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Effects of Stress on Tumor Growth and Metastasis in Mice Bearing Lewis Lung Carcinoma

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Abstract—*The progression of Lewis lung carcinoma has been examined in mice under the stress of different housing and experimental conditions. The maintenance of the animals in a low stress environment decreased the weight of spontaneous lung metastases in comparison with conventional housing. The handling of mice in the low stress environment for intraperitoneal saline administration increased metastasis formation, whereas the application of a psychological stressor (spatial disorientation) to these animals increased both primary tumor growth and metastasis formation. These results indicate that psychological and experimental stressors can modulate, presumably via neuroendocrine mechanisms, the host's antitumor responses which can control metastases and primary tumor independently from each other.*

INTRODUCTION

THE STRESS of housing and handling laboratory animals is a usually disregarded experimental factor, which may influence the results obtained in laboratory investigations [1, 2]. Indeed, the maintenance of mice in a low stress environment, in comparison with a conventional animal room, has been shown by Riley *et al.* to reduce the growth of transplantable tumors [3]. The stress of shipment, as well as that of animal isolation or overcrowding, has been similarly found to alter tumor growth in rodents [1, 3, 4]. These findings are consistent with the notion that psychological and physical stress affects tumor progression (for reviews see [1, 4–8]; in this connection an almost total lack of data in the literature on the effects of pyschological stress on tumor metastasis is noteworthy [6].

We thought it therefore worthwhile to examine the effects of housing conditions, as well as those of handling the animals for drug administration, on tumor growth and specifically on the formation of spontaneous metastases of Lewis lung carcinoma in mice. The variables examined have been the housing in a conventional room vs. a low stress environment, as well as handling for intraperitoneal injections, also in comparison with a defined paradigm of psychological stress (spatial disorientation), using conditions essentially similar to those basically devised by Riley *et al.* [3].

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MATERIALS AND METHODS

Animals, housing and spatial disorientation

The animals used are female C57BL/6 and BD2F1 mice weighing 18–20 g, purchased from Charles River, Calco, Como, Italy. They were kept five per cage in order to avoid the effects of overcrowding or isolation on tumor progression [3, 4]. Where appropriate to the experimental design, the cages were placed in a protected environment for 2 weeks before tumor inoculation in order to allow the animals to recover from the stress of shipment [3, 4, 9], and to adapt them to the new housing conditions. The protected environment consisted of a cabinet containing the animal cages with laminar air flow, minimizing acoustic, olfactory and visual communication among the cages, and also with events outside of the cabinet [3] (Fig. 1). The cabinets were contained in a room distant from other animal rooms, where staff entered only once every 5 days for water and food supply to the animals, which were given *ad libitum*. The light cycle in the room was 12–12 h, with an intensity in the cages of approximately 5 lux; constant temperature and relative humidity were 20°C and 60% respectively.

Spatial disorientation (rotational stress) was applied to the animals in the low stress environment when indicated by spinning the cages at 45 rpm for 10 min every hour for 6 days starting from tumor inoculation.

Tumor transplantation

Lewis lung carcinoma was originally provided by the National Cancer Institute, Bethesda MD, U.S.A., and is maintained in C57BL/6 mice by subcutaneous injection in the axillary region of 50 mm^3 of minced tumor tissue aseptically prepared from donors similarly inoculated 2 weeks before [10]. For experimental purposes, the tumor was propagated in BD2F1 mice by subcutaneous implantation as described for tumor line maintenance.

Measurement of tumor growth and metastasis formation

Primary tumor weight (g) was determined at 14 days after tumor inoculation by caliper measurements of short (a) and long (b) axes (cm), taking tumor density equal to 1:

$$\text{Tumor weight} = \pi/6 \times a^2 \times b. \quad (1)$$

The number of metastases was determined at sacrifice on day 22 from tumor inoculation by examining the surface of the lungs by a low-power stereo-microscope. The weight of metastases was determined as the sum of their individual weights calculated according to Eqn (1) after determination of their dimensions by an ocular micrometer [11].

RESULTS

The results obtained under the experimental conditions used are presented in Table 1: the replication of the experiment in three further occasions provided substantially identical results. When Lewis lung carcinoma is implanted in mice adapted before tumor implantation, and further maintained afterwards in the protected environment, the weight of spontaneous pulmonary metastases is significantly and markedly reduced in comparison with mice kept in conventional housing for the whole duration of the experiment. The application of a controlled paradigm of psychological stress (spatial disorientation) to animals continuously kept in the protected housing before and after tumor implantation significantly and markedly increases the number and weight of pulmonary metastases. No statistically significant difference ($P < 0.05$) results for the values obtained in mice continuously kept in conventional housing, as compared with mice placed in the protected environment after tumor implantation, and eventually subjected to spatial disorientation. The handling for the daily intraperitoneal administration of physiological saline of mice constantly kept in the protected environment significantly increases the weight and number of lung metastases to a value not significantly different ($P < 0.05$) from that observed after application of spatial disorientation. The effects of handling for daily intraperitoneal administration of saline are not additive with those caused by the application of spatial disorientation to animals kept in the protected environment, and are not significant in the other experimental groups, including animals constantly kept in the conventional housing.

