Reports

Localization of S-100 Protein in Müller Cells of the Retina— 1. Light Microscopical Immunocytochemistry

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S-100 is an acidic brain protein previously found to be present in glial cells of the brain and the nervous system of gut and respiratory tract. Immunocytochemistry at the light microscopical level localized immunoreactivity for S-100 in the Müller cells in the retina of rat, guinea pig, and Chinese hamster. The Müller cells represent the main glial component of the retina, with a structural role in the support and insulation of neurons and sensory elements. The use of S-100 protein as an immunocytochemical marker of Müller cells may be useful in the study of pathologic conditions of the retina where glial cell proliferation could reflect the index of neuronal injury. Invest Ophthalmol Vis Sci 24:976–980, 1983

S-100 is an acidic protein that was isolated originally from bovine brain by Moore[1] and subsequently was found to be widely distributed in the nervous system of many mammals.[2] It is generally thought that in both central and peripheral nervous systems, the main localization of this protein is in glial elements, as recently demonstrated in the nervous system of the gut[3] and the respiratory tract.[4]

The morphologic identification of the glial cells is of great importance for the study of their functional relationship with neurons in normal and diseased conditions. The retina, because of its relatively simple organization and the particularly abundant glial component, represents an unique model for the study of neuronal and non-neuronal elements of the nervous system. Although various retinal neuronal elements have been recognized by immunocytochemistry,[5] the identification of glial cells of the retina is still based on silver impregnation methods as originally used by Cajal,[6] who supplied the classic anatomic description.

The retina contains various forms of glial cells, such as astroglia, microglia, perivascular glia, and Müller cells. In most species, it is this last type that constitutes the main structural framework.[7] Although Müller cells have mainly a structural role, it is thought that they could also provide nutrients to the surrounding cells and modulate neuronal sensitivity.[7–9] It is evident that Müller cells are an important retinal constituent and could be involved in the functions of normal and diseased retina[10,11]; their identification by the use of a suitable marker is thus of primary relevance. In this study, we report on the immunocytochemical visualization at light microscopic level of Müller cells in the mammalian retina by using the presence of S-100 protein as a marker for glial cells.

Materials and Methods. Albino rats (n = 5), guinea pigs (n = 5), and Chinese hamsters (n = 4) were used. The animals were killed by exsanguination under ether anaesthesia; the eyeballs were removed and immediately processed. The whole eye was fixed by immersion in a solution of 0.4% benzoquinone in 0.01 M phosphate-buffered saline (pH 7.1–7.4) at 4°C for 30 to 45 min and then rinsed several times in phosphate-buffered saline. After fixation, an incision was made at the level of the ora serrata and the posterior half of the eye was separated from the anterior ocular structures.

Cryostat blocks were prepared from the eyecup with the retina in situ and sections were cut at 10 μm and 20 μm thickness. Immunocytochemistry was carried out according to the indirect immunofluorescence method,[12] using rabbit S-100 antiserum[13] at a dilution of 1/400 in phosphate-buffered saline. The sections were incubated with primary antibody for 16 to 20 hours at 4°C. Control sections were incubated with non-immune rabbit serum or first layer antiserum preabsorbed with purified S-100 protein (20 μg/ml diluted antiserum).

Results. In all the animals, the immunoreactive S-100 appeared to be localized in those retinal cells corresponding to the classical description of Müller cells (Fig. 1). Since these cells appeared evenly distributed in all retinal regions and presented a similar morphologic appearance in all three species, a single description of the results will be given. However, the frequency of the cells varied between species, being most numerous in the retina of Chinese hamster and least numerous in the guinea pig retina.

