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Restraint Stress Reduces the Antitumor Efficacy of Cyclophosphamide in Tumor-Bearing Mice

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Treatment with the cytotoxic antitumor drug cyclophosphamide is highly effective in mice bearing Lewis lung carcinoma, causing the absence of macroscopically detectable tumors at necroscopy after sacrifice. When the effects of the treatment on survival are determined, a significant increase in survival time and in the proportion of long-term survivors is observed. When restraint stress is further applied, tumors develop in all of the mice treated with cyclophosphamide, and survival time and the fraction of long-term survivors are significantly reduced. Flow cytometry of splenic T-lymphocyte subsets in normal mice indicates a significant decrease in the number of $CD3^+$, $CD4^+$, and $CD8^+$ subsets after treatment with cyclophosphamide and after application of restraint stress; the interaction of the two treatments is significant for $CD3^+$ and marginally significant for $CD4^+$ subsets. The attenuation by restraint stress which was observed for the effects of cyclophosphamide on the presence of tumors at necroscopy and for the survival of the treated mice might thus be interpreted as follows: restraint stress attenuates the immune functions of the host directed toward the weakly immunogenic tumor, an effect which, in the absence of restraint stress, interacts effectively with the cytotoxic action of cyclophosphamide toward tumor cells. The results obtained using this animal model thus indicate that experimental stress reduces the therapeutic efficacy of a cytotoxic antitumor drug; experimental and clinical implications are discussed.

Key Words: stress; physical restraint; tumor; metastasis; antitumor drug; cyclophosphamide; antitumor efficacy; lymphocyte subpopulations; psychoneuroimmunology.

INTRODUCTION

Cancer chemotherapy with cytotoxic antitumor drugs is remarkably effective in patients with numerous types of malignant tumors (Frei, 1985, 1987). Experimental evidence from animal tumor systems indicates that immune functions of the host cooperate in determining the curative action of cytotoxic antitumor agents when the tumor possesses significant antigenic and/or immunogenic properties. Indeed, low dosages of cyclophosphamide and melphalan were equally as effective as, or more effective than, higher dose levels of the same drugs at curing mice bearing MOPC-315 plasmacytoma (Ben-Efraim, Bocian, Mokyr, & Dray, 1983; Berko, Seissman, Colvin, Bocian, Ben-Efraim, & Dray, 1988; Mokyr & Dray, 1983). Mice cured with low-dosage schedules showed a strong acquired immune resistance to further tumor challenges, but this did not occur in mice given high dosages (Ben Efraim et al., 1983; Hengst, Mokyr, & Dray, 1981; Mokyr & Dray, 1983; Mokyr, Brundett, Colvin, & Dray, 1986). Moreover, the acquired immune resistance was shown to depend on the appearance of Lyt 2^+ T-cells (Mokyr et al., 1986; Mokyr, Baker, Weiskirch, Takesue, & Pyle, 1989). Findings consistent with a dosage-dependent immunoregula-

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tory action have also been found in rats bearing KMT-17 fibrosarcoma upon treatment with bleomycin (Morikawa, Hosokawa, Hamada, Sugawara, & Kobayashi, 1985).

In addition, the immune functions of the host have been shown to be modulated via neuroendocrine activation by the application of stress in experimental animals (Hayden-Hixson & Nemeroff, 1993). The application of stress also facilitates the growth of transplanted tumors (Amkraut & Solomon, 1972; Justice, 1985; Moynihan, Brenner, Cocke, Karp, Breneman, Dopp, Ader, Cohen, Grota, & Felten, 1994; Pradhan & Ray, 1974; Riley, Spackman, McClanahan, & Santisteban, 1979; Steplewski, Vogel, Ehya, Poropatich, & McDonald Smith, 1985). The development of secondary tumor metastatic foci can be specifically enhanced by the application of experimental stress in animal models (Giraldi, Perissin, Zorzet, Piccini, & Rapozzi, 1989; Giraldi, Perissin, Zorzet, Rapozzi, & Rodani, 1994; Moynihan et al., 1994).

