Endothelial inclusions in dermatomyositis

WALTER L. NORTON,* EDWARD VELAYOS, AND LOWELL ROBISON†

From the Sections of Clinical Immunology and Rheumatology, Department of Medicine, University of Tennessee College of Medicine, Memphis, Tennessee

Abnormalities of the microvasculature of muscle from patients with polymyositis have been reported by González-Angulo, Fraga, and Mintz (1968). These changes were similar to those of systemic lupus erythematosus (SLE) and scleroderma (Norton, Hurd, Lewis, and Ziff, 1968). Although the nature of the vascular injury is unknown, the capillaries of active lesions of SLE have recently been shown to contain characteristic endothelial inclusions (Gyorkey, Min, Sinkovics, and Györkey, 1969; Norton, 1969). The present report describes the occurrence of similar inclusions in skin and muscle of dermatomyositis. Attempts to define the nature of the inclusions are described and their possible significance is discussed.

Methods

Subjects

Patients included in this study are listed in Table I.

Table I Particulars of five patients studied

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Duration of disease (mths)</th>
<th>Major clinical manifestations</th>
<th>Clinical impression of disease activity at time of biopsy</th>
<th>Prednisone therapy at time of biopsy (mg./day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54</td>
<td>M</td>
<td>12</td>
<td>Proximal muscle weakness, heliotrope rash, dysphagia</td>
<td>Active, progressive</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>F</td>
<td>2</td>
<td>Proximal muscle weakness and severe tenderness, heliotrope rash, dysphagia</td>
<td>Active, progressive</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>F</td>
<td>33</td>
<td>Muscle weakness, heliotrope rash, growth failure</td>
<td>Chronically active, progressive</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>58</td>
<td>M</td>
<td>4</td>
<td>Muscle weakness and tenderness, macular rash</td>
<td>Partial remission</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>M</td>
<td>30</td>
<td>Muscle weakness</td>
<td>Early remission</td>
<td>40*</td>
</tr>
</tbody>
</table>

*Also receiving methotrexate, 100 mg. weekly.

All but one of them (Patient 5) were hospitalized in the Clinical Research Center of the University of Tennessee. All had a primary history compatible with the diagnosis of dermatomyositis or polymyositis. In addition to a thorough clinical evaluation, a number of studies were carried out, including serum transaminase, creatine phosphokinase, aldolase, latex and sheep cell tests for rheumatoid factor, L.E.-cell test, fluorescent anti-nuclear antibodies, and 24-hr urine creatine. Of the patients in the present report, four were considered to have typical dermatomyositis with active skin involvement. Three of these had severe muscle weakness (Patients 1 to 3) and the other had moderate muscle weakness (Patient 4). Patient 5 was considered to have steroid resistant chronic polymyositis which responded promptly to intravenous Methotrexate therapy.

Open muscle biopsies were obtained in four patients and needle muscle biopsy in one (Patient 5). Open biopsies were obtained from the muscles showing maximum electromyographic changes on the contra-lateral side. Biopsies of skin lesions were obtained in Patients 2 and 4. Control observations from other

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*Special Investigator, Arthritis Foundation.

†Trainee in Arthritis, National Institute of Arthritis and Metabolic Disease.
diseases and normal individuals have been reported previously (Norton, 1969). These included normal skin and muscle, muscle and kidney from scleroderma, kidney from Goodpasture's syndrome, amyloidosis and chronic glomerulonephritis, pericardium from idiopathic pericarditis, and synovium from rheumatoid arthritis.

**ELECTRON MICROSCOPY**

Tissues were immediately fixed in 2.5 per cent. cacodylate buffered glutaraldehyde for 1 hour and post-fixed in 1 per cent. osmium tetroxide for 30 minutes. Tissues were embedded in Maraglas-Cardolite mixture. Sections were stained with uranyl acetate and lead and viewed in an AEI-6B electron microscope.

**IMMUNOFLUORESCENCE**

Tissues were frozen immediately in isopentane precooled in liquid nitrogen and were stored at −75°C until sectioned. Direct or indirect immunofluorescence was performed, using the patients’ own sera, the sera of other patients, and antisera to a variety of viral antigens. Antiviral sera used were directed against influenza A, B, and C, Parainfluenza 1, 2, and 3, measles, mumps, respiratory syncytial, rubella, New Castle disease, canine distemper, and rinderpest. The last three sera were kindly provided by Drs. R. A. Bankowski and Max Appel, and the Plum Island Animal Disease Laboratory, respectively.

**COMPLEMENT-FIXATION TESTS**

Complement-fixation tests for several myxoviruses and rubella were performed using a micromethod (Sever, 1962) (Table II).

