Institute of Experimental Morphology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 15
Sofia • 2010

Distribution of the Tomato Lectin-Reactive Objects in Healthy and Degenerative Hamster Brain

D. Kadiysky, M. Svetoslavova

Institute of Experimental Morphology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia

The distribution, topography and morphology of the TL-reactive objects in hamster cerebral hemispheres were studied by histochemical procedure with tomato lectin –TL (*Lycopersicon esculentum*) immunohistochemistry. The analysis reveals TL-positive objects in the healthy and degenerative cortex, hippocampus and thalamus of hamster central nervous system (CNS). A little number of them are sparse lectin-reactive cells identified as ramified microglia exhibiting elongated forms and branched processes. The bigger number of TL(+) objects are components of brain microvasculature. Details of morphology and topographical distribution of the TL(+) objects in hamster CNS are described.

Key words: TL (tomato lectin) immunohistochemistry, ramified microglia, brain microvasculature.

Introduction

Tomato lectin (TL) from *Lycopersicon esculentum* as neurobiological marker was proposed earlier [1] but its specificity remains till now non-well determined. It was known that the lectin of *Lycopersicon esculentum* has affinity for poly-N-acetyl lactosamine sugar residues [23]. Tomato lectin binding in CNS is related to some of the glial cells [6]. Microglial cells are basic potential target for TL binding in CNS [1, 3, 6, 22, 23]. Now microglial reactivity usually is estimated by a number of other markers as for example monoclonal antibodies 5D4 against keratan-sulfate [9] or wide-used macrophage specific markers (MAC-1). At the same time many investigators propose TL for this aim [5, 8, 12, 15, 21]. In several cases they use to confirm the results with a second specific microglial marker like MAC-1, RCA-1 or Isolectin B4 [7, 17, 20]. Equally TL is proposed for electronmicroscopical immunohistochemisry [19]. A high specificity of TL for microglial cells in the normal adult brain is presumed [4, 11]. Here, we demonstrate that TL immunohistochemistry applied in healthy and degenerative brain tissue is marker simultaneously for several objects of CNS microanatomy.

Material and Methods

Adult five-week-old female outbred golden Syrian hamsters (45 animals) were source of healthy and degenerative brain tissue. A procedure for obtaining degenerative brain tissue was described previously [14]. Commercially available biotinilated – *Lycopersicon esculentum* (tomato) lectin (VECTOR Labs, Cat.No. B-1175) diluted in working solution of 10 micrograms/ml buffer PBS (phosphate buffer saline), enriched with $CaCl_2$ (1mM), $MgCl_2$ (0.1 mM) and stabilized with Natrium azide (0,08%) was used for procedure. Incubation of the brain sections with TL was performed at 20°C for two hours followed by application of ABC reagent (VECTOR Labs, Cat.No.PK-7100). DAB substrate kit for peroxidase (VECTOR Labs, Cat.No. SK-4100) was used as diaminobenzidine chromogen source for 2-10 min.

Light microscopy and interferential contrast microscopy (Nomarski optics) was performed.

Results

In healthy or degenerative hamster cortex, thalamus and hippocampus of hamster CNS TL immunohistochemistry reveals different kinds of positive objects – ones with determined cellular shape bodies and others obviously non-cellular elements of the brain microanatomy. TL(+) cells are very irregularly shaped with ramified and amoeboid morphologies (Fig. 1). Elongated cells with several processes and rounded cells are labelled generally. These microglia-like cells are seen abundantly in the degenerative hamsters brains. A microgliosis could be registered during the terminal stage (after 80th day of the agent inoculation) of the experimental transmissive spongiform encephalopathy, provoked by the strain scrapie 263K (Fig. 2). Detailed study of the immunostained CNS fields in cortex, thalamus and hippocampus reveals many other objects positive to TL

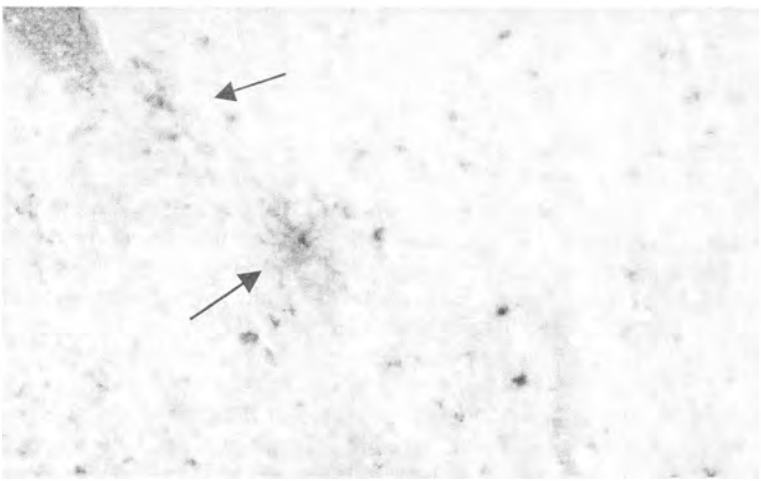

Fig. 1. TL(+) cellular objects are very irregularly shaped with ramified and amoeboid morphologies. Cortex, × 200

