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Expression of Procollagen α1 type Ⅲ mRNA in Rotator Cuff Tears

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The purpose of this study was to investigate the relationship among expression of procollagen α1 type Ⅲ mRNA, subacromial adhesion, and limited shoulder elevation in rotator cuff tears. Procollagen α1 type Ⅲ mRNA was analyzed in the torn supraspinatus tendons and synovial tissue of 42 patients with rotator cuff tears. As a control, four normal supraspinatus tendons of patients who had undergone shoulder surgery other than rotator cuff repair served as controls. Cells stained positively for procollagen α1 type Ⅲ mRNA were more abundant in the adhesive synovium of the subacromial bursa and in the torn supraspinatus tendons than in controls. Patients with complete-thickness tears who had undergone surgery less than 10 months after trauma were found to have more signal-positive cells than those later than 10 months. We conclude that the adhesive synovium of the subacromial bursa contains abundant cells stained positively for procollagen α1 type Ⅲ mRNA. We suggest that the production of type Ⅲ collagen by these cells could contribute to the limitation of shoulder motion in patients with rotator cuff tears, and recommend release and excision of the excess adhesive synovium during rotator cuff surgery.

Key words : Rotator cuff tear, Adhesion, Procollagen α1 type Ⅲ mRNA

INTRODUCTION

Type Ⅲ collagen, which is abundant in the tissue of patients with Dupuytren's disease, was related to adhesion and contracture (Brickley 1981). Type Ⅰ and Ⅲ collagens are of fibrillation type in all kinds of collagens. The collagen in the normal tendon is mainly type Ⅰ (95%), although Type Ⅲ, Ⅳ, and Ⅴ are also present (5%) (Von der Mark 1981). Type Ⅲ collagen is found in the endotenon and epitenon, and near the blood vessels in tendons (Epstein 1975 and Sandberg 1987). Williams et al. (1980, 1984) have reported that the scarred tendon had 20 to 30 per cent of type Ⅲ collagen in addition to type Ⅰ and this abnormal condition continued until 3 months after injury. Riley et al. (1994) reported that 17% of normal cadaveric supraspinatus tendons and 82% of cadaveric supraspinatus tendons with tendinopathy including rotator cuff tears contained more than 5% type Ⅲ collagen, and that this proportion increased after inflammation, minor trauma, or microscopic fiber damage to the tendon.

Disabling rotator cuff tears frequently occur after age 40 and are accompanied by pain and weakness. In addition, some cases show limited shoulder motion, which has been and still is a most nagging problem to be overcome in order to achieve satisfactory functional results. The limitation of rotation and elevation of the shoulder joint has been considered to be due to the adhesion of the subacromial bursa (McLaughlin 1951 and Neviaser 1945) and/or to the inflammation and contracture of the coracohumeral ligament and the rotator interval (Ozaki et al. 1989) (Fig. 7). Type Ⅲ collagen is composed of three α1 type Ⅲ chain, which are produced from α1 type Ⅲ procollagen mRNA. Thus, the cells expressing procollagen α1 type Ⅲ mRNA are in the active process of type Ⅲ collagen production. The purpose of this study is to verify the hypothesis that the limitation of shoulder elevation is associated with the active production of procollagen

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type Ⅲ in the subacromial bursa and the torn supraspinatus tendon.

