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Stressor-Induced Alterations of the Splenic Plaque-Forming Cell Response: Strain Differences and Modification by Propranolol

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KERR, L., L. DRUMMOND, M. ZAHARIA, J. CLELFORD AND H. ANISMAN. *Stressor-induced alterations of the splenic plaque-forming cell response: Strain differences and modification by propranolol.* PHARMACOL BIOCHEM BEHAV **53**(2) 235-241, 1996.—The effects of stressor application on the splenic plaque-forming cell (PFC) response was assessed in two strains of mice: the BALB/cByJ strain, which is highly responsive to stressors; and the more hardy DBA/2J strain. Both strains exhibited a peak PFC response 120 h following administration of sheep red blood cells (SRBC; 5 × 106 cells). Stressor exposure reduced the immune response; however, the appearance of such an outcome was dependent upon the time at which the stressor was applied relative to SRBC inoculation. In DBA/2J mice, foot-shock applied either immediately after SRBC inoculation or at the time of the peak immune response (120 h) resulted in suppression of the PFC response. In BALB/cByJ mice, both stressor severities provoked an immunosuppression when applied 120 h after inoculation, but when applied 96 h after immunization only foot-shock reduced the PFC response. At other intervals, the stressors were without effect. Pretreatment with the β-norepinephrine antagonist propranolol precluded the immunosuppression elicited by a stressor applied 96 h after inoculation, but did not affect the reduction of the PFC response elicited by a stressor applied 120 h after inoculation. It is suggested that several factors may contribute to stressor-provoked alterations of the immune response, and that the contribution of these factors vary over the course of an immune response being mounted.

Stress Norepinephrine SRBC Plaque-forming cell response

STRESSORS may compromise various aspects of immune functioning in infrahuman animals, including splenic and serum natural killer (NK) cell activity (1,41,45), the proliferative response to T- and B-cell mitogens (11,21,24,26), as well as the splenic plaque-forming cell (PFC) response to sheep red blood cells (SRBC), and plasma antibody titers (15,29). Moreover, stressors have been shown to reduce the secretion of various cytokines [e.g., interleukin (IL)-1, IL-2, IL-6] (2,6, 14,32). In contrast to the immunosuppressive effects of relatively intense stressors, mild stressors may enhance the PFC response (8,19), the splenic mitogen response (9,31), NK cell activity, and IL-2 production (44). In addition to stressor severity, the PFC response may be influenced by the timing of stressor application relative to antigen administration. For instance, the peak PFC response (measured 96 h after SRBC administration) was markedly reduced in CD-1 mice that had been exposed to a stressor 72 h following immunization. In contrast, a stressor applied at other times following immunization (0, 24, 48, or 95 h) induced no such effect (46,47). Enhancement of the immune response associated with mild stressors also appear to be dependent on the timing of stressor application relative to antigen exposure. Croiset et al. (9) noted that the immunoenhancing effects of a mild stressor were evident when SRBC was administered within 15 min of

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the stressor; however, when longer periods (1, 4, or 8 h) intervened between the two treatments, the immune enhancement was not evident.

Although profound genetic differences exist across strains of mice and rats with respect to basal hormonal, neurochemical, and immunologic activities, there are few data evaluating the impact of stressors as a function of the genetic variable. It has been demonstrated, for instance, that in contrast to the suppression of the mitogen response typically observed in rats, stressor exposure increased the mitogen response in three of four strains of mice (C3H/HeJ, C57BL/6N, and HLA-SW/ICR) and had no effect on a fourth strain, C3H/HeN (25). The extent of the immunoenhancement was directly related to stressor severity. In a subsequent study using the HLA-SW/ICR strain, it was observed that with repeated stressor exposure an adaptation developed such that the enhanced mitogen response was no longer evident. In a sense, the latter finding is reminiscent of studies conducted in rats, where the immunosuppressive effects of an acute stressor were abated with repeated environmental insults (11,26). It is unclear, however, why acute stressors enhanced the mitogen response in mice, but had an opposite effect in rats (30). With respect to other immune parameters, the effects observed in mice were reminiscent of those seen in rats. In particular, foot-shock stress produced a reduction of NK cytotoxicity in mice, although the time course for this effect varied across several strains (23,45).

