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Cellular fibronectin and tenascin in an orbital nylon prosthesis removed because of infection caused by *Staphylococcus aureus*

Taru Päällysaho[1], Kaarina Tervo[1], Tero Kivelä[2], Ismo Virtanen[1], Ahti Tarkkanen[2], Timo Tervo[2]

Departments of [1] Anatomy, University of Helsinki, Siltavuorenpenger 20A, 00170 Helsinki and [2] Ophthalmology[2], University of Helsinki, Haartmanninkatu 4C, 00290 Helsinki, Finland

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Abstract. An orbital nylon prosthesis was removed because of an infection caused by *Staphylococcus aureus* that was resistant to antimicrobials. It was processed for histopathology and immunohistochemistry. Within 3 weeks the implant had an extensive ingrowth of fibrovascular tissue containing chronic inflammatory cells, foreign body giant cells, and myofibroblasts. By using the indirect immunofluorescent method, this tissue was found to react with monoclonal antibodies (Mabs) against extradomain A of cellular fibronectin (EDA-cFN) and tenascin (TN). The presence of EDA-cFN and TN within the implant are indicative of an active healing process, since both of these proteins, scarce in adult tissues, have been shown to be reexpressed during tissue regeneration. The findings suggest that fibronectin plays a definite role in bacterial adherence and foreign body infections.

Introduction

Fibronectin (FN) and tenascin (TN) are large extracellular matrix (ECM) glycoproteins [10, 23, 31, 39]. There are two types of FN, soluble plasma FN (pFN) secreted by hepatocytes and cellular FNs (cFN) synthesized by local tissue cells [46]. The extradomain A (EDA)-containing form of cFN that is widespread in the embryo [11, 34], is reexpressed during wound healing [12]. TN is expressed in developing and regenerating tissue, such as sites of wound healing [6, 27, 32, 45], but not to the same extent in corresponding adult tissues [27, 30]. These two ECM proteins may play a role in the migration of cells into the wound granulation tissue and in the proliferation of cells [6].

In eukaryotic cells, FN and TN bind to specific cell surface receptors [1, 3, 10, 39, 40]. The adherence of pathogenic bacteria to the host cell surface or in the ECM is also mediated by receptors on the bacterial cells [4, 17, 20]. In fact, bacterial adherence to specific substrates is considered to be the first step in the colonalization and development of many bacterial infections [37]. The FN receptor of *Staphylococcus aureus* is especially well characterized [13, 14, 28, 35, 42]. The ability of *S. aureus* to bind to FN has been shown to be important for its adherence and for the initiation of infections [16, 29, 36, 38]. As a component of bacterial biofilm, FN may play a role in blocking the access of antimicrobial agents to the infection site [7, 8, 15].

In the present study, we obtained an orbital nylon prosthesis implanted 3 weeks earlier. The prosthesis was extracted because of a therapy-resistant orbital discharge and infection. *In vitro* the recovered causative agent, *S. aureus*, was sensitive to the antimicrobial therapy used. Sections of the nylon prosthesis were processed for routine histopathology and for immunohistochemical detection of the ECM glycoproteins FN and TN.

Materials and methods

The patient was an 88-year-old female with long-term complications from an extracapsular cataract extraction performed in 1982, resulting in absolute glaucoma and a painful blind right eye. Because various medical treatments failed, the right eye was enucleated and an orbital nylon prosthesis was implanted in October 1990. The preoperative conjunctival smear revealed both *S. aureus* and micrococci. Three weeks later the orbital discharge increased and a fistula was observed in the apex of the socket. Orbital cultures revealed a heavy growth of *S. aureus* with an antibiotic resistance spectrum similar to that observed prior to enucleation. The nylon prosthesis was extracted because the infection did not respond to treatment despite the *in vitro* sensitivity of the bacteria to the antibiotic used.

