Study on the Cytotoxic T Lymphocytes of Mice Treated with Leukemia Vaccine

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Abstract **Objective** To study the cytotoxicity of cytotoxic T lymphocyte (CTL) derived from C57BL/6 mice after being treated with the leukemia vaccine prepared by us. **Methods** We established the leukemia burden mice and prepared three types of leukemia vaccine, which were administered on the mice respectively. The MTT colorimetric method was adopted 2 and 4 weeks later to measure the cytotoxicity of CTL derived from the mice accept immunotherapy or prevention, and compared with control group. **Results** ①With the growth of leukemia cells in the mice, the cytotoxicity of CTL was seriously depressed. ② The tumor vaccine prepared from inactivated tumor cells, IFA and cytokines(rGM-CSF, rIL-2 and rIL-6)promote the cellular immunity of tumor burden mice more efficiently than tumor vaccine made from either inactivated tumor cells, IFA or inactive tumor cells themselves alone. **Conclusion** The tumor vaccine simply prepared with inactive tumor cells, IFA and cytokines (rGM-CSF, rIL-2 and rIL-6) can activate the specific cellular immunity. So the tumor vaccine provides a promising future in the active specific immunotherapy against hematopoietic tumor.

Key Words active specific immunotherapy; leukemia; tumor vaccine; cytotoxic T lymphocyte

At present, chemotherapy is still important to treat the malignant hematopoitic tumor. This therapy has not only many side effects but also its survival rate is very low. Now, the research of active specific immunotherapy has become a research hot spot after the progress of molecules-immunology and tumor-immunology. It has been accepted as a new way to treat the leukemia in the 42nd America Hematology Meeting. So the immunotherapy of leukemia has been very noticing. On the basis of some treatment effects on treating some leukemia patients with our leukemia vaccine[1], we established leukemia burden mice model and investigated the cytotoxicity of CTL derived from it after the leukemia vaccine was injected subcutaneously.

MATERIALS AND METHODS

Materials

C57BL/6 mice: female, 6~8 weeks, were obtained from animal center of Shanxi Academy of Traditional Chinese Medicine. FBL-3 erythroleukemia cell line, which was derived from C57BL/6 mice, was purchased from Department of Immunology of Shanghai Medical University. Leukemia vaccine was prepared by the institute of immuopathology, medical college of Xi'an Jiaotong University.

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Methods

Establishing Mice model

The tumor-burden mice were prepared according to the methods described in the literature [2]: C57BL/6 mice were injected subcutaneously with 0.2ml FBL-3 cells (1×10^6/ml), then tumor nodes were touched after 3-7 days.

Active immunotherapy

The leukemia burden mice were divided into 5 groups: group A, B, C, D, and E. 8 mice in each group. Mice of group A were injected with the vaccine of inactivated tumor cells, as well as IFA and cytokines (IL-2, IL-6 and GM-CSF), and the Group B's with the vaccine of inactivated tumor cell and IFA; the Group C's with the vaccine of inactivated tumor cells; the Group D's with PBS; The group E's with nothing. The mice in group A, B, C and D were injected 0.2 ml vaccine subcutaneously once a week for 4 weeks. Half mice of each group were killed at the beginning of the third week and the rest were killed at the beginning of the fifth week. The cytotoxicity of CTL was tested respectively.

Acquiring CTL

The suspensions of the spleen cells were obtained and the CTL were prepared as reported [2]. C57BL/6 mice's spleen lymphocytes were co-cultured with FBL-3 cells (pre-inactivated with mitocin-C, 30ug/L) (1:20) in RPMI1640 with addition of 20% FCS, 10ug/ml rIL-2, 3% L-glutamine, 0.24% HEPES, 100U/ml peni-

cillin, 100ug/ml streptomycin, at 37℃ in a humidified environment containing 5% CO_2 . Then the CTL was acquired after 6 days.