In summary, it seems that several variables can be identified that may be fundamental in determining the immunologic alterations provoked by stressors. Of course, the nature of the stressor effect depends on the immune compartment being examined (e.g., in blood vs. lymphoid organs, such as spleen) (12). In addition, it seems likely although while acute stressors tend to provoke immunosuppressant effects, chronic stressors either may have no effect or may enhance the immune response (5). Likewise, there is reason to suppose that the impact of acute stressors will vary with the species or strain being examined, the severity of the insult, and the timing of its application relative to antigenic challenge. The present investigation assessed the contribution of the latter three variables in determining the effect of stressors on the PFC response. To this end, the effects of stressors were assessed in BALB/cByJ mice and in DBA/2J mice. The BALB/cByJ strain of mouse exhibits basal levels and turnover of neurotransmitters and endocrines that are not remarkable. However, mice of this strain appear to be particularly vulnerable to stressor-induced behavioral and neurochemical alterations, and also exhibit marked alterations of plasma corticosterone in response to stressors (4,37–40). Thus, this strain, together with somewhat more hardy animals such as the C57BL/6J or DBA/2J, may serve as an ideal preparation to evaluate the impact of stressors on immune system activity. In addition, because considerable information is already available for these strains concerning corticosterone and central amine variations engendered by stressors, it may be possible to ascertain whether correspondence exists between the immunologic, neurochemical, and adrenocortical alterations engendered by stressors.

EXPERIMENT 1

It will be recalled that there exists in the CD-1 mice a critical period following antigen administration during which a stressor will come to affect the peak splenic PFC response. Inasmuch as this time-dependent effect has as yet only been demonstrated in the CD-1 mouse, it is unclear whether it is in fact unique to this strain. Moreover, if critical periods exist in other strains, they may well differ from that seen in CD-1 mice, particularly because the development of the peak splenic PFC response might be strain dependent. Accordingly, before evaluating the effects of stressor exposure on the PFC response, we conducted a preliminary study in two strains of mice, BALB/cByJ and DBA/2J, to evaluate the time course and magnitude of the PFC response to sheep red blood cells.

Method

Subjects. A total of 60 male mice each of the BALB/cByJ and DBA/2J strain, approximately 6 weeks of age, were obtained from the Jackson Laboratory (Bar Harbor, Maine). Mice were housed by strain in groups of four or five in standard polypropylene cages, and permitted to acclimatize to the laboratory for 4–6 weeks before being used as experimental subjects. Mice were permitted free access to food and water and were maintained on a 12 L : 12 D cycle (light on a 0700–1900 h). Testing procedures were conducted between 0800 and 1200 h.

Immunization and PFC assay. Sheep red blood cells were prepared by washing citrated sheep's blood three times in sterile saline. Animals were immunized intraperitoneally (IP) with 5×106 cells in a volume of approximately 0.18 ml. Determination of the PFC response was made using a slight modification of the method of Cunningham and Szenberg (13). Mice were decapitated and their spleens were removed and dispersed to a single cell suspension in Hank's balanced saline solution (HBSS) medium (Gibco, Burlington, Ont.) supplemented with Hepes buffer (1.0 M solution), gentamycin sulphate (40 mg/ml), and penicillin G (100 U/ml; Sigma Chemical Co., St. Louis, MO). The spleen cells were washed by centrifugation at $400 \times g$ for 10 min and resuspended in 2 ml of HBSS medium. The cells were then layered on a Ficoll-Hypaque gradient (density = 1.1) and centrifuged at $700 \times g$ for 30 min. After centrifugation, the mononuclear cells at the interface were removed, resuspended in 4 ml HBSS, and washed three times at $400 \times g$. We combined 20 μl of the cell suspension (at 107 cells/ml) with 20 ml of guinea pig complement (absorbed with SRBC at a ratio of 2 : 1), 20 ml of HBSS medium, and 20 ml of SRBC (approximately 2.5×108 cells/ml). The suspension was introduced by capillary action into microslides (Canlab, Pointe Claire, Quebec, Canada) with dimensions of $0.22 \times 4 \times 100$ mm at a volume of 80 ml. The ends of the microslides were sealed with a 50% paraplast embedding medium and 50% vaseline mixture. The slides were incubated at 37°C for approximately 15 min. Plaques were counted by microscopic examination of the microslides at a magnification of $\times 10$. Data were expressed as PFC/106 mononuclear cells.