Our group has investigated the influence of stress on the effects of the antitumor cytotoxic drug cyclophosphamide and the selective noncytotoxic antimetastatic agent razoxane (Perissin, Zorzet, Piccini, Rapozzi, & Giraldi, 1991). In mice bearing a weakly immunogenic tumor, Lewis lung carcinoma, the application of rotational stress (spatial disorientation) significantly decreased the magnitude of the effects of both drugs, determined as the reduction of primary tumor size at the end of treatment and reduction of metastatic nodules at necroscopy after sacrifice.

The aim of the present study was to determine whether the application of a different form of stress would also reduce the efficacy of an antitumor drug. We therefore determined the effects of physical restraint on tumor progression and survival of the hosts in relation to immune function (splenic T-cell subpopulations) in mice bearing Lewis lung carcinoma and treated with cyclophosphamide.

MATERIALS AND METHODS

Animals and Tumor Transplantation

The animals used were female C57BL/6 and C57BL/6 × DBA/2F$_1$ (BD2F1) mice weighing 18–20 g, purchased from Charles River, Calco (Como, Italy). Lewis lung carcinoma, originally provided by the National Cancer Institute (Bethesda, MD) is maintained in C57BL/6 mice by subcutaneous injection into the axillary region of 50 mm^3 of minced tumor tissue aseptically prepared from donors similarly inoculated 2 weeks before the experiment. For experimental purposes, the tumor is propagated in BD2F1 mice by intramuscular injection of a tumor cell suspension containing 10^6 viable tumor cells (Giraldi et al., 1989).

Measurement of Tumor Growth and Metastasis Formation

Primary tumor volume was determined 14 days after tumor inoculation by caliper measurements of its short and long axes. The volume of metastases was determined at sacrifice on day 21 after tumor inoculation by examining the surface of the lungs with a low-power stereo microscope. For the details of these procedures see Giraldi et al. (1989).

Experimental Stress

To reduce the uncontrolled stress from shipment and housing (Labarba, 1970; Riley, Fitzmaurice, & Spackman, 1981a; Riley, Fitzmaurice, & Spackman, 1981b), animals were kept in a protected environment for 2 weeks before each experiment

and throughout its duration. To avoid overcrowding or isolation effects on tumor progression (Labarba, 1970; Riley et al., 1981a), the animals were kept in groups of five in plastic cages measuring 27 × 21 × 14 cm with a stainless steel grid cover (Techniplast Gazzada Srl, Buguggiate Varese, Italy). To minimize acoustic, olfactory, and visual communication, the cages were kept in cabinets allowing laminar air flow in between them and placed in a room remote from the animal rooms; staff entrance was limited to delivering water and food. The light:dark cycle in the room was 12:12 hr, with a light intensity of approximately 5 lux in the cages. Temperature and relative humidity were constant at 20°C and 60%, respectively. Restraint stress consisted of tying the animals' legs with string fixed to small plastic boards; daily sessions lasting 1 h were repeated on days 1–6 after tumor inoculation.

Measurement of Splenic T-Lymphocyte Subpopulations

Spleens were removed immediately following sacrifice by cervical dislocation. They were disaggregated and then passed through a double layer of gauze to obtain single-cell suspensions. The cells were washed and lymphocytes were separated from red blood cells by Ficoll–Hypaque centrifugation (Sigma, St. Louis, MO) (Hunt, 1987). The final suspension of splenic lymphocytes was labeled with anti-mouse monoclonal antibodies using a PBS staining medium, pH 7.4, containing 0.5% BSA and 0.1% NaN_3. Aliquots of 10^6 viable cells in 0.5 ml of staining medium, counted by trypan-blue exclusion test, were incubated in the dark for 30 min at 4°C with 50 μl of rat anti-mouse monoclonal antibodies to CD3 (0.5 μg), CD4 (1 μg), or CD8 (1 μg) (Pharmingen, San Diego, CA). Stained cells were examined using an EPICS flow cytometer (Coulter, Miami, FL); each analysis consisted of 10,000 events counted (Dasic, Pacor, Bergamo, Salerno, Vranesic, Jukic, Tomasic, & Sava, 1994). Results for single-color analysis are expressed as the total number of positive cells collected from the spleen of each animal.

