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Keratan-Sulfate Immunohistochemistry: a New Tool for Characterization of Microglial Morphology and Topography

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Recently discovered anti-keratan-sulfate monoclonal antibody 5D4 selectively stains ramified microglia *in situ* both in paraffin and cryostat sections. Using this antibody are studied and discussed in this work both the structural polymorphism of microglia and their heterogenous density throughout the CNS (topography).

Key words: keratan-sulfate immunohistochemistry, microglia, 5D4 monoclonal antibodies, central nervous system (CNS).

Introduction

Microglia are distinct cell type in the CNS [5] but at the same time many evidences indicate that they are one of the components of the brain mononuclear phagocytes [7, 10]. Regularly spaced microglia in brain as "resting" type under physiological conditions they adopt ramified morphological appearance and serve the role of immune surveillance and host defence [4]. Microglia are the sole cells in CNS expressing immune functions [11]. Since Del Rio Hortega's studies on microglia in 1932 [6] till now many questions about cellular size, exact shape or about not so-uniform microglial morphology aren't dissolved clearly. Keratan-sulfate immunohistochemistry using 5D4 monoclonal antibodies was proposed last decade as specific marker for the most numerous subgroup of brain mononuclear phagocytes-ramified microglia [8, 9]. 5D4 monoclonal antibodies are directed against human keratan sulfate [2]. Keratan sulfate as a sulfated mucopolysaccharide is found in skeletal tissues and

cornea, and it contains D-galactose and D-glucosamine-6-O-sulfate. Our study shows the specific labelling features of keratan sulfate immunohistochemistry in CNS and some new data about microglial morphology.

Material and Methods

Animals: 10 adult female hamsters 5 weeks old are used. Anaesthetized hamsters were sacrificed and the brains were fixed in Carnoy's solution at room temperature overnight. After embedding in paraffin transversal sections 7 μm were obtained using. Commercially available monoclonal *antibodies* 5D4 (Calbiochem-San Diego CA), clone 5-D-4, isotype IgG1, k, were used for immunohistochemical procedure. Optimal working dilution of monoclonal antibodies is 1:1000 for overnight incubation at 4°C. The next day a procedure, using PicTure Polymer Detection System — a horseradish peroxidase/Fab polymer conjugate (ZYMED), was performed for 45 min at room temperature. DAB substrate kit for peroxidase (VECTOR) is applied as diaminobenzidine chromogen for 5-10 min. *Studied CNS regions*: immunohistochemistry of cerebral cortex, hippocampus, cerebellum, thalamus. *Controls*: Whole immunostaining procedure without 5D4 mAb and whole procedure with 5D4 mAb 1:1000 in PBS with 100 μg type I and II keratan sulfate.

Results

Keratan-sulfate immunohistochemistry applied in healthy hamster CNS shows that 5D4(+) cells are located in all studied by us regions: cortex, hippocampus, thalamus and cerebellum (Fig. 1). 5D4(+) microglial cells are characteristic with their ramified shape and multiple branched cellular processes and immunopositivity is expressed equally by highly ramified and by not so well ramified microglial cells (Fig. 2). In the cerebellum, cortex and thalamus are distributed smaller (8-12 μm), oval, and visually non-well ramified 5D4(+) microglial cells. The density of this type microglia increases essentially in molecular layer of cerebellum (Fig. 3). Keratan sulfate immunohistochemistry visualizes a specific for the brain parenhyma cellular association: cell-to-cell direct contact between a highly ramified and widespread microglial cell with one (or several) big neuron(s). A centrally located very thin blood vessel (8-10 μm in diameter) is always found in the middle of this cellular complex (Fig. 4). This finding that cellular and vascular elements interact in clusters is equally demonstrated in hamster brain cortex, hippocampus and thalamus.

Discussion

The specificity of this immunohistochemical procedure for ramified microglia is confirmed previously [1, 2, 3]. Keratan-sulfate epitope is expressed only by this microglial subpopulation [2] but not by all microglia. According to Wilms H. et al. [12] the smaller 5D4(+) cells (with not-well ramified shape) are probably microglia in the early stages of morphological transformation into ramified state. Our finding that many 5D4(+) microglial cells in the mature brain are in direct contact with neuronal population shows the large variety of the cell interactions in CNS. The specific localization of the neuron/microglia complexes next to adjacent microvascular network point to their eventual activities in blood brain barrier functions but this remains an open issue for further studies.