Procedure. Mice of each strain were individually housed and assigned to six treatment conditions. Mice were inoculated IP with SRBC (5×106 cells) and at various times thereafter (1, 2, 3, 4, 5, and 6 days). They were then decapitated and their spleens were taken for determination of the PFC response.

Results and Discussion

Figure 1 depicts the PFC responses of the BALB/cByJ and DBA/2J mouse strains at various times following SRBC administration. Analysis of variance (ANOVA) confirmed that the splenic PFC response varied as a function of the Strain $\times$ Day interaction [$F(5, 107) = 4.782$, $p < 0.01$]. Newman-Keuls multiple comparison ($\alpha = 0.05$) of the simple main effects indicated that the immune response in both

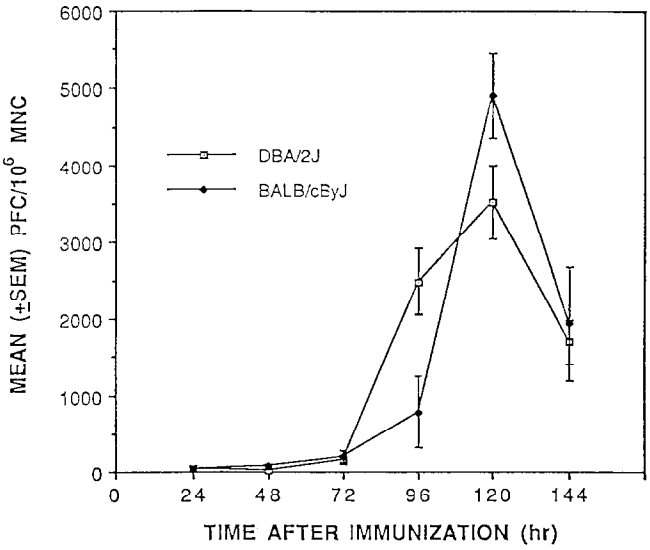

FIG. 1. Mean (±SEM) splenic plaque-forming cell (PFC) response of BALB/cByJ and DBA/2J mice at various times (24, 48, 72, 96, 120, or 144 h) following intraperitoneal administration of SRBC (5 × 106 cells).

strains of mice peaked 5 days following SRBC inoculation. In the DBA/2J mice the PFC response became apparent earlier than in BALB/cByJ mice, such that 4 days after inoculation the PFC response was significantly greater in the former strain. However, at the time of the peak immune response (5 days), the PFC response in BALB/cByJ mice exceeded that of DBA/2J.

EXPERIMENT 2

Experiment 2 was conducted to evaluate the effects of stressor application on the peak immune response in the BALB/cByJ and DBA/2J strains of mice. Given that the immune response may depend on stressor severity, the effects of two levels of stress were assessed (foot-shock of 150 μA and novel apparatus exposure) at various times following SRBC inoculation.

