Research Article:
Effect of Immune Responses Against Hydatid Cyst Antigens on Growth of Melanoma Tumor

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ABSTRACT
Background: Hydatid cyst is the larval stage of the tape worm parasite, Echinococcus granulosus. Human is infected by ingestion of parasite ova excreted in dog feces. The anticancer activity of different antigens of hydatid cyst has been reported in the previous works. In this research, the role of immune system in induction of this anticancer activity has been investigated.

Materials and Methods: Spleen cells of mice immunized with hydatid cyst antigens were transferred to the recipient mice that had already been challenged with melanoma cells. Also, mice with melanoma cancer were injected with antisera raised against hydatid cyst antigens. In all above mice, tumor growth was measured using a caliper.

Results: Passive transfer of spleen cells resulted in significant inhibition of melanoma cancer growth. However passive transfer of antisera did not affect cancer growth in the recipient mice.

Conclusion: According to the results of this work, cellular immunity but not humoral immunity may be involved in induction of anticancer activities of hydatid cyst.

Keywords: Hydatid cyst, Cellular immunity, Humoral immunity, Melanoma

Introduction
Hydatid cyst is the larval stage of the tape worm parasite, Echinococcus granulosus. Human is infected by ingestion of parasite ova excreted in dog feces. The cysts are seen in various viscera mainly in liver and lungs. Hydatid cyst consists of germinal and laminate layers, brood capsules containing protoscolices and hydatid cyst fluid [1]. Anticancer effect of hydatid cyst has been reported in different investigations. In a retrospective study, it has been shown that in patients with hydatid cyst, incidence of different cancers was much lower than what exist in normal population [2]. Also the effect of different hydatid cyst antigens on inhibi-
bition of the melanoma cancer growth has been reported in animal model [3-5] and in cell culture medium [6-8].

In this context, it has been reported that in experimental animals, hydatid cyst fluid induces antitumor activity against colon cancer [3] and immunization of mice with live protoscolices resulted in significant reduction of melanoma cancer growth in C57/black mice [4]. Also in different investigations, the existence of common antigens such as the Tn antigen, TK antigen and Sial Tn antigen, has been shown between cancer and hydatid cyst [9].

Immunotherapy with microorganisms has been used for the treatment of different cancers. For example, Mycobacterium bovis Bacillus Calmette-Guérin (BCG) has been reported to be effective in immunotherapy of bladder cancer [10, 11]. Immunotherapy with antisera has also received special attention since long time ago. For example, polyspecific antisera are now used for the treatment of different diseases, especially hematologic malignancy [12].

Cancer uses different strategies to suppress the host immune system [13]. Thus, it is worthwhile to use exogenic antigens to induce effective immune response against cancer cells. Because of some common antigens between cancers and parasites [14-16], parasite antigens can be considered as candidates for cancer immunotherapy [9]. Thus, the effect of passive transfer of spleen cells of mice immunized with hydatid cyst antigens and antisera against different hydatid cyst antigens on the growth of melanoma tumor in animal model was investigated.

Materials and Methods

The study population consisted of inbred C57/black mice purchased from Pastor Institute, Tehran, Iran. To prepare different antigens, hydatid cysts of Echinococcus granulosus were collected from Fasaran slaughter house in Isfahan, Iran. Hydatid fluid of cysts was aspirated and then examined under microscope for the presence of protoscolices. The fluids were then centrifuged, and the supernatant was concentrated and kept at -20°C as hydatid cyst fluid antigen. To prepare protoscolices crude antigen, pellet of protoscolices were sonicated and centrifuged, then the supernatant kept at -20°C as protoscolices crude antigen. The cyst wall collected from animal cyst, washed and sliced in Phosphate Buffered Saline (PBS) and then disrupted by sonication. The mixture was centrifuged, and the supernatant kept at -20°C as cyst wall antigen.

Each of the three prepared antigens was emulsified in Freund’s adjuvant and injected to three rabbits. For the first injection, complete and for the boosters, incomplete adjuvants were used. Following the third booster, the blood samples were collected from the rabbits and presence of specific antibody in each rabbit serum was checked by ELISA test. In case of existence of high titer of specific antibodies, the rabbits were then bled and their sera were collected in -20°C until further use. Prior to passive transfer, the antisera were inactivated in 56°C for one hour. Melanoma cells were purchased from Pastor Institute and cultured in RPMI medium enriched with fetal bovine serum. The cells were then harvested for injection to mice as we did before [5].

Spleen cell transfer experiment

To prepare donor mice for cell transfer experiment, three groups of mice were immunized with the cyst fluid, protoscolex crude antigen and cyst wall antigen, respectively. All antigens were then injected with Freund’s adjuvant. Group 4 of mice received no injection and kept as control. In groups 1-3, two boosters were given to each mouse and one week after the last booster, antibody responses against these antigens were investigated using ELISA test. Spleens of mice with appropriate antibody response were exited and the pooled spleen cells of each group was prepared. For passive transfer of spleen cells, every mouse was received 10^6 melanoma cells under chest skin. Three hours later, every mouse was injected with 10^6 spleen cells intravenously.

