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Effect of cationic groups on the adhesivity of peritoneal macrophages to polymeric beads

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This study demonstrates the adhesion of macrophages to polymer beads from a copolymer of hydroxyethyl methacrylate and dimethyl aminoethyl methacrylate intraperitoneally injected into the rat. Dimethyl aminoethyl methacrylate exhibited a stimulatory effect on macrophage adhesion 5 h after injection of the beads. Electron microscopy demonstrated protein adsorption on the surface of the beads. Protein fibres between the cell surface and beads were observed. The experimental system used minimizes the influence of tissue damage at macrophage recognition of polymers. This method seems to be suitable for short-term investigation of macrophage–polymer interaction.

1. Introduction

The chemical structure of polymers, and especially their surface properties, strongly influence their biological properties, including the effect on macrophages behaviour [1, 2]. Our previous studies demonstrated a remarkable effect of the functional chemical groups in certain polymeric matrices on the adhesion, spreading and fusion of macrophages on the surface of subcutaneously implanted polymers. Anionic groups were found to have an inhibitory influence on these parameters (–COOH > $-SO_3H$) while cationic groups stimulate macrophage fusion [3–5]. Cationized albumin is more actively internalized by macrophages than untreated albumin and anionic sugars of the macrophage surface were identified as the binding site for cationic molecules [6]. This effect of cationic molecules on macrophage biology was supported by the observation that polyamines are efficient activators of macrophage function [7].

The explanation of the stimulatory effect of cationic molecules on macrophage function is not clear. Bacterial cells with sialic or polysialic acid anionic coat are only poor activators of complement, and the ability of macrophages to engulf and kill these bacteria is minimal [8]. These bacterial strains are usually highly pathogenic, in contrast to the unsialiled strains of the same species.

Our previous studies with subcutaneously implanted polymers were able to demonstrate only the relatively late response, because the early observations were influenced by surgical trauma [3–5]. This paper considers the behaviour during the first 5 h after intraperitoneal injection of beads of a copolymer containing an increased concentration of dimethyl aminoethyl methacrylate (DMAEMA) in mixture with 2-hydroxyethyl methacrylate (HEMA).

2. Material and methods

Polymeric beads of 58.2 ± 15 μm diameter were prepared by radical suspension copolymerization according to Čoupek and coworkers [9]. The properties of the copolymers are given in Table I. 50 mg of dried beads were suspended in 50 ml of PBS (phosphate buffered saline) and 1 ml of 3.8% (w/v) of sodium citrate. The beads were injected intraperitoneally into 150–180 g male Wistar rats (in-bred in the Department of Toxicology, 1st Faculty of Medicine, Charles University, Prague) under sterile conditions. The rats were sacrificed after either 20 min, 1 h or 5 h after injection by prolonged aether anaesthesia, and the beads were harvested according to the method of Lentz and coworkers [10]. Five rats for each type of bead and for each time interval were used (total 60).

TABLE I Physical properties of beads used in experiments

DMAEMA (%)	*Wr*[a] (g)	*N*[b] (%)
0	1.215	0.00
10	1.235	0.67
20	1.405	1.24
30	1.426	1.79

[a] *Wr*: Water retention per 1 g of dry beads
[b] *N*: percentage of N atoms in copolymer measured by ESCA procedure

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The harvested beads were washed three times in PBS and divided into four aliquots, as follows:

1. Specimens of the first were smeared on supporting glass, fixed by Baker's fixative and stained by hematoxylin-eosin. These were used for the calculation of the number of macrophages adhering to the bead surface. The macrophages were distinguished on the basis of cytological criteria, and other cell types were not included.

2. The second group was fixed by 2% paraformaldehyde in PBS and used for immunohistochemical detection of fibronectin. The polyclonal antibody (Dakopatts, Glostrup, Denmark) was used as a first step and FITC-labelled swine anti-rabbit serum (USOL, Prague, Czech Republic) as a second step antibody. The smeared beads were mounted in 90% (v/v) glycerin in PBS containing 0.1% (w/v) of paraphenylendiamine (Aldrich, Milwaukee, USA) at pH 9. The specificity of the reaction was tested by incubation of beads with non-immune rabbit serum and then with the second step antibody, or with the second step antibody only.

3. The third set of specimens were routinely prepared for transmission electron microscopy (fixation with 2% glutaraldehyde, postfixation in osmium tetroxide, dehydration and embedding in Epon). The ultrathin sections were stained with lead nitrate and uranyl acetate and observed with a JEOL JEM-200 CX transmission and scanning electron microscope.

