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Distribution of polylactosamines and vinculin in macrophages and foreign body giant cells colonizing the cellophane implant surface

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The membrane poly-N-acetyllactosamines participate in the control of the adhesivity of macrophages and/or monocytes. They have been visualized by FITC labelled tomato lectin in macrophages and multinucleate foreign body giant cells colonizing a cellophane implant in this study. The maximal positivity was found in the periphery of poorly spread macrophages. The multinucleate cells exhibited positivity also in the dorsal surface. The cytoskeletal protein vinculin represents a part of adhesive plaques and participates in anchoring actin fibres to the integrin receptor. The immunohistochemical detection of this protein in the same model indicated poor accumulation of vinculin in the ventral membrane of mononuclear macrophages and multinucleate cells. The increased incidence of this protein was observed in the area of mutual contact between some macrophages.

1. Introduction

Macrophages which colonize the implant surface may control the course of a foreign body reaction, including the implant encapsulation [1].

The implant colonization by macrophages include recognition of the implant by these cells, their adherence to the implant and fusion into multinucleate foreign body giant cells. The macrophage surface oligosaccharides are of a great importance in the recognition and first contact with other surfaces including those of endothelial cells. The membrane poly-N-acetyllactosamines seem to be related to their adhesive behaviour and they are recognized by selecting molecules of the endothelial cells [2–4]. Their expression reflects very well macrophage activation [5].

It is well known that cells adhering to synthetic substrates form adhesive plaques in their ventral surface as a specialized structure responsible for their adhesion. Vinculin is the cytoskeletal protein representing a part of the integrin–actin membrane complex [6, 7] which is accumulated in the adhesive plaques under *in vitro* conditions. The pattern of the vinculin positive adhesive plaque reflects the functional activity of the cell [8]. The molecular structure of vinculin indicates its participation in the transfer of the information on the integrin–ligand interaction to the nucleus [6, 7] and in the control of gene expression. Its distribution in the ventral cell surface has been very intensively studied *in vitro* but little information is available from *in vivo* conditions.

The cellophane foil induces a foreign body reaction with maximal intensity 9 days after surgery [9–11]. This study demonstrates the distribution of poly-N-acetyllactosamines and vinculin in macrophages and giant cells colonizing the biologically characterized cellophane implant. It represents a basis for the study of the influence of the chemical design of polymers on the pattern of both molecules in macrophages colonizing its surface and for better understanding of the molecular mechanism of the recognition of cross-linked macromolecules by macrophages.

2. Material and methods

The pieces of cellophane foil (5 × 5 mm, CHEMOSVIT, Svit, Slovak Republic) were subcutaneously implanted into 10 Wistar rats as described previously [9]. The strips were removed at 5 or 9 days after the surgery. Three implants of each time interval were stained with hematoxylin-eosin and used for the calculation of the density of macrophages and fusion index (*FI* = number of nuclei in MGC/number of nuclei in MF and MGC [10]). Four implants (9 days) were fixed with 1% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.2–7.4) for 5 min and washed for 10 min in PBS. Each strip was divided in two parts. One was stained for poly-N-acetyllactosamines and second for vinculin.

2.1. pL detection

2.1.1. Lectin preparation

The tomato lectin was isolated from Lycopersicon esculentum fruit according to the method of Nachbar

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and coworkers [12] and liophilized. The lectin redissolved in Tris-buffered saline (TBS, pH 7.4) containing 0.001 M $CaCl_2$ showed the agglutination of human erythrocytes and exhibited a single band (71 kDA) in SDS-polyacrylamide electrophoresis. The lectin was FITC conjugated and then separated on a Sephadex G-25 column.

2.1.2. Lectin histochemistry

The lectin was diluted in TBS (5 μm/ml) supplemented with 0.001 M $CaCl_2$. The strip containing cells was incubated for 60 min at room temperature. The preincubation of specimen with unlabelled lectin fully inhibited the binding of the labelled form.

