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The accumulation of inflammatory cells in synovial sheath and epitenon during adhesion formation in healing rat flexor tendons

B. WOJCIAK & J. F. CROSSAN* *Cell Biology Department, Glasgow University, and *Department of Orthopaedic Surgery, Western Infirmary, Glasgow, UK*

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SUMMARY

The accumulation of inflammatory cells in synovial tissue was studied using indirect immunofluorescence assays on cell cultures and frozen tissue sections of healing rat digital flexor tendons. Flexor tendons were collected from rats 3, 7 and 14 days after crush injury. Tendon sheath and epithenon cells were isolated by sequential enzymic digestion and cultured for 2 days. Subpopulations of synovial and inflammatory cells were identified with MoAbs against cell surface glycoproteins present on B lymphocytes (CD45), T lymphocytes (CD2, CD4, CD8), macrophages (CD14) and endothelial cells. A phagocytosis assay was also used to identify macrophages. We report a substantial increase in the number of T lymphocytes (mainly helper/inducer) and phagocytotic cells with monocyte/macrophage surface markers in tendon sheath and epitenon 3 days after crush injury. The infiltration of inflammatory cells into synovial sheath and epitenon preceded an increase in fibronectin production by tendon cells which was seen 7 days after injury. To study the interaction between T lymphocytes and synovial cells *in vitro*, we established synovial fibroblast-like type B cell cultures and used stimulated and non-stimulated T lymphocytes in cell binding assays. We observed increased adhesiveness between unstimulated synovial cells and synovial cells previously cultured with activated and non-activated T lymphocytes. ELISA inhibition studies have shown an increase in fibronectin production by synovial fibroblasts co-cultured with stimulated $CD4^+$ T lymphocytes. We suggest that the presence of inflammatory cells in synovial sheath and epitenon during tendon healing induces synovial fibroblasts and epitenon cells to increase their production of fibronectin, which provides a scaffold for subsequent adhesion formation.

Keywords tendon repair adhesion formation inflammatory cells lymphocytes macrophages monocytes

INTRODUCTION

The formation of restrictive adhesions between the site of tendon injury and the surrounding tissues remains the single greatest obstacle to restoring normal tendon function [1,2].

For many years adhesion formation was regarded as an essential component of tendon repair. It was suggested that sheath or extrinsic fibroblasts are the main sources of cells producing scar tissue [3,4]. It was then observed that the initial response to injury appears in the epitenon, when cells start to migrate into the gap between the ends of the divided tendon and form a type of callus [5].

According to recent reports, increased synovial cell proliferation and migration is accompanied by increases in fibronectin levels, mainly in epitenon [6]. In some studies the presence of inflammatory cells in healing tendons was noted, but little attention was given to their role in the pathogenesis of tendon repair [7].

In vivo experiments on the repair of tendon segments within synovial pouches or within synovial environments which were isolated from the site of injury have demonstrated that tendon healing can occur without adhesion formation [8,9]. More recently, *in vitro* culture of injured tendon segments has demonstrated conclusively that tendons have the capacity for intrinsic repair which proceeds as a result of the proliferation and migration of epitenon cells to the site of injury [10]. It may therefore be possible to abolish adhesion formation without impairing the process of primary tendon healing.

In this study we have produced adhesion formation between tendon and its synovial sheath by administering a crush injury to the rat digital tendon and its sheath. The role of the inflammatory cell response has been studied in relation to fibronectin production and adhesion formation, with the aim of clarifying the biological mechanisms involved.

Correspondence: Dr Beata Wojciak, Cell Biology Department, Glasgow University, G128QQ Glasgow, UK.

MATERIALS AND METHODS

Experimental model

Twelve male Sprague Dawley rats were anaesthetized using halothane. A small transverse incision was made at the base of the middle digit of the hind paw under tourniquet control. The synovial sheath and tendon were identified. A crush injury to the tendon and synovium was administered using Adson forceps applied 10 times over 3 mm. The wound did not require suturing. At intervals of 3, 7 and 14 days, four rats were killed. The flexor tendons and adjacent synovial sheath were removed for cell culture experiments in one group, and in a further group the paw was preserved for tissue section. The operation was repeated eight times.

Isolation and primary culture of synovial sheath and epitenon cells

Tendon sheath cells. Tendon sheath and epitenon cells were isolated according to the method described by Banes *et al.* [11] with some modifications. Briefly, in step 1, tendons free of attachments to bone and muscle were washed in Ham's serum-free medium (GIBCO BRL, Life Technologies, Paisley, UK), then treated with 0·5% collagenase (Clostridiopeptidase A; EC 3.4.24.3; Sigma Chemical Co., Poole, UK) for 10 min at 37°C. The freed cell population was centrifuged at 200 ***g*** for 6 min and plated into Petri culture dishes with inserted glass coverslips at a cell density of 1×10^5 cells/ml. Then cells were incubated in culture medium containing a 3:1 (v/v) mixture of Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% tryptose-phosphate broth (GIBCO) and Ham's F-12 nutrient mixture with 10% calf serum (GIBCO-Biocult, Paisley, UK) and antibiotics.

