Pathogenesis of soft-tissue contracture in club foot
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We investigated the pathogenesis of soft-tissue contracture in club foot, using immunohistochemistry to study 41 biopsy specimens and 12 normal deltoid ligaments from cadavers. Five biopsy specimens were studied by electron microscopy (EM) to determine the presence of myofibroblasts.

All 41 specimens of club foot stained positively for vimentin as against only one of the 12 control specimens. By contrast, there was no difference in staining for desmin or α-smooth muscle actin. EM showed some variability in the appearance of ligamentous cells. Most contained bundles of microfilaments in the cytoplasm and many had abundant pinocytotic vesicles, but no basal lamina or plasmalemmal attachment plaques.

Cells of the medial ligamentous tissue in patients with club foot contain vimentin and others have myofibroblastic characteristics. Both features may contribute to recurrence after soft-tissue release.

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The pathogenesis of club foot is unknown despite numerous hypotheses.1,2 There have been many studies of the soft tissues on the medial side of the foot.3-8 In 1959, Fried3 described marked thickening of the posterior tibial tendon with a hard fibrous mass surrounding the whole of the medial side of the talus. He believed that contraction of the posterior tibial tendon was responsible for club foot. Hersh6 described a disc-like mass of fibrous tissue lying between the end of the navicular and the medial malleolus and recommended its excision. Turco7 considered that medial contracture was due to a fused dense mass of scar tissue which involved the deltoid and spring ligaments, the posterior tibial tendon, and the talonavicular capsule. Ippolito7 and Ippolito and Ponseti8 investigated specimens of fetal club foot histologically and described fibrous tissue penetrating muscle, fasciae, ligament and the tendon sheath on the posterior and medial sides of the club foot. They believed that retracting fibrosis could be responsible for the deformity. Fukuhara et al9 examined specimens of fetal club feet for the presence of the cytocontractile proteins desmin, vimentin and α-smooth muscle actin, which have been shown to represent differentiation markers during development and pathological conditions.10 They found cells staining positively for desmin and also myofibroblast-like cells. Myofibroblasts are believed to contribute to contraction in wound and ligament healing,11,12 infantile desmoid-type fibromatosis13 and Dupuytren’s disease.14,15 Zimny et al16 used electron microscopy (EM) to study fascia from the medially contracted mass and found myofibroblast-like cells.

To clarify the immunohistological characteristics of the medial soft tissues, we studied biopsy specimens from patients with club foot and compared them with normal deltoid ligaments from cadavers to provide a control group. We also used EM to identify myofibroblasts.

Patients and Methods

Specimen collection. We collected ligamentous tissue from the medial side of the foot during operations on 31 patients with club foot at the Children’s Hospital of Eastern Ontario, Ottawa, Canada, the Bustamante Hospital for Children and the University Hospital of the West Indies, Kingston, Jamaica. All patients had had serial casting before operation but none had had previous surgery. There were 19 boys and 12 girls; their mean age was 14 months (6 to 30). Fifteen patients had bilateral operations (30 feet) and 16 unilateral (16 feet), giving material from 46 feet. Four patients (6 feet) had spina bifida and one (two feet) had arthrogryposis. The other 26 patients
(38 feet) were diagnosed as having idiopathic club foot.

The control group was 12 normal deltoid ligaments collected from eight postmortems at the Bustamante Hospital for Children. There were four boys and four girls; their mean age was two months (0 to 5). Their cause of death was not associated with any bone or joint disorder.

**Light microscopy.** Forty-one specimens of club foot and all the control specimens were fixed in 10% neutralised formalin and embedded in paraffin. Sections 7 μm in thickness were cut, stained with haematoxylin and eosin and assessed for the shape of the nucleus and the spatial arrangement of collagen fibres.

Monoclonal antibodies against desmin (mouse IgG1, clone 33; BioGenex, San Ramon, California), vimentin (mouse IgG1, clone V9; BioGenex) and α-smooth muscle actin (mouse IgG2a; Sigma, Oakville, Canada) were used for immunohistochemical staining. Sections were hydrated and then washed in phosphate-buffered saline (PBS). Antibodies against desmin and vimentin were used for 30 minutes at room temperature followed by a PBS wash. A supersensitive immunodetection system (Biogenex) was then used, incubating the link and label for 20 minutes each with a PBS wash step between. Liquid dianminobenzidine (Biogenex) was applied for five minutes followed by a counterstain with haematoxylin. For staining of α-smooth muscle actin, the slides were predigested with 0.025% trypsin at 37°C for 30 minutes.

**Assessment of immunohistochemical staining.** Care was taken to examine only ligamentous tissue and avoid surrounding loose connective tissues and their small vessels. Each specimen was assessed as either positive or negative, with faintly stained cells considered to be negative. These histological evaluations were performed blind by two observers to assess the interobserver reliability. The principal observer then scored all specimens again to assess the intraobserver reliability. When two observers disagreed, a consensus was reached.

**Electron microscopy.** Five specimens of club foot were examined; three were from idiopathic cases and two from club feet in patients with spina bifida. The tissue samples obtained at operation were immediately minced with razor blades to fragments of about 1 mm³ in size and fixed in cold, 2.5% buffered glutaraldehyde; the samples were then embedded in epoxy resin. Semi-thin sections of about 1 μm in thickness were cut, stained with toluidine blue and scanned. From the selected blocks, thin sections were cut on a Reichert OmU2 microtome, mounted on copper grids, contrasted with uranyl acetate and lead citrate and examined on a Zeiss EM109 electron microscope.