The perikarya of the Müller cells appeared to be

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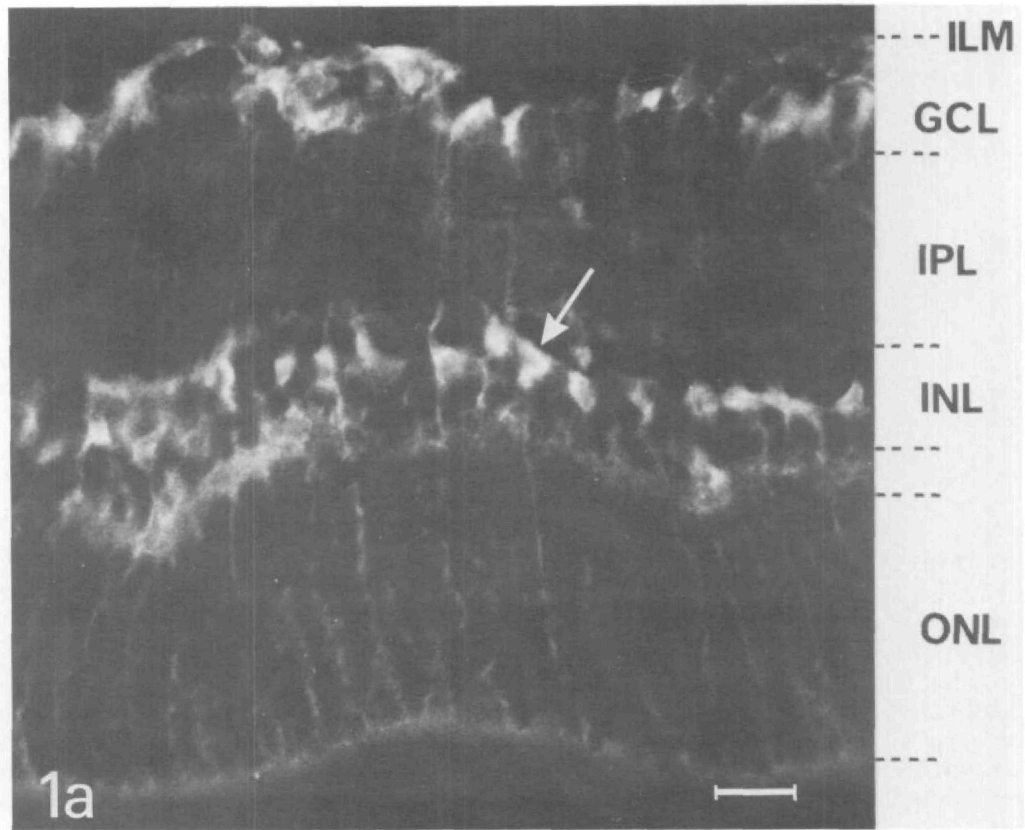
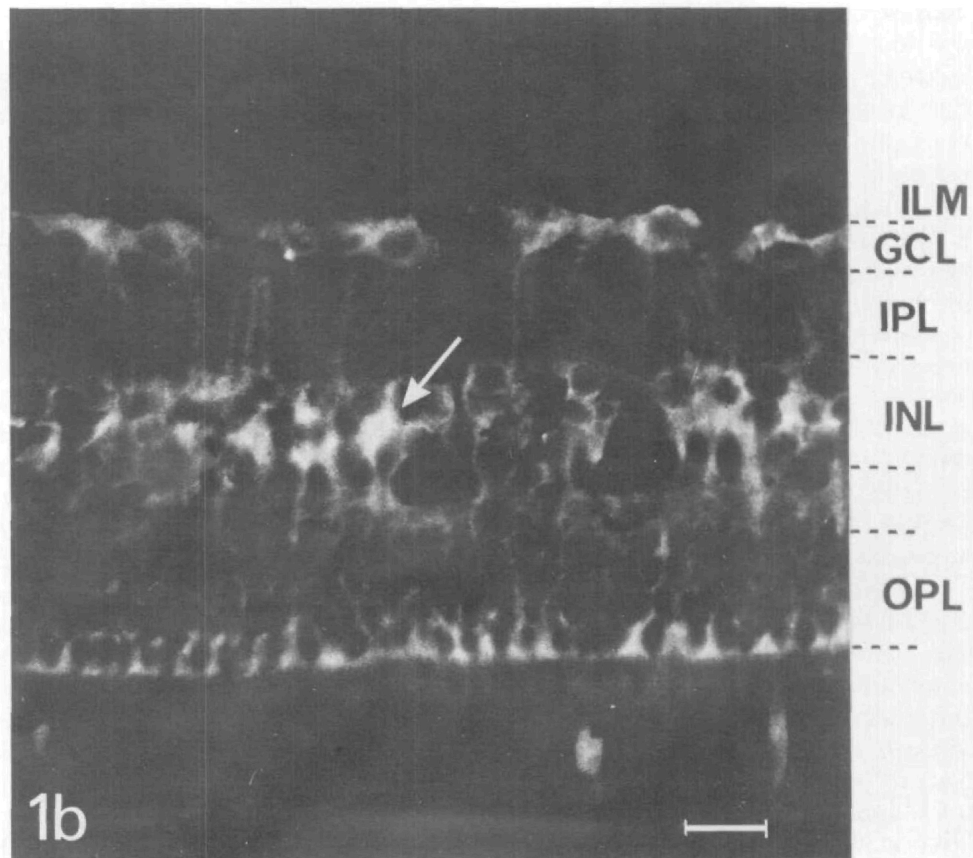


