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Identification of astrocyte- and oligodendrocyte-like cells of goldfish optic nerves in culture

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Fish glial cells were obtained from cultivated segments of the optic nerve and raised in vitro. Two types of cells were identified as astrocyte- and oligodendrocyte-like glia by the monoclonal antibody Mab O_1 (specific for oligodendrocytes) and the rabbit serum anti-goldfish glial fibrillary acidic protein (anti-G-GFAP). Cells of compact morphology were rare, and anti-G-GFAP positive and O_1 negative. Multipolar cells in 5-day-old cultures were anti-G-GFAP but rarely O_1 positive. In 5-week-old cultures, however, roughly 75% of the multipolar cells were double-labeled with both anti-G-GFAP and O_1; 10% were single labeled with Mab O_1 and 15% with anti-G-GFAP, respectively.

Since goldfish retinal axons regenerate readily after injury it has been supposed that the glial cells of the fish optic nerve possess growth-promoting properties [5, 8]. Compared to mammals very little is known about glial cells in fish, one reason being that it has been difficult to identify fish astrocytes by immunohistochemistry [3, 5]. To test the interaction of regenerating axons with glial cells in vitro requires that glial cells can be raised and identified in culture. This was the goal of the present study. We developed and describe here a simple method how to obtain and raise fish glial cells of goldfish optic nerves in culture. Of those cells two types are astrocyte- and oligodendrocyte-like cells.

Previously transected and normal optic nerves from goldfish (7–10 cm) were isolated, divided into small pieces (1 mm^3) and incubated in Ca^{2+}-free phosphate-buffered saline (PBS) for 5 h at 4°C. These pieces were transferred onto polylysine/laminin-coated coverslips, covered with Leibowitz medium (L15, complemented with 10% fetal calf serum) and kept at 22°C. Cells began to emigrate and were cultured up to 6 weeks.

Immunostaining of cultured cells: living cells were incubated with the monoclonal

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antibody O_1 [9] (Mab O_1, supernatant of a hybridoma cell line), diluted 1:2 in L15, for 30 min at 20°C. Cells were permeabilized in methanol (−20°C, 5 min) fixed in formaldehyde (2% in PBS, 10 min), and subsequently incubated with a polyclonal rabbit antiserum [6] which was raised against a gel band of 51 kDa from a goldfish brain filament preparation (diluted 1:400 in PBS, 1 h at 20°C) (anti-goldfish GFAP (anti-G-GFAP)). The secondary antibodies (goat anti-mouse IgM rhodamine-coupled, and goat anti-rabbit IgG fluorescein-coupled) (Dianova) were applied for 1 h at 37°C.

Immunostaining on frozen sections: frozen sections (10 μm) of unfixed eyes, optic nerves and brains on polylysine-coated slides were fixed with methanol (−20°C). They were incubated simultaneously with Mab O_1 (diluted 1:2 in PBS) and anti G-GFAP (diluted 1:400) for 1 h at 37°C, and then with the secondary antibodies as described above. In some experiments, anti-G-GFAP was replaced by rabbit antiserum against bovine GFAP (kindly provided by L. Eng).

Cocultures of glial cells and goldfish retinal axons were obtained by explanting a segment of an isolated goldfish retina onto the coverslips which carried the cells. Contacts of growing axons with living cells were monitored with time lapse videomicroscopy. Immunostaining of glial cells in these cocultures was as described above.

In sections, Mab O_1 showed intense and specific staining on the myelinated axons in the optic fiber layer of the retina, in the optic nerve and in the tectum. These results

Fig. 1. Phase contrast micrographs of living multipolar glial cells after 4 weeks in culture. Cells have veil-like membraneous protrusions on their processes (arrows). Bar in a = 50 μm, in b = 100 μm.

suggest that the antibody binds to processes of fish oligodendrocytes as it does in mammals [9]. Anti-bovine GFAP labeled a few radial glia processes and, occasionally, processes in the fish optic nerve [3]. Anti-G-GFAP [6], however, intensely stained numerous radial glia processes throughout their extent, Müller glia in the retina and an abundance of glial cell processes which partition the bundles of O_1 positive retinal axons in the regenerating optic nerve and tectum. Anti-G-GFAP obviously detects cell processes which were, based on morphological criteria, identified as astrocytes [4, 10].

Within 24 h after explantation, cells emigrated from nerve explants and spread on the polylysine/laminin-coated glass surface. Two types of cells, positive for anti-G-GFAP and Mab O_1 are considered here. The majority of these cells are bi- or multipolar emitting long processes of up to 300 μm in length, and with veil-like membraneous protrusions (Fig. 1a). Cells were either aligned in parallel or randomly oriented and in contact with other cells (Fig. 1b). These cells increased steadily in number during the first 2 weeks in culture. The compact cells were morphologically distinct and always only anti-G-GFAP positive (Fig. 2a, b). They were rare in 5-day- as well as in 5-week-old cultures.

