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Brain lesion-induced alteration of selected phenotypic properties of spleen macrophages and their partial restoration in the course of foreign body reaction against intraperitoneally implanted polymers

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A lesion in the dorsoposterior part of the rat brain septum is known to exert an inhibitory effect on the delayed skin hypersensitivity and incorporation of radiolabeled thymidine into the lymphoid organs. To determine whether distinct properties of macrophages will also be modulated by this type of injury, we have focused upon the monitoring of expression of sugar receptors (lectins). In this study we show a reduction in the number of macrophages expressing carbohydrate-binding sites for asialoglycoproteins (β-D-galactoside), α-D-mannoside and α-D-mannoside-6-phosphate in spleen macrophages after the lesion of the dorsoposterior septum of the brain in the rat. The number of ED-1$^+$ macrophages was not influenced. The intraperitoneal injection of beads prepared from the copolymer of 2-hydroxyethyl methacrylate with dimethyl aminoethyl methacrylate (30 wt%) elevated significantly the number of ED-1$^+$ spleen macrophages and number of macrophages with binding site(s) recognizing asialoglycoproteins and α-D-mannoside-6-phosphate, respectively. These results indicate that a foreign-body reaction appears to be able to mediate a phenotypic restoration of lectin expression by spleen macrophages altered by the brain lesion. It can be suggested that, for example, a probable production of cytokines by the inflammatory cells colonizing the implanted beads plays a role in this process.

1. Introduction

Neuroimmunomodulation represents a rapidly burgeoning topic in immunology [1, 2]. The central nervous system (CNS) can apparently influence the immune response by two different pathways. The first is usually defined as the hypothalamo–pituitary–adrenal axis, the second is represented by the innervation of the primary and secondary lymphoid organs such as thymus, spleen, and lymph nodes [1]. By measuring the extent of activity, i.e. in delayed skin hypersensitivity and incorporation of radiolabeled thymidine into the lymphocytes, certain regions of the rat brain such as prefrontal medial cortex, septum nucleus amygdalae and parabrachialis were characterized, where a deliberate injury entails the elicitation of biochemically defined responses that lead to quantitative alterations on the level of the immune system [3, 4].

Polymers implanted in the form of biomedical devices into an organism are usually recognized as being non-self and therefore induce a local foreign-body reaction. The synthetic implants are colonized by polymorphonuclear leukocytes and subsequently by macrophages [5, 6]. If the biocompatibility of the implanted polymer is poor, the macrophages fuse into the multinucleate foreign-body giant cells and produce multifunctional cytokines such as interleukin 1 and 6 and tumor necrosis factor-α [7–10]. Due to their pleiotropic nature the analysis of the systemic effect of the foreign body response mediated by

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these cytokines warrants experimental efforts. Morphologically, the last step of the foreign-body reaction is the encapsulation of the implant by connective tissue [5, 6].

In our previous study, we demonstrated that the chemical structure of implanted hydrogel clearly influences the extent of local foreign-body reaction [7]. Moreover, intraperitoneal injection of polymer beads has been proven to affect phenotypic properties of spleen red pulp macrophages, which are not in direct contact with the implant. The secretion of bioactive cytokines by inflammatory cells colonizing the surface of intraperitoneally implanted beads could hypothetically be responsible for the change of the phenotype of spleen macrophages [11]. The copolymer of 2-hydroxyethyl methacrylate (HEMA) with 30 wt % of dimethyl aminoethyl methacrylate (DMAEMA) was the most potent inductor of the local foreign body reaction and activator of spleen macrophages in comparison with pure poly-HEMA and a copolymer of HEMA with the sodium salt of methacrylic acid [12, 13]. These results on long-range effects with assumed participation of immune mediators encourage the study of responses to manipulations on the level of the CNS or the immune reaction to the ''non-self'' device.

In this paper we show the effect of the stereotactic lesion of the septum in the rat brain on the number of red pulp spleen macrophages (ED-1$^+$) and expression of the binding sites for asialofetuin (ASF), α-D-mannoside (Man) and α-D-mannoside-6-phosphate (Man-6P), respectively. These parameters were chosen because the respective C- and P-type lectins are involved in intercellular interactions and in endocytosis (for review, see [14, 15]). The same parameters were also evaluated after the additional intraperitoneal injection of beads prepared from the copolymer of HEMA with 30 wt % of DMAEMA. This part of the experimental design addresses the question whether cellular responses in the course of a foreign body reaction can work upon the same glycobiological features which are monitored after inflicting the stereotactic brain injury.

