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Ultrastructural Study of Human Natural Killer (NK) Cell*)

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ABSTRACT

An electron microscopic study of human natural killer (NK) cells showed abundant cytoplasm and relatively large mitochondria. They also had various kinds of granules in their cytoplasm. The mode of binding to the tumor cell (K 562) was tight, and villi protruded from NK cells to the target cells. Some traget cells were already lysed after incubation for 5 minutes at 4°C.

INTRODUCTION

It has long been known that normal lymphocytes, in the absence of disease, sensitization, or deliberate immunization of the lymphocyte donor, can destroy a variety of target cells cultured in vitro. This natural cell-mediated cytotoxicity has recently been extensively documented in humans and rodents. However, there are few reports on the morphological characteristics of NK cells.

In this communication, we describe the ultrastructural features of human NK cells binding to target cells (K 562).

MATERIALS AND METHODS

Tumor cell line (target cells):

Tumor cells (K 562) were maintained by continuous in vitro culture. They were established from leukemic blast cells from the pleural effusion of a patient with chronic myelogenous leukemia[3].

Isolation of peripheral blood lymphocytes (effector cells):

Mononuclear leukocytes were isolated from the heparinized peripheral blood of healthy human donors by centrifugation with Ficoll-diatrizoate sodium gradient at 400×G for 30 minutes at room temperature.

Cells were washed 3 times in phosphate-buffered saline (PBS) and suspended in Eagles minimal essential medium (MEM No. 1, Nissui) supplemented with 10% fetal calf serum (FCS, Gibco). In order to remove monocytes, 6–8×10^6 mononuclear cells suspended in 3 ml MEM with 10% FCS were incubated at 37°C for 2 hours in Falcon plastic dishes (65 mm×15 mm). Non-adherent cells were harvested and used as effector cells, which were more than 96% lymphocytes[7].

Preparations for electron microscope:

To conjugate NK cells with target cells, 1×10^7 of K 562 cells (target cells) were mixed with 1×10^6 lymphocytes (effector cells) in 3 ml culture medium (E/T ratio=1 : 10) at 4°C for 5 min and were centrifuged at 100×G for 10 min. Then, cells on the bottom of the test tube were fixed with 3% glutaraldehyde in phosphate buffer for 2 hours and postfixed with 1% osmium tetroxide. The samples were dehydrated in ethanol and embedded in Poly Bed 812 (Polyscience, Inc.). Ultrathin sections were prepared on a Porter-Blum ultramicrotome MT-1. The sections were stained with uranyl acetate and lead acetate and examined in a HITACHI H-300 transmission electron microscope.

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RESULTS AND DISCUSSION

In this experiment, we used a 1 : 10 E/T ratio in order to exclude many lymphocytes which formed nonspecific conjugates with target cells. Fig. 1 shows a non-binding lymphocyte. This cell was a small lymphocyte (6 μm. in diameter) with a high N/C ratio and poorly developed cytoplasmic organelles.

Fig. 2 and 3 show lymphocytes (NK cells) binding to the tumor cell. The NK cells were medium-sized or large (8 to 10 μm. in diameter). It was characteristic that their surface was mostly villous. They had relatively abundant cytoplasm containing characteristic organelles. The granules were of varied appearance.

Some granules were round, homogeneously dense (100 to 200 nm. in diameter). Others were oval (300 to 600 nm. in diameter). There was also a large granule (1,000 nm. in diameter) which contained dense deposits and myelin-like figures (Fig. 2, arrow).

As shown in Fig. 3, NK cells also had numerous large mitochondria which were elongated. The nucleus was slightly irregular and had peripherally dense chromatin.

These findings had some similarities to the $T_\gamma$cells (T cells with receptors for IgG) or null cells (non-T, non-B cells with high avidity receptors for IgG) described by Grossi et al.[1,2] It is not known precisely what kind of lymphocytes exhibit natural killer activity.

The purified human NK cells that Saksela et al designated as large granular lymphocytes had many distinct membrane-coated granules[5,6,8]. In this study, however, we could not find as abundant granules in the cytoplasm of NK cells as they reported.