MATERIALS AND METHODS

Patients

Forty-two patients with 28 cases of complete- and 14 cases of incomplete-thickness rotator cuff tears (6 bursal-side, 4 intratendinous and 4 joint-side tears), documented at surgery, were studied. The age of the patients with complete-thickness tears ranged from 36 to 77 years (mean 55.9 years) and those with incomplete-thickness tears were 26 to 72 years of age (mean 54.6 years). Eighteen of the 28 patients with complete-thickness tears and 7 of the 14 patients with incomplete-thickness tears had history of apparent trauma (mean age 56.2 and 54.3 years, respectively). The mean duration of pain was 10 months (3 weeks to 4 years) in complete-thickness tears and 1 year and 7 months (1 month to 9 years) in incomplete-thickness tears (Table 1). The degree of adhesion in the subacromial bursa was divided into four levels at the operation: no adhesion, slight adhesion, which can be released easily with fingers, moderate adhesion, which can be released with fingers with difficulty, and severe adhesion, which can not be released with fingers. The specimens were obtained as follows: The synovial tissue of the subacromial bursa was excised in the vicinity of the greater tuberosity from both patients with torn cuff and normal controls. The rotator cuff was excised from the margin of the proximal stumps of the supraspinatus tendon in complete-thickness tears. In incomplete-thickness tears, the cuff tissue was obtained from the en bloc resection of the supraspinatus tendon with the torn area in its center. For control cuff specimens, a longitudinal strip of the supraspinatus tendon of normal appearance, measuring approximately 10 × 2 mm, was excised from the following four patients; two patients with humeral shaft fractures before intramedullary nailing from the proximal end of the humerus, one with brachial plexus palsy before multiple muscle transfer, and one with an extensive skin cancer before glenohumeral disarticulation. The age of the control patients ranged from 19 to 73 years (mean 40.8 years). The sampling of the supraspinatus tendon was agreed by the patients and approved by the intramural ethics board of the Tokai University.

Tissue preparation

All specimens were fixed in 10% neutral buffered formalin (Wako, Osaka, Japan) for 48 hours at 4℃. They were then dehydrated and embedded in paraffin (Wako, Osaka, Japan). Sections 6 mm thick were mounted on silane coated slides (Matsunami Glass Ind., Ltd., Osaka, Japan) and dried for 24 hours on a slide warmer at 42℃.

Histologic Study

Hematoxylin-eosin and azan stainings were used. Results were compared with those of in situ hybridization studies.

In Situ Hybridization Study

In situ hybridization procedures were as described by Hamada et al. (1994, 1995, 1997). The Tm values of the probes were calculated using the equation (Baldino & Davis, 1986): Tm = 16.6(logM) + 0.41(%GC) + 81.5-675/L-0.65(%F)-%MM, where M is molarity, L is length of the oligonucleotide, F is formamide concentration, and MM is mismatch of bases. After deparaffinization and rehydration, the sections were soaked in 0.2 N hydrochloric acid at room temperature for 10 minutes. Sections were then incubated with 5 mg/mL of Protease K (Sigma Chemical, St. Louis, MO, U.S.A. type XXVIII) in 0.1M Tris-HCl (pH 7.5) buffer at 37℃ for 20 minutes and soaked in 0.25% acetic anhydride in 0.1M triethanolamine (pH 8.0) for 10 minutes. After being air-dried, samples were hybridized with a digoxigenin-labeled oligonucleotide probe at a concentration of 150 pg/mL in hybridization mixture [50% deionized formamide, 6 × standard saline-sodium citrate (SSC, pH 7.0), 50 mM Tris-HCl (pH 7.0), 2 × Denhardt's mixture (0.02% w/v), 0.2% sodium dodecyl sulfate (SDS), and 250 mg/mL yeast transfer RNA (Sigma Chemical)]. The hybridization mixture containing the 24mer oligonucleotide probe (5'-GGTTCATCTCCATAATACGGGGCA-3') (Hamada et al. 1995) was spread on the dried specimens, which were covered with parafilm and incubated in a moist chamber containing 50% formamide at room temparature for 18 hours. After hybridization, sections were washed in 2 × SSC for 30 minutes (two changes) and 0.1 × SSC for 30 minutes (three

changes) at room temparature. After the slides were soaked in Tris-buffer [100 mM tris-HCl, 150 mM NaCl, and 0.02% (w/v) sodium azide (pH 7.7)] for 2 minutes, they were preincubated with Tris-buffer containing 0.6% (w/v) carrageenan (Sigma Chemical) and 0.05% (v/v) Triton × −100 (Sigma Chemical) for 30 minutes at room temperature in a moist chamber. The hybridization signals were amplified using

Table 1 Patients (n = 42)

