Expression of a Wide Range of Extracellular Matrix Molecules in the Tendon and Trochlea of the Human Superior Oblique Muscle

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PURPOSE. To show that the molecular composition of the extracellular matrix of the trochlea and its associated tendon may explain the link between some cases of acquired Brown syndrome and rheumatoid arthritis.

METHODS. One trochlea and its tendon from 11 dissecting-room cadavers were fixed in methanol, cryosectioned, and immunolabeled with a panel of monoclonal antibodies against types I, II, III, V, and VI collagens, chondroitin-4 and -6, keratan and dermatan sulfates, aggrecan, link protein, versican, and tenascin. Labeling was detected with an avidin-biotin-peroxidase detection kit.

RESULTS. The trochlea had a central core of hyaline cartilage surrounded by a band of fibrocartilage, but the tendon had no cartilage cells. Significantly, however, both structures immunolabeled for aggrecan, link protein, and type II collagen—molecules typical of articular cartilage.

CONCLUSIONS. The presence of aggrecan, link protein, and type II collagen may account for the coincidence between transient attacks of acquired Brown syndrome in patients with juvenile and adult forms of chronic rheumatoid arthritis. Cleavage of aggrecan by aggrecanase in articular cartilage characterizes cartilage degeneration in rheumatoid arthritis. Thus, it is possible that aggrecan cleavage also occurs in the trochlea and tendon and contributes to their degeneration or to a local inflammatory reaction that may swell and thicken the tendon. In this context, it is also significant that link protein and type II collagen are now regarded as relevant antigenic targets for autoimmune responses in rheumatoid arthritis. (Invest Ophtalmol Vis Sci. 2002;43:1330–1334)

The tendon of the superior oblique muscle changes its direction of pull by wrapping around a fibrocartilaginous pulley (the trochlea) attached to the medial wall of the orbit. A loss of function in this region impairs the ability of a patient to elevate the eye in adduction—a condition known as Brown syndrome, or superior oblique tendon sheath syndrome.1–4 Although the trochlea is frequently described as cartilaginous5,6 or fibrocartilaginous,7–9 little is known of its molecular composition or that of the tendon itself. Such information is important to establish, however, because of the link that is now known to exist between the acquired form of Brown syndrome and rheumatoid arthritis (RA).10–15 In some patients with RA, there is an autoimmune response against aggrecan, link protein, and type II collagen.16–19 Herein, we argue that if such molecules are also present in the trochlea and/or tendon, it may explain the connection between the diseases. Significantly in this connection, the transverse ligament of the atlas contains all these molecules and can also be affected by RA with an autoimmune response.20 Similar to the tendon of the superior oblique muscle, this ligament is subject to both compressive and tensile loading as it changes direction by wrapping around a pulley—the dens. The purpose of the present study therefore was to provide an immunohistochemical profile of the superior oblique tendon and its trochlea, using a wide range of antibodies directed against molecules found in the extracellular matrix (ECM) of connective tissues, including cartilage.

METHODS

Tissue Preparation

The trochlea and its associated tendon were removed from 11 unfixed human cadavers (both sexes; ages 17–39 years) within 36 hours of death and fixed for 24 hours in 90% methanol at 4°C. Institutional Review Board and Ethics Committee approval was not required for this study, because all human material was obtained from bodies donated to the Department of Anatomy at Munich University for research purposes, before death. The protocol adhered to the tenets of the Declaration of Helsinki for research involving human tissue.

Immunohistochemical Procedures

Details of the immunohistochemical procedures have been published previously,20,21 but briefly, the procedure was as follows. Specimens were stored at −20°C as necessary, rinsed in phosphate-buffered saline (PBS), infiltrated overnight with 5% sucrose in PBS, and cryosectioned at 12 µm. Sections were stained with toluidine blue to highlight the presence of any cartilage and fibrocartilage by its metachromasia and with a panel of monoclonal antibodies (Table 1). The antibodies were directed against collagens (types I, II, III, V, VI), glycosaminoglycans (GAGs; chondroitin-4 and -6-sulfates, dermatan sulfate and keratan sulfate), and proteoglycans (versican, tenascin, together with aggrecan and its link protein). Sections immunolabeled for aggrecan and for link protein were treated with 10 mM dithiothreitol in 50 mM Tris-HCl and 200 mM sodium chloride (pH 7.4) for 2 hours at 37°C and then alkylated with 40 mM iodoacetamide in PBS for 1 hour at 37°C. The sections were subsequently incubated at 37°C with chondroitinase AC (0.25 U/mL; Sigma, Diesenhofen, Germany). Endogenous peroxidase activity was blocked in all sections by pretreatment with 0.3% hydrogen peroxide in methanol for 30 minutes and nonspecific binding of the secondary antibody was reduced with an appropriate serum block.

