Cell death and phagocytosis in the neuroepithelium of the developing retina. A TEM and SEM study

J.A. García-Porrero and J.L. Ojeda[1]

Department of Anatomy, Faculty of Medicine of Santander, Santander (Spain), 12 July 1978

Summary. Phagocytosis by neuroepithelial cells independent of the presence of pseudopodes is observed as the main mechanism of elimination of physiologically degenerating cells during the early development of the retina in the chick embryo.

During the early development of the retina, a phenomenon of morphogenetic cell death takes place[2-4]. Dead cells are grouped to form necrotic areas, one of which is located in the ventral half of the inner wall of optic cup[5]. It has been suggested that these dead cells are eliminated by phagocytosis[6]. In this paper, we present the morphologic characteristics of the phagocytic process in this necrotic area of the chick embryo by means of light, transmision and scanning electron microscopy.

Materials and methods. Normal chick embryos ranging from stages[7] 13 to 15 were fixed for 4 h in glutaraldehyde in 0.2 M cacodylate buffer at pH 7.3 and then transferred to buffer solution where the head region was dissected free.

For light and electron transmision microscopy (TEM), the tissue blocks were postfixed in 1% osmium tetroxide for 1 h, stained in block with uranyl acetate, dehydrated through a graded series of acetones and propilene oxide, and embedded in araldite. Serial semithin sections were cut in a Jeol JUM-7 ultratome and stained with 0.1% toluidine blue in 1% sodium borate solution. Ultrathin sections of the necrotic area were stained with lead citrate[8] and examined with a Philips EM 201.

For scanning electron microscopy (SEM), the head region of the embryos were carefully fractured frontally through the level of ocular anlage under a binocular microscope. The pieces obtained were dehydrated through a series of acetones, dried by the critical point method, sputtering coated with gold and observed with a Philips SEM-501.

Results and discussion. In the course of the stages studied here, the optic vesicle undergoes invagination forming the optic cup in which an outer wall and an inner wall can be distinguished. The inner wall consists only of neuroepithelial cells which form a thick pseudostratified epithelium similar to that of the neural tube. Some of these neuroepithelial cells undergo degeneration and are clustered in the ventral half of the presumptive retina, forming a necrotic area similar to that reported in mammals[5] (figure 1). This cell death process might well be involved in the invagination which undergoes the optic vesicle, but the problem is still not clear[6] and further experimental studies should be done to confirm this hypothesis. The degenerative events of these cells under TEM are characterized by an initial increase of the electron density both of the nucleus and the cytoplasm being followed by chromatin condensations and dramatic changes of the cells shape. SEM observations confirm the cell shape changes, showing that fragmentation is a constant feature of the degenerating cells[9]. The resulting cell fragments are rounded and contrast with the high columnar shape of the healthy neighbouring cells (figures 2 and 3). Similar changes were observed in other studies of cell death, both by TEM[10-13] and by SEM[14].

Semithin and ultrathin sections show cell fragments both

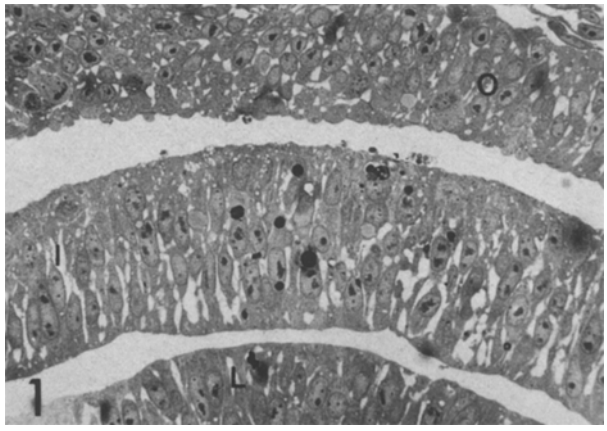

Fig. 1. Panoramic view of the necrotic area of the inner wall (I) of the optic cup. Lens placode (L), outher wall (O). Stage 14. Toluidine blue. ×400.

