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EFFICIENT ACTIVATION OF ANTITUMOR IMMUNITY BY IL-6 GENE-MODIFIED LEUKEMIA VACCINE IN COMBINATION WITH LOW DOSE CYCLOPHOSPHAMIDE AND LOW DOSE IL-2

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Objective: To investigate the antitumor effect of the IL-6 gene-modified erythroleukemia cells combined with low dose cyclophosphamide (Cy) and low dose IL-2. Methods: Mice inoculated with FBL-3-IL-6 in combination with low dose IL-2 and low dose cyclophosphamide (Cy). Results: Mice received combined therapy of FBL-3-IL-6, IL-2 and Cy developed tumors most slowly and survived much longer when compared with mice in control groups, with 5 out of 8 leukemia-bearing mice being tumor free 100 days after the combined treatment. To further explain the mechanism of the antitumor effects by the combined therapy. It was found that combined therapy with low dose Cy, low dose IL-2 and FBL-3-IL-6 achieved maximal cytotoxic effects of nature killer cells and specific cytotoxic T lymphocytes, increased production IL-2, TNF and GM-CSF from spleen lymphocytes in tumor-bearing mice. Vaccination with the FBL3-IL-6 also enhanced the cytotoxic activity of the peritoneal macrophages. The results demonstrated that administration of low dose Cy and low dose IL-2 in combination with IL-6 gene-modified leukemia vaccine could elicit potent anti-leukemia effects, and the mechanisms involved in the antitumor process may include the induction of specific and nonspecific antitumor immunity, reversal of T suppressor cells that mediated local immuno-suppression in tumor bearing mice. Conclusion: The combined therapy with cytokine gene-modified tumor vaccine, low dose of Cy and IL-2 might be a promising approach for the treatment of leukemia.

Key words: Interleukin 6, Immunotherapy, Gene therapy, Leukemia, Interleukin 2, Cyclophosphamide

Produced IL-6 is a cytokine with multifunction. It is porduced by a number of cell types and involved in the responses of immune response, hematopoiesis and inflammation.[1] IL-6, alone or in combination with other cytokines like IL-1, IFNγ, GM-CSF and TNF, could elicit obvious differentiation effects on various leukemia cell both *in vitro* and *in vivo*.[2-4] Recombinant IL-6 was found to induce in vivo generation of CTL against syngeneic transplantable FBL-3 in lymph node cells and peritoneal exude cells in mice.[5] Recently cytokine gene therapy for cancer has been studied intensively with several approaches of gene delivery developed.[6] Tumor vaccine engineered to secrete various cytokines has been proved to be of particular interests for the treatment of certain kinds of tumors.[7] In these studies, the genes of IL-2, IL-4, IFNγ, TNF, GM-CSF, etc. were introduced into tumor cells to increase the immunogenicity of the tumor cells, and vaccination with the cytokine gene-modified tumor vaccine could induce systemic immunity against a subsequent challenge with parent tumor cells.[8,9]

In the present study, therapeutic effects of the IL-6 gene-modified erythroleukemia cells combined with low dose cyclophosphamide (Cy) and low dose IL-2 were observed *in vivo*. Our results demonstrated that IL-6 gene-modified tumor vaccine, with the synergy of immuno-modulating effects of IL-2 and Cy, could

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elicit potent anti-leukemia effects, mainly through the induction of antitumor immunity.

MATERIALS AND METHODS

Medium and reagents

All culture media were purchased from Gibco-BRL, USA, and fetal calf serum (FCS) provided by Shanghai Institute of Biological Products, Shanghai, China. G418, Con A and actinomycin D from Sigma USA. Mitomycin C was from Kyowa Hakko Kogyo Japan. Na_2CrO_4 was purchased from Amersham, Arlington Heights, USA. Recombinant human IL-2 (specific activity 1×10^7 IU/mg, purity>98%) was expressed in E. coli. and purified in our laboratory. Recombinant human IL-6 was kindly provided by Genentech, USA.

