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Acute and Chronic Stressor Effects on the Antibody Response to Sheep Red Blood Cells

STEVE ZALCMAN* AND HYMIE ANISMAN†[1]

**Institute for Cell Biology, Winnipeg, Manitoba, and †Department of Psychology, Carleton University, Ottawa, Ontario K1S 5B6, Canada*

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ZALCMAN, S. AND H. ANISMAN. *Acute and chronic stressor effects on the antibody response to sheep red blood cells.* PHARMACOL BIOCHEM BEHAV 46(2) 445–452, 1993.—Exposure to inescapable foot-shock 72 h following immunization with sheep red blood cells resulted in a marked suppression of the peak splenic immunoglobulin (Ig)M plaque-forming cell response and plasma antibody titers in CD-1 mice. However, the nature of this effect was influenced by the animal's stressor history. In particular, if mice were initially exposed to a single stressor session immediately or 24 h following antigen treatment subsequent reexposure to the stressor (72 h following inoculation) did not provoke the immunosuppression. Moreover, reexposure to the stressor-related cues elicited a marked immunoenhancement. In contrast, if animals were exposed to a single stressor session 48 h prior to inoculation then later reexposure to the stressor-related cues provoked an immunosuppression. Among mice that had been exposed to a repeated stressor regimen on successive days prior to inoculation, the immunosuppression ordinarily elicited by an acute stressor was absent. Indeed, chronic stressor exposure typically favored potentiation of the immune response. However, the immunofacilitation elicited by the chronic stressor treatment likely was unrelated to the immunoenhancing effects of pairing a stressor with antigenic challenge.

Immunity Stress Adaptation Neuroimmunomodulation

THE impact of environmental stressors on immunocompetence appears to be dependent upon, among other things, the characteristics of the stressor regimen employed. For instance, acute exposure to stressors such as foot-shock or restraint reduce natural killer (NK) cell activity (4,23,28,32), cellular proliferation in response to a mitogen (12,18), splenic cytokine secretion (29,31), and the antibody response to T-cell-dependent antigens (13,22,33). In contrast, repeated exposure to aversive stimulation does not result in a diminished immune response (10,11,14) and may, in fact, provoke a significant immunoenhancement (21).

In addition to the stressor regimen employed, it appears that the timing of a stressor relative to antigen administration may influence some aspects of the immune response. In particular, in CD-1 mice a stressor applied immediately, 24, 48, or 95 h following inoculation with sheep red blood cells (SRBCs) did not influence either plasma antibody titers or the splenic immunoglobulin (Ig)M plaque-forming cell (PFC) response measured 96 h after inoculation. However, if the stressor was applied 72 h after inoculation a marked suppression of these responses was evident (33). This effect appeared to be subject to conditioning-like effects in that reexposure to cues that had been paired with a stressor 2 weeks prior to inoculation elicited an immunosuppression (34). A different effect was observed, however, if the initial stressor session was applied immediately after inoculation. In particular, in animals that had been exposed to the stressor on the day of inoculation subsequent reexposure to stressor-related apparatus cues at the 72-h interval provoked an immunoenhancement. Moreover, among mice that had initially been exposed to the stressor at the time of inoculation subsequent exposure to the stressor at the 72-h interval did not lead to the immunosuppression that was otherwise observed (34). Along the same lines, it has been reported that restraint on 2 successive days prior to immunization resulted in a suppression of the PFC response, whereas no such effect was apparent if restraint was applied on 2 days following immunization (23). Moreover, Esterling and Rabin (7) reported an adrenal-independent reduction of the PFC response in mice exposed to rotation stress on 3 consecutive days commencing 1 day following SRBC inoculation. Curiously, the PFC response was unaffected when the stressor regimen was begun soon after immunization. The latter findings, like those of Zalcman et al. (34), suggest that a stressor applied in close temporal proximity with antigen administration may limit the immunosuppressive effects of a subsequently applied stressor. On the basis of these findings, it was suggested that when a stressor was applied in close temporal congruity with an antigen a compensatory immunologic response was engendered that was subject to conditioning processes. This compensatory response effectively increased the

[1] To whom requests for reprints should be addressed.

immune response upon exposure to stress-related cues and served to antagonize the immunosuppression that would ordinarily be elicited by a stressor applied 72 h after inoculation (34).

In view of the finding that a stressor applied soon after antigen administration proactively influenced the response to a subsequently applied stressor (34), the possibility was considered that the adaptation associated with a repeated stressor regimen was related to such processes. In effect, it is conceivable that a chronic stressor results in an abatement of the immunosuppression owing to the development of compensatory immunologic changes, rather than the provocation of a genuine adaptation associated with repeated stressor exposure. The present series of experiments assessed in greater detail the impact of acute and chronic stressor application on plasma antibody titers and the splenic PFC response elicited by SRBC inoculation. In particular, it was determined whether the proactive effects of a chronic stressor, like those of acute insults, were related to the timing of stressor application relative to antigen administration.

EXPERIMENTS 1A AND 1B

As already indicated, the proactive influence of stressors on immune activity may be dependent upon the time at which the stressor was applied relative to antigen administration. In contrast to the immunosuppression elicited by cues that had been associated with a stressor 2 weeks prior to SRBC inoculation, presentation of cues that had been associated with a stressor that had been applied soon after immunization provoked a marked immunoenhancement (34). Experiment 1A was conducted to assess in further detail the relationship between the timing of acute stressor application (relative to antigen administration) and the subsequent PFC and antibody responses engendered by reexposure to the stressor or stressor-related cues. Moreover, because the effects of a stressor on the PFC response is influenced by prior application of an acute stressor at the time of antigen administration (34) a second study (Experiment 1B) assessed whether such an effect would likewise be evident in mice that were exposed to the stressor on each day following SRBC inoculation.