Drug Treatment

Cyclophosphamide was generously provided by Schering SpA (Milan, Italy). When the antitumor effects of cyclophosphamide were examined in combination with the application of restraint stress, the drug was administered orally admixed in powdered food to avoid the stress of repeated handling and intraperitoneal injections (Moynihan et al., 1994; Perissin et al., 1991). Such treatment was performed for 6 days starting 24 h after tumor implantation; the control animals also received powdered food. Drug concentration was selected to provide 240 mg/kg/day on the basis of a measured average daily food consumption of 5.0 ± 0.1 g per mouse. The amount of food supplied daily to the animals was 5 g per mouse, and food consumption remained constant throughout the duration of the experiment in both the controls and the group receiving cyclophosphamide. When indicated, in order to determine the effects of cyclophosphamide on the host and prevent possible interference with the results due to contact between the drug and the tumor cells, the animals were treated with a single intraperitoneal injection of 200 mg/kg cyclophosphamide 24 h before tumor inoculation.

Statistical Analysis

Table values are group means ± SD. Data were subjected to the appropriate factorial ANOVAs assessing significance against an α level of $p < .05$. All analyses were

TABLE 1
Effects of Restraint Stress and Cyclophosphamide on Primary Tumor Growth and Lung Metastasis Formation in Mice Bearing Lewis Lung Carcinoma

Stress	Cyclophosphamide before tumor[a]	Cyclophosphamide after tumor[b]	Tumor volume (cm^3)	Metastasis volume (mm^3)	No. of mice with tumor/total no.
−	−	−	3.2 ± 1.4	208 ± 102	10/10
+	−	−	4.7 ± 0.9	328 ± 95	10/10
−	−	+	0	0	0/10
+	−	+	1.0 ± 0.6	8 ± 5	10/10
−	+	−	3.3 ± 0.5	326 ± 133	10/10
+	+	−	3.5 ± 0.7	302 ± 70	10/10
−	+	+	0.8 ± 1.1	3 ± 5	10/10
+	+	+	0.6 ± 0.9	5 ± 6	10/10

Note. Each value is the mean (±SD) obtained using groups of 10 mice. The data were subjected to ANOVA analysis, and the findings are reported under Results.

[a] The animals received a single dose of cyclophosphamide (200 mg/kg ip) 24 h before tumor implantation.

[b] The animals received cyclophosphamide (240 mg/kg day orally) for 6 days starting 24 h after tumor implantation.

performed using standard procedures implemented in the Systat package (SYSTAT Inc., Evanston, IL).

RESULTS

Effects of Restraint Stress and Cyclophosphamide on Primary Tumor and Metastasis

A first series of experiments determined the effects of the treatments in terms of primary tumor volume measured 2 weeks after implantation and in terms of lung metastasis volume determined at necroscopy 1 week later. The treatment with cyclophosphamide performed after tumor implantation appears highly effective, reducing the proportion of mice with macroscopically detectable tumors at necroscopy from 10/10 for controls to 0/10 in the treated group, Pearson $\chi^2 = 80.0$, $df = 7$, $p < .0001$. Separate 2 (presence or absence of stress) × 2 (presence or absence of cyclophosphamide before tumor implantation) × 2 (presence or absence of cyclophosphamide after tumor implantation) factorial ANOVAs confirmed that the effects of cyclophosphamide administered after tumor implantation on the primary tumor and on metastasis volume are significant, $F_{(1,72)} = 242.9$, $p < .0001$ and $F_{(1,72)} = 315.9$, $p < .0001$, respectively. The effect of restraint stress on primary tumor volume is significant, $F_{(1,72)} = 8.13$, $p = .006$. However, treatment with cyclophosphamide before tumor implantation did not affect either the primary tumor or the metastases, $F_{(1,72)} = 2.23$, $p \leq .139$. The two-way interaction of treatment with cyclophosphamide before and after tumor implantation was significant for primary tumors, $F_{(1,72)} = 5.80$, $p = .019$, as was the interaction of treatment before implantation with restraint stress, $F_{(1,72)} = 7.65$, $p = .007$. The interaction of treatment before tumor implantation with stress was significant for metastasis volume, $F_{(1,72)} = 5.02$, $p = .03$, as was the three-way interaction of stress with treatment before and after tumor implantation, $F_{(1,72)} = 4.93$, $p = .03$ (see Table 1).

