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# Effects of social isolation stress on immune response and survival time of mouse with liver cancer

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## Abstract

**AIM:** To investigate the effects of isolation stress on mouse with liver cancer and possible associated mechanisms.

**METHODS:** Transplantable murine hepatoma22 (H22) model was used to evaluate the effects of social isolation stress on murine liver cancer. Mice were immunized with sheep red blood cell (SRBC) and intraperitoneally inoculated with H22 cell, then divided into two groups, one reared individually as group (I) and the other reared in groups as group (G). Titer of antibody to SRBC and interleukin 2 (IL-2) in serum was monitored. The survival time of mouse with liver cancer was observed.

**RESULTS:** The titer of antibody to SRBC in group (G) was 1:24.5 and that in group (I) was 1:11.2. There was a significant difference between these two groups ($t$ = 2.60, $P$ = 0.02). A significant difference in IL-2 concentration was observed between group (G) (39.6 ng/L) and group (I) (47.1 ng/L, $t$ = 2.14, $P$ = 0.046). The survival time in group (G) (16.5 d) was markedly longer than that in group (I) (13.2 d, $t$ = 3.46, $P$ = 0.002).

**CONCLUSION:** Our study suggests that survival time of the mouse bearing H22 tumor is affected by the social isolation stress and the associated mechanism may be the immunological changes under the social isolation stress.



**Key words:** Liver cancer; Psychoimmunology; IL-2

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## INTRODUCTION

In humans, stressful life events have been shown to play a role in increased cancer risk and metastasis[1-3]. However, a number of studies have reported little or no support for the association among stressful life events, coping, biological changes, and cancer progression. The complex relationships between psychosocial stressors and the progression of cancer are difficult to investigate in humans because a number of issues, such as the stage of treatment in which the patients are examined as well as tumor and host factors, may affect interpretation of the data. Animal models allow investigation of the relationship among stressors, coping mechanisms, and tumor growth under more controlled conditions. In animals, psychosocial stressors such as housing condition and psychological stressors such as forced restraint and rotation have been shown to affect tumor growth rates or metastasis of both transplantable and chemically induced tumors[4,5].

There are now some evidences in animals and humans that psychological stress may affect many aspects of the integrative network among the central nervous system, endocrine system and immune system. The complex effects of psychological stress on the interactions among these three systems have been subject to study in the developing field of psychoneuroendocrinoimmunology. There is a large body of literature on the effects of psychological stress on immune function in humans[6-8]. Supposing that immunological changes play an important role in the survival time of mouse with liver cancer under the social isolation stress, we designed this experiment to understand the association among isolation stress, immunological response, and survival time of mouse with liver cancer.

## MATERIALS AND METHODS

### *Animal management*

Mice (KM strain, 18-22 g) were used for this study. All mice were offered the same commercial diet and tap water *ad libitum* in a temperature-controlled (22 ℃) holding room.

### *Immunization with SRBC*

Each male mouse was injected intraperitoneally with 0.2 mL of 200 mL/L sheep red blood cell (SRBC). On the $10^{th}$ d, blood samples were obtained by decapitation and sera were prepared by routine methods.

### *Inoculation with tumor cell*

After 2 d, male mice were inoculated intraperitoneally with liver cancer cells ($5\times10^4$ cells/mouse) obtained from murine hepatoma22 (H22).

### *Grouping*

One male mouse with two females, avoiding aggression, was reared in the same cage (21 cm×32 cm×14 cm) with wood shavings for group (G) or society group. One male

mouse reared individually in each cage to induce stress by isolation condition for group (I) or isolation group. Half of them were used to detect immunological parameter on the 10th d and the other half were used to observe survival time.

### *Detection of anti-SRBC antibody[9]*

Normal saline (25 μL) was added in each cup of microtiter plate, and then was the sera (25 μL) in the first cup, followed by doubling dilution. After incubation with 10 mL/L SRBC (25 μL) for 1 h at 37 ℃, titers of the antibody were determined by the maximum dilution when the cells form a continuous carpet on the base of the cup.