After 5 days in vitro, most multipolar cells were anti-G-GFAP positive and Mab O_1 negative (Fig. 3a, b). This situation was different in 5-week-old cultures. Roughly 75% of the cells were double-labeled displaying intensively stained anti-G-GFAP-positive fibrils while the surfaces of their membraneous extensions were O_1 positive (Fig. 3c, d). The number of multipolar cells which were either anti-G-GFAP or O_1 positive comprised approximately 15% and 10% respectively. The intensity and distribution of anti-G-GFAP and O_1 staining in double-labeled cells was variable. Cells with numerous brightly stained anti-G-GFAP-positive fibrils had membraneous extensions which were weakly O_1 positive. Cells with few anti-G-GFAP-positive fibrils were stained with Mab O_1 over their entire extent (Fig. 3e, f). Thus, the multipolar glial cells of the regenerating goldfish optic nerve coexpress molecules which are usually found separately on either differentiated oligodendrocytes or in astrocytes.

When cocultured with 5-week-old glial cells, retinal axons grew readily over all cells and their processes. The cells and their processes then associated closely with

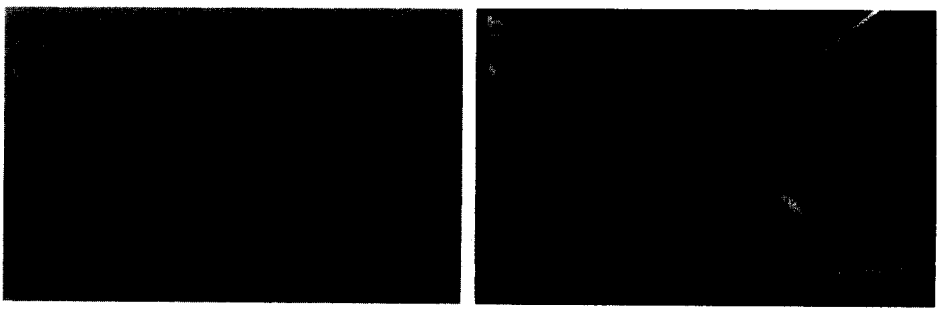

Fig. 2. Compact cell in phase contrast (a). This cell exhibits brightly stained anti-G-GFAP-positive fibrils (b), but is Mab O_1 negative. Bar = 40 μm.

