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Changes in cellular ultrastructure induced by gamma-interferon in K562 cells may be prerequisite for apoptosis.

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Abstract

We report here the time-course of electron microscopic changes induced by gamma-interferon (IFN-gamma) in the human erythromyeloid leukemia cell line K562. In K562 cells treated with IFN-gamma for 6h, the nuclei were polygonal in shape and microvilli were far more abundant on cell membranes compared with control K562 cells, and invaginations were often seen in the cell membranes. There was a reduction in the number of cell-membrane microvilli and an increase in the number of lysosomal bodies in the cytoplasm of K562 cells treated with IFN-gamma for 12h. After treatment with IFN-gamma for 24h, the cell membrane microvilli disappeared, large numbers of cellular organelles were observed, such as mitochondria and lysosomes, and the cytoplasm became electron-dense. Cytoplasmic vesicles and vacuoles were also observed. These vesicles may correspond to an intermediate step in the ultimate cellular disintegration associated with apoptosis caused by IFN-gamma.

KEYWORDS: IFN-?, cellular ultrastructure, K562

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Brief Note

Changes in Cellular Ultrastructure Induced by Gamma-Interferon in K 562 Cells may be Prerequisite for Apoptosis

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We report here the time-course of electron microscopic changes induced by gamma-interferon (IFN-γ) in the human erythromyeloid leukemia cell line K562. In K562 cells treated with IFN-γ for 6h, the nuclei were polygonal in shape and microvilli were far more abundant on cell membranes compared with control K562 cells, and invaginations were often seen in the cell membranes. There was a reduction in the number of cell-membrane microvilli and an increase in the number of lysosomal bodies in the cytoplasm of K562 cells treated with IFN-γ for 12h. After treatment with IFN-γ for 24h, the cell membrane microvilli disappeared, large numbers of cellular organelles were observed, such as mitochondria and lysosomes, and the cytoplasm became electron-dense. Cytoplasmic vesicles and vacuoles were also observed. These vesicles may correspond to an intermediate step in the ultimate cellular disintegration associated with apoptosis caused by IFN-γ.

Key words: IFN-γ, cellular ultrastructure, K562

Gamma-interferon (IFN-γ) is the most potent known lymphokine for activating cells of the mononuclear phagocyte lineage (1). It augments the expression of MHC class I and class II antigens (2, 3) and IgG-Fc receptor in mononuclear phagocytes (4), enhances cytotoxicity against tumor target cells *in vitro* (5), and induces both oxygen-dependent and -independent antimicrobial states (6, 7). IFN-γ exerts its action by binding to a unique receptor, distinct from that for IFN-α and -β (8), and triggering processes requiring *de novo* RNA and protein synthesis result in biological activities. The mechanisms of signal transduction after IFN-γ binds to its receptor are well known, but few studies have been done which focus on the electron microscopic changes induced by IFN-γ. To understand the mechanism of IFN-γ action, it is also important to know the ultrastructural changes.

Materials and Methods

Cells. K562 cells, a human chronic leukemia cell line, were maintained in RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% fetal calf serum (Gibco, NY, USA), 10U/ml penicillin-G and 0.1mg/ml streptomycin at 37°C in 5% CO_2 in a humidified atmosphere. Cells in the exponential growth phase were harvested for the experiments.

Cytokine. IFN-γ was produced by lipopolysaccharide-stimulated human myelomonocytic HBL-38 cells and was purified to over 99% homogeneity (9). The activities of IFN-γ were determined using a cytopathic effect inhibition assay that employed human FL cells challenged with Sindbis virus. The results were standardized against the international reference preparations of human IFN-γ (Ga23-901-530), and the activities of IFN-γ were found to be 2.1×10^7 IU/mg protein.

