Synthetic Hydrogel Capacity to Induce Formation of Foreign-Body Giant Multinucleate Cells Differs *in Vivo* and *in Vitro*

(foreign-body multinucleate giant cell / macrophage / synthetic polymer / parasite / biocompatibility)

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Abstract. The granulomatous reaction accompanied with MGC formation represents the most striking feature of the non-favourable biological tolerance of implanted devices. We compared MGC formation in the course of the granulomatous reaction *in vitro* and *in vivo* employing three types of hydrogels whose biocompatibility had been well studied earlier. The efficiency of the *in vitro* assay for the granulomatous reaction, including MGC formation, was verified employing the nematode *Nippostrongylus brasiliensis*, a well-known inductor of MGC formation *in vitro*. The *in vitro* results demonstrated a very low level of MGC formation in reaction against all three types of hydrogels without polymer-specific differences in comparison with the nematode experiment characterized by a high extent of MGC formation. On the other hand, the extent of MGC formation was implant type-specific *in vivo*: pHEMA-*co*-DMAEMA > pHEMA > pHEMA-*co*-NaMA. These results indicate that in the *in vitro* assay it was not possible to discriminate among the types of polymers used in the experiment in comparison with the animal experiment. They also indicate potential differences between granuloma formation induced by parasites and by foreign bodies.

Implanted devices must be biologically safe, which includes a low level of their immune recognition by the host. It is known that poorly tolerated implants induce the so-called foreign-body reaction. It can be characterized as a chronic granulomatous reaction with the occurrence of foreign-body multinucleate giant cells (MGCs) as a characteristic feature of the granuloma (Coleman et al., 1974). These cells are formed by fusion of specialized macrophages (MPhs) – epitheloid cells – and they are present in granulomas induced by different types of infectious agents such as selected bacteria (*Mycobacterium tuberculosis*) or parasites (*Schistosoma mansoni*), as well as by poorly tolerated foreign bodies (Papadimitriou et al., 1973; Smetana, 1987). The extent of MPh fusion into MGCs seems to be related to physicochemical properties of the implanted polymer (Smetana et al., 1990). Cytokines such as interleukin-4 and tumor necrosis factor-α seem to be stimulatory agents in the fusion of MPhs into MGCs (Shikama et al., 1989; McNally and Anderson, 1995; Sorimachi et al, 1995; Ikeda et al., 1998).

In addition to pathological findings and *in vivo* experiments, the granulomas can be generated also *in vitro*. The yeast *Candida albicans* (Heinemann et al., 1997) or the larval stages of the nematode *Nippostrongylus brasiliensis (NB)* (Seitzer et al., 1997) introduced to culture with human mononuclear cells induce formation of granulomas, including the fusion of MPhs into MGCs.

In this study we compared the formation of MGCs induced by synthetic hydrogels and larval stages of *NB in vitro*. Three types of hydrogel that differ in their fusogenic capacity *in vivo* were employed in this study: poly(2-hydroxyethyl methacrylate) (pHEMA) inducing a moderate fusion of MPhs, copolymer of HEMA with 30 wt% of dimethylaminoethyl methacrylate (pHEMA-*co*-DMAEMA) with a very high level of fusogenic activity, and copolymer of HEMA with 3 wt% of sodium methacrylate (pHEMA-*co*-NaMA), which induces no fusion of MPhs *in vivo* (Smetana et al., 1990; Smetana et al., 1993). Characterization of the *in vitro* assay of granuloma formation for the testing of the biocompatibility of polymers was the main purpose of this study. However, acquisition of new data for better understanding of molecular mechanisms of polymer biocompatibility has also been considered.

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Abbreviations: FI – fusion index, MGC – foreign-body multinucleate giant cell, MPh – macrophage, *NB - Nippostrongylus brasiliensis*, PBMCs – peripheral blood mononuclear cells, pHEMA – poly(2-hydroxyethyl methacrylate), pHEMA-*co*-DMAEMA – copolymer of HEMA with 30 wt% of dimethylaminoethyl methacrylate, pHEMA-*co*-NaMA – copolymer of HEMA with 3 wt% of sodium methacrylate.

Material and Methods

Polymer preparation

The pHEMA and copolymers pHEMA-*co*-DMAEMA (30 wt%) and pHEMA-*co*-NaMA (3 wt%) were prepared in the form of beads (150 ± 40 μm per diameter) or strips (4 × 8 mm) as described previously (Smetana et al., 1990; Smetana et al., 1993; Smetana et al., 1995; Smetana et al., 1996).

Preparation of human peripheral blood mononuclear cells (PBMCs)

PBMCs containing predominantly the monocyte and lymphocyte pool were prepared by Ficoll-Hypaque (Pharmacia, Freiburg, Germany) gradient centrifugation of heparinized blood samples from healthy donors followed by washing as described (Seitzer et al., 1997).