### *Detection of interleukin-2 (IL-2)*

Concentrations of IL-2 in sera were measured with ELISA kit (Jingmei Biotech Co., Ltd). All the procedures and conditions were consistent with the instructions of the kit.

### *Statistical analysis*

Using Student's *t*-test with statistics package SPSS10.0, the differences in the survival time and titers of anti-SRBC antibody as well as concentration of IL-2 from isolation and society mice were evaluated. Differences were considered statistically significant at *P* values <0.05.

## RESULTS

Survival time of mouse with liver cancer is shown in Table 1. There was a significant difference in survival time between the two groups ($P<0.01$).

**Table 1** Survival time of mice of two groups (mean±SD)

| Groups | *n* | mean±SD (d) | *t* | *P* |
|---|---|---|---|---|
| Society | 12 | 16.5±2.7 | 3.46 | 0.002 |
| Isolation | 12 | 13.2±1.9 | | |

Quantities of anti-SRBC antibody are shown in Table 2. The titers were converted to logarithms for analysis. We observed a significant difference in anti-SRBC antibody titers between the society and the isolation groups ($P<0.05$).

**Table 2** Titers of anti-SRBC antibody

| Groups | *n* | Titers | Ig, mean±SD | *t* | *P* |
|---|---|---|---|---|---|
| Society | 10 | 1:24.5 | 1.39±0.38 | 2.60 | 0.02 |
| Isolation | 10 | 1:11.2 | 1.05±0.16 | | |

Quantities of IL-2 in sera are shown in Table 3. Serum IL-2 of the mice in the society group was significantly lower compared to the isolation group ($P<0.05$).

**Table 3** Concentrations of serum IL-2

| Groups | *n* | mean±SD (ng/L) | *t* | *P* |
|---|---|---|---|---|
| Society | 10 | 39.6±6.3 | 2.14 | 0.046 |
| Isolation | 10 | 47.1±9.1 | | |

## DISCUSSION

The effects of psychic factors on somatic diseases and rehabilitation have been more and more appreciated. Several clinical reports have demonstrated that psychic influences work on human bodies mainly through immune system[10-12]. However, many interfering factors in experiments on humans, especially on psychic activities, impair the rigor of the results, and retard further studies. Therefore, we used mice for this experimentation.

Ever since the introduction of the concept of the "animal model" into psychiatric medicine, various experimental models have been applied to clinic oncology trying to reveal the different effects of various psychic states on patient with cancer[4,5,13,14]. Mice are social or gregarious animals; so living alone will leave stressful effects on mice, while living in a society with appropriate sex ratio will not[15]. Our results showed that survival time of mouse with liver cancer in the society group was significantly longer than that in the isolation group ($P<0.01$, Table 1), suggesting that survival time of the mouse bearing H22 tumor was affected by the isolation stress.

Stressor-induced changes in hormones and cytokines have been demonstrated in various studies[6-8]. Our results revealed that the immune response to SRBC of mice in the isolation group was significantly lower than that of the mice in society group ($P<0.05$, Table 2), while IL-2 showed the opposite change ($P<0.05$, Table 3). Thus, differential immune activity in mice in the different experimental housing conditions could alter survival time of mouse with liver cancer.

IL-2 is an important immunoregulatory factor[16,17]. In our experiment, the average serum level of IL-2 in the isolation group mice was obviously higher than that in society group mice, which might be due to the compensative increase of IL-2 to maintain the necessary immune response under stress of social isolation. Even in animal models, data are complex, factors such as the type of tumor, strain or the species have been shown to influence results, and the mechanisms underlying the differential biological change under the stress may also be mediated in survival time of liver cancer. However, further studies are needed for the detailed psychoimmunological pathway and regulation.

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