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THE HUMORAL ANTITUMOR RESPONSES INDUCED BY IL-4 GENE-MODIFIED TUMOR VACCINE[1]

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It is well known that IL-4 plays important roles in the induction of humoral immunity. In the present study, the humoral antitumor responses induced by IL-4 gene-modified tumor vaccine were investigated. The mice were vaccinated with the IL-4 gene-modified B16 melanoma cells. Then the proliferative capacity of the splenic lymphocytes, the levels of the antibodies in the murine serum against wild-type B16 cells and the cytotoxicity of the serum to wild-type melanoma cells were detected. Our data showed that the LPS-induced proliferation of the splenic lymphocytes from the mice vaccinated with the IL-4 gene-modified tumor vaccine increased more significantly than that from mice vaccinated with wild-type tumor vaccine. The cytotoxicity of the serum to wild-type melanoma cells also increased markedly when detected. It was also observed that the number of pulmonary metastases decreased more obviously when the mice were intravenously injected with the mixture of wild-type B16 cells and the serum from the mice vaccinated with the IL-4 gene-modified B16 cells. Our data demonstrated that humoral immunity might contribute to the antitumor effect of IL-4 gene therapy.

Key words: Interleukin 4, Melanoma, Gene therapy, Vaccine, Humoral immunity.

A large number of studies on cytokine gene therapy of cancer showed that the cellular immune mechanisms were involved in the antitumor responses induced by cytokine gene-modified tumor cellular vaccines. But the humoral immune mechanisms were often ignored. It is well known that Interleukin-4 (IL-4) plays important roles in the induction of humoral immunity.[1] In our previous studies, we observed that not only many T lymphocytes and macrophages but also a large number of plasma cells infiltrated into the tumor nodes when the mice were inoculated with IL-4 gene-modified tumor cells.[2,3] The purpose of the present study is to determine whether the humoral immune mechanisms were involved in the antitumor immune responses induced by IL-4 gene-modified tumor vaccines.

MATERIALS AND METHODS

Animals and Cell Lines

Male or female C57BL/6 mice, 6-8 weeks of age, purchased from Joint Ventures SIPPR-BK Experimental Animal Co., Shanghai, China, were housed in a specific pathogen-free state for any experiment. B16F10, a subclone of B16 melanoma cell line from C57BL/6 mice, B16-IL-4 which was engineered to secrete IL-4, B16-Neo which was transfected with control Neo gene[2] were maintained in RPMI-1640 medium supplemented with penicillin 100 U/ml, streptomycin 100 μg/ml, 2-mercaptoethanol 50mmol/L and 10% fetal calf serum(FCS).

Immunizations

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The wild-type B16 cells, B16-IL-4 cells and B16-Neo cells were inactivated by irradiation and then suspended at a concentration of 5×10^6 /ml in RPMI-1640 respectively as tumor vaccines. The C57BL/6 mice were divided into four groups and were injected i.p. with 0.5 ml of RPMI-1640 or the tumor vaccines respectively for 3 times during 3 weeks. The mice were killed 40 days after the first immunization, their sera and splenocytes were obtained for further experiments.

Assay for B Lymphocyte Proliferation

The B lymphocytes separated from the slpenocytes of the mice vaccinated with tumor cellular vaccines were seeded at 4×10^6 /ml in RPMI-1640 containing 10% FCS and 10 μ g/ml LPS. 100 μ l of cell suspension was added to each well of a 96 well tissue culture plate and then the plate was incubated for 72 h at 37°C. Cell proliferation was determined by MTT method as described previously.[1]

Complement-Mediated Cytotoxicity

Specific cytotoxicity of sera was assessed in a ^{51}Cr release assay. In briefly, wild-type B16 cells were resuspended in RPMI-1640 and the $Na_2{}^{51}CrO_4$ (500 μ Ci per 1×10^7cells) were added. The cells were incubated for 60 minutes at 37 °C with occasional shaking. Then the cells were washed at least three times by centrifugation at 300 × g for 10 minutes at room temperature. The cells were then resuspended in RPMI-1640 containing 10%FCS as target cells. 10^4 labeled target cells were incubated in round-bottomed microplates with serial twofold dilutions of serum in 0.2 ml total volume at 4°C for 1 hour and subsequently with guinea pig complement diluted 1:20 for 30 min at 37°C. 0.1 ml of supernatant was collected at the end of incubation period and was counted for gamma radioactivity. The percentage of cytotoxicity was calculated with the following formula: 100×[(cpm released in serum + C–cpm released in C)/(cpm released in detergent–cpm released in C)]

