## *Short Communication*

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

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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*.[1] 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.*[2] Bone marrow cells were cultured for 2 days in DM-160[3] 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.[2] Macrophages cultured in the control medium were round as shown in Fig. 1A. The shape of HUB-15 cells[4] 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)[4] 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.[5] 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.[6] Recently, it was shown that EP3 inhibited the cytopathic effects of human immunodeficiency virus (HIV),[7] herpes simplex virus, western equine encephalitis virus, and polyovirus *in vitro*.[8] 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.

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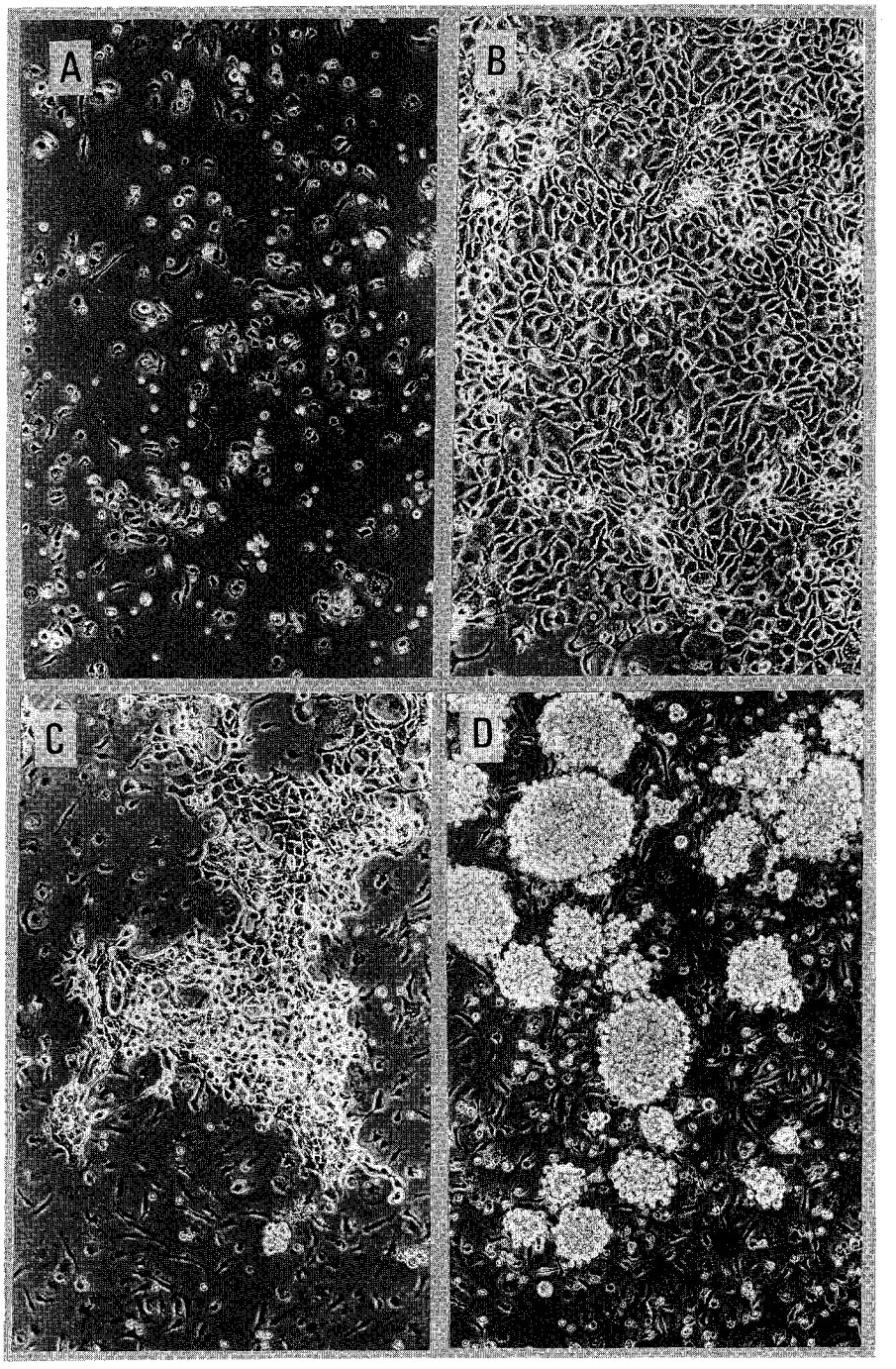


**Fig. 1.** Phase Contrast Micrographs of HUB-15 Cells Co-cultured with Macrophages in the Presence and Absence of the Lignin Derivative EP3.

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 ($3 \times 10^4$) were cultured for 5 days (B). Bone marrow cells ($3 \times 10^6$) 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 $\mu$g/ml for 3 days. The magnification was $\times$ 100.

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