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Antitumor Effects of IL-6 on Murine Liver Tumor Cells in vivo

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Key Words

Liver tumor · Interleukin-6 · Antitumor effects · Host immunity

Abstract

IL-6 is a pleiotropic cytokine that is capable of modulating the diverse functions of hepatocytes such as acute phase responses and inflammation in the liver. To learn its antitumor effects in vivo, the cDNA of IL-6 was transfected into murine liver cells, TIB cells. IL-6-transfected TIB cells (TIB73-IL-6 or TIB75-IL-6) produced much higher levels of IL-6 compared with vector-transfected TIB cells (TIB73-vec or TIB75-vec). To investigate the effects of IL-6 on TIB tumor growth in vivo, IL-6-transfected TIB cells or vector-transfected TIB cells were injected subcutaneously into syngeneic mice. Vector-transfected TIB cells grew rapidly 3 weeks after injection, but IL-6-transfected TIB cells did not grow at all for up to 6 weeks. Pathologically, IL-6-transfected TIB cells demonstrated a severe necrosis and apoptotic pattern. Taken together, these results indicate that IL-6 functions as a growth inhibiting factor in vivo, and another biological role of IL-6 in the liver is suggested.

Many cytokines are involved in regulating biophysical homeostasis of the liver. They regulate the biomolecule metabolism, development, and regeneration of liver cells. IL-6 has diverse effects on liver functions including differentiation and inflammation in the liver. Kopf et al. [4] reported that IL-6-deficient mice showed impaired immune and acute phase response, whereas liver failure and defectiveness in hepatocyte regeneration also has been demonstrated in IL-6-deficient mice [2]. These in vivo studies further suggest that IL-6 has an essential role in liver functions. In addition, IL-6 has been reported to induce host immunity to regress tumors such as melanoma [7], lung carcinoma [8], sarcoma [5], and B lymphoma [1].

To test the antitumor effects of IL-6 on liver tumors, human IL-6 cDNA (kindly provided by Dr. T. Kishimoto, Osaka University, Japan) was amplified by polymerase chain reaction with primers containing an SmaI restriction site. After cutting with SmaI and ligation with Bam HI linker, IL-6 cDNA was subcloned into the Bam HI site of the pNeoSRall expression vector [1]. To obtain a stable transfected clone, the IL-6 cDNA was transfected into murine liver tumor cells, TIB73 cells (ATCC TIB73) or TIB75 cells (ATCC TIB75), and selected in 0.75 mg/ml G418 containing medium for three weeks. Subsequently resistant cells were limit-diluted for single cell cloning. TIB73 or TIB75 clones (5×10^3) were maintained in

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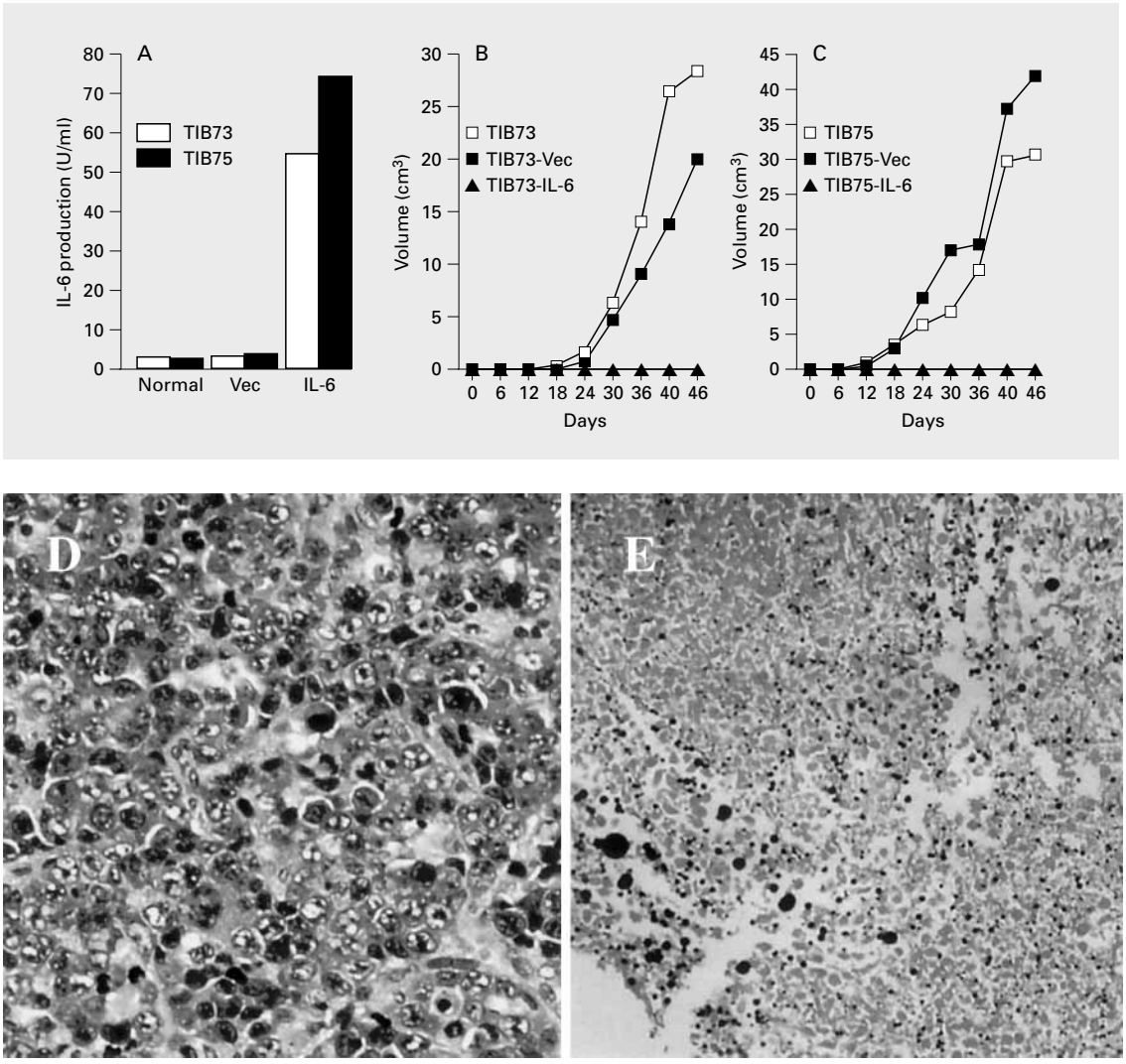