CASE	AGE, SEX	TYPE	TRAUMA	DURATION OF PAIN	LOSS OF ELV
1	58 M	CMPL	+	4mos	+
2	47 M	CMPL	+	1yr2mos	+
3	65 M	CMPL	−	1yr	−
4	58 F	CMPL	+	5mos	−
5	51 M	CMPL	−	4mos	−
6	51 M	CMPL	−	2yrs	+
7	65 F	CMPL	+	9mos	−
8	64 M	CMPL	−	1yr6mos	−
9	64 M	CMPL	+	1yr5mos	+
10	60 M	CMPL	−	8mos	+
11	52 F	CMPL	+	11mos	+
12	77 F	CMPL	+	3yrs	−
13	39 M	CMPL	+	1yr9mos	−
14	71 F	CMPL	−	4mos	−
15	62 M	CMPL	+	2mos	−
16	54 M	CMPL	−	1yr3mos	−
17	61 F	CMPL	−	4yrs	−
18	57 M	CMPL	+	3wks	−
19	58 M	CMPL	−	8mos	−
20	52 F	CMPL	+	1mo2wks	+
21	63 M	CMPL	+	2mos	+
22	58 F	CMPL	+	4mos	−
23	36 M	CMPL	+	4mos	−
24	58 M	CMPL	+	2mos	+
25	68 M	CMPL	+	5mos	+
26	70 F	CMPL	+	11mos	+
27	48 F	CMPL	+	3wks	−
28	57 F	CMPL	+	5mos	+
29	48 M	B	−	3mos	−
30	64 F	J	+	8mos	+
31	72 M	B	+	11mos	+
32	52 F	J	+	9mos	+
33	63 M	B	−	8mos	−
34	59 M	J	−	1yr6mos	+
35	61 M	I	+	4yrs	+
36	43 M	B	−	9yrs	−
37	61 F	B	−	8mos	+
38	61 M	I	−	1yr10mos	−
39	26 M	I	+	4mos3wks	−
40	49 M	B	−	1yr2mos	−
41	53 M	J	+	1mo	−
42	52 M	I	+	3mos	+

CMPL = complete-thickness tear B = bursal-side tear I = intratendinous tear J = joint-side tear ELV = elevation

the sandwich method of Hamada et al. (1995). The sections were sequentially treated with: (1) mouse monoclonal anti-digoxin (1:10,000, Sigma Chemical); (2) biotinylated anti-mouse F(ab)2' fragment (1:200, Mississauga, ON, Canada); and (3) avidin conjugated with alkaline phosphatase (1:100, Dako A/S, Denmark). Following each incubation with (1) and (2), the slides were washed with Tris-buffer [100 mM tris-HCl, 150 mM NaCl, and 0.02% (w/v) sodium azide (pH 7.7)] for 10 minutes (two changes) at room temperature. Following incubation with (3), they were washed with Tris-buffer (100 mM Tris-HCl, 150 mM NaCl pH 7.5) for 10 minutes (two changes). The visualization of the probe mRNA hybrids was done by applying 4-nitroblue tetrazolium chloride (Boehringer Mannheim, Mannheim, Germany) and 5-bromo-4-chloro-3-indolyl-phosphate (Boehringer Mannheim) at room temperature in the dark for 60 minutes. The slides were washed with Tris-buffer [0.8 mM EDTA and 8 mM Tris-HCL (pH 8.0)], fixed with Crystal/Mount (Biomeda, Foster City CA, U.S.A) and mounted with Eukitt (O. Kindler, Germany). For the control experiments, a probe omission test, a competition test, ribonuclease (RNAse) treatment and incubation with sense probe were performed to confirm the probe specificity. The number of cells stained positively for procollagen α1 type Ⅲ mRNA (signal-positive cells－SPCs) was determined in a 250 mm × 250 mm area at 5 randomly selected areas in the torn portion, bursal-side layer, intratendinous layer, and joint-side layer of the torn rotator cuffs, and in the synovial tissues in the subacromial bursa.