**Statistical analysis.** We used the chi-squared test to compare the results of the staining properties between control and club-foot specimens (Statview for Macintosh; Abacus Concept Inc, Berkeley, California).

**Results**

Interobserver agreement was found in 141 of 159 assessments (88.7%) and intraobserver agreement in 149 (93.7%).

In the club-foot specimens, the ligamentous cells were arranged more haphazardly than those of the control group and had nuclei varying from spindle-shaped to plump. The collagen fibres were disorganised (Fig. 1). In the control group ligamentous cells showed a regular spatial arrangement and most of the nuclei were spindle-shaped or oval (Fig. 2); the wavy pattern of the collagen fibres was always clear.

**Immunohistochemistry.** All 41 club-foot specimens stained positively for vimentin (Fig. 3) compared with only one of the 12 control specimens (p < 0.0001, Table I). By contrast, there was no difference in the staining for desmin and α-smooth muscle actin between club-foot and control specimens. In contrast to our previous study of fetal specimens, neither club-foot nor control specimens stained positively for desmin, but most stained positively for α-smooth muscle actin (Table I). We found no differ-
ence in staining properties between the idiopathic cases and those from children with spina bifida or arthrogryposis (Table II).

**Electron microscopy.** The appearance of the ligamentous cells varied. Most contained bundles of microfilaments in the cytoplasm, usually parallel to the longitudinal axis of the cell. No dense bodies were noted in any of these cells. The granular endoplasmic reticulum was well developed, as was the Golgi zone. In many cells the endoplasmic reticulum contained flocculent material. Some showed abundant pinocytotic vesicles, but a basal lamina and dense bodies are absent (×8000); and b) fibroblasts with pyknotic and fragmented nuclei; that with a pyknotic nucleus has pinocytotic vesicles (arrow, ×9900).

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<th>Table II. Comparison of the immunohistochemical staining properties in idiopathic club feet and specimens from children with spina bifida and arthrogryposis</th>
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**Discussion**

Cytokontracile proteins and myofibroblasts are present during soft-tissue contraction. Myofibroblasts are known to have phenotypic characteristics of both fibroblasts and smooth muscle cells and immunohistochemistry has shown that they have differing combinations of the staining properties of desmin, vimentin and of α-smooth muscle actin. There is strong evidence that localised soft-tissue contraction is involved in the pathogenesis of club foot. Our results suggest that the process continues after birth, since we observed the presence of both myofibroblasts and vimentin in club-foot specimens obtained from patients of six to 30 months of age.

In our earlier study of seven severely deformed prenatal club feet, we observed the presence of desmin in six, but in this study, neither club-foot nor control specimens...
showed desmin-positive cells. Vimentin-positive cells were seen in all club-foot specimens but only in one of the 12 controls. Desmin is believed to be a marker of general differentiation of smooth and skeletal muscle,19 while vimentin is a marker of mesenchymal differentiation. Kocher et al20 studied smooth muscle cells in the intima and media of the rat aorta at 15 and 75 days after endothe-

dial injury. They reported that cells which had migrated into the intima contained decreased amounts of desmin and increased amounts of vimentin at 15 days after injury. Shum and McFarlane15 observed desmin-positive cells in proliferative Dupuytren’s nodules, which decreased signifi-
cantly in the fibrous phase of the disease. Based on their findings, we suggest that the proliferative activity in the medial ligamentous tissue of club feet during the intra-
uterine period may differ from that in the postnatal phase. It is likely that the proliferation of fibrous tissue is active during the intrauterine phase and that it decreases gradually thereafter. Increasing vimentin and decreasing desmin seem to reflect this change.

By contrast, α-smooth muscle actin was usually positive in both club-foot and control specimens, which confirms the results of our previous study on fetal specimens.4 As Fletcher et al19 and Gabbiani, Csank-Brassert and Schnee-
berger23 described, the contractile protein actin is ubiqui-
tous and therefore cannot be used as a marker of a specific differentiation.

No difference in immunohistochemistry could be found between idiopathic club feet and those in patients with spina bifida and arthrogryposis. This suggests that the cytocon-
tractile proteins may be responsible for the local soft-tissue contracture irrespective of the underlying pathological con-
dition. Primary factors which may induce the production of cytocontractile proteins remain to be elucidated.

There is a gradation of cells from fibroblasts to myo-

fibroblasts. Our ultrastructural findings showed many cells with features of myofibroblasts such as the pinocytotic vesicles described by Schürch, Seemayer and Gabbiani.22 Other features were compatible with metabolically inactive fibroblasts such as a marked increase in heterochromatin, as observed by Ghadially.23 We agree with the latter that the pyknosis, also observed in our study, is probably associated with apoptosis.23 This suggests that the process may evolve to a stationary, purely fibrotic stage, similar to that occurring during the later period of wound healing.5 Darby et al17 noted that during experimental wound healing, myo-

fibroblasts modulated to fibroblasts after about three weeks and that the number of fibroblasts diminished by apoptosis. Although, like Zimny et al,16 we could not find typical myofibroblasts, we believe that the cells from the medial soft tissue of club feet have many myofibroblastic charac-
teristics and that some recurrences after soft-tissue release could be attributed to the continued presence of cytocon-
tractile elements.

Our results do not allow speculation on the role that serial casting may have played in the development of the observed features.

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References