Cell lines

FBL-3, a erythroleukemia cell line derived from C57BL/6 mouse, was kindly provided by Prof. Wei Chen in Cleveland Clinic Foundation, Cleveland, USA. FBL-3 cells, transduced with IL-6 gene, control Neo gene or without transduction, were maintained in RPMI-1640 medium supplemented with penicillin 100 U/ml, streptomycin 100 μg/ml, 2-mercaptoethanol 50 mmol/L and 10% fetal calf fetal calf serum (FCS). YAC-1 cells, obtained from the Cell Bank, Chinese Academy of Science, Shanghai, China, were maintained in RPMI-1640 medium supplemented with above agents. CTLL-2, B9, and L929 cells were routinely maintained in our department.

Animals

Male or female C57BL/6 mice, 6-8 weeks of age, purchased from Joint Ventures Sippr-BK Experimental Animal Co., Shanghai, China, were housed for at least one week in a specific pathogen-free state before their use in any experiment.

Preparation of tumor vaccines

FBL-3 cells were transduced with recombinant expression vector BMGNeo and BMGNeoIL-6 containing human IL-6 cDNA by the calcium phosphate co-precipitation method and the transduced cells were collected by selecting G418-resistant cells.[10] IL-6-secreting FBL-3 cells or parental FBL-3 cells were propagated *in vitro* and resuspended in RPMI-1640 medium with 10% FCS. Mitomycin C was added to the leukemia cells with a final concentration of 80 μg/ml. The cells were then cultured at 37°C in 5% CO_2 for 1 h. The cells were washed three times with PBS and resuspended in RPMI-1640 with a density of 1×10^7 cell/ml and used as tumor vaccines.

Treatment of leukemia-bearing mice with tumor vaccine

C57BL/6 mice were given 2×10^6 FBL-3 cells s.c.. Three days after the tumor inoculation the leukemia-bearing mice were divided in to 12 groups and the following therapy were treated twice with one week interval. Each group received an intraperitoneal injection of any of the following preparations: In group A (Hanks), mice were given injection of Hanks solution 3 times a day for 3 consecutive days. The mice in group B (IL-2) received 3 injections of IL-2 (2000 U) a day for 3 days. For mice in group C (Cy), mice were given an injection of Cy at 20 mg/kg and in group D (Cy+IL-2), Cy 20 mg/kg was given one day prior to the administration of IL-2 2000 U/mouse. In group E (FBL-3), 0.5 ml of leukemia vaccine equivalent to 5×10^6 FBL-3 cells were injected. The mice in group F (FBL-3 + IL-2) received the injection of wild type of FBL-3 vaccine and IL-2 (2000 U, 3 times a day for 3 days). In group G (FBL-3 + IL-2), the mice were administered with the pre-injection of Cy (20mg/kg) and subsequent injection of FBL-3 vaccine (5×10^6 cell in 0.5 ml). In group H (FBL-3 + Cy + IL-2). The mice were injection with Cy (20 mg/kg) one day prior to the injection of FBL-3 leukemia vaccine (equivalent to 5×10^6 cells) and low dose IL-2 (2000 U, 3 times a day for 3 days). In group I to group L, inactivated FBL-3-IL-6 leukemia vaccine were used to replace the wild type FBL-3 cells in groups E to G and are assigned as Group I (FBL-3IL-6), Group J (FBL-3-IL-6 + IL-2), Group K (FBL-3-IL-6+Cy), Group L (FBL-3-IL-6+IL-2+Cy).

Preparation of effector cells

Two weeks after various treatments, the mice in each groups were killed and splenocytes were isolated from the sacrificed mice. The freshly prepared splenocytes were washed three times in Hanks

solution and the cells were used as NK (natural killer) cells. For CTL preparation, the cells were co-cultured with mitomycin C-inactivated FBL-3 cells at a ratio of 20:1 for 6 days and then the viable lymphocytes were used as CTL. The cytotoxicity assay at effector: target (E:T) ratio of 50:1 was carried out by using the standard ^{51}Cr-release method. YAC-1 and wild type FBL-3 were used as targets for the evaluation of NK and CTL cytotoxic activities, respectively.