The nylon prosthesis was removed and divided into two halves. One half was fixed in formalin and embedded in paraffin for routine histopathology and immunoperoxidase staining [26]. The other half was fixed in 96% ethanol for 60 min and rehydrated in descending series of ethanol. The tissue was then frozen and 6-μm sections were cut with a cryostat.

The following monoclonal antibodies (Mabs) were used: The Mab 100EB2 against human TN was used to detect TN-like immunoreactivity [21]. FN-like immunoreactivity was detected by using

Correspondence to: T. Päällysaho

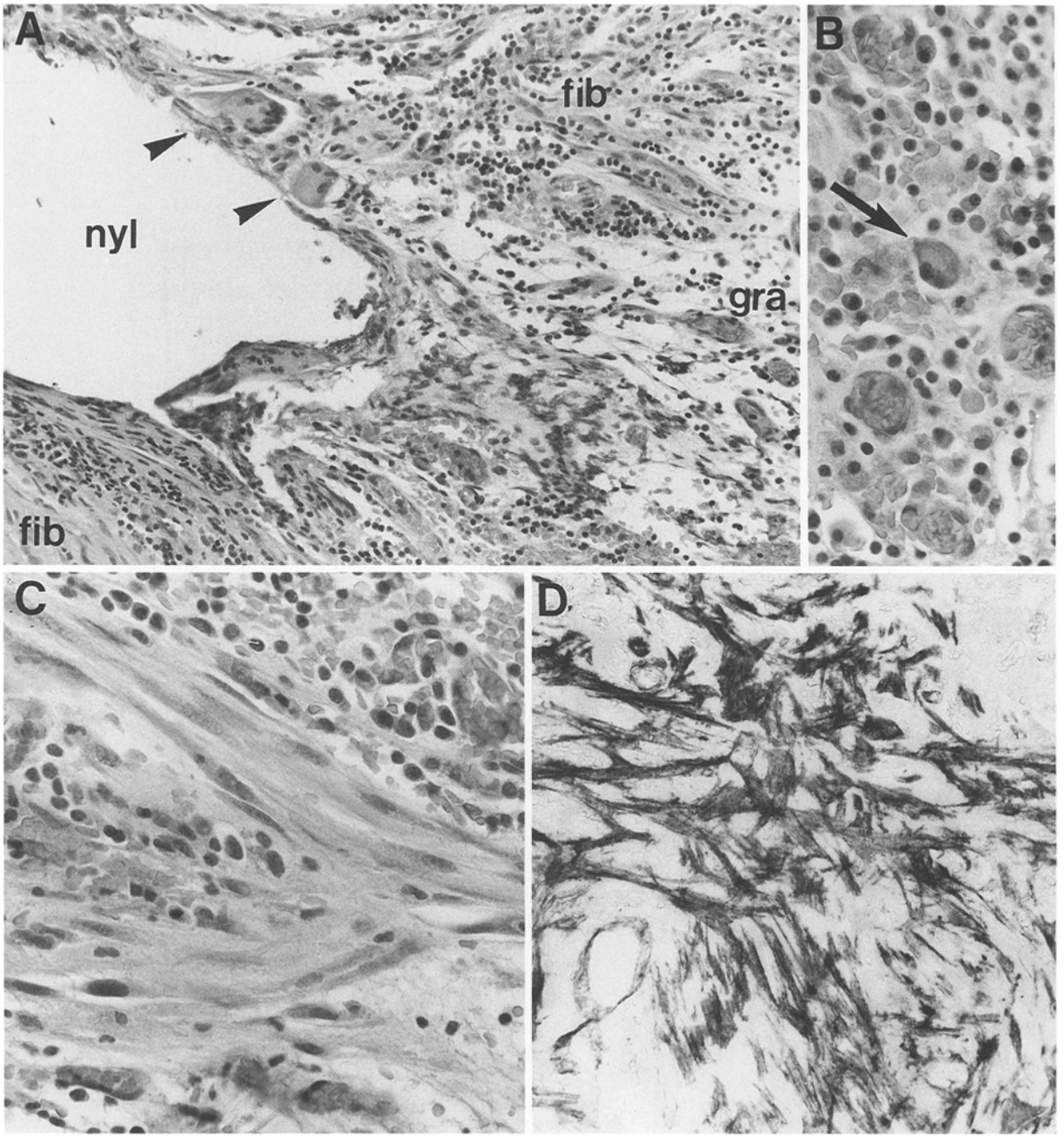

Fig. 1. **A** The spaces left by the nylon threads are surrounded by giant cells (*arrowheads*) of the foreign body type. The spaces between them have been filled with loose granulation tissue, which is invaded by activated fibroblasts. *nyl*, nylon thread; *gra*, granulation tissue; fib, fibroblast (H&E, ×190). **B** Loose granulation tissue is mainly composed of plasma cells and mononuclear leukocytes, with occasional immunoblasts (*arrowhead*) and histiocytes (H&E, ×460). **C** Granulation tissue is largely invaded by spindle-shaped activated fibroblasts (H&E, ×460). **D** Activated fibroblasts give a fibrillary reaction pattern with antibodies to α-smooth muscle actin, indicating that they are myofibroblasts with contractile properties (immunoperoxidase staining, ×460)

the following Mabs: the Mab 52BF12 was used to detect both forms of FN, pFN and cFN, whereas the Mab 52DH1 recognizing the extradomain A (EDA) present in the cellular form of FN only was used to detect EDA-cFN [46]. A Mab against α-smooth muscle actin was used to detect actin (Clone 1A4, Sigma Chemical, St. Louis, Mo.) [43].

For indirect immunofluorescence, the sections were first incubated with 4% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) at pH 7.4 in order to block nonspecific staining. They were then incubated with the Mabs for 60 min, followed by three 10 min washes in PBS. Thereafter, the sections were incubated with FITC-conjugated anti-mouse IgG (Jackson Immunoresearch Laboratories, West Grove, Pa.) for 60 min. After three 10 min washes in PBS, the sections were mounted in sodium veronal-glycerol buffer (1:9, pH 8.4) and viewed with a Leitz Diaplan 20 fluorescence microscope. In control sections incubated with the secondary antibody alone, no immunostaining was seen. The procedure for the immunoperoxidase staining used to detect α-smooth muscle actin has been previously described [26].