TABLE 2
Effects of Restraint Stress on the Increase in Survival Time Caused by Cyclophosphamide in Mice Bearing Lewis Lung Carcinoma

Cyclophosphamide	Stress	Mean survival time (days)[a]	Long term survivors (no.)
−	−	26.4	0/15
−	+	27.2	0/18
+	−	37.9	4/17
+	+	24.9	0/20

Note. Survival time of the animals was recorded and analyzed by the Kaplan–Meier method. Values are reported as mean survival time and the fraction of mice with a survival time >60 days.
[a] Logrank analysis, $p < .0001$.

Effects of Restraint Stress and Cyclophosphamide on Survival

A second series of experiments determined experimental effects in terms of the survival time of immunocompetent mice that did not receive treatment with cyclophosphamide before tumor implantation. In these animals, the application of restraint stress did not modify survival time. Cyclophosphamide treatment in mice not subjected to restraint stress significantly increased their mean survival time and 4 of 17 mice were cured, displaying a survival time longer than 60 days, Pearson $\chi^2 = 13.2$, $df = 3$, $p = .004$. When cyclophosphamide treatment was combined with the application of restraint stress, the survival time and the proportion of long-term survivors were reduced to the same values observed in untreated controls, log-rank $\chi^2 = 22.4$, $df = 3$, $p < .0001$ (see Table 2 and Fig. 1). A bivariate Cox proportional hazard analysis confirms that restraint stress constitutes a significant negative prognostic factor (hazard ratio = 4.36), whereas treatment with cyclophosphamide contributes positively to survival (hazard ratio = 0.145).

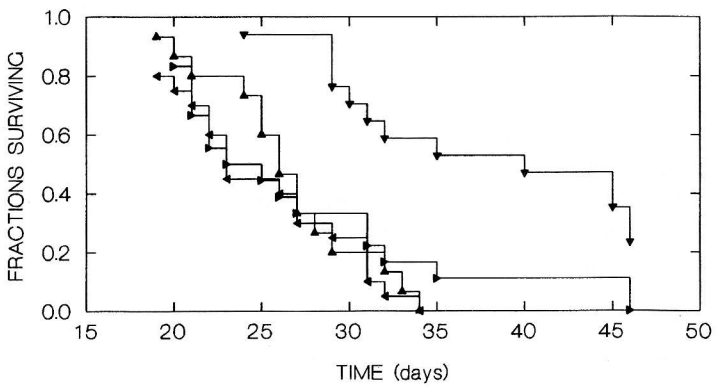

FIG. 1. Effects of restraint stress on the increase in survival (Kaplan–Meier plot) caused by cyclophosphamide in mice bearing Lewis lung carcinoma, whose data are also reported in Table 2. ▲, Group 1 ($n = 15$) without stress without cyclophosphamide; ◀, group 2 ($n = 18$) with stress without cyclophosphamide; ▼, group 3 ($n = 17$) without stress with cyclophosphamide; ▶, group 4 ($n = 20$) with stress with cyclophosphamide.

TABLE 3
Effects of Restraint Stress and Cyclophosphamide on Splenic T-lymphocyte Subpopulations in Normal Mice

Cyclophosphamide	Stress	$CD3^+$	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$ ratio
−	−	20.5 ± 1.3	11.1 ± 1.4	6.4 ± 1.2	1.78 ± 0.37
−	+	13.6 ± 1.1	6.7 ± 1.1	5.5 ± 1.2	1.23 ± 0.08
+	−	9.3 ± 0.7	5.9 ± 0.7	3.3 ± 0.5	1.82 ± 0.31
+	+	5.6 ± 0.4	3.3 ± 0.3	2.3 ± 0.4	1.49 ± 0.37

Note. Each value is the mean $\times 10^6$ (±SD) obtained using groups of 5 mice. The data were subjected to ANOVA analysis, and the findings are reported under Results.

