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Effect of Passive Transfer of Spleen Cells from Immunized Mice with Hydatid Cyst Antigens on the Growth of Melanoma Cancer in C57/Black Mice

Seyedeh Tala Ramazninia[1], Seyedeh Maryam Sharafi[2], Mehran Bahadoran[1], Fereshteh Jafaee Nodeh[1], Mehdi Mahmoudzadeh[3] and Hossein Yousofi Darani[1*]

[1]Department of Medical Parasitology and Mycology, Isfahan University of Medical Sciences, Isfahan, Iran.
[2]Infectious Disease and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
[3]Department of Internal Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Author STR contributed in designing the study, performed the experimental work and wrote the first draft of the manuscript. Author SMS contributed in performance of experimental work and writing the manuscript. Author MB helped with data analysis. Author FJN contributed in doing experimental works. Author MM was cancer consultant of the project. Author HYD designed the study, supervised the research project and write the manuscript.

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ABSTRACT

Background: Anticancer effect of hydatid has been shown in previous investigations. However the mechanism of anticancer effects of hydatid cyst has not been clarified. So in this work the effect of spleen cell transfer immunized by the hydatid cyst antigens on melanoma cancer growth in animal model has been investigated.
Methods: Spleen cells of mice immunized with hydatid cyst fluid, cyst wall and protoscoleces were transferred to different group of mice and subsequently challenged with melanoma cells. Then the

**Corresponding author: E-mail: yousofidarani@gmail.com;*

tumor size, tumor growth rate and survival time of mice were compared with those of control groups.
Results: Tumor size, tumor growth rate and mice survival time were significantly lower than what observed in control mice.
Conclusion: Immune response to hydatid cyst antigens may be involved in Anti-cancer effect of this parasite.

Keywords: Hydatid cyst; spleen cell transfer; melanoma.

1. INTRODUCTION

Due to importance of melanoma cancer in the world, different efforts such as Surgery [1,2], hemotherapy [2,3], Radiotherapy [2,4,5], Hormone Therapy [2,6,7] and Immunotherapy [2] have been used for teaetment of this cancer. However, search for other treatment is important [8]. Immunotherapy using microorganisms has bee used for treatment of different cancers for example *Salmonella typhimurium* has been used for liver cancer treatment [9]. Also *Bifidobacterium adolescentis* has recently been used as antiangiogenic protein [10]. The cell wall skeleton of Mycobacterium bovis *Bacillus Calmette-Guérin* (BCG) has been used as an effective adjuvant for immunotherapy of bladder cancer [11,12]. Moreover some parasites such as *Trypanozoma cruzy, Toxoplasma gondii*, *Plasmodium falciparum, Strongyloides stercoralis* and hydatidcyst fluid have been shown that inhibit growth of Ehrlich's adenomacarcinoma, fibrosarcoma, Lewis lug, leukemia and colon cancer respectively [13-16]. Anti cancer effect of hydatid cyst which is larval stage of *Echinococcus granulosus* has been shown in different investigations. Akgul and his colleagues in a retrospective study showed that in patients with hydatid cyst, incidence of different cancers was much lower than that of the normal population [17]. Also the effect of different hydatid cyst antigens on inhibition of the melanoma cancer growth has been shown in animal model or *in vitro* studies [18,19]. Moreover, in various studies existence of common antigens such as the Tn antigen, TK antigen and Sial Tn antigen, between cancer and hydatid cyst has been shown [20]. Tn antigen is a glycoprotein that is expressed during the early phases of various malignancies, including carcinomas of the breast, pancreas, lung, gastrointestinal tract. Although hydatid cyst has anticancer activity, the involved mechanism has not been yet clarified. It is possible that the immune response raised against hydatid cyst is effective in inhibiting the cancer growth. So this study was performed to evaluate the effect of spleen cell transfer immunized by parasite antigens on melanoma cancer growth in animal model.

2. MATERIALS AND METHODS

In this experimental study, population study consisted of C57/ Black inbred mice and the sample size was 66. These mice were purchased from Pasteur Institute of Iran. The cyst fluid, protoscolex, and cyst wall antigens were prepared as we published before [19]. Briefly cyst fluid was aspirated and collected and then centrifuged and the supernatant was collected and stored at -20 as hydatid cyst fluid antigen. The sediment was washed with PBS and then disrupted by sonication. The mixture was then centrifuged and the supernatant was collected, and stored at -20 as protoscolex antigen. The cyst membrane excised from the adventure layer and washed and sliced in PBS. The mixture was then disrupted by sonication and centrifuged, and kept at – 20 as laminated layer antigen.

