Phagocytic Activity of HeLa Cells after Thymidine Treatment

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Summary. HeLa cells demonstrated a marked phagocytic activity towards degenerating cells in their own population after single thymidine blocking. The thymidine treatment caused the high frequency of cell death at the time of thymidine release, but these dead cells were cleared by phagocytosis or autolysis in 7 hr after release. Then a prominent increase in the phagocytic activity of non-mitotic cells towards degenerating cells occurred in the mitosis-rich stage 10 hr after the release, but this was not associated with a high frequency of cell death.

The non-mitotic cells in this stage had many microplicae and microvilli on their surface, while those in the other stages were relatively smooth in surface morphology. HeLa cells, epithelial in origin, are considered as non-professional phagocytes which retain a primitive phagocytic activity and manifest this function when necessary.

Phagocytosis is a system of cell activities for the ingestion of particulate matter, and is related to cell nutrition or to clearing off foreign materials and necrotic tissue debris in the animal body. Phagocytic activity is retained, to a greater or lesser extent, by all immature cell types. When the cells mature, they manifest their own specialized functions and usually lose their primitive phagocytic activity. Cells with a highly developed capacity for phagocytosis differentiate to play an essential role in the removal of microorganisms, exogenous materials, or necrotic tissue debris. These cells are called “macrophages” and “microphages” as METCHNIKOFF described (1892) and represent professional phagocytes. Other cells retain the primitive capacity and may manifest this function when necessary. This process has been observed in a variety of cells in different organs and the cells are usually called facultative or non-professional phagocytes (RAVINOVITCH, 1969; KAPLAN et al., 1975; HURLE et al., 1978).

During the course of ultrastructural studies of HeLa cells cultured semi-synchronously with single thymidine blocking, we observed a remarkable increase in phagocytic activity towards degenerating cells in the mitosis-rich stage after the thymidine treatment. The HeLa cells originated from human uterine cancer and are not considered professional phagocytes. Using these HeLa cells as an example we here discuss the non-professional phagocytosis of various cell types.
MATERIALS AND METHODS

Cell culture and thymidine treatment
Logarithmically growing HeLa cells in a suspension culture were resuspended at a concentration of $4 \times 10^6$ cells/ml in a total volume of four liters in MEM (minimum essential medium) containing 10% fetal calf serum, 200 μg/ml of streptomycin, 200 U/ml penicillin, 4 mM L-glutamine, 1 mM sodium pyruvate, and 0.1 mM non-essential amino acids. The cell cycle was blocked in the S phase by incubating the cells with the medium containing 2.5 mM thymidine, for 20 hr. They were then released and allowed to progress into the S phase by washing the cells free from thymidine and resuspending them in fresh medium (thymidine release). The number of cells per ml of suspension was counted in a hemocytometer. Cell smears were stained with 0.025% crystal violet for 2 min, and the cells in mitotic stages were counted.

Electron microscopy
For morphological studies, 1 ml aliquots of cell suspension were taken out at 0, 4, 7, 10 and 24 hr after the thymidine release, and the cells were collected by centrifugation for 10 min at 2,300 x g. Cell pellets were fixed in 2% glutaraldehyde buffered at pH 7.4 with 0.1 M sodium cacodylate for 2 hr at 4°C. They were postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) for 90 min at room temperature. After dehydration with graded ethanol, they were embedded in Epon. Ultrathin sections were cut on a Porter Blum MT-2 ultramicrotome using a diamond knife. Three ultrathin sections from different areas of one Epon block were cut and stained with uranyl acetate and lead citrate, then examined in a Hitachi electron microscope (HU 11DS, 12A or H-800).

RESULTS

The growth pattern of HeLa cells after thymidine release is shown in Figure 1. An increase in the number of cells occurred 10-14 hr after release. At the same time, a
marked increase of mitotic cells was noticed, constituting 13%. The value indicates that synchronization of the cell cycle was still incomplete by a single thymidine treatment, and thus the term "mitosis-rich stage" is used for this stage. In a preparative experiment, incorporation of \(^{3}\)H-thymidine into the cells was measured by precipitating cells with 10\% TCA in a Packard-Tri Carb liquid scintillator at several time points after the initial addition of \(^{3}\)H-thymidine. There was an increase of \(^{3}\)H-thymidine incorporation, i.e. DNA synthesis, 4-5 hr after thymidine release.