RESULTS

I. Histologic Findings

The synovial tissue was classified into three histologic types: fibrous (or collagenic), areolar, and adipose (Key 1932). The synovial tissues of the subacromial bursa taken during surgery composed mainly of areolar and adipose types. The synovium adhered to the joint-side layer of the suprasupinatus tendon was composed mainly of fibrous and areolar types. No apparent increase in the number of lining cells was noted, although an accumulation of lymphocytes and plasma cells was observed.

II. In Situ Hybridization Findings

1) Probe specificity and normal supraspinatus tendons

SPCs were detected in the tenocytes and

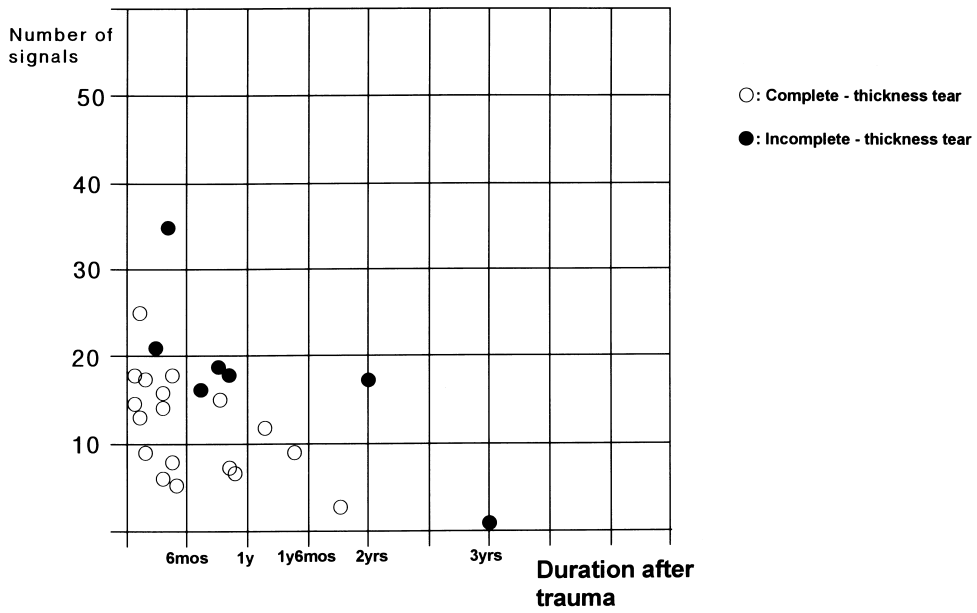

Fig. 1 Graph showing the relationship between the number of cells positive for procollagen α1 type Ⅲ mRNA in the torn suprasupinatus tendon and the duration after trauma.

undifferentiated mesenchymal cells of the supraspinatus tendons from the 4 control patients. The four control experiments (probe omission, probe competition, RNAse treatment, and incubation with sense probe) yielded no positive signals. SPCs indicating the presence of procollagen α1 type Ⅲ mRNA were detected mainly in the bursal-side layer and in the vicinity of capillary vessels, and were present with a frequency of 2 to 6 cells (mean 3.5) per 250 mm × 250 mm. SPCs in the mid-layer of the tendon, joint-side layer of the tendon, and synovium of the subacromial bursa and joint capsule were present with a frequency of from 1 to 4 cells (mean 2 cells) per 250 mm × 250 mm.