METHOD

Subjects

A total of 163 male, CD-1 mice, 3 months of age (Charles River Canada, Inc., St. Constant, Quebec), served as subjects in Experiment 1A, while Experiment 1B involved 25 naive CD-1 mice. Animals were acclimatized to laboratory for 2 weeks before being used as experimental subjects. Mice were housed in groups of five in standard polypropylene cages, maintained on a 0700–1900 h light/dark cycle, and permitted ad lib access to food and water.

Immunization

Sheep red blood cells were prepared by washing citrated sheep's blood (Woodlyn Laboratories Ltd., Guelph, Ontario) three times in sterile saline. Animals were immunized intraperitoneally with 10^6 cells in a volume of approximately 0.15 ml. It was previously observed (34) that this dosage resulted in a PFC response such that experimental manipulations could provoke either a decrease or increase of the response without the problem of ceiling or floor effects precluding such outcomes.

PFC Assay

Determination of the IgM PFC response was made using a slight modification of the method of Cunningham and Szenberg (6). Mice were decapitated, spleens removed, and dispersed to a single cell suspension in RPMI medium (Grand Island Biological) supplemented with Hepes buffer (1.0 M solution), gentamycin sulphate (40 mg/ml), and streptomycin G (5,000 U/ml; John's Scientific). The spleen cells were washed by centrifugation at 400 × *g* for 10 min and resuspended in 2 ml medium. The cells were then layered on a Ficoll-Hypaque gradient (density = 1.1) and centrifuged at 700 × *g* for 30 min. After centrifugation, the mononuclear cells at the interface were removed, resuspended in 5 ml RPMI, and washed three times at 400 × *g*. Twenty microliters of the cell suspension (at 10^7 cells/ml) were combined with 20 μl SRBCs (approximately 2.5×10^8 cells/ml) and 20 μl guinea pig complement (absorbed with SRBCs at a ratio of 2 : 1). The suspension was introduced by capillary action into microslides (Canlab, Pointe Claire, Quebec) with dimensions of 0.22 × 4 × 100 mm at a volume of 80 μl. 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 1 h. Plaques were counted by microscopic examination of the microslides (in duplicate) at a magnification of 10×. Data were expressed as PFC/10^6 mononuclear cells.

Determination of Antibody Titer

Mice were decapitated, trunk blood collected, and allowed to clot. Samples were subsequently centrifuged at 400 × *g* for 10 min and the supernatant collected and spun at 600 × *g*. Serum complement was then inactivated at 56°C for 30 min. Twofold serial dilutions of inactivated serum, saline, and a 1% SRBC solution were then made in glass microwells. The highest dilution at which aggregation of SRBCs was still evident was considered to be the antibody titer and expressed in $\log_2$ units.

Apparatus

Five identical black Plexiglas chambers, measuring 30 × 14 × 15 cm, were employed to deliver inescapable footshock. 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 μA, 60 Hz, AC) was delivered through a 3,000-V source, thereby providing a relatively constant current.

Procedure

Mice of Experiment 1A were individually housed and assigned to one of five treatment conditions. Mice of each group (n = 8–12/group) were inoculated IP with SRBCs (10^6 cells) at various times relative to stressor application, that is, the stressor was applied either 24 or 48 h prior to, immediately after, or 24 after immunization. An additional group of animals was not exposed to the stressor. The stressor session consisted of mice being individually placed in the shock apparatus for a 1.1-h period, during which they received 360 footshocks [150 μA, 2-s duration, 9-s intertrial interval (ITI)]. Animals in the nonshocked group were handled but were not

placed in the shock apparatus. Immediately after the stressor session, mice were returned to their home cages. Each of the groups was subdivided into three treatment conditions such that 72 h after SRBC treatment mice received either foot-shock (360 shocks of 150 μA, 2-s duration at intervals of 9 s), reexposure to the apparatus in which shock had been previously delivered (1.1 h), or no treatment. Animals were sacrificed 24 h after the reexposure treatment (i.e., 96 h after immunization). Spleens were taken for subsequent determinations of the PFC responses and trunk blood was collected for evaluations of serum antibody titers.

In Experiment 1B, mice were immunized with SRBCs (10^6 cells). Independent groups of mice then received either a) 360 foot-shocks applied over a 1.1-h period (150 μA, 2-s duration, 9-s ITI) commencing 72 h after immunization (n = 9), b) 360 foot-shocks (150 μA, 2-s duration, 9-s ITI) on five occasions (0, 24, 48, 72, and 95 h after immunization; n = 8), or c) left undisturbed in their home cages (n = 8). Animals were sacrificed 96 h after inoculation, whereupon spleens were taken for subsequent determinations of the PFC responses and trunk blood collected for evaluations of serum antibody titers.

RESULTS AND DISCUSSION

In this and each of the ensuing experiments, the stressor treatments were not found to influence the number of mononuclear cells. Several samples were lost during the course of the experiment, and hence the degrees of freedom for the two variables differed in Experiment 1A, as well as in the ensuing experiments. The PFC response for each of the treatment groups is shown in Fig. 1. Analysis of variance (ANOVA) revealed that the PFC scores varied as a function of the initial stressor treatment × reexposure treatment interaction, $F(8, 148) = 3.96$, $p < 0.01$. Subsequent Newman-Keuls multiple comparisons ($\alpha = 0.05$) of the simple effects comprising this interaction revealed that among mice that had initially not been exposed to the stressor subsequent application of foot-shock 72 h after inoculation provoked a significant suppression of the PFC response. In contrast, in animals that received a single session of foot-shock within 48 h of inoculation, but received no further stressor treatment, the PFC scores were comparable to those of nonstressed mice. These data confirm our previous findings (33) that a critical period existed after SRBC immunization in CD-1 mice (i.e., 72 h) during which a stressor was most likely to induce an immunosuppression.