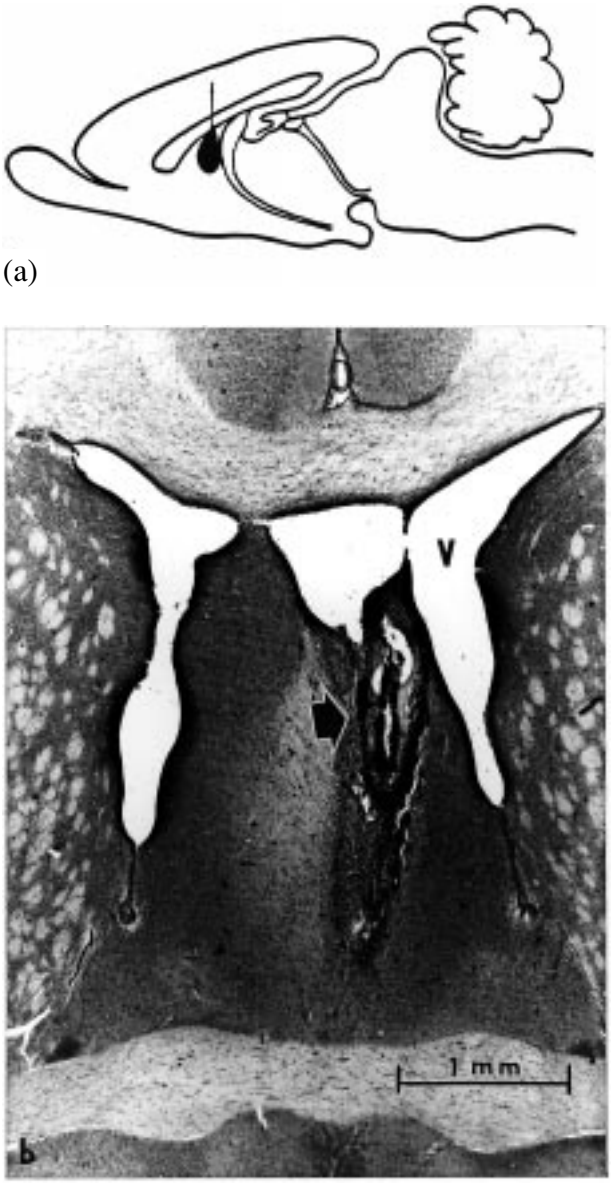

Figure 1 Scheme (a) of the site of lesion in the posterodorsal region of rat septum (asterisk) and its histological verification (b). V, lateral ventricle, arrow, site of lesion. Cresyl violet, magnification × 60.

2. Material and methods

2.1. Polymer preparation, surgery and tissue preparation

A group of 10 rats of the Lewis strain of both sexes (breeding colony of the Institute of Anatomy) weighing 250–300 g were used for the experiments. The skull of two control animals was opened without damage to the meninges and brain to exclude the possibility of non-specific irritations from the general surgical procedure being false-positively evaluated. The stereotactic electrolesion (1–2 mA of direct current for 5–10 s) of the septum (Fig. 1) was set for seven animals, one animal received the injury in the striatum as further control. The surgical procedure was performed under ether anesthesia without exception.

The beads from the copolymer poly HEMA-*co*-DMAEMA (30 wt %) of approximately 150 mm in diameter were prepared as described previously [16]. The beads (150 mg of dry weight per dose) as suspension in physiological saline solution were intraperitoneally injected into three rats 14 days after the brain injury. The rats were sacrificed 48 h later. The spleens were infiltrated by Tissue-tek (Ames, Naperville, USA) and frozen. Following the preparation of the spleens the animals were perfused with physiological saline solution with heparin and the fixative containing 4% (w/v) of paraformaldehyde.

2.2. Brain histology

The brains were removed and the fixation was accomplished in 4% (w/v) paraformaldehyde. Serial frozen sections (40 μm) of the brain were made on a freezing microtome and then stained with cresyl violet. The extent of lesion was assessed by projection into standardized brain section drawings. Using the method of superpositioning of standardized sections we could determine those septum areas whose injury appeared to

have a bearing on selected properties of the spleen macrophages (Fig. 1).

