The Influence of Stress on Murine Leukemia Virus Infection* (32754)

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The induction of malignant transformation of animal cells by viruses is well documented. Often the association between host and tumor virus is of long duration before signs of disease, if ever, are seen. Environmental and hereditary factors have been suggested as influencing the outcome of such host–parasite relationships.

Both clinical and experimental observations have suggested an influence of psychological stress on susceptibility to infectious diseases (1). Our previous studies have shown that animals which are subjected to emotional or nonspecific stress have an altered resistance to various cyto-destructive virus infections (2–4). Extending these studies to tumor viruses, it was found that stress applied late in the course of polyoma infection had little influence on tumor development (5). However, an increased rate of tumorigenesis was noted when stress was applied immediately after inoculation of young mice with polyoma virus (6).

The purpose of the following study was to determine the influence of nonspecific stress on the course of Rauscher murine leukemia virus infection.

Materials and Methods. Virus. The Rauscher virus used in the early experiments was supplied by Dr. F. J. Rauscher as a crude 10% extract of leukemic spleens from BALB/C mice. This preparation induced palpable spleens in our Swiss mice 12–14 days after iv injection of 0.2 ml of a 1:40 dilution. The inoculum used in the later experiments was a 10% cell free extract of leukemic spleens prepared in our laboratory from Swiss mice inoculated with the above virus. A 1:20 dilution of this latter preparation produced results similar to those with Rauscher's material. These dosages were used in all experiments, unless otherwise stated.

Mice. All mice were 6- to 8-week-old females from our inbred colony of Swiss Webster-BRVS strain.

Stressing procedures. Both sound stress (7) and avoidance-learning stress (2) were employed. Sound stress consisted of daily 3-hour exposures to an 800 cps sine wave sound at about 120 db intensity. Avoidance-learning stress was effectuated by a shuttle box apparatus automatically programmed to require the mice to jump a barrier to the opposite side of a compartment once every 5 min on the presentation of a warning buzzer and light. Mice failing to jump would receive an electric shock. Mice soon learned to avoid the electric shock and were thus exposed primarily to the stress of anticipation of pain and fear. Daily 6-hour periods of this stressor were used.

Experiments and Results. Influence of stress on spleen size. In the first series of experiments mice were subjected to about 10-daily periods of sound stress before being injected iv with virus. Following injection, daily stress exposure was continued for 10–17 days before the mice were sacrificed. The whole body and spleen weights were determined, and the spleens were fixed as described by Pluznik and Sachs (8); when possible, the number of spleen colonies were counted. Plasma was also collected for viral assay (9). Equal numbers of nonstressed control mice were always injected and sacrificed at the same time as the stressed mice. The results in 3 experiments (Table I, Expts. 1, 2, and 4) show a significant retardation of splenic enlargement in sound stressed mice. The degree of retardation was not significant in Expt. 3, but the results were in the same direction.

In Expt. 4 an additional group of mice was subjected to avoidance-learning stress (stress-

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TABLE I. The Influence of Stress on Spleen Size in Intact Mice Inoculated with Rauscher Leukemia Virus.[a]

Exp. no.	Group	No. of mice	Days of stress: Before inoculation	Days of stress: After inoculation	Mean spleen wt.[c]	p value
1	Stress (S)	49	10	10	13.5 ± 4.1	.01
	Control	49			17.4 ± 3.7	
2	Stress (S)	42	10	17	18.8 ± 6.3	.01
	Control	43			25.7 ± 7.1	
3	Stress (S)	56	10	15	12.0 ± 4.4	NS
	Control	56			13.1 ± 5.3	
4	Stress (S)	42	9	13	14.5 ± 4.8	.05
	Stress (AL)	48	9	13	12.2 ± 5.0	.01
	Control	42			18.0 ± 6.2	
5	Stress (S)	49	0	10	11.6 ± 2.2	.01
	Control	49			12.8 ± 2.3	
6	Stress (S)	42	10	0[b]	11.2 ± 3.6	NS
	Control	42			11.1 ± 3.3	

[a] Abbr.: (S) = sound stress; (AL) = avoidance-learning stress; NS = not significant at 5% level.

