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ANTITUMOR EFFECTS INDUCED BY B7-1 GENE MODIFIED EL-4 LYMPHOMA COOPERATED WITH IL-2 *IN VIVO* AND *IN VITRO**

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Costimulation plays very important role in T cells activation and tumor immunity. B7-1 is alone of the major costimulatory molecules both in human and rodents. Previous work indicated that B7-1 gene transfected EL-4 lymphoma can induce antitumor immunity *in vivo*. This paper showed that inoculation of EL-4B7-1$^+$ plus IL-2 could elicit an antitumor effects *in vivo* and *in vitro*. Immunization with EL-4B7-1$^+$ tumor cells or EL-4B7-1$^+$ tumor cells plus IL-2 could significantly prolong the survival time of the EL-4 tumor-bearing mice and also delay the occurrence of the tumor node *in vivo*. A strong proliferation response and CTL activity as well as the increased IL-2 and TNF production of the splenocyte from the immunized mice with EL-4B7-1$^+$ or EL-4B7-1$^+$ plus IL-2 were seen in the *in vitro* TLMC system. These finding indicated that the costimulatory molecule is necessary for inducing an effective antitumor immunity and IL-2 optimal production.

Key words: B7-1 gene, IL-2, EL-4 lymphoma

T cells are the major effector cells in antitumor immune response.[1] Activation of tumor specific T cells is therefore crucial for immune intervention against tumors. It is well established that signals transmitted merely by TCR is insufficient for optimal IL-2 production, proliferation and cytotoxic effects.[2] In the last few years it is well known that combination of B7-1 (CD80) and its counter receptor on T cells provides the major costimulatory signals for optimal T cells activation.[3]

B7-1 costimulation play a very important role in the induction of T cell immunity against tumors. Absence of costimulation has been suggested to contribute to the progressive growth of tumors in immunocompetent hosts. Previous study has shown some tumors no longer grew after B7-1 gene transfection.[4] We previously reported that B7-1 gene modified EL-4 lymphoma could induced more effective immunity against wild-type EL-4 *in vivo*.[5] In this report, we demonstrated the antitumor effects of immunization by B7-1 gene transfected EL-4 lymphoma plus IL-2 *in vitro* and *in vivo*.

MATERIALS AND METHODS

Cell Lines

EL-4 lymphoma, a tumor induced in C57BL/6 by DMB (kindly gift from Dr. Chen W, Division of Oncology, Washington University), EL-4B7-1$^+$ tumor cells were prepared previously,[5] CTLL-2, IL-2 sensitive tumor cells (kindly gift from Ma BL, Shanghai Secondary Medical University), Wehi 164, TNF sensitive fibroblast (kindly gift from Dr. Disis M, Division of Oncology, Washington University, USA).

Animal

C57BL/6 mice (female, 6 — 8 weeks) are from Shanghai Sippr-BK lab animal Co. Ltd.

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Antibody

anti-mouse CD80, FTTC labeled, is from Pharmgen Co. Ltd, USA.

Immunotherapy Experiment

2×10^5 EL-4 tumor cells were inoculated subcutanously into 24 syngeneic C57BL/6 mice. 5 days later, these mice were divided into 4 groups and ip immunized separately with inactivated EL-4 cells or EL-4B7-1$^+$ cells (5×10^6 /mouse) with or without IL-2. The procedure of experiments described in Table 1. The tumor growth and the survival days of the hosts were observed.

Table 1. The procedure of immunotherapy experiments

Inoculation of tumor cells	Immunized with inactivated	Combination of IL-2 usage (i.p. IL-2 2000IU/mouse)
EL-4	EL-4B7-1$^+$	IL-2 at 0,3,7,14,21,28,35,42 day
EL-4	EL-4B7-1$^+$	
EL-4	EL-4	IL-2 at 0,3,7,14,21, day
EL-4	EL-4	

Tumor Cells and Lymphocytes Mixed Culture (TLMC)

C57BL/6 mice were immunized with mitomycin C inactivated EL-4 or EL-4B7-1$^+$ cells, 14 days later, the splenocytes from the immunized mice were harvested and dispensed into 96 well plate at a concentration of 2×10^5 per well. The inactivated EL-4 or EL-4 or EL-4B7-1$^+$ cells mixed with or without 10IU/ml IL-2 were added to each wells at a concentration of 2×10^4 per well. After 72 hrs mixed culture at 37 ℃, 5% CO_2, the mixed cells in each well were added of 10μg MTT, harvested 3 hrs later, add DMSO to solute the crystal, read the OD on MC516 auto reader with 540nm filter. The supernatants are removed for cytokines assay.

Cytotoxic T Cell Assay

The cytotoxicity of T cells was detected by ^{3}H-TdR release assay. The splenocytes from immunized mice were boosted *in vitro* with the inactivated tumor cells. After 2 times restimulation, these splenocytes were harvested and dispensed into 96 well plate mixed with ^{3}H-TdR labelled EL-4 tumor cells at a E:T ratio of 10:1. 4 hrs later, the cells were harvested and the results were detected by β liquid scintillation accouter. The cytotoxic activity was indicated by lysis percentage which was caculated as following formula.

$$\text{Specific lysis } \% = \left(\frac{\text{release}_{\text{control}} - \text{release}_{\text{exp}}}{\text{release}_{\text{control}}} \times 100\%\right)$$

Cytokines Assay

The concentration of IL-2 in the supernant of TLMC was detected by CTLL-2 assay, and results recorded by MTT assay as TLMC assay described above. The TNF assay were determined in alamar blue assay.[6] Wehi 164 fibroblast cells, a specific sensitive cell line for TNF assay, were cultured at 96 well plate with the supernants sample at a concentration of 2.0×10^4 cells per well for 24 hrs. The media of the damole were removed from the plate and replaced with medium containing 10% alamar blue. The cells were continuously incubated with dye for 12 hrs and the result were read on MC516 auto reader with a 620 nm filter.

