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A specific histochemical marker (lectin *Ricinus communis* agglutinin-1) for normal human microglia, and application to routine histopathology

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Summary. Microglia were demonstrated in paraffin-embedded human nervous tissues with an avidin-biotin peroxidase method and *Ricinus communis* agglutinin-1 (RCA-1). Specific staining was observed in cell bodies and processes of microglia. Although endothelial cells and blood cells reacted with RCA-1, they were easily distinguished morphologically from microglia. Astrocytes, oligodendrocytes, and neurons did not react with RCA-1. These results suggest that RCA-1 can be used as a new histochemical marker for microglia in normal human brain.

Key words: Microglia – *Ricinus communis* agglutinin-1 – Lectin – Histochemistry

Over 65 years ago, del Rio-Hortega (1919) first stained microglia in the central nervous system using a silver impregnation method. Since then, microglia in a variety of normal and pathological tissues have been demonstrated by silver impregnation (Penfield 1932; Weil and Davenport 1933; Gallyas 1963; Scott 1971) and by histochemical (Smith and Rubinstein 1962; Ibrahim et al. 1974; Kreutzberg and Barron 1978) and immunohistochemical methods (Mathew et al. 1983; Esiri and Booss 1984; Pesce et al. 1985). However, histochemical and immunohistochemical methods were not always superior to the conventional (Weil and Davenport 1933) silver impregnation methods for identifying microglia in formalin-fixed material.

With the introduction of lectin histochemistry, it is possible to delineate some of the cell types in nervous tissue (Hori et al. 1982; Liwnicz 1982; Schwechheimer et al. 1984). When it is combined with the rapid and highly specific biotin-avidin marker system, increased labeling specificity is possible. This report shows that microglia in normal human brain can be specifically demonstrated using the lectin RCA-1, as detected with the avidin-biotin peroxidase method.

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Material and methods

Normal brains were obtained from ten patients aged 2–21 years who had no evidence of neurological disease or neuropathology. The regions examined included frontal cortex, basal ganglia, cerebellum, brain stem (midbrain, pons and medulla), and spinal cord. Formalin-fixed paraffin-embedded tissues were sectioned at 5–7 μm.

Avidin D-horseradish peroxidase (HRP) and the following biotinylated lectins were obtained from Vector Laboratories (Burlingame, CA, USA): *Ricinus communis* agglutinin-1 (RCA-1), *Ulex europaeus* agglutinin-1 (UEA-1), concanavalin A (Con A), wheat germ agglutinin (WGA), soybean agglutinin (SBA), *Dolichos biflorus* agglutinin (DBA), peanut agglutinin (PNA), *Griffonia siplicifolia* agglutinin-1 (GSA-1), *Pisum sativum* agglutinin (PSA), and *Helix pomatia* agglutinin (HPA). Lectin binding was detected with an avidin-biotin peroxidase method (Hsu and Raine 1984).

Staining was performed as follows: Sections were deparaffinized, rehydrated, and incubated for 30 min in 0.3% hydrogen peroxide in methanol and then for 15 min with 1% bovine albumin in Hepes-buffered saline (0.01 M Hepes buffer, 0.15 M sodium chloride, 0.1 mM calcium chloride, 0.01 mM magnesium chloride). Then the sections were incubated for 45 min with one biotinylated lectin at a concentration of 25 μg/ml diluted in the above buffer and followed by three 5-min washes in this buffer. After exposure to the lectin the sections were incubated for 50 min with avidin D-HRP at a concentration of 50 μg/ml diluted in a bicarbonate buffer (0.05 M sodium bicarbonate and 0.15 M sodium chloride), followed by three 5-min washes in this buffer. The final incubation was for 4 min in 0.05 M Tris-HCl buffer (pH 7.4) containing 3.3'-diaminobenzidine (DAB) and hydrogen peroxide. The sections were counterstained with hematoxylin.

The specificity of the staining obtained with RCA-1 was tested by preincubating the lectin with 0.2 M lactose before application to the sections. Negative controls consisted of avidin alone and biotinylated lectins without avidin D-HPR. Adjacent 15-μm sections were stained for microglia with the ammoniacal silver nitrate method of Scott (1971). Serial sections were also examined for glial fibrillary acidic protein (GFAP) using the avidin-biotin peroxidase complex (ABC) technique (Hsu and Raine 1984). The monospecificity of our antiserum against GFAP has been described elsewhere (Mannoji et al. 1981).