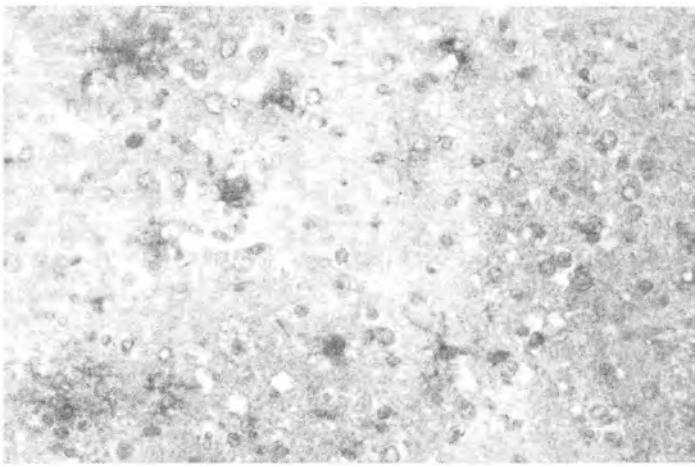

Fig. 2. Microgliosis could be registered by TL immunohistochemistry during the terminal stage (after 80th day of agent inoculation) of the experimental transmissive spongiform encephalopathy, provoked by the strain scrapie 263K. Thalamus, ×200

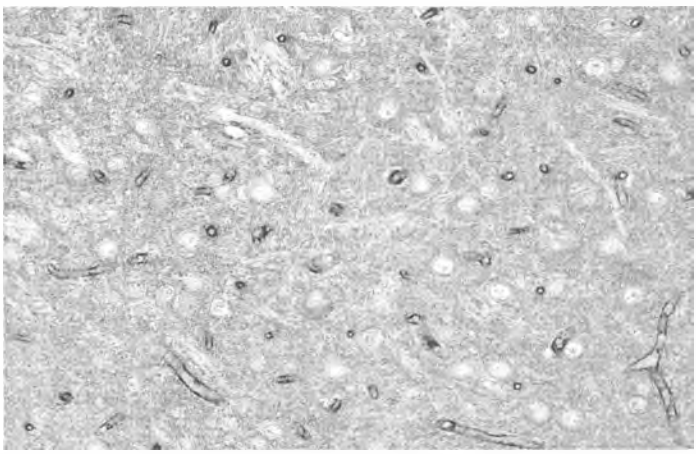

Fig. 3. Other CNS objects positive to TL are components of the brain microvasculature. Thalamus, ×100

(Fig. 3). The light microscopy reveals rounded and prolonged objects situated everywhere in the brain tissue both in healthy and degenerative CNS. The interferential contrast microscopy shows that lectin positive objects in studied regions are components of the brain microvasculature.

Discussion

TL immunohistochemistry reveals a lower density of labelled microglia in comparison with other procedures for microglial visualization (5D4 monoclonal antibodies) [14]. Second, a great number of the TL(+) objects in hamster brain are components of the thinnest network of the brain microvasculature.

During degenerative changes in hamster CNS a marked response of the microglia occurred in the terminal stages of the experimental scrapie 263K in the cortex, hippocampus and thalamus. The universal phenomenon of microgliosis could be registered with TL immunohistochemistry as it is proposed earlier [13]. The widespread extracellular TL labelling found by us in the hamster CNS corresponds to fine microvasculature components. A sure explication of this fact is that tomato lectin is specific for blood brain capillary endothelium [10, 16, 18].

The staining of the vascular network by TL immunohistochemistry in healthy and diseased CNS is higher efficient than this with commonly used vascular markers as EMS laminin or PECAM-1 [16].

