

PATHOLOGICAL STUDIES ON THE ANTI-INVASIVE CHARACTER BY RECOMBINANT HUMAN INTERLEUKIN-6 GENE-TRANSFECTED MOUSE LEUKEMIA CELLS

Ge Linfu 葛林阜 Cao Xuetao* 曹雪涛 Ma Dalie* 马大烈 Li Jinsong 李劲松
Zhang Fuzhou 张福州 Yu Yizhi* 于益芝 Ye Tianxing* 叶天星

General Hospital of Jinan Command, PLA, Jinan, 250031

* Second Military Medical University, Shanghai, 200433

Mouse FBL-3 erythroleukemia cells were transfected with recombinant human interleukin-6 (rhIL-6) gene and a clone secreting IL-6 was selected (i.e., FBL-3-IL-6⁺). The cell clone secreting IL-6 was inoculated into C57BL/6 mouse. The growth of tumors was observed and histologic analyses of the tumors *in situ*, liver, spleen and bone marrow were performed after inoculation. The mice inoculated with wild-type FBL-3 erythroleukemia cells were used as the control. The results showed that the later the tumor occurrence, the slower the tumor development, the lower the pathological changes degree and the longer the survival time in experimental group compared to that of the control. The results demonstrated that the inoculation of the FBL-3 cell clone secreting IL-6 can inhibit the invasion of leukemia cells, suggesting that the FBL-3-IL-6⁺ cells can be used as a vaccine to treat leukemia.

Key words: Leukemia, Experimental interleukin-6 gene transfer, Invasion of leukemia cells

The effect of recombinant human interleukin-6 (rhIL-6) on leukemia has been observed in animal experiment. Mouse FBL-3 erythroleukemia cells were transfected with IL-6 gene and a clone secreting rhIL-6 was selected (i.e. FBL-3-IL-6⁺) in our laboratory. Previous work showed that the tumor cell growth was

inhibited, the tumorigenicity was reduced and immunogenicity was enhanced in mice inoculated with the cell line. In this paper, the histologic examinations of the tumors in mice inoculated with FBL-3 erythroleukemia cells transfected with IL-6 gene were performed. The aims were to know whether the clone could inhibit the invasion of the leukemia cells.

MATERIALS AND METHODS

Mice

In this study, male C57BL/6 strain mouse (n=80) from the Shanghai SIPPR/BK Experimental Animal Ltd. Col., between the ages of 6 and 8 weeks were eligible. Mice were fed with a standard pellet diet in clean shield system.

Tumor Cells

The FBL-3 erythroleukemia cells obtained from Prof. He Qizao (Department of Immunology, Shanghai Medical University). FBL-3-IL-6⁺ cell line were established in our laboratory as described previously.¹

Experimental Design

Mice were divided into two groups at random and treated as follow: FBL-3-IL-6⁺ (2×10^6 cells) was inoculated into each mouse subcutaneously in experimental group (n=40). In the second group (n=40) each mouse was inoculated in same situs with wild-type FBL-3 erythroleukemia cells (2×10^6 cells). The tumor growth of each mouse was observed. 8 mice of each group were killed at day 14, 21, 28 and 35 after the inoculation, and histologic examinations of the tumors in situs and the livers, spleens and bone marrows were performed, respectively. And, the tumors in situs were removed and the diameter of tumors were measured. Then the samples were fixed in 10% buffered formalin and embedded in paraffin wax. Sections (4 μ m) were cut and routinely stained with Hematoxylineosin (HE) for routine histologic

evaluation. The survival time for 60 days was observed in the remainder mice.

RESULTS

The Tumor Growth Characteristics

As shown in Table 1, consisted mainly of later the tumor occurrence, slower the growth speed and lower the pathological changes degree in experimental group compared with that of the control. There were 40 days survival time 6 mice in experimental group, and one mouse of this group was still alive at 60 days after inoculation. All control mice were dead within 40 days.

Table 1. The tumor growth characteristics after inoculation in two groups

Day	wild-type tumor			FBL-3-IL-6 ⁺ tumor		
	n	diameter (mm, $\bar{x} \pm s$)	section	n	diameter (mm, $\bar{x} \pm s$)	section
7th	8	show		8	no	
14th	8	7.5 \pm 0.7	grey nodular, spotty foci	8	show	white nodular
21th	8	10.5 \pm 0.8	patchy foci, liquefaction in center	8	2.0 \pm 0.5	white nodular
28th	8	13.0 \pm 0.9	liquefaction, destruction of bone	8	5.0 \pm 0.7	grey nodular, spotty foci
35th	8	16.0 \pm 0.6	tumor rupture, liquefaction necrosis	8	8.0 \pm 0.9	grey nodular, liquefaction in center

The Invasion of Leukemia Cells after Inoculation

The visible tumors around inoculated situs has been shown from the 7th day in mice administered with wide-type FBL-3 cells. In control mice, the obvious invasion of leukemia cells in the specimens of musculi skeleti were detected at day 14 (Figure 1), the invasive foci of the bone and bone marrow were observed at day 21 (Figure 2), dominant pathological lesions in erythrocytoblast system of bone marrow turned to leukoblast system and the invasive foci of the liver and spleen were shown at day 28 (Figure 3-5). In the experimental group, a slight invasion of leukemia cells in the specimens of musculi skeleti were found at day 14 (Figure 6), but the invasion of leukemia cells in the bone marrow weren't shown at

day 21 (Figure 7). The invasive foci of the bone, bone

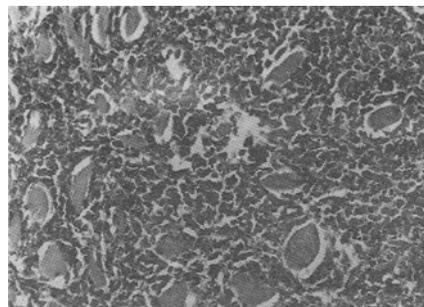


Fig 1. The obvious invasion of leukemia cells in the musculi skeleti of mice at day 14 after inoculated with wide-type FBL-3 cells.