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for 40 minutes. We controlled for nonspecific binding of antibodies by omitting the primary antibody or by incubating the sections with normal mouse immunoglobulins (5 μg/mL for all monoclonal antibodies). Antibody binding was detected with an avidin-biotin-peroxidase complex kit (Vectastain ABC Elite; Vector Laboratories, Burlingame, CA).

**Monoclonal Antibodies**

The monoclonal antibodies CIICI,22 5C6,50 and 12C5,23,28 were obtained from the Developmental Studies Hybridoma Bank maintained by the Department of Biological Sciences, University of Iowa (under contract NO1-HD-7-3263 from the National Institute of Child Health and Human Development [NICHD], Bethesda, MD).

**RESULTS**

The tendon was largely fibrous (Fig. 1a) and separated from the trochlea by a bursa (Fig. 1b). Although the tendon had no obvious fibrocartilage cells, its ECM was slightly metachromatic, especially near the surface where it faces the trochlea. The latter varied greatly in appearance from the center to the periphery. Its central part more closely resembled hyaline cartilage, but its periphery was more fibrous (Fig. 1a) and separated from the fibrocartilaginous (Figs. 1c–e) and hyaline cartilage (compare Figs. 1c, 1d) of the zone of hyaline cartilage in the trochlea. The absence of typical fibrocartilage cells in the center of the tissue, labeling for versican was absent in the territorial and interterritorial matrix (Fig. 2i). There was greater variability in GAG and proteoglycan (PG) labeling between tendons. Chondroitin-6-sulfate (Figs. 2a, 2b) and keratan sulfate (Figs. 2c, 2d) were less commonly detected in tendons than in their pulleys, but tenascin and versican were present in most tendons (Fig. 2i). Aggrecan was seen in 10 of the 11 tendons (Fig. 2f), but link protein was detected in all (Fig. 2h).

**Collagens**

Types I, III, and VI collagens were found in all regions of the tendon (Figs. 1a–d). However, labeling for types I and II collagen varied substantially in different parts of the trochlea. Both the peripheral region of fibrocartilage and the outer parts of the zone of hyaline cartilage in the trochlea labeled for type I collagen, but the inner part of the hyaline cartilage zone did not (Figs. 1c, 1d). In contrast, labeling for type II collagen was absent from the fibrocartilage (compare Figs. 1c, 1e) and present throughout the zone of hyaline cartilage. Local patches of type II collagen labeling were seen in the tendon in 7 of the 11 specimens (Fig. 1f). Although there was no difference in the labeling intensity for type VI collagen in the different parts of the tendon, labeling was more pericellular in the trochlea (Fig. 1b).

**Glycosaminoglycans and Proteoglycans**

With the exception of a single specimen that failed to label for keratan sulfate, all the trochleae examined labeled for all GAGs (Figs. 2a–d). Aggrecan and link protein were more prominent in the center of the trochlea and most evident pericellularly (Figs. 2e–h). In some specimens where there were large cartilage cells in the center of the tissue, labeling for versican was absent in the territorial and interterritorial matrix (Fig. 2i).

**DISCUSSION**

The results show that the trochlea is a mixture of fibrocartilage and hyaline cartilage and that the superior oblique tendon has a fibrous morphology but a profile of ECM molecules more typical of fibrocartilage.20,21,31–33 Biomechanical data suggest that during adduction-elevation of the eye, the superior oblique muscle generates a force of 17.4 g and that its tendon has a longitudinal excursion of approximately 5.6 mm as it slides through the trochlea.34 According to Helveston et al.,35 the longitudinal excursion of the central part of the tendon is greater than the remainder and may reach 8 mm. The values are only slightly lower for isolated elevation (12.7 g and 4.1 mm34). However, under conditions of tetanic stimulation, forces may reach 40 to 100 g.34,36 Because the angle through which the tendon changes direction is steep (53°), such forces may be enough to trigger the production of some molecules typical of a fibrocartilaginous ECM near its articulating-sliding surface.

The absence of fibrocartilage cells in the superior oblique tendon clearly contrasts with their presence in many of the large tendons that wrap around bony pulleys in the foot (e.g., peroneus longus).37 The absence of typical fibrocartilage from the tendon makes sense biomechanically, in that the central part of the tendon is more mobile than the remainder,38 and its...
fascicles must thus slide over each other for any relative movement to occur. Such a sliding of tendon fascicles would be impossible in many of the fibrocartilaginous tendons of the foot, because their parallel fascicle arrangement is interrupted.