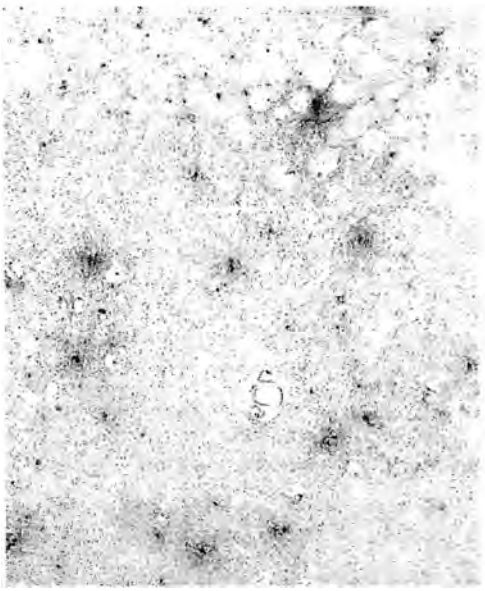

Fig. 1. Topographical distribution of 5D4(+) ramified microglia in mature hamster CNS. Hippocampus area (× 200)

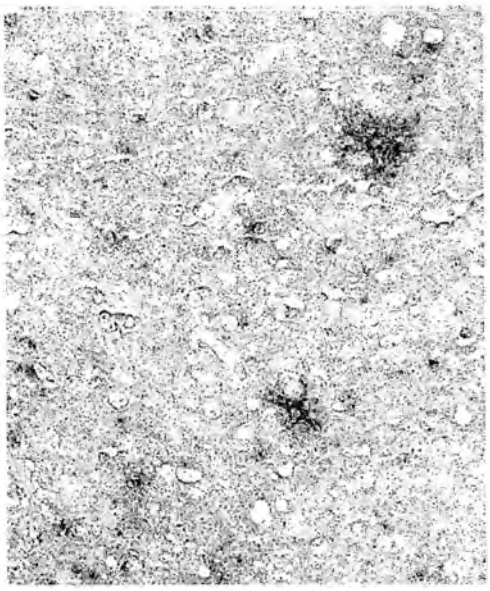

Fig. 2. Keratan-sulfate immunohistochemistry of two kind 5D4(+) microglia: large, highly ramified and smaller (not so well ramified) cells. Thalamus area. Adult hamster (× 400)

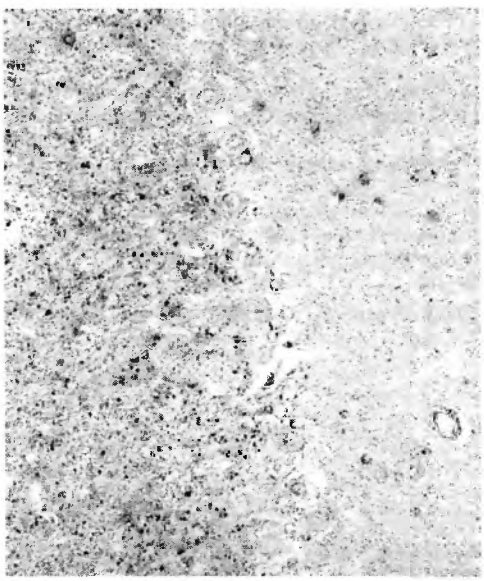

Fig. 3. Density of the smaller (not so well ramified) subclass of 5D4(+) microglia throughout molecular layer of cerebellum. Adult hamster (× 200)

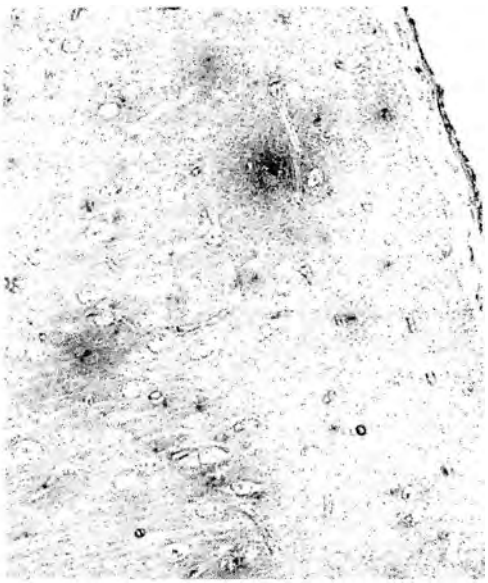

Fig. 4. Microglia/neuron cell-to-cell complexes associated to fine brain vasculature. Occipital cortex. Adult hamster (× 400)

Conclusion

Our data obtained by keratan-sulfate immunohistochemistry for ramified microglia reinforce the knowledge about morphology, tissue distribution (topography) and microenvironmental interrelationships of ramified microglia — a basic member of exclusively heterogenous family of brain mononuclear phagocytes.

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