Acta Anatomica
Editors-in-Chief: W. Lierse, Hamburg; A.W. English, Atlanta, Ga.

Separatum
Publisher: S. Karger AG, Basel
Printed in Switzerland

Acta Anat 1991;140:250–253

0001–5180/91/1403–0250 $ 2.75/0

Microglial Cells in the Central Nervous System of the Rabbit and Rat: Cytochemical Identification Using Two Different Lectins

J. Boya, A.L. Carbonell, J.L. Calvo, A. Borregon

Department of Histology and General Embryology, Faculty of Medicine, University Complutense, Madrid, Spain

Key Words. Microglia · Lectin histochemistry · *Ricinus communis* agglutinin-120 · *Griffonia simplicifolia* B_4 isolectin · Cerebrum · Cerebellum · Rabbit · Rat

Abstract. Microglial cells were selectively demonstrated in the central nervous system of adult rabbits and rats using lectin histochemistry. Biotinylated *Ricinus communis* agglutinin-120 (RCA-1) and biotinylated *Griffonia simplicifolia* B_4 isolectin (GSA I-B_4) were used as histochemical markers on sections of Bouin-fixed paraffin-embedded cerebrum and cerebellum. Results were quite similar using both lectins and both species. GSA I-B_4 resulted in a better staining in the rat, while RCA-1 labelling was superior in the rabbit. Neither neurons nor glial cells other than microglia were stained with our technique. Lectin histochemistry applied for the detection of microglial cells appears to be of sufficient selectivity and may be considered as an important tool in the morphological and neurobiological study of these cells.

Introduction

Microglial cells were formerly described by del Rio-Hortega [1], who established a mesodermic nature and phagocytic properties for these cells. Since then, these neural tissue elements have been the centre of a considerable and permanent controversy. Their origin, functions and relationships with macrophages appearing in lesional foci in nervous tissue have been largely debated [2, 3; for a review, see 4, 5].

Besides the classical histological techniques of silver impregnation, a certain amount of procedures for the detection of microglial cells has been lastly developed. Among these procedures, cytochemical [6–8] or immunocytochemical methods [9; for a review, see 10] have provided outstanding results.

Recently, lectin histochemistry has been applied as a new tool for the study of the microglia. Thus, *Ricinus communis* agglutinin-120 (RCA-1) and mistletoe lectin-1 selectively bind to human [11, 12] and rat microglial cells [12], and *Griffonia (Bandeiraea) simplicifolia* B_4 isolectin (GSA I-B_4) binds to rat microglia [13, 14] and rabbit retinal microglia [15]. Nevertheless, according to Suzuki et al. [12], microglia staining showed qualitative changes in relation with different species and lectins.

The purpose of the present paper is to study the application of two different lectins, RCA-1 and GSA I-B_4, for the detection of microglial cells in two different mammalian species, the rabbit and rat. Results between these species have also been compared.

Materials and Methods

Tissue Preparation

Adult New Zealand white rabbits and Wistar albino rats of both sexes were used. Animals were kept in an environment of constant temperature, humidity and day-night cycle and had free access to food and water. Under ether anaesthesia animals were killed by decapitation, and fragments of cerebrum and cerebellum were quickly removed and immersed in Bouin's fixative (75 ml of a saturated solution of picric acid in distilled water, 25 ml of pure formalin, 5 ml of glacial acetic acid) overnight at 4 °C. After washing in tap water, samples were dehydrated in ethanol, cleared in xylene and embedded in paraffin. Sections (7 μm thick) were cut and mounted on glass slides.

Lectin Histochemistry

Tissue sections were dewaxed and rehydrated, and endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol (15 min). After a rinse in phosphate-buffered saline (PBS; 0.01 *M*, pH 7.3) for 15 min, the sections were incubated overnight with the biotinylated lectin diluted in PBS, in a humid chamber. Both lectins were obtained from Sigma (St. Louis, Mo., USA) and used at an optimum concentration of 2.2 µg/ml for RCA-1 and 5 µg/ml for GSA I-B_4. After the incubation, sections were rinsed in PBS for 15 min and incubated in extravidin-horseradish peroxidase (extravidin-HRP, Sigma) at a concentration of 5 µg/ml in PBS, for 1 h in a humid chamber. Sections were rinsed in PBS for 15 min and treated with a solution of 3-3′-diaminobenzidine tetrahydrochloride (Sigma) and hydrogen peroxide (5 mg diaminobenzidine, 10 ml PBS, 30 µl 0.3% H_2O_2) under light-microscopical control. After a rinse in distilled water, sections were dipped in 1% osmium tetroxide in distilled water for 20 min to intensify the reaction product. Sections were washed in tap water and briefly counterstained with Carazzi's haematoxylin for 20–30 s. Finally, they were washed in tap water, dehydrated, cleared and mounted in Eukitt (Kindler, Freiburg, FRG). The histochemical procedure was performed at room temperature.

