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Neurons and glia in purified retinal cultures identified by monoclonal antibodies to intermediate filaments

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Two monoclonal antibodies known to bind intermediate filaments were used to identify neurons and glia from embryonic chick neural retina. Neurofilament specific antibody RT-97-F1, bound neuroepithelial cells, migrating neurons, as well as the photoreceptor layer, plexiform layers and optic fiber layer. The other, 3A7, bound intermediate filaments of Müller cells. In purified neuronal cultures, RT-97-F1 bound 75, 83 and 98% of the cells present at 24, 48 and 72 h respectively, while 3A7 bound 26, 15 and 0.3% for the same times *in vitro*. In purified glial cultures, RT-97-F1 showed a weak perinuclear binding, while 3A7 strongly bound intermediate filaments of nearly 100% of the cells. These antibodies confirmed and quantitated the high purity of our cultures.

Cultures of purified neurons or purified glia from the retina have been widely used in the study of neuronal development and function [3, 5–7, 11, 12]. Monoclonal antibodies have proven to be an important tool in the identification of *in vitro* cell types [4, 9, 10, 13]. An earlier description of purified neuronal cultures utilizing tetanus toxin binding and thymidine labeling suggested that 80–90% of the cells in these cultures were neuronal [2]. On the other hand, using these markers and glia specific monoclonal antibodies, the ‘flat’ cells of mixed cultures have been identified as glia [2, 8, 13]. We have now undertaken to reexamine the purity of our neuronal and glial cultures using two antibodies. The monoclonal antibody RT-97-F1, binds specifically to the 210,000 subunit of neurofilaments [16, 17]. The monoclonal antibody 3A7 has been shown to bind specifically to intermediate filaments of Müller cells in the retina [8].

Purified cultures of retinal neurons or glia were prepared from E8 White Leghorn chick retina as previously described [2] and seeded on polyornithine-coated, serum-

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treated glass coverslips at 600 cells/mm² in our previously described defined culture medium, R6 [18]. Retina from E6 and E12 chicks were fixed in 4% paraformaldehyde–0.01% glutaraldehyde and prepared for cryostat sectioning or paraffin embedding. Sections or cultures were fixed at the times indicated, washed in phosphate-buffered saline (PBS), treated with 0.05% Triton-X 100 before being incubated either with hybridoma supernatant for 3A7 or a 1:100 dilution of the RT-97-F1 antibody. Samples were then washed and incubated with a fluorescein-labeled goat anti-mouse IgG (Cappel) diluted 1:500 in PBS/10% horse serum before viewing or with a 1:400 dilution of a biotinylated mouse IgG (Vector Labs.) followed by incubation with avidin–biotin–peroxidase (Vectastain, Vector) and diaminobenzidine as a peroxidase substrate. Sections treated as above, but without the incubation with the primary antibody showed no staining. The number of labeled cells was determined as previously described [2].

In sections of E6 retina, RT-97-F1 antibody labeled cells in the outer layer of the neural retina and the optic fiber layer (Fig. 1a). Based on their morphology and position, these cells appeared to be neuroblasts or migrating neurons. Presumptive neuroblasts were also identified by the binding of RT-97-F1 to the mitotic apparatus (Fig. 1a, insert). The binding of RT-97-F1 to the mitotic apparatus is likely due to either a protein meshwork surrounding the mitotic apparatus or histones. The nuclear lamina contains intermediate-type filament proteins [1] and histones share antigenic determinants with neurofilaments [17]. In the case of migrating neurons, labeling could be seen in both the soma and the leading processes of these cells (Fig. 1a). In sections of E12 retina, RT-97-F1 bound to the photoreceptor layers, the inner and outer plexiform layers, and the optic fiber layer (Fig. 1b). There was generally no somal labeling observed in the inner nuclear or ganglion cell layer of E12 retina, except for an occasional cell which was probably a late migrating neuron. As previously reported [8] 3A7 antibody bound only to Müller cells of the retina (Fig. 1c).

In purified neuronal cultures, RT-97-F1 bound to most of the cells observed (Fig. 2a, b), staining both the soma and neuritic processes. This was similar to what has been reported for cultured hypothalamic neurons [14]. The antibody 3A7 bound to a limited number of cells in purified neuronal cultures, with staining primarily, but not exclusively in the soma (Fig. 2c). In glial containing cultures, as previously noted, 3A7 bound to flat cells, indicating their glial nature (Fig. 2d), whereas RT-97-F1 did not bind to these glial cells, except for a weak perinuclear binding similar to what is seen elsewhere [17] (data not shown). This perinuclear staining was not seen in the intact retina suggesting it is unique to glia only when they are flat cells and is likely due to histones or the nuclear lamina of these cells.