A statistically significant increase in primary tumor growth is observed in the group of animals continuously kept in the protected environment and subjected to spatial disorientation, as compared

Table 1. Effects of housing conditions, spatial disorientation and handling for paraenteral saline adminstration on primary tumor growth and spontaneous lung metastasis formation in mice implanted s.c. with Lewis lung carcinoma

SD (days)	Housing before tumor inoculation	Housing after tumor inoculation	Intraperitoneal saline adminstration	Tumor weight at 14 days (g)	Metastasis at 22 days: Number	Metastasis at 22 days: Weight (mg)
—	Conventional	Conventional	−	1.6 ± 0.2□△	44.2 ± 5.8*□	220.2 ± 61.6*
—	Conventional	Conventional	+	1.9 ± 0.2□△	47.3 ± 7.6*□	205.3 ± 30.1*
—	Conventional	Protected	−	1.8 ± 0.3□△	60.2 ± 11.3*	354.2 ± 123.4*
—	Conventional	Protected	+	1.4 ± 0.1□△	54.0 ± 11.0	332.4 ± 72.4*
0–6	Conventional	Protected	−	1.7 ± 0.5□△	47.8 ± 11.8	266.0 ± 122.0*
0–6	Conventional	Protected	+	2.1 ± 0.4□△	68.7 ± 16.0*	255.7 ± 106.5*
—	Protected	Protected	−	2.0 ± 0.3†	32.6 ± 6.7†	70.7 ± 25.6†
—	Protected	Protected	+	2.0 ± 0.4□	57.8 ± 9.0*	278.0 ± 76.7*
0–6	Protected	Protected	−	4.0 ± 1.7*■	80.5 ± 7.0*■	281.2 ± 80.8*
0–6	Protected	Protected	+	3.7 ± 1.2*▲	59.0 ± 8.8*	223.0 ± 44.8*

Adaption to housing conditions lasted 15 days before tumor inoculation. Spatial disorientation (SD) was applied to mice in the protected housing for 7 (0–6) days from tumor inoculation. Each value is the mean (± S.E.) obtained using groups of five mice. The animals were implanted s.c. with Lewis lung carcinoma on day 0. When indicated, the animals received i.p. on days 1–21 0.1 ml of isotonic NaCl solution (9 g/l). t-test for grouped data [26] $P < 0.05$: * : means significantly different from † ; ■ : means significantly different from □ ; ▲ : means significantly different from △.