B16F10 melanoma cell line was purchased from Pasteur Institute of Iran and cultured on RPMI (Roswell Park Memorial Institute) medium.

Four groups of mice were immunized as followings: group 1 immunized with the cyst fluid, group 2 immunized with protoscolex antigen, group 3 immunized with the cyst wall antigens. All antigens were injected with equal volume of Freund's adjuvant. Group 4 received no immunization. Two boosters were given to each mouse of group 1- 3. Three days after the last booster, antibody responses against these antigens were investigated by ELISA test. Mice with appropriate antibody response were killed and their spleens were minced in PBS. To prepare spleen cells the minced spleens were then sieved to remove the large debris [12]. The cells were then injected to groups of mice as shown in Table 1. Three hours after the injection of spleen cells, each mouse was injected with a million of melanoma cells (B16F10) under chest skin (study groups). The control group did not

receive spleen cells but like the other groups were injected with melanoma cells.

Tumor diameter was measured using a caliper every 4 days. Monitoring of umor growth in all groups was followed until their deaths. Tumor area was estimated as we published before [21] by using the following formula.

$$\text{Tumor area} = \pi \times \frac{(Diameter_{1} + Diameter_{2})^{2}}{4}$$

Then tumor growth rate was calculated and analyzed using SPSS and Koruskal-Wallis statistical test. Death of any of the mouse was recorded every day and using Kaplan-Meier analysis lifetime of different groups was compared. This research project was performed by permission from research ethic committee of Isfahan university of medical sciences.

Table 1. Arrangement of mice receiving spleen cells of mice immunized with different hydatid cyst antigens

Group 1 (control group 1)	Mice that have not received spleen cells
Group 2 (control group 2)	Mice that have received normal spleen cells (without any injection)
Group 3	Mice that have received spleen cells of mice immunized with cyst wall antigens
Group 4	Mice that have received spleen cells of mice immunized with cyst liquid antigens
Group 5	Mice that have received spleen cells of mice immunized with protoscolex antigens

3. RESULTS

Tumor nodules were observed 15 days after melanoma cells (B16F10) injection in the chest area and every four days tumor diameter was measured and tumor area was calculated for every mouse in each measurement. Tumor size of the groups that received immunized cells with hydatid cyst antigens was significantly lower than that of the control group (Table 2).

In experimental animals tumor growth rate was also calculated for mice in different groups, the details have been summarized in Table 3. Tumor growth rate was significantly lower in mice that received spleen cells immunized with hydatid cyst fluid or cyst wall antigens.

Transfer of spleen cells of immunized mice with hydatid cyst fluid resulted in 20% increase in survival time in recipient mice. Also cell transfer with spleen cells of mice that immunized with cyst wall antigen resulted in 40% increase in survival time in recipient mice. However all mice in control group 1 died after 31 days and in group 2 after 35 days. The details of data regarding survival time of mice in groups 1-5 summarized in Table 4.

Table 2. Mean tumor size in mice injected with spleen cells immunized with hydatid cyst wall (group 3), hydatid cyst fluid (group 4) and protoscolicex antigens (group 5) in comparison with mean tumor size of mice injected with normal spleen cells (group 2) or mice that received no spleen cells (group 1)

Groups	P		Mean±SD
	Control group 1	Control group 2	
Control group 1	-	-	1690±1003
Control group 2	.199	-	1222±909
Group 3	.0001	.001	575±850
Group 4	. 001	. 01	622±702
Group 5	.003	.05	691±565

p<0.05 is significant

Table 3. Tumor growth rate in mice injected with spleen cells immunized with hydatid cyst wall (group 3), hydatid cyst fluid (group 4) and protoscolex antigens (group 5) in comparison with mean tumor size of mice injected with normal spleen cells (group 2) or mice that received no spleen cells (group 1)

Groups	P		Mean±SD
	Control group 1	Control group 2	
Control group 1	-	-	136±126
Control group 2	.753	-	115±84
Group 3	.001	.001	36±58
Group 4	.001	.001	46±65
Group 5	.135	.130	70±55

p<0.05 is significant

Considering tumor size, tumor growth rate and survival time, the differences between the control groups 1 and 2 were not significant.