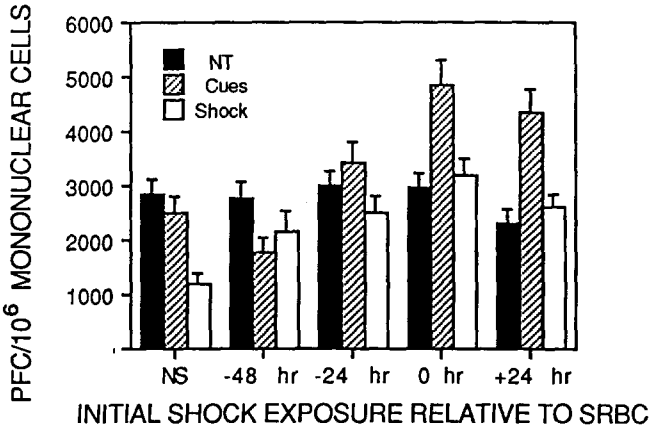

FIG. 1. Mean (±SEM) splenic immunoglobulin (Ig)M plaque-forming cell (PFC) responses as a function of the timing of the initial stressor treatment relative to sheep red blood cell (SRBC) immunization and the reexposure treatment applied 72 h after SRBC immunization (i.e., no treatment, exposure to shock-related cues, or reexposure to foot-shock).

The effects of reexposure to the stressor or stressor-related cues was dependent upon the time at which the initial stressor was applied. In particular, if the first stressor session was applied either immediately or 24 h after inoculation then the immunosuppression ordinarily induced by shock applied 72 h after immunization was prevented. Likewise, if the stressor was initially administered 24 or 48 h prior to inoculation the suppression of the immune response engendered by a subsequent stressor was largely attenuated, although the PFC response remained 20–25% below that of nonstressed animals. The time-dependent effects of apparatus reexposure in previously stressed animals were considerably more pronounced than that seen after reexposure to the initial stressor. In particular, as previously observed (34), in animals initially exposed to the stressor immediately after inoculation subsequent exposure to the stress apparatus (without shock being delivered) led to a significant enhancement of the PFC response. It was similarly observed that if the stressor was initially applied 24 h after antigen administration then subsequent exposure to the apparatus cues provoked a significant immunoenhancement. No such effect was evident, however, if the initial stressor session occurred 24 h prior to SRBC inoculation, and a significant reduction of the immune response was engendered by reexposure to apparatus cues associated with a stressor applied 48 h prior to immunization.

The antibody titers for each of the groups of Experiment 1A, shown in Table 1, paralleled the PFC scores. ANOVA revealed that the antibody titers varied as a function of the Initial stressor treatment × reexposure treatment interaction, $F(8, 136) = 3.16$, $p < 0.05$. As in the case of the PFC response, the Newman-Keuls multiple comparisons of the simple effects comprising the interaction confirmed that in previously nonstressed animals exposure to foot-shock 72 h after inoculation significantly reduced the antibody titers. Exposure to the shock apparatus alone, however, was without effect. In mice that had been exposed to the foot-shock treatment immediately or 24 h following inoculation, a treatment that did not affect antibody titers itself, later reexposure to the stressor-related cues provoked a significant increase of antibody titers. Moreover, upon reexposure to the stressor antibody titers were not reduced relative to animals that had not initially been exposed to the stressor. In contrast to these findings, if the stressor was applied 48 h prior to inoculation then reexposure to the stressor-related cues provoked a significant reduction of antibody titers. Finally, if the initial stressor was applied 24 h following inoculation then neither reexposure to the stressor nor the stressor-related cues influenced the immune response.

The mean PFC response for each of the groups in Experiment 1B are shown in Fig. 2. ANOVA revealed that the stressor treatment influenced both the PFC response, $F(2, 22) = 8.07$, $p < 0.05$, and antibody titers, $F(2, 18) = 3.67$, $p < 0.05$. Consistent with our earlier reports, Newman-Keuls multiple comparisons ($\alpha = 0.05$) confirmed that a single session of foot-shock applied 72 h after inoculation reduced both indices of the immune response relative to either nonstressed mice or mice that received repeated foot-shock exposure. The latter two groups did not differ from one another. These data suggest that the effects of an acute stressor may be eliminated

TABLE 1

ANTIBODY TITER TO SRBCs (LOG_2 ± SEM) IN MICE AS A FUNCTION OF THE INITIAL STRESSOR TREATMENT AND REEXPOSURE TREATMENT

	Reexposure Treatment		
Initial Treatment	No Treatment	Apparatus	Shock
Nonstressed	4.22 ± 0.40	4.50 ± 0.40	2.40 ± 0.45*
−48 h	4.11 ± 0.51	2.75 ± 0.46*	3.36 ± 0.51
−24 h	3.90 ± 0.59	4.60 ± 0.48	4.44 ± 0.53
0 h	4.10 ± 0.60	5.60 ± 0.34*	4.50 ± 0.53
+24 h	3.81 ± 0.27	5.66 ± 0.37*	4.72 ± 0.47

*$p < 0.05$ relative to mice that received no reexposure treatment.

by repeated exposure to foot-shock or by a stressor applied on the day of inoculation. As such, the possibility exists that the apparent adaptation may be a result of the stressor applied on the day of inoculation as opposed to effects attributable to repeated stressor exposure.

