

## Characterization of immune alterations induced by a conditioned aversive stimulus

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In this study, we investigated the immune alterations induced in rats by an aversive conditioned stimulus that had been developed through pairings with electric shock. The results showed that the conditioned stimulus induced a pronounced suppression of the mitogenic responsiveness of splenic and blood lymphocytes and a reduction in splenic natural killer cell activity. In contrast, the conditioned stimulus did not induce any alteration in the mitogenic responsiveness of lymphocytes from the mesenteric lymph nodes. The reduction in the mitogenic responsiveness of splenic lymphocytes was not related to a reduction in the level of interleukin-2 (IL-2) production, as splenic lymphocytes from subjects exposed to the conditioned stimulus showed normal levels of IL-2. Plasma corticosterone measurements showed that glucocorticoid secretion was related to the alteration of the mitogenic responsiveness of blood lymphocytes. However, plasma corticosterone levels were not related to any of the other immune measures. These findings establish that a signal for an aversive event can have a pronounced effect on immune function, but that such an effect is dependent on the particular compartment of the immune system studied. These results support the claim that glucocorticoids can induce immune alterations, but they suggest that additional pathways must be involved in the immune alterations induced by a conditioned aversive stimulus.

It is well documented that physically aversive stimuli have a pronounced effect on immune function. For example, presentations of electric shock to rats decrease the responsiveness of lymphocytes to stimulation by plant lectins (Keller, Weiss, Schleifer, Miller, & Stein, 1981, 1983; Lysle, Lyte, Fowler, & Rabin, 1987), decrease natural killer cell activity (Cunnick, Lysle, Armfield, & Rabin, 1988; Shavit, Lewis, Terman, Gale, & Liebeskind, 1984), and increase susceptibility to tumor challenge (Lewis, Shavit, Terman, Gale, & Liebeskind, 1983-1984; Lewis et al., 1983). There is also evidence that the immune alterations produced by a physically aversive stimulus can be modulated by psychological factors. Laudenslager and colleagues (Laudenslager, Ryan, Drugan, Hyson, & Maier, 1983) have demonstrated that rats permitted to control electric shocks behaviorally by escaping them show little, if any, suppression of lymphocyte reactivity, as compared with yoked subjects receiving the same, but uncontrollable, shocks.

Recent studies in our laboratory have shown that a conditioned aversive stimulus can also alter immune function (Lysle, Cunnick, Fowler, & Rabin, 1988). A conditioned aversive stimulus is an environmental event that

is not inherently aversive, but which acquires that property by reliably predicting an event (such as electric shock) that is inherently aversive. Our studies have shown that presentations of the conditioned aversive stimulus by itself suppress the responsiveness of splenic lymphocytes to the T-cell mitogens concanavalin-A (Con-A) and phytohemagglutinin (PHA). As further evidence of a conditioned effect, the suppression of lymphocyte responsiveness was attenuated by extinction and preexposure manipulations that reduced the effectiveness of the conditioned stimulus as a signal for the shock.

Although our research provides strong evidence that an aversive conditioned stimulus can alter immune function, it is limited in that our assessment of immune function was restricted to the mitogenic responsiveness of splenic lymphocytes. There is evidence that physically aversive stimuli do not have a comparable effect on all compartments of the immune system. For example, although both blood and splenic lymphocytes are suppressed following the initial presentations of electric shock or the first injection of 2-deoxy-D-glucose (an antimetabolic glucose analogue that produces an acute intracellular glucoprivation), the suppression for the splenic lymphocytes, but not for the blood lymphocytes, attenuates with continued presentation of those stimuli (Lysle, Cunnick, Wu, et al., 1988; Lysle et al., 1987). Furthermore, other studies have indicated that electric shock can have different effects on different types of lymphocytes within an immune compartment (Cunnick et al., 1988; Irwin & Livnat, 1987). In particular, those studies have shown that the mitogenic responsiveness of splenic lymphocytes and splenic natural

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killer cell activity are differentially affected by the presentation of electric shock, and may even be influenced by different mechanisms. Collectively, these findings indicate that different compartments of the immune system, as well as different cells within compartments, are differentially sensitive to aversive stimulation.

