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Modification of the Antitumor Action of *Corynebacterium parvum* by Stress

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TURNEY, T. H., A. G. HARMSEN AND M. A. JARPE. *Modification of the antitumor action of* Corynebacterium parvum *by stress.* PHYSIOL BEHAV 37(4) 555–558, 1986.—Social grouping and isolation of mice, in the presence of an acute stressor, were found to differentially affect the antitumor action of the immunological adjuvant *Corynebacterium parvum*. Socially grouped DBA/2j mice were injected intradermally with P815 mastocytoma ascites cells. Half the mice had a threshold dose of *C. parvum* admixed with the P815 cells. Half the mice in each of those conditions were given acute, inescapable electric footshock. In a second experiment, the stressed mice were socially isolated prior to the acute stress. Tumor growth itself was not affected by the stress procedures. *C. parvum* inhibited tumor growth in non-stressed and socially isolated, stressed mice. However, social grouping selectively negated the *C. parvum* effect resulting in tumor growth and mortality equivalent to mice not given the adjuvant. Psychological factors may be important to the development of concomitant immunity and the efficacy of immunotherapies.

Corynebacterium parvum   Stress   Antitumor action   P815 mastocytoma cells

THE importance of concomitant immunity in oncogenesis, while of general interest, remains uncertain. In order for concomitant immunity to a tumor to occur, the tumor must be antigenic to the host. However, there is currently no means of determining the antigenicity of a tumor in man [9]. In addition, animal experiments have shown that increasing quantities of tumor antigens generate tumor specific suppressor T-cells [9,11]. Transfer of these suppressor cells to immune animals makes the animals more susceptible to the tumor growth [8,10]. It has been suggested that the mounting quantity of tumor antigen, produced by a growing tumor, causes the generation of the suppressor T-cells which inhibit antitumor effector mechanisms.

Immunosuppression can also be affected by neuroendocrine mechanisms initiated by psychologically stressful conditions [3,12]. Pituitary and adrenal hormones, which may be released in response to stress, have been shown to modify activities of the immune system, including producing an immunosuppression [1, 2, 7]. Stress has also been shown to affect natural killer cell function [13], T-cell mitogen responses [6], and the growth of transplanted tumors in animals [15]. Psychological stress has also been implicated as a factor contributing to the development of some human cancers [13,14]. Since stress is known to affect tumor growth and immunosuppression, and since immunosuppression inhibits concomitant immunity to tumors, we studied the effect of stress on concomitant immunity to tumors.

Concomitant immunity to tumors can be induced by the injection of *Corynebacterium parvum* together with transplantable tumor cells [11]. In this model, the tumor grows normally for about 10 days and begins to regress. Animals in which the tumor is caused to regress by the *C. parvum* are then resistant to subsequent challenges with the tumor cells. The *C. parvum* is thought to increase T-cytolytic activity and may inhibit the development of suppression [8,10]. This *C. parvum* model of concomitant tumor immunity was utilized in the present study.

We first determined the minimum dose of *C. parvum* which inhibited tumor growth. Using this dose, P815 ascites tumor cells with or without *C. parvum* were injected intradermally into two groups of mice. Twenty-four hours after tumor cell transplantation, half the mice in each group were stressed with acute, inescapable, electric footshock. In two experiments, the mice were either socially grouped or isolated prior to tumor cell transplantation and acute stress. Thus, in the two experiments we examined psychological effects on the ability of *C. parvum* to enhance tumor resistance.

METHOD

Subjects

One hundred-five male, DBA/2j mice were used as subjects. The mice were obtained from Jackson Laboratories, Bar Harbor, ME, at the age of eight weeks. The mice were housed in translucent plastic cages on hardwood chip bedding (Sanichip, Murphy Forest Prod., Rochelle, NJ). Wayne Lab Blox chow and tap water were provided ad lib. A 12/12