Cytotoxic assay

Four-hour ^{51}Cr release assays were performed as described previously. Briefly, 2×10^6 B16F10 or YAC-1 in 0.5 ml RPMI-1640 with 20% FCS were labeled with 200 μCi $Na^{51}CrO_4$ for 2 h. The labeled cells were washed three times in serum-free medium. 10^4 target cells were then mixed with effector cells for 4 h at 37°C at the ratio indicated. For the maximal ^{51}Cr release control, 0.1 ml of 0.1 N HCl was added to the target cells, and for the spontaneous ^{51}Cr control, 0.1 ml medium was added to the labeled cells. The amount of ^{51}Cr released was determined by γ counting on a 1275 Minigamma Counter (LKB-Wallac, Finland), and percentage of specific lysis was calculated as follows:

$$\% \text{ of specific lysis} = \frac{\text{Experimental cpm - spontaneous cpm}}{\text{Maximal cpm - spontaneous cpm}} \times 100$$

Induction of cytokine

Splenocytes freshly prepared from the sacrificed mice were co-cultured at 5×10^6 cells/ml in complete RPMI-1640 medium containing 10 μg/ml of Con A. 24 h later, the culture supernatants were collected for IL-2 assay, 48 h later the supernatants were collected for the activity analysis of GM-CSF. The splenocytes were cultured at 5×10^6 cells/ml with 10 μg/ml PHA for 48 h at 37 °C in 5% CO_2, and the supernatants were collected for TNF assay.

IL-2 or IL-6 bioassay

IL-2 or IL-6 activity was determined utilizing IL-2-depedent CTLL-2 cells or IL-6-dependent B9 cells. Briefly, 2×10^4 cells in a volume of 50 μl of serial dilutions of the supernatants or recombinant cytokines were added and cultivated in 5% CO_2 at 37°C for 36 h. The proliferation of cells was measured by MTT method. The unit of IL-2 and IL-6 was defined as standard recombinant IL-2 or IL-6.

TNF bioassay

TNF bioassay was performed utilizing L929 cells as targets. Briefly, L929 cells at 2×10^4/well were plated in a 96-well plate to form monolayer. Supernatant in two-fold dilutions was added to the cell monolayer in combination with 1 μg/ml actinomycin D. One day later the remaining live L929 cells after TNF lysis were determined by staining with 0.5% crystal violet and the absorbance of the dye dissolved in extraction solution was read on a BIO-RAD model 2550 microplate spectrophotometer. The cytotoxic activity (in units/ml) was defined as the reciprocal of the dilution resulting 50% cytotoxicity determined by plotting the regression line of log dilution against absorbance.

GM-CSF assay

The GM-CSF activity in the supernatants of splenocytes 48 h after co-culture with PHA was determined using murine bone marrow cell proliferation methods. Briefly, murine bone marrow cells prepared from mice were plated in 96-well plate and dilutions of supernatants or standard recombinant murine GM-CSF were added, and the cultures have incubated in 5% CO_2 at 37°C for 36 h. Proliferation of bone marrow cells was measured by MTT method. The amounts of GM-CSF were defined as standard recombinant murine GM-CSF.

Cytotoxicity of peritoneal macrophages

Resident macrophages were washed from the peritoneal cavity with 5 ml RPMI-1640 after centrifugation at 1000 rpm for 10 min at 4°C, the cell pellet was resuspended in complete media and the adherent macrophages were obtained by plating the cells into 96-well plastic trays (Corning Glass Works, USA). For macrophage cytotoxicity assay, macrophages were incubated with L1210 cells at E/T = 10 in a total volume of 0.1 ml medium per well in 96-well flat-bottomed plate. After 20 h of co-incubation at 37°C, 5% CO_2 and 95% relative

humidity, 10 μl MTT was added to L1210 cells removed from macrophages. The plates were cultured for 4 h followed by the addition of 0.1 ml 10% SDS in 0.01 N HCl. The formazan crystals were dissolved in 10% SDS and the absorbance was read at 540 nm on a BIO-RAD model 2550 microplate reader. Percentage of cytotoxicity was calculated relative to the calibration standard L1210 as follows:

Percentage of cytotoxicity =

$$1 - A_{L1210\ remained} / A_{L1210\ standard}$$

Statistics

All experiments were run in triplicate and the results are means ± SD of triplicate determinations (or representative data from one or two independent experiments). Statistical analysis was performed using the Student's *t* test.