[b] Mice sacrificed 12 days after inoculation.

[c] Expressed as mg of spleen to gm of body wt. ± SD.

AL); a significant retardation in the rate of splenic enlargement was also induced in these mice. Experiments 5 and 6 (Table I), demonstrated the influence of daily sound stress given either before or after virus inoculation. When daily stress was applied only after inoculation (Expt. 5), a significant retardation in spleen size was seen. When stress was given only before inoculation (Expt. 6), no difference in spleen size was induced. Also, when the incubation periods were longer than about 21 days, the spleen sizes became very large, 600 to >2000 mg, and differences between stress and control mice were generally obliterated.

Stress also induced a slight decrease in total body weight. The average weight was 20.9 gm for stressed and 21.3 gm for control mice. This difference was significant at the 5% level in Expts. 1, 2, and 4 and at the 1% level in Expts. 3 and 6 (Table I).

The data were analyzed by means of a two-way analysis of variance; the two factors were treatments and cages. Multiple animals within a cage were treated as replicates. All F tests were against the error mean square.

Role of adrenal glands. As the stress induced hyperactivity of the adrenal glands was the most probable response which could account for the retardation of spleen size, the following experiment was conducted. Mice were first subjected to avoidance-learning stress for 5 days, then adrenalectomized, rested for 1 day, and then injected iv with virus. Stress was continued to 13 days after inoculation at which time the mice were sacrificed. Control mice received the same surgical and inoculation procedures. Intact stressed and control mice were simultaneously tested. No significant difference in spleen size was found between stressed and control adrenalectomized mice, while the difference between the stressed and control intact mice was highly significant (Table II).

Influence of stress on death rate. In the following experiments, death rates were used to measure the influence of stress on infected mice. A 1:250 dilution of our virus preparation, which produced a milder infection compared to the above experiments, was used. Mice were injected after 7 days of avoidance-learning stress and daily stress was continued

TABLE II. The Influence of Stress on Spleen Size in Adrenalectomized Mice Inoculated with Rauscher Leukemia Virus.

Group	No. of mice	Mean spleen wt.[a]	p value[b]
Adrenalectomized Stress	21	13.2 ± 2.9	NS[c]
Control	20	14.1 ± 4.3	
Intact Stress	23	8.3 ± 2.3	.01
Control	24	11.3 ± 3.3	

[a] Expressed as mg spleen to gm of body wt. ± SD.
[b] Compared to own controls.
[c] NS = Not significant at 5% level.

through the experiment. Two such experiments were conducted, each used 49 stress and 49 control mice. The combined results are shown in Fig. 1. The accumulative death rate was slightly but consistently higher in the control groups. Death rates were significantly higher in the control mice of both experiments at the termination time of 290 days (p values were <0.01 and <0.02, respectively).

Influence of stress measured by other parameters. The counting of colony forming units on the infected spleens (8) and the measurement of virus content of the plasma (9) were not found to be useful criteria for measuring differences in the response of stress and control mice to this infection. With both parameters no consistent correlation was found with spleen sizes and wide variations were seen within each group of mice.

Histological sections and smears of the spleens were examined from both stress and control mice. No apparent differences were seen in the types of cells involved.

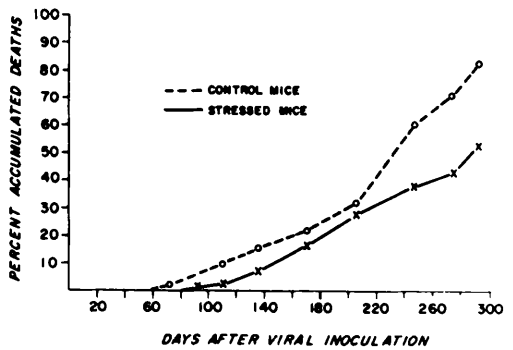

FIG. 1. Death rates in mice inoculated with Rauscher leukemia virus and subjected to daily avoidance-learning stress exposures.