Epitenon cells. In step 2 tendons were incubated in trypsin/EDTA solution (trypsin, 300 BAEE (Nα-benzoyl-L-arginine ethyl ester) U/ml; EDTA, 0·001 M EDTA) for 1·5 h, then the released cells were centrifuged and plated out as in step 1. After 24 h, non-attached cells and cells growing on coverslips inserted into culture dishes were fixed and used for immunofluorescent staining.

Identification of cell populations

Cell fixation and immunofluorescent staining. Cells growing on glass coverslips were fixed for 10 min in 4% paraformaldehyde solution in PBS at 4°C. Slides were washed three times with PBS and incubated with MoAbs for 2 h at 4°C. The following mouse MoAbs were used: anti-rat thymocytes and T lymphocytes (CD2) code no. MCA 444, anti-rat T helper/inducer lymphocytes (CD4) code no. MCA 372, anti-rat CD4 activated T lymphocytes code no. MCA 730, anti-rat T suppressor/cytotoxic cells (CD8) code no. MCA 48, anti-rat B lymphocytes (CD45) code no. MCA 340, anti-rat monocytes and macrophages (CD14) code no. MCA 341, anti-rat endothelium code no. MCA 276 (all from Serotech, Oxford, UK). Antibodies were diluted in 0·5% bovine serum albumin (BSA) in PBS at 1:200. As a second antibody, sheep anti-mouse immunoglobulin FITC-labelled antibody was applied at dilution 1:100 (Scottish Antibody Production Unit (SAPU), Law Hospital, Carluke, UK).

To identify the population of T lymphocytes in epitenon cell cultures, cells after isolation were maintained in suspension for 1 h in BHK21 medium and then fixed and stained using the same procedure as for cells on coverslips.

Phagocytosis assay

To determine the number of phagocytotic cells we used the method of Van Furth [12]. FITC-labelled polystyrene beads (Polysciences Fluoresbrite Microspheres), 0·94 μm in diameter, were suspended in culture medium containing 1% calf serum at the density $4{\cdot}5 \times 10^7$ beads/ml. Cells were washed once with Ham's F10 medium and incubated with fluorescent bead suspension for 6 h at 37°C. Then slides were rinsed thoroughly with PBS to remove the excess of beads, and cells were fixed with methanol for 10 min and allowed to air dry. Slides were then flooded with xylene, air dried, mounted in 50% glycerol and examined under a fluorescent microscope. The percentage of cells which phagocytosed fluorescent beads was recorded as the number of macrophage-like cells.

Preparation and immunofluorescent staining of frozen tissue sections

Frozen tissue sections were prepared as described in [13]. Fixation and immunostaining procedure was as described for cultured cells. Antibodies used were mouse monoclonal anti-rat monocytes/macrophages (Serotech), 1:500, rabbit anti-rat fibronectin antiserum (Calbiochem, La Jolla, CA), 1:20. Second antibodies used were sheep anti-mouse immunoglobulin FITC labelled (SAPU, Law Hospital), donkey anti-rabbit immunoglobulin Texas red labelled (SAPU), diluted 1:100.

Isolation and activation of T lymphocytes

Thymocytes were teased from thymus obtained from four control rats, aged 3–5 months, and were purified as described [14]. Activated T lymphocytes were prepared by the incubation of thymocytes with RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS) and 20 μg/ml concanavalin A (Con A; Sigma) for 3 days, and were then washed with 0·3 M α-methylmannoside (Sigma) to remove residual Con A [14]. Activated T lymphocytes were selected using plastic surfaces coated with anti-CD8 antibodies [15]. Activated and non-activated T lymphocytes were suspended in 5 ml of serum-free BHK21 medium and seeded onto synovial fibroblast cultures at cell density 1×10^6 cells/ml. To obtain medium from cultures of non-activated and activated $CD4^+$ T lymphocytes, cells were incubated in serum-free BHK21 medium for 3 days at density 1×10^6 cells/ml. After that time cells were spun, medium was collected and filtered through 0·2 μm pore size filters.

Fibronectin assay

For estimating the levels of fibronectin produced by synovial fibroblasts we used an ELISA inhibition assay described in [16], with some modifications. Briefly, polystyrene plates (Corning Glass Works, Corning, NY) were coated with purified bovine plasma fibronectin by placing 200 μl of 5 μg/ml fibronectin solution diluted in 0·05 M carbonate-bicarbonate buffer pH 9·6 in each well and incubating the plate overnight at 4°C. Plates were washed twice with PBS pH 7·2 and filled with 250 μl/well of 1% casein solution in PBS for 1 h. Two hundred microlitres of each test sample were incubated at room temperature with rabbit anti-rat fibronectin antiserum (Calbiochem) at final dilution 1:500 for 30 min, then samples were added to polystyrene wells precoated with fibronectin and incubated for another 30 min. Then ELISA assay was carried out as described [15]. As a second antibody, donkey anti-rabbit horseradish peroxidase-labelled immunoglobulin (SAPU, Law Hospital)