We chose to quantitate the number of cells, in purified neuronal cultures, which bound RT-97-F1 or 3A7 antibodies as a function of time in culture. After 24 h in culture, we found that RT-97-F1 bound approximately 75% of the cells, at 48 h the percentage of labeled cells rose to 83% and at 72 h 98% of the cells in culture were labeled with the RT-97-F1 antibody (Fig. 3). In contrast, at 24 h, 3A7 bound approximately 26% of the cells in culture, this fell to 15% at 48 h and decreased to 0.3% at 72 h. Thus, the presence of glial cells in the purified neuronal cultures is limited and

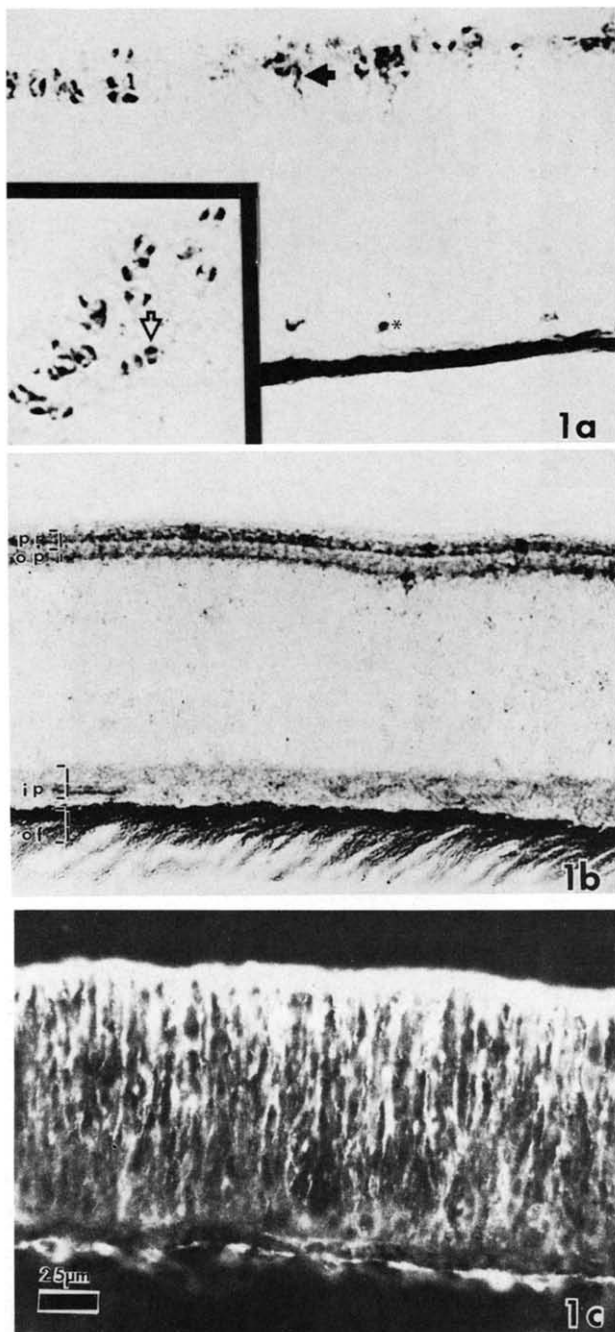


Fig. 1. Intermediate filament staining of retina. (a) E6 retina showing RT-97-F1 positive labeling in the outer cell layer and the optic fiber layer. Note a stained neuron with a vitreal process (solid arrow) and stained cells in the ganglion cell layer (*). The insert is a more peripheral area of the same retina with stained mitotic apparatus (open arrow). (b) E12 retina showing RT-97-F1 positive staining of the photoreceptor layer, outer and inner plexiform layer, and the optic fiber layer. (c) E6 retina showing 3A7 staining of Müller cells. pr, photoreceptor layer; op, outer plexiform layer; ip, inner plexiform; of, optic fiber layer.