The NK cells were bound to K 562 target cells with broad irregular contacts or interdigitation. In the interdigitated form, the microvilli protruded from the binding lymphocytes to their target cells and were tightly conjugated (Fig.3). In the broad contact form, the binding lymphocytes were often triangular (Fig. 2). Under the conditions employed in this study (incubation for 5 minutes at 4°C), K 562 target cells conjugating with NK cells showed various stages of lytic degeneration with cytoplasmic vacuolation or total cell death.

Roder et al described murine NK cells binding to YAC cells (Molony virus-induced lymphoma of A/Sn mice[4]). They reported that the general ultrastructural appearance of NK cells was consistent with that of resting lymphocytes, with a high N/C ratio, scanty cytoplasm, relatively numerous large mitochondria, inconspicuous Golgi zones, a lack of endoplasmic reticulum and polyribosomes, and a predominantly heterochromatic nucleus with a cresent nucleolus. The contact between lymphocyte and target cell was similar to that in the present report. However there were some differences in ultrastructural appearance of human NK cells. These may reflect a species difference.

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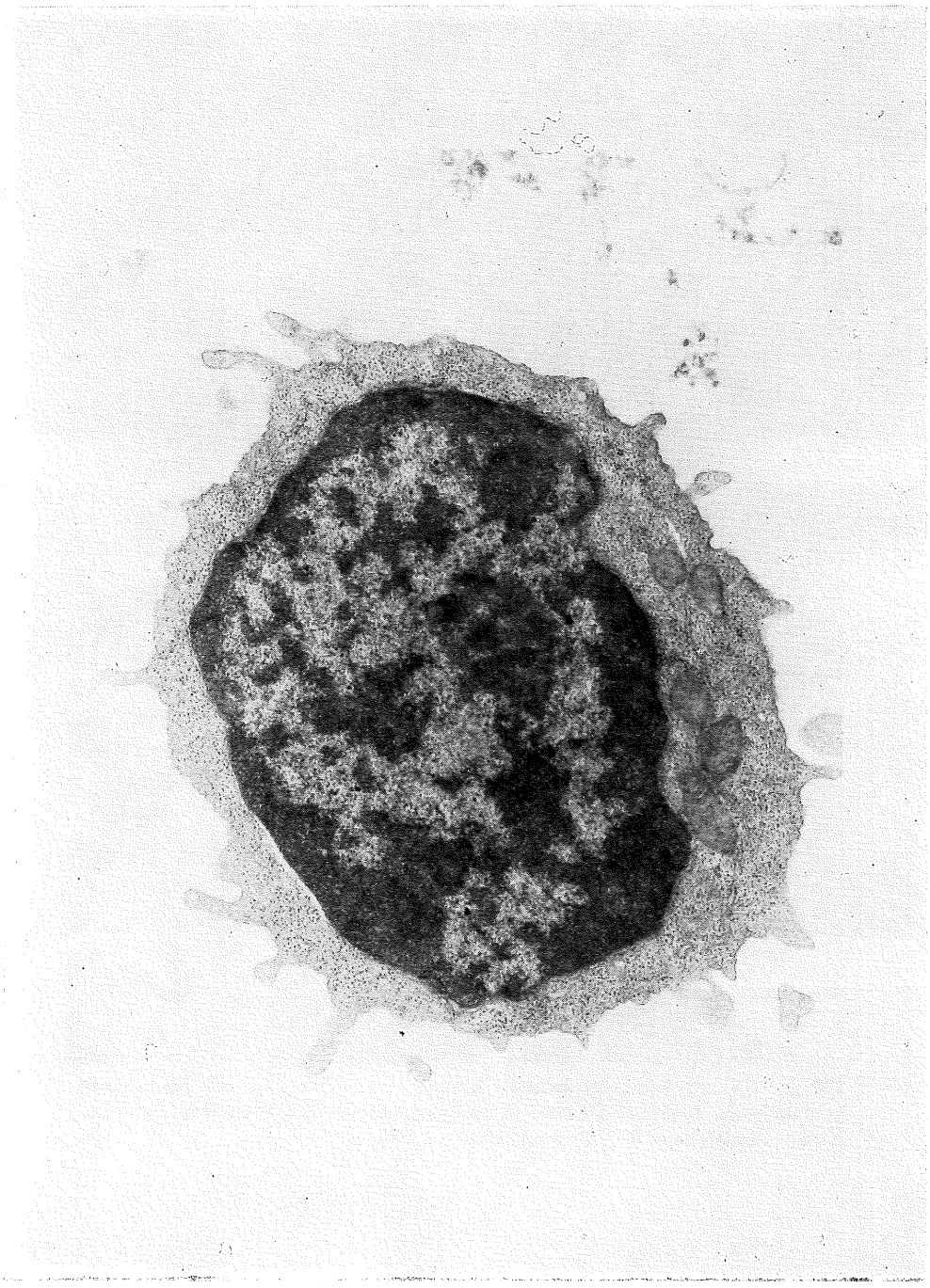