2) Torn supraspinatus tendons with apparent trauma

SPCs were detected in supraspinatus tendons of 28 patients with complete-thickness and 14 with incomplete-thickness cuff tears. Eighteen of 28 patients with complete-thickness tears had history of apparent trauma, and 17 of those 18 patients had undergone surgery 2 to 18 months after trauma. These 17 patients showed significantly more SPCs among the tenocytes and undifferentiated mesenchymal cells at the torn portion of the tendon than the control ($p = .0017$ by Mann-Whitney U test) (Fig. 1). The patients with complete-thickness tears who had undergone surgery in less than 10 months after trauma were found to have more SPCs than those who had undergone surgery later than 10 months. This difference was statistically significant ($p = .0429$ by Mann-Whitney U test). SPCs were more abundant in both groups than the control ($p = .0022$ for patients undergoing surgery in less than 10 months, and $p = .0204$ for those undergoing surgery later than 10 months after trauma by Mann-Whitney U test). In the 7 patients with the incomplete-thickness tears and history of apparent trauma, the population of SPCs ranged from 0 to 35 cells (Fig. 1). SPCs at the proximal stump of the torn portion of the supraspinatus tendon in the 6 patients who had undergone surgery within 2 years after injury were more abundant than the control ($p = .0420$ by Mann-Whitney U test) (Fig. 2). The number of SPCs was not statistically different among the bursal-side, intratendinous and joint-side layers of the torn portion ($p = .7147$ by ANOVA).

3) Torn supraspinatus tendons without apparent trauma

In 10 patients with complete-thickness tears who did not have history of apparent trauma, the number of SPCs at the torn por-

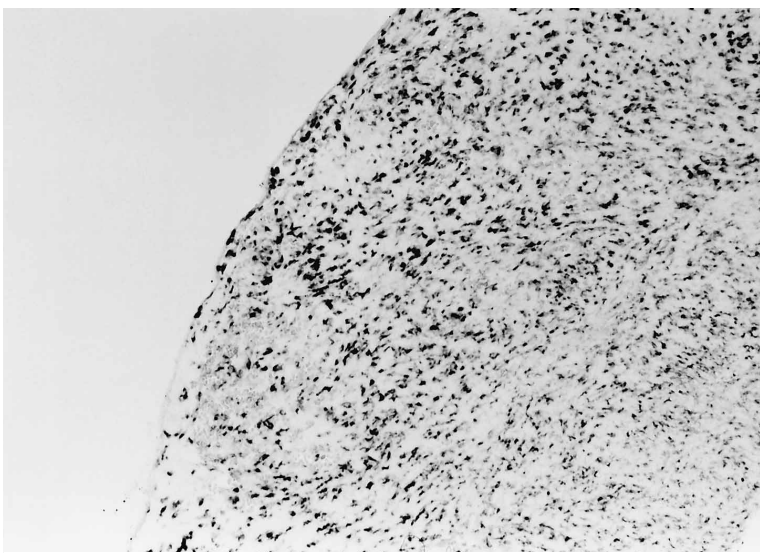

Fig. 2 Bursal-side tear in a 26-year-old male, 5 months after trauma. In situ hybridization with an antisense probe. Intense signals for procollagen α1 type Ⅲ mRNA were detected in the tenocyte. (× 50)

tion was not different from that in controls ($p = .0615$ by Mann-Whitney U test). In 7 patients with incomplete-thickness tears who did not have history of apparent trauma, the number of SPCs at the proximal stump of the torn tendon did not exceed that in controls ($p = .0624$ by Mann-Whitney U test).

4) Synovium on the bursal and joint surfaces of the rotator cuff tears

In the synovium of the subacromial bursa and joint capsule of all 42 cases, SPCs were detected among fibroblasts (Fig. 3). SPCs were more numerous in the synovium of subacromial bursa than on the joint capsule in all 42 cases ($p = .0427$ by Mann-Whitney U test). The synovium of the subacromial bursa demonstrated a far greater number of SPCs than the controls, and that in the synovium of joint capsule was not significantly different ($p = .0137$, $p = .0752$, respectively, by Mann-Whitney U test). There was no statistically significant correlation between the number of SPCs in the synovium of the subacromial bursa and history of trauma in complete- and incomplete-thickness tears ($p = .0752$ and $p = .0646$, respectively, by Mann-Whitney U test). The number of SPCs in the proliferative and adhesive (moderate and severe) synovial tissues in the subacromial bursa with complete- and incomplete-thickness tears were greater than 8 cells per 250 mm × 250 mm, while there were less than 4 SPCs in the synovium without adhesions (Fig. 4). The number of SPCs in moderate and severe adhesions was significantly greater than in the control ($p = .0375$ and $p = .0032$, respectively, by Mann-Whitney U test). However, there was no statistical difference between the number of SPCs in the synovial tissue with no or slight adhesions and that in the control. In complete- and incomplete-thickness cuff tears, a positive correlation was found between the number of SPCs in the synovium of the subacromial bursa and the degree of adhesion ($p = .0306$ for complete-thickness tears, and $p = .0065$ for incomplete-thickness tears by Spearman's rank correlation) (Fig. 4). Moreover, a significant correlation was found between the degree of the subacromial adhesion and the limitation of the shoulder elevation as compared to the normal side. The limitation was defined as more than 30 degrees of difference in elevation compared to the normal side. ($p = .001$ for complete-thickness tears, and $p = .0119$ for incomplete-thickness tears by Mann-Whitney U test) (Fig. 5). The synovium of the subacromial bursa in patients with limited shoulder elevation showed more abundant SPCs than those without. ($p = .0067$ for complete-thickness cuff tears,

