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Antinuclear factor, smooth and striated muscle antibodies in Duchenne-type muscular dystrophy

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Antinuclear factors and antibodies to smooth and striated muscle were studied by the indirect immune fluorescence method in the sera of 19 children suffering from progressive muscular dystrophy.

In 47% of the patients antinuclear factor positivity, in 65% anti smooth muscle antibody positivity, and in 26% antistriated muscle antibody positivity was found.

Antibody to striated muscle was present in patients with serious advanced dystrophy and in patients unable to walk, while anti-smooth muscle antibody occurred in less serious cases, too. On the basis of the results, it is concluded that in genetically determined progressive muscular dystrophy a secondary autoimmune process develops owing to the degeneration of muscles as the disease progresses.

Duchenne muscular dystrophy (DMD) is a genetically determined, X-bound recessive hereditary myopathy, the primary biochemical defect of which is not known. There is some evidence pointing to a membrane defect, as for instance is the case of muscle tissue, platelets, and erythrocytes [1, 8, 15, 16, 17, 21]. Several authors [20, 24] have investigated the properties of the erythrocyte membrane in DMD patients and obtained conflicting results. Percy and Miller [19] found pathological deformability, Fisher et al. [10] and Lloyd and Nunn [14] increased fragility, Howland [12], Dise et al. [7] increased permeability for the ions K and Ca.

The immunological condition of DMD patients is unclear. As a working hypothesis we assumed that in the genetically determined myopathy the myoglobin which reaches the blood from the disintegrating muscle fibres, would trigger a secondary immunological process.

Materials and Methods

Examination of the antinuclear factor (ANF) and the antibodies to smooth muscle (SM) and striated muscle (STM) was carried out by the indirect immune fluorescence method (IIF) [4] in the sera of 19 children with DMD and 24 control children free from autoimmune disease and DMD. For determination of antinuclear factor, rat liver and kidney tissue, for determination of antibodies to striated muscle the quadriceps muscle of rats were used as substrate, and for the demonstration of antibodies to smooth muscle the fundus ventriculi of the rat was used. The immune fluorescent kit of Hyland Co., Costa Mesa, Calif. Cat. No.

TABLE I

Anti-nuclear-antibody (ANF), smooth-muscle-antibodies (SMA) and striated-muscle-antibodies (STMA) in control children

Number	Age, year	ANF		SMA		STMA	
		+	—	+	—	+	—
24	6.6±2.3	1 (4%)	23 (96%)	1 (4%)	23 96%)	0 (0%)	24 (100%)

TABLE II

ANF, SMA and STMA in the serum of Duchenne muscular dystrophy patient

Case No.	Age, year	ANF		SMA		STMA		Duration of DMD, years
		+	—	+	—	+	—	
1	13	+		+		±		5
2	11	+		++			—	4
3	11		—		—		—	6*
4	8	+			—		—	4
5	12	+			—		—	4
6	10		—	++		++		5*
7	8		—	+		++		5*
8	12		—	++			—	5
9	9		—	+		++		6*
10	8		—	+			—	4**
11	12		—	+		++		8**
12	8	+		++			—	3
13	18	+		+		++		15*
14	2		—		—		—	3
15	12	+			—		—	4
16	8	+		++	±	±		4
17	5		—		—		—	2
18	12		—		—		—	3
19	5	+		++			—	1
	$\bar{x} = 9.94$ S.D. = 3.11	n = 9 47.36%	n = 10 52.64%	n = 12 64.5%	n = 7 35.84%	n = 5 26.31%	n = 12 63.16%	$\bar{x} = 4.79$ S. D. = 2.92
		$\chi^2 = 8.37$		$\chi^2 = 14.19$		±n=2		
		$p < 0.01$		$p < 0.01$		10.53%		
		$K_{coef.}$ −0.01		$K_{coef.}$ 0.31		$K_{coef.}$ 0.74		
		T = 0.081		T = 1.389		T = 4.57		
		$p > 0.1$		$p > 0.10$		$p = 0.00031$		