Fig. 1. Transmission electron micrographs of non-binding human lymphocyte.(×10,000)

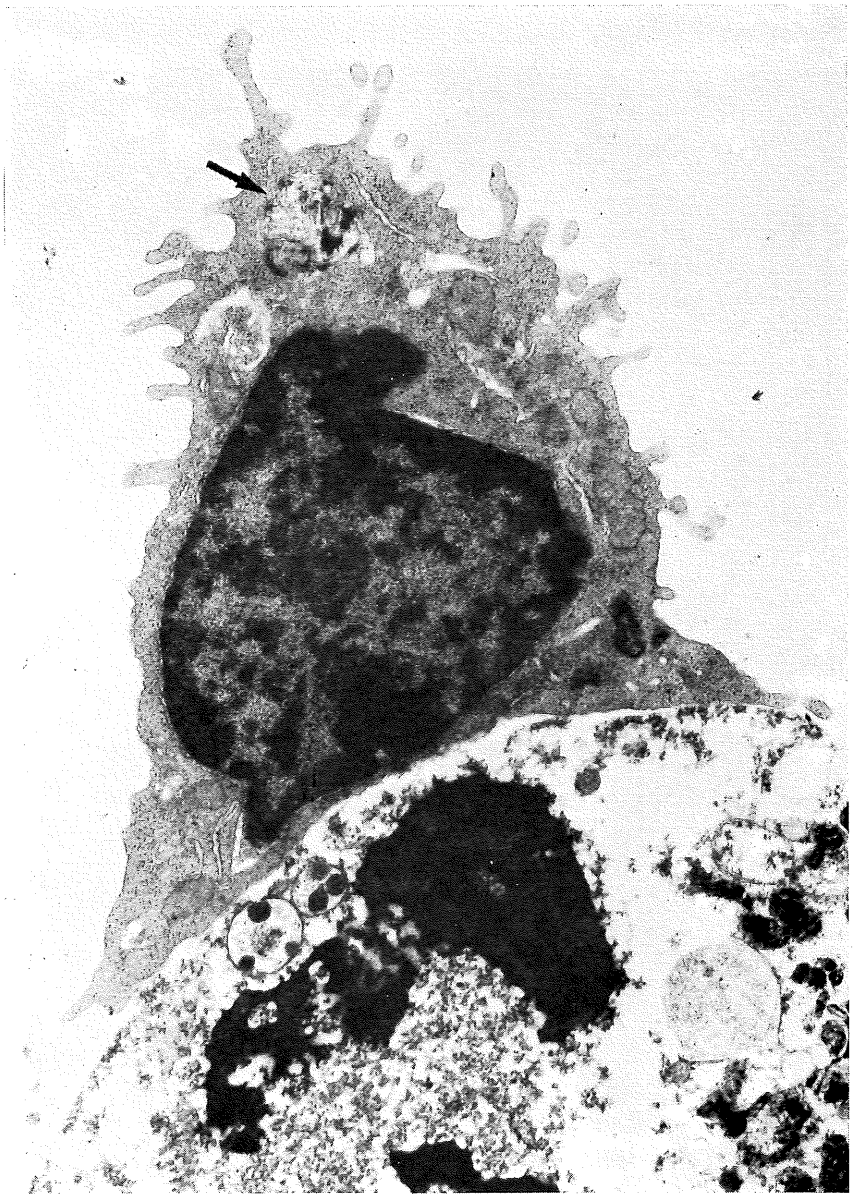

Fig. 2. NK cell with broad irregular contact with K 562. This cell has a large granule in the cytoplasm(1,000nm. in diameter) (arrow). The NK cell is triangular. K 562 already shows destructive changes.(×7,000)