Method

Subjects and apparatus. A total of 130 mice each of the BALB/cByJ and DBA/2J strains were employed. The subject characteristics and housing procedures were the same as those of Experiment 1. Five identical black Plexiglas chambers, which measured 30 × 14 × 15 cm, were employed to deliver inescapable foot-shock. The chamber floors consisted of 0.32-cm stainless-steel rods spaced 1.0 cm apart (center to center) and were connected in series by neon bulbs. In addition, the end walls of the chambers were lined with stainless-steel plates that were connected in series with the grid floor. The lids of each box consisted of red translucent Plexiglas providing limited illumination. Foot-shock (150 or 300 mA, 60 Hz, AC) was delivered through a 3000-V source, thereby providing a relatively constant current.

Procedure. Mice of each strain received IP SRBC (5 × 106) injection as described in Experiment 1. Independent groups of each strain were exposed to either foot-shock or a novel apparatus (n = 10/group) either immediately (0), 1, 2, 3, 4, or 5 days (119 h) after inoculation. Mice were individually placed in the shock apparatus for a 1.1-h period. During this time, mice of one group received 360 shocks of 150 μA (2-s duration at 9-s intervals), whereas mice of the second group were placed in the shock boxes (novel environment), but did not receive the foot-shock treatment. An additional group of mice of each strain were not exposed to the apparatus and served as an external control condition. At 120 h following inoculation, which was the time of the peak immune response in both strains, mice were decapitated and their spleens were collected for the PFC assay as described earlier. An additional study was conducted to confirm our previous finding (39) that stressor exposure provoked a greater increase of plasma corticosterone in BALB/cByJ than in DBA/2J mice. Accordingly, mice of each strain received either foot-shock, as described earlier, or no treatment (n = 7/group). Immediately thereafter, mice were decapitated and trunk blood was collected. Corticosterone concentrations were determined by radioimmunoassay using kits obtained from ICN Biomedicals (Costa Mesa, CA).

Results and Discussion

A 2 (strain) × 2 (stressor) × 6 (days) between-groups ANOVA was performed to assess the effects of the stressor treatments applied at various times following SRBC inoculation. Dunnett's tests were used to compare specific treatments to the outside control groups (i.e., animals that received none of the stressor treatments). To compare the relative effects of the stressor treatments in the two strains of mice, a separate analysis of variance was conducted comparing individual scores as a proportion of the mean nonstressed PFC scores for each of the strains.

The mean PFC scores for the BALB/cByJ and DBA/2J as a function of the stressor condition are shown in Fig. 2. ANOVA of the PFC scores among nonstressed BALB/cByJ and DBA/2J mice revealed that the two groups did not differ from one another, although as in Experiment 1, the PFC values were somewhat higher in the BALB/cByJ mice. Analysis of the PFC scores revealed a significant main effect of the day of treatment [$F(5, 337) = 3.13$, $p < 0.05$]. Newman-Keuls multiple comparisons ($\alpha = 0.05$) revealed that this main effect was attributable to the PFC scores of mice stressed either 96 or 120 h after inoculation being significantly lower than those of mice exposed to a stressor 48 h after inoculation.

Dunnett's tests revealed that in the BALB/cByJ mice, foot-shock applied either 96 or 120 h after inoculation provoked a significant reduction of the PFC response relative to nonstressed control mice. Moreover, when applied 120 h after inoculation, the mild stressor of apparatus exposure reduced the PFC response relative to that of nonstressed mice. Neither apparatus exposure nor foot-shock administered at other times influenced the PFC response. In contrast to the effects seen in the BALB/cByJ mice, in the DBA/2J strain, foot-shock applied immediately or 120 h after inoculation reduced the PFC response relative to nonstressed mice.

The ANOVA of PFC scores as a proportion of the control (unhandled, nonstressed) scores revealed that the change in PFC scores induced by the stressor in BALB/cByJ mice was more pronounced than it was in DBA/2J mice [$F(1, 337) = 4.56$, $p < 0.05$]. Moreover, the change in the PFC scores relative to control values were more pronounced at 96 and 120 h after inoculation than at other intervals [$F(5, 337) = 3.12$, $p < 0.05$].