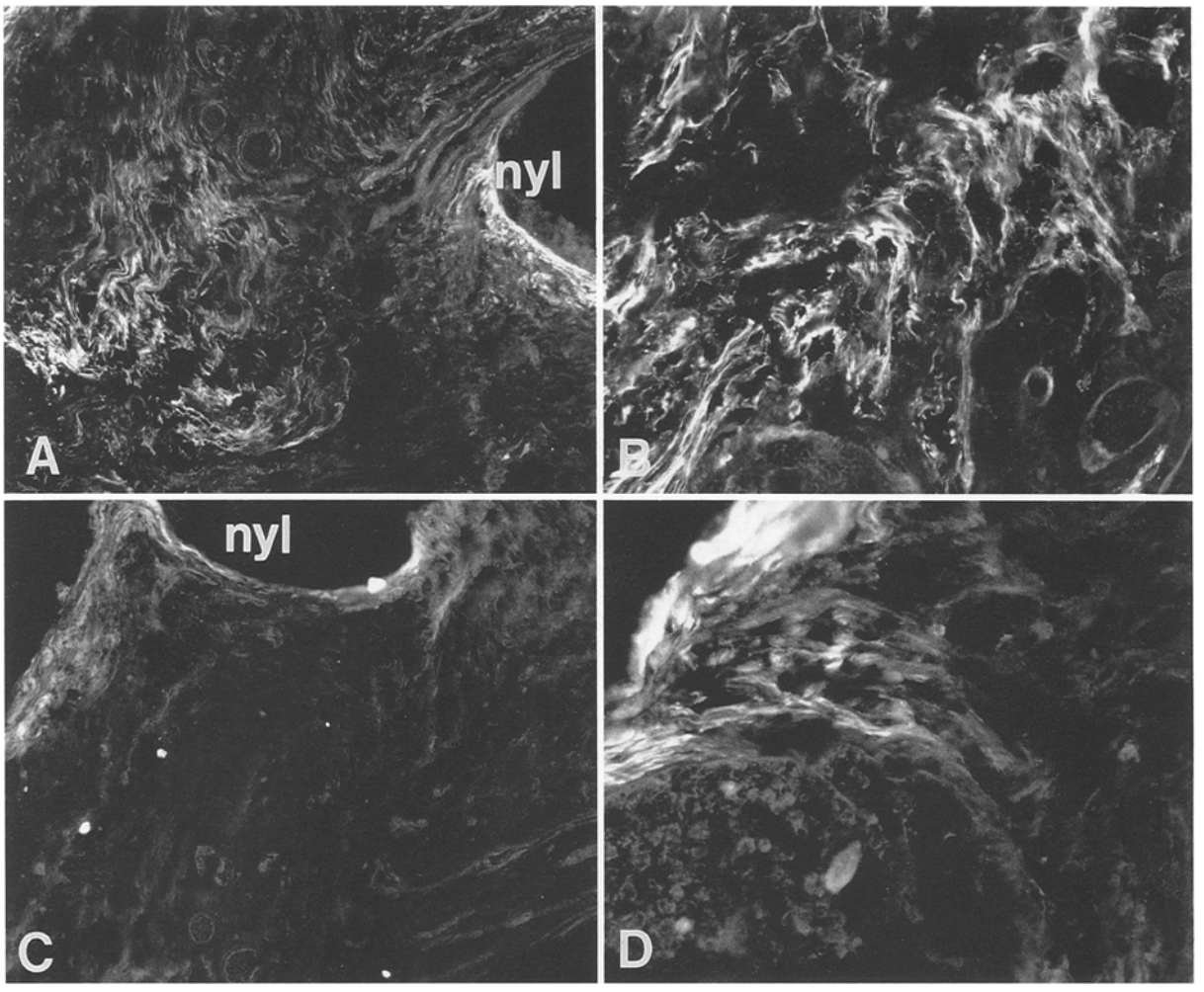

Fig. 2A–D. Immunofluorescent labelling for EDA-cFN (**A, B**) and for TN (**C, D**). *nyl*, nylon thread (**A** and **C** ×200; **B** and **D** ×400)

Results

In routinely stained sections, all spaces between the nylon threads were completely filled with granulation tissue (Fig. 1A). It consisted mainly of chronic inflammatory cells, in particular plasma cells (arrow in Fig. 1B), small lymphocytes, and histiocytes with occasional immunoblasts, resembling a pyogenic granuloma. The nylon threads themselves had incited a significant foreign body reaction, with abundant giant cells surrounding them (arrowheads in Fig. 1A). Close to the threads, remnants of polymorphonuclear granulocytes (PMN) were observed. Otherwise, acute inflammatory cells were already rare. Giemsa and Alcian blue stainings revealed a few, largely degranulated mast cells. The granulation tissue was richly vascularized and in the process of actively organizing into a fibrotic scar. The tissue had been largely invaded with fibroblasts that streamed into it from adjacent orbital connective tissue (Fig. 1A, C). These cells had either a spindle or a more flattened shape and were labelled with α-smooth muscle actin antibodies, indicating that they were activated myofibroblasts with contractile properties (Fig. 1D).

When using the Mab against EDA-cFN, intense immunofluorescence was observed in the areas organizing into a fibrous scar (Fig. 2A, B). Reaction was also detected in the regions surrounding the nylon threads. Less intense, but yet distinct labelling was detected in the regions where scar formation was less advanced. Faint immunofluorescence was also observed in the subendothelial regions of blood vessels invading the nylon prosthesis (Fig. 2B). Labelling with the Mab recognizing both cFN and pFN was identical to that with the Mab specific for EDA-cFN (not shown).