Proliferation assay. Cells were seeded in triplicate into 96-well microtitre plates at 5×10^3 cells/well in a volume of 0.2ml in the presence of various concentrations of IFN-γ. After incubation at 37°C for 6, 12 and 24h, the cells were stained with 0.05% crystal

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Table I Cell growth of K562 cells with gamma-inteferon (IFN-γ) for 6, 12 or 24h

IFN-γ (IU/ml)	Incubation time (h)	Optical density	Relative cell growth (%)
0	6	0.394 + 0.073	100.0
	12	0.831 + 0.029	96.7 + 7.4
	24	1.302 + 0.060	330.5 − 15.3
10	6	0.436 + 0.019	110.6 − 4.8
	12	0.402 + 0.021	102.0 + 5.3
	24	1.325 + 0.030	336.3 + 7.6
100	6	0.423 + 0.059	107.4 + 14.9
	12	0.394 + 0.013	100.0 + 3.3
	24	1.182 + 0.136	300.0 + 34.5

Values are the Mean + SD from seven different experiments. Cell growth (mean + SD) compared with control cells cultured for 6h.

violet. The dye was eluted with Sorenson's buffer and the absorbance at 540nm was determined with a FT-2 plate analyser. The number of viable cells was estimated from the absorbance, based on the correlation between the amount of dye eluted and the number of cells. Cell growth was calculated according to the following formula: Percentage growth = (mean absorbance of the treated group)/(mean absorbance of the control group) × 100. Each experiment was performed in triplicate and was repeated at least three times.

Histochemical electron microscopic investigation. The number of viable cells was determined by the exclusion of trypan blue. Cells were suspended and cultured in RPMI 1640 medium in the presence of IFN-γ (100IU/ml) for 6, 12 and 24h. Medium alone was used for control cultures. The IFN-γ- treated cell suspension