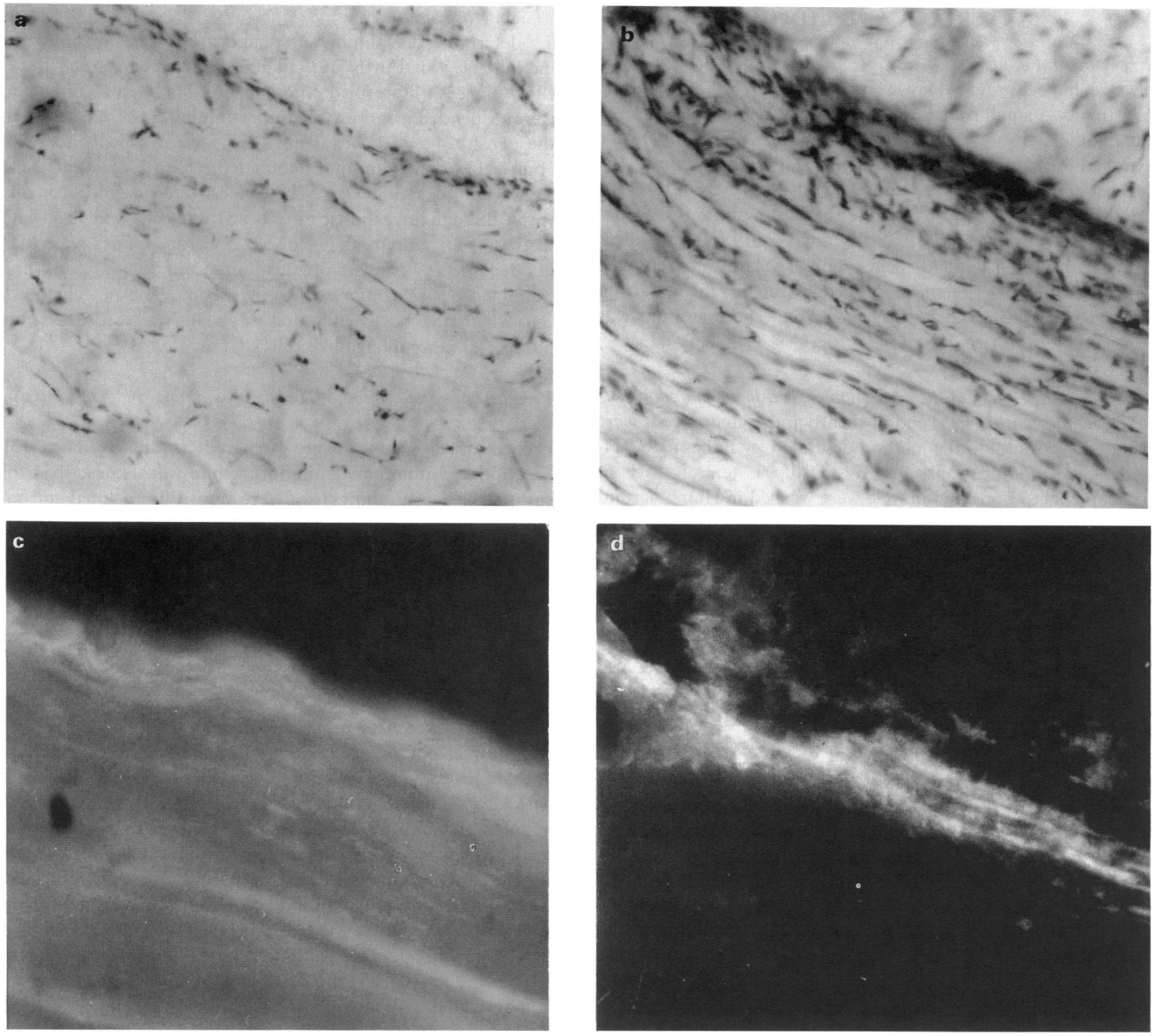

Fig. 1. Adhesion formation between tendon sheath and epitenon. (a) Control. (b) Seven days after crush injury. Haematoxylin and eosin staining. (c) Staining of epitenon with rabbit anti-rat fibronectin MoAbs 7 days after injury. Texas red, double immunofluorescence. (d) Staining of epitenon with mouse MoAbs against rat monocyte/macrophage cell surface markers (CD14). Texas red, double immunofluorescence. × 120.

was used at a dilution 1:1000, as a substrate: 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulphonic acid) (Sigma). The optical density of the wells was read on a Micro ELISA plate reader.

The concentration of fibronectin in culture supernatants was determined by comparison of the optical density of test wells with wells containing known amounts of fibronectin (diluted in 0·05% PBS-Tween 20).