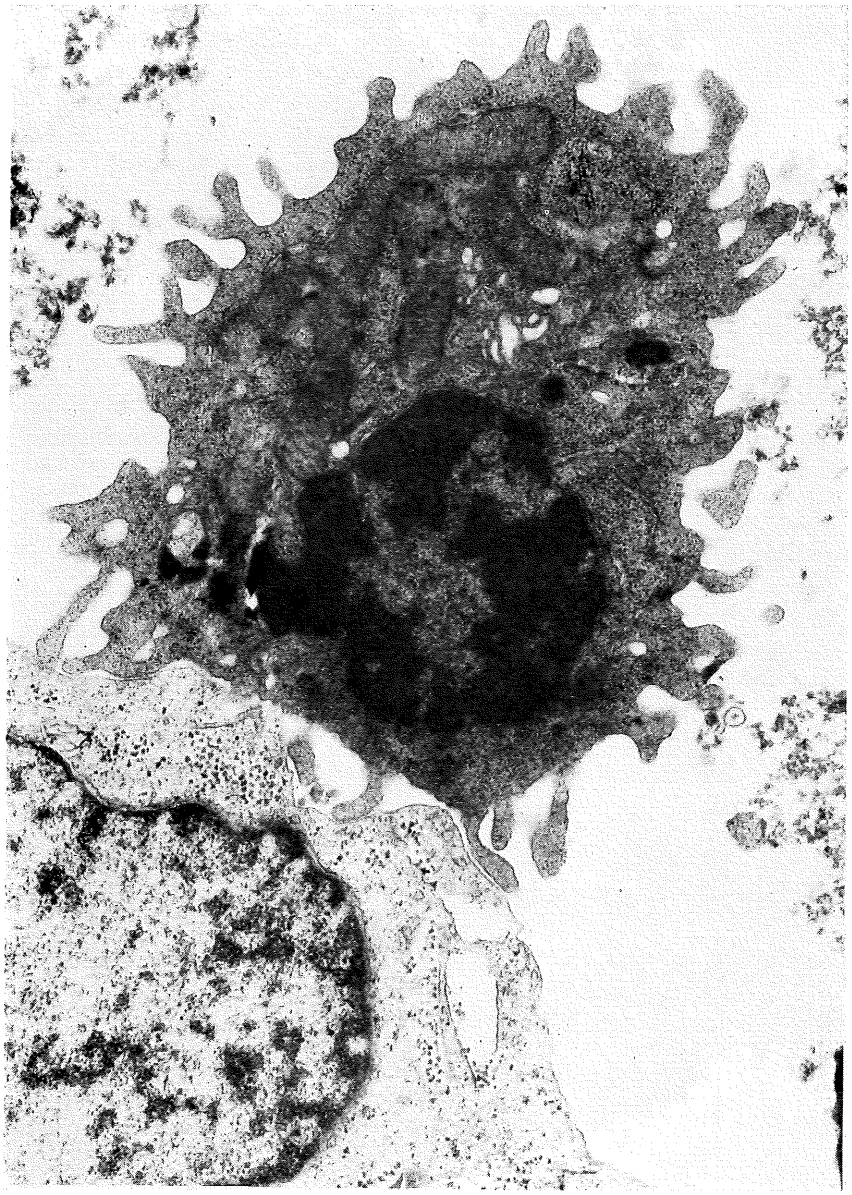

Fig. 3. NK cell bound to K 562 by interdigitation. This cell has elongated mitochondria.($\times 10,000$)