References

1. Acarin, L., J. Vela, B. Gonzales, B. Castellano. Demonstration of poly-N-acetyl lactosamin residues in ameboid and ramified microglial cells in rat brain by tomato lectin binding. – J. Histochem. Cytochem., **42,** 1994, No 8, 1033-1041.
2. Acarin, L., B. Gonzales, B. Castellano, A. Castro. Microglial response to N-methyl-D-aspartate-mediated excitotoxicity in the immature rat brain. – J. Comp. Neurol., **367,** 1996, No 3, 361-364.
3. Acarin, L., B. Gonzales, B. Castellano, A. J. Castro. Quantitative analysis of microglial reaction to a cortical lesion in the early postnatal brain. – Exp. Neurol., **147**(2), 1997, 410-417.
4. Acarin, L., B. Gonzales, B. Castellano. – Triflusal posttreatment inhibits glial nuclear factor-kB downregulates the glial response ant is neuroprotective in an excitotoxic injury model in postnatal brain. – Stroke, **32**, 2001, No 10, 2394-2402.
5. Acarin, L., B. Gonzales, Castro A. J., B. Castellano. Primary cortical glial reaction versus secondary thalamic glial response in the excitotoxically injured young brain: microglial/macrophage response and MHC class I and class II expression. – Neuroscience, **89**, 1999, No 2, 249-265.
6. Ambalavanar, R., R. Morris. The binding of a poly-N-acetyllactosamine specific lectin from the tomato to primary afferens in the rat trigeminal system. – Eur. J. Neurosci., **4**(suppl.), 1991, 27-29.
7. Anjelkovoc, A. V., B. Nikolic, J. S. Pachter, N. Zecevic. Macrophages/microglial cells in human central nervous system during development: an immunohistochemical study. – Brain Res., **814**, 1998, No 1-2, 13-25.
8. Bass, W. T., G. A. Singer, F. J. Liuzzi. Transient lectin binding by white matter tract border zone microglia in the foetal rabbit brain. – Histochem. J., **30**,1998, No 9, 657-666.
9. Bertolotto, A., B. Katerson, G. Canavese, A. Migheli, D. Schiffer. Monoclonal antibodies to keratan sulfate immunolocalize ramified microglia in paraffin and cryostat sections of rat brain. – J. Histochem. Cytochem., **41**, 1993, 481-487.
10. Facla, I., A. Hever, J. Molnar, J. Fisher. Tomato lectin labels the 180 kD glycoform of P-glycoprotein in rat brain capillary endothelia and mdr tumor cells. – Anticancer Res., 1998, No 18(4C), 3107-3111.
11. Fujita, S. C., K. Sakuta, R. Tsuchiya, H. Hamanaka. Apolipoprotein E is found in astrocytes but not in microglia in normal mouse brain. – Neurosci. Res., **35,** 1999, No 2, 122-133.
12. Ignacio, A., Y. Muller, M. Carvalho, E. M. Nazari. Distribution of microglial cells in the cerebral hemispheres of embryonic and neonatal chicks. – Braz. J. Med. Biol. Res., **38**, 2005, No 11, 1615-1622.
13. Jimeno, D., A. Velasco, C. Lilo, J. Lara, J. Aijon. Response of microglial cells after a cryolesion in the peripheral proliferative retina of tench. – Brain Res., **816**, 1999, No 1, 175-189.
14. Kadiysky, D., M. Svetoslavova, N. Sales, C. Lasmezas, J-P. Deslys. Keratan-sulfate immunohistochemistry: a new tool for characterization of microglial morphology and topography. – Acta Morphologica and Anthropologica, **10**, 2005, 5-8.
15. Kong, G. Y., K. Kristensson, M. Bentivoglio. Reaction of mouse brain oligodendrocytes and their precursors, astrocytes and microglia, to proinflamatory mediators circulating in cerebrospinal fluid. – Glia, **37**, 2002, No 3, 191-205.
16. Mazzetti S., S. Frigerio, M. Gelati, A. Salmaggi, L. Vitellaro-Zuccarello. Lycopersicon esculentum lectin: an effective and versatile endothelial marker of normal and tumoral blood vessels in the central nervous system. – Eur. J. Histochem., **48**, 2004, No 4, 423-428.
17. Moffett, J. R., M. G. Espey, S. A. Walter, W. J. Streit, M. A. Namboodiri. Quinolinate immunoreactivity in experimental rat brain tumors is present in macrophages but not in astrocytes. – Exp. Neurol., **144**, 1997, No 2, 287-301.

18. Molnar, J., A. Hever, I. Fakla, J. Fisher, I. Ocsovski, A. Aszalos. *Inhibition of the transport function of membrane proteins by some substituted phenothiazines in E. coli and multidrug resistant tumor cells.* – Anticancer Res., **17**, 1997, No 1A, 481-486.
19. Nacher, J., C. Ramirez, J. J. Palop, P. Artal, A. Molowny, C. Lopez-Garcia. Microglial cells during the lesion-regeneration of lizard medial cortex. – Histol. Histopathol., **14**, 1999, No 1, 103-117.
20. Pelaez, B., J. L. Blazquez, F. E. Pastor, A. Sanchez, P. Amat. Lectin histochemistry and ultrastructure of microglial response to monosodium glutamate-mediate neurotoxicity in the arcuate nucleus. – Histol. Histopathol., **14**, 1999, No 1, 165-174.
21. Peng, Z. C., K. Kristensson, M. Bentivoglio. Distribution and temporal regulation of the immune response in the rat brain to intracerebroventricular injection of interferon-gama. – Exp. Neurol., **154**, 1998, No 2, 403-417.
22. Velasco, A., E. Caminos, E. Vecino, J. Lara, J. Aijon. Microglia in normal and regenerating visual pathways of the tench: a study with tomato lectin. – Brain. Res., **705**, 1995, No 1-2, 315-324.
23. Zhu, BC-R., R. Laine. Purification of acetyllactosamine specific tomato lectin by erythroglycan-sepharose affinity chromatography. – Prep. Biochem., **19**, 1989, No 4, 341-350.