Culture of synovial fibroblasts

Rat synovial fibroblasts were cultured in BHK21 medium (20 mM HEPES buffered Glasgow modified MEM supplemented with 0·5% bicarbonate, 10% calf serum, 10% tryptose broth, 2·85 mM glutamine, antibiotics). For experiments they were used between 15 and 25 passages. Cells grown in plastic 25-cm^2 Falcon culture flasks until confluence were then washed twice with serum-free BHK21 medium and cultured for 7 days in 5 ml of the following media: serum-free BHK21 medium (control), medium from cultures of non-activated and activated CD4$^+$ T lymphocytes, serum-free BHK21 medium with suspended activated and non-activated T lymphocytes at density 1×10^6 cells/ml. After that time the medium was collected and fibronectin levels measured with an ELISA inhibition assay.

Cell binding assays

Synovial fibroblasts were cultured until confluence in 5 ml BHK21 medium which was then replaced by medium from cultures of activated and non-activated T lymphocytes, or BHK21 medium containing activated and non-activated T lymphocytes at density 1×10^6 cells/ml. After 24 h, medium was removed and a suspension of synovial fibroblasts was seeded

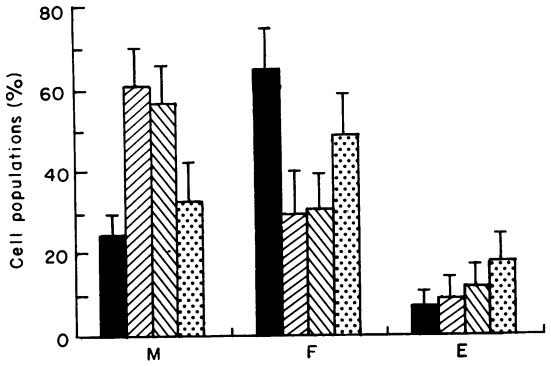

Fig. 2. Percentage of cell populations in epitenon cell cultures: control tendons, 3, 7, 14 days after injury. M, Macrophage-like cells; F, fibroblasts; E, endothelial cells. Cell types were identified with the use of MoAbs against cell surface markers and phagocytosis assay (see Materials and Methods). Only cells which were attached to the bottom of culture dish were stained and counted for cell populations. Each bar represents mean ± 1 s.d., $n = 15$. ■, Control; ▩, 3 day; ▧, 7 day; ⊡, 14 day.

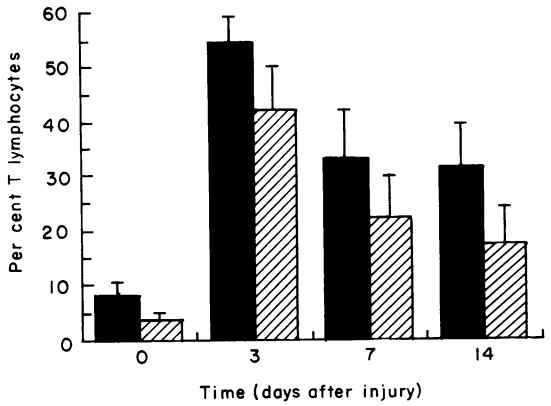

Fig. 3. Percentage of T lymphocytes in epitenon cell cultures 3, 7 and 14 days after injury. ■, Percentage of T lymphocytes ($CD2^+$) in total cell population; ▩, percentage of T helper/inducer cells ($CD4^+$) in total cell population. Each bar is a mean ± 1 s.d., $n = 12$.

over a synovial cell monolayer at density 1×10^5 cells/ml. After 15, 30 and 60 min, medium was removed and the number of non-adherent cells was counted. The percentage of adherent cells was calculated as 100% − percentage non-adherent cells.

Statistical analysis

All results are expressed as means ± 1 s.d. The unpaired Student's *t*-test was used to compare differences between groups. In all experiments in which cell populations were counted, $n \geq 12$. For ELISA assay, six independent samples of each group were used ($n = 6$). The assay was repeated three times.

RESULTS

Macroscopic appearance of the site of injury; frozen sections

Following crush injury the digits showed slight swelling for 2 days. The wounds healed by primary intention within 14 days with no infection. Adhesion formation between the synovial sheath and tendon was present within 7 days of injury. At this time, epitenon thickened to a 5–7 cell layer and stained brightly for fibronectin and for monocyte/macrophage cell markers (Fig. 1). The staining of control tendons for fibronectin was very weak and was limited to the single-cell layer of epitenon (not shown).

Identification of cell populations in cell cultures

Control tendons. Collagenase treatment released cells from the synovial sheath and left the tendons with a smooth, shiny surface. Treatment with trypsin, which released mainly epitenon cells, was discontinued at a stage when the collagenous structure of the tendons became visibly loosened. The number of cells obtained from collagenase treatment was approximately $2–3 \times 10^5$ cells, and from trypsin treatment $3–4 \times 10^5$ cells (four tendons).