Table 4. Survival time of mice injected with spleen cells immunized with hydatid cyst wall (Group 3), hydatid cyst fluid (Group 4) and protoscolex antigens (Group 5) in comparison with mean tumor size of mice injected with normal spleen cells (Group 2) or mice that received no spleen cells (Group 1)

Groups	P		Means and medians for survival time			
			Mean		Median	
	Control group 1	Control group 2	Estimate	Std. error	Estimate	Std. error
Control group 1	-	-	28	1.39	29	1.09
Control group 2	.613	-	29	2.85	31	1.09
Group 3	.001	.001	60	11.47	55	21.9
Group 4	.002	.006	50	9.6	39	21.19
Group 5	.6	.6	30	1.36	31	3.2

p<0.05 is significant

4. DISCUSSION

In this study, it was shown that the transfer of spleen cells immunized with hydatid cyst fluid and cyst wall antigens to the mice that subsequently injected with melanoma cancer cells resulted in reduction of the size and the growth rate of tumors. Moreover these interventions also resulted in increasing mice longevity. When protoscoleces were used as antigen the size of tumor reduced in experimental mice but did not cause significant reduction either on the tumor growth rate or mice life time.

Results of this study were in agreement with previous works results about effect of hydatid cyst on reducing the growth of cancer. In this context it has been shown that hydatid cyst protoscolices induce cell death in WEHI 164 fibrosarcoma cells [18]. Also inhibitory effect of different hydatid cyst molecules on Hela and Vero cell lines has been shown [22,23]. In another studies effect of hydatid cyst fluid on growth of melanoma [19] and colon [16] cancers has been shown. Also in agreement with our work Braille et al. [16] demonstrated that injection of human hydatid fluid into mice with colon cancer increased their survival time [16].

Related to results of our study, Zenina et al. [24] and Zhigunova et al. [25], investigated the effect of spleen cell transfer of sensitized mice with *Trypanosoma cruzi* to the mice that were injected with cancer cells of Ehrlich's carcinoma. They showed that transmission of spleen cells sensitized with *Trypanosoma cruzi* antiges to the experimental group of mice significantly reduced the speed of tumor growth in comparison with the control mice. Also they showed that the generation of cellular immune response by *T. cruzi* may be responsible for this anti-cancer effect [24,25].

In another study Woodruf et al. [26] showed that the transmission of spleen cells immunized with breast tumor antigen had been effective in the decreasing of the growth of breast cancer tumor and increasing of mice life time in the experimental animals in comparison with the control group.

Considering the anticancer effect of hydatid cyst it has been shown that the immune responses raised by hydatid cyst fluid (HCF) can change the immature dendritic cells (DC) into mature dendritic cells which took place simultaneously with an increase in IL-6 and IL-12 secretions [27-29]. Also IL-12 is stimulating factor of NK-Cell, and contribute in differentiation of Th0-lymphocytes to Th1 [30]. Both of these are protective against cancer growth.

There are some important molecules in the HCF that act as constituents of the adjuvant or antigen [16]. Several antigens including antigen 5 antigen B, [31,32], *creatine kinase* and mortalin [16], are present in hydatid cyst fluid. Previous studies showed that mortalin expression associated with poor prognosis in patients with colorectal cancer [33]. Mortalin is one of the hsp70 family in the cell membrane that has about 60% similarity with the hsp70 which is present in the *Echinococcus granulosus* [16]. So, it is possible that anti- hsp70 immune response of hydatid cyst inhibits HSRR70 of cancer cells and reduce tumor growth. In this context existence of common antigens such as Tn antigen, Tk antigens and Sialyle Tn antigen between hydatid cyst and some cancers has been shown [20]. These common antigens may

be responsible for induction of protection against cancer growth in our study.

5. CONCLUSION

The results of this study showed that passive transfer of spleen cells from immunized mice with antigens of hydatid cyst to mice with melanoma cancer resulted in reduction in tumor size, tumor growth rate and also increasing the survival time in comparison with the control groups. So cellular immune response to hydatid cysts antigens may also has anti-tumor activity.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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