HeLa cells in the suspension culture could be divided into four types on the basis of their cytological features (Fig. 2): "Mitotic cells" demonstrated features characteristic of the mitotic stages including prophase, metaphase, anaphase, and telophase. "Resting cells" had a nucleus of normal appearance and showed no phagocytic activity toward dead cells. "Dead cells" or "degenerating cells" demonstrated various stages of disintegration of the nuclear and cytoplasmic structures. "Phagocytic cells" contained inclusion bodies from phagocytized dead cells in their cytoplasm.

The frequency distributions of these cell types in the stages after the thymidine release are shown in Figure 3. The number of each cell type was counted under the electron microscope; from three different ultrathin sections a total of 700 to 1,000 cells of the four types of cells was counted in each stage. Immediately after the thymidine release (at 0 time) there were 10.4\% dead cells in the cell suspension. This value was much higher than that in the untreated control suspension (3.0\%). Incubation with thymidine may be the primary cause of this high frequency of the dead cells. Of all the cells, 3\% were phagocytic and this value was about 6 times higher than that of the untreated control (0.5\%). At 4 hr after the thymidine release, the percentages of dead cells and phagocytic cells were 5.7\% and 2.0\%, and they were still higher than the untreated control. At 7 hr after the thymidine release, the percentage of dead cells decreased to 3.4\% and that of phagocytic cells decreased to 0.8\%, showing a frequency distribution pattern similar to that of the untreated control. These decreases suggest that most of the dead cells at 0 time of thymidine release were cleared by phagocytosis or autolysis by the time of 7 hr after the release. At 10 hr after the thymidine release, when a marked increase of mitotic cells was observed (4.3\%), phagocytic cells markedly increased to 5.6\%, while 4.0\% of the total cells were dead cells. At 24 hr after the thymidine release, the percentages of dead cells, phagocytic cells and mitotic cells were 2.7\%, 1.0\% and 0.4\% respectively. This frequency distribution pattern was similar to that in the untreated control which had 3.0\% dead cells, 0.5\% phagocytic cells, and 0.6\% mitotic cells. The phagocytic cell/dead cell ratio was lower than 0.38 in all stages except the mitosis-rich stage where the ratio was 1.4. This indicates that the high frequency of phagocytosis in the mitosis-rich stage was not associated with the increase in dead cells.

The stages of phagocytosis by HeLa cell are shown in Figure 4 A-D. At first, extending cytoplasmic processes, the HeLa cell enveloped a mass of degenerating cell (A) and then engulfed it completely (B). The engulfed degenerating cell material was further disintegrated within the cytoplasm (C). Many lysosomes were observed in the phagocytic cells at this stage. Finally, the engulfed material was digested and disappeared from the cytoplasm (D).

Inclusion bodies, presumably from phagocytized cellular materials, were also found in the cytoplasm of dividing cells (Fig. 5). These bodies were found in 4 out of 69 mitotic figures observed by electron microscopy in the mitosis-rich stage. From our pictures it is, however, not clear whether the cells phagocytized dead cells during the mitotic phase or the cells containing phagocytized inclusion bodies went into mitosis.
Fig. 2. Legend on the opposite page.
There were some morphological differences between the resting cells in the mitosis-rich stage and those in the other stages. In the mitosis-rich stage, resting cells had many microplicae and microvilli extending from the surface. These cells were separate from each other, showing very wide intercellular space even in the pellets sedimented by centrifugation (Fig. 6). In the other stages, with low mitotic incidence, the resting cells were usually round in shape with poorly developed folds and microvilli. In the pellets, the cells were packed closely to each other. Dead cells or their debris were often present in the intercellular space of the resting cells, but most of these cells did not show any phagocytic activity towards the dead cells (Fig. 7).