Cells isolated from the synovial sheath of intact tendons comprise three main populations: macrophage-like type A cells (rounded, with surface markers for monocyte/macrophage cells and phagocytotic activity), fibroblast-like type B cells (elongated, non-phagocytotic) and endothelial cells (positive staining with anti-rat endothelium MoAbs, forming small colonies in culture), classification criteria based on [17,18].

Epitenon cell cultures consisted mainly of fibroblast-like type B cells (70%) and macrophage-like type A cells (Fig. 2). The respective cell types in the synovium and epitenon were morphologically indistinguishable.

Third day following injury. The number of cells obtained from digested tissue increased 1–2 times. Cell populations isolated after collagenase treatment were highly enriched in T lymphocytes (mainly $CD4^+$ helper/inducer), monocytes and endothelial cells (Figs 2 and 3). In contrast to control samples, lymphocytes adhered to the surface of monocytes, fibroblast-like and macrophage-like cells (Fig. 4). In cell cultures, over 18 ± 6% of spread cells had T lymphocytes adherent to their surface; for the control it was only 6 ± 4% ($n = 12$, $P < 0.005$).

Seventh day following injury. The size of cell population isolated from healing tissue increased 4–5 times compared with controls. The number of cells with macrophage/monocyte markers increased 1·7 times over control values ($n = 12$, $P < 0.001$) (Fig. 2). The number of cells with adherent T lymphocytes reached maximal value of 40 ± 10% of total population of spread cells.

Fourteenth day after injury. The total number of cells was reduced to control values. The number of T lymphocytes and monocyte/macrophage cells present in cultures had declined, while the number of endothelial cells had increased progressively (Fig. 2). Also, in cell cultures the percentage of cells with T lymphocytes adherent was reduced to 11 ± 7%.

Fibronectin production in cell cultures; ELISA assay

Synovial fibroblasts cultured with non-activated thymocytes and Con A-activated thymocytes produced more fibronectin than control cells, although the stimulatory effect of activated T lymphocytes was more significant (respectively 2·7 and 4 times over control value, $n = 6$, $P < 0.001$) (Fig. 5). Medium obtained from the culture of activated T lymphocytes also increased the level of fibronectin secreted by synovial fibroblasts.

Synovial fibroblasts adhesion assay

After 15 passages, cells isolated from normal rat epitenon cell population comprised 98 ± 10% synovial type B fibroblasts.

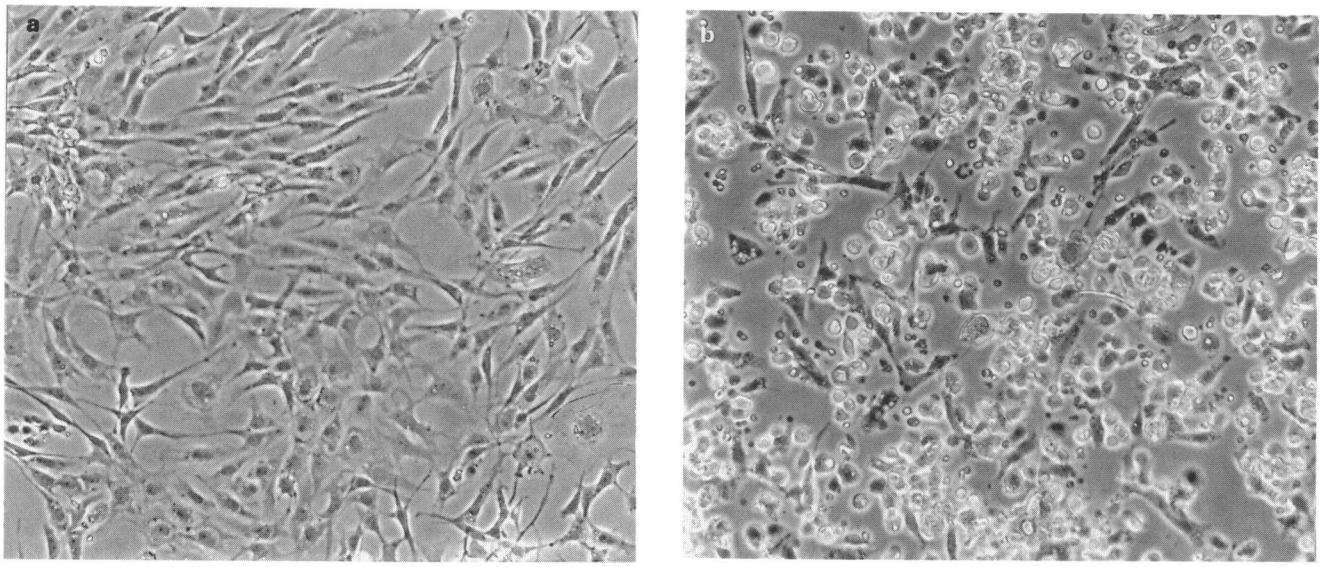

Fig. 4. Culture of epitenon cells. (a) Control. (b) Healing tendon 3 days after injury. Numerous inflammatory cells adhere to fibroblast-like and macrophage-like cells in cultures of epitenon from healing tendons. Phase contrast, ×200.