Antiseras passive transfer experiment

For passive transfer of antiseras groups of mice challenged with melanoma cells (10^6 cells for every mouse) and three hours later, mice groups 1-3 were injected with antisera against hydatid cyst fluid, crude protoscolices antigen or cyst wall antigen, respectively. Control groups were injected with normal rabbit antisera (control group 1) or left intact (control group 2). Every mouse was injected intravenously with 300 µL antisera. After two weeks, tumor size of each mouse in all groups was measured using a caliper (Measuring each tumor in 2 tumor diameters) and tumor area for each mouse was calculated as we published before [18].

Results

In the first experiment, passive transfer of spleen cells immunized with hydatid cyst fluid, crude protoscolices antigen and cyst wall antigen to mice resulted in significant inhibition of tumor growth in comparison with the
control groups. Results of this experiment are summarized in Table 1. In the second experiment passive transfer of antisera against hydatid cyst fluid, crude protoscolices antigen or cyst wall did not result in significant tumor growth control. The mean tumor area of mice that received those antisera was not statistically different from mean tumor area of mice that either received normal rabbit serum or left intact (Table 2).

**Discussion**

In this study, the first experiment shows that the transfer of spleen cells immunized with hydatid cyst fluid, crude protoscolices antigen or cyst wall antigens to the mice that had already been challenged with melanoma cancer cells resulted in inhibition of tumor growth. However, results of the second experiment revealed that passive immunization of mice with antisera against hydatid cyst fluid, crude protoscolices antigen, or cyst wall antigen do not have a significant effect on tumor growth of mice.

In agreement with the result of the first experiment, Zenina, et al. and Zhigunova et al. reported that transmission of mice spleen cells immunized with antigens of Trypanosoma cruzi to the recipient mice that had already been challenged with Ehrlich’s carcinoma resulted in reduction of tumor size. They also showed cellular immune response generated by T. cruzi antigens may be responsible for this anti-cancer effect [17, 18].

In another study, Woodruf et al. transferred mice spleen cells sensitized by breast cancer antigens to the recipient mice. They reported that this passive cell transfer resulted in inhibition of breast cancer growth and also increased the mice life time in comparison with control mice [19]. Also in agreement with the results of the first experiment it has been shown that immune response raised by hydatid cyst fluid is associated with an increase in IL-6 and IL-12 secretions [20-22]. IL-12 is stimulating factor of natural killer cells which may be protective against tumor growth [23]. Moreover, existence of common antigens such as Tn antigen, Tk antigens and Sial Tn antigen between cancers and extractions of hydatid cyst has been shown [18]. Immune response to common antigens of parasite origin may be responsible for induction of protection against cancer growth in our study or other similar works.

Considering the results of our second experiment, passive transfer of antisera against hydatid cyst antigens did not have any significant effect on melanoma cancer growth. Contrary to our results, Carter et al. showed that infection of murine monoclonal antibodies against growth factor receptors specifically inhibits proliferation of human tumor cells [24].

In another work, Kallinikova et al. reported that antibody response against Trypanosoma cruzi may have protective effect against some forms of cancers [25]. It is not clear why passive transfer of antisera was not effective in our experiment. The volume of injection to each

| Mice Group | Group A | Group B | Group C | Control 1 | Control 2 |
|------------|---------|---------|---------|-----------|-----------|
| Mean tumor area (mm²) | 622 | 691 | 575 | 1222 | 1690 |

| | | | | | |
| | <0.05 | <0.05 | <0.05 | - | - |

Mean tumor area of mice in comparison with control groups.

| Mice Group | Antiserum Against Hydatid Cyst Fluid | Antiserum Against Protoscolices Crude Antigen | Antiserum Against Cyst Wall | Normal Rabbit Serum | Isotonic Saline |
|------------|-------------------------------------|-----------------------------------------------|-----------------------------|---------------------|----------------|
| Mean tumor area (mm²) | 508.67 | 732.01 | 949.45 | 769 | 908 |

Comparison with controls: Not significant.

Mean tumor area of mice in comparison with controls.

| Mice Group | Antiserum Against Hydatid Cyst Fluid | Antiserum Against Protoscolices Crude Antigen | Antiserum Against Cyst Wall | Normal Rabbit Serum | Isotonic Saline |
|------------|-------------------------------------|-----------------------------------------------|-----------------------------|---------------------|----------------|
| Mean tumor area (mm²) | 508.67 | 732.01 | 949.45 | 769 | 908 |

Comparison with controls: Not significant.

Mean tumor area of mice injected with melanoma cells and then received antiserum against hydatid cyst fluid, crude protoscolices antigen, cyst wall antigen and normal rabbit serum or isotonic saline (control).
mouse or the number of injections may be responsible for this result. Results of this investigation revealed that cellular immune response has a more prominent role in anticancer effects of hydatid cyst antigens.

Ethical Considerations

Compliance with ethical guidelines

This research project was performed by approval from Research Ethic Committee of Isfahan University of Medical Sciences.

Funding

This work was supported by grant from Isfahan University of Medical Sciences.

Conflict of interest

The authors declared no conflict of interest.

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