Observation In Situ of Lymphocyte and Macrophage–Mediated Tumor Cell Lysis in Nephroblastomas

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Abstract **Objective** To investigate the interaction between tumor cells and tumor infiltrating lymphocytes or macrophages in nephroblastomas. **Methods** Fresh tissues of 11 cases of nephroblastoma were observed by electron microscope. **Results** Lymphocyte infiltration with vary degree was found in 9 of 11 cases, and macrophage infiltration in 2 cases. The damage of varying degree had been seen in the tumor cells contacted with lymphocytes and macrophages, and the infiltrating lymphocytes and macrophages remained intact. **Conclusions** the infiltrating lymphocytes and macrophages could be cytotoxic to the tumor cells in some nephroblastoma cases.

Key Words Nephroblatoma; Immunity; cellular; Lymphocytes; Macrophages;

Lymphocytic infiltration was present in many malignant tumors[1~5]. But little is known about the function of tumor infiltrating lymphocytes and macrophages in nephroblastomas. Here, we observed the interaction between tumor cell and tumor infiltrating lymphocytes or macrophage in fresh tissues of 11 cases of nephroblastomas with light and electron microscope.

MATERIALS AND METHODS

11 case of nephroblastoma were studied. The patients' ages ranged from 7 months to 5.2 years. None of them received chemotherapy or radiotherapy before surgery. All the specimens were obtained immediately after operation. The pathologic diagnosis was confirmed by light microscopy.

Light microscopy

In each cases, the tissues were fixed in 10 % formalin and embedded in paraffin. Sections 5μm thick were stained with hematoxylin-eosin.

Electron microscopy

11 cases of nephroblastoma were processed for transmission electron microscopic examination. In each case, the fresh tumor tissues were immediately fixed in 3 % phosphate-buffered glutradehyde, postfixed in 1 % osmium tetroxide, dehydrate in graded alcohols and embedded in EPON. One-micron thick sections were cut and stained with the toluidine blue and examined under the light microscopy to select the tumor tissues with lymphocytic infiltration. For ultrastructural study, Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined with an electron microscope.

RESULTS

Light microscopic findings

In 9 cases of 11 patients with nephroblastoma, lymphocytic infiltration with various degrees was found in tumor tissues. The lymphocytes were present among the tumor cells.

Electron microscopic findings

The infiltrating lymphocytes in the tumor tissues had round or oval nucleus, with dense chromatins. Their cytoplasm was scanty and contained some mitochondria and a few other organelles. On their surface, some projections or pseudopodia were present. The lymphocytes were found to be in intimate contact with tumor cells. The tumor cells were damaged in various degrees (Fig 1). In two cases, it was found that a few macrophages were in contact with tumor cells, which had also shown severe damage (Fig 2, 3). The damage of the tumor cells included: the focal loss of the cell membrane at the regions in contact with the lymphocyte; partial loss of both cell membrane and cytoplasm at the contact regions; the lymphocytes and macrophages penetrated into

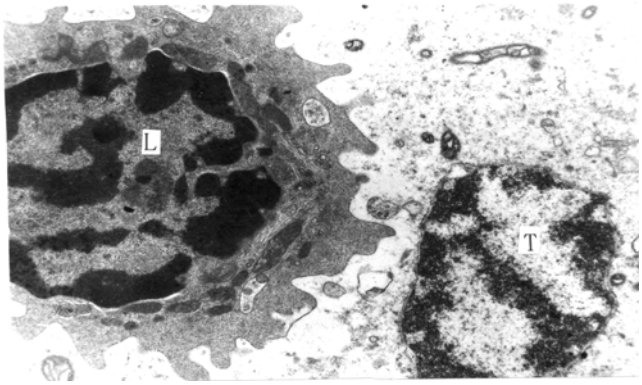

Fig 1. The tumor cell (T) showed complete loss of membrane at the contact region with the lymphocyte (L), whereas the latter remained intact.(×10,000)