EXPERIMENT 2

Experiment 1B indicated that repeated exposure to a stressor on a daily basis throughout the period between inoculation and spleens being taken prevented the immunosuppression ordinarily provoked by an acute stressor applied 72 h after immunization. Experiment 2 was conducted to determine whether repeated stressor exposure prior to inoculation would likewise result in an adaptation of the immunosuppressive effect ordinarily associated with an acute stressor. Inasmuch as a stressor applied soon after inoculation may limit the immunosuppression ordinarily elicited by a stressor applied 72 h after inoculation, in Experiment 2 the stressor regimen terminated 24 h prior to SRBC treatment.

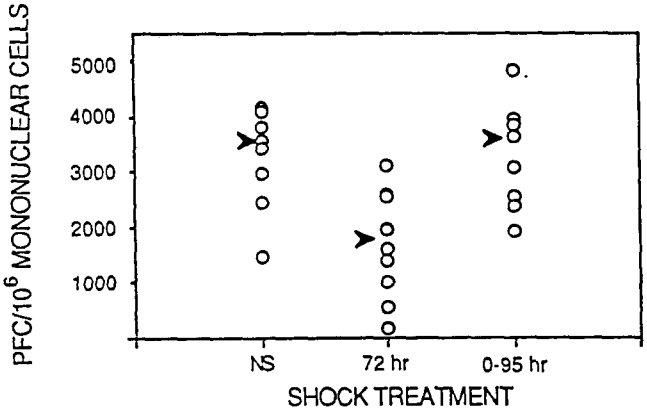

FIG. 2. Individual (○) and mean (arrowheads) splenic immunoglobulin(Ig)M plaque-forming cell (PFC) responses in mice immunized with sheep red blood cells (SRBCs) and exposed to either no shock (NS), one session of shock 72 h after immunization (72 h), or shock on 5 days commencing immediately after immunization (0-95 h). Plasma antibody titers for these three groups were 4.67 ± 0.80, 3.12 ± 0.44 and 4.86 ± 0.26, respectively.

METHOD

Subjects and Apparatus

A total of 71 mice were utilized in Experiment 2. The subject characteristics and apparatus specifications were identical to those described in Experiment 1A.

Procedure

Mice were assigned to one of three treatment conditions and either a) received exposure to 360 foot-shocks on each of 15 successive days (150 μA, 2-s duration, 9-s ITI), b) were left undisturbed in their home cage for 14 successive days and exposed to one session of 360 shocks on day 15, or c) were left undisturbed in their home cages for 15 successive days. Twenty-four hours following the last stressor session, mice were inoculated with SRBCs (10^6 cells) and 72 h thereafter mice in each group (n = 11 or 12/group) were subdivided and exposed to either one shock session (150 μA, 2-s duration, 9-s ITI) or were exposed to the shock apparatus without shock being delivered (1.1 h). Spleens were taken 96 h after inoculation for PFC determinations and trunk blood was collected for determinations of antibody titers.

RESULTS AND DISCUSSION

The mean PFC response as a function of the shock treatment is shown in Fig. 3. ANOVA confirmed that the PFC response varied as a function of the initial shock treatment × reexposure treatment interaction, $F(2, 65) = 6.31$, $p < 0.01$. Newman-Keuls multiple comparisons ($\alpha = 0.05$) of the means comprising this interaction confirmed that in previously nonstressed mice exposure to foot-shock 72 h after SRBC inoculation resulted in a marked reduction of the PFC response. In contrast, the immunosuppression was absent in mice that were acutely exposed to the stressor 24 h prior to inoculation and reexposed to the stressor 72 h after immunization. In mice that received repeated exposure to a stressor over 15 days, subsequent reexposure to the stressor did not result in the immunosuppression seen after acute foot-shock. In these animals, a small, although significant, increase of the PFC response was evident relative to that of animals that had not been reexposed to the stressor. In effect, the repeated shock treatment not only prevented the reduction of the PFC response ordinarily associated with acute foot-shock but actually conferred a modest immunoenhancing effect in mice that were reexposed to the stressor.

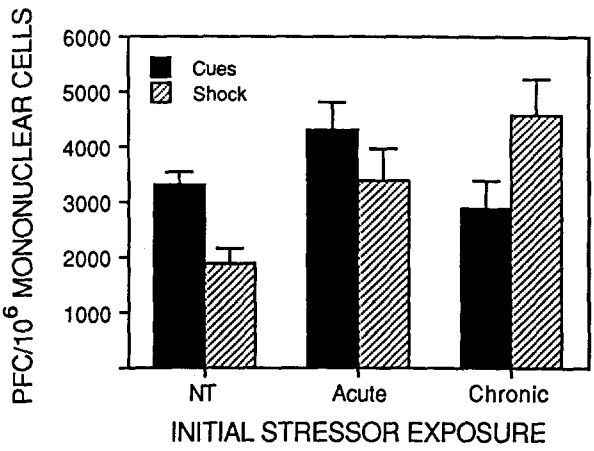

FIG. 3. Mean (±SEM) splenic immunoglobulin (Ig)M plaque-forming cell (PFC) responses in mice exposed to either 15 sessions of foot-shock on successive days (chronic), a single session of foot-shock (acute), or no shock treatment (NT). Mice were immunized with sheep red blood cells (SRBCs) 1 day after the final shock treatment and 72 h later exposed to either a single shock session (shock) or to the shock apparatus without the shock being applied (cues).

The reduction of antibody titers induced by acute foot-shock were smaller than that previously observed (33,34). In fact, while acute foot-shock applied 72 h following inoculation produced a small reduction of antibody titers, this effect was not statistically significant (see Table 2). The ANOVA did reveal, however, that the initial shock manipulation influenced antibody titers, $F(2, 68) = 4.78$, $p < 0.05$. Multiple comparisons confirmed that a single session of foot-shock applied 24 h prior to inoculation or repeated exposure to the stressor increased antibody titers relative to animals that had not been exposed to the stressor prior to SRBC treatment.