Fig. 2. SEM micrograph of the inner wall of the optic cup showing cell fragments free (F) and at different phases of internalization by the healthy neuroepithelial cells (large arrows). Pseudopode presence is not observed. Note the presence of cell junctions between the phagocytes and neigbouring cells (small arrow). Stage 14. ×4000.

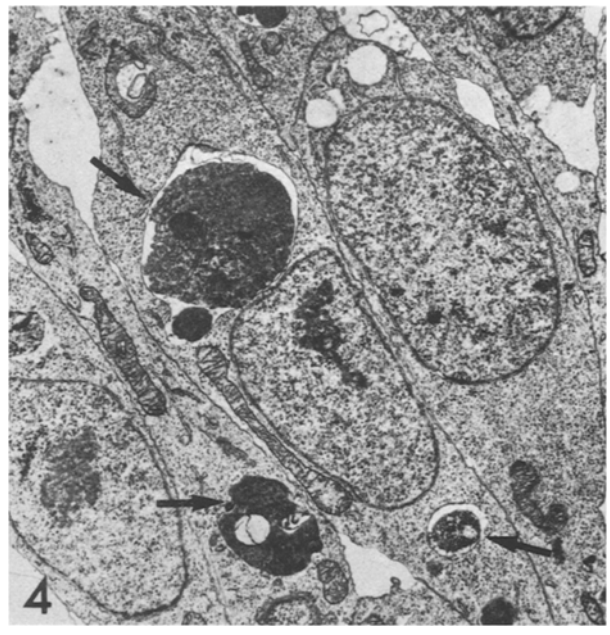

Fig. 3. SEM micrograph of the inner wall of the optic cup showing numerous cell fragments free in the extracellular space (F). Note a neuroepithelial cell containing a rounded cell fragment into a cytoplasmic invagination (arrow). Stage 15. × 4000.

Fig. 4. TEM micrograph showing cell fragments phagocytosed by neuroepithelial cells (arrows). Note that phagosomes are located close to the nuclei. Stage 14. × 5000.

free in the extracellular space and phagocytosed by the neighbouring neuroepithelial cells. The phagocytosed fragments are contained in phagosomes up to 5 μm in diameter. These phagosomes are mainly located in the perinuclear zone (figure 4) and only in some instances were they found in the cell poles. Different stages of deterioration of the phagocytosed cell fragments were observed, suggesting that they undergo progressive digestion within the phagosomes.

The SEM allowed us to follow the phagocytic uptake of cell fragments (figures 2 and 3). In the initial stage of internalization, the cytoplasm of the neuroepithelial cells which contacts the cell fragments appeared to sink in, pulling the fragments into invaginations. In the following stages, the edges of the cytoplasmic invagination undergo progressive closure over the cell fragments, these being finally enclosed into a phagosome. This process of internalization was observed mainly in the perinuclear area of the neuroepithelial cells. These phagocyting cells did not show modifications of their columnar shape and remained joined to their neighbouring cells by cell junctions.

Phagocytosis has been extensively studied, mainly in tissue culture conditions. From these studies it is now well known that 2 different mechanism of phagocytosis can be distinguished. One of them involves inmunological factors and is typical of the macrophages[15]. The other is not inmunological-dependent and takes place in numerous cell types[16–18]. The term of 'non-professional phagocytes' was proposed for these cells[19]. In our study we founded that the elimination of the dying cells is carried out by the neuroepithelial cells, where macrophages are not involved, as has been reported in the neural tube[20]. These findings are in agreement with the idea that all the cell types might behave as 'non-professional phagocyte' under the appropiate stimulus[21]. Recent 'in vitro' studies[19] showed that, while the immunological dependent uptake of particles occurs in the peripheral zone of the cytoplasm and requires involvement of pseudopodes, the non-inmunological process, on the contrary, takes place in the perinuclear region without pseudopodes involvement. Our observations in SEM are in agreement with this hypothesis, suggesting that these results can be extended to the 'in vivo' conditions.

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