

Images in Cellular / Molecular Medicine

Phagocytosis of apoptotic cells by microglia *in vitro*

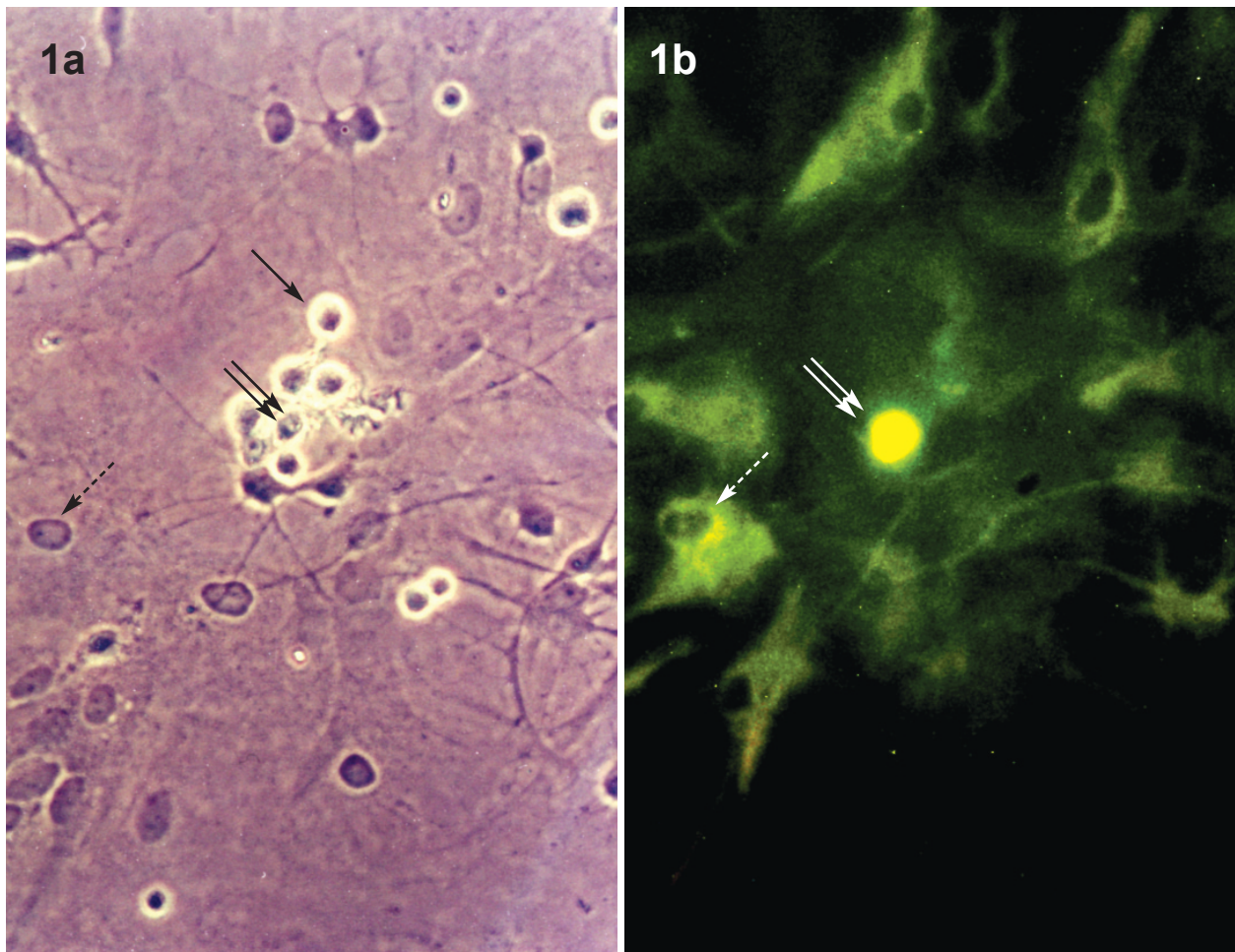


Fig. 1 Phagocytosis of apoptotic cells in glial cell culture. **a.** Phase-contrast microscopy; **b.** Fluorescence microscopy. Some apoptotic astrocytes (double arrow) from glial cell cultures (obtained after 8-10 days from rat brain cell cultures), were phagocytized *in vitro* by microglia (arrow). Astrocytes (dotted arrow) were evidenced by intracellular staining for glial fibrillary acidic protein (GFAP) after fixation, permeabilization and incubation with monoclonal anti-GFAP antibody and anti-mouse IgG-FITC conjugated secondary antibody (green). Nikon-Eclipse TE 300 Microscope, Ob. 40.