RESUTS

Tumor growth after combined therapy

Mice inoculated with FBL-3 cells would develop palpable tumor nodules within 2 weeks after tumor inoculation. The results in Figure 1 showed that FBL-3 vaccine treatment inhibited the growth of subcutaneous tumor and the inhibiting effects were enhanced when accessory low dose Cy or low dose IL-2 were combined. The mice in group H which received the injection of FBL-vaccine, IL-2 and Cy grew more slowly than the mice in groups A-G. When IL-6 gene transduced FBL-3 cells (FBL-3-IL-6) were used instead of wild type FBL-3 cells, the growth of tumors in the mice (group H-L) were greatly arrested compared with the mice treated with wild FBL-3 cells (group E-H). The mice that received triplicate combination therapy of FBL-3-IL-6, IL-2 and Cy developed tumors most slowly when compared with mice in all other 11 groups ($P<0.01$).

Eight mice in each of 12 groups were observed through the experiments for their survival evaluation after various treatments. The mice in groups A-G, which received treatments of Hanks, low dose Cy, low dose IL-2 and/or FBL-3 vaccine, continuously died within 50 days. Slight protective effect was observed with FBL-3 vaccine. The introduction of IL-6 gene into FBL-3 greatly increased the protective effects of FBL-3-IL-6 cell vaccine. The mice treated with FBL-3-IL-6 vaccine lived much longer than all the mice which did not received the therapy with IL-6 gene-modified leukemia vaccine. The co-injection of low dose Cy and low dose with FBL-3-IL-6 vaccine markedly increased the survival period of leukemia-bearing mice, with 25%--50% of the mice lived more than 100 days after experiments and were calculated as tumor free ($P<0.01$). The first mice died 47 days after the tumor inoculation in group L, which were treated with low dose Cy low dose IL-2 and GBL-IL-6 vaccine, and 5 of 8 mice were found to be tumor free 100 days after the therapy (Figure 2).

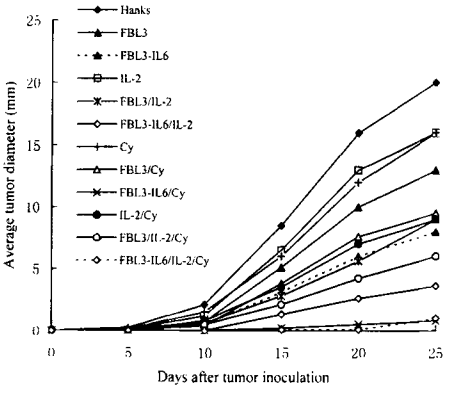

Fig. 1. Effect of combined therapy with IL-6 gene-modified FBL-3 vaccine, low dose IL-2 and low dose cyclophosphamide on tumor growth and survival rate of tumor-bearing mice. C57BL/6 mice were inoculated with 2×10^6 FBL-3 tumor cells s.c. in the rear leg, and received treatment 3 days after the inoculation. The tumor volume was expressed as average diameter of tumor size.

Pathological changes

FBL-3 cells were high invasive and will infiltrate into the bone marrow, liver, spleen and muscle of the leukemia-bearing mice. We observed the infiltration of FBL-3 cells in the muscle, liver, spleen and bone marrow to evaluate the therapeutic effects of FBL-3IL-6 vaccine. The results showed that the muscle, spleen, liver and bone marrow in mice in groups A-G, which received treatments of Hanks, low dose Cy, low dose IL-2 and /or FBL-3 vaccine, were all seriously infiltrated with live proliferating FBL-3 cells. The mice treated with FBL-3-IL-6 vaccine showed slighter infiltration in above organs, and the combination of low dose Cy or low dose IL-2 further decreased the infiltration with only small piece of infiltration area

being found in muscle. After combined therapy with low dose Cy, low dose IL-2 and FBL-3-IL-6 vaccine, no obvious tumor infiltration was found in spleen, liver, bone marrow and muscle (photographs not shown).