EXPERIMENT 3

The results of Experiment 2 confirmed that a chronic stressor regimen applied prior to antigen administration (terminating 24 h prior to immunization) prevented the immunosuppression ordinarily elicited by foot-shock administered 72 h after inoculation. In fact, this treatment promoted a significant immunoenhancement. Yet, in animals that received acute foot-shock treatment 24 h prior to inoculation the immunosuppressive effect of subsequent foot-shock was also prevented. Thus, it is possible that the timing of the initial stressor treatment relative to inoculation may have contributed to the adaptation associated with a chronic stressor regimen. Accordingly, an additional experiment was conducted to establish whether the effects of a chronic stressor that terminated 2 days prior to inoculation would influence the immunosuppression engendered by a stressor applied 72 h after SRBC administration.

TABLE 2

ANTIBODY TITER (LOG_2 ± SEM) TO SRBCs IN MICE INITIALLY EXPOSED TO NO TREATMENT, ACUTE SHOCK, OR CHRONIC SHOCK AND REEXPOSED TO EITHER APPARATUS OR SHOCK

	Reexposure Treatment	
Initial Treatment	Apparatus	Shock
No treatment	3.54 ± 0.41	2.50 ± 0.51
Acute shock	4.84 ± 0.46	4.23 ± 0.57
Chronic shock	4.23 ± 0.54	4.50 ± 0.61

METHOD

Subjects and Apparatus

A total of 62 mice were employed in Experiment 3. The subject characteristics and apparatus specifications were identical to those described in Experiment 1A.

Procedure

In Experiment 3, mice were divided into one of three groups and were either a) exposed to foot-shock (parameters described in Experiment 1A) on 13 successive days, b) left undisturbed in their home cage for 12 successive days and exposed to one session of shock on day 13, or c) left undisturbed in their home cage. Forty-eight hours following the final shock session, mice were inoculated with SRBCs as previously described and 72 h later were subdivided and exposed to either one session of shock (as described in Experiment 2), placed in the shock apparatus without shock being delivered (1.1 h), or left undisturbed in their home cage (n = six or seven/group). Spleens were taken 96 h after inoculation for PFC determinations and trunk blood was collected for determinations of antibody titers.

RESULTS AND DISCUSSION

ANOVA confirmed that the PFC response varied as a function of the initial shock treatment × reexposure treatment interaction, $F(4, 53) = 3.63$, $p < 0.05$. As seen in Fig. 4 and confirmed by Newman-Keuls multiple comparisons ($\alpha = 0.05$), in the absence of any prior shock treatment exposure to foot-shock 72 h after SRBC inoculation resulted in a marked reduction of the PFC response relative to the non-

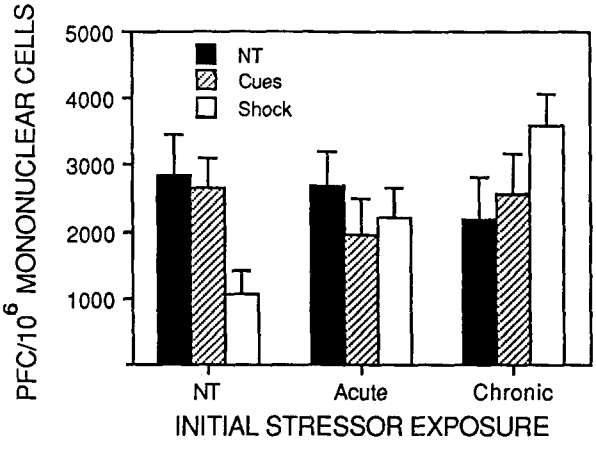

FIG. 4. Mean (±S.E.M) IgM-PFC response in mice exposed to chronic footshock on each of 13 successive days (Chronic), one session of shock on Day 13 (Acute) or no shock treatment (NT). Mice were immunized with SRBC on Day 15 and 72 hr later exposed to either a single session of shock (Shock), the shock apparatus without the shock being applied (Cues) or no treatment (NT).

stressed groups. Among mice that received acute foot-shock 48 h prior to inoculation, neither subsequent apparatus exposure nor foot-shock treatment influenced the PFC response. In contrast, in mice that received the repeated foot-shock treatment reexposure to foot-shock 72 h after inoculation resulted in a small, but significant, enhancement of the PFC response. Evidently, if the initial stressor session and SRBC inoculation are separated by 48 h later exposure to stressor-related cues will not provoke the immunoenhancement ordinarily elicited when the initial stressor and inoculation are administered in closer temporal congruity. In acutely stressed mice, later reexposure to the stressor at the 72-h interval did not elicit the immunosuppression, while in chronically stressed animals reexposure to the stressor itself resulted in a significant immunoenhancement relative to chronically stressed mice that had not been reexposed to the stressor.

The effects of the stressor on antibody titers were in most respects comparable to the effects on the PFC response (see Table 3). The ANOVA revealed that antibody titers varied as a function of the initial stressor × reexposure treatment, $F(4, 57) = 5.43$, $p < 0.05$. Newman-Keuls multiple comparisons ($\alpha = 0.05$) indicated that in mice that had not been stressed prior to inoculation a marked reduction of antibody titers was induced by foot-shock applied 72 h after SRBC administration. As in the case of the PFC response, the shock treatment applied to mice that had been exposed to the stressor 48 h prior to inoculation did not differ from that of mice that had not been reexposed to the stressor. However, reexposure to the apparatus in which mice had initially been shocked prior to inoculation resulted in a significant reduction of the antibody titers. Finally, in chronically shocked mice reexposure to either the stressor or the cues associated with the stressor did not result in a reduction of antibody titers. In fact, upon reexposure to the stressor antibody titers exceeded those of mice that had initially received acute shock or no shock and were then exposed to foot-shock or animals that had received the chronic shock treatment and then were either not stressed or exposed to the cues associated with the stressor. Thus, it seems that an acute stressor applied 48 h prior to inoculation can, at most, limit the immunosuppression provoked by a subsequently applied stressor, while a chronic stressor treatment may favor the development of an immunoenhancement.

