Morphological Change in Tumor Endothelial Cells Induced by Natural-Type Human Tumor Necrosis Factor

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ABSTRACT. The effects of natural-type human tumor necrosis factor (nh-TNF) on tumor endothelial cells of experimental brain tumors were investigated electron microscopically. Tumor vessels with hypertrophic endothelial cells were observed 12 and 24 hr after an intralesional administration of 5,000 U of nh-TNF. Increased biosynthetic organelles such as the Golgi complex and rough endoplasmic reticulum were evident in the plump cytoplasms. These endothelial cells resembled those in high endothelial venules (HEV) functionally characterized by the high permeability of leukocytes. In addition, close interactions between these endothelial cells and leukocytes were observed. Our findings indicated that nh-TNF could promote the morphological change in tumor endothelial cells into HEV-like cells.

— KEY WORDS: high endothelial venule, tumor endothelial cell, tumor necrosis factor (TNF).

Tumor necrosis factor (TNF) is believed to have potent antitumor activity in some experimental tumors, and its clinical application in patients with glioblastoma is expected [1, 12, 14, 15]. TNF has been reported to have various effects on tumor vasculature leading to regressions of tumor tissue [1, 11, 15]. Meth A sarcoma is a commonly used rodent tumor model to be investigated the activity of TNF where hemorrhage necrosis is demonstrated after an administration of TNF. Direct cytotoxicity to tumor endothelial cells was believed to be attributable for the regression of this tumor model [7, 10, 15, 16].

In our previous report on the antitumor activity of natural-type human TNF (nh-TNF) on experimental brain tumors in rats [9], repeated injections of nh-TNF successfully prolonged the survival rate of the tumor-bearing rats, in which coagulative necrosis was induced in the tumor tissues. In that study, marked congestion of the tumor tissues was observed, suggesting vascular effect of nh-TNF. However, degeneration of the tumor endothelial cells as reported in the experiment using Meth A sarcoma was not observed. Though the effect of TNF on tumor endothelial cells has been extensively investigated, definitive conclusion has not yet been obtained. In this study, we investigated electron microscopic changes in endothelial cells of experimental brain tumors after an intraleseional administration of nh-TNF.

Twelve three-week-old male Wistar rats purchased from Japan SLC (Shizuoka, Japan) were used in this study. They were fed ad libitum and had free access to water throughout the experiment. This nh-TNF was supplied from Mochida Pharmaceutical Co., Ltd. (Tokyo, Japan). This nh-TNF was prepared from the culture medium of human leukemic B cell line (BALL-1) stimulated by Sendai virus.

The experimental brain tumor was produced by an intracranial inoculation of C6 cells as described previously [9]. In brief, the rats were anesthetized with an intraperitoneal injection of pentobarbital (40 mg/kg), then were placed to a stereotaxic frame. A burr hole was made in the skull, through which 5.0 × 10^5 of C6 cells suspended in 10 µl of PBS was injected into the nucleus caudate.

On the 10th day after the implantation, nine tumor-bearing rats were anesthetized and placed to the stereotaxic frame. After the exposure of the burr hole, 5,000 U of nh-TNF diluted with PBS into a total volume of 17 µl was injected into the tumor tissue directly through the hole using a microsyringe.

Two, 12 and 24 hr after the single injection of nh-TNF, each three rats were subjected to transcardiac perfusion as described previously [17]. The rats were anesthetized, fixed in the supine position and received cannulation into the left ventricle. The rats were initially perfused with heparinized saline solution for a few minutes, and then sacrificed by whole-body perfusion fixation with 4% glutaraldehyde with a total volume of 200 ml for about 30 min. The whole brain was removed and the tumor tissue at a distance of 5 mm from the injection site was cut into small pieces. The tumor tissues were additionally fixed in 4% glutaraldehyde for 3 hr. Post-fixation was performed for 2 hr in osmium tetroxide. Following osmication, the tissue samples were dehydrated in graded series of ethanol and propylene oxide, and embedded in Epon 812.

The remaining three tumor-bearing rats without treatment of nh-TNF were also subjected to transcadiac perfusion and used as controls.

Thin sections of each sample were cut with a microtome and mounted on copper grids. The grids were stained with uranyl acetate, followed by lead citrate. The tissues were examined and photographed in a Hitachi H-700 electron microscope.