Analysis of the plasma corticosterone concentrations con-

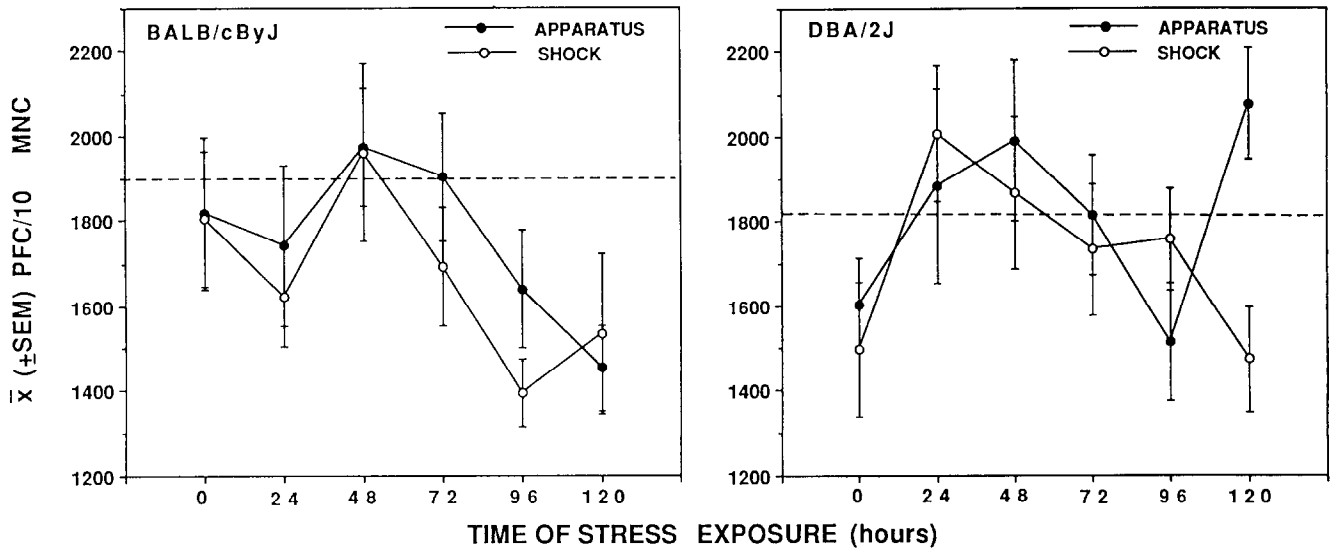

FIG. 2. Mean (±SEM) splenic plaque-forming cell (PFC) responses in BALB/cByJ (left panel) and DBA/2 J (right panel) mice that received either a single session of apparatus exposure or 150 mA foot-shock at various times following (immediately, or at 24, 48, 72, 96, or 120 h) SRBC (5 × 106) administration. The horizontal dashed lines represent the PFC response of mice of each strain that had not been exposed to a stressor. All animals had their spleens removed 120 h after antigen inoculation.

firmed our earlier reports showing that the effects of stressor exposure on corticosterone concentrations varied between the two strains of mice [$F(1, 24) = 4.33$, $p < 0.05$]. Multiple comparisons indicated that in both strains the stressor treatment increased corticosterone concentrations ($x \pm$ SEM = 3.59 ± 0.40 μg/dl and 37.56 ± 5.51 μg/dl in nonshocked and shocked BALB/cByJ mice; 3.65 ± 0.46 μg/dl and 24.21 ± 3.28 μg/dl in nonshocked and shocked DBA/2J mice). Moreover, following shock treatment, the corticosterone levels were higher in the BALB/cByJ than in similarly treated DBA/2J mice.