*unable to walk
** serious walking difficulty

6–73–00–10 was employed. Cryostat sections 2–4 μm thick were prepared from the organs. The sections were incubated first in serum diluted 1:28, then with FITC-labelled polyvalent antihuman immune globulin conjugate (5E-ShAu–IgGAM–FITS, Human, Budapest) diluted 1:32 at room temperature in a wet chamber. In the periods of incubation and after staining with fluorescein, the preparations were washed with Nairn buffer, and finally a mixture of glycerol and water 1:1 was added and the preparation was covered with a coverglass. Optimum dilution of the serum samples and the fluorescein conjugate was determined by chessboard titration. Evaluation was made with a Zeiss–Fluoval type immune fluorescence microscope (Zeiss–Fluo II. No. 685193, Carl Zeiss, Jena, GDR) with a B-221 g filter as primary filter, and a D-287 g filter as secondary filter.

Unstained preparations and preparations incubated only with fluorescein conjugate were used as controls.

Results

Table I shows the data of the control group. It can be seen that while ANF and SM positivity was found in 4.17% of the cases, all the serum samples proved negative for anti-STM antibodies. The data for the DMD patients as well as the age of the patients and the period of existence of DMD as reckoned from the appearence of the clinical symptoms are given in Table II. The sera of the diseased children showed ANF positivity in 47.36% (Fig. 1), SM positivity in 65.4% (Fig. 2), and STM positivity in 26.31% (Figs 3 and 4). The percentile incidence of ANF positivity and SM positivity proved significantly higher in the χ^2 test than in the control group. Among the antibodies examined, the correlation coefficient, calculated with the period of existence of DMD showed a significant correlation with the anti-STM antibodies.

Discussion

Indirect immune fluorescence examination showed that the occurrence of anti-ANF and anti-SM antibodies

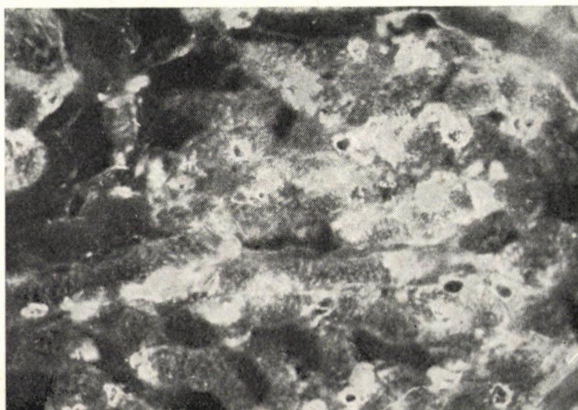

Fig. 1. ANF-indirect immune fluorescence (IIF) of rat lip muscle. ×500

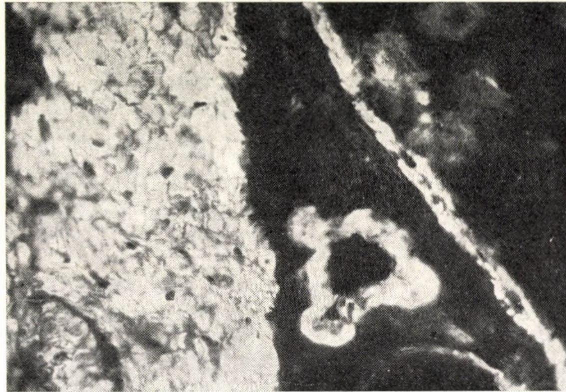

Fig. 2. Smooth muscle IIF positivity in fundus ventriculi of rat. ×500

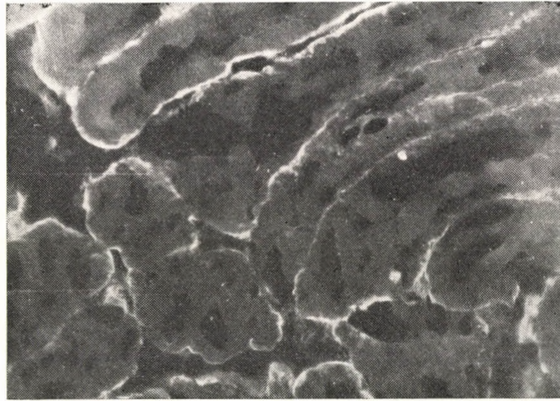

Fig. 3. Striated muscle (sarcolemmal, subsarcolemmal) IIF positivity in sternocleidomastoid muscle of rat. ×500