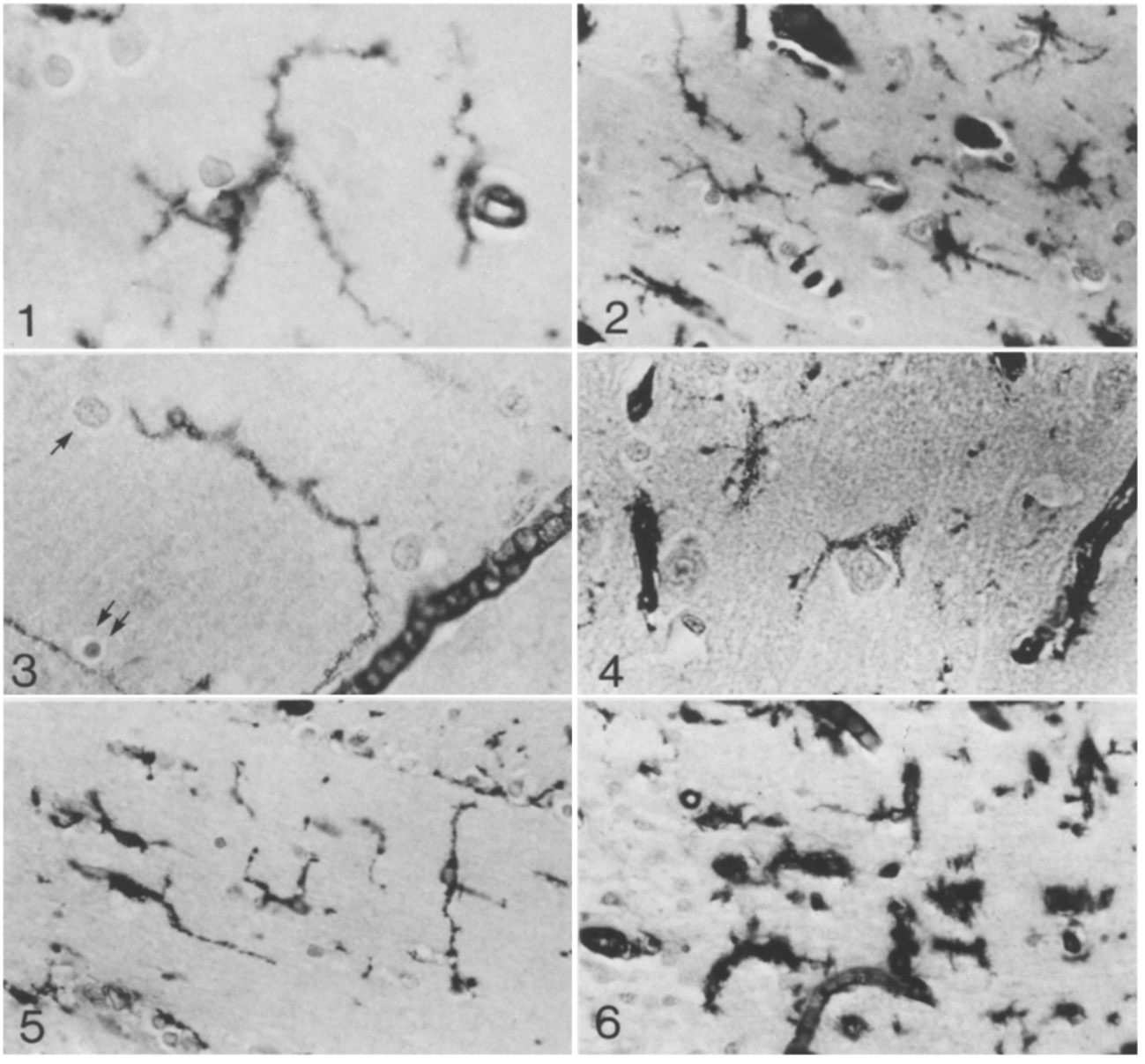

Fig. 1. Photomicrograph of an RCA-positive cell morphologically identical to microglia. Hematoxylin counterstain, ×640

Fig. 2. Photomicrograph of many RCA-positive microglia and blood vessels. Hematoxylin counterstain, ×280

Fig. 3. Photomicrograph of an RCA-1-positive microglial process directed toward a blood vessel. An astrocyte (*arrow*) and oligodendrocyte (*double arrow*) are negative. Hematoxylin counterstain, ×580

Fig. 4. Photomicrograph showing RCA-1-positive staining microglia surrounding a neuron, which is RCA-1-negative. Hematoxylin counterstain, ×320

Fig. 5. Photomicrograph showing RCA-1-positive microglial processes in white matter. Hematoxylin counterstain, ×320

Fig. 6. Photomicrograph of numerous ameboid microglia in the cerebellum of a 2-year-old child. Hematoxylin counterstain, ×285

Results

Of the ten lectins used in this study, only RCA-1 specifically labeled microglia. RCA-1 binding was completely inhibited by 0.2 M lactose. Specific staining was observed in the cell bodies and processes of microglia in all specimens (Figs. 1, 2). Although endothelial cells and blood cells also reacted with RCA-1 (Figs. 1, 3), they were easily distinguished morphologically from microglia. Astrocytes, oligo-

dendrocytes, and neurons did not react with RCA-1 (Figs. 3, 4).