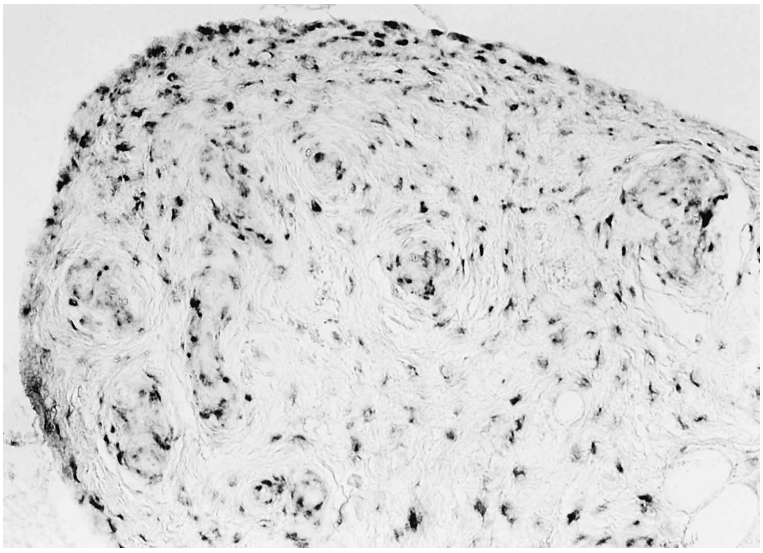

Fig. 3 Complete-thickness tear in a 58-year-old man with severe subacromial adhesion. In situ hybridization with an antisense probe revealed abundant procollagen $\alpha 1$ type Ⅲ mRNA signal-positive cells in the synovium. (× 50)

and p = .0326 for incomplete-thickness cuff tears by Mann-Whitney U test) (Fig. 6).

DISCUSSION

Observing the same specimens of the present study, Hamada et al. (1994. 1997) have reported that cells expressing procollagen α1 type Ⅰ mRNA in the torn suprasupinatus tendon decreased in number after 4 months following trauma. Compared to these reports, procollagen α1 type Ⅲ appears to be produced for a longer time than procollagen α1 type Ⅰ after tearing of the rotator cuff. This could be one reason for the observed higher extent of type Ⅲ collagen in degenerated torn tendons of long standing (Williams 1980).