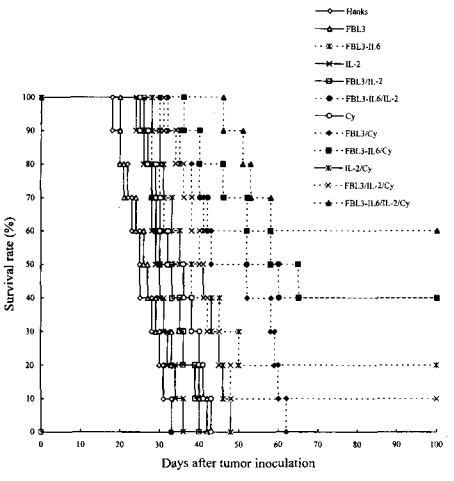

Fig. 2. Survival curve of mice received combined therapy with IL-6 gene-modified FBL-3 vaccine, low dose cyclophosphamide and low dose IL-2. C57BL/6 mice were inoculated with 2×10^6 FBL-3 tumor cells s.c. in the rear leg, and received treatment 3 days after the inoculation.

NK and CTL cytotoxic activity

The fresh splenocytes were directly used as NK effector cells, and the splenocytes were co-cultured with mitomycin C inactivated FBL-3 cells at a ratio of 20 : 1 for 6 days and then the lymphocytes were used as CTL effector cells. The results in Table 1 demonstrated that IL-2, alone or in combination with Cy, or FBL-3 vaccine, could augment the cytotoxicity of NK and CTL. NK and CTL activities were all showed to be greatly elevated after the leukemia-bearing mice were treated with FBL-3-IL-6 vaccine. And these cytotoxic effects could be further augmented by the combination of low dose Cy or low dose IL-2. After combined therapy with low dose Cy, low dose IL-2 and FBL-3-IL-6, the maximal cytotoxic effects of NK and CTL were achieved. These data strongly showed that IL-6, the maximal cytotoxic effects of NK and CTL were achieved. These data strongly showed that IL-6 gene transduction could obviously increase the cytotoxicity of NK and CTL activity *in vivo*, and these cytotoxic effects could be further increased by the combined therapy with Cy and low dose IL-2 ($P<0.01$).

Cytokine release

Splenocytes prepared from sacrificed mice were-co-cultured with Con A to induce IL-2 and GM-CSF, and were cultured with PHA for TNF induction. IL-2 activity was determined utilizing IL-2-depedent

Table 1. The NK and CTL activities induced from splenocytes of leukemia-bearing mice treated with combined therapy of IL-6 gene-modified FBL-3 vaccine, low dose IL-2 and cyclophosphamide. YAC-1 and FBL-3 cells were used as target cells for the cytotoxic activity assay of the NK and CTL cells at E: T of 50: 1.

Groups	NK activity ($\bar{\chi} \pm s$)	CTL activity ($\bar{\chi} \pm s$)
Hanks	12.6 ± 1.7	9.2 ± 2.1
IL-2	16.8 ± 2.7	16.8 ± 2.6
Cy	13.7 ± 1.9	9.1 ± 2.1
IL-2+ Cy	17.3 ± 3.3	17.1 ± 2.7
FBL-3	17.8 ± 2.4	8.5 ± 2.8
FBL- 3+IL-2	25.2 ± 3.7	24.3 ± 3.1
FBL-3+ Cy	18.1 ± 3.0	18.6 ± 2.9
FBL-3+ IL-2 + Cy	26.4 ± 3.6	27.8 ± 3.0
FBL-3-IL-6	27.3 ± 3.9	38.6 ± 3.1
FBL-3-IL-6+ IL-2	36.8 ± 5.9	46.2± 5.1
FBL-3-IL-6+ Cy	28.1 ± 4.0	39.2 ± 3.2
FBL-3-IL-6+ IL-2+ Cy	42.6 ± 4.2	59.8 ± 6.3

CTLL-2 cells. The GM-CSF activity in the supernatants of splenocytes was determined using murine bone marrow cell proliferation methods, and INF activity in supernantants were determined according to the cytolysis activity of TNF to L929 cells. As illustrated in Table 2, the vaccine prepared with FBL-3-IL-6 cells increased the release of IL-2, TNF and GM-CSF from spleen lymphocytes in tumor-bearing mice. Co-administration of low dose Cy, low dose IL-2 and FBL-3-IL-6 leukemia vaccine most magnificently stimulated the production of these three cytokine from splenocytes ($P<0.01$).