4. With the fourth group, the beads were placed on egg albumin treated coverslips, fixed with 2% glutaraldehyde, postfixed with osmium tetroxide, dehydrated and dried via critical point drying (Balzers), covered with gold (Balzers) and observed with a JEOL JEM-200 CX transmission and scanning electron microscope.

The adsorption of C3 protein of the complement system was studied *in vitro*. The beads of each sample were incubated in routinely citrated blood plasma of AB group (Institute of Hematology and Blood Transfusion, Prague, Czech Republic) at 37 °C for 60 min. After centrifugation the samples were suspended in cold PBS and washed three times for 10 min. The C3 fragments were visualized with swine anti human C3 FITC-labelled serum (USOL, Prague, Czech Republic). The specificity was tested by incubation of beads with human albumin, fraction V (SIGMA, St. Louis, USA) and with IgG fraction of human serum (USOL, Prague, Czech Republic) instead of whole plasma.

3. Results and discussion

The increased concentration of DMAEMA exhibited only minimal influence on macrophage adhesion to the beads 20 min and/or 1 h after the injection. 5 h after application, there was good correlation between the DMAEMA concentration and macrophage adhesion as shown in Figs 1 and 2. This result is in good agreement with our previous observation [4]. Similarly, the cationized albumin is more actively internalized than the untreated native molecule [6]. The finding that cationic polyamines are able to activate

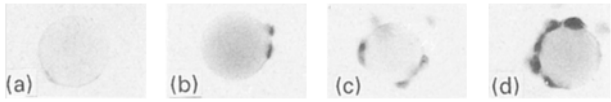

Figure 1 Samples of beads 1 h after the intraperitoneal injection. (a) poly HEMA, (b) copolymer of HEMA with 10% DMAEMA; (c) copolymer of HEMA with 20% DMAEMA, (d) copolymer of HEMA with 30% DMAEMA. Hematoxylin-eosin, magnification × 170.

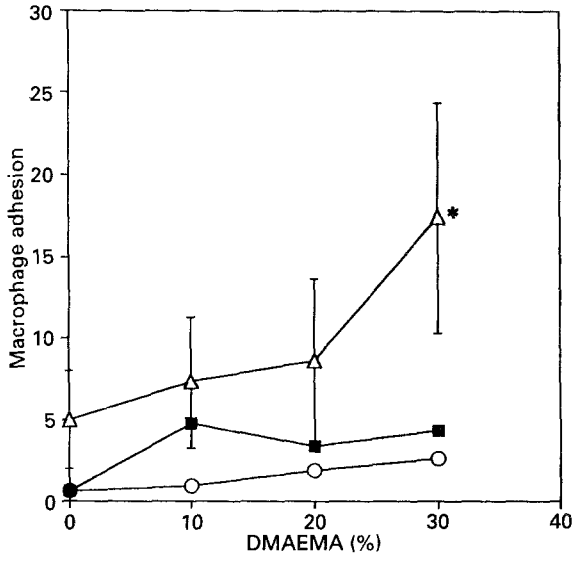

Figure 2 Number of peritoneal macrophages adhered to beads after time 20 min (○); 1 h (■); 5 h (△). Statistically significant difference from poly HEMA at 0.05 significance level as shown (*).

the macrophage functions [7] is also in good agreement with this finding.

The protein deposits on the surface of the beads and the network of complicated supramolecular protein fibres in the macrophage–bead interface was observed by use of electron microscopy (Figs 3 and 4). The immunohistochemical detection of fibronectin demonstrated the presence of this adhesive glycoprotein

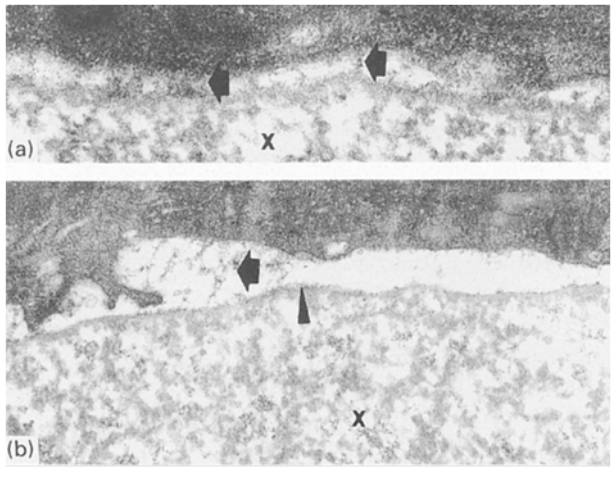

Figure 3 Macrophage-bead (×) interface: (a) copolymer of HEMA with 20% DMAEMA; and (b) HEMA with 30% DMAEMA. Proteins between cell and the bead surface (arrow) and proteins deposited on the polymer surface (arrowhead); uranyl acetate and lead nitrate; magnification × 40 000 (a) and × 13 000 (b)