Fig. 1. S-100 immunoreactivity localized to Müller cells of: **A**, rat retina (×540; scale bar = 20 μm); **B**, guinea pig retina (×540; scale bar = 20 μm); **C**, Chinese hamster retina (×460; scale bar = 30 μm). Twenty-micrometer sections. The cell bodies are clearly visible in the inner nuclear layer (INL) (arrows), with long processes radiating in opposite directions, forming a network of fibers in the outer nuclear layer (ONL) and terminating on the inner limiting membrane (ILM) with a pedicule-shaped ending. ILM = inner limiting membrane; GCL = ganglion cell layers; IPL = inner plexiform layer; INL = inner nuclear layer; ONL = outer nuclear layer.



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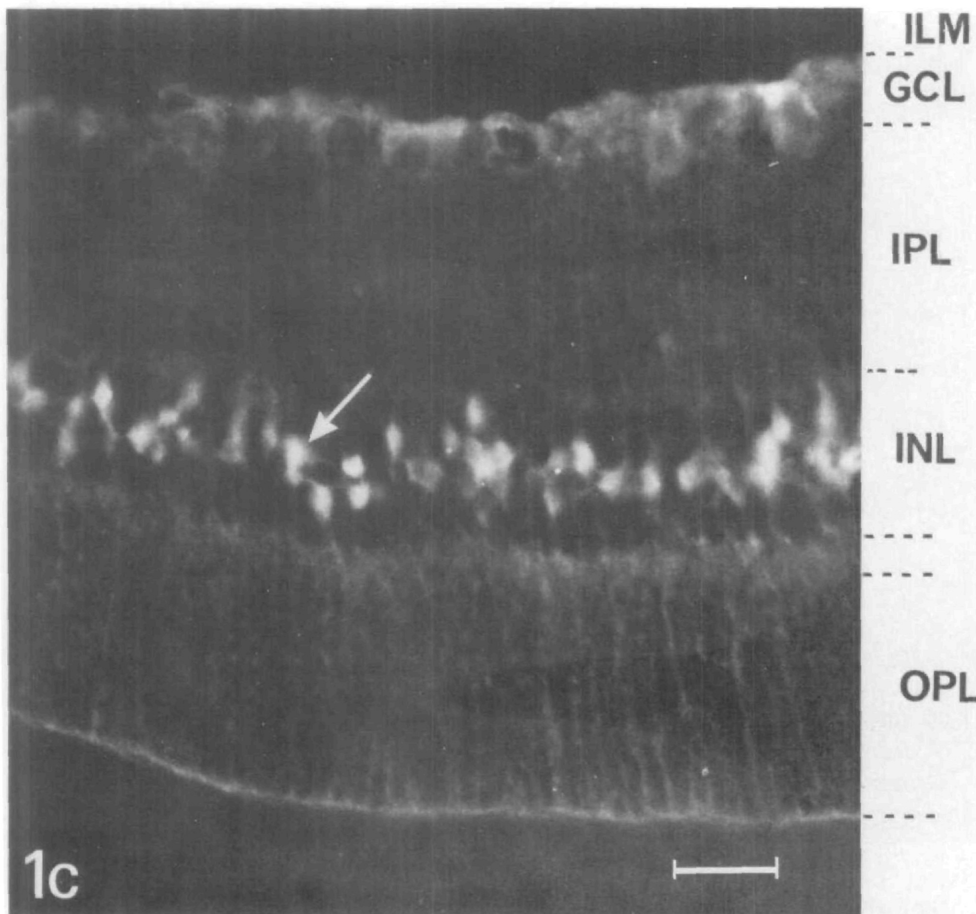