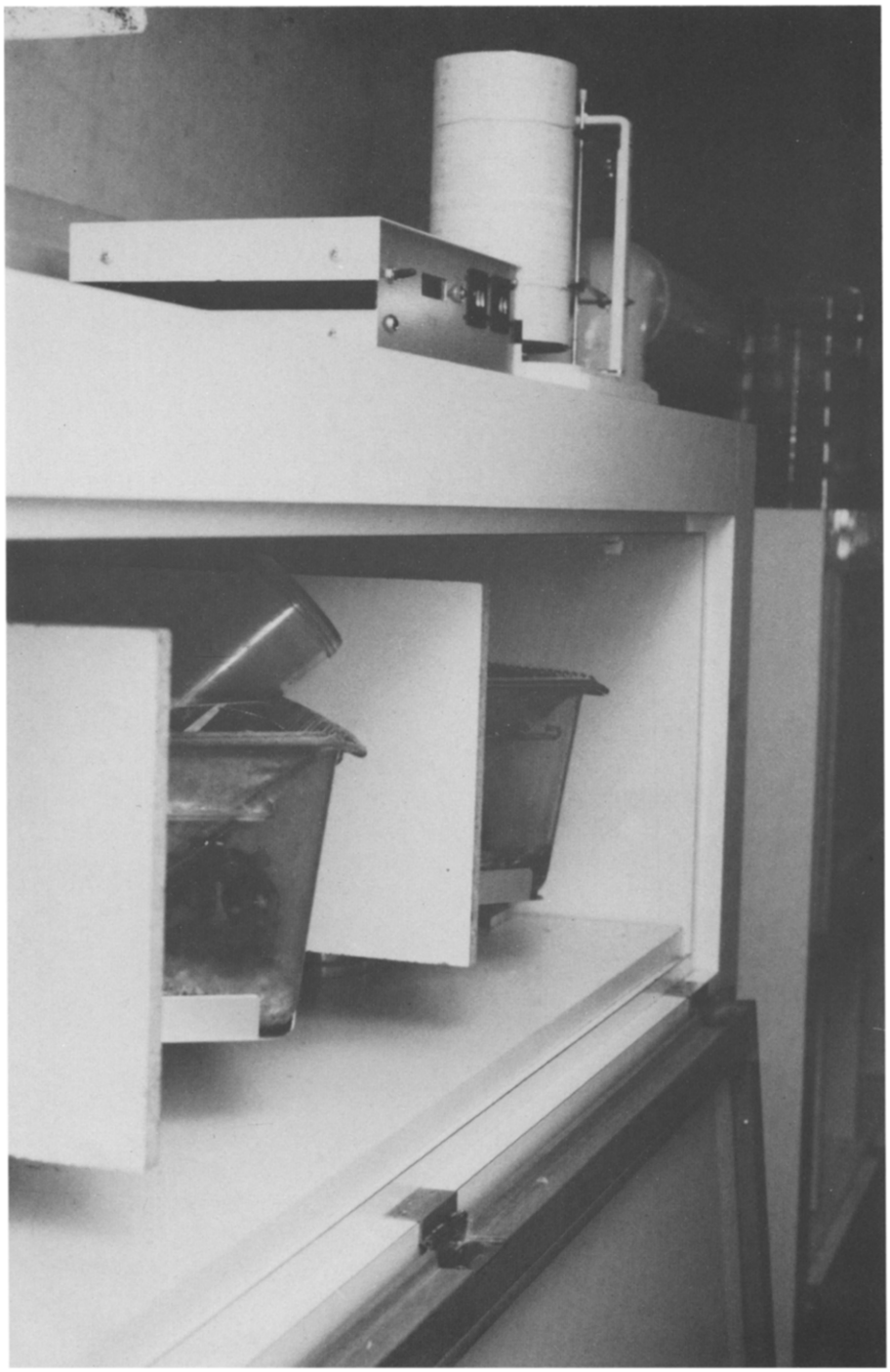

Fig. 1. Illustration of the protected animal housing, including details of the cabinet and of the mechanisms for periodic application of spatial disorientation, as described in Materials and Methods.

with the other experimental groups, which is maintained also when handling is combined with spatial disorientation.

DISCUSSION

A relatively large number of reports exists in the literature, showing that stress can influence the incidence and progression of tumors in laboratory animals [1–9]. The data available indicate that the physical stress produced by therapeutic procedures, such as radiotherapy [12–15], chemotherapy [16–18], anesthesia [19] and surgery [15, 19–21], increases tumor metastasis in experimental animal-tumor systems. Psychological stressors, including isolation or overcrowding during animal housing, have also been shown to influence the incidence and growth of tumors in laboratory rodents [1, 3, 4]. The results obtained with psychological stressors are rather conflicting, and appear to depend on the experimental conditions chosen, i.e. the animal—tumor system used, the acute or chronic application of the stressor, as well as its nature [6]. Moreover, the majority of the animal-tumor systems employed in these studies were allogeneic, and metastasis was rarely examined [6] in spite of the clinical relevance of this phenomenon.

An absolute distinction between physical and psychological stressors appears to be difficult; for instance, a certain degree of subjective cognitive and emotional reaction is presumably always present during coping with physical stressors. In the present investigation the stressor variables examined have been housing conditions, handling the animals for intraperitoneal drug administration and the periodic application of spatial disorientation. These stressors appear mainly to have a psychological nature, and spatial disorientation in particular was suggested to be an emotional stressor (anxiety) [3], since the centrifugal acceleration caused in the cages by rotation is smaller than 0.15 g.

Using these conditions, and Lewis lung carcinoma implanted into syngeneic BD2F1 mice as the animal–tumor system, the results reported show that tumor metastasis is reduced independently from primary tumor growth by housing the animals in a low stress environment. The application of spatial disorientation, and handling the animals for the intraperitoneal administration of physiological saline, increases metastasis and primary tumor growth in animals kept in the low stress housing. This increase is not additive when spatial disorientation and handling are combined, and it is not observed when the mice are not adapted before, and kept after, tumor implantation in the low stress housing. These results might be interpreted considering that the central nervous system modulates the natural antitumor responses of the host via neural vegetative and endocrine mechanisms [22]; coping with stressors may lead to a reduction in these responses [23, 24]. The tumor responses presently caused by housing conditions, handling and spatial disorientation are of a similar magnitude; their observed non-additive effect might depend on the fact that each of them is the maximal degree of control that the host exerts in response to the stressors applied. This interpretation is consistent with the data obtained in several other studies, which show that in certain circumstances the effect of one stressor can be non-additive with, modified by, and even reversed by, the combined application of further stressors, depending on their nature and timing of application [7, 8].

Further work is in progress, aiming to experimentally examine this hypothesis, and to determine the mechanism(s) of the tumor enhancing effects of the stressors used, in the perspective of the experimental and clinical implications of these findings.

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