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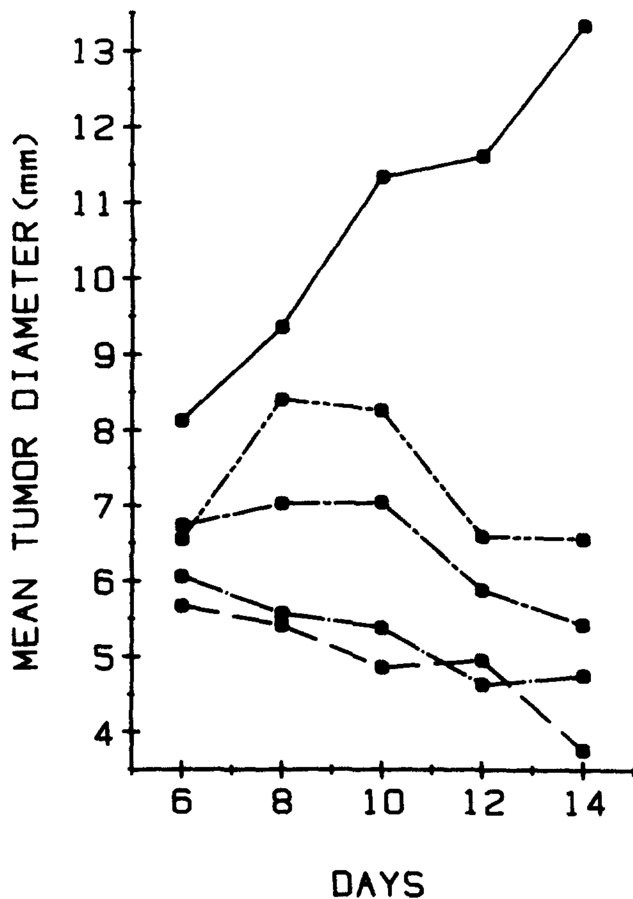

FIG. 1. Mean tumor diameter over days for mice receiving P815 cells only (——) and those receiving P815 cells admixed with *C. parvum* doses of 1 μg (— - - —), 10 μg (— - —), 50 μg (— · —) and 100 μg (— —).

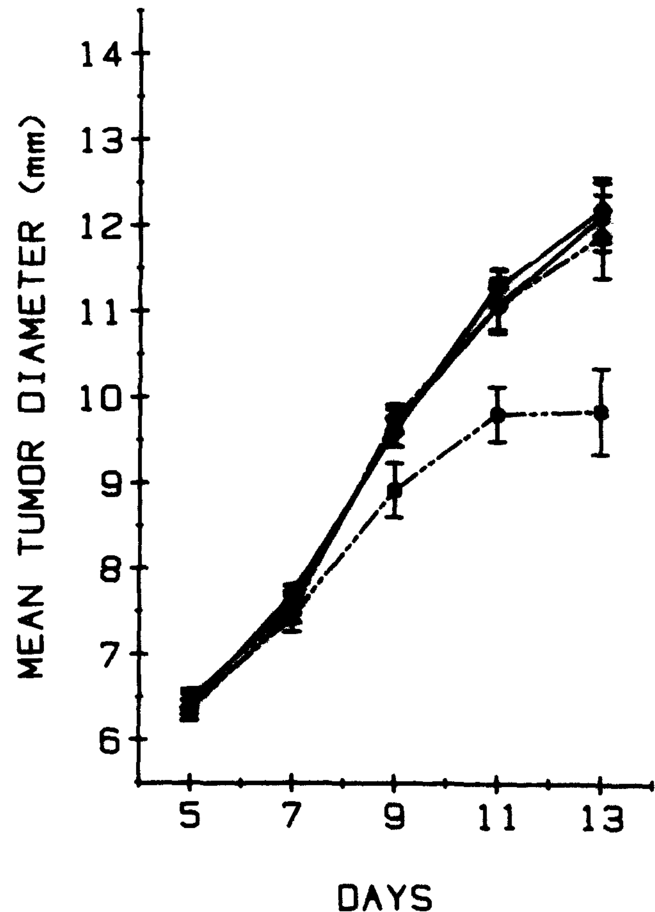

FIG. 2. Mean and SEM of tumor diameter over days for social grouping experiment. All mice were injected with P815 tumor cells. Only non-stressed mice receiving *C. parvum* (——) showed tumor regression. Stressed mice receiving *C. parvum* (●—●) showed the same rate of tumor growth as stressed mice (◆—◆) and non-stressed mice (◆———◆) receiving only P815 cells.

TABLE 1

MEAN (±SEM) DAY OF SURVIVAL AFTER P815 INJECTION AS A FUNCTION OF ACUTE STRESS AND *C. PARVUM*

	C. parvum	No. *C. parvum*
stressed	22.92* ±1.87	20.73* ±1.46
not stressed	32.27 ±2.57	20.25* ±2.42

*Indicates 100% mortality.

hr light/dark cycle was used with light onset at 0700 hours. All experimental procedures occurred between 0900 and 1700 hr.

Tumor Cells

The P815 mastocytoma cells, syngeneic in DBA/2j mice, were obtained from American Type Culture, Rockville, NC. The cells were maintained in culture in RPMI 1640 (Gibco Laboratories, Grand Island, NY) with 10% fetal bovine serum (Gibco Laboratories) and 20 μg/ml gentamycin (Sigma, St. Louis, MO). Prior to tumor transplantation, the tumor cells were passaged intraperitoneally through DBA/2j mice for 12 days. For transplantation, the ascites cells were harvested in RPMI 1640, washed twice with Hank's Balanced Salt Solution (HBSS, Gibco Laboratories), and suspended to an appropriate number in HBSS.