Effects of Restraint Stress and Cyclophosphamide on Splenic T-Lymphocyte Subpopulations

In normal mice, the application of restraint stress and treatment with cyclophosphamide following tumor implantation caused a significant decrease in the number of $CD3^+$, $CD4^+$, and $CD8^+$ lymphocyte subsets, as indicated by separate 2×2 ANOVAs, $F_{(1,16)} = 152.9$, $p < .0001$; $F_{(1,16)} = 66.8$, $p < .0001$; and $F_{(1,16)} = 5.1$, $p = .038$, respectively.

The treatment with cyclophosphamide following tumor implantation also caused a significant decrease in the number of $CD3^+$, $CD4^+$, and $CD8^+$ lymphocyte subsets $F_{(1,16)} = 503.3$, $p < .0001$; $F_{(1,16)} = 99.2$, $p < .0001$; and $F_{(1,16)} = 55.3$, $p < .0001$, respectively. The two-way interaction between stress and cyclophosphamide proved significant for $CD3^+$ cell number, $F_{(1,16)} = 13.6$, $p < .002$, and marginally significant for $CD4^+$ cell number, $F_{(1,16)} = 4.3$, $p < .055$), whereas the interaction of the treatments for $CD8^+$ cell number was not significant, $F_{(1,16)} = .019$, $p = .892$. The $CD4^+/CD8^+$ ratio was significantly reduced only by restraint stress, $F_{(1,16)} = 10.2$, $p = .006$. Finally, the interaction of the two treatments was not significant (see Table 3).

When mice implanted with Lewis lung carcinoma were examined, the application of restraint stress significantly lowered the number of $CD3^+$ and $CD8^+$ splenic lymphocytes, $F_{(1,16)} = 7.6$, $p = .014$ and $F_{(1,16)} = 13.3$, $p = .002$, respectively; the number of $CD8^+$ splenic lymphocytes was significantly reduced by the treatment with cyclophosphamide, $F_{(1,16)} = 6.6$, $p = .02$. The two-way interactions between restraint stress and cyclophosphamide treatment were significant and indicated an increase in the number of $CD3^+$, $CD4^+$, and $CD8^+$ subsets when the treatments were combined, $F_{(1,16)} = 51.0$, $p < .0001$; $F_{(1,16)} = 27.1$, $p < .0001$; and $F_{(1,16)} = 35.7$, $p < .0001$, respectively. The effects on $CD4^+/CD8^+$ ratio were significant in the case of cyclophosphamide treatment, $F_{(1,16)} = 7.88$, $p = .013$, but not in the case of the two-way interaction between the treatments (see Table 4).

DISCUSSION

The aim of the present study was to determine the possible influences of stress on the effectiveness of antitumor chemotherapy with a cytotoxic drug in a laboratory animal tumor system. Mice bearing Lewis lung carcinoma have thus been treated with cyclophosphamide, by providing the animals with powdered food containing the desired amount of the drug. This modality of administration was chosen because the stress resulting from animal handling (Moynihan et al., 1994; Brenner, Cohen,

TABLE 4
Effects of Restraint Stress and Cyclophosphamide on Splenic T-lymphocyte Subpopulations in Mice Bearing Lewis Lung Carcinoma

Cyclophosphamide	Stress	$CD3^+$	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$ ratio
−	−	34.4 ± 4.2	23.7 ± 3.6	10.6 ± 1.2	2.24 ± 0.32
−	+	23.5 ± 3.9	15.9 ± 3.0	7.9 ± 1.7	2.05 ± 0.34
+	−	18.7 ± 2.6	11.5 ± 1.9	6.6 ± 0.8	1.77 ± 0.37
+	+	43.2 ± 9.1	25.3 ± 7.8	17.9 ± 4.7	1.47 ± 0.59

Note. Each value is the mean × 10^6 (±SD) obtained using groups of 10 mice. The data were subjected to ANOVA analysis, and the findings are reported under Results.