In contrast, morphological changes in fine structures of
Fig. 1. 1A (× 3,500) shows the electron microscopic findings of the tumor vasculature in the untreated C6 glioblastoma and 1B (× 2,500) shows those in the tumor 2 hr after the nh-TNF administration. The surfaces of the vascular lumens are very smooth and the endothelial cells have thin cytoplasms. In contrast, the endothelial cells are pleomorphic and have rich cytoplasms 12 hr after the nh-TNF administration (Fig. 1C, × 6,000 and 1D, × 7,000). 1E (× 18,000) shows a highly magnified picture of cytoplasms of the pleomorphic endothelial cells appearing after the nh-TNF administration. The cytoplasms contained many synthetic organellae such as rough endoplasmic reticulum and Golgi complex. Occasionally, endothelial cells with many villous processes projecting into the vascular lumen were observed (Fig. 1F, × 3,500).
tumor endothelial cells were observed 12 hr after the administration of nh-TNF. Some of the capillaries revealed narrow lumen and their cytoplasms were hypertrophic (Figs. 1C and 1D). Increased synthetic organelles such as rough endoplasmic reticulum, Golgi complex and free ribosomes were observed in the plump cytoplasms, which were not observed in control tumors and those 2 hr after the nh-TNF injection (Fig. 1E). In addition to the pleomorphic edothelial cells, the endothelial cells with a large number of villous processes projecting into the capillary lumen were observed (Fig. 1F). These endothelial cells were also observed 24 hr after the nh-TNF administration.

Furthermore, leukocytes were increased in number in the lumen of the tumor vessels (Fig. 2A). These leukocytes mostly consisted of lymphocytes and polymorphonuclear leukocytes. Some leukocytes had close contact with the plump endothelial cells (Figs. 2B and 2C), and in some areas, leukocytes penetrating the pleomorphic endothelial cells were observed in the section that was independent of the intercellular junction. Leukocytes between the endothelial cells and basement membrane were also observed (Fig. 2D). No degeneration of tumor endothelial cells were observed 12 or 24 hr after nh-TNF administration.

In the case of Meth A sarcoma, TNF has been believed to have direct cytotoxicity to tumor endothelial cells [7, 10, 15, 16]. However, in the present electron microscopic study, no degeneration of the endothelial cells in C6 glioblastoma was observed. The most important findings after the nh-TNF administration were marked morphological changes in the tumor endothelial cells. Hypertrophy of the endothelial cells, which acquired plump cytoplasms and marked biosynthetic organelles, was observed in many tumor vessels 12 or 24 hr after nh-TNF administration, whereas those morphological features of the endothelial cells were not observed in the control tumors. Therefore, nh-TNF may have potential of inducing morphological change to the tumor endothelial cells by direct injection into the brain tumor.

Some endothelial cells existing in lymph nodes are believed to be responsible for the recirculation of lymphocytes [5, 13]. These endothelial cells are highly permeable for immune-mediated cells and are called as high endothelial venules (HEV) from their peculiar morphology of plump cuboidal appearance [6, 13]. Fine structures of these cells have been shown to be prominent in biosynthetic organellaes [4]. Since the appearance and fine structures of
the endothelial cells in nh-TNF-treated tumors closely resembled those in HEV, one of the effects of nh-TNF on tumor endothelial cells was thought to be the promotion of the morphological change into HEV-like cells.

In addition to those changes, some of the endothelial cells exhibited many villous processes projecting into the capillary lumen. These endothelial cells did not exhibit the typical morphological features of HEV. However, some investigators reported that these villous processes were highly positive for adhesion molecules, and played an important role in the initial phase of the extravasation of leukocytes [2, 8, 13]. We previously reported that the expression of intercellular adhesion molecule-1 (ICAM-1) on the tumor endothelial cells was increased after the administration of nh-TNF [9]. In this study, these endothelial cells frequently had close contact with leukocytes. Therefore, the endothelial cells with villous processes were considered to be positive for adhesion molecules and to function as HEV, as well as morphologically HEV-like vessels.

TNF is known to be one of the important inflammatory cytokines and to modulate functions of endothelial cells in subcutaneous tissues [3]. Permeability of these vessels for immune-mediated cells increases after an exposure to TNF. Our findings suggested that one of the biological activities of nh-TNF as an inflammatory cytokine was exerted on the tumor endothelial cells, at least in C6 glioblastoma, and consequently, tumor vessels became highly permeable to immune-mediated cells. We previously reported that dense infiltration of macrophages and polymorphonuclear leukocytes were observed after the nh-TNF administration [9]. These immune-mediated cells were major effector cells in antineoplastic immunotherapy. The promotion of the morphological and functional changes in tumor endothelial cells into HEV-like was considered to be important in these leukocytic infiltrations.

REFERENCES