Determination of C. parvum *Dosage*

Twelve-week old male mice were anesthetized with CO_2 and their abdomens were shaved. Immediately, injections of 1.0×10^6 P815 ascites tumor cells in a volume of 0.05 ml PBS were given intradermally in the abdomen. The mice received

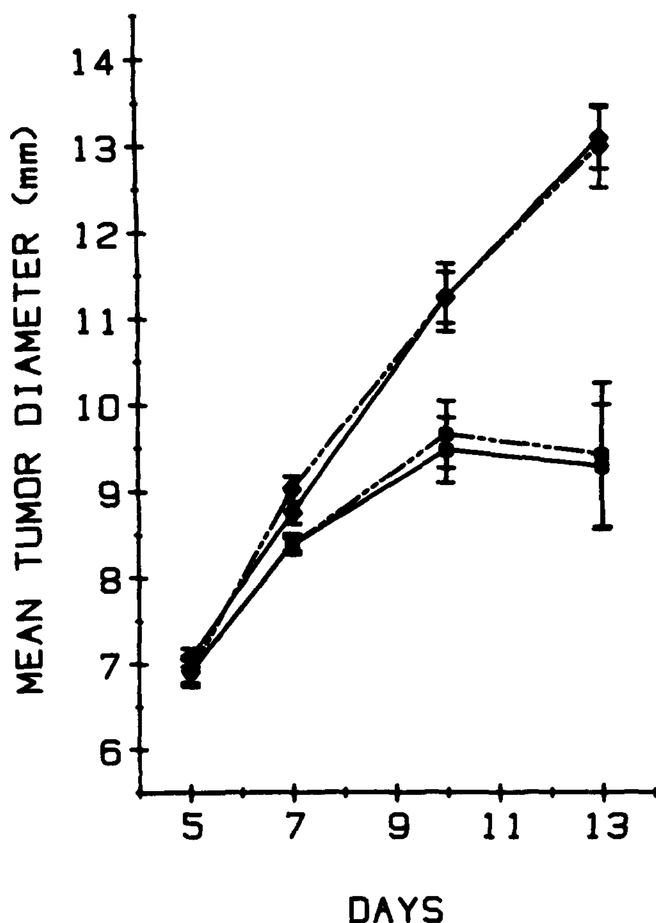

FIG. 3. Mean and SEM of tumor diameter over days for the social isolation experiment. All mice were injected with P815 tumor cells. Both stressed mice (●—●) and non-stressed mice (●------●) receiving *C. parvum* showed tumor regression. Stressed mice (◆—◆) and non-stressed mice (◆------◆) injected only with P815 cells showed typical tumor growth.

either 2 μg, 10 μg, 50 μg, or 100 μg of washed, formalin-killed *Corynebacterium parvum* (Burroughs Wellcome Co., Research Triangle Park, NC) admixed with the tumor cells. Two mice received each dosage. A control animal received only tumor cells. Tumor growth was determined from daily measurement of the average of the longest tumor length and the length perpendicular to it. Measurments were obtained with a dial caliper over a two week period while the mice were lightly anesthetized with CO_2.

Figure 1 displays mean tumor lengths over days for different *C. parvum* dosages. Dosages of 50 and 100 μg produced complete regression of the tumors. The 10 μg dosages produced slower regression, while 1 μg resulted in only partial regression. Because we sought a threshold dosage which would be both sensitive to behavioral manipulations and yet produce some regression, we chose a 2 μg dosage for the subsequent experiments.

Effects of Social Grouping

Forty-eight DBA/2j mice were obtained at eight weeks of age and housed for two weeks in groups of four to alleviate shipping stress. Four treatment conditions were used: P815, P815/stress, P815 + *C. parvum* and P815 + *C. parvum*/stress. On Day One of the experiment, all the mice were injected intradermally with 1.0×10^6 P815 ascites tumor cells in 0.05 ml PBS as described above. Half of the mice had 2 μg of fomalin-killed *C. parvum* admixed with the tumor cells. The mice were returned to their original groups and cages.

On Day Two, half of the mice in each of the P815 and P815 + *C. parvum* treatments were given one hour of intermittent, inescapable, electric footshock. The unscrambled shock averaged 0.5 mA, but varied depending upon the movements of the mice. Observation of the mice showed no signs of escape during sessions. Sixty shocks of six second duration were delivered by a Lafayette constant current shocker at a fixed interval of sixty seconds. Each mouse was shocked individually in a 13×12×27 cm Plexiglas chamber. Three chambers were connected in series. The mice remained in their original cage groups for the remainder of the experiment. All four mice within a cage were given the same treatment.