DISCUSSION

A high frequency of phagocytosis towards dead cells was observed in the non-mitotic HeLa cells in the mitosis-rich stage after thymidine treatment, and this was not as-
Fig. 4. Legend on the opposite page.
Phagocytic Activity of TdR-Treated HeLa Cells

associated with a high frequency of cell deaths. Thymidine treatment caused the highest frequency of cell deaths at the time of thymidine release, but the dead cells were cleared by activated phagocytosis or autolysis before the mitosis-rich stage. Although synchronization of the cell cycle was not complete after a single thymidine treatment, the increase in phagocytic activity of HeLa cells (phagocytic cell/dead cell ratio) was specifically associated with the mitosis-rich stage. The non-mitotic cells in this stage had many microplicae and microvilli on their surface, suggesting surface activity, while those in other stages had a relatively smooth surface morphology. These differences may account for the differences in phagocytic activity in these cells. Rapidly growing cells in culture might produce some factors to regulate the cellular activity of the whole population, but this remains for further study.

Kihlstrom and Nilsson (1977) have shown that HeLa cells possess the ability to take up bacteria added into the culture medium. Schweichel and Merker (1973) has reported that during prenatal development, physiological or so-called programmed cell death occurs in various organs, and the dead cells are phagocytized by mesenchymal macrophages. In addition, epithelial and other cells in various developing organs are known to take up and dispose of debris from neighboring dead homologous cells. Examples of this phenomenon have been reported in the embryonic thymus (Hoshino et al., 1969), epithelial tooth germ (Moe and Jessen, 1972; Kindaichi, 1980), kidney (Pipan, 1976), testis (Black, 1971), ovary (McNamara and Black, 1975), and central nervous

Fig. 4. The stages of phagocytosis by HeLa cells. Extending cell processes envelope a mass of degenerating cell (A). The degenerating cell material is engulfed completely (B). The engulfed material is disintegrated and digested to disappear from the cytoplasm (C and D). × 6,300

Fig. 5. An inclusion body (I), presumably from phagocytized cellular materials, is seen in the cytoplasm of a dividing cell. × 4,700
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system (MATSUYAMA et al., 1975; LANGMAN and CARDELL, 1977, 1979; HAYASHI and KAMEYAMA, 1979). HURLE et al. (1978) reported that myocardial cells in the developing heart also have phagocytic ability. In adults, epithelial cells in some organs also have phagocytic ability. Pigmented epithelial cells in the retina phagocytize the tip of the rod cells' outer segment (YOUNG and BOK, 1969). Keratinocytes in the epidermis actively take up foreign materials such as ferritin, horse-radish peroxidase or latex particles (WOLFF and SCHREINER, 1970, 1972; SAGEBIEL, 1972). Paneth's cells in the small intestine phagocytize and digest bacteria (ERLANDESEN and CHASE, 1972a, b). Epithelial reticular cells of the thymus have the ability to take up degenerating lymphocytes (KLUG, 1965). Microhemorrhagic red blood cells in the thyroid follicular lumen are engulfed by the follicular epithelial cell (ZELIGS and WOLLMAN, 1977). In the periodontal ligament of incisor teeth of rodents, fibroblasts play a role in collagen turnover by phagocytosis of collagen fibrils (TEN CATE and DEPORTER, 1974; GARANT, 1976). These phagocytic functions of cells in the developing as well as adult organs are the same as those of macrophages, but seem to be additional to their specific functions.

Fig. 6. Cells in the mitosis-rich stage. In this stage, resting cells (R) have many folds and microvilli extending from the surface, and they are separate from each other showing very wide intercellular spaces. × 5,400

HeLa cells originated from epithelial cells are maintained in vitro as a single cell line. It is noteworthy that these cells show phagocytic activity, and for this function they can be categorized in the group of non-professional phagocytes.

In a previous paper, KOBAYASHI and HOSHINO (1979) discussed cells with phagocytic and/or immune response mediating functions, and classified these cells into 3 groups: 1) Nonspecific scavenger macrophages: cells that phagocytize a variety of materials
including foreign substances and endogenous cellular or tissue debris, and make antigens non-immunogenic by intracellular degradation. 2) Immune macrophages: cells that have both non-specific scavenger type phagocytic and immune response-mediating functions. 3) Immune response-mediating cells: cells that mediate immune responses by taking or carrying antigens, but do not perform non-specific scavenger type phagocytosis.

The non-professional phagocytes discussed above do not seem to have immune response mediating functions and thus their phagocytic functions are considered to be analogous to those of non-specific scavenger macrophages. The latter macrophages are mesenchymal in origin while many non-professional phagocytes are epithelial cells. Non-professional phagocytes in various organs may take part in local defense and be scavengery by their phagocytic ability under certain physiological or pathological conditions.

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