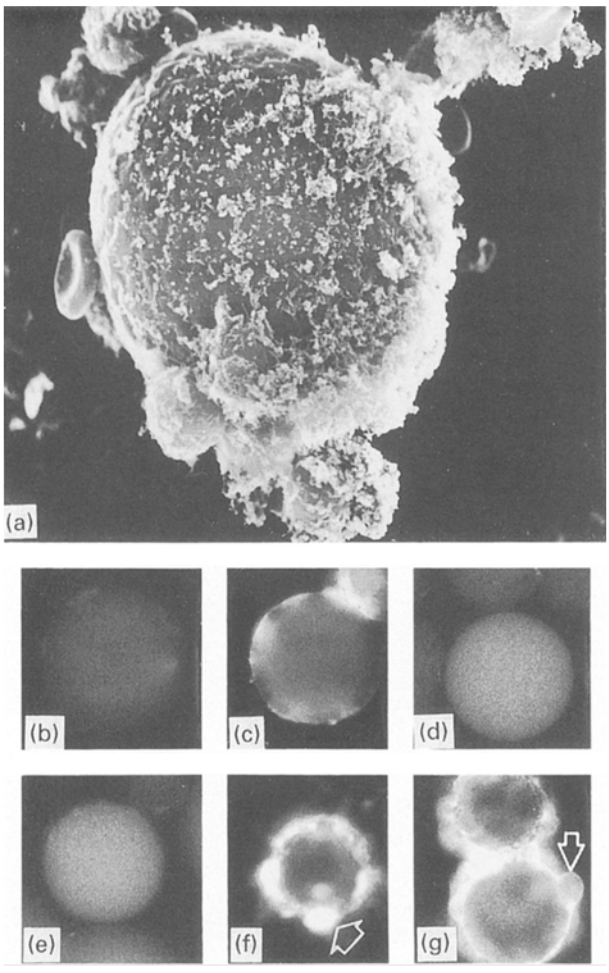

Figure 4 Adsorption of proteins to the surface of beads detected by scanning electron microscopy (a) and by immunohistochemistry (b–g). C3 detection (b–e); (b) poly HEMA; (c) copolymer of HEMA with 10% DMAEMA; (d) copolymer of HEMA with 20% DMAEMA; (e) copolymer of HEMA with 30% DMAEMA. Fibronectin detection (f, g): (f) poly HEMA; (g) copolymer of HEMA with 30% DMAEMA. Fibronectin positive macrophages (arrows). Magnification ×2000 (a) and ×370 (b–g).

on the polymer surface (Fig. 4). The visualization of C3 complement adsorption *in vitro* showed a higher intensity of reaction on the surface of beads containing DMAEMA (Fig. 4). The morphological pattern of the macrophage–bead interface was very different from the interface of a macrophage–anionic polymer [11], where the space between the cell and polymer was only very small and the quantity of proteins was minimal. On the other hand, it must be mentioned that in this case, the polymers were implanted subcutaneously and harvested 9 days after the surgery. It is generally known that certain active plasma proteins (fibronectin, vitronectin, C3b complement) are of great importance in the control of macrophage adhesion and activation. The immunohistochemical results obtained in this study are only able to confirm the low sensitivity of immunohistochemistry in this situation. They are able to provide little evidence of complement activation (the antibody against whole C3 molecule and citrated blood plasma was used). However, the agreement between the adsorption of C3 (the C3b is usually not covalently bound to the artificial surface [12, 13]) on the surface containing DMAEMA and higher adhesion of macrophages (5 h) to these beads is noticeable. Moreover, it is known that –OH and $-NH_2$ groups are good activators of the alternative pathway of the complement cascade [13].

4. Conclusions

The cationic DMAEMA stimulates the peritoneal macrophage adhesion under *in vivo* conditions. This phenomenon is dependent on the DMAEMA concentration and time of incubation. The injection of beads minimizes tissue damage during implanation and it seems to be very convenient for the study of the early response of macrophages against the implant.

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