2.3. Reversed glycohistochemistry and immunocytochemistry on spleen sections

The cryostat sections were washed in phosphate-buffered saline (PBS) and immediately fixed with 4% (w/v) paraformaldehyde in PBS. The carbohydrate-binding sites were detected as described previously [17–20]. Briefly, the binding site for asialoglycoproteins (α-D-galactoside specificity) was detected by biotinylated asialofetuin. The binding sites for α-D-mannoside (Man) and α-D-mannoside-6-phosphate (Man-6P) were detected by biotinylated neoglycoproteins with the following molecular design: saccharide ligand–bovine serum albumin–biotin. The peroxidase-labeled avidin (ABComplex-Px, Dako, Glostrup, Denmark)/hydrogen peroxide/diaminobenzidine tetrahydrochloride (Sigma, Prague, Czech Republic) were used for the visualization of specifically bound probes, completing the glycohistochemical processing.

The macrophages were detected with the ED-1 monoclonal antibody [21] (Serotec, Oxford, UK) in a routine immunocytochemical procedure using peroxidase-labeled swine antimouse immunoglobulins (SWaM-Px, SEVAC, Prague, Czech Republic)/diaminobenzidine tetrahydrochloride/hydrogen peroxide as signal-generating system. One half of the sections was counterstained by hematoxylin and all specimens were dehydrated by routine procedure and mounted to solacryl.

The specificity of the glyco- and immunohistochemical reactions was ascertained by the preincubation of sections with preimmune serum or label-free ASF or neoglycoprotein as competitive inhibitor as a first step or by the omission of labeled probe or antibody to disclose the level of background staining. The number of macrophages positive for ED-1 and binding sites for ASF, Man and Man-6P was calculated in the area of $0.27\,mm^2$. The difference between the control rats without lesion and rats with damaged septum without or with the injected poly HEMA-*co*-DMAEMA beads were statistically compared by using Student's *t*-test.

3. Results

The circumstances of this experimental series were evaluated to present no apparent deviation from the normal situation. As generally seen, the intraperitoneally injected beads were colonized by inflammatory cells, prevalently macrophages (not shown). The macrophages positive for the tested markers were observed in the typical site of the red pulp of the spleen. The lesion in the septum had no influence on the number of ED-1^+ macrophages (Fig. 2). The number of macrophages which express the binding sites for ASF, Man and Man-6P was significantly lower in rats with the lesion in the septum than in injury-free controls which nonetheless underwent the stress of a surgery (Figs 2 and 3). The intraperitoneal injection significantly elevated the number of ED-1^+ - positive macrophages in comparison with the control animals and animals after the septum lesion (Fig. 2). Since no other factor was altered, the injection of the ''non-self'' beads can account for the increase in the number of macrophages positive for the binding sites for the β-galactoside structures of ASF and for Man-6P (Figs 2 and 3). This stimulus, however, did not influence the number of macrophages which express the binding site for Man, at least preferentially a tandem-repeat C-type lectin (Fig. 2). The tested parameters in the spleen macrophages in the second control rat with the striatum lesion exhibited no significant changes, emphasizing the importance of inflicting an injury to distinct areas and excluding a non-specific reaction to any type of brain lesion.

4. Discussion

The results show the significant influence of a targeted CNS lesion in the area of the septum on the number of macrophages in the red pulp of the spleen expressing binding sites for the β-galactosides of ASF, for Man and Man-6P. The number of ED-1^+ macrophages was not affected by the injury in the brain septum, mainly affecting the dorsal and posterior parts of septum (i.e. nucleus septalis lateralis, pars intermedia et dorsalis, nucleus septohippocampalis, nucleus triangularis septi and the nucleus septofimbriatus). These septal areas are spatially in close neighborhood or identical to the region, where a loss of integrity diminished certain immune responses (delayed hypersensitivity, DNA synthesis in immunocompetent organs measured by extent of utilization of ^{3}H-thymidine) in a previous series of experiments [22].