Testing the cytotoxicity of CTL by MTT assay[3]

The living CTL was super 90% tested by 0.4% Cone worm blue, then the CTL were collected and adjusted to 1×10^6/ml. 100ul volume (1×10^5CTL cells) were distributed into 96-well v-bottom culture plates in each well, 3 wells for one mice. Target cells (living FBL-3 cells) were added at the desired effect to target ratios (20:1, 10:1, 5:1). Also, the control wells of effectors and target cells were done. After incubating for 16-18hours at 37℃ in a humidified environment containing 5%CO_2, 20UL MTT (5ug.ml) was added into each wells and incubated for another 4 hours. Finally 100ul DMSO was added into wells and slightly shacked for15 minutes to lyses the purple crystal. The OD value was obtained by colorimetric assay (the wave-length was 490nm). The cytotoxicity was calculated according to the following formula: cytotoxicity = (1-(OD of the mixture of the effect and target cells- OD of effect cells)/OD of target cells) ×100%.

Vaccine preventing

The normal C57BL/6 mice were divided into 4 groups: group A, B, C and D, 4 mice in each group. Mice of group A were injected the vaccine of inactivated tumor cells, IFA and cytokines (IL-2, IL-6 and GM-CSF). Group B's were injected the vaccine of inactivated tumor cell and IFA. Group C's were injected the vaccine of inactivated tumor cells. Group D's were injected PBS. The mice in each group were injected 0.2 ml vaccine subcutaneously once a week for 2 weeks and attacked by injecting 0.2ml living FBL-3 cells (1×10^6/ml) subcutaneously in the third week. Then mice were killed at the beginning of the fifth week. The cytotoxicity of CTL was tested respectively.

Statistical analyses The Student t test was applied, $P<0.05$ was set as significant difference.

RESULTS

The cytotoxicity of CTL after 2 weeks

From table 1, there isn't statistic difference in the cytotoxicity of CTL between group D (PBS control) and E (normal rats) after 2 weeks. The cytotoxicity of CTL has risen in some degree by injecting 3 kinds of vaccines after 2 weeks, there is statistic difference between the group A, B and D, E group. This implies that A and B vaccine can induce active immunity in vivo.

The cytotoxicity of CTL after 4 weeks

From table 1, the cytotoxicity of CTL from group D is obviously lower compared with group E ($p<0.01$), it tells that the function of T lymphocyte was depressed obviously after tumor grew for 5 weeks. The cytotoxicity of CTL has risen in some degree after 3 kinds of vaccines inoculated 4 times, and the vaccine of inactivated tumor cells, IFA and cytokines (IL-2, IL-6 and GM-CSF) has the best effect. The inactivated tumor cells alone (C group) can't induce effective anti-tumor immunity compared with group E ($p>0.05$). Compared with that of 2 weeks ago, the cytotoxicity of group D's CTL is much lower ($p<0.01$) after the growth of tumor cells, the cytotoxicity of group A is obviously higher ($p<0.05$), but the cytotoxicity of group B, C are not statistically different compared with 2 weeks ago, which mean that the vaccine of inactivated tumor cells, IFA and cytokines (IL-2, IL-6 and GM-CSF) can effectively induce the active specific immunity with addition of injection times.

The cytotoxicity of CTL after inoculation

The cytotoxicity of CTL from group A, B, C were obviously higher after prevention, there were statistically different from group D, especially that of group A were much higher than that of group B, C. But there were no difference between group B and C. These meant that, af-

Table 1 The cytotoxicity of CTL after 2 weeks and 4 weeks (E: T=20:1) ($\bar{x} \pm s$)

G	N	2 weeks			4 weeks		
		OD_{E+T}	OD_E	Cytotoxicity/%	OD_{E+T}	OD_E	Cytotoxicity/%
A	4	0.44±0.02	0.22±0.01	39.39	0.14±0.00	0.01±0.00	59.48
B	4	0.35±0.03	0.16±0.001	38.39	0.19±0.01	0.00±0.00	42.85*
C	4	0.30±0.02	0.10±0.01	33.88	0.21±0.01	0.00±0.00	36.88**
D	4	0.31±0.02	0.07±0.00	26.24*△	0.32±0.01	0.01±0.00	4.55**△□
E	4	0.31±0.02	0.07±0.00	27.39*△□	0.25±0.01	0.01±0.00	27.39**△•