2.2. Vinculin detection

The vinculin was detected by monoclonal antibody clone No. VIN-11-5 (SIGMA) diluted 1:100 with PBS for 45 min at room temperature. The cells were permeabilized by Triton X-100 (SERVA) diluted with PBS to a final concentration 0.5% (v/v) for 5 min before the antibody application. The swine anti-mouse FITC-labelled serum (SWaM-FITC, USOL, Prague, Czech Republic) was used as a second step. The incubation of cells with non-immune mouse serum instead of anti-vin antibody and with a second step antibody exhibited no positive reaction.

The stained specimens were mounted in glycerine containing paraphenylendiamine as a whole mount preparation, as described in [11].

After the detection of both molecules by UV microscopy, the specimens were stained by hematoxylin-eosin for study of the cytological appearance of the implant surface.

3. Results and discussion

The number of macrophages was similar on the surface of the implants at 5 as well as 9 days after surgery (Fig. 1) in contrast to the fusion index (demonstrating the ability of macrophages to fuse) which was significantly higher 9 days after the implantation (Fig. 2). This result is in good agreement with our previous observation [9]. Cytological evaluation of the surface of the implants showed no significant differences, except the number and size of giant cells between the 5th and 9th day after the implantation.

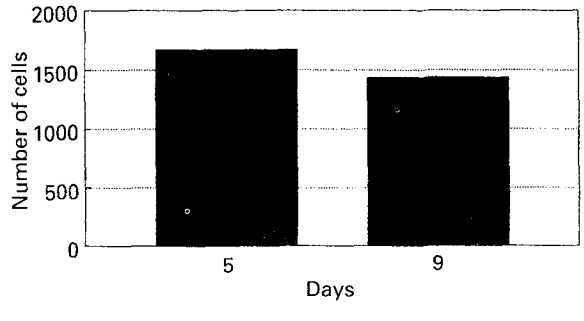

Figure 1 Number of macrophages per mm^2 adhering to the cellophane implant 5 and 9 days after surgery.

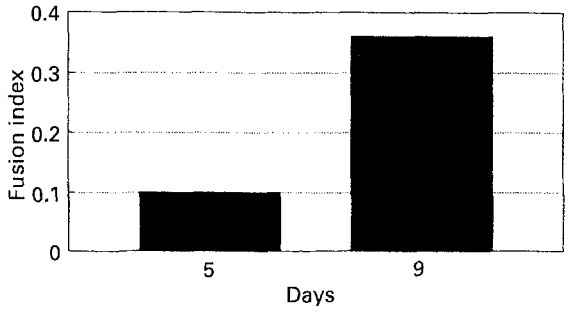

Figure 2 Fusion index (FI) in macrophages adhering to the cellophane implant 5 and 9 days after the surgery.

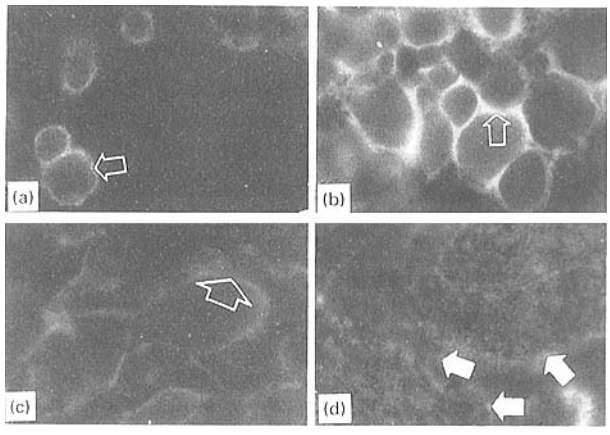

Figure 3 Expression of poly-N-acetyllactosamines on surface of macrophages and giant multinucleate cells colonizing a cellophane implant 9 days after the surgery: (a) individual cells and/or cells in minimal contact; (b) poorly spread cells with extensive mutual contacts; (c) widely spread cells in contact; (d) MGC-dorsal surface. Open arrow: positive periphery of mononuclear cells, compact arrow periphery of MGC. Magnification × 900.

