Diminished Cap Formation in Lymphocytes from Patients and Carriers of Duchenne Muscular Dystrophy

Harland L. Verrill,1 Nathan A. Pickard,2,3 and Hanns-Dieter Gruemer2,3

Currently, the most useful clinical laboratory aid in establishing the carrier state of Duchenne muscular dystrophy is to determine creatine kinase (EC 2.7.3.2) activity in the plasma. The considerable overlap between plasma creatine kinase activities of controls and of carriers at the childbearing age contributes appreciable difficulty to genetic counseling of potential carriers. The consistent failure of lymphocyte cap formation in Duchenne muscular dystrophy patients and carriers in this study suggests a valuable tool for the confirmation of the carrier state.

Additional Keyphrases: inherited disorders • diagnostic aids • systemic membrane abnormality in Duchenne muscular dystrophy

Results of several biochemical and histological studies were suggestive of a membrane defect in DMD4 (1, 2). An abnormality has been observed by electron microscopy in the morphology of the sarcolemma of skeletal muscle biopsies (3), and biochemical studies demonstrated an alteration in a membrane-bound protein kinase (4). Basal adenylyl cyclase (EC 4.6.1.1) activity of erythrocyte membranes in Duchenne patients was twice that of controls, and ouabain has been observed to enhance, in contrast to its classic inhibitory action, the (Na+ + K+)-stimulated ATPase activity of erythrocyte membranes from DMD patients (5). Matheson and Howland (6) observed an above-normal number of distorted erythrocytes in patients and carriers of DMD and in patients with limb-girdle and fascioscapulo-humeral muscular dystrophy.

Imipramine has been successfully used in conjunction with serotonin to simulate a Duchenne-like model myopathy in rats (7, 8). The drug-induced changes in vitro and in vivo were membrane directed, as evidenced by alteration in the activities of enzymes bound to muscle membrane, increased release of intracellular macromolecules, and inhibition of the capping process of lymphocyte membrane proteins (8–10). The close resemblance between the imipramine-serotonin rat model and human DMD led us to investigate the membrane capping function in DMD patients and carriers.

Methods and Materials

Ten male DMD patients were selected who had a history of early onset of progressive weakness of the proximal extremities, grossly increased values for plasma CK, and other clinical, histological, or electrophysiological findings pathognomonic for this disease. The mothers of these DMD patients were regarded as carriers. Normal male age-matched children were used as controls for both capping experiments and plasma CK determinations. No physical activity restrictions were imposed before conducting the study. The collection of blood, isolation of lymphocytes, labeling of the membrane-bound immunoglobulins, fluorescent microscopy, and surface pattern classification were performed as previously described (10). Plasma CK activity was determined by the SMA 12/60 method (Technicon Instruments Corp., Tarrytown, N.Y. 10591) (11).

Results and Discussion

As may be seen in Table 1, the plasma CK activities in the DMD patients were in every instance higher than those observed for the age-matched controls, resulting in a significant difference between groups by the sign test (p < .01). There is no significant difference, however, by the run test between the DMD carriers and the controls, supporting well-documented earlier observations of overlapping distributions between these two groups (12). For the DMD patients there was a decrease of CK activity in plasma with increasing age, paralleling the decrease in the availability of skeletal muscle as the source of CK.

Table 1 also shows the proportion of fluorescein-labeled lymphocytes in each of the established classifications: uniform, clustered, patched, and capped (13). There was only negligible progression from the clustered to the capped state in the lymphocytes of DMD patients and their carriers, as compared to non-afflicted control patients, with no difference in the number of capped lymphocytes. Thus, the defect of progression from the clustered to the capped stage appears to be equally expressed in the membranes of both DMD patients and female carriers.

Intramembrane protein mobility, as indicated by the capping process, requires a triad of normalcy: normal membrane fluidity (14), proper function of the ultrastructural microf...
laments as well as microtubules, and adequate energy supply in the form of ATP (12). Abnormalities of capping have been observed in vitro by alterations of each of these structural or functional prerequisites (15-17). The only other report of aberrant cap formation in human lymphocyte membranes has been in chronic lymphocytic leukemia (15), and the proportion of cells failing to cap in that study agrees well with our results for DMD patients and carriers.

The failure to progress to the cap formation reflects a basic defect in the membranes of all cells in both carriers and DMD-affected sons. The DMD carriers usually have only a subclinical myopathy accompanied by marginally increased enzyme activity; the affected hemizygotes demonstrate high plasma enzyme activities with rapid degeneration of muscle tissue, with death common during the second decade of life (18). One would therefore expect, as we did at the beginning of the study, that the severity of the disease in each group would be reflected in the number of cells capped: the sons showing little or no capping and the mother’s values intermediate between their sons and the control group. However, mothers and sons demonstrated equally severe abnormalities in the numbers of capped lymphocytes, in contrast to the general observation of qualitative differences between homo- and heterozygotes. One must also consider the possibility of additional conformational membrane changes in the sons to explain the large amount of enzyme released, which is believed to be a membrane phenomenon. These and other additional mechanisms require further clarification. Our present findings and reports by other authors cited above support the hypothesis of a systemic membrane abnormality in DMD that is incompatible with the maintenance of normal muscle function.

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Addendum

Since submission of this manuscript, we have observed, in cooperation with Drs. Edward R. Isaacs and Edwin C. Myer, additional patients with proximal muscular dystrophies (Duchenne, limb-girdle, facioscapulohumeral, and congenital muscular dystrophy). All of them showed a diminished capping ability of B lymphocytes, indicating the occurrence of a systemic membrane disease for each of these entities with muscle as the site of least resistance to functional requirements. These findings will be reported elsewhere.

References


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**Corrections**

**Volume 19**

p 152: There are errors in the placement of two closed parentheses in the equation that follows equation 6. It should read

\[
K([Ag_i] - [Ag_i](B/F)/(B/F + 1)) \times ([Ab_i] - [Ag_i](B/F)/(B/F + 1)) = [Ag_i](B/F)/(B/F + 1)
\]

These errors were pointed out by Drs. C. L. Partain (Imaging Division) and John E. Hammond (RIA-Endocrine Laboratory) of the School of Medicine, the University of North Carolina, Chapel Hill, N. C., who supplied a detailed mathematical derivation of the equation, which is available on request either directly to them or to the Editorial Office of this journal.

The first equation in the right-hand column was corrected in a subsequent reprinting. It should read: logit \((Y) = \log_e \left[ \frac{Y}{100 - Y} \right] = a + b \log_e X.\) This error was detected by D. Rodbard (NIH) and E. A. Sugden (Animal Diseases Research Institute, Hull, Quebec).

**Volume 23**

p 1398: The last two sentences in the paragraph on Tumor Extracts are incomplete. The sentence should read

"The tissue is homogenized and then centrifuged to give a clear supernatant fluid, which is deproteinized either by precipitation with sulfosalicylic acid (50 g/liter) or by passage through an ultrafilter that retains compounds with molecular weight exceeding 1000 (Type PSAC; Millipore Corp.). Both procedures gave identical chromatograms. The deproteinized extract is applied directly to the column."

p 515: footnote 1: Remove the words "current address." Mr. Canos was (and is) at this address when the work was begun.