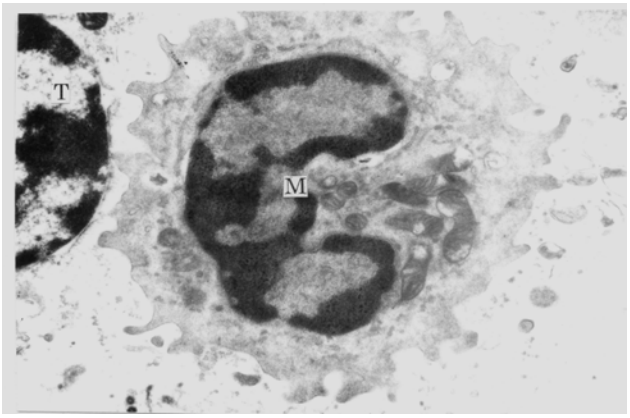

Fig.2 The macrophage (M) had directly penetrated into the nucleus of the tumor cell(T). The loss of both cell membrane and cytoplasm of the tumor cell (T) were present. (×6,000)

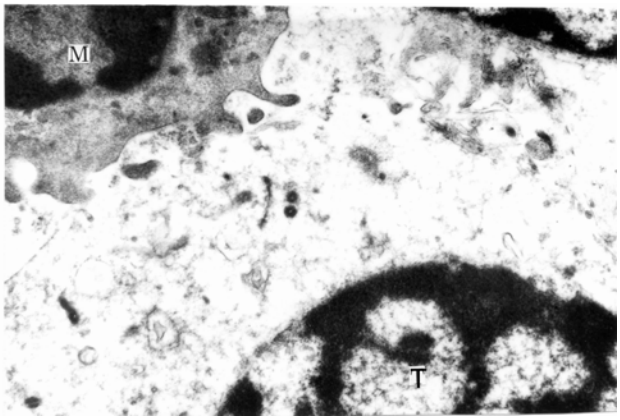

Fig.3 The macrophage (M) was the same one as showed in Fig 2. The other two tumor cells (T) contacted with the macrophage were also destroyed. (×22,000)

the cytoplasm and sometimes contacted with the nucleus of the tumor cell (Fig 2). The most severe damage of the tumor cells was the lose of both cell membrane and cytoplasm and the destruction of nucleus. In spite of severe damage of the tumor cells, the infiltrating lymphocytes and macrophages remained intact.

DISCUSSION

Some investigators reported that seminoma was often heavily infiltrated by mononuclear cells, whose presence was associated with an improved survival rate [6]. But it was described that the lymphocytes isolated from the tumor tissues of seminomas were not cytotoxic to autochthonous tumor cells in the ^{51}Cr release cytotoxicity in vitro[4]. However, some lymphocytes in several tumors such as lung cancer and seminomas were in direct contact with tumor cells that exhibited damage with various degree[2, 3, 5]. Our present study also demonstrated that the lymphocytes and macrophages penetrated into the cytoplasm and resulted in local loss of the membrane and cytoplasm. The pseudopodia of the inflammatory cells even reached the nucleus of the tumor cells. As a result, the membrane of the nucleus of the tumor cells was damaged. In spite of the presence of damage to the tumor cells, the lymphocytes and macrophages remained intact.

Wei et al[5] found that the great majority of the lymphocytes were UCHL 1-positive cells and the macrophages were positive for MAC 387. Our study revealed a direct evidence that the infiltrating lymphocytes and macrophages might be cytotoxic to the tumor cells in some nephroblastoma cases. Leek et al[6] reported that tumor-associated macrophages (TAMs) produced angiogenic factors and was associated with high vascular grade and poor survival in breast cancer. Recently, Tsung et al[7] found that interleukin (IL)-12 could activate a T-cell-dependent antitumor immune response that was able to eradicate established large tumors in a number of immunogenic tumor models. The mechanisms of these dramatic antitumor responses have not yet been identified, but the macrophages was the predominant immune infiltration cell in the ascites of the tumor models. Thus, the exact mechanism and clinical significance of tumor infiltrative lymphocytes and macrophages should be further investigated.

Acknowledgement

We would like to thank Prof. Zhenbiao Hang from Dept. of Pathology of West China hospital of Sichuan University for his contributions to this article.

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