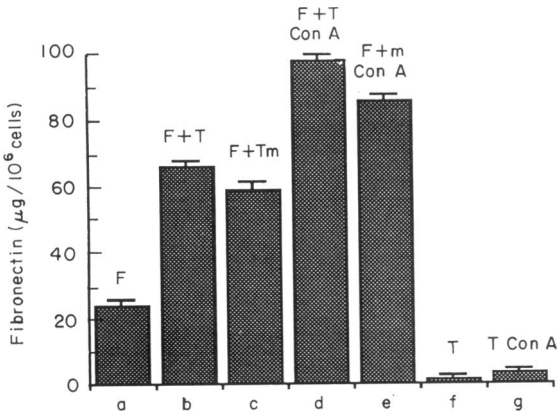

Fig. 5. Increase in fibronectin production by synovial fibroblasts (the total amount of fibronectin produced during 7 days in culture, ELISA assay, see Materials and Methods). a, Control (fibroblasts alone); b, fibroblasts cocultured with non-stimulated thymocytes; c, fibroblasts cocultured with conditioned medium from cultures of thymocytes; d, fibroblasts cocultured with concanavalin A (Con A)-stimulated T lymphocytes; e, fibroblasts cultured in conditioned medium from cultures of Con A-activated T lymphocytes; f, T lymphocytes alone; g, Con A-activated $CD4^+$T lymphocytes. Each bar represents a mean ±1 s.d., $n=6$.

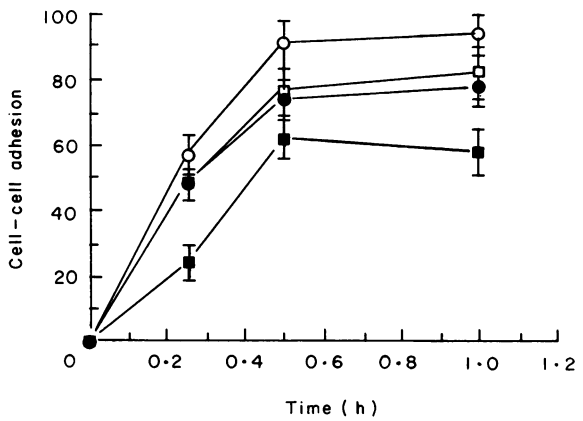

Fig. 6. Adhesion of synovial fibroblasts to synovial cell monolayer. ○, Fibroblasts were preincubated with Con A-activated T lymphocytes; □, fibroblasts were preincubated with non-activated T lymphocytes; ●, fibroblasts were preincubated with conditioned medium from cultures of Con A – activated lymphocytes (see Materials and Methods); ■, control. Results are expressed as a percentage of total synovial cell population that was plated onto synovial cell monolayer. Each point represents mean ±1 s.d., $n=10$.

Adhesion of synovial fibroblasts to a synovial fibroblast monolayer was increased in cultures where the cell monolayer had been preincubated with isolated activated and non-activated thymocytes (Fig. 6). Medium from cultures of stimulated and non-stimulated thymocytes also increased the ability of synovial cells to adhere to synovial cell monolayers.

DISCUSSION

We have demonstrated that an infiltration of inflammatory cells into synovial sheath and epitenon during tendon healing precedes an increase in fibronectin production by synovial and epitenon cells. We have also shown that activated T lymphocytes induce increased adhesion between synovial fibroblasts *in vitro*, and also stimulate their production of fibronectin.

Fibronectin production by synovial fibroblasts was also significantly increased by factors in medium derived from cultures of activated $CD4^+$ lymphocytes.

Whilst our model conforms to previous observations of increased numbers of monocyte/macrophage cells appearing around damaged tissues within 48–96 h of injury [19], we identified high numbers of T lymphocytes in the tissues 3 days following injury. T cells adhered to fibroblast-like and macrophage-like cells *in vitro*, and over 80% of them were identified as helper/inducer cells. This contrasts with the observations of

Fishel *et al.* [20], who noted that the ratio of CD4/CD8 lymphocytes migrating into polyvinyl alcohol sponges implanted into rat dermal wounds was less than 1, and that this was greatly decreased compared with the ratios in peripheral blood and lymph nodes. On the other hand, several authors reported the selective accumulation of $CD4^+$ helper-inducer T lymphocytes in connective tissue disorders and inflammatory lesions [21,22]. This suggests that the involvement of different subsets of T lymphocytes in the healing process may be tissue-specific.