The movement of the tendon in the trochlear region is compromised in Brown syndrome, often necessitating surgical treatment. In such cases, the most prominent features are deficient elevation in adduction and positive forced motions for both elevation in adduction and exocyclotorsion of the globe. Although this pattern of ocular deviation is congenital in many patients, there is also a rare form of acquired Brown syndrome that is associated with RA. However, the etiology of this acquired condition is unclear. Although it has long been known that autoantibodies against a 23-kDa protein of human fibroblasts arise in Graves disease, there is also a rare form of acquired Brown syndrome that is associated with RA.

In view of the association between acquired Brown syndrome and RA, it is important to note that aggrecan and link protein are consistently present in the trochlea and are generally also present at the sliding surface of the tendon. Aggrecan is the large aggregating proteoglycan that is responsible for the compression–tension properties of articular cartilage, and link protein is an extracellular glycoprotein that stabilizes the

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**Table 2. Summary of the Immunolabelling Characteristics of the Tendon of the Superior Oblique Muscle and the Trochlea**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Tendon</th>
<th>Fibrocartilage</th>
<th>Hyaline Cartilage</th>
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<tbody>
<tr>
<td>Type I collagen</td>
<td>11</td>
<td>11</td>
<td>11*</td>
</tr>
<tr>
<td>Type II collagen</td>
<td>7</td>
<td>0</td>
<td>11</td>
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<tr>
<td>Type III collagen</td>
<td>11</td>
<td>11</td>
<td>11</td>
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<tr>
<td>Type V collagen</td>
<td>9</td>
<td>10</td>
<td>11</td>
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<tr>
<td>Type VI collagen</td>
<td>11</td>
<td>11</td>
<td>11</td>
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<tr>
<td>DS-C4S (2B6 + ChABC)</td>
<td>11</td>
<td>11</td>
<td>11</td>
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<tr>
<td>C4S (2B6 + ChAC)</td>
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<td>11</td>
<td>11</td>
</tr>
<tr>
<td>C6S (3B3 + ChABC)</td>
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<td>11</td>
<td>11</td>
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<tr>
<td>KS (5D4)</td>
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<td>10</td>
<td>10</td>
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<tr>
<td>C6S-oversulfated (7D4)</td>
<td>6</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Versican (12C5 + ChAC)</td>
<td>9</td>
<td>11</td>
<td>11*</td>
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<tr>
<td>Tenascin (T2H5 + ChAC)</td>
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<tr>
<td>Aggrecan (1C6 + ChAC)</td>
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<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Link Protein (1C6 + ChAC)</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

Each column entry shows the number in 11 specimens examined in which positive labeling was seen. ChAC, chondroitinase AC; ChABC, chondroitinase ABC; C6S, chondroitin-6-sulfate; C4S, chondroitin-4-sulfate; DS, dermatan sulfate; KS, keratan sulfate.

* Variability in labeling within the region.
The interaction between aggrecan and hyaluronan. The two epitopes recognized by the monoclonal antibody 8A4 for link protein lie in the tandem-repeat domains that are involved in hyaluronan interactions. In articular cartilage, it is the high charge density of sulfated GAGs in aggrecan that attracts water into the tissue. The aggrecan and water are then held in place by the fibrous network of type II collagen. To our knowledge, this is the first time that aggrecan and link protein have been demonstrated in the human trochlea. The significance of the finding relates to clinical observations that report a coincidence between transient attacks of acquired Brown syndrome in patients with juvenile and adult forms of chronic RA. Because cleavage of aggrecan by aggrecanase in articular cartilage is a characteristic feature of cartilage degeneration in RA, it seems possible that such cleavage occurs in the trochlea and perhaps in the tendon as well. This would contribute to the degeneration of these structures or at least to a local inflammatory reaction that may cause swelling and thickening of the tendon. It is significant therefore that link protein is now also regarded as one of the major antigenic targets for autoimmune responses in juvenile and adult RA. Because RA is a systemic disease predominantly affecting tissues with a cartilage phenotype and because the trochlea immunolabels strongly for link protein, aggrecan, and collagen type II (which is also a major target in RA), this region should also be regarded as a preferred target for inflammatory reactions. Thus, in the course of the disease, the trochlea might be expected to lose its mechanical function of promoting free tendon movement more readily than if it were pure fibrous tissue instead. The partial disappearance of enthesis fibrocartilage in patients with RA has already been reported at the attachment of the central slip of the extensor tendon to the intermediate phalanx.

References