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Identification of fibronectin in tissue implant interfaces

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This study demonstrates the presence of fibronectin in cells and extracellular deposits on the surface of a cellophane implant nine days after surgery. The mononuclear cells with epithelioid or fibroblastoid morphology were positive for fibronectin. A small quantity of this fibronectin contained fibrillar network-like extracellular deposits, which are typical of fibroblasts. Multinucleate foreign-body giant cells were only poorly positive for fibronectin. These results indicate the prevalence of macrophages on the surface of cellophane implants, contrasting with the low incidence of fibroblasts.

1. Introduction

Implanted materials are obviously not part of the human body. The foreign-body reaction represents a complicated chronic granulomatous inflammation [1] including encapsulation of implants by connective tissue [2].

Fibronectin participates in numerous physiological as well as pathological conditions, including tissue healing and scar formation [3]. This study demonstrates the presence of fibronectin in cells and extracellular deposits in the tissue implant interface with respect to the distinction between macrophages and fibroblasts.

2. Material and methods

Cellophane foil (Chemosvit, Svit, CSFR), a hydrophilic material based on cellulose, may induce a typical foreign-body reaction [4, 5]. Strips of this material were subcutaneously implanted in 10 female Wistar rats (VELAZ, Prague, CSFR) under ether anaesthesia, in sterile conditions, and removed nine days after surgery. The implants were fixed by 4% paraformaldehyde in PBS at pH 7.2 for 5 min. Part of each strip was stained by hematoxylin-eosin as whole mount preparations. The cell membrane of cells on the other part was permeabilized by absolute acetone at $-20\,^{\circ}C$ for 3 min and washed by absolute acetone at room temperature. The fibronectin was detected by rabbit antihuman plasma fibronectin serum diluted 1:15 (Dakko, Glostrup, Denmark) for 45 min at room temperature. The implants were washed three times for 10 min and were incubated with swine antirabbit fluorescein isothyocyanate conjugated antibody diluted 1:10 (SWaR-FITC, ÚSOL, Prague, CSFR) as a second step antibody for 45 min. The specificity of reaction was tested by incubation of strips with non-immune rabbit serum and with second step antibody. The washed strips were mounted in 90% glycerine diluted with PBS containing 0.01% of paraphenylendiamine at pH 9.0.

3. Results

The cellophane strips induced a typical foreign-body reaction (Fig. 1). The implants were colonized pre-

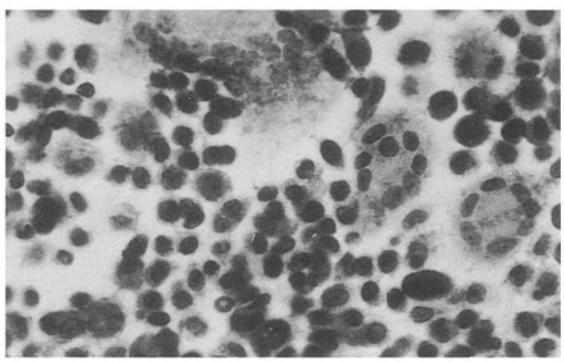

Figure 1 Foreign-body reaction against cellophane nine days after surgery. Whole mount preparation. Hematoxylin-eosin. (Magnification × 160.)

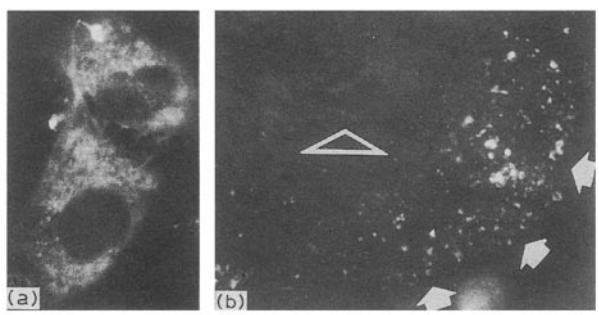

Figure 2 Mononuclear macrophages (a) and multinucleate foreign-body giant cell (b) on the implant surface. Centre of multinucleate cell (△), periphery of multinucleate cell (arrows). Whole mount preparation. Fibronectin detection. (Magnification × 900.)

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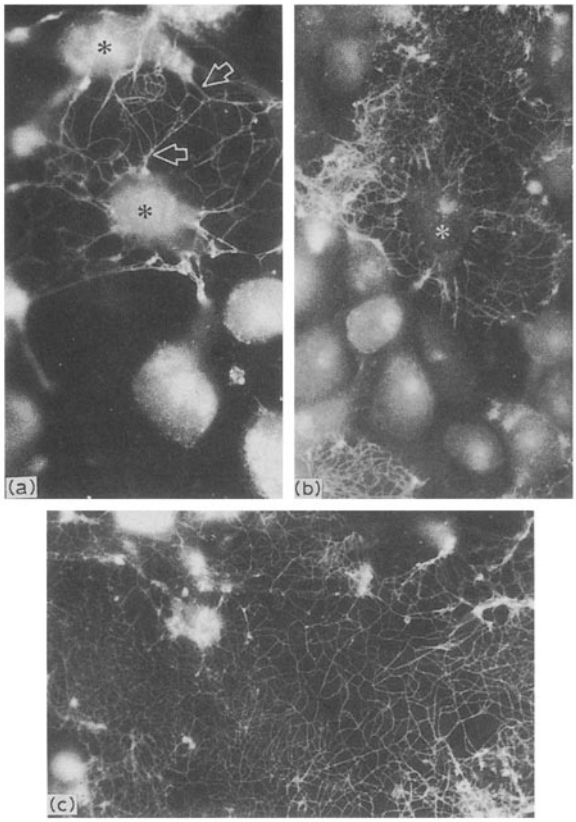

Figure 3 Fibroblasts (*) producing fibronectin containing network-like structure (a, b) and well-developed network covering the part of implant surface (c). Cooperation of fibroblasts producing the network (arrows). Whole mount preparation. (Magnification ×900.)

dominantly by mononuclear cells with epithelioid or fibroblastoid morphology and with numerous foreign-body giant multinucleate cells. Both types of mononuclear cells were positive for fibronectin in contrast to the multinucleate cells, which exhibited only the intensively fluorescing spots (Figs 2 and 3). Some mononuclear cells of both types produced fibrillar network-like fibronectin containing deposits (Fig. 3). Parts of the implant surface were covered by this network with a low incidence of cells.

4. Discussion

The cytological appearance of the cellophane implant was similar to that described previously [4, 5]. Mononuclear cells with epithelioid or fibroblastoid morphology were positive for fibronectin, but only exceptional cells produced fibronectin containing network-like deposits. The morphology of macrophages adhering to the artificial support is extremely variable [6] and, therefore, their distinction from fibroblasts is problematic when using conventional staining. Both macrophages and fibroblasts release fibronectin, but only fibroblasts are able to create extracellular deposits characterized by a fibrillar network-like appearrance [7].

The results indicate the prevalence of macrophages and the low incidence of fibroblasts at the surface of cellophane nine days after surgery. They produce fibronectin that probably participates in the early stage of the implant encapsulation.

References

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