Fig. 1. Antitumor effects of IL-6 on TIB cells. Constructed pNeoSRall containing IL-6 cDNA was transfected into TIB cells. After selection with 0.75 mg/ml G418, stable TIB73-IL-6 and TIB75-IL-6 cells were obtained. Culture supernatants from normal cells, vector-transfected cells (vec), or TIB-IL-6 cells (IL-6) were tested for IL-6 activity using B9 bioassay (**A**). Five syngeneic BALB/C mice were injected with 1×10^5 TIB-vec, TIB73-IL-6 cells (**B**), or TIB75-IL-6 cells (**C**). Palpable tumor growth was measured every day up to 46 days after injection. Tumors growing subcutaneously were excised, fixed in formalin, stained with hematoxylin and eosin, and examined by light microscopy. TIB-vec tumors (× 400), well-developed liver cell tumors, were observed (**D**). TIB-IL-6 tumor (× 400): necrotic and apoptotic cells were observed in the tumor area (**E**).

RPMI medium containing 10% fetal bovine serum and the culture supernatants were assayed for IL-6 activity using B9 bioassay [3]. IL-6 transfected TIB73 or TIB75 produced more than 20 times the IL-6 as compared with the control or with vector-transfected TIB cells (fig. 1A). Moreover, there was no apparent difference in growth rates between the IL-6 transfectants and control cells (data not shown).

To learn the growth pattern of TIB cells in vivo, 1×10^6 TIB cells were subcutaneously injected into syngeneic BALB/c mice (6–8 weeks old). Tumor growth was then examined daily and palpable tumors were measured on two perpendicular axes. Tumor volume was calculated assuming spherical growth using the formula, $4/3\ \pi\gamma^3$. Three weeks after injection, tumor growth was detectable in the case of control or vector-transfected cells (fig. 1B,

C). However, complete tumor regression occurred in the IL-6-transfected cells up to 46 days after injection, suggesting IL-6 itself had an antitumor effect on TIB cells in vivo. Histological analysis indicated that IL-6 induced severe tumor necrosis and apoptosis (fig. 1E) compared with control cells (fig. 1D).

Many cytokines are known to induce host immunity to suppress tumors in vivo. IL-2 and IFN-γ have induced the infiltration of cytotoxic T lymphocytes to inhibit the growth of fibrosarcoma, bladder carcinoma, and mastocytoma. IL-4 abrogated the growth of renal cell carcinoma by activating T cells and eosinophils. Recently cloned IL-18 also inhibited Meth A sarcoma and melanoma [6].

This is the first report that IL-6 inhibits murine liver tumors in vivo. We do not yet know what mechanisms are involved in liver tumor regression in vivo. The lack of apparent effects of IL-6 on tumor cell growth in vitro and in previous in vivo studies using IL-6 [1, 5, 7, 8] strongly suggests that IL-6 induces host immunity in vivo. Further characterization will be required on this point.

In conclusion, our results imply that IL-6 has diverse effects on normal heptocytes in mainting their functions and regeneration as well as inhibiting liver tumor cells growth in vivo.

Acknowledgments

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