**Results**

In general, the morphology of muscle at the electron microscopic level was similar to that described by Mintz, González-Angulo, Fraga, and Zavella (1968). The capillaries of five biopsies contained inclusions (Figs 1 to 3, opposite) which appeared to be identical with inclusions previously reported in systemic lupus erythematosus (Fig. 4, overleaf) by Györkey and others (1969) and Norton (1969).

The inclusions were bounded by membranes which were frequently in continuity with outer nuclear membrane and rough surfaced endoplasmic reticulum. They were usually found within endothelial cells, but were also noted occasionally within fibroblasts and macrophages. Intranuclear inclusions were not noted, and inclusions were not detected within muscle cells.

In general, the frequency of inclusions paralleled the degree of histologic involvement of the tissues biopsied, the only exception being the biopsy of Patient 3 (Table III, overleaf).

Immunofluorescence of inclusion positive tissues failed to reveal evidence of specific myxovirus or rubella antigens, or of antibody in patients’ sera directed against the inclusions.

Antimyxovirus antibodies in patients’ sera did not differ from that expected in the general population (Table III).

**Discussion**

The inclusions in the present series appear identical to those noted in SLE, scleroderma, Goodpasture’s syndrome, idiopathic thrombocytopenia, discoid lupus erythematosus, and congenital rubella syndrome (Norton, 1969; Györkey and others, 1969; Fresco, 1968; Hashimoto, 1969). However, they differed significantly in location from those reported in polymyositis by Chou (1967), who found inclusions within muscle cells—both cytoplasmic and intranuclear. The filamentous structures reported here were found to be membrane bounded, and to be more coiled than the structures reported in polymyositis by Chou (1967), in subacute sclerosing panencephalitis (SSPE) by Chen, Watanabe, Zeman, and Mealey (1969), and in typical myxovirus infections reported by Howe, Morgan, de Vaux St. Cyr, Hsu, and Rose (1967). Although the segregation of viral RNP within endoplasmic reticulum would be an unusual feature of viral infection, a similar phenomenon has been reported in polio virus infection of capillaries by Blinzinger, Simon, Magrath and Boulger (1969).

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**Table II** Complement-fixation titres against myxovirus and rubella antigens

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Influenza</th>
<th>Parainfluenza</th>
<th>Measles</th>
<th>Mumps</th>
<th>RSV</th>
<th>Rubella</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1/16</td>
<td>0</td>
<td>1/128</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1/8</td>
<td>0</td>
<td>1/4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1/8</td>
<td>1/16</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1/2</td>
<td>1/128</td>
<td>1/8</td>
<td>0</td>
<td>1/64</td>
<td></td>
</tr>
</tbody>
</table>
FIG. 1 Multiple cytoplasmic inclusions (open arrows) within a capillary endothelial cell in skin lesion of Patient 2. Inclusions are at least partially membrane bounded, the limiting membrane often being related to endoplasmic reticulum (er). × 41,500. approx. m = mitochondria; N = nucleus.

FIG. 2 Inclusions (*) within cytoplasm of muscle macrophage from Patient 2. m = mitochondria. × 41,500 approx.

FIG. 3 Filamentous inclusion (arrow) in perinuclear space of endothelial capillary of muscle from Patient 1. N = nucleus. × 41,500 approx.

The diameter of the filamentous structures within the inclusions were larger than that usually given for the ribonucleoprotein portion of paramyxoviruses. Their diameter (20 to 25 mμ) was similar, however, to that reported for the measles-like virus of SSPE (20 to 22 mμ).
forms of the same infectious agent, that they are different viruses, or are completely unrelated. Positive identification of both types of structures will be necessary before any conclusions can be drawn. The immunological evidence obtained in these studies gives no support for myxovirus infection, but the evidence is indirect and does not absolutely rule out this possibility. Nonetheless, it does suggest that other possibilities should be sought.

If the data presented here is postulated to represent chronic virus infection of the microvasculature, it seems likely that the organ localization of such an infection would be a major determinant of the syndrome produced. The role of immune mechanisms in the production of tissue injury would have to be re-examined in this light.

The distribution of these endothelial inclusions in active lesions of several diseases would also be compatible with a non-viral origin. They may represent a reactive or regenerative phenomenon, perhaps related to a special type of tissue insult (Chandra, 1968). However, the inclusions are not simply a non-specific concomitant of tissue injury, since a number of lesions from several diseases fail to demonstrate them, and they occurred in large numbers in several related syndromes. Hence it must be concluded that the finding is a manifestation of some common process, the nature of which is unknown.

Whatever interpretation should subsequently be proven, the findings are in accord with the concept that injury to small blood vessels is an important, and perhaps the major mechanism, in the genesis of several of the rheumatic diseases.