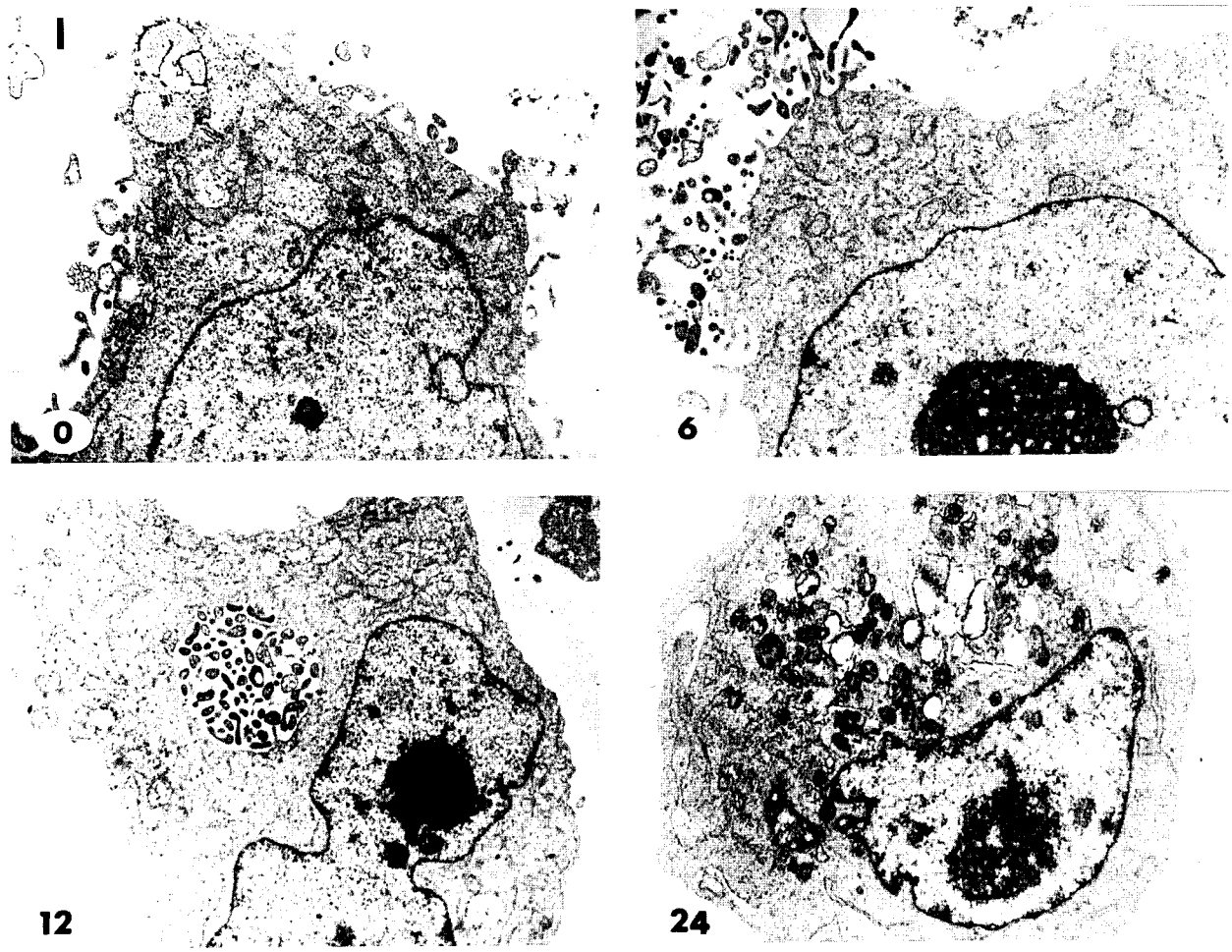

Fig. 1 Electron micrographs showing K562 cells after exposure to gamma-interferon for 0, 6, 12 or 24h.

(5 ml containing 5.0×10^6 cells) in a phosphate-buffered saline solution was incubated in a test tube for several hours. These cell suspensions were then centrifuged at 2,000 rpm for 5 min at 4°C, and the sediments were removed and 20 ml of 4% paraformaldehyde and 1% glutaraldehyde fixative solution was added to the sediments. After fixation for 2 h, the sediment was washed twice with 0.1 M phosphate buffer (pH 7.4) and then postfixed with 1% osmium tetroxide for 2 h. The sediments were dehydrated using a graded series of acetones rinses and embedded in EPON resin. Ultrathin sections were cut on an ultramicrotome and examined with a JEOL 100 CX electron microscope and multiple sections were viewed in order to examine the morphological changes in response to IFN-γ treatments.

Results and Discussion

The cell growth of IFN-γ-treated K562 cells did not differ from that of non-treated K562 cells at any time or at any concentration (Table 1). The cell viability of IFN-γ-treated K562 cells determined by the exclusion of trypan blue also did not differ from that of control K562 cells (data not shown).

In contrast, IFN-γ induced remarkable ultrastructural changes in K562 cells and these changes became increasingly prominent in proportion to the incubation time (Fig. 1). In control K562 cells, most of the cells had well-developed cell organelles such as mitochondria, large clear nuclei and cell membranes with few microvilli. In K562 cells treated with IFN-γ for 6 h, the nuclei were polygonal in shape and microvilli were far more abundant on cell membranes as compared with control K562 cells, and invaginations were often observed in the cell membranes.

There was a reduction in the number of microvilli in cell membrane and an increase in the number of lysosomal bodies in the cytoplasm of K562 cells treated with IFN-γ for 12 h. After treatment with IFN-γ for 24 h, the cell membrane microvilli disappeared, large numbers of organelles were observed such as mitochondria and lysosomes, and the cytoplasm became electron-dense. Cytoplasmic vesicles and vacuoles were also observed (Fig. 1).

Similar cytoplasmic vesicles and vacuoles can occasionally be observed in irradiated cells or in cells sufferring from hypoxia; in all these situations the formation of vesicles precedes the ultimate cellular disintegration associated with apoptosis. A possible explanation for the appearance of cytoplasmic vesicles and vacuoles is that they originate from mitochondrial reactive oxygen metabolites when cellular repair mechanism are paralysed and precede the changes of apoptosis and cellular disintegration (10).

We have previously reported that IFN-γ exposure resulted in a compensatory increase in mitochondrial membrane potential with accerelated oxidative respiration, resulting in increased production of oxygen radicals (11). The present research demonstrated that IFN-γ induced striking cytoplasmic ultrastructural changes and striking increases in the number of cells containing large numbers of cytoplasmic vesicles and vacuoles might precede the changes of activation-induced cell death, *i.e.* apoptosis.

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