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Amniotic fluid mononuclear phagocytes: Phenotypes and functions

K Polgár[1], G Ábel,[2] S Sipka[3], J Laczkó,[4] Z Papp[1]

[1]Department of Obstetrics and Gynecology [2]Institute of Pathophysiology [3]IIIrd Department of Internal Medicine [4]Central Research Laboratory University Medical School of Debrecen, Debrecen, Hungary

Sutherland et al [7] reported that amniotic fluid (AF) from pregnancies with fetuses having open neural tube defects (NTDs) had contained a cell type resembling macrophages. These cells appear as rapidly adhering cells in culture vessels 12–24 hours after cultivation.

More than a century ago in 1882 Metchnikoff discovered the function of phagocytosis by observing macrophages. We also turned to Metchnikoff's discovery and applied neutral red as a target for amniotic fluid phagocytes.

Instead of using culture technique, we have developed and introduced a rapid test for detecting viable amniotic fluid macrophages in suspension as a novel method for diagnosis of open neural tube defects. It has been found that these phagocytic cells selectively ingest neutral red dye and are easily identified as "red cells" by microscopical examination [1, 2, 4].

The question was, where these phagocytic cells of the amniotic fluid are originated from? In order to characterize them we applied the following strategy (Table I.) This conception is mainly based on the macrophage characteristics described previously by van Furth [8, 9].

Table I

The strategy of characterization of mononuclear amniotic fluid cells

Morphology
- light microscopy
- phase-contrast microscopy
- electron-microscopy

Cytochemistry
- endogeneous peroxidase
- non-specific esterase

Immunocytochemistry and immunofluorescence

detection of various cellular antigens:
- GFAP
- AFP
- lysozyme
- MO1–MO2
- EPA

Cell membrane receptors
- CR1 (C3b) receptor
- Fc receptor

Functional properties
- pinocytosis
- non-specific phagocytosis
- receptor mediated phagocytosis
- chemiluminescence
- NBI reduction

Characteristics in culture
- adherence
- division and survival

Among *morphologic properties* we studied light and ultrastructural

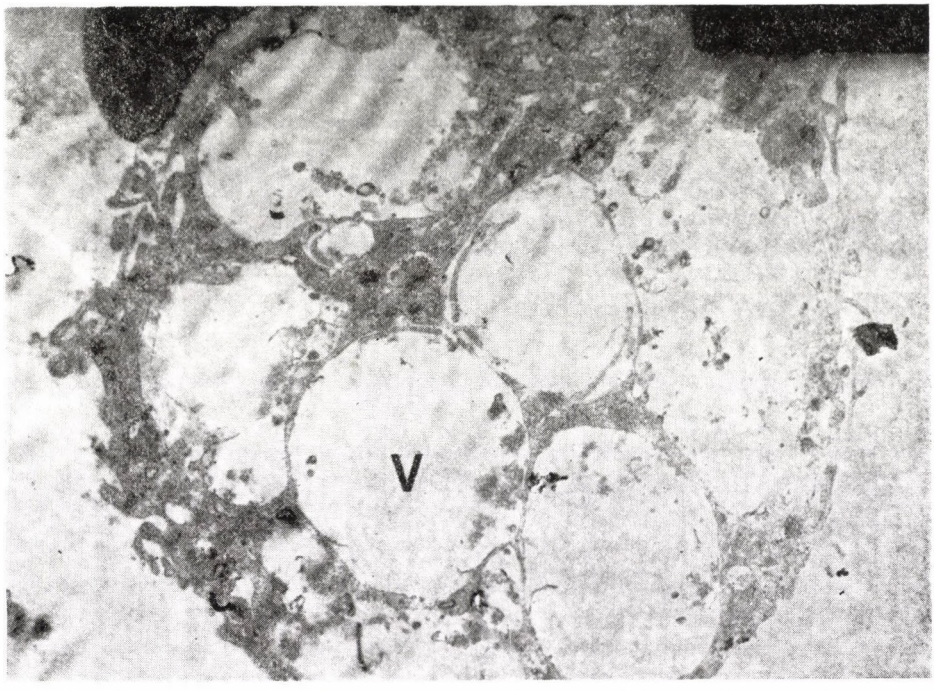

FIG. 1. Electron micrograph of an uncultured AF macrophage. In the cytoplasm several very large, irregularly shaped vacuoles (V) and intense phagocytic activity can be seen. (×20,000)

characteristics. As shown by the electron micrograph AF macrophages exhibited typical ultrastructural features. In the cytoplasm several very large, irregularly shaped vacuoles are seen with intense phagocytic activity on the surface, with large variety of phagosomes and phagolysosomes and with ingested red blood cell fragment (Fig. 1).

As it was demonstrated in our earlier studies we found *cytochemical* evidence for endogeneous peroxidase and nonspecific esterase activity in one part of these mononuclear cells [3]

We could also detect of various cellular antigens by using *immunocytochemistry* and *immunofluorescence*. Glial fibrillary acidic protein (GFAP), alpha-fetoprotein (AFP) [3], lysozyme, MO1 – MO2 [4] embryonal prealbumin (EPA) were detected by means of peroxidase-antiperoxidase method and immunofluorescence.