Cytotoxicity of peritoneal macrophages

The cytotoxic activity of macrophages was determined utilizing L1210 as target cells. The data in Figure 3 illustrated that FBL-3 vaccine treatment increased the cytotoxicity of macrophages and the inhibiting effects were enhanced when additional low dose Cy or low dose IL-2 were combined. When IL-6 gene transduced FBL-3 cells (FBL-3-IL-6) were used instead of wild type FBL-3 cells, macrophages from peritoneum in the mice (group H-L) showed enhanced cytotoxic effects when compared with mice in group A to group K ($P<0.01$).

Table 2. Cytokine release from splenocytes of leukemia-bearing mice treated with combined therapy of IL-6 gene-modified FBL-3 vaccine, low dose IL-2 and cyclophosphamide.

Groups	IL-2 (U/ml) ($\bar{\chi}\pm s$)	TNF (U/ml) ($\bar{\chi}\pm s$)	GM-CSF (ng/ml) ($\bar{\chi}\pm s$)
Hanks	34.1±4.1	21.4±5.4	14.8±3.2
FBL-3	31.6±3.9	20.8±5.7	9.4±2.8
IL-2	38.2±5.1	22.6±5.3	10.6±2.9
Cy	32.4±4.3	21.8±5.8	11.4±3.1
IL-2+ Cy	39.3±5.2	22.9±6.1	11.6±3.8
FBL-3-IL-6	52.4±6.2	28.4±4.7	24.8±4.7
FBL-3-IL-6+ IL-2	54.6±6.5	31.2±5.6	26.6±5.4
FBL-3-IL-6+IL-2+Cy	58.9±7.1	38.4±6.1	59.8±5.6

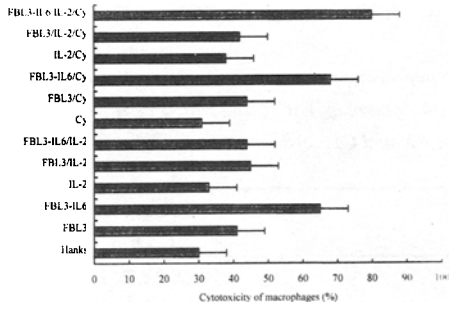

Fig. 3. The cytotoxicity of peritoneal macrophages in leukemia-bearing mice treated with IL-6 gene-modified FBL-3 vaccine, low dose cyclophosphamide and low dose IL-2.

DISCUSSION

Tumors grow out of control mainly through the escape from the immune surveillance of the host. So an important therapy for tumors was to prepare tumor vaccine which might taken from the biopsy of the tumor patients, directly used after inactivation or transduced with various cytokine genes or co-stimulator genes with increased immunogenicity and decreased tumorigenicity. This approach might also be proved to be useful for the treatment of leukemia. A number of investigators have transduced the genes of IL-2, IL-4, IL-6, IFNα, GM-CSF, TNF etc into various tumor cells and their potency as antitumor vaccines tested.[11,12] Very encouraging results were obtained from these new types of tumor vaccine, with satisfactory therapeutic effects and sustained systemic immunity against tumor achieved. The antitumor therapeutic efficacy induced by cytokine gene-modified tumor cells could elicit potent antitumor effects against subsequent challenge with parental tumor cells, but they are not strong enough to eradicate the established tumors. In the host with pre-established tumors, the immunity of the host was often suppressed by the invasion of the tumors. So it would be of great importance to reverse the immuno-suppression when

tumor vaccine were used for the therapy of established tumors.