All macrophages exhibited poly-N-acetyllactosamines mainly on the periphery of the cell ventral surface. The positivity was decreased with increased diameter (spreading) of the cell. In contrast to the macrophage, the dorsal surface of all giant cells were also highly positive with signs of clustering (Fig. 3).

The surface carbohydrates of normal and/or malignant cells participate in the recognition of natural and artificial surfaces including adhesion of leukocytes to the endothelial cells of blood vessels [2–4, 13–15]. The inhibitory effect of the macrophages spreading on the expression cannot be explained but it is known that spreading of the cells participates in the control of the cell function including gene expression [16].

The typical vinculin positivity in adhesive plaques of cells was observed in 10% of macrophages only. Numerous macrophages (50%) which were in mutual contact were clearly vinculin positive in the contact area of neighbouring cells (Fig. 4). Some giant cells (up to 5%) showed a very weak vinculin positivity in the periphery of the cell.

The majority of cultivated cells exhibit the typical vinculin positive adhesion plaques in their ventral surface. However, the adhered cells cultivated without serum showed no accumulation of vinculin in the plaques even if they were cultivated on the surface of

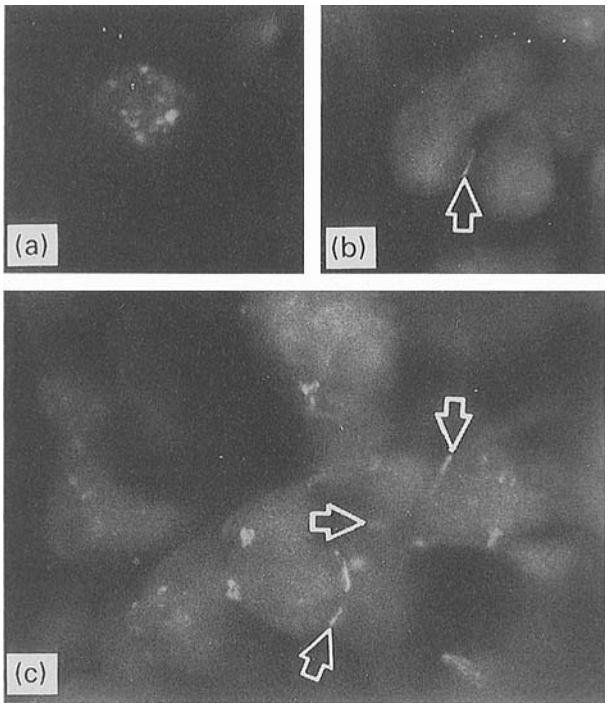

Figure 4 Vinculin pattern in macrophages on the cellophane implant 9 days after surgery: (a) single cell exhibiting vinculin positive adhesive plaque; (b), (c) group of cells with vinculin positive accumulation in the intercellular contact area. Arrow: the vinculin positive accumulation in the intercellular contacts. Magnification × 900.

fibronectin precoated coverslips [17]. The stimulation of granulosa cells by gonadotropins *in vitro* has an inhibitory effect on vinculin accumulation in the plaque [8]. Similarly, no accumulation of vinculin (in contrast to the talin protein of the integrin–actin complex) were observed during the adherence of cytotoxic T-lymphocytes to the target cells [18]. These findings suggest that the accumulation of vinculin in the plaque could reflect the functional state of the cell. The tyrosine proteinkinase function of vinculin is suggested, except its role in the anchor of actin to the integrins [6, 7] and the pattern of the vinculin positivity in the plaque may be related to the control of cell activity. The accumulation of vinculin in the contact area of macrophages has not been observed before. A similar accumulation, but of integrins (vinculin participates in the formation of the integrin–actin complex), was observed in the contact area of the keratinocytes [19] *in vitro*.

This paper provides information about the distribution of both molecules important in the cell adhesion in macrophages *in vivo* in a well characterized model of foreign body reaction against cellophane. It represents a basis for study of the influence of the chemical design of polymer on its recognition by macrophages.

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