Table III  Biopsy findings

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Tissue biopsied</th>
<th>Histological appearance</th>
<th>Presence of inclusions*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Muscle</td>
<td>Muscle fibre fragmentation, perivascular inflammations, variation in individual fibre size</td>
<td>++++</td>
</tr>
<tr>
<td>2</td>
<td>Muscle</td>
<td>Muscle fibre fragmentation, occasional perivascular inflammation</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Skin</td>
<td>Minimal perivascular inflammation</td>
<td>++++</td>
</tr>
<tr>
<td>3</td>
<td>Muscle</td>
<td>Muscle fibre atrophy with vacuolization and homogenization. Acute and chronic inflammation</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Muscle</td>
<td>Muscle fibre replacement by fat, no inflammation</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Skin</td>
<td>Moderate perivascular round cell infiltration and oedema</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Muscle</td>
<td>Fibrosis</td>
<td>0</td>
</tr>
</tbody>
</table>

*+++ = Very frequent, present in the majority of vessel cross-sections.
++ = Intermediate between +++ and +.
+ = Extremely rare, found only after extensive searching
0 = None.
Summary
Characteristic inclusions have been observed in muscle and skin biopsies from four of five patients with dermatomyositis or polymyositis. The inclusions were composed of intertwined tubules which measured from 20 to 25 μm in diameter. The inclusions were bounded by membranes continuous with endoplasmic reticulum or outer nuclear membrane. They appear to be identical to the endothelial inclusions of systemic lupus erythematosus and several other syndromes.

Attempts to identify myxovirus and rubella antigens in involved tissues, antibodies in patients' sera directed toward the same viruses or against the endothelial inclusions were negative.

The observations do not permit conclusions about the nature of the inclusions. Although they do not appear to represent myxoviruses, other viral aetiology has not been excluded. They could also represent some secondary cytological change reflecting a common process in the production of tissue damage in dermatomyositis and other diseases in which similar inclusions have been noted. The localization of inclusions to endothelial cells suggested that the microvasculature was intimately involved in the pathological process.

References

Résumé
Les inclusions endothéliales dans la dermatomyosite
Les inclusions caractéristiques ont été observées dans la biopsie des muscles et de la peau chez quatre des cinq malades atteints de dermatomyosite ou de polymyositis. Les inclusions étaient composées de tubules entrelacés qui mesuraient 20 à 25 μm de diamètre. Les inclusions étaient circonscrites par des membranes continues au reticulum endoplasmique ou à la membrane nucléaire.

SUMARIO
Inclusiones endoteliales en la dermatoalmiositis
Se han observado inclusiones características en biopsias de músculo y piel en cuatro de cinco pacientes con dermatomiositis y polimiositis. Las inclusiones estaban formadas por túbulos entrelazados que median de 20 a 25 μm de diámetro. Las inclusiones estaban envueltas en membranas continuas con retículo endoplásmico o membrana nuclear exterior, y parecen ser idénticas a las
Elles semblaient être identiques aux inclusions endothéliales du lupus érythémateux disséminé et d'autres syndrômes.

Des essais faits pour identifier les myxovirus et les antigènes de rubéole dans les tissus affectés, les anticorps du sérum des malades contre les mêmes virus ou contre les inclusions endothéliales ont été négatifs.

Les observations n'ont pas permis d'arriver à des conclusions quant à la nature de ces inclusions. Malgré qu'elles ne semblent pas représenter des myxovirus, d'autres étiologies virales n'ont pas été exclues. Elles pourraient aussi représenter quelque changement cytologique secondaire reflétant un processus commun dans la production des lésions au tissu dans la dermatomyosite et d'autres maladies où des inclusions semblables ont été notées. La localisation des inclusions dans les cellules endothéliales a suggéré que la microvasculature était intimement liée au processus pathologique.

Inclusiones endoteliales de lupus eritematoso diseminado y de varios otros síndromes.

Tuvieron resultados negativos las tentativas encaminadas a identificar antígenos de mixovirus y rubéola en los tejidos afectados, anticuerpos en el suero de los pacientes, dirigidos contra los mismos virus o contra las inclusiones endoteliales. Las observaciones no permiten llegar a ninguna conclusión acerca de la naturaleza de las inclusiones. Si bien no parecen representar mixovirus, no se ha excluido otra etiología viral. También podrían representar cierto cambio citológico secundario que refleja un proceso común, que ocasiona daños en tejidos como en la dermatomiositis y otras enfermedades en las cuales se han notado inclusiones similares. El hecho de que las inclusiones se localicen en las células endoteliales sugiere que la microvasculatura se hallaba intimamente afectada en el proceso patológico.
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