RCA-1-positive microglia were observed throughout the central nervous system, more frequently in white than gray matter, and around blood vessels (Fig. 3) and neurons (Fig. 4). In white matter, the microglial processes followed an undulating and tortuous course along or across myelinated fibers (Fig. 5). In cerebellum, microglia were observed more frequently in molecular than granular layers. In one patient (2 years old), numerous ameboid and pseudopodic microglia (Rio-Hortega 1932) were stained (Fig. 6). All microglia from adjacent sections impregnated by silver were reactive with RCA-1. On serial sections, RCA-1-positive cells did not react with antisera to GFAP.

Discussion

The present study shows that RCA-1 binds specifically to microglia of formalin-fixed paraffin-embedded human brain. Microglia have recently been demonstrated using a monoclonal macrophage antibody that reacted with resting microglia (Mathew et al. 1983), and also using macrophage markers (Esiri and Booss 1984; Pesce et al. 1985). However, these investigators concluded that the conventional silver impregnation method (Weil and Davenport 1933) was superior for demonstrating resting and reactive microglia in formalin-fixed material. We believe our system is preferable to other methods for the following reasons: the binding of RCA-1 is not affected by formalin fixation of the tissues, as might occur with immunohistochemical markers; the biotin-avidin complex is highly specific and permits further amplification with the ABC method, if desired; and inhibition controls can be easily included to demonstrate specificity.

Our results clearly demonstrate that RCA-1 can be used as a histochemical marker for microglia in normal human brain. Since the tissues were routinely formalin-fixed and paraffin-embedded, our labeling method may be suitable for routine histopathology.

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References

Esiri MM, Booss J (1984) Comparison of methods to identify microglial cells and macrophages in the human central nervous system. J Clin Pathol 37:150–156

Gallyas F (1963) Silver impregnation method for microglia. Acta Neuropathol (Berl) 3:206–209

Hori T, Nishiyama F, Matsutani N, Teramoto A, Takakura K, Sano K, Hirano H (1982) Lectin-binding sites of the human pituitary adenoma cells by means of the ferritin-labeling technique. Acta Neuropathol (Berl) 56:67–74

Hsu S-M, Raine L (1984) The use of avidin-biotin-peroxidase complex (ABC) in diagnostic and research pathology. In: DeLellis RA (ed) Advances in immunohistochemistry. Masson Publishing USA, New York, pp 31–42

Ibrahim MZM, Khreis Y, Koshayan DS (1974) The histochemical identification of microglia. J Neurol Sci 22:211–233

Kreutzberg GW, Barron KD (1978) 5′-Nucleotidase of microglial cells in the facial nucleus during axonal reaction. J Neurocytol 7:601–610

Liwnicz BH (1982) Mitogenic lectin receptors of nervous system tumors. Study of gliomas, neural crest tumors, and meningiomas in vitro using phytohemagglutinin and concanavalin A. J Neuropathol Exp Neurol 41:281–297

Mannoji H, Takeshita I, Fukui M, Ohta M, Kitamura K (1981) Glial fibrillary acidic protein in medulloblastoma. Acta Neuropathol (Berl) 55:63–69

Mathew RC, Gupta SK, Katayama I, Curtis J, Turk JL (1983) Macrophage-specific antigen is expressed by resting microglia in the CNS but not by Langerhans cells in the skin. J Pathol 141:435–440

Penfield W (1932) Neuroglia and microglia, the interstitial tissue of the central nervous system. In: Cowdry EV (ed) Special cytology, 2nd edn, vol 3. Hoeber, New York, pp 1445–1482

Pesce C, Tobia F, Scott T (1985) Microglia in teratomas. Acta Neuropathol (Berl) 67:332–336

Rio-Hortega P del (1919) El tercer elemonto de los centros nerviosos. I. La Microglia en estado normal. II. Intervencion de la microglia en los procesos patologicos. III. Naturaleza probable de la microglia. Bol Soc Esp Biol 9:69–120

Rio-Hortega P del (1932) Microglia. In: Penfield W (ed) Cytology and cellular pathology of the nervous system, vol 2. Hoeber, New York, pp 482–534

Schwechheimer K, Weiss G, Schnabel P, Möller P (1984) Lectin target cells in human central nervous system and the pituitary gland. Histochemistry 80:165–169

Scott T (1971) A rapid silver impregnation technique for oligodendrocytes, microglia, and astrocytes. J Clin Pathol 24:578–580

Smith B, Rubinstein LJ (1962) Histochemical observations on oxidative enzyme activity in reactive microglia and somatic macrophages. J Pathol Bacteriol 83:572–575

Weil A, Davenport HA (1933) Staining of oligodendroglia and microglia in celloidin sections. Arch Neurol Psychiatry 30:175–178

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