Ader, & Moynihan, 1990) or intraperitoneal administration of isotonic saline solution (Giraldi et al., 1989) has been shown to facilitate metastasis formation in mice implanted with solid malignant tumors. The possible effects of aversion to the food containing cyclophosphamide, which might lead to restricted food intake and hence to immune system consequences (Theofilopoulos & Dixon, 1985), can be ruled out because the amount of food consumed by the animals was measured and remained constant during the period of treatment. The examination of the proportion of animals with primary tumors and metastasis at sacrifice indicated that treatment with cyclophosphamide after tumor implantation was highly effective, causing the disappearance of macroscopically detectable tumors as determined at necroscopy. The apparently curative action of cyclophosphamide was abolished when the animals were subjected to restraint stress and was similarly abrogated when the animals were treated before tumor implantation with a single intraperitoneal dose of cyclophosphamide capable of depressing T-lymphocyte-mediated immune responses (Mantovani, Polentarutti, Alessandri, Vecchi, Giuliani, & Spreafico, 1977; Milton, Carpenter, & Addison, 1976), as well as with the combination of cyclophosphamide and restraint stress. The results of ANOVA further show significant effects on primary tumor volume, of cyclophosphamide administered after tumor implantation, of restraint stress, and of the interactions between cyclophosphamide administered before and after tumor implantation, as well as of the interaction between cyclophosphamide administered after tumor implantation and restraint stress. With respect to metastasis volume, ANOVAs indicated a significant effect of cyclophosphamide administered after tumor implantation and also of the interaction of cyclophosphamide with restraint stress as well as of the interaction of both treatments with cyclophosphamide and restraint stress. The currently reported attenuation of cyclophosphamide efficacy by restraint stress is in agreement with a similar reduction in the effectiveness of cyclophosphamide and razoxane, which has been observed at necroscopy after application of rotational stress in mice bearing the same tumor, Lewis lung carcinoma (Perissin et al., 1991).

The effectiveness of cyclophosphamide treatment has been also examined in terms of survival time of the host rather than in terms of primary tumor and metastasis size at sacrifice. This confirms the effectiveness of cyclophosphamide administered according to the schedule used: it resulted in 4/17 long-term survivors cured by the drug, and the mean survival time of the mice that were not cured was significantly increased. The application of restraint stress, which by itself has no effects on sur-

vival, completely abolished the increase in survival time caused by the treatment with cyclophosphamide.

The results presented above might be interpreted by assuming that restraint stress in some way modulates the immune responses of the host, which are normally effective in controlling primary tumor growth and metastatic development. In fact, data in the literature have shown that stress-induced reduction of T-lymphocytic functions leads to a significant increase in metastasis formation (Moynihan et al., 1994). Moreover, the data currently reported indicate that the fraction of immunocompetent animals that develop tumors (0/10) after cyclophosphamide treatment is increased to 10/10 when the animals are treated before tumor implantation with a single intraperitoneal dose of cyclophosphamide capable of causing immunodepression (Mantovani et al., 1977; Milton et al., 1976).

This view is also consistent with the data obtained from determination of splenic lymphocyte subpopulations in the treated animals. Indeed, the application of restraint stress to non-tumor-bearing mice significantly reduced the number of $CD3^+$, $CD4^+$, and $CD8^+$ T-lymphocyte subsets, as well as the $CD4^+/CD8^+$ ratio. A reduction in each of the $CD3^+$, $CD4^+$, and $CD8^+$ T-lymphocyte subsets was also caused by treatment with the antitumor cyclophosphamide schedule. The interaction between restraint stress and cyclophosphamide treatment was significant for $CD3^+$ cell number and marginally significant for $CD4^+$ cell number.

