodies are undetectable using indirect immunofluorescence techniques. Indeed the situation may be analogous to that previously documented in measles encephalitis or subacute sclerosing

panencephalitis where discrepant results between detectable serum and cerebrospinal fluid antibody have been attributed to primary production of certain oligoclonal antibodies within the central nervous system (18). In addition since clinical signs of chorea are often extremely subtle and rapidly masked by therapy such as that with phenobarbital or reserpine, complete correlation of clinical activity and presence of antibody may prove difficult. Studies are now in progress to assay cerebrospinal fluid and serum for presence of anti-neuronal antibody. If antibody to basal ganglia neurons were the primary cause of the chorea syndrome, then it is difficult to reconcile the occurrence of the antibody in sera from children with carditis who are known on clinical grounds never to have exhibited detectable chorea.

The positive-staining reactions recorded with neurons in medullary centers such as hypoglossal nuclei or trigeminal nuclei indicate that the neuronal distribution of antigen is not unique to the caudate, subthalamic, or substantia nigra system. It is conceivable that IgG antibody to cytoplasmic structures in subthalamic and caudate nuclei is somehow involved in the genesis of chorea but that the clinical syndrome itself is modulated by other factors than merely the presence of antibody itself. Thus, extracellular streptococcal products, genetic background, or other parallel features may also be involved.

The higher titers recorded in sera from children with chorea as compared to those with carditis coupled with the clear correlation of positive reactions with duration and clinical severity of chorea point to a significant relationship of the antibody and the disease process. The precise nature of the cross-reacting antigens in caudate and subthalamic nuclei and Group A streptococci remains unknown. There is some experimental evidence, however, that the central nervous system structures involved in the pathogenesis of some forms of chorea may depend on alterations in catecholamines – specifically, dopamine or norepinephrine (19, 20). Precise chemical definition of the basis of the positive immunofluorescent reactions described here must await further study.

A recent report by Kingston and Glynn (21) presents findings which are in some ways similar to the approach outlined in this paper; however, these authors recorded what appeared to be cross-reactions between streptococcal antigens and limiting brain membrane, ependymal tissue, and fibrous astrocytes. Rabbit antisera made against streptococci showed reactions with these neural structures in their hands. No such cross-reactions have yet been noted during our studies. It may be that a spectrum of antigens is present in neural tissues which cross-react with streptococcal materials. The precise relationship to clinical phenomena such as chorea remains unclear.

Many features of Sydenham's chorea still remain poorly defined and the chorea syndrome has always stood out as an enigmatic but clear manifestation of rheumatic activity. During our studies in Egypt, it appeared that the incidence of chorea was higher than that observed in other parts of the world where acute rheumatic fever is still clinically prevalent, such as India (22). While other factors may be operative, it is possible that the occurrence of chorea itself may be conditioned or somehow modulated by *Ir* genes related to HL-A phenotype. Such a situation might explain the presence of subthalamic and caudate nuclear cytoplasmic antibodies in both chorea and active carditis.

Summary

46% of sera from 30 children with rheumatic chorea showed IgG antibody reacting with neuronal cytoplasm of human caudate and subthalamic nuclei. The antibody was also detected in 14% of 50 children with active rheumatic carditis. 55 normal control sera, as well as 148 sera from a broad variety of other disease states showed a low prevalence (1.8–4.0%) of positive reactions. In rheumatic chorea the presence of anti-neuronal antibody appeared to correlate with severity and duration of clinical attacks. Antibody reacting with neuronal cytoplasm was completely removed by absorption with Group A streptococcal membranes or with isolated human neurons from caudate nucleus. Partial absorption of antibody was also recorded using Group A cell wall preparations but not with Group A carbohydrate. No absorption of positive reactions was seen with streptococcal Group D membranes or cell walls. In rheumatic chorea, anti-neuronal antibody appeared to represent cross-reaction with antigens shared by Group A streptococcal membranes.

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