Culture of PBMCs with Nippostrongylus and polymer beads

In vitro granulomas were generated in cultures of *NB* larvae or polymer beads with human PBMCs as described previously (Heinemann et al., 1997). Briefly, culture of 0.75×10^6/ml PBMC and 20 worms/well or 20 polymer beads/well was performed in a volume of 2 ml in 24-well tissue culture plates. Cell culture medium was Iscove's modified Dulbecco's medium (Gibco, Karlsruhe, Germany) supplemented to a final concentration of 100 U/ml penicillin G sodium salt, 100 mg/ml streptomycin (Gibco), and 5% heat-inactivated autologous human serum. At time intervals of 1, 4, 8 and 15 days in culture

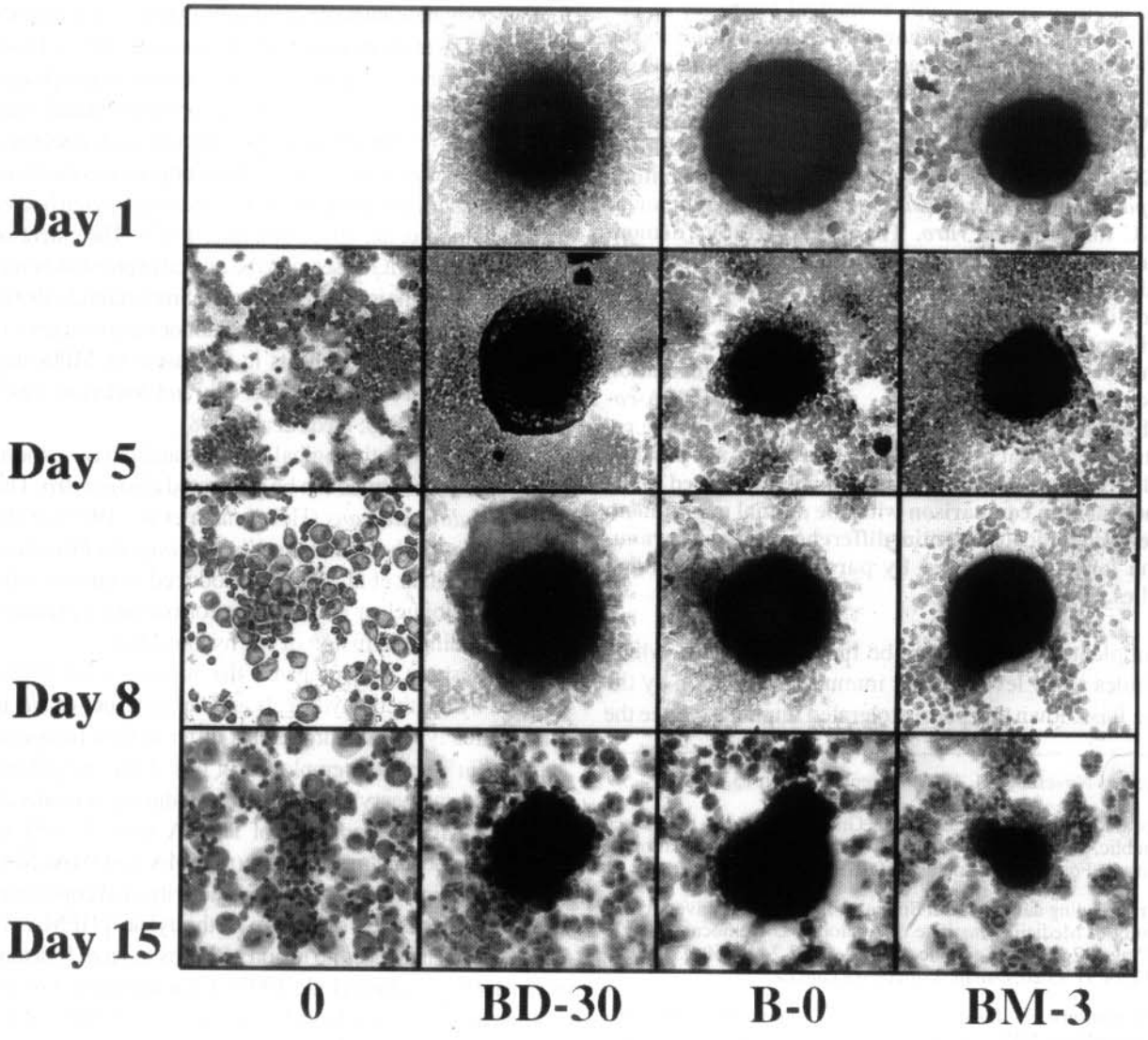

Fig. 1. In vitro granulomatous reaction against beads prepared from pHEMA-*co*-DMAEMA (BD-30), pHEMA (B-0) and pHEMA-*co*-NaMA (BM-3). The control experiment is designated "0". Staining according to Pappenheim, magnification 200×.