Fig. 1. (*Continued*)

localized in approximately the middle of the inner nuclear layer; the cell bodies were irregular in shape, very often showing a concave outline (Fig. 2). This feature is characteristic of the Müller cells and is consistent with their supportive role and their ability to fill the gaps between the rounded neuronal cell bodies. The main long processes radiated from the cell body in opposite directions, occupying the full thickness of the retina. In the centrifugal direction, the processes presented numerous ramifications at the level of the outer nuclear layer, giving rise to a meshwork of fibres which completely surrounded the photoreceptor cell bodies. In the opposite direction, the processes reached the more inner layers of the retina, generally terminating in a pedicle that appeared to be in contact with the inner limiting membrane (Fig. 3). At the level of the ganglion cell layer, the processes showed ramifications that surrounded most of the ganglion cells. In this layer, a few small cells immunoreactive for S-100 were also observed, probably corresponding to astroglial elements (Fig. 3); they appeared sporadically distributed throughout the retina and showed irregular cell bodies with comparatively short ramifications mainly confined to the same layer.

Discussion. We report here on the immunocytochemical localization at light microscopic level of S-100 in the retina of three types of small mammals. The immunoreactivity for S-100 is localized mainly to cells corresponding to Müller cells, as described by Cajal.[6] No immunoreactivity was observed in ganglion cells, as reported recently by Linser[14] in avian retina.

The possibility that S-100 immunoreactivity could be localized to either bipolar or interplexiform cells, both retinal elements with centrifugal and centripetal neuronal ramifications, was ruled out on the basis of the characteristic morphological appearance of the immunoreactive cells. These immunoreactive cells resembled Müller cells but not bipolar or interplexiform cells, as they showed indented nuclei, processes occupying the full thickness of the retina with pedicle-shaped terminations on the inner limiting membrane and extensive networks of fibers surrounding the sensory cell bodies.

Our results are consistent with the findings of S-100 protein in glial cells of other areas of the central[2,15] and peripheral nervous system[3,4,13] and emphasize the similarities of Müller cells and glial elements.

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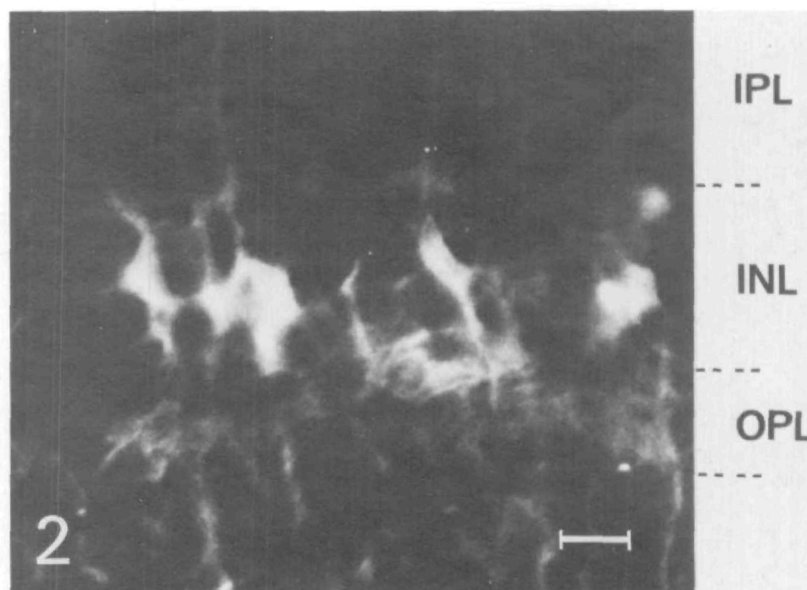

Fig. 2. Rat retina, 10-μm section, S-100 immunoreactivity in Müller cell bodies, showing details of the irregular and indented outline (×880; scale bar = 10 μm). IPL = inner plexiform layer; INL = inner nuclear layer; OPL = outer plexiform layer.

Although the Müller cells form a supportive and insulating framework for the neuronal elements, it is thought that they participate actively in the complex functional mechanism of the retina. The high content of glycogen and of enzymes related to glycolytic metabolism indicate clearly their probable role in providing energy to the surrounding neurons.[8] It has also been suggested that by occupying all the spaces between neurons, they contribute in reducing the extent of optical damage to the incident retinal image.[9] In more general terms, if injury in neuronal parenchyma can be visualized as a proliferation of glial elements, immunostaining for S-100 protein might provide a useful means of assessing the extent of the damage. As an example, Müller cells have been shown to be involved in the accumulation of increased glycogen deposits in the retina of Chinese hamsters affected by severe diabetes.[10] The potential use of S-100 protein as a diagnostic marker of retinal diseases is also shown by the finding of glial elements in retinoblastoma, which is thought to originate from retinal glial cells.[11]

In conclusion, the Müller cells, like glial elements of other systems, contain S-100 protein, which could represent a useful immunocytochemical marker for the investigation of pathologic conditions of the retina.