Lundberg (1969) and Risk (1983) defined

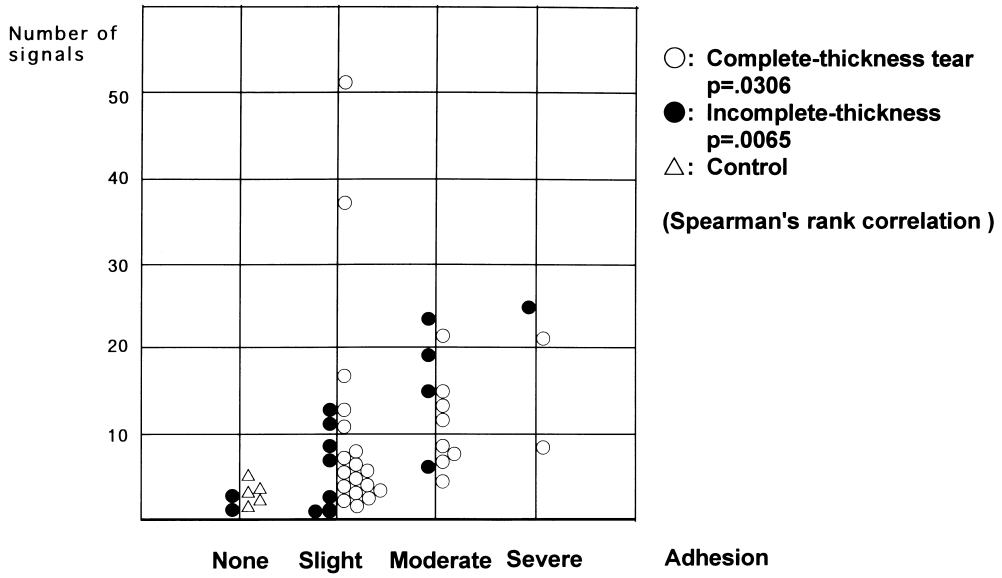

Fig. 4 Graph showing the relationship between adhesion and the signals for procollagen α1 type Ⅲ mRNA in the synovium of the subacromial bursa. Positive correlation was found between the number of SPCs and the degree of adhesion. Most patients with moderate or severe adhesion had limitation of shoulder elevation.

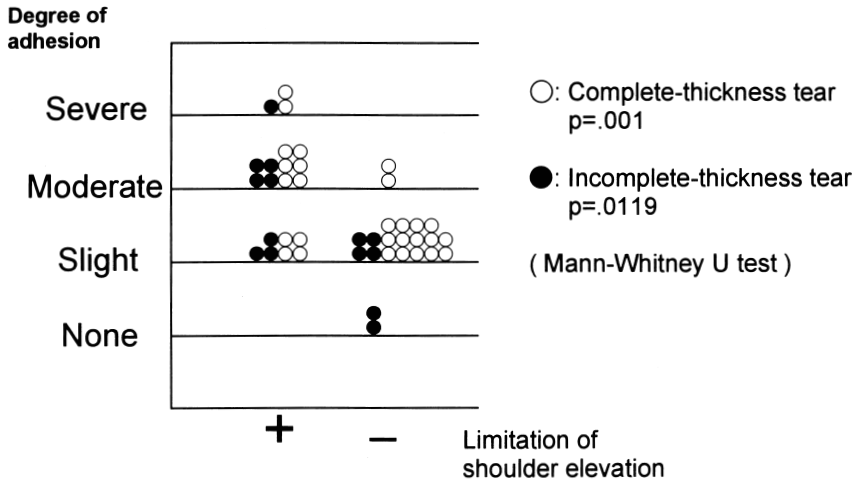

Fig. 5 Graph showing the relationship between limitation of shoulder elevation and the degree of adhesion in the subacromial bursa.

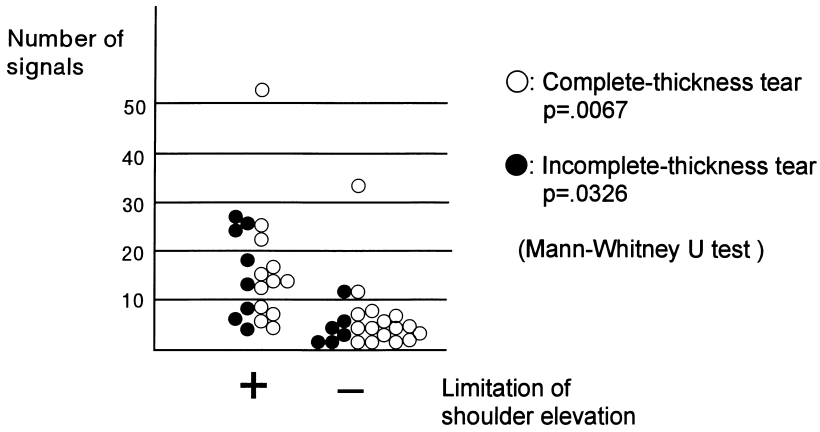

Fig. 6 Graph showing the relationship between limitation of shoulder elevation and the number of signals of the subacromial bursa.