EXPERIMENT 3

The effects of a stressor on immune activity in different strains of mice, or at various times following immunization, need not involve the same mechanisms. For example, stressor exposure has been shown to influence activity within the interleukin 1 (IL-1) and corticotropin releasing factor (CRF) interface (36). Following antigenic challenge, central IL-1 excites CRF release from the paraventricular nucleus, which in turn provokes both the secretion of pituitary adrenocorticotropic hormone (ACTH), as well as sympathetic innervation of splenic lymphocytes (14,16,17,36). Accordingly, changes in immune functioning associated with stressors may stem from either CRF effects on pituitary, and hence adrenocortical functioning, or sympathetic innervation of the spleen. Likewise, the influence of catecholamines on immune functioning may vary over time following inoculation or across strains of mice. Given that the BALB/cByJ strain is particularly vulnerable to stressor effects, exhibiting marked increases in plasma corticosterone, as well as central transmitter alterations (38–40), any number of mechanisms may be responsible for the greater immunosuppressant effects of the stressor experience in this strain. Experiment 3 was conducted to assess the impact of norepinephrine (NE) receptor blockade by propranolol on the stressor-provoked alterations of the PFC response in BALB/cByJ mice stressed either 4 or 5 days following SRBC inoculation. Inasmuch as only BALB/cByJ mice exhibited suppression of the PFC response on both days 4 and 5 following SRBC inoculation, only this strain was assessed in Experiment 3.

Methods

Subjects and apparatus. A total of 160 mice of the BALB/cByJ strain served as subjects. The subject characteristics and the apparatus were the same as those of Experiments 1 and 2.

Procedure. Experiment 3 was conducted as two independent replications. Mice received IP administration of SRBC (5 × 106) cells, and either 4 days (96 h) or 5 days (119 h) later (times at which stressor exposure provoked a suppression of the peak splenic PFC response) exposed to either 150 mA foot-shock or no treatment as described in Experiment 2. Then, 20 min before the shock or no treatment, mice received IP injection of either DL-propranolol hydrochloride (10 mg/kg in a volume of 10 ml/kg) or an equivalent volume of saline. Spleens were taken at 120 h following inoculation as described in Experiment 1 and 2.

Results and Discussion

The mean PFC response of the BALB/cByJ strain across days, stressor, and drug treatments is shown in Fig. 3. Both replications of the experiment yielded comparable results. The ANOVA confirmed that the PFC response varied as a function of the Drug × Stressor treatment interaction [$F(1, 112) = 8.69$, $p < 0.01$]. Newman-Keuls multiple comparison ($\alpha = 0.05$) of the simple effects comprising this interaction confirmed the presence of a shock-induced immunosuppression in saline-treated animals. Moreover, these comparisons demonstrated that propranolol alone (in the absence of any stressor treatment) provoked a suppression of the immune response, compared with saline-treated controls. As a result, the

propranolol-treated mice that were exposed to the stressor did not differ from saline animals that were likewise stressed. Although the Shock Treatment × Days × Drug Treatment interaction was not significant, Newman-Keuls multiple comparison of the simple effects for this interaction were conducted, because an a priori prediction had been made concerning specific comparisons between the stressor treatment and the timing of this treatment. These comparisons confirmed that a stressor applied at either 4 or 5 days after inoculation produced an immunosuppression relative to nonstressed, saline-treated mice. In addition, these comparisons revealed that 4 days after SRBC inoculation, treatment with propranolol reduced the effects of the stressor. In contrast, as seen in Fig. 3, when the treatments were applied at the 5-day interval, propranolol did not antagonize the effects of the stressor.

GENERAL DISCUSSION

The results of the present investigation confirmed that genetic variability exists in the development of the immune response mounted to SRBC. In contrast to CD-1 mice, where the peak immune response occurred 4 days after SRBC inoculation (46), in both the BALB/cByJ and the DBA/2J inbred strains the peak splenic immune response occurred 5 days after inoculation. Furthermore, the course of the immune response in the latter two strains could be distinguished from one another. Whereas the emergence of the T-cell-dependent immune response occurred earlier in DBA/2J than in BALB/cByJ mice, the peak response ultimately attained was greater in the BALB/cByJ strain.