Role of stress in drug induced suppression of leukemic cells. As spleen size is used as a criterion to evaluate chemotherapeutic agents against tumor viruses (9), the question arose as to whether such drugs acted as stressors which might stimulate the pituitary–adrenal axis, which in turn would retard splenomegaly independent of the antitumor action of the drug. To answer this question infected intact and adrenalectomized mice, 14 per group, were treated with anticancer drugs and spleen sizes were measured after 3 weeks. The following 3 compounds (obtained from the

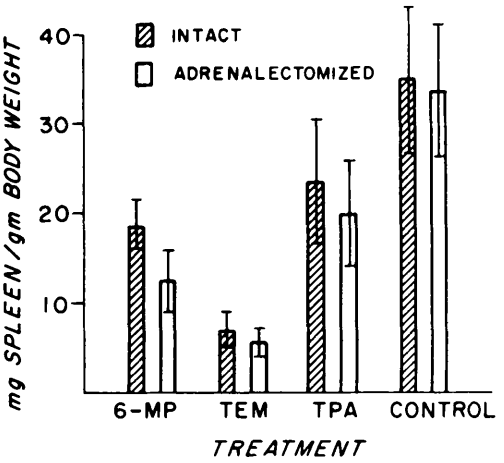

FIG. 2. Spleen size in intact and in adrenalectomized mice infected with Rauscher leukemia virus and treated with antitumor compounds. Abbr. 6-MP = 6-mercaptopurine; TEM = triethylene melamine; TRA = terephthalanilide. Controls received saline. Vertical lines represent standard deviations.

Cancer Chemotherapy National Service Center) were used: (i) 6-mercaptopurine at a dosage of 30 mg/kg per injection, (ii) triethylene melamine at 0.5 mg/kg, and (iii) terephthalanilide (2-chloro-4,4″-di-2-imidazolin-2-ylterephthalanilide) at 50 mg/kg. Drug injections were given subcutaneously every other day through day 16. Noninfected mice were also injected with the drugs and their adrenal weights were determined after 3 weeks. As no significant differences were seen between intact and adrenalectomized mice (Fig. 2), these experiments clearly demon-

strated a lack of involvement of the adrenal glands in drug induced retardation of leukemic spleen size; in fact, the adrenalectomized mice had slightly smaller spleens throughout. No changes were seen in adrenal gland size in the noninfected drug treated mice, thus indicating no stressor effect by these drugs.

Discussion. The host response to Rauscher leukemia virus was shown to be altered by nonspecific or psychological stressors. Infected mice subjected to stress had significantly smaller spleens than nonstressed infected mice when they were sacrificed 10–18 days after viral inoculation. Death rates were likewise slightly lower in stressed mice. Stress applied before the viral injection had no influence on spleen size, while stress given after the virus markedly retarded splenic enlargement. As both avoidance-learning and sound stress produced essentially the same response, the induced changes are assumed to have been mediated by a common nonspecific pathway. The adrenal glands appeared to play a major role in the splenic retardation, presumably as a result of increased glucocorticoids secretion stimulated via the hypothalamic–pituitary ACTH pathway.

Our previous studies on stress and susceptibility demonstrated responses different from the present findings with Rauscher virus. Stress given over several weeks induced an accumulative response (10), which predisposed mice to greater susceptibility to such cyto-destructive viral infections as herpes simplex (2) and Coxsackie B (4). In other experiments using vesicular stomatitis virus (3), stress induced a transitory biphasic response in susceptibility which was related to the daily stress period. Neither the accumulative predisposing nor the transitory biphasic responses were manifested in the presently reported leukemia virus infection, but the influence was on virus induced cellular proliferation which occurred after the initiation of infection.