Preparation of the Tumor-Bearing Mice with Pulmonary Metastases

0.5 ml of wild-type B16 cells (4×10^6/ml) were mixed with 0.5 ml of sera from the mice vaccinated with B16-IL-4 or B16-Neo or wild-type B16 cells for 30 min respectively. 100 μ l cell suspensions were injected into C57BL/6 mice via the tail vein respectively. The mice were killed 15 days later and the pulmonary metastases were counted.

Statistical Analysis

The significance of differences was analyzed by Student's *t* test.

RESULTS

The Proliferative Capacity of the B Lymphocytes from the Mice Immunized with IL-4 Gene-Modified Tumor Vaccines

B cells respond to most antigens by proliferating as well as by differentiating to plasma cells, so we detected the proliferative ability of B cells to obtain the first evidence that B cells were stimulated by tumor antigen. The B lymphocytes from the spleens of mice immunized with various vaccines were isolated and then stimulated with LPS. The proliferative ability were assayed by MTT method. As shown in Figure 1, the LPS-stimulated B lymphocytes from the mice vaccinated with IL-4 gene-modified tumor vaccine was significantly enhanced when compared with that of the B lymphocytes from the normal mice or the mice vaccinated with wild-type B16 or B16-Neo($0.05>P>0.01$).

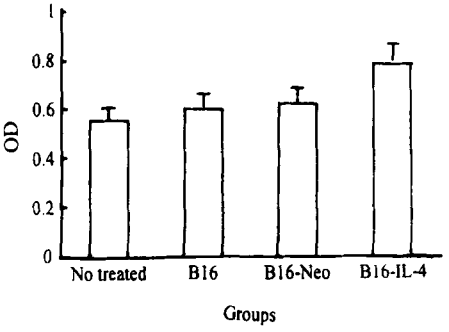

Fig. 1. The proliferative capacity of LPS-stimulated B lymphocytes from the mice immunized with various tumor vaccines detected by MTT method.

The Cytotoxicity of the Sera from the Mice Immunized with IL-4 Gene-Modified Tumor Vaccines

The complement system plays important roles in host defense and it can be activated by antigen-antibody dependent classical pathway, so the specific cytotoxic activity to the tumor cells of the sera becomes the another important evidence that the humoral immunity is involved in the antitumor responses induced by tumor cellular vaccines. Figure 2 showed that the cytotoxicity to B16 melanoma cells of the sera from the mice vaccinated with IL-4 gene-modified tumor vaccines increased markedly when compared with controls($0.05>p>0.01$).

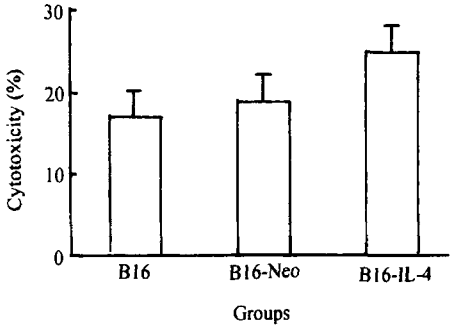

Fig. 2. The cytotoxicity of the serum from the mice immunized with IL-4 gene-modified tumor vaccine.

The Metastatic Potential of Wild-Type B16 Cells after Treated with the Serum from the Mice Immunized with IL-4 Gene-Modified Tumor Vaccine

Table 1 showed that the number of pulmonary metastases decreased significantly in mice injected with wild-type B16 cells mixed with the serum from the mice vaccinated with IL-4 gene-modified tumor vaccine, when compared with that of the mice injected with wild-type B16 cells mixed with the serum from normal mice or from the mice vaccinated with control gene-modified tumor vaccine ($0.05> P>0.01$). The results indicated that the serum from the mice immunized with IL-4 gene-modified tumor vaccine inhibited the growth of wild-type B16 melanoma cells *in vivo*. The effect of the serum might be mediated by complement.