Controls

Controls were performed by incubation either with RCA-1 in the presence of 0.2 *M* lactose (Sigma) or with GSA I-B_4 in the presence of 0.2 *M* melibiose (6-O-α-*D*-galactopyranosyl-*D*-glucose; Sigma), to saturate lectin-binding sites thus preventing interaction with sugars of tissue components. In addition, controls not previously treated with the biotinylated lectin were incubated in extravidin-HRP in order to discard a nonspecific tissue binding.

Results

Rabbit

Lectins RCA-1 (fig. 1) and GSA I-B_4 (fig. 2) clearly demonstrated positive cells with classical microglial cytomorphology in both the cerebrum and the cerebellum, with slight preponderance in the grey matter.

Cells stained for RCA-1 showed small, triangular, spindle- or round-shaped cell bodies and 2–4 elongated and tortuous cell processes branching and thinning out distally (fig. 3, 4). The cell nucleus, devoid of reaction product, occupied most of the cell body volume. Frequently, microglial cells were located near blood capillaries (fig. 4), though cell processes such as astroglial endfeet were never seen. Sometimes, microglial cells were seen abutting on neuronal or astroglial somata (fig. 4).

Neurons, astrocytes and oligodendrocytes did not react with RCA-1 and were easily identified by haematoxylin counterstaining.

Results comparable to those described above were obtained by using lectin histochemistry for GSA I-B_4. Yet, the intensiveness of the reaction product was slightly weaker with this lectin (fig. 2, 5).

Rat

In this species, positive microglial cells were less abundant in both the cerebrum and the cerebellum. Furthermore, these cells were more intensely stained with GSA I-B_4 (fig. 6) than with RCA-1 (fig. 7) lectin histochemistry. Rat microglial cells, with shorter and less ramified processes, showed simpler morphological features compared with rabbit microglia. Positive cells showed tissue distribution and relationships similar to those described for the rabbit with respect to astrocytes, neurons and blood capillaries.

The incubation of RCA-1 in the presence of lactose and with GSA I-B_4 in the presence of melibiose resulted in a complete absence of histochemical staining in both the rabbit and the rat nervous tissue.

Discussion

The present study demonstrates that lectin histochemistry using RCA-1 or GSA I-B_4 on paraffin sections of Bouin-fixed material is suitable to detect microglial cells in the rabbit and rat central nervous system.

Morphological criteria alone are enough to consider these cells as microglial cells. The cells discussed in our study shared identical morphological features with microglial cells described classically in the silver-impregnated central nervous system. It deserves mention that paraffin sections used in our study are considerably thinner than those used for classical metallic impregnations, whereby it is more difficult to follow the course of the cell processes.

In our study, cells stained with lectins were clearly distinguishable from other lectin-negative glial cells. Lectin-positive microglial cells never showed processes similar to astrocytic end-feet and were never seen bordering the nervous parenchyma. Furthermore, lectin-positive cells in the white matter were never seen forming rows as is characteristic of oligodendrocytes.

In our material, we have found some morphological differences between microglial cells of the rabbit and the rat. Thus, rabbit microglia exhibit cell processes which are slenderer and more delicate than those of the rat microglia. This difference confirms classical observations on silver-impregnated frozen sections.

Different lectins have been used on several species to identify microglial cells: human (RCA-1 [11, 12]), rat (RCA-1* and mistletoe lectin-1 [12, 13]) and rabbit cells (retinal microglia, GSA I-B_4 [15]). Histochemical methods used in these studies have also been diverse: biotinylated lectin and avidin-HRP [11]; lectin, antilectin anti-