TABLE 3

ANTIBODY TITER TO SRBCs ($LOG_2 \pm SEM$) IN MICE INITIALLY EXPOSED TO NO TREATMENT, ACUTE SHOCK, OR CHRONIC SHOCK AND THEN EXPOSED TO NO TREATMENT, THE APPARATUS OR TO FOOTSHOCK

	Reexposure Treatment		
Initial Treatment	No Treatment	Apparatus	Shock
No treatment	5.28 ± 0.84	5.14 ± 0.26	2.71 ± 0.56*
Acute shock	4.86 ± 0.55	2.71 ± 0.78*	3.55 ± 0.53
Chronic shock	5.14 ± 0.51	4.62 ± 0.42	6.71 ± 0.52†

*$p < 0.05$ relative to mice initially left undisturbed in their home cage.

†$p < 0.05$ relative to mice that had initially received acute shock or no shock and were then exposed to foot-shock or animals that had received the chronic shock treatment and then were either not stressed or exposed to the cues associated with the stressor.

GENERAL DISCUSSION

As described earlier, although a stressor may alter the PFC response and antibody titers to SRBCs the nature of the immunologic changes are dependent upon the time at which the stressor was applied, as well as the animal's stressor history. In particular, in the CD-1 strain of mouse a marked immunosuppression was induced when foot-shock was administered 72 h after SRBC inoculation, whereas no such effect was evident when the stressor was applied at other intervals prior to or following inoculation (33). As indicated earlier, similar observations were noted by other investigators (7,23) and there is reason to suppose that the timing of stressor administration may also be important in the growth of transplanted or virus-induced tumors (20,30) as well as the development of adjuvant arthritis (17).

Although a stressor administered at or near the time of immunization did not affect the immune response, such a treatment influenced the response engendered by a subsequent stressor applied 72 h after immunization. If foot-shock was first applied immediately or 24 h following immunization, then the immunosuppressive effects of a stressor applied 72 h postinoculation was prevented. A similar, although less pronounced, effect was apparent if the initial stressor session was administered 48 h prior to SRBC inoculation. Interestingly, if animals were exposed to the stressor immediately or 24 h after immunization then reexposure to stressor-related cues 72 h after inoculation resulted in a significant immunoenhancement. In contrast, a stressor applied 48 h prior to inoculation resulted in an immunosuppression, just as such an effect was elicited by cues associated with a stressor that had been applied 2 weeks prior to inoculation (34). It is conceivable that pairing a stressor with antigen exposure resulted in the establishment of a compensatory immune response, which was subject to conditioning-like processes (34). Accordingly, when animals were later reexposed to the cues this compensatory immunologic response was elicited. These results, like those of other investigators (8,16,19), indicate that stressor-related environmental cues may come to provoke immunologic changes. However, insofar as the PFC and antibody responses to SRBCs are concerned the proactive effect of a stressor is critically dependent upon its timing relative to antigen administration.

In assessing the effects of a stressor on the IgM PFC response, it should be considered that antigen-related sympathetic and central neurochemical alterations can occur at various time frames after immunization. For example, sympathetic (β-adrenergic) signals/stimulation occur during the early phases of the IgM PFC response and influence the peak immune response (25). Moreover, we previously observed that around the time of the peak IgM response to SRBCs the utilization of norepinephrine (NE) was increased in brain areas such as the hypothalamus and hippocampus (35), which may influence immune functioning (2). Hence, stressor administration, which is known to affect central and peripheral monoamine activity (2), when applied at some intervals following immunization might interact with antigen-related sympathetic and neurochemical changes that would be occurring simultaneously and might thus come to affect the subsequent PFC response.

In contrast to the immunosuppressive effect of acute stressor exposure, an adaptation occurred following a chronic stressor regimen. Similar outcomes were previously noted with respect to mitogen-induced T-cell proliferation (10,11,18) and NK cell activity (10,14). It will be recalled that stressor expo-

sure on successive days following immunization prevented the immunosuppression ordinarily associated with a stressor applied 72 h after immunization. However, the extent of such an effect was not different from that of mice initially stressed on the day of immunization. These data might be taken to suggest that the apparent adaptation associated with repeated stressor application was, in fact, simply a consequence of a compensatory response associated with the stressor being applied on the day of immunization, rather than effects associated with the repeated application of the insult. It might likewise be argued that compensatory mechanisms might account for the adaptation that is evident when the chronic stressor was applied prior to inoculation; that is, a chronic regimen that terminated within 24–48 h of inoculation might engender a compensatory immunologic response upon subsequent reexposure to the stressor (i.e., 72 h after inoculation). In the present investigation, the chronic stressor did not simply result in immune functioning returning to levels equivalent to that of nonstressed animals. Rather, this treatment actually potentiated immune activity well beyond that observed in the absence of a stressor. In effect, it seems that the chronic stressor provoked dynamic variations that served to enhance immune activity.