The role of T lymphocytes in wound healing has not been clearly characterized. Several experiments suggest that T cells may regulate cell proliferation and the deposition of extracellular matrix components by cells during wound healing. T lymphocytes have also been observed to play a key role in several models of fibrosis. Wahl [23] demonstrated that the continued presence of an antigenic stimulus at a healing site leads to excessive fibrosis, presumed to be due to T cell activation and uncontrolled production of fibroblast activating factor (FAF). IL-2, a cytokine produced solely by T cells, has also been shown to enhance collagen deposition when administered to rats, and may also play a role in the production of fibrosis [24]. Many studies have also demonstrated the involvement of transforming growth factor-beta (TGF-β), growth factor secreted by activated T lymphocytes and other inflammatory cells, in the deposition of extracellular matrix by cells in healing wounds and the formation of granulation tissue [25,26]. TGF-β has been shown to induce fibrosis in fetal wounds, and to stimulate collagen and fibronectin production in cultured fibroblasts [25,26]. Our results suggest that one of the cytokines secreted by T lymphocytes, most likely TGF-β or interferon-gamma (IFN-γ), may be responsible for the increase of cell–cell adhesion in cultures of synovial fibroblasts, and for the stimulation of fibronectin production.

Tissue fibrosis induced in mice by the injection of streptococcal cell wall has been shown to be T cell-dependent, since it did not develop in T cell-deficient nude (*nu/nu*) mice [27]. Mice depleted of T lymphocytes before wounding or up to 1 week following wounding exhibit reduced collagen synthesis [28]. Whilst the depletion of $CD4^+$ cells had no effect on collagen synthesis, depletion of CD8 cells significantly increased collagen synthesis by cells in healing wounds [29].

Our results demonstrate that fibronectin is deposited by cells in the region of synovial sheath and epitenon after tendon crush injury. The prior co-localization of T cells seen in the immunohistological sections suggests that the *in vitro* observations of T cell-mediated fibronectin production by synovial fibroblasts may also occur *in vivo*. Fibronectin was shown to stimulate cell migration and proliferation in several wound healing models [30,31]. It has been shown that fibronectin secreted by wound fibroblasts may act as a template for the deposition of collagen fibrils and for the organization of the fibronexus [32,33]. The role of fibronectin as a scaffold for connective tissue deposition has also been shown in experimental models of hepatic cirrhosis and in experimentally produced interstitial pulmonary fibrosis [34,35].

It has been shown that the inflammatory response is not necessary for primary tendon healing, as tendon segments have been shown to heal under artificial conditions *in vitro* [10] and in synovial pouches [36]. In the latter experiments a small number of tendon segments became adherent to the scar of the incision, which is the only site within the synovial pouch where inflammatory cells were present in large numbers. Further experimental evidence for the role of inflammatory cells in the production of adhesions can be found in the canine model, in which the injured tendon was enclosed in an artificial sheath [37]. Adhesions did not form at the site of tendon injury immediely, but did so only when granulation tissue began to invade the inside of the artificial sheath.

We propose that the mechanism of adhesion formation involves the accumulation of T helper/inducer cells, monocytes and macrophages to the site of tendon crush injury. The involvement of inflammatory cells in the adhesion formation suggests several possible methods of preventing this process. The use of anti-inflammatory agents or mechanical barriers may help to reduce the infiltration of inflammatory cells into the site of injury and improve the healing process.

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REFERENCES

1 Gelberman RH, Vande Berg JS, Lundborg GN *et al.* Flexor tendon healing and restoration of the gliding surface. J Bone Joint Surg 1983; **65**:70–9.
2 Rothkopf DM, Webb S, Szabo RM *et al.* An experimental model for the study of canine flexor tendon adhesions. J Hand Surg 1991; **16A**:694–700.
3 Potenza AD. Tendon healing within the flexor digital sheath in the dog. J Bone Joint Surg 1962; **44A**:49–64.
4 Linsday WK, Birch JR. The fibroblast in flexor tendon healing. Plast Reconstruct Surg 1964; **34**:223–32.
5 Linsday WK, Thomson HG. Digital flexor tendons: an experimental study. Part 1. The significance of each component of the flexor mechanism in tendon healing. Br J Plast Surg 1961; **12**:289–316.
6 Gelberman RH, Steinberg T, Amiel D *et al.* Fibroblast chemotaxis after tendon repair. J Hand Surg 1991; **16A**:686–93.
7 Kang HJ, Park BM, Hahn SB *et al.* An experimental study of healing of the partially severed flexor tendon in chickens. Yonsei Med J 1990; **31**:264–73.
8 Lundborg GN, Hansson HA, Rank F, Rydevik B. Superficial repair of severed flexor tendons in synovial environment—an experimental study on cellular mechanisms. J Hand Surg 1980; **5**:451–61.
9 Matthews P, Richards F. The repair potential of digital flexor tendons. An experimental study. J Bone Joint Surg 1976; **56B**: 618–25.
10 Mass DP, Tuel RJ. Participation of human superficialis flexor tendon segments in repair *in vitro*. J Orthop Res 1990; **8**:21–34.
11 Banes AJ, Link WG, Bevin AG *et al.* Tendon synovial cells secrete fibronectin *in vivo* and *in vitro*. J Orthop Res 1988; **6**:73–82.
12 Van Furth R, Diesselhoff-Den Dulk MM. Method to prove ingestion of particles by macrophages with light microscopy. Scand J Immunol 1980; **12**:265–9.
13 Aaron JE, Carter DH. Rapid preparation of fresh-frozen undecalcified bone for histological and histochemical analysis. J Histochem Cytochem 1987; **35**:361–9.
14 Haynes BF, Grover BJ, Whichard LP. Synovial microenvironment—T cell interactions. Arthritis Rheum 1988; **31**:947–55.
15 Hudson L, Hay FC. Practical immunology, 3rd edn. Oxford: Blackwell Scientific Publications, 1980.
16 Gerdes JS, Douglas SD. Decreased fibronectin biosynthesis by