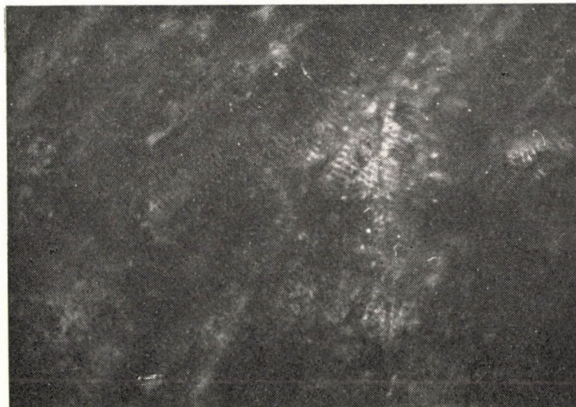

Fig. 4. Striated muscle IIF positivity in rat muscle. ×500

was significantly more frequent in the sera of DMD-patients than in the control group. With progression of the disease, anti-STM circulating antibodies could be demonstrated in the sera of the patients. Persisting SM antibodies can be demonstrated in titres above 1:800 in chronic aggressive hepatitis; temporary low-titre SM occurs in upper respiratory infections [22], acute viral hepatitis [9], infectious mononucleosis [1], and CMV infection [2]. SMA and ANF are pathogenic for lupoid hepatitis.

Anti-striated muscle antibodies occur in titres above 1:60 in myasthenia associated with thymoma, less frequently in polymyositis [13, 18, 23].

Peers et al. [18] testing glycerinated myofibrils by immune fluorescence technique, demonstrated anti-skeletal and heart muscle antibodies in the sera of patients suffering from myasthenia gravis. Inside the muscle they observed a striational reaction. These antibodies are heterogeneous in thymoma; they can be demonstrated also in penicillamine-treated rheumatoid arthritis. Strauss et al. [23] found a complement-fixing globulin fraction bound to striated muscle in the sera of myasthenia gravis patients. The serum of one patient suffering from paroxysmal myoglobinuria also bound the complement. The serum of a patient with acute dermatomyositis produced sarcolemmal fluorescence on skeletal muscle in the presence of guinea pig complement and fluorescein-labelled rabbit anti-guinea-pig complement. Storch [22] emphasized that the cytoplasmic components of skeletal and heart muscle and the subsarcolemmal and sarcoplasmatic reticulum can be regarded as skeletal muscle antagonizing target antigens; biochemically myosin, heavy meromyosin and actin come into consideration as antigens. The antibodies are considerably tissue-specific, but at the same time not species-specific. Antibodies of the SH type reacting to skeletal and heart muscle do not bind complement, while those of the S type react only to skeletal muscle and do bind complement [3]. Caspary et al. [4] detected no statistically significant difference in regard to antimyosin antibodies between the controls and the patients suffering from polymyositis, muscular dystrophy (myotonic dystrophy, facio-scapulohumeral dystrophy, limb-girdle dystrophy) and neurogenic muscle atrophy. Immunoconglutinin was more frequent in the sera of patients with polymyositis and neurogenic muscle-wasting than in the controls, whereas the muscular-dystrophy sera showed no significant variation from the normal. In the absence of evidence for an immunological basis of these disorders, the antibody response is not specific; it rather seems to reflect the indirect effect of a damage to muscle tissue.

The present results seem to prove our assumption that in primarily genetically determined DMD a secondary immunological process develops in connection with the degeneration of the muscles as the disease progresses.

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