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BRIEF COMMUNICATION

Effects of *Toxoplasma gondii* and *Toxocara canis* Antigens on WEHI-164 Fibrosarcoma Growth in a Mouse Model

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Abstract: Cancer is the main cause of death in developed countries. However, in underdeveloped countries infections and parasitic diseases are the main causes of death. There are raising scientific evidences indicating that parasitic infections induce antitumor activity against certain types of cancers. In this study, the effects of *Toxoplasma gondii* and *Toxocara canis* egg antigens in comparison with Bacillus Calmette Guerin (BCG) (known to have anticancer distinctive) on WEHI-164 fibosarcoma transplanted to BALB/c mice was investigated. Groups of 6 male BALB/c mice injected with *T. gondii* antigen, BCG, or *T. canis* egg antigen as case groups and alum alone as control groups. All mice were then challenged with WEHI-164 fibrosarcoma cells. The mice were examined for growth of the solid tumor and the tumor sizes were measured every other day up to 4 wk. The mean tumor area in *T. gondii*, BCG, or alum alone injected mice in 4 different days of measurements was 25 mm^2, 23 mm^2, and 186 mm^2 respectively. Also the mean tumor area in *T. canis* injected mice in 4 different days was 25.5 mm^2 compared to the control group (alum treated) which was 155 mm^2. *T. gondii* parasites and *T. canis* egg antigens induced inhibition of the tumor growth in the fibrosarcoma mouse model. We need further study to clarify the mechanisms of anti-cancer effects.

Key words: *Toxoplasma gondii*, *Toxocara canis*, BCG, fibrosarcoma, neoplasm

Data regarding the cause of death in different countries revealed that while cancers were the main causes of death in developed countries, infections and parasitic diseases were considered as the main causes of death in underdeveloped countries. In the year 2002, the mean estimated death due to infectious and parasitic diseases per 100,000 population in 15 developed and developing countries was 12.6 and 962.6, respectively, whereas due to cancers it was 230.6 for developed and 59.8 for developing countries in the same year [1]. The difference in the cause of death in those countries was statistically significant. Moreover, in the United States, public health interventions for control of infectious diseases have been associated with a parallel increase in mortality rates for cancers [2]. Various factors such as air pollution and dietary habits may be responsible for the increase in the cancer mortality following control of infectious and parasitic diseases. However, there are raising scientific evidences indicating that parasitic or bacterial infections induce antitumor activity against certain types of cancers [3-8]. For example, anticancer activity of *Trypanozoma cruzi* parasites has been demonstrated [3], and it has been shown that Bacillus Calmette Guerin (BCG) is an effective immunotherapy for carcinoma of the bladder, as reviewed by Herr et al. [8]. The present study aimed to examine the above hypothesis on the effects of *Toxoplasma gondii* and *Toxocara canis* egg antigens in comparison with BCG, which is known to have antitumor characteristics, on fibosarcoma development in BALB/c mice.

For 2 separate experiments, 8-wk-old male inbred BALB/c mice (5-6 mice in each group) were used. In the first experiment, each mouse (group 1) was injected with 120 mg *T. gondii* antigen in 100 μl isotonic saline absorbed on 100 μl alum adjuvant. In the case of group 2 (positive control), every mouse was injected in food pad with 20 μl BCG and in the negative control group every mouse was injected with 100 μl saline absorbed on 100 μl alum as the adjuvant. In the second experiment, each mouse was injected with 120 mg *T. canis* egg antigen in 100 μl isotonic saline absorbed on 100 μl alum as the adjuvant. The control group was injected as the negative control group of the first experiment. All mice were received 2 boosters fortnightly with the same antigens in case groups or alum alone in control groups.

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Two weeks after the last booster all mice in case and control groups were challenged with 5×10^5 WEHI-164 fibrosarcoma cells suspended in 200 μl culture medium. The cells were injected subcutaneously in the chest place of the animals. Three days after the tumor cell inoculation mice were examined for detection of solid tumors in the injected locations. When the tumor was detectable in the examination, its size was measured in 2 diameters every 2 days up to 1 month. It was supposed that the tumors have sphere shape and so the tumor area was calculated according to the following formula which is the modification of the sphere area formula. This formula was first used for the tumor area calculation in a PhD thesis at University of Newcastle, Australia, by Shirzadeh, H. 1n 1995.