human cord blood mononuclear phagocytes *in vitro*. Leukoc Biol 1984; **35**:91–9.

17 Edwards JC. The origin of type A synovial lining cells. Immunobiol 1982; **161**:227–31.

18 Banes AJ, Donlon K, Link GW *et al.* Cell populations of tendon: a simplified method for isolation of synovial cells and internal fibroblasts: confirmation of origin and biologic properties. J Orthop Res 1988; **6**:83–94.

19 Barbul A. Role of the immune system. In: Cohen K, Diegelman RF, Lindbled WJ, eds. Woundhealing. Philadelphia: Saunders Co. 1992: 282–91.

20 Fishel RS, Barbul A, Beschorner WE *et al.* Morphologic assessment using monoclonal antibodies. Ann Surg 1989; **20b**:25–9.

21 Becker H, Langrock A, Federlin K. Imbalance of $CD4^+$ lymphocyte subsets in patients with mixed connective tissue disease. Clin Exp Immunol 1992; **88**:91–5.

22 Pitzalis C, Kingsley G, Haskard D, Panayi G. The preferential accumulation of helper-inducer T lymphocytes in inflammatory lesions: evidence for regulation by selective endothelial and homotypic adhesion. Eur J Immunol 1988; **18**:1397–404.

23 Wahl SM. Host immune factors regulating fibrosis. In: Evered D, Whelan J, eds. Fibrosis. Ciba Foundation Symposium 114, London: Pitman 1985:175–95.

24 Barbul A, Knud-Hansen J, Wasserkrug HL. Interleukin-2 enhances wound healing in rats. J Surg Res 1986; **40**:315–19.

25 Krummel TM, Michna BA, Thomas BL *et al.* Transforming growth factor beta induces fibrosis in a fetal wound model. J Pediatr Surg 1988; **23**:647–52.

26 Kehrl JH, Wakefield LM, Roberts AB *et al.* Production of transforming growth factor β by human T lymphocytes and its potential role in the regulation of T cell growth. J Exp Med 1986; **163**:1037–50.

27 Angeli M, Wahl SM. Cytokines and fibrosis. Clin Exp Rheumatol 1986; **4**:379–88.

28 Peterson JM, Barbul A, Breslin RJ *et al.* Significance of T lymphocytes in wound healing. Surgery 1987; **102**:300–5.

29 Barbul A, Breslin RJ, Woodyard JP. The effect of *in vivo* T helper and T suppressor lymphocyte depletion in wound healing. Ann Surg 1989; **209**:479–83.

30 Clark RAF, Quinn JH, Winn HJ *et al.* Fibronectin is produced by blood vessels in response to injury. J Exp Med 1982; **156**:646–51.

31 Babu M, Diegelmann R. Fibronectin is overproduced by keloid fibroblasts during abnormal wound healing. Mol Cel Biol 1989; **9**:1642–50.

32 McDonald JA, Kelley DG, Broekelman TJ. Role of fibronectin in collagen deposition: Fab′ to the gelatin binding domain of fibronectin inhibits both fibronectin and collagen organisation in fibroblast extracellular matrix. J Cell Biol 1982; **92**:485–92.

33 Singer II, Kawka DW, Kazazis DM *et al. In vivo* co-distribution of fibronectin and actin fibres in granulation tissue. Immunofluorescence and electron microscope studies of the fibronexus at the myofibroblast surface. J Cell Biol 1984; **98**:2091–106.

34 Martinez-Hernandez A. The hepatic extracellular matrix. I. Electron immunohistochemical studies in rats with CCl_4-induced cirrhosis. Lab Invest 1985; **53**:166–86.

35 Raghow R, Lurie S, Seyer JM *et al.* Profiles of steady state levels of messenger RNAs coding for type I procollagen, elastin and fibronectin in hamster lungs undergoing bleomycin-induced interstitial pulmonary fibrosis. J Clin Invest 1985; **76**:1733–9.

36 Potenza AD, Herte MC. The synovial cavity as a 'tissue culture *in situ*'—science or nonsense. J Hand Surg 1982; **7**:196–200.

37 Potenza AD. Critical evaluation of flexor tendon healing and adhesion formation within artificial digital sheaths. J Bone Joint Surg 1962; **45A**:1217–33.