As a marker for macrophage functionality, e.g. in intercellular interactions and endocytosis, the expression of members of certain lectin families by (neo)glycoconjugates has been chosen. The intraperitoneal injection of beads prepared from poly HEMA-*co*-DMAEMA led to an increase in the number of the macrophages with binding sites for ASF and Man. This hydrogel had already been used in a series of previous experiments, where the implants prepared from this copolymer induced a very high extent of the foreign-body reaction [6, 12, 13]. Moreover, the beads prepared from this copolymer and intraperitoneally injected into the rat significantly elevated the number of macrophages expressing binding sites for the neoglycoproteins and ASF, although the effect on the number of ED-1^+ was negligible [11].

In conclusion, the injury of the septal region of the rat brain intimates that this region can procure a stimulatory influence on the expression of selected carbohydrate-binding sites in spleen macrophages. Such a reactivity is seen in response to the inflammation induced by a foreign body. At present, a precise answer concerning the nature of the stimulators is not unequivocally possible. It should certainly be noted that intraperitoneally injected beads were strongly colonized with inflammatory cells, mainly macrophages. This phenomenon had been observed and emphasized in a previous study [11]. The inflammatory cells colonizing the biomaterials can produce bioactive cytokines such as interleukin-1 and 6 or tumor necrosis factor-α [8–10]. Since lectin expression is regulatable by

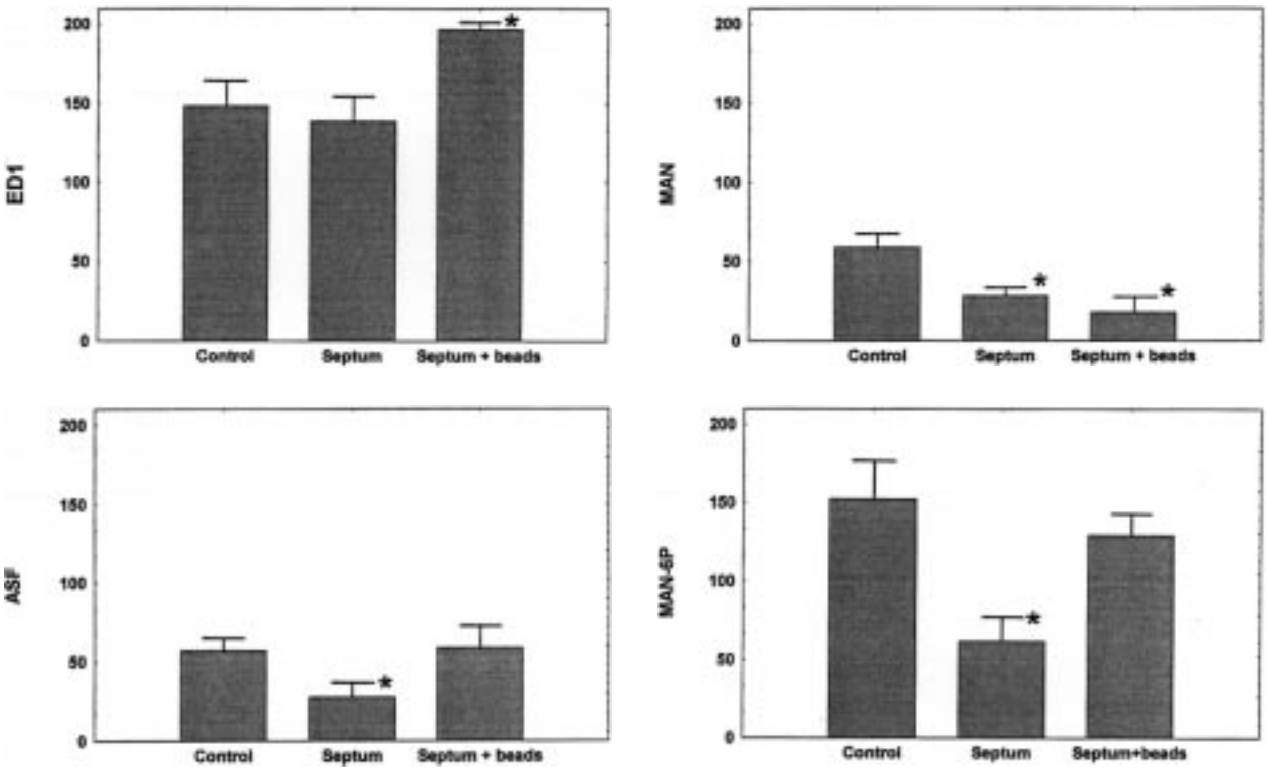

Figure 2 Mean number of red pulp spleen macrophages ($\pm$ SD) in control rats, rats with injured septum and rats with injured septum and intraperitoneally injected beads. The ED-1$^+$ macrophages were visualized immunohistochemically. The binding sites for asialoglycoproteins were detected by biotinylated ASF and binding sites for Man and Man-6P by the respective biotinylated neoglycoproteins. The asterisk indicates the statistically significant difference with respect to the control experiment at the 5% significance level (Student's *t*-test).

changes in the composition of the microenvironment [23–25], it is reasonable to propose a hypothetical scheme as a stimulus and guideline for further research, presented in Fig. 4.

