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BRIEF COMMUNICATION

Immunofluorescence Staining Patterns in Skeletal Muscle Using Serum of Myasthenic Patients and Normal Controls

J. M. VETTERS

*University Department of Pathology, Western Infirmary, Glasgow, W.*1

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Summary. Using indirect immunofluorescent tests upon the sera of patients with myasthenia gravis and of normal subjects, two types of staining pattern are found. The first, A band staining, is seen only with myasthenic sera. The second, or 'I band' pattern has not previously been described and is seen in an equal proportion of normal controls and of myasthenic patients. 'I band' staining is a possible source of confusion in immunofluorescent tests for muscle antibody.

INTRODUCTION

In 1960 Simpson produced clinical evidence suggesting an autoimmune basis for myasthenia gravis. Almost simultaneously, Strauss, Seegal, Hsu, Burkholder, Nastuk and Osserman (1960) using immunofluorescent techniques, demonstrated in the sera of some myasthenic patients an autoantibody which bound specifically to the A bands of skeletal muscle. This has been subsequently confirmed, using similar techniques, by the following workers: Beutner, Witebsky, Hicken and Adler (1962), two out of ten cases positive, Feltkamp, van der Geld, Oosterhuis, den Oudsten and Hijmans (1964), 33 per cent (thirty-seven out of 111) cases positive and by Djanian, Beutner and Witebsky (1964), 18 per cent (eight out of forty-five) cases positive. In the present series two types of staining reaction are found and these are described below.

METHODS

Normal human ocular muscle was obtained within 12 hours of death. It was then either snap-frozen on a chuck or sealed in polythene tubing and immersed in acetone/CO_2 slush. The material was sectioned at 4μ in a cryostat at $-20°$. All sections not used immediately were kept at $-70°$ for periods of up to a fortnight.

The sera of sixty-eight myasthenic patients and sixty-eight normal controls matched by sex and 5-year age grouping were diluted prior to use 1/10 with physiological saline and tested using Coons's indirect immunofluorescent technique with commercially prepared fluorescein labelled anti-human γ-globulin (Burroughs Wellcome Batch Nos. K4533 and K5145).

1. A drop of diluted serum was placed on each section which was incubated at 17° for 60 minutes.

2. The serum was washed off with veronal buffered saline (pH 7·2) and the sections then agitated in veronal buffered saline for 60 minutes.

3. After removing excess buffer one drop of fluorescein-conjugated anti human γ-globulin was added to each section and left 30 minutes.

4. As in 2 above, agitation for 30 minutes.

5. The sections were then mounted in buffered glycerol and examined using a dark ground fluorescence microscope.

RESULTS

Two types of reproducible striational staining are identified (see Fig. 1).

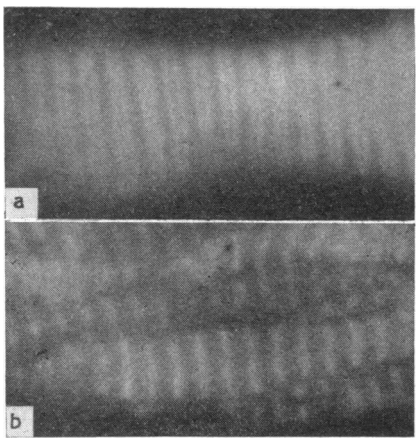

FIG. 1. Immunofluorescent staining of skeletal muscle. (a) A-band staining, (b) 'I-band' staining.

1. The first type, A band staining, is seen in 16 per cent of myasthenics and in none of the controls. This pattern is identified by the relatively greater ratio of widths of fluorescent/non-fluorescent bands. The fluorescent bands are also split by a non-fluorescent area corresponding to the H disc.

2. A second type is found in approximately 70 per cent of control sera and of those myasthenics who do not have anti-A band antibody. Here the fluorescent areas are narrower than the non-fluorescent bands and are not bifid.

As a result of comparison between photomicrographs of the striational patterns it is considered that the structures demonstrated in the second type of staining are probably I bands.

The intensity of 'I band' staining by myasthenia gravis and normal control sera is, generally, less than that seen in the A band staining observed with the positive myasthenic sera but in approximately 5 per cent of both myasthenic and normal control sera intense 'I band' staining comparable with that of the A band staining is seen.

These findings have been repeated using acetone-fixed human muscle and both acetone-fixed and unfixed rat diaphragm. Fluorescein-conjugated anti-human γ-globulin prepared in this laboratory gave results comparable with the commercially prepared reagent. There is no relationship between the ABO groups of the sera and of the donors of the skeletal muscle in the occurrence of 'I band' staining. When the results of an initial survey were reviewed it was found that several patients previously thought to have A band antibody in fact showed the second 'I band' staining pattern which is not of diagnostic significance.

Other workers have not commented on this finding but it is clear that confusion may arise in examination of myasthenic sera unless this is considered. It is hoped to publish a more detailed study of these observations at a later date.

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