Table 1. The number of pulmonary metastases in mice after intravenous injection with the mixture of wild-type B16 cells and the sera

The sera against the different vaccines	No. of pulmonary metastases
Wild-type B16	149± 24
B16-Neo	141± 20
B16-IL-4	108± 19

DISCUSSION

Many experimental studies in murine tumor models have demonstrated that the tumor immune responses can be induced efficiently by cytokine gene-modified tumor vaccines. Although the exact mechanism remains unclear, some results suggested that secretion of cytokines by those tumor cells was crucial. IL-4 is a kind of immunoregulatory cytokine.[4,7] It influences the activity of lymphocyte-activated killer(LAK) and natural killer(NK) tumor effector cells and increases the tumoricidal activity of macrophages. Several groups have demonstrated that tumor cells engineered to secrete IL-4 don't grow in nu/nu mice or MHC-match mice.[5] The role of IL-4 in tumor immunity explained the observations in our previous study that non-specific immune responses and T cell-mediated specific antitumor immune responses were induced by IL-4 gene- modified tumor vaccines. It is suggested that the IL-4 secreted by tumor vaccines is involved in induction of the host cellular antitumor immunity.

IL-4 also displays potent roles in humoral immunity. It can promote proliferation and differentiation of B cells and regulate antibody production.[6,7] Our results showed that the LPS-induced proliferation of the B lymphocytes from the mice immunized with the IL-4 gene-modified tumor vaccine increased more significantly than that from mice vaccinated with wild-type tumor vaccine and the cytotoxicity of the serum to wild-type melanoma cells also increased markedly when detected. It was also observed that the metastases of wild-type B16 melanoma cells decreased obviously when treated with the aerum against the IL-4 gene-modified B16 melanoma cells. Our previous study also showed that a large number of plasma cells infiltrated into the tumor nodes when the mice were inoculation with IL-4 gene-modified tumor cells. These data demonstrated that humoral immunity might contribute to the antitumor effects of IL-4 gene therapy. The humoral antitumor responses might be induced by IL-4 gene-

modified tumor vaccine through following pathway. The immunigenicity of tumor cells was augmented by IL-4 gene modification and the level of the specific antibody to tumor cells increased when the mice were immunized by the vaccine. The potential of *in vivo* antibody production was also enhanced by the IL-4 secreted by IL-4 gene-modified tumor vaccine. The exact mechanisms are further under investigation.

REFERENCES

1. 于益芝, 曹雪涛, 徐志工, 等. 白细胞介素 4 基因转移的肿瘤细胞体外生物学特性及体内致瘤的研究. 中国免疫学杂志 1995;11:26.
2. 于益芝, 曹雪涛, 陶群, 等. 白细胞介素 4 基因转染的肿瘤细胞体内生长病理学特性的研究. 中国免疫学杂志 1994;10(增刊):57.
3. 曹雪涛, 于益芝, 徐志工, 等. 白细胞介素 4 基因转染瘤苗体内抗肿瘤转移作用及其免疫机理的初步研究. 中华微生物学和免疫学杂志 1995;15:53
4. Suk K,Somer SD and Ekickson KL. Regulation of murine macrophage function by IL-4: IL-4 and IFN-γ differently regulate macrophage tumoricidal activity. Immunol 1993; 80:617
5. Golumbek PT, Lazenby AJ, Levitsky HI, et al. Treatment of established renal cancer by tumor cells engineered to secrete IL-4. Science 1991; 254:273
6. Constant S, Schweitzer N, West J, et al. B lymphocytes can be competent antigen-presenging cells for priming $CD4^+$T cells to protein antigens *in vivo*. J Immunol 1995; 155: 3734
7. Brown MA and Hural J. Functions of IL-4 and control of its expression. Critical Reviews in Immunology 1997; 17:1