Notes: ODE was the average OD value of the effect cells; in this research ODT was 0.33±0.00 ; *$P<0.05$ as compared with the group A; **$P<0.01$ as compared with the group A; △$P<0.05$ as compared with the group B; □ $P<0.05$ as compared with the group C; •$P<0.01$ as compared with the group C.

ter injecting 3 kinds of vaccines, normal mice could induce obvious active special immunity when being attacked by tumor cells, especially the vaccine made of inactivated tumor cells, IFA and cytokines was the more obvious than others (from table 2).

Table 2 The cytotoxicity of CTL after prevention (E:T=20:1) ($\bar{x} \pm s$)

Group	n	OD_{E+T}	OD_E	OD_T	ytotoxicity/(%)
A	4	0.05±0.01	0.01±0.00	0.33±0.00	85.85
B	4	0.15±0.01	0.01±0.00		58.18**
C	4	0.23±0.10	0.02±0.00		36.44**
D	4	0.28±0.08	0.01±0.00		19.30**△□

Notes **P<0.01 as compared with the group A; △P<0.01 as compared with the group B; □ P<0.05 as compared with the group C.

DISCUSSION

Why can't host acquire effective anti-tumor immunity? Recently, many researchers suggest that:(1) there is no special antigen on the tumor cell surface. (2)The function of presenting antigen is not strong in vivo. For example, there are no B7, MHC molecules on the tumor cell surface. (3) The function of immune active cells is inhibited or the signal conduction route inhibited. (4) Tumor cells release immunity inhibitor; the immunity inhibition cells are active and so on. All of these could suppress the host's immunity and make tumor cells escape immune supervision. Our experiments show that the cytotoxicity of CTL were suppressed obviously as the growth of FBL-3 cells in the C57BL/6 mice. This phenomenon suggested cellular immunity was suppressed as the growth of malignant cells in vivo.

For this reason, the active specific immunotherapy (ASI) is noticing now. In the past, people took much more energy to the research of the tumor antigen than that of the immune microenvironment system. But now, as the progress of the molecular immunology, people discover that many cytokines, such as GM-CSF, IL-2, IL-6, IL-10, IL-12, IFN-α , B7, MHC and so on, could regulate immune reaction. Dendritic cell (DC) could present antigen. These make the ASI be researched deeply. For example, to improve the immunogen of tumors cell, people transduced the gene B7, IL-2, IL-6, GM-CSF into tumor cells [4-7], co-incubated tumor cells with DC to prepare the DC vaccine and so on [8,9]. We prepare this leukemia vaccine, which included inactivated leukemia cells, IFA, IL-2, IL-6, GM-CSF, and inject it into the tumor burden mice subcutaneously. We observe that the CTL's cytotoxicity increased from 39.39% after the second injection to 59.48% after the fourth injection. Our experiments show that this vaccine could improve the nonspecific and specific cellular immunity of tumor burden mice. On the other hand, this vaccine could protect the normal C57BL/C mice from the attack of the wild FBL-3 cells if the vaccine inoculated early.

To malignant hematopoietic tumors, there is an important clinical idea that host's immunity would be built again after the disease obtains complete remission by chemotherapy or bone marrow transplantation. This may be a chance for ASI, which could reverse the multiple-resistance drug and avoid the tumor relapse. In conclusion, the cellular immunity of the leukemia burden mice is suppressed as the growth of the tumor cells in the host; the suppression of the specific cellular immunity is later than that of the non-specific. The leukemia vaccine made from inactivated tumor cells, IFA and cytokines could raise the cellular immunity of leukemia burden mice after repeated injection, especially the specific cellular immunity of leukemia burden mice.

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