Short Report

Lymphocyte capping in myotonic dystrophy

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SUMMARY Lymphocyte capping with antihuman immunoglobulin was studied in 16 cases of myotonic dystrophy, including two cases with the congenital form. Percentage capping after 1 hour incubation was reduced and the time course of the capping sequence was apparently delayed. The significance of these findings remains to be determined.

Myotonic dystrophy is a dominantly inherited disorder with involvement of skeletal muscles, heart, eyes, testes, central and peripheral nervous system as well as other tissues. Recent findings of abnormalities of erythrocyte membranes, of monocyte and skeletal muscle adrenergic receptors, of smooth muscle adrenergic receptors and of neutrophil function have emphasised the multisystemic nature of the disorder and have led to the suggestion that an intrinsic cell membrane defect may underlie the various manifestations of the disease.

A number of abnormalities of immune function have been recognised in muscular dystrophy. These include reduced serum IgG and IgA levels, depressed antibody responses to antigenic challenge and impaired delayed hypersensitivity reactions and T cell responsiveness to mitogenic stimulation. The significance of these findings remains uncertain.

Lymphocyte capping with antihuman IgG and with concanavalin A has been reported to be abnormal in patients with Duchenne muscular dystrophy and in carriers of the gene, and as in patients with other forms of muscular dystrophies, and on the basis of this finding it was suggested that there is an abnormality of the lymphocyte membrane in these disorders. The abnormality of lymphocyte capping in Duchenne muscular dystrophy could not be confirmed in other studies. As part of a study of a large group of patients with various forms of muscular dystrophy, Pickard et al reported normal lymphocyte capping in six cases of muscular dystrophy. We here report our findings in a group of 16 cases of muscular dystrophy in whom lymphocyte capping was studied.

Patients and methods

Fourteen patients (five males and nine females) 13–62 years of age (mean age 35.8 years) with typical myotonic dystrophy diagnosed on clinical and electromyographic grounds, who were not taking drugs, were studied together with 13 normal volunteers (seven males and six females) aged 18–42 years (mean age 27.8 years). Lymphocyte capping was also studied in two cases of congenital muscular dystrophy aged 2 and 3 years. The method used to study capping was a modification of that described by Pickard et al. Ten millilitres of heparinised blood were mixed with 5 ml of Dulbecco's phosphate buffered saline (PBS) and were then layered onto 7.5 ml of Lymphoprep (sodium metrizoate/Ficoll solution). After centrifugation for 25 minutes at 450 g the lymphocyte band was removed, washed twice in PBS to remove passively adsorbed immunoglobulins, centrifuged at 250 g for 10 minutes and then resuspended in PBS. Four million lymphocytes were mixed with 0.1 ml of a 1:10 dilution of FITC-conjugated polyvalent antihuman immunoglobulin (DACO immunoglobulins, Copenhagen) and were incubated in the dark at 4°C for one hour. The cells were then washed twice in ice-cold PBS, centrifuged at 200 g for 10 minutes, resuspended in 0.1 ml PBS and incubated at 37°C for one hour. A wet mount slide was then prepared and scanned under a Vickers photoplan fluorescence microscope. Approximately 100 labelled lymphocytes were counted and classified into the following categories according to the distribution of fluorescence: uniform, clustered or patchy, and fully capped. Cells with indistinct labelling, nonviable cells with dull diffuse labelling, cells thought to be too large to be lymphocytes, and cell clusters were not included in the count. Blood samples were taken in pairs from a patient and a control subject and were coded so that...
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Table 1  Proportions of lymphocytes counted showing different patterns of fluorescent staining in myotonic dystrophy patients and normal controls; mean percentages and standard deviations are shown; p values calculated using Student’s t test

<table>
<thead>
<tr>
<th></th>
<th>Controls (13)</th>
<th>Myotonic dystrophy (14)</th>
<th>Difference from controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniform</td>
<td>0.3 ± 0.5</td>
<td>2.6 ± 4.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Clustered</td>
<td>23.7 ± 13.6</td>
<td>34.2 ± 15.8</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(0-2)</td>
<td>(0-18)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(2-39)</td>
<td>(5-59)</td>
<td></td>
</tr>
<tr>
<td>Patchy</td>
<td>28.0 ± 23.3</td>
<td>40.1 ± 12.6</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>(2-66)</td>
<td>(26-62)</td>
<td></td>
</tr>
<tr>
<td>Capped</td>
<td>47.5 ± 14.8</td>
<td>23.1 ± 6.7</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(27-64)</td>
<td>(10-39)</td>
<td></td>
</tr>
</tbody>
</table>

the procedure was carried out in a blind fashion by the same person.

Results

No qualitative differences were observed in the patterns of cell labelling in the muscular dystrophy and control subjects. However, the mean percentage of capped cells after one hour incubation was significantly lower in the muscular dystrophy group than in the normal subjects (23.1 ± 6.7 vs. 47.5 ± 14.8) (table) although there was some overlap between the two groups (fig). The proportion of cells showing a uniform, clustered or patchy pattern of staining was not significantly different in the two groups. However, when the proportions of clustered and patchy cells in individual cases were combined the mean percentage value in the muscular dystrophy group was significantly higher than in the control group (74.3 ± 4.9 vs. 51.7 ± 14.0; table). No significant correlation was found between percentage of fully capped cells and age or sex in either the muscular dystrophy or control groups. The lowest values for capping were found in the two cases of congenital myotonic dystrophy (9% and 11% fully capped cells at one hour) but no age-matched controls were available.

Discussion

The present finding of reduced lymphocyte capping contrasts with the finding of normal capping in six cases of myotonic dystrophy by Pickard et al.14 The reasons for this discrepancy are not clear but could be methodological; for example the source of the antisera and incubation times which were different in the two studies. Capping of B lymphocytes results from the formation of surface antigen/antibody complexes and their subsequent redistribution in the membrane from a dispersed state through various stages of aggregation or clustering, leading finally to the formation of a single large aggregate (cap) at some point on the cell surface.20 21 The rate at which this process occurs is thought to reflect intracellular membrane properties and to be a measure of membrane fluidity, but is also dependent on other factors. The present finding of a reduced percentage of capped cells with an increased percentage of cells at the intermediate stages (that is cells showing clustered or patchy staining) suggests that the time course of the capping process is prolonged in muscular dystrophy. Whether this is due to a conformational abnormality of the lymphocyte membrane, to an abnormality of membrane associated contractile elements involved in the lateral movement of proteins within the membrane, or to impaired generation of energy required for the process (in the form of ATP)14 remains to be determined. Similarly, the relationship between the finding of an altered capping sequence and the disturbances of humoral immune function in muscular dystrophy requires further investigation.

Fig 1  Percentage of fully capped lymphocytes and cells showing intermediate patterns of staining (clustered or patchy) in control and myotonic dystrophy (MD) subjects. Each point represents an individual subject. Mean values are represented by horizontal bars.

References

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