It should be underscored that the development of the adaptation in response to repeated insults may be dependent upon the background conditions upon which the stressor was applied. It was reported (15) that the adaptation of a mitogen response associated with a chronic stressor was not evident when the test stressor was administered in an environment different from that in which the adaptation had developed. Thus, these investigators suggested that the adaptation reflected a situation-specific compensatory response that opposed the effect ordinarily provoked by the stressor. The adaptation in the present investigation may likewise reflect such a compensatory process. However, the nature of the immunologic changes observed in the present investigation were also dependent upon the timing of stressor application relative to antigen administration. Accordingly, the adaptation apparent in the present investigation should be distinguished from the immunologic changes reported by Lysle and Cunnick (15) following chronic stressor exposure.

The mechanisms operative in provoking the stressor-provoked alterations of immune functioning remain to be identified. While adrenal corticosteroids are certainly known to influence immune functioning, there is reason to suspect that corticosterone alterations associated with a stressor were not responsible for the results observed in the present investigation. In particular, using stressor parameters identical to those employed in the present investigation we observed that such an adaptation was not evident with respect to plasma corticosterone concentrations (9,27). This should not be taken to imply that other adrenal factors do not contribute to stressor-provoked variations of some components of the immune system. Indeed, it has been reported (7) that although adrenalectomy did not affect stressor-provoked alterations of the PFC response, Ly-2 lymphocyte activity varied as a function of the interaction of time of stressor application and adrenalectomy. Moreover, it has been reported that the antibody response to SRBCs can be modified by sympathectomy elicited by systemic 6-hydroxydopamine (6-OHDA) treatment, but such an effect was pronounced only in animals that had undergone adrenalectomy (3). Further to the same issue, it has been reported that while acute administration of the synthetic corticoid, dexamethasone, resulted in a suppression of splenic lymphocytes no such effect was evident following repeated treatment with this compound (24). Thus, these investigators suggested that chronic stressor exposure may result in the desensitization of lymphocytes to corticosterone. In contrast to this conclusion, however, it was reported that the adaptation of the lymphoproliferative response following a chronic stressor was not accompanied by alterations in the sensitivity of lymphocyte receptors for hypothalamic-pituitary-adrenal hormones, including corticotropin-releasing factor (CRF), corticotropin (ACTH), corticosterone, or dexamethasone (26). While corticosterone may not be solely responsible for the immunosuppressive effects of an acute stressor and the adaptation associated with a repeated stressor regimen, it may still be premature to reject a secondary or modulatory role for adrenal corticoids in affecting immune activity.

It has been reported that while the mitogen response in blood may be adrenal dependent, the stressor-induced alterations of the splenic mitogen response may be adrenal independent but influenced by catecholamines (5). Interestingly, the adaptation of immune activity associated with a chronic stressor likewise appeared to be dependent upon the immune compartment examined (18). In particular, while an adaptation was evident with respect to the splenic mitogen response no such adaptation was apparent in blood. Hence, it is conceivable that corticoids may play a role in accounting for the variations of the mitogen response in blood, while catecholamines are more intricately involved in determining the response in the spleen. Indeed, the central and peripheral NE changes associated with a stressor are subject to an adaptation with a chronic stressor regimen, while the variations of plasma corticosterone are typically not diminished or are reduced to a lesser extent with repeated stressor application (1,2). Whether similar differentiations exist with respect to the splenic PFC response and plasma antibody titers remains to be determined. Given the parallels between the neurochemical (including NE, dopamine, and serotonin) and immunologic consequences of acute and chronic stressors (2), it is tempting to speculate that these physiological changes might be related to one another. Of course, at this juncture there is no evidence suggesting a causal relationship between the two, nor is it possible to differentiate the relative contributions of the various neurotransmitter alterations engendered by stressors.

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REFERENCES

1. Anisman, H.; Zacharko, R. M. Multiple neurochemical and behavioral consequences of stressors: Implications for depression. Pharmacol. Ther. 46:119–136; 1990.
2. Anisman, H.; Zalcman, S.; Shanks, N.; Zacharko, R. M. Multisystem regulation of performance deficits induced by stressors: An animal model of depression. In: Boulton, A. A.; Baker, G. B.; Iversen, M. T., eds. Neuromethods. vol. 20. Animal models in psychiatry. Clifton, NJ: Human Press; 1991:1–55.
3. Besedovsky, H. O.; Del Rey, A.; Sorkin, E.; Da Prada, M.; Keller, H. H. Immunoregulation mediated by the sympathetic nervous system. Cell Immunol. 48:346–355; 1979.
4. Cunnick, J. E.; Lysle, D. T.; Armfield, A.; Rabin, B. Shock-