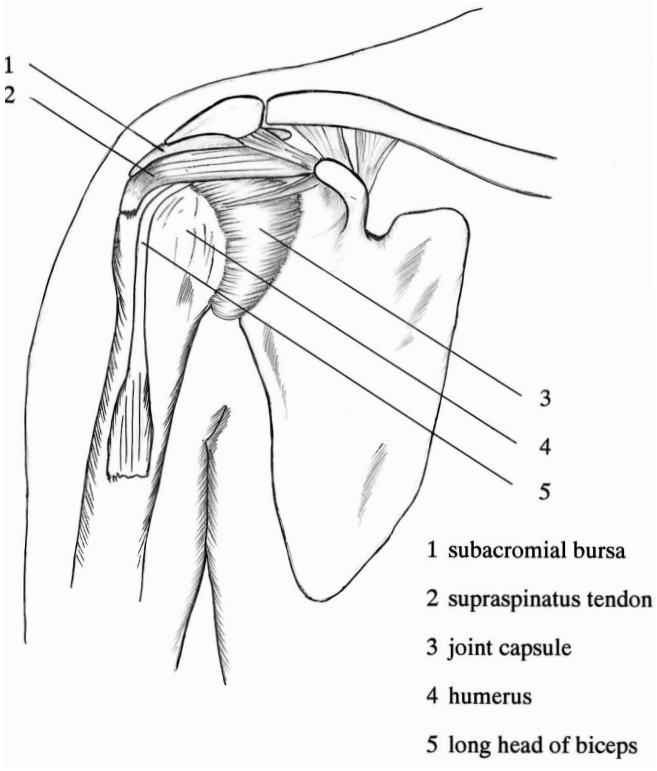

Fig. 7 Illustration showing the shoulder joint.

loss of shoulder elevation as less than 135 and 140 degrees of elevation, respectively. We defined it as a 30 degree difference in elevation compared to the normal side, and evaluated it for potential relationship with the degree of adhesion in the subacromial bursa assessed during operation. An increase in type Ⅲ collagen is usually accompanied by scarring and adhesion of the collagenous tissue as evidenced by abundant deposits in the palmar fascia and aponeurosis in Dupuytren's disease (Bailey 1977, Brickley-Parsons 1981). It is necessary to detect the expression of procollagen α1 type Ⅲ mRNA

in assessing the on-going process of synovial adhesion formation. In our study of both complete- and incomplete-thickness cuff tears, the signals for procollagen α1 type Ⅲ mRNA were detected in fibroblasts in the subacromial bursa, and increased in proportion to the degree of adhesion. We have shown that the number of cells producing procollagen α1 type Ⅲ mRNA in the synovium of the subacromial bursa correlates with the extent of subacromial adhesion and the limitation of shoulder elevation. Well known Codman's assertion describes that complete-thickness cuff tears did not form adhesions, while many incomplete-thickness tears did (Codman 1934). However, in our study, there was no clear distinction between complete- and incomplete-thickness cuff tears regarding the degree of adhesion. Our separate clinical study, comparing the degrees of passive elevation and external rotation between 60 complete- and 60 incomplete-thickness cuff tear patients, confirmed this finding (Fukuda 1998). The validity of Codman's assertion can be questioned because definite diagnosis of incomplete-thickness cuff tear was not possible then without surgery and without modern imaging techniques such as ultrasonography and MRI. Investigators have had varying opinions on whether the bursal tissue should be preserved (Uhthoff et al. 1991) or excised (Neer 1990, Ellman 1991). We found that SPCs in proliferating bursa in patients with severe limitation of shoulder elevation were greater in number than the control. Based on our study, we advocate excision of the surplus adhesive synovium in rotator cuff tears, because this tissue is strongly related to limitation of shoulder elevation.

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