Short Communication

Morphological Changes of Tumor Cells Caused by Macrophages Treated with Lignin Derivatives

Kenji Sorimachi, Sunao Yamazaki,* Shozo Toda* and Yosihiro Yasumura

Department of Microbiology, Dokkyo University School of Medicine, Mibu, Tochigi 321-02, Japan
* Department of Agricultural Chemistry, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan

Received March 19, 1990

Macrophages were activated with a lignin derivative (EP3) purified from the water extract of the culture medium of Lentinus edodes mycelia (LEM). When human urinary bladder carcinoma cells (HUB-15) were co-cultured with lignin-activated macrophages, HUB-15 cell almost completely came off from the culture surface.

Recently, it was reported that LEM activated macrophages in vitro. This led us to examine whether or not macrophages activated with lignin derivatives affect co-cultured tumor cells. Macrophages were prepared from rat bone marrow by the method of Saotome et al. Bone marrow cells were cultured for 2 days in DM-160 supplemented with 10% fetal bovine serum. The cell cultures were then washed to remove floating cells. After these procedures, about 95% of the attached cells are macrophages.21 Macrophages cultured in the control medium were round as shown in Fig. 1A. The shape of HUB-15 cells was epithelial as shown in Fig. 1B. Bone marrow cells and HUB-15 cells were co-cultured at 37°C for 2 days. The culture was then washed to remove floating cells and further cultured for 3 days. The shape of HUB-15 cells changed as shown in Fig. 1C. A significant morphological change was observed after 1 day of culture after cell washing. Furthermore, when EP3 at 10 µg/ml was added to the culture medium HUB-15 cells markedly changed to a round shape and tended to come off from the culture surface within one day. After 3 days of culture in the presence of EP3, most of the HUB-15 cells came off as shown in Fig. 1D. Other human urinary bladder carcinoma cell lines (HUB-4 and HUB-31) derived from different tumors were also affected by macrophages treated with EP3. Neither EP3 alone nor the conditioned medium of macrophages treated with EP3 affected the shape of HUB-15 cells. This suggests that the EP3-activated macrophages may directly attack tumor cells or the responsible factor in the conditioned medium may be unstable.

Another lignin derivative, LS (lignosulfonate), is known to activate macrophages. In this study, macrophages treated with LS also affected HUB-15 cells. This effect was quite similar to that of EP3. An in vivo study showed that the oral administration of LEM containing EP3 improved hepatic function of hepatitis-B patients. Recently, it was shown that EP3 inhibited the cytopathic effects of human immunodeficiency virus (HIV), herpes simplex virus, western equine encephalitis virus, and polyovirus in vitro. Judging from these results, the cytotoxic effect of macrophages activated with lignin derivatives is of interest not only to basic research but also to clinical research.

Acknowledgment. We thank Dr. J. Enami of Dokkyo University School of Medicine for revising this manuscript.

References

Bone marrow cells cultured for 2 days were washed three times to remove floating cells, and the attached cells were further cultured for 3 days (A). HUB-15 cells (3 x 10^4) were cultured for 5 days (B). Bone marrow cells (3 x 10^6) and HUB-15 cells co-cultured for 2 days and the culture was then washed three times to remove floating cells, and further cultured in the absence (C) and presence (D) of EP3 at 10 μg/ml for 3 days. The magnification was x 100.