Commensurate with earlier reports (46,47), foot-shock elicited a reduction of the peak splenic PFC response to SRBC. Zalcman et al. (46) reported that the reduced PFC response was only apparent when shock was applied 72 h after inoculation of CD-1 mice (for which the peak immune response occurs 96 h after inoculation). The present results, however,

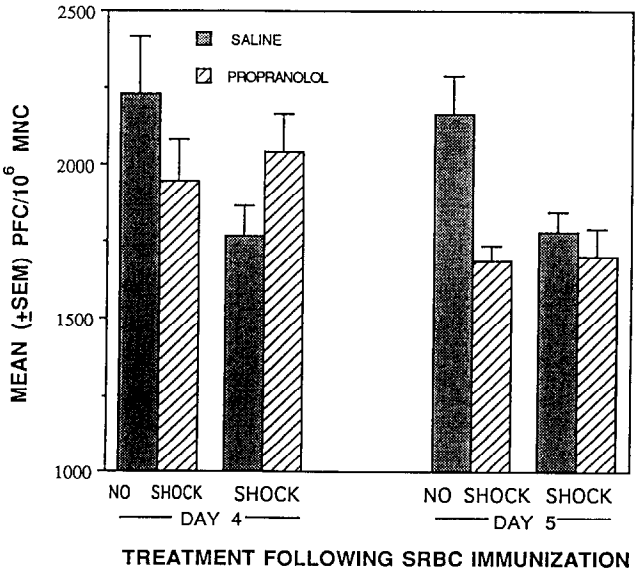

FIG. 3. Mean (±SEM) splenic PFC responses in BALB/cByJ mice as a function of the drug (propranolol vs. saline) and stressor (150 μA shock vs. no shock) treatment. These treatments were applied either 4 or 5 days (119 h) after SRBC (5 × 106) injection. All mice had spleens removed 120 h after SRBC administration.

suggest that the window for the stressor-induced immunosuppression is greater than that reported by Zalcman et al. (46), although this effect was strain dependent. In the BALB/cByJ strain, a single session of foot-shock (150 μA) applied either 96 or 120 h following antigen administration elicited a significant immunosuppression relative to nonstressed animals. Novel apparatus exposure 96 h after SRBC inoculation provoked a modest, nonsignificant immunosuppression in this strain, whereas this manipulation applied 120 h following SRBC inoculation (just before the peak PFC response) provoked a significant suppression of the PFC response. Stressor exposure at other times did not influence the magnitude of the peak immune response in the BALB/cByJ strain.

The stressor-induced immune alterations in DBA/2J were modest relative to those seen in the BALB/cByJ strain. Novel apparatus exposure was without effect, whereas foot-shock applied immediately or 120 h after inoculation reduced the immune response. The DBA/2J strain has generally been shown to be more resilient to stressor effects than the BALB/cByJ mice, exhibiting less profound behavioral impairments, and smaller alterations of neuroendocrine functioning after acute stressor exposure (3,37,38,42). Thus, the limited stressor effects on the immune response of this particular strain may provisionally be attributed to moderate stressor responsivity of DBA/2J mice.

Contrary to the immunosuppressive effects of severe stressors, mild stressors have been shown to elicit an immunoenhancement (19,25). Fujiwara and Orita (19) suggested that mild stressors provoke epinephrine-stimulated T-cell activation, thus leading to an immunoenhancement, whereas severe stressors lead to immunosuppression as a result of the involvement of alternative systems, such as pituitary-adrenal activation. In the present investigation a mild stressor (apparatus exposure) did not lead to an increase of the PFC response in either strain, irrespective of the timing of stressor application. To the contrary, exposure to the mild stressor just before spleens were taken resulted in a reduction of the PFC response in BALB/cByJ mice. Several investigators (9,11) demonstrated differential immunoreactivity to mild environmental challenges, which were dependent upon organismic and experiential factors. Thus, although an immunoenhancement was not apparent in the present investigation, it is premature to assume that such an outcome would not be evident under appropriate conditions (e.g., using different stressor parameters, the backdrop upon which the stressor was applied, and the particular aspect of the immune response monitored).