* GSA I-B_4

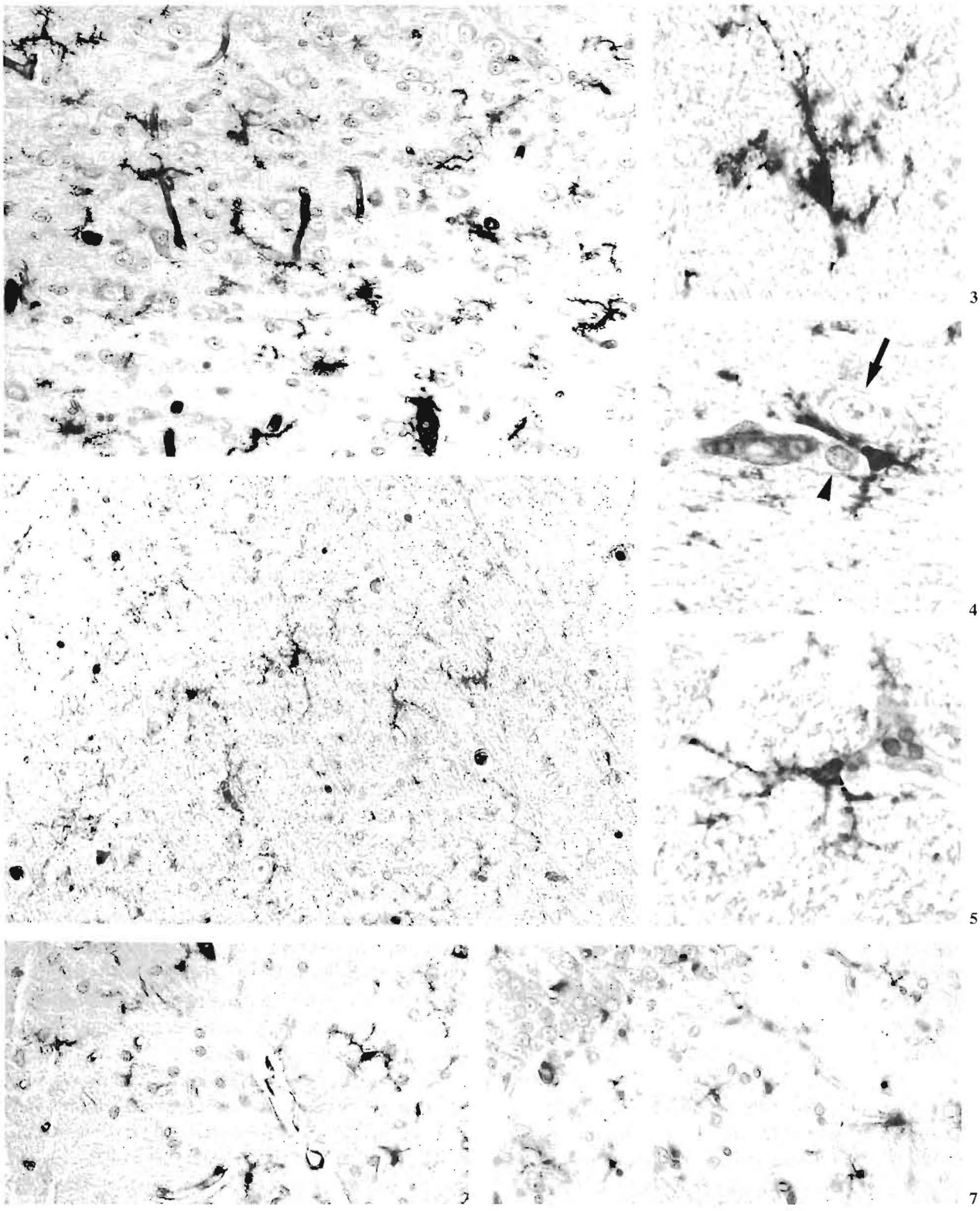

Fig. 1. Cerebrum. Rabbit. RCA-1-positive microglial cells interspersed throughout the nervous parenchyma. ×295.
Fig. 2. Cerebrum. Rabbit. GSA-I-B_4-positive microglial cells. ×295.
Fig. 3. Cerebrum. Rabbit. RCA-1-positive ramified microglial cell. ×800.
Fig. 4. Cerebrum. Rabbit. RCA-1-positive ramified microglial cell near a blood vessel. Arrow = Neuronal cell body; arrowhead = astrocyte. ×800.
Fig. 5. Cerebrum. Rabbit. GSA-I-B_4-positive microglial cell with slender and branching processes near a blood vessel. ×800.
Fig. 6. Cerebellum. Rat. GSA-I-B_4-positive microglial cells in the plexiform layer. ×300.
Fig. 7. Cerebrum. Rat. RCA-1-positive microglial cells. ×300.

body, biotinylated anti-antilectin antibody and avidin-biotin-HRP complex [12], or HRP-conjugated lectin [13, 15]. We have used two species, the rabbit and rat, two biotinylated lectins (RCA-1 and GSA I-B_4) and extravidin-HRP. According to the product specifications, the extravidin is a modified form of purified avidin that combines the highly specific activity of avidin with the low background staining of streptavidin. Our results demonstrate that there are no strong differences in the histochemical labelling with both lectins. However, RCA-1 provides better results in the rabbit whereas GSA I-B_4 is better in the rat. As indicated by Suzuki et al. [12], such differences could be due to subtle variations among species in the conformation of the microglial surface glycoproteins.