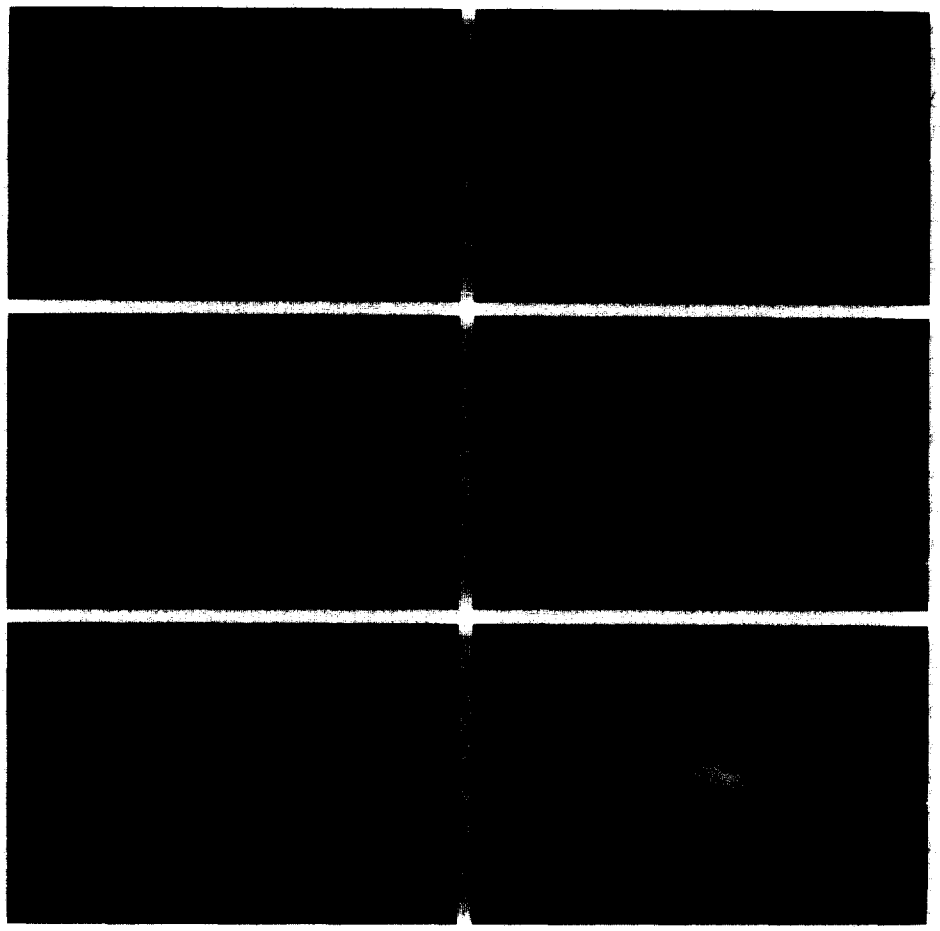

Fig. 3. Examples of multipolar cells which were treated with Mab O_1 and anti-G-GFAP. a, b: 2 cells which are G-GFAP positive (a) and Mab O_1 negative (b). c, d: this cell is labeled with anti-G-GFAP (c) and Mab O_1 (d). e, f: cell containing a few G-GFAP-positive fibrils (e) but bright Mab O_1 staining on its surface (f). Bar = 100 μm for all panels.

the axons. Using these cocultures we tested whether the presence of retinal axons would influence the expression of the antigens recognized by anti-G-GFAP and Mab O_1. Regardless of whether staining was carried out 3 or 14 days after addition of the retina explant, cells contacting axons were still double labeled, containing anti-G-GFAP-positive fibrils and O_1-positive cell surfaces (Fig. 4). Obviously, the association with axons did not drive the cells to express exclusively one of these antigens.

We have established a method which allows to cultivate glial cells from the goldfish optic nerve. The presence of Mab O_1 and anti-G-GFAP immunoreactivity on cells in culture suggests that these are astrocyte- and oligodendrocyte-like cells. Many cells coexpress O_1- and anti-G-GFAP-positive sites, suggesting that these cells in culture do not develop into either astrocytes or oligodendrocytes but maintain characteristics

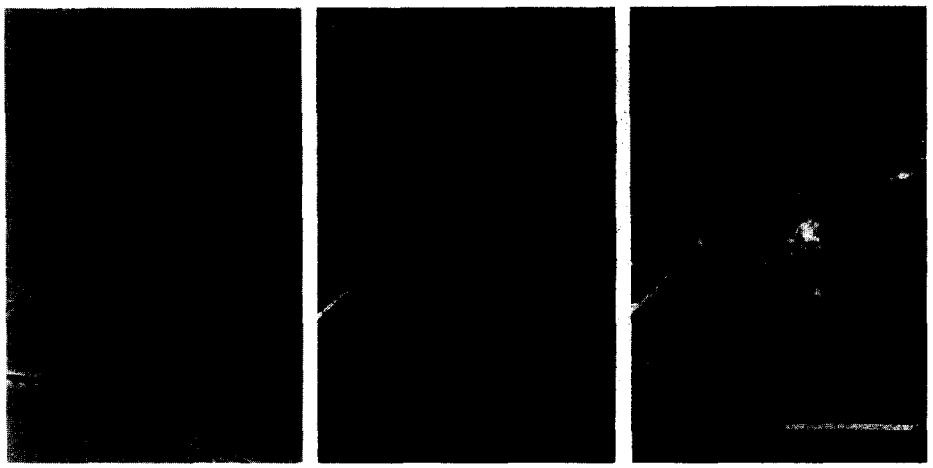

Fig. 4. Coculture of retinal axons and glial cells. Phase contrast micrograph. Some axons are marked by arrows (a). Cells are in contact with axons and are anti-G-GFAP positive to variable extents (b). Cells except the one marked by an arrowhead are O_1 positive (c). Bar = 100 μm.

of both cell types. According to morphological criteria, the fish optic nerve seems to possess distinct astro- and oligodendrocytes [4, 10]. It is possible that the multipolar cells in culture require a specific factor to differentiate into astro- and oligodendrocytes and that their association with growing axons is not sufficient for this process.

In mammals the progenitor cells for astrocytes type II and oligodendrocytes occasionally express astrocyte- and oligodendrocyte-specific markers [7]. Our information on the fish glial cells is too sparse to determine whether the multipolar cells are equivalent to the mammalian progenitor cell. Moreover, glial cells in the fish optic nerve divide and increase in number after nerve injury and it remains to be investigated whether such lesions may provoke a change in glial cell fate in fish.

Mammalian astrocytes type I derive from a separate progenitor cell [7]. No information is available on whether the fish optic nerve has equivalents to astrocytes type I and II. The compact cells, which are morphologically different from the multipolar cells, are solely anti-G-GFAP positive and never O_1 positive, and apparently do not proliferate in culture. To decide whether they are descendants of the multipolar cells or whether they represent a separate type of astrocyte requires further investigation. Glial cells from injured fish optic nerves were reported to produce factors which stimulate axonal regeneration, not only in fish but also in mammals [8]. Glial cell cultures as those described here may help to identify the molecules involved in promoting axonal regrowth. We have demonstrated earlier that fish retinal axons collapse and retract upon contact with O_1-positive rat oligodendrocytes [1], known to possess unfavourable substrate properties [2]. We are now interested in monitoring the encounter of retinal axons with O_1-positive glial cells of fish.

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