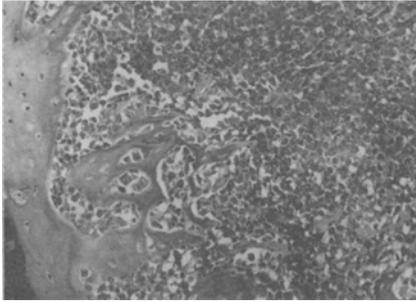


Fig 2. The invasive foci of the bone and bone marrow in mice at day 21 after inoculated with wide-type FBL-3 cells.

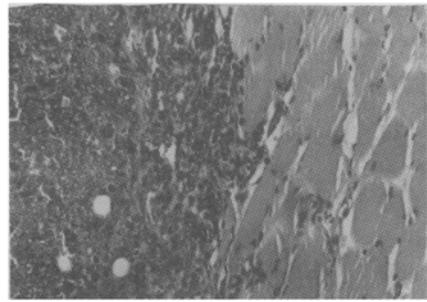


Fig 6. The slight invasion of leukemia cells in the musculi skeleti of mice at day 14 after inoculated FBL-3-IL-6⁺ cells.

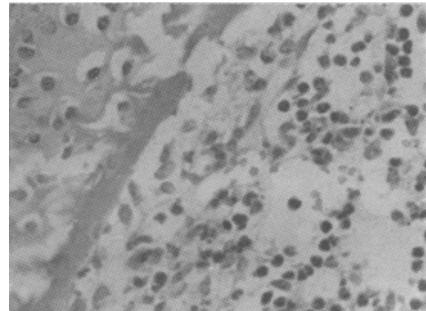
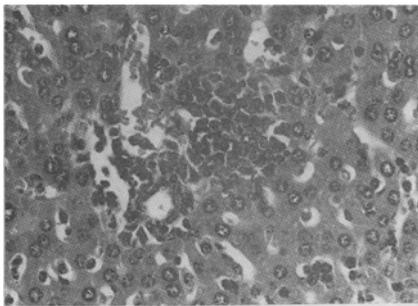


Fig 7. The normal bone marrow of mice at day 21 after inoculated FBL-3-IL-6⁺ cells.

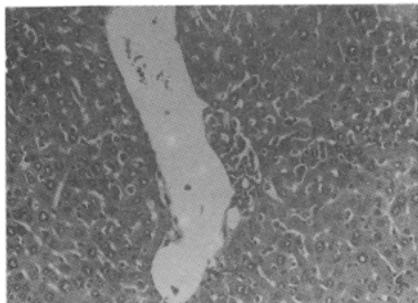
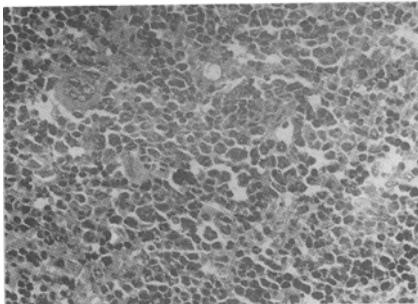


Fig 3 – 5. The invasive foci of the liver and spleen in mice at day 28 after inoculated with wide-type FBL-3 cells.

marrow, liver and spleen were detected only in 3 experimental mice at day 35. There as a survival time of 60 days without the invasiveness of the bone, bone marrow, liver and spleen in one experimental mouse.

DISCUSSION

IL-6 is a pleiotropic cytokine which has been shown to have many biologic activities. IL-6 may be an important cytokine in the host's immune and metabolic responses to leukemia.² IL-6 had a direct inhibitory effect on the growth of certain tumor cells *in vitro*.^{3,4} Mule, et al. have shown that C57BL/6 mice were injected intravenously with MCA-105, -106 or -203 sarcoma or MC-38 colon adenocarcinoma cells or intrasplenically with MCA-203 sarcoma cells to

induce pulmonary and hepatic metastases, respectively. In their experiment metastases from all-four-tumors were significantly reduced by the administration of rhIL-6 alone without apparent toxicity.⁵ In this report, we have shown here that FBL-3-IL-6⁺ anti-invasion of leukemia cells effect was observed in mice. The mechanism may be as follows: IL-6 may be secreted by FBL-3-IL-6⁺ cells in situ, secondary cytokines may be released or cellular immune mechanisms may be activated following FBL-3-IL-6⁺ inoculation, and directly or indirectly result in the anti-invasion effect.

The organs invasion of leukemia cells is one of the major causes resulting in the deterioration or death in the patients with leukemia. Anti-invasive characteristics in mice inoculated FBL-3-IL-6⁺ (2×10^6 cells) were observed in present study; nevertheless, the organs invasion of leukemia cells could be found and lead to death in the most of experimental mice within 60 days. The results presented in this paper suggested that the antitumor effect of FBL-3-IL-6⁺ was not enough to kill all of tumor cells and that IL-6 gene may be lost or the IL-6 level decreased in body after inoculation with FBL-3-IL-6⁺.

The results demonstrated that inoculation of the FBL-3 cell clone secreting IL-6 can inhibit the invasion of leukemia cells, suggesting that the FBL-3-

IL-6⁺ cells can be used as a vaccine to treat leukemia.

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