RCA-1 is a lectin obtained from *R. communis* (castor bean) with a molecular weight of 120 kD. This lectin binds specifically to cell surface β-*D*-galactose residues [12]. GSA I-B_4 is obtained from *Bandeiraea simplicifolia (G. simplicifolia)*, has a molecular weight of 114 kD and binds specifically to α-*D*-galactose terminal residues [13]. The binding of lectins to microglia cells is inhibited by the incubation in the presence of lactose (for RCA-1) or melibiose (for GSA I-B_4). This points out the specific binding of these lectins to terminal carbohydrate residues on the cell surface.

Heretofore, lectin histochemistry has been scarcely used to investigate the origin of the microglial cells [14, 15] or the reactions of this cell type to different insults, both in experimental [16] and human pathology [17–19]. In our opinion, lectin histochemistry provides sound advantages for the study of the microglia. These techniques are very specific, can be applied on paraffin-embedded tissue sections and are suitable for electron microscope studies [13]. These properties highlight lectin histochemistry as an outstanding tool for the research on the neurobiology of this controverted cell type.

References

1 Rio-Hortega P del: El tercer elemento de los centros nerviosos. I. La microglia en estado normal. II. Intervención de la microglia en los procesos patológicos. III. Naturaleza probable de la microglia. Bol Soc Esp Biol 1919;9:69–120.

2 Boya J, Calvo J, Carbonell AL, García-Mauriño E: Nature of macrophages in rat brain. A histochemical study. Acta Anat 1986;127: 142–145.

3 Boya J, Carbonell AL, Calvo J, Borregon A: Ultrastructural study on the origin of rat microglia cells. Acta Anat 1987;130:329–335.

4 Ling EA: The origin and nature of microglia; in Fedoroff S, Hertz L (eds): Advances in Cellular Neurobiology. New York, Academic Press, 1981, vol 2, pp 33–82.

5 Jordan FL, Thomas WE: Brain macrophages: Questions of origin and interrelationship. Brain Res Rev 1988;13:165–178.

6 Kreutzberg GW, Barron KD, Schubert P: Cytochemical localization of 5′-nucleotidase in glial plasma membrane. Brain Res 1978; 158:247–257.

7 Murabe Y, Sano Y: Thiaminepyrophosphatase activity in the plasma membrane of microglia. Histochemistry 1981;71:45–52.

8 Vorbrodt AW, Wisniewski HM: Plasmalemma-bound nucleoside diphosphatase as a cytochemical marker of central nervous system (CNS) mesodermal cells. J Histochem Cytochem 1982;30:418–424.

9 Miles JM, Chou SM: A new immunoperoxidase marker for microglia in paraffin section. J Neuropathol Exp Neurol 1988;47: 579–587.

10 Streit WJ, Graeber MB, Kreutzberg GW: Functional plasticity of microglia: A review. Glia 1988;1:301–307.

11 Mannoji H, Yeger H, Becker LE: A specific histochemical marker (lectin *Ricinus communis* agglutinin-1) for normal human microglia, and application to routine histopathology. Acta Neuropathol (Berl) 1986;71:341–343.

12 Suzuki H, Franz H, Yamamoto T, Iwasaki Y, Konno H: Identification of the normal microglial population in human and rodent nervous tissue using lectin-histochemistry. Neuropathol Appl Neurobiol 1988;14:221–227.

13 Streit WJ, Kreutzberg GW: Lectin binding by resting and reactive microglia. J Neurocytol 1987;16:249–260.

14 Ashwell KWS, Holländer H, Streit W, Stone J: The appearance and distribution of microglia in the developing retina of the rat. Vis Neurosci 1989;2:437–448.

15 Ashwell K: Development of microglia in the albino rabbit retina. J Comp Neurol 1989;287:286–301.

16 Streit WJ, Kreutzberg GW: Response of endogenous glial cells to motor neuron degeneration induced by toxic ricin. J Comp Neurol 1988;268:248–263.

17 Dickson DW: Multinucleated giant cells in acquired immunodeficiency syndrome encephalopathy. Origin from endogenous microglia? Arch Pathol Lab Med 1986;110:967–968.

18 Michaels J, Price RW, Rosenblum MK: Microglia in the giant cell encephalitis of acquired immune deficiency syndrome: Proliferation, infection and fusion. Acta Neuropathol (Berl) 1989;76: 373–379.

19 Sangruchi T, Sobel RA: Microglial and neural differentiation in human teratomas. Acta Neuropathol (Berl) 1989;78:258–263.

Received: June 27, 1990
Accepted: October 5, 1990

J. Boya
Department of Histology and General Embryology
Faculty of Medicine
University Complutense
E–28040 Madrid (Spain)