The tumor-induced immuno-suppression is mainly thanks to the presence of T suppressor cells, which give an obstable to the activatiuon on antitumor immunity of the host. Low dose Cy or low dose IL-2 were effective agents for the elimination of suppressor T cells.[13-16] In our previous reports we employed an experimental tumor model with murine B16F10 melanoma pulmonary metastasis and confirmed the hypothesis that the antitumor response could be induced more efficiently by the administration of low dose Cy, or/and low dose IL-2 prior to the therapy with cytokine gene-transduced tumor cells. Cy, being one of the most widely used drugs for chemotherapy, could augment the cell-mediated immunity and act as potent immuno-modulating agents when used in low dose. Therapeutic effects had been reported in mice bearing small immature tumors with a single low dose of Cy. Clinical trials with low dose Cy confirmed its immuno-modulating effects and positive antitumor response could be obtained in melanoma patients with combined therapy of tumor vaccine, low dose of Cy and recombinant IL-2. We have reported previously that vaccination with inactivated IL-2 gene-modified vaccine, in combination with IL-1 as adjuvants and low dose Cy as immuno-modulating agent, could exert potent anti-metastatic effects in mice inoculated with B16F10 melanoma. It has been soundly demonstrated that low dose IL-2 may enhance T cell-mediated immune response against weakly immunogenic tumors and can make immuno-suppressed non-responders become responsive to vaccination. Obvious antitumor effects has been achieved with the immunotherapy by IL-3, IL-4, TNF gene-trasduced tumor vaccine combined with low dose of Cy and low dose of IL-2 in our department.

Recombinant IL-6 has been shown to be an effective agent for the treatment of primary tumor and metastases in tumor-bearing micel.[16,17] Combined therapy with IL-6 and inactivated tumor cells was found to suprress metastasis in mice bearing 3 LL lung carcinomas.[18] Different studies in experimental animals have been performed to investigate the biological characteristics and antitumor effect of IL-6 gene-transfected tumor cells.[19,20] IL-6 gene-transfected D122 lung carcinoma cells showed *in vitro* growth inhibition, which was directly correlated with the levels of IL-6 secretion. Fibrosarcoma cells transduced with IL-6 gene exhibited reduced tumorigenicity, increased immunogenicity and decreased metastatic potential.[21] So tumor cells transduced with IL-6 gene generally showed reduced tumorigenicity, enhanced immunity of the host, and could employed as useful tumor vaccine dose of Cy and low dose IL-2 prior to the therapy with IL-6 gene modified leukemia vaccine could elicit potent anti-leukemia effects on leukemia bearing mice, the pathological analysis found that the muscle, liver, spleen and bone marrow in mice treated with Hanks, low dose Cy, low dose IL-2 and/or FBL-3 vaccine were all seriously infiltrated with live proliferating FBL-3 cells, but after combined therapy with low dose Cy, low dose IL-2 and FBL-3-IL-6 vaccine, no obvious tumor inmfiltration was found. Several mechanisms could be involved in the antitumor effects of IL-6, including direct effects on leukemia cells, immune-enhancing effects on the host, being a co-stimulator of T and B lymphocytes, induction of tumor specific cytotoxic T lymphocytes.[22,23] To further explain the mechanism of the antitumor of the combined therapy, we found that combined therapy with low dose Cy, low dose IL-2 and FBL-3-IL-6 achieved maximal cytotoxic effects of NK and CTL, increased the production IL-2, TNF and GM-CSF from spleen lymphocytes in tumor-bearing mice. Treatment of the leukemia-bearing mice with FBL-3-IL-6 also enhanced the cytotoxic activity of the peritoneal macrophages. So the anti-leukemia mechanisms of the combined therapy involved in the antitumor process may include the induction of specific and nonspecific antitumor immunity, reversal of T suppressor cells mediated local immuno-suppression in tumor bearing mice.

In conclusion, our results demonstrated that adinistration of IL-6 gene modified leukemia vaccine incombination with low dose Cy and low dose IL-2 could greatly improve the therapeutic efficacy in the treatment of established leukemia. The mechanisms involved in the antitumor process may include the induction of specific and nonspecific antitumor immunity, reversal of T suppressor cells mediated local immuno-suppression in leukemia-bearing mice.

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