On Day Five and alternate days thereafter, each mouse was anesthetized with CO_2 and its tumor length calculated as described above. Each cage was checked daily for deaths for forty-five days. Mice living at the end of the experiment were assigned a death date of forty-five days for analyses.

Effects of Social Isolation

Forty-eight DBA/2j mice were obtained at eight weeks of age. The mice were then socially isolated in individual cages for three weeks followed by housing in groups of three for one week prior to the experiment. Such changes in housing conditions have been shown to affect tumorigenesis [14,15]. On Day One, the mice were treated identically to those of the social grouping experiment above. Thus, the mice were acutely stressed with electric shock in the same manner. The same four treatment conditions were given. Mice were housed in groups of three for the experiments unlike the grouping experiment. Cages in which social dominance was established and subordinate mice wounded were discarded and not included in the results. Some initial fighting occurred in most groups, but quickly ended. On Day Five and at two to three day intervals thereafter, tumor sizes were obtained using the procedure above.

RESULTS

Social Grouping

Figure 2 displays the mean tumor lengths over days for the treatment conditions. The P815 + *C. parvum*/stress group did not differ from the P815 and P815/stress groups. Since these groups did not differ, the three groups were combined for analyses. The P815 + *C. parvum* group showed significantly less tumor growth on Day Eleven, $t(45)=2.68$, $p<0.02$, as compared to the combined groups.

The mean days of deaths are shown in Table 1. Longevity was not different between P815, P815/stress and P815 + *C. parvum*/stress groups. These groups were combined for comparison to the P815 + *C. parvum* group. The P815 + *C. parvum* group lived significantly longer than the three combined groups, $t(45)=3.90$, $p<0.001$. Two mice from the P815 + *C. parvum* group were alive 60 days following termination of the experiment. Autopsies of the mice that died during the experiment revealed peritoneal metastases, especially of the liver as found by other investigators [14].

Social Isolation

Figure 3 displays the mean tumor length over days for the four treatment conditions. Social isolation followed by acute stress did not have any effect on tumor growth. The P815 and P815/stress groups did not differ from each other; nor did the P815 + *C. parvum* and P815 + *C. parvum*/stress groups differ from each other. The *C. parvum* did inhibit tumor growth however. By Day Seven, the combined P815 groups had significantly larger tumors than the combined P815 + *C. parvum* groups, $t(21)=3.97$, $p<0.001$. On subsequent days, the tumors grew larger in P815 groups, but not in groups given *C. parvum*.

DISCUSSION

These experiments demonstrated that acute stress within a given social context can reduce the antitumor action of a minimal dose of *C. parvum*. In the context of social grouping, acute stress negated the immunopotentiation affect of *C. parvum* injection. This inhibitory effect of acute stress on the antitumor activity of *C. parvum* treatment was alleviated by prior isolation of the mice. Sklar and Anisman [16] have shown that the growth of P815 mastocytoma tumors in DBA/2j mice can be facilitated by the stress of acute, inescapable electric footshock. Chronic electric shock mitigates this effect as does making acute shock escapable [16]. These effects of electric shock are dependent upon the social stresses of prior housing conditions as in the present study [14]. Unlike Sklar and Anisman, a tumor enhancement *per se* did not occur with the present acute stress procedure as indicated by the control groups. Although Sklar and Anisman had suggested that immunological mechanisms were not important for this stress effect upon P815 growth itself, immunological mechanisms are implicated by the present immunoadjuvant results. While the P815 mastocytoma is syngeneic in DBA/2j mice it is also considered to be, at least weakly, immunogenic [9].

The immune mechanism affected by our stress procedures were not determined. Stress is known to inhibit natural killer cell function [13], but stress had no effect on tumor growth in animals not treated with *C. parvum*. This suggests that natural killer cell function was not affected by the acute stress. Cytolytic T-cell function has been shown to be enhanced by *C. parvum* treatment [8]. There is also evidence that T-cell function is adversely affected by stress [6]. It is possible that the acute stress affected suppressor cell function in the tumor bearing mice. Stress related neurochemicals are known to affect suppressor cell function [10,16] and suppressor cells are known to be generated in animals bearing actively growing, antigenic tumors [2,6]. It is possible that the stress caused the enhancement of suppressor cell function that was normally down-regulated by *C. parvum* treatment. Presumably, the different effects produced by social grouping and isolation were mediated by endocrinological and neural factors associated with those treatments [14].

In summary, we found that behavioral treatments differentially modified the antitumor activity of an immunoadjuvant. This suggests that psychological factors may play an important role in oncogenesis by inhibiting the development of concomitant immunity to an antigenic tumor. Our results also implicate psychological conditions as potentially important in modifying the efficacy of immunotherapies.

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