A simple explanation for the time- and severity-dependent effects of the stressor treatments is not readily available, at least not one that accounts for the data using a single mechanism. There is, however, no a priori reason to believe that only a single mechanism is operative in determining the impact of stressors on the PFC response. The processes involved in generating the immune response may vary over time following activation of the immune system, and these developmental processes may be differentially influenced by stressors applied at various times over the course of the immune response being mounted. Both Sanders and Munson (35) and Roszman and Carlson (34) indicated that norepinephrine receptors present on the lymphocyte cell surface are particularly amenable to neurotransmitter manipulations, but that the effectiveness of catecholamine stimulant or blocking agents varied over time following immunologic challenge, as well as the specific immune response examined (e.g., T- or B-cell-dependent immune response; mitogen or PFC assays) and the specific dose

of agonist or antagonist employed (18,34,35). Given the varied neuroendocrine and neurochemical effects that occur after exposure to an environmental insult, it would not be surprising to observe similar time-dependent variations on immune activity.

In support of a role for NE in subserving the effects of stressors, it has been demonstrated that the foot-shock-induced suppression of the splenic PFC responses, as well as the mitogen-provoked proliferative response, involved sympathetic nervous system activation (43). Likewise, administration of propranolol to rats 30 min before foot-shock protected against the stressor-induced suppression of cell proliferation in response to a T-cell mitogen (ConA) (12). Interestingly, cold stress has also been reported to provoke enhancement of SRBC-specific murine IgM production by splenocytes, and 4 consecutive days of treatment with propranolol augmented this effect (7). These studies suggest that β-NE receptor antagonism may act against the immunosuppressive consequences of stressors or augment the immunoenhancing actions of some forms of environmental insult. In accordance with the effects of propranolol on cell proliferation in response to a mitogen, in the present investigation propranolol attenuated the foot-shock-induced suppression of the PFC response induced by stress applied 4 days after SRBC injection. Interestingly, propranolol administered 5 days after SRBC inoculation (just before the time of the peak immune response) did not prevent the immunosuppression observed after foot-shock. Thus, it is tempting to speculate that whereas β-NE stimulation might have a protective role in the immunosuppression provoked by a stressor, the effectiveness of this manipulation was dependent on the time of its of administration relative to SRBC inoculation. Yet, it ought to be underscored that in the absence of foot-shock, propranolol administered on day 5 reduced the PFC response to a greater extent than it did on day 4. Thus, it is conceivable that the inability of propranolol to reverse the effects of the stressor on day 5 stemmed from the marked immunosuppressive action induced by the NE-receptor antagonist at that time.

The mechanisms that might be operative in accounting for the immunosuppression induced by stressors applied 5 days after inoculation remain to be determined. Certainly pituitary-derived hormones, such as ACTH, influence immune functioning (10) as do glucocorticoids (20,27), presumably by binding to ligand-specific receptors on lymphocytes, thereby causing an intracellular increase of second messengers. Inasmuch as stressful events reliably influence these hormones, it is certainly conceivable that they may subserve the stressor-provoked immune alterations. However, it should be noted that the specific second messenger produced depends on the timing of the increased ACTH levels with respect to the development of the immune response (33). Thus, the effects of ACTH on immune functioning may vary over the course of an immune response being mounted (22). Although we have suggested previously that ACTH and glucocorticoids likely do not account for the immunosuppression induced by a stressor applied 24 h before spleens are taken, corticosterone release, which is particularly pronounced in the BALB/cByJ strain, ought to be considered a possible candidate for the immunosuppressive action of stressors applied immediately before spleens are taken.

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