Key words: glia, immunocytochemistry, Müller cells, retina, S-100 protein

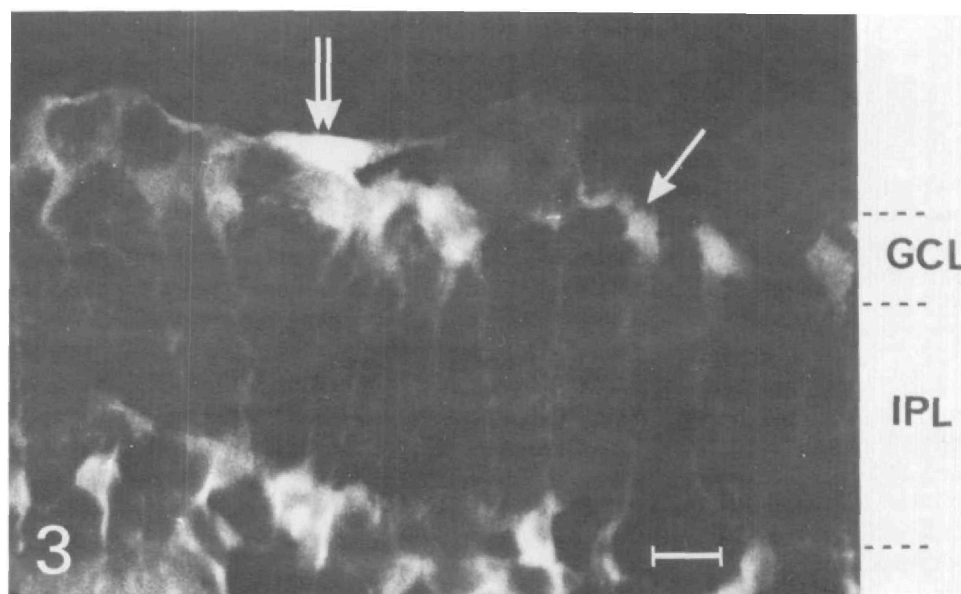

Fig. 3. Rat retina, 10-μm section. S-100 immunoreactivity in the pedicule-shaped processes of the Müller cell terminating on the inner limiting membrane (arrow). An astroglial cell (double arrow) also shows S-100 immunoreactivity (×880; scale bar = 10 μm). GCL = ganglion cell layer; IPL = inner plexiform layer.

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Localization of S-100 Protein in Müller Cells of the Retina— 2. Electron Microscopical Immunocytochemistry

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The cellular and subcellular distribution of S-100 protein was investigated at the ultrastructural level in the rat retina by the immunocytochemical PAP method. S-100 appeared to be localized in the cytoplasm and nucleus of Müller cells, offering conclusive evidence that in the mammalian retina, the protein is confined to glial cells. S-100, as a marker for Müller cells, may be a useful tool in order to study the cytoarchitecture of the retina in normal as well as in pathologic conditions. In addition, the retina may represent a suitable model for further investigation on the biologic role of S-100. Invest Ophthalmol Vis Sci 24:980–984, 1983

S-100[1] is an acidic Ca^{2+}-binding protein originally isolated from the brain where it is found primarily in the cytoplasm and nucleus of glial cells,[2] although additional locations outside the nervous system have also been described.[3-9] The previous paper[10] reports the specific localization of S-100 in Müller cells in the retina of three rodent species as studied by light microscopic immunocytochemistry. We report here on the ultrastructural distribution of the protein in the rat retina, conclusively stating that S-100 may be regarded as a marker specific to Müller cells in the mammalian retina.

Materials and Methods. Adult male Wistar rats (n = 5) were anesthesized with sodium penthobarbital (3–4 mg/100 g) and perfused through the heart with 4% paraformaldehyde (0.1 M phosphate buffer, pH 7.4). The eyeball was sectioned along the equatorial

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