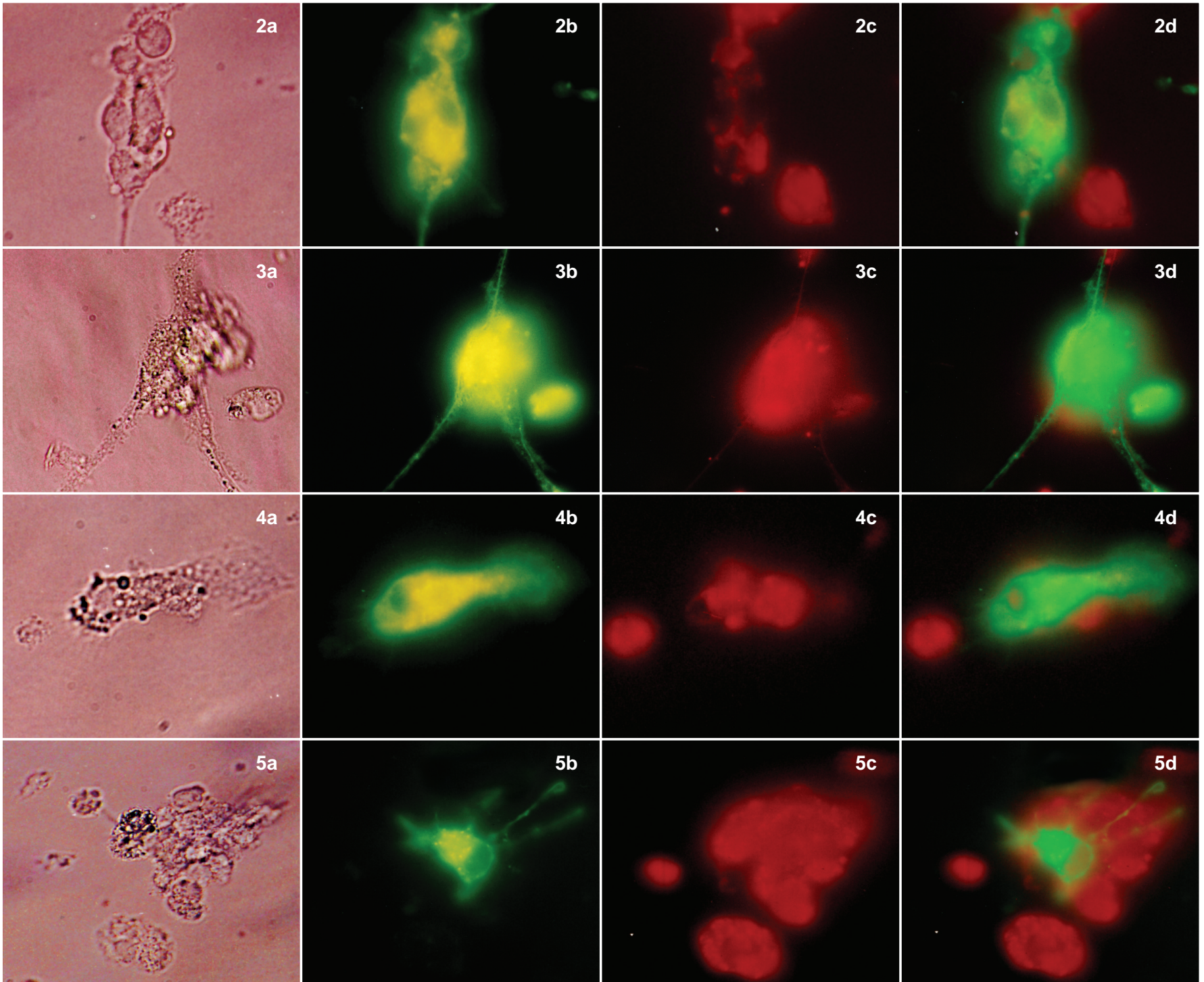


Fig. 2, 3, 4, 5 Phagocytosis of apoptotic cells by microglia in co-cultures of microglia and apoptotic astrocytes. Using a shaking and adhesion procedure we obtained two kinds of cultures: microglial cell cultures (by shaking the culture dishes, microglia were separated and cultured in another dish) and astrocyte cell cultures (the cells that remain after microglia removal). Apoptosis was induced with Staurosporine, 4 μ M/ml for 3 hours. Apoptotic cells were evidenced with annexin V-Cy3 (red). Apoptotic astrocyte cells from astrocyte cultures were co-cultured with microglia from microglial cultures (5:1). Phagocytosis was evidenced by fluorescent and phase-contrast microscopy after 2-3 hours in cultures. Microglial membranes (green) were stained with PKH67. **a.** Phase-contrast microscopy. **b, c, d.** Fluorescence microscopy. Nikon-Eclipse TE 300 Microscope, Ob. 60.

Microglia, mononuclear phagocytes present in the CNS play a crucial role in recognition and phagocytic removal of apoptotic neurons or glial cells. Apoptotic cells generate a complex surface signal recognized by different receptor systems on microglia [1]. When activated, these macrophage-like cells assume a more amoeboidal form and actively phagocytose cell and tissue debris [2]. This occurs in a number of situations ranging from

normal development, where microglia phagocytize apoptotic neurons, to a variety of degenerative, traumatic or inflammatory conditions in the CNS [3]. Using a shaking and adhesion procedure, microglia can be isolated from the brain cell cultures [4].

We revealed the phagocytosis of apoptotic glial cells (apoptotic bodies) by microglia in two types of cultures: mixed culture of glial cells and co-culture of microglia with apoptotic astrocytes.

Keywords: phagocytosis • apoptosis • microglia • astrocytes • GFAP • annexin

References:

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