Labelling with the anti-TN antibody gave immunofluorescence in the region surrounding the nylon threads only (Fig. 2C and D). This labelling was more diffuse than that with the Mab against EDA-cFN and did not show a clear fibrillar nature. Areas more distant from the threads did not show distinct labelling.

Discussion

The histopathologic examination of the nylon prosthesis revealed the ingrowth of richly vascularized fibrous tis-

sue within the implant in a period of three weeks. This is in agreement with the recent study by Shields et al., where the ingrowth of fibrovascular tissue in an orbital hydroxyapetite implant was detected within 4 weeks [41]. The inflammatory cells present were typical for a chronic inflammation although a few PMNs were still observed. Activated α-smooth muscle actin positive myofibroblasts, spindle-shaped or flattened, were moving in from the adjacent orbital tissue.

FN is a disulfide-linked dimeric glycoprotein present in a soluble form in blood plasma and other body fluids and as fibrils in ECM [23, 46]. The major function of FN is probably related to its ability to mediate substrate adhesion of eukaryotic cells by binding to specific cell-surface receptors [1, 39, 40]. The molecular biology of FNs is interesting; there is only one gene, but with the capacity to code for a large number of variants based on differential splicing of the mRNA [22]. In the present study the detection of EDA-cFN was notable, especially in light of the reported reappearance of this form of FN during tissue regeneration [12].

TN is a large ECM glycoprotein. It is prominent in the embryonic development of many tissues [2, 9, 18, 33], but is present only in restricted locations in adult tissues [10]. However, it is reexpressed during wound healing [6, 32, 45] and in some tumors [5, 25]. Both TN and EDA-cFN expression has also been shown in epiretinal membranes [24]. Similarly, TN, probably synthesized by the activated fibroblasts, was found to be a component of the granulation tissue within the nylon prosthesis. The association of TN with embryonic tissues, wound healing, epiretinal membranes, and ingrowth of fibrovascular tissue into an implant suggests a definite role in tissue growth and remodelling.

The infection of the orbital nylon prosthesis was a typical foreign body infection. It was localized, resistant to antimicrobial treatment indicated effective by routine sensitivity testing, and subsided only after extraction of the prosthesis. Bacteria appear to adhere preferentially to biomaterials and traumatized tissue surfaces. In the present study, the bacteria were introduced into the sterile nylon prosthesis, which later became infiltrated with FN- and TN-containing granulation tissue. The causative organism *S. aureus* is known to possess a FN receptor [13, 14, 28, 35, 42]. The binding of *S. aureus* to FN has been shown to be a prerequisite for colonization by the bacteria and thus for the initiation of the infection [16, 29, 36, 38]. Also, the presence of an FN-containing bacterial biofilm may have interfered with the penetration of the antimicrobials into the site of infection, which was richly vascularized and thus accessible to drugs from this point of view [7, 8, 15].

The relatively rapid ingrowth of fibrovascular tissue into a nylon prosthesis indicates that it will become an integrated part of the orbital tissues, which makes it less likely to extrude. The risk of infection may also possibly be reduced because of the tissue integration and rich vasculature. However, as in this case, if bacteria from the patient's normal conjunctival flora gain access into the implant during or immediately after its introduction, the risk of infection may be increased because of FN-supported bacterial adherence, colonization, and biofilm formation [15, 19, 20, 44]. This is to be taken into account when introducing intraocular implants.

In light of the current observations and the broad literature on the role of bacterial adherence as a prerequisite for colonization and initiation of infections, the use of nylon balls composed of bundles of nylon threads seems somewhat questionable. Such a structure not only enables the ingrowth of tissue components in the implant, but also creates a tremendous contact area for adherence and colonization of microorganisms. These may not be accessible to systemic antimicrobials, or they may be protected by the biofilm covering them [7, 8, 15].

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