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

Toxoplasma antigen was prepared by centrifugation of RH tachyzoites to make a concentration containing 6×10^5 parasites per ml. The suspension was frozen and thawed 5 times with the presence of Fenil metil solfonil floraid. The antigen was then stored at -20. The BCG vaccine is a live attenuated lyophilized bovine mycobacterium containing $1.5\text{-}6.0 \times 10^6$ bacteria per ml. This vaccine was purchased from Pasture institute, Tehran, Iran. WEHI -164 cells are fibrosarcoma cells of BALB/c inbred mice purchased from the cell bank of Pasture Institute, Tehran, Iran.

T. canis egg antigen was prepared by harvesting the eggs from the uterus of female worms. These eggs were kept in isotonic saline for 2 wk and then sonicated. The sonicated mixture was kept at -20℃ for use as the antigen. Kruskal Wallis and Mann-Whitney tests were used for statistical analysis.

In the first experiment, the solid tumors were detectable 17 days after the cell injection. The tumor sizes were measured on days 17th, 21th, 26th, and 31th after the cell inoculation. The mean area of tumors in *T. gondii*, BCG, or alum alone injected mice in 4 different days of measurements were 25 mm^2, 23 mm^2, and 186 mm^2, respectively (Table 1).

In the second experiment the solid tumors were detectable 15 days following the cell challenge. The tumor size was measured on days 15th, 17th, 22th, and 26th and the mean tumor area in *T. canis* injected mice in 4 different days was 24.5 mm^2 compared to the control group (alum treated) which was 155 mm^2 (Table 2).

The results of this study demonstrated that injection of 2 parasite antigens, *T. gondii* and *T. canis*, associated with a significant reduction in the tumor size in comparison with the control group (no antigen injected). There are scientific evidences indicating that some parasitic and microbial infections interfere with tumor growth and have anticancer activities [3-8]. Plumelle et al. [6] showed that patients with leukemia who were infected with *Strongyloides stercoralis* parasites survived longer compared with the same patients without *S. stercoralis* infection. In another investigation, tumoricidal potential of a pathogenic ameba in cell cultures has been demonstrated [7]. It has been shown that *T. cruzi* infection confers resistance to the tumor development in mice, and also in vitro studies have shown toxic effects of parasite extracts on the cancer in cell cultures [3,4]. *T. gondii* infection inhibits the tumor growth in certain types of cancers in mouse

Table 1. The tumor area (mean and SD; mm^2) of mice injected with *Toxoplasma gondii* antigen absorbed on alum (case 1), BCG (case 2) and alum alone (control) following 17, 21, 26, and 31 days after challenge with fibrosarcoma cells

Groups	Day after cell injection			
	Day 17th	Day 21th	Day 26th	Day 31th
Case 1				
Mean	3.18	22.68	35.36	41.02
SD	7.11	45.54	47.05	54.60
Case 2				
Mean	0.00	5.66	40.22	47.62
SD	0.00	12.65	57.23	67.38
Control				
Mean	42.48	117.40	267.10	319.18
SD	82.33	96.32	168.32	206.10
P	Not significant	Not significant	0.023	0.032

Data are from 5 mice in each group.
BCG, Bacillus Calmette Guerin.

Table 2. The tumor area (mean and SD; mm^2) of mice injected with *Toxocara canis* egg antigen absorbed on alum (case group) in comparison with mice injected with alum alone (control group) following 15, 17, 21, and 26 days after challenge with fibrosarcoma cells

Groups	Day after cell injection			
	Day 15th	Day 17th	Day 22th	Day 26th
Case				
Mean	4.41	21.33	35.58	36.50
SD	6.86	45.21	55.15	54.63
Control				
Mean	49.98	93.33	222.58	255.03
SD	108.75	87.09	101.55	117.37
P	Not significant	Not significant	0.004	0.004

Data are from 6 mice in each group.

models through induction of Th1 immure responses and antiangiogenic activities [5]. However, there are also some evidences indicating that parasitic infections enhance cancer growth or predispose patients to malignancies [9-13].

How parasite antigens interfere with the tumor growth and what is the mechanism of anticancer effects of those antigens are not understood. One possibility is that immune responses provoked by parasite antigens may be non-specifically effective toward tumor cells. In this context, it has been shown that immunization of mice against *T. cruzi* provided co-protective effects against the transplanted tumor [14]. In another study, Kim et al. [5] demonstrated that *T. gondii* infection inhibits the tumor growth in the Lewis lung carcinoma mouse model through induction of Th1 immune responses and antiangiogenic activities. Moreover, natural killer (NK) cells which are activated in some parasitic and microbial infections [15-18] may nonspecifically affect the tumor cells [17,18]. In 2007, the involvement of NK cells against tumors and parasites has been reviewed by Papazahariadou et al. [17]. However, further work is necessary to find out the exact mechanisms involved in antitumor activities of those 2 parasite antigens.

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