The GFAP specific immunostaining of rounded mononuclear cells supported the hypothesis that one part of the amniotic fluid macrophages originate from the nervous system. Pathological development of the neural tubes in fetal open lesions may be due to the direct contact between the neural tissue and the amniotic fluid. In cases of NTDs the uptake of AFP by macrophages or activated glial cells via endocytosis cannot be excluded [3].

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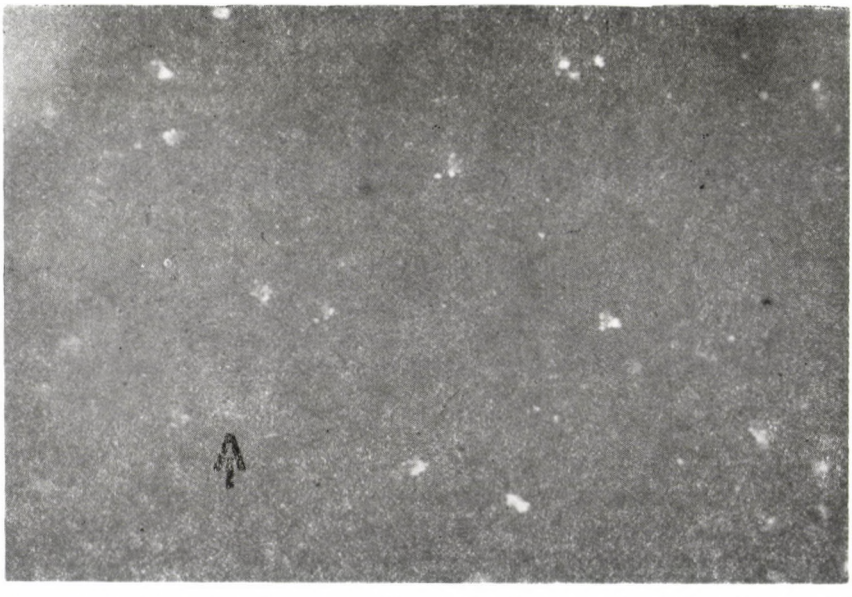

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FIGS. 3 AND 4. Positive nitroblue-tetrasolium reduction test of rapidly adhering AF cells in a case of anencephaly. The same microscopic field under light microscope (Fig. 3.) and fluorescence microscope (Fig. 4.). Note the same cell (arrows). (×200)

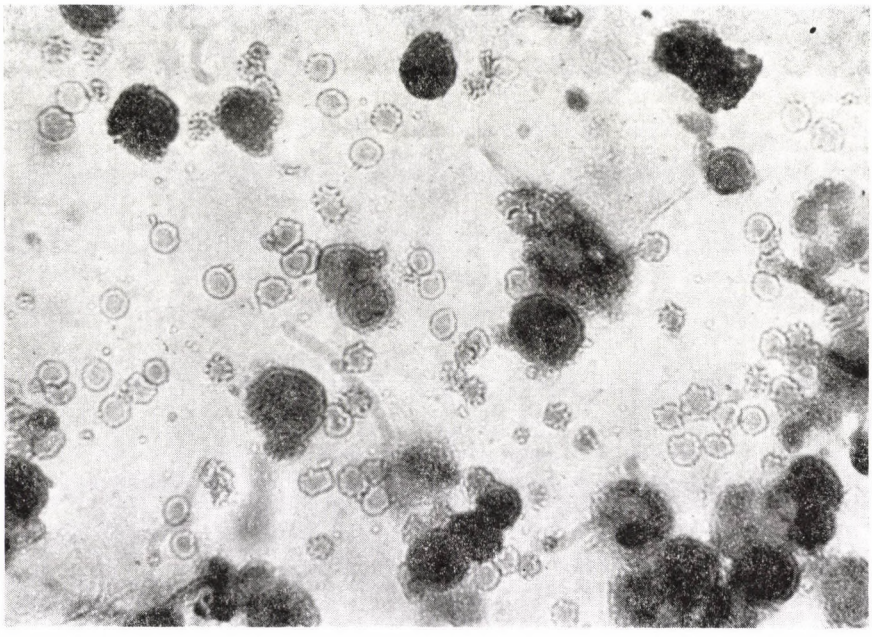

FIG. 2. Cytopsin preparation of amniotic fluid cells in a case of anencephaly: note the intense lysozyme specific immunoreactivity of rounded mononuclear cells. (×400)

Lysozyme positivity of AF macrophages (Fig. 2.) provided further evidence for the monocytic origin of one part of these cells. The findings that pathognomonic cells has MO1 – MO2 positivity suggest that AF mononuclear cells belong to the human monocyte/macrophage cell lineage [4].

In order to prove Fc and C3b *receptor activities* on these pathognomonic cells we applied hemolysin sensitized sheep red blood cells (sSRBC) and opsonized zymosan (Mannozym) particles as targets for receptor-mediated phagocytosis [5].

Among *functional properties* we could demonstrate aspecific phagocytosis ingesting colloidal carbon granules (6), chemiluminescence activities [5], nitroblue-tetrazolium reduction test (Figs 3, 4) [6].

These pathognomonic cells are able to adhere within 24 hours to the solid surface. *Division and survival* is characteristic. Interestingly we could maintain already four different AF rapidly adhering cell line more than five month.

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