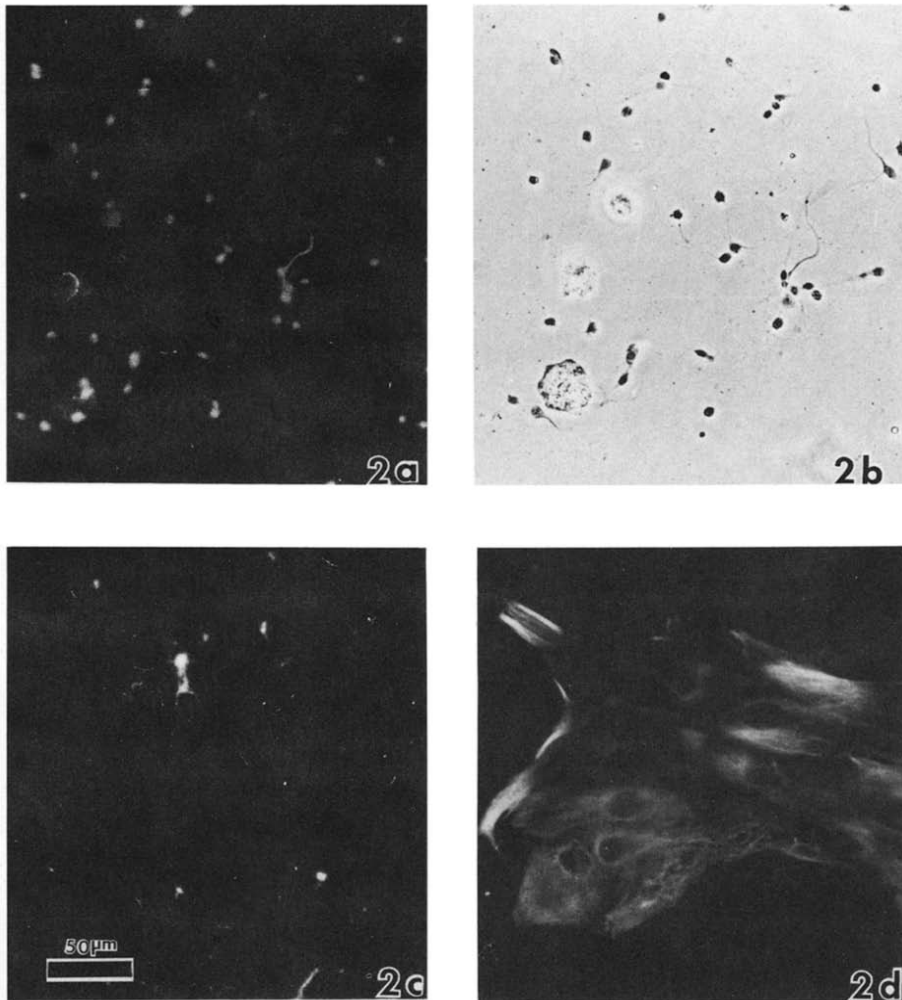
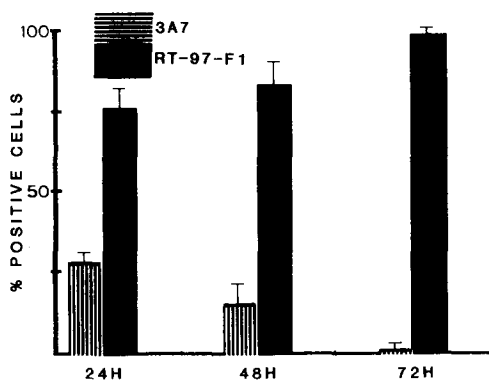


Fig. 2. Intermediate filament staining in purified neuronal cultures. (a, b) Fluorescence and phase micrographs of 48 h neuronal cultures stained with RT-97-F1 antibody. (c) Fluorescence micrograph of a 48 h neuronal culture stained with 3A7 antibody. (d) Fluorescence micrograph of a 7 day in vitro glial cultures stained with 3A7 antibody.

glia fail to survive beyond 48 h in vitro when cultured under the conditions used to produce purified neuronal cultures.

In conclusion, the antibody RT-97-F1 in the retina binds to immature neurons, the photoreceptor layer, the plexiform layers, and the optic fiber layer. The use of the RT-97-F1 neural antibody and the 3A7 glial antibody indicates that our purified neuronal cultures are significantly free of glial cells. It is of interest to note that the number of glial cells in the culture falls dramatically within the first 48 h in vitro. It was previously observed that multicellular clumps are an absolute requirement for the generation of glial flat cells in retina monolayer cultures [2]. It now appears that



ANTIBODY LABELED CELLS IN NEURONAL CULTURES

Fig. 3. The percent of positive antibody labeled cells in neuronal cultures. Neuronal cultures were stained at 24, 48 and 72 h with RT-97-F1 or 3A7 antibody. The total number of cells and the number of labeled cells were quantitated by strip counting 2-4, 0.1×20 mm strips using light and fluorescence microscopy. The average number of cells per culture was 47.2×10^3 , 40.5×10^3 and 35.5×10^3 for 24, 48 or 72 h cultures respectively. Results represent the percentage \pm S.E.M. of labeled cells per culture from an average of 3 experiments.

the techniques used to generate our purified neuronal cultures (highly adhesive substratum and low seeding density), designed to prevent the formation of multicellular clumps, also are unfavorable for the initial survival of glia *in vitro*. This study has further demonstrated the high degree of purity of our neuronal cultures, particularly after 48 h *in vitro*. This information serves as an important guideline in the study of neuronal development *in vitro*.

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