The influence of stress on host responses to malignant diseases has been demonstrated in varying forms. An early period of reduced resistance followed by a period of increased resistance to Walker 256 cells was noted in rats subjected to the stress of celiotomy, a response not thought to be related to an adrenal response (11). Similarly the stress of early separation of rats from their mother seemed to increase their susceptibility to Walker 256 cells (12). Surgical trauma was shown to increase the carcinogenic effects of methylcholanthrene in mice, while audiogenic or electric shock stress had an opposite effect, i.e., caused a prolongation of the latent period and time of death (13). The retardative effects of emotional stressors, i.e., fear (14) and avoidance-learning (15), have been shown against several types of tumors in experimental animals. On the other hand, Chang and Rasmussen (6) demonstrated a greater incidence of tumorigenesis in stressed polyoma infected mice, with a reduced time to the development of palpable tumors. From these observations it is seen that stress influences the host response to malignant diseases in different ways, depending on the mechanisms involved and the parameters measured.

Our findings with Rauscher murine leukemia infection appear to represent a stress-induced, adrenal mediated, reduction of cellular proliferation during the early erythroid phases of the disease, and might be compared to other anti-inflammatory influences of stress on nonmalignant tissues (16,17). Whether or not the stress had any influence on the induction of the malignant state at the cellular level is not known.

Summary. Nonspecific or psychological stressors were shown to alter the host response to Rauscher leukemia virus. Infected mice subjected to sound or avoidance-learning stress developed significantly smaller spleens than nonstressed infected mice. Stress applied before viral injection had no influence on spleen size, while stress given after the virus markedly retarded splenic enlargement. This phenomenon was not seen in adrenalectomized mice. Death rates were slightly lower in stressed mice. The stress response had no influence on the action of antitumor drugs.

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Friend Disease and a Tumor Variant in Hybrid (BDF_1) Mice* (32755)

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Transplantable reticulum cell sarcoma variants of Friend virus leukemia reportedly have been induced only in inbred mice highly susceptible to Friend virus infection (1,2). However, only tissue from an occasional mouse with terminal Friend disease appeared capable of inducing these tumors. The tumors were readily transplantable in normal mice of the same inbred strain and invariably contained large quantities of virus. The latter has complicated *in vivo* antigenic studies because it rapidly induced fatal leukemia which is unrelated to the development of the tumor. One approach to the problem is to nullify the lethal activity of the virus. This could be accomplished if it were possible to induce a transplantable reticulum cell sarcoma in a host of low susceptibility to Friend disease. Adult BDF_1 mice, the offspring of Friend virus resistant C57BL/6 females and susceptible DBA/2 males, represent such a host because they are highly, but not totally, resistant to Friend disease (3). The unusual course of Friend disease, and the properties of an induced transplantable tumor in BDF_1 mice, are the subjects of this report.

Materials and Methods. The BDF_1 mice, obtained from Simonsen Laboratories, Gilroy, California, were young adult females. The BALB/c mice were 6- to 8-week-old animals of either sex from our own colony.

The strain of Friend virus, the method of preparing pools and the procedure for carrying out neutralization tests were the same as described previously (4). Virus was inoculated intraperitoneally (ip).

For the initial induction of tumors, spleen and liver from a Friend virus infected BDF_1 mouse were individually minced with iris scissors to a brei, suspended in Hanks' BSS and drawn into a 10-ml Luer-Lok syringe. The suspension was then expelled through an 18-gauge needle and the procedure repeated until a "heavy" homogeneous suspension was obtained. Initially BDF_1 mice were inoculated subcutaneously (sc) with 0.2 ml. When tumors appeared, they were removed aseptically and suspended in the same manner as the original tissue. Serial transplantation was carried out by either the sc or ip route.

For quantitative studies tumors were minced, washed 3 times with phosphate buf-

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