In mice bearing Lewis lung carcinoma, the effects of restraint stress were significant for $CD3^+$ and $CD8^+$ T-lymphocyte number, whereas those of cyclophosphamide were significant for $CD8^+$ cell number. The effects of the treatments on the $CD4^+/CD8^+$ ratio indicate a tendency toward reduction in the experimental groups, which is significant in the case of the cyclophosphamide antitumor schedule. The interaction between stress and cyclophosphamide indicates a significant increase in the number of T-lymphocyte subsets for the combined treatments. This increase in the number of splenic T-lymphocyte subsets caused by restraint stress and cyclophosphamide in tumor-bearing mice, and the reduction conversely observed in $CD3^+$ and marginally in $CD4^+$ subsets in non-tumor-bearing mice, might be explained by the presence or absence of tumors in the different experimental groups. In fact, lymphocytic and immune functions have been shown to be stimulated by the presence of tumors and to be inhibited by large tumors (Maccubbin, Mace, Ehrke, & Mihich, 1989). The progression of malignant tumors in laboratory animals has been shown to be controlled also by NK cell responses of the host (Page, Ben-Eliyahu, & Liebeskind, 1994); in mice bearing Lewis lung carcinoma that were subjected to rotational stress, the number of spleen cells positive for NK1.1 monoclonal antibody was determined using flow cytometry and showed no significant effect for the treatments or for their interaction (unpublished results). It is therefore likely that NK cells are factors in the results currently reported.

In the light of the results and the considerations presented above, it is thus proposed that the antitumor effects observed for cyclophosphamide actually depend on the cytotoxicity of the drug toward tumor cells accompanied by T-lymphocytic antitumor responses of the host, the latter of which is amenable to modulation (reduction) by stress. This view is further supported by the following findings. Lewis lung carcinoma is a weakly immunogenic tumor (Sava, Giraldi, Zupi, & Sacchi, 1984), and a small inoculum of this tumor, but not a larger one, is rejected in normal syngeneic mice maintained in the protected environment; tumor take occurs in all the mice implanted

with the smaller inoculum upon application of rotational stress (Perissin et al., 1991). Moreover, when mice are cured by surgical removal of early primary Lewis lung tumors combined with chemotherapy, the cured hosts resist a further challenge with the same tumor, indicating that an effective immune antitumor response has occurred (Giraldi, Sava, Cherubino, Lassiani, Bottiroli, & Mazzini, 1983).

One possible explanation of the effect of stress on drug action might be a modification of drug pharmacokinetics, as has been observed for vibration stress with rifampicin as well as for immobilization or foot shock stress with theophylline and caffeine in rats (Lavicky & Raskova, 1989; Okazaki, Eto, Furuno, Oishi, & Gomita, 1995). Since cyclophosphamide requires metabolic activation to exert cytotoxic effects (Chang, Weber, Crespi, & Waxman, 1993), this possibility has been considered. Plasma levels of cyclophosphamide and its activated alkylating metabolites have been determined in mice using the same treatment schedule used in this study and were not significantly modified by the application of rotational stress (unpublished results). It is therefore presumed that the pharmacokinetics of cyclophosphamide are also not modified by restraint stress.

In conclusion, the data presented indicate that the application of restraint stress reduces the magnitude of the antitumor effects of cyclophosphamide in mice. Restraint stress does not modify the survival time of tumor-bearing mice, but markedly attenuates the number of cured animals and increases survival time induced by cytotoxic antitumor treatment with cyclophosphamide. This may be relevant to human clinical situations. There is, indeed, evidence to show that various psychosocial factors, including stress, contribute to determining the progression of cancer in patients (Forsén, 1991; Greer, Morris, & Pettingale, 1979; Greer, Morris, Pettingale, & Haybittle, 1990; Morris, Pettingale, & Haybittle, 1992; Pettingale, Morris, Greer, & Haybittle, 1985; Ramirez, Craig, Watson, Fentiman, North, & Rubens, 1989; Giraldi, Rodani, Cartei, & Grassi, 1997); these results were obtained in patients who almost invariably received cytotoxic antitumor chemotherapy. Future investigation aimed at discovering the specific relevance of psychosocial factors in determining the outcome of cancer chemotherapy in comparison with the effects of these factors on the natural history of disease progression per se thus appears warranted. The experimental model currently employed might provide a useful animal system for such investigations.

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