Statistical Analysis and Calculation

All data are expressed as means ± SD. Significance were assessed by *t* test.

RESULTS

Therapeutic Effect by Vaccination with B7-1 Positive Tumor Cells Plus IL-2

The results (Table 2) showed therapy by vaccination with B7-1 positive tumor cells alone or combined with IL-2 could significantly delay the occurrence and prolong the survival time of tumor bearing hosts.

Proliferation Response of Splenocytes in *in vitro* TLMC System by Vaccination with EL-4B7-1$^+$ Cells

The proliferation of splenocytes from EL-4B7-1$^+$ and EL-4B7-1$^+$ plus IL-2 immunized mice in TLMC system showed much stronger than that from

those mice immunized with EL-4 or EL-4 cells plus IL-2 (Figure 1). The difference was not seen between the two groups immunized with EL-4B7-1$^+$ and EL-4B7-1$^+$ plus IL-2 mice (Figure 2).

Table 2. Therapeutic effects by vaccinatiion with B7-1 positive tumor cells plus IL-2

Group by different vaccinatioin	Incidence (%)	Occurrence dates (MT±SD, day)	Survival dates (MST±SD, day)
EL-4B7-1$^+$+IL-2	100	19 ± 1	46. 5 ± 15
EL-4B7-1	100	14 ± 1	29. 5 ± 12
EL-4+IL-2	100	8 ± 1	25. 2 ± 2
EL-4	100	7 ± 1	23.5 ± 1. 5

n=6, P<0.01 (1,2vs 3,4)

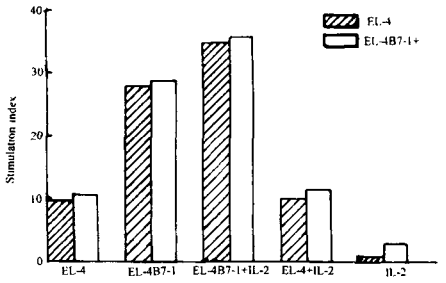

Fig 1. The proliferation of splenocytes of TLMC with different stimuli.

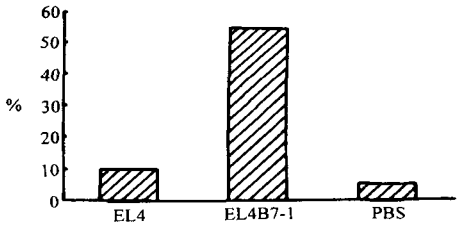

Fig 2. CTL activity of immunized mice

Cytotoxic Effect of Splenocytes from B7-1 Positive Tumor Cells Vaccinated Mice

A strong cytotoxic activity of splenocytes from the immunized mice was seen in the TLMC system *in vitro* which couldn't be shown in the control experiment

IL-2 and TNF Production in the Supernatant of *in vitro* TLMC System

The results showed that both IL-2 and TNF production was significantly increased from the splenocytes of EL-4B7-1$^+$ cell immunized mice in a TLMC system (Figure 3).

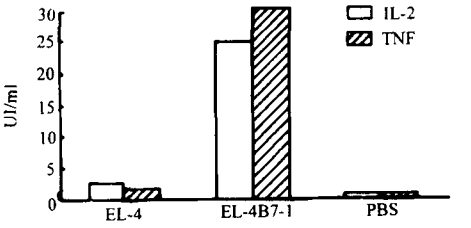

Fig 3. IL-2 and TNF production in the supernatant of *in vitro* TLMC system.

DISCUSSION

A successful antitumor T cell response involves induction, recruitment and effect function of T cells. Previously we have confirmed that vaccination with EL-4B7-1$^+$ tumor cells could induce a protective effect to a subsequent challenge with wildtype EL-4 tumor and have a limited therapeutic effect in the tumor-bearing host. In present study we found that combined application of EL-4B7-1$^+$ tumor cells vaccination with IL-2 significantly augment the immunogenecity of the tumor cells and also the therapeutic effect. Our experiments demonstrated that immunized with EL-4B7-1$^+$ tumor cells plus IL-2 could significantly prolong the survival time of the tumor bearing hosts but also delayed the occurrence of the tumor node. *In vitro* experiments showed a strong CTL activity and cytokines (IL-2, TNF) production of the splenocytes from those animals treated in the same way. Two reasons may be considered about the role of IL-2 in

our experiments. The first is that IL-2 could maintain the stable expression of B7-1 on tumor cells, and provide the sufficient secondary signal for T cell activation. The another reason is that IL-2 provide an optimal micro-environment for T cell activation. In a local micro-environment, the existence of IL-2 could conjugate its receptor on T cells, which would augment T cells response to antigen especially cooperate with costimulatory signals. To some extent, IL-2 itself has the ability as a costimulatory molecule.

In vitro assay of TLMC showed that B7-1 costimulatory molecule expression or not is important for splenocytes proliferation despite the original stimuli. It is to say if T cells primarily activated *in vivo*, it would be amplified *in vitro* in the existence of B7-1 molecule. By the assay of cytotoxic effect of T cells, we can see this T cells showed high cytotoxic effect than control, and it may take some advantage for tumor therapeutic effects.

Taken together, IL-2 can augment the immune response induced by B7-1 gene transfected EL-4 tumors *in vivo*, but take little effect *in vitro* proliferation and function. The primary activated T cells in vivo can be activated and proliferation in vitro in the existence of B7-1 molecule, and showed high cytotoxic effect. These findings may provide a potentially useful way for tumor therapy.

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