induced modulation of lymphocyte responsiveness and natural killer activity: Differential mechanisms of induction. Brain, Behav. Immunol. 2:102–113; 1988.
5. Cunnick, J. E.; Lysle, D. T.; Kucenski, B. J.; Rabin, B. S. Evidence that shock-induced immune suppression is mediated by adrenal hormones and peripheral β-adrenergic receptors. Pharmacol. Biochem. Behav. 36:645–651; 1990.
6. Cunningham, A. J.; Szenberg, A. Further improvements in the plaque technique for detecting single antibody-forming cells. Immunology 14:599–600; 1968.
7. Esterling, B.; Rabin, B. S. Stress-induced alteration of T lymphocyte subsets and humoral immunity in mice. Behav. Neurosci. 101:115–119; 1987.
8. Gorczynski, R. M.; Kennedy, M. Associative learning and regulation of immune response. Prog. Neuropsychopharmacol. Biol. Psychiatry 8:593–600; 1984.
9. Irwin, J.; Ahluwalia, P.; Zacharko, R. M.; Anisman, H. Central norepinephrine and plasma corticosterone following acute and chronic stressors: Influence of social isolation and handling. Pharmacol. Biochem. Behav. 24:1151–1154; 1986.
10. Irwin, M. R.; Hauger, R. I. Adaptation to chronic stress: Temporal pattern of immune and neuroendocrine correlates. Neuropsychopharmacology 1:239–242; 1988.
11. Jessop, J. J.; Gale, K.; Bayer, B. M. Enhancement of rat lymphocyte proliferation after prolonged exposure to stress. J. Neuroimmunol. 16:261–271; 1987.
12. Keller, S. E.; Weiss, J. M.; Schleifer, S. J.; Miller, N. E.; Stein, M. Suppression of immunity by stress: Effect of a graded series of stressors on lymphocyte stimulation in the rat. Science 213: 1397–1400; 1981.
13. Laudenslager, M. L.; Fleshner, M.; Hofstadter, P.; Held, P. E.; Simons, L.; Maier, S. F. Suppression of specific antibody production by inescapable shock: Stability under varying conditions. Brain Behav. Immunol. 2:92–101; 1988.
14. Livnat, S.; Irwin, J.; Ader, R.; Gallo, K. L.; Anisman, H. immunologic reactivity following acute and chronic stress. Soc. Neurosci. Abstr. 11:861; 1985.
15. Lysle, D. T.; Cunnick, J. E. Stressor-induced modulation of immune function: Evidence for context dependent habituation process. Soc. Neurosci. Abstr. 16:1197; 1990.
16. Lysle, D. T.; Cunnick, J. E.; Fowler, H.; Rabin, B. S. Pavlovian conditioning of shock-induced suppression of lymphocyte reactivity: Acquisition, extinction, and preexposure effects. Life Sci. 42: 2185–2194; 1988.
17. Lysle, D. T.; Luecken, L. J.; Maslonek, K. A. Suppression of the development of adjuvant arthritis by a conditioned aversive stimulus. Brain Behav. Immunol. 6:65–73; 1992.
18. Lysle, D. T.; Lyte, M.; Fowler, H.; Rabin, B. S. Shock-induced modulation of lymphocyte reactivity: Suppression, habituation, and recovery. Life Sci. 41:1805–1814; 1987.
19. Lysle, D. T.; Maslonek, K. A. Immune alterations induced by a conditioned aversive stimulus: Evidence for a time-dependent effect. Psychobiology 19:339–344; 1991.
20. Monjan, A. A. Stress and immunologic competence: Studies in animals. In: Ader, R., ed. Psychoneuroimmunology. New York: Academic Press; 1981:185–228.
21. Monjan, A. A.; Collector, I. I. Stress-induced modulation of the immune response. Science 196:307–308; 1977.
22. Moynihan, J. A.; Ader, R.; Grota, L. J.; Schactman, T. R.; Cohen, N. The effects of stress on development of immunologic memory following low-dose antigen priming in mice. Brain Behav. Immunol. 4:1–12; 1990.
23. Okimura, T.; Nigo, Y. Stress and immune responses. I. Suppression of T-cell function in restraint-stressed mice. Jpn. J. Pharmacol. 40:505–511; 1986.
24. Paciotti, G. F.; Skwerer, R. G.; Tamarkin, L. Differential response of rat splenic lymphocytes to short-term and long-term neuroendocrine challenges: Possible desensitization of the cellular immune response to corticosteroids. J. Neuroimmunol. 16:253–259; 1987.
25. Sanders, V. M.; Munson, A. E. Norepinephrine and the antibody response. Pharmacol. Rev. 37:229–248; 1985.
26. Sandi, C.; Cambronero, J. C.; Borrell, J.; Guaza, C. Effects of HPA hormones on adapted lymphocyte responsiveness to repeated stress. Brain Res. Bull. 28:581–585; 1992.
27. Shanks, N.; Griffiths, J.; Zalcman, S.; Zacharko, R. M.; Anisman, H. Mouse strain differences in plasma corticosterone following uncontrollable foot-shock. Pharmacol. Biochem. Behav. 36:515–519; 1990.
28. Shavit, Y.; Lewis, J. W.; Terman, G. W.; Gale, R. P.; Liebeskind, J. C. Opioid peptides mediate the suppressive effect of stress on natural killer cell cytotoxicity. Science 223:188–190; 1984.
29. Sheridan, J. F.; Feng, N.; Bonneau, R. H.; Allen, C.; Huneycutt, B. S.; Glaser, R. Restraint-induced stress differentially affect anti-viral cellular and humoral immune responses. J. Neuroimmunol. 31:245–255; 1991.
30. Sklar, L. S.; Anisman, H. Stress and cancer. Psychol. Bull. 89: 369–406; 1981.
31. Weiss, J. M.; Sundar, S. K.; Becker, K. J.; Cierpial, M. A. Behavioral and neural influences on cellular immune responses: Effects of stress and interleukin-1. J. Clin. Psychiatry 50:43–55; 1989.
32. Zalcman, S.; Irwin, J.; Anisman, H. Stressor-induced alterations of natural killer cell activity and central catecholamines in mice. Pharmacol. Biochem. Behav. 39:361–366; 1991.
33. Zalcman, S.; Minkiewicz-Janda, A.; Richter, M.; Anisman, H. Critical periods associated with stressor effects on antibody titers and on the plaque forming cell response to sheep red blood cells. Brain Behav. Immunol. 2:254–266; 1988.
34. Zalcman, S.; Richter, M.; Anisman, H. Alterations of immune functioning following exposure to stressor-related cues. Brain Behav. Immunol. 3:99–109; 1989.
35. Zalcman, S.; Shanks, N.; Anisman, H. Time-dependent variations of central norepinephrine and dopamine following antigen administration. Brain Res. 557:69–76; 1991.