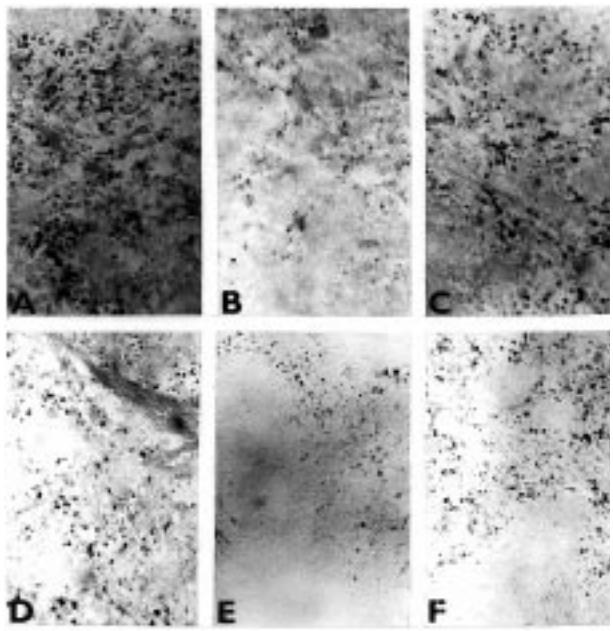

Figure 3 Visualization of binding sites for asialoglycoproteins (A, B, C) and Man - 6P (D, E, F) in spleen macrophages of animals without the septum lesion (A, D), animals with injured septum (B, E) and animals with injured septum and intraperitoneally injected beads (C, F). Magnification $\times$ 80.

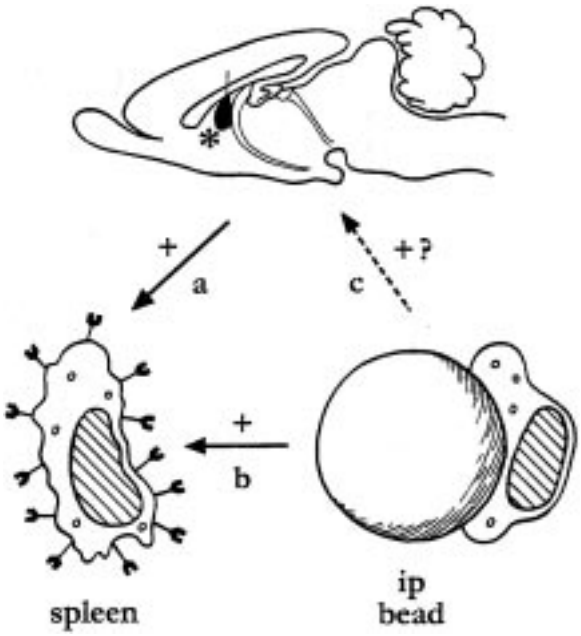

Figure 4 Hypothetical depiction of the processes which may be responsible for the measured effects. The dorsoposterior part of the rat septum positively influences the number of macrophages expressing binding sites for asialoglycoproteins (β-D-galactoside), α-mannoside and α-D-mannoside-6P. If this brain region is damaged, the foreign body inflammatory reaction and the number of macrophages (ED-1$^+$, ASF$^+$ and Man-6P$^+$) are in a temporal relationship, which is suggestive for a functional dependence. The assumption that multifunctional cytokines produced by the inflammatory cells which colonize the implants can act on the splenic cell populations and may also exert an impact on regulatory reactions on the level of the CNS is graphically shown. (a) Chain of polyneural pathways running from the CNS (septum) to the spleen. (b) Cytokines produced by inflammatory cells in response to the intraperitoneal injection of the polymer beads. (c) Speculative (retrograde) influence of these cytokines on the CNS.

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