The purpose of the present research was to extend our investigation of the immune alterations that are produced by a conditioned aversive stimulus. We evaluated the mitogenic responsiveness of lymphocytes from the blood and lymph nodes, as well as from the spleen. The evaluation included both T- and B-cell mitogens. In addition, we assessed the cytotoxic activity of natural killer cells derived from the spleen. We also examined whether the reduced mitogenic responsiveness of splenic lymphocytes was related to a decrease in the production of interleukin-2 (IL-2), a T-cell growth factor produced by activated T lymphocytes. As a general index of the aversiveness of our conditioning and control treatments, we examined the level of plasma corticosterone and assessed its relation to the immune alterations that were observed, because there is evidence to suggest that cortical steroids induce immunosuppression (Crabtree, Munck, & Smith, 1980; Johnson et al., 1982; Onsrud & Thorsby, 1981; Robbins & Gershwin, 1978).

## METHOD

### Subjects

Male rats of the Lewis strain, 65 days old and 250–300 g in weight, were purchased from Charles River Laboratories. Upon arrival, the subjects were individually caged in a colony room where a reversed day-night (12-h) cycle was maintained by artificial illumination. The subjects received free access to both food (Wayne Rodent Blox) and water throughout the experiment and a 2-week acclimation period prior to the experimental manipulations. All manipulations were initiated 1 h into the dark phase of the cycle.

### Conditioning Apparatus

Eight rodent chambers (Coulbourn Instruments Model E10-10), measuring 25 × 30 × 33 cm, served as the conditioning apparatuses. The chambers had clear Plexiglas side walls, sheet-metal top and end walls, and a grid floor consisting of bars 0.24 cm in diameter, spaced 0.87 cm apart. Timer circuitry to the output of a shock generator and scrambler (BRS/LVE Models 903 and SC 902, respectively) was used to provide the aversive unconditioned stimulus: a 5.0-sec, 1.6-mA footshock. The chambers were individually housed in identical sound-attenuating cubicles, 50 × 60 × 88 cm, which were located in a room adjacent to the programming equipment. A 100-W, 120-V bulb, recessed behind a frosted plate in the ceiling of each cubicle, was operated at 85 V ac to provide diffuse illumination of the chamber. An ambient sound level of 72 dB was provided by operating the cubicle's ventilating fan at 57 V ac.

### Conditioning Procedure

The subjects were randomly assigned to four groups ( $n = 6$ ). Two groups were given 2 days of training in the conditioning chambers (designated C), which involved 10 daily presentations of a 5-sec, 1.6-mA footshock (designated +), on a 4-min variable-time schedule. This procedure established the chamber cues as a conditioned aversive stimulus. Following conditioning, the subjects were kept in their home cages for 6 days, and then a single test session was administered. For the test session, one group (C+/C-) received

a 40-min exposure to the conditioning chamber (without the shock, designated -) prior to their sacrifice for the immunological assays; the other group (C+/HC) was not exposed to the conditioning chamber but remained in their home cages (designated HC) until sacrifice. Thus, both groups received the same conditioning experience, but only one group was exposed to the conditioned aversive stimulus on the test day.

To control for handling and exposure to the conditioning apparatus, a third group (C-/C-) was given the same training and testing as group C+/C-, except that the shock was never presented. A fourth group served as a general control and was kept in the home cages during both training and testing (HC/HC).

Immediately following the test or home-cage control treatment, each subject was rapidly sacrificed by cervical dislocation with a clamp. The animal was then placed on its back and a mid-abdominal incision was made to expose the abdominal aorta. Blood from all subjects was collected into heparinized syringes through 21-ga needles. The spleen and mesenteric lymph nodes were removed and placed in polypropylene tubes containing 7 ml of RPMI-1640 tissue culture medium (Gibco), which was supplemented with 10 mM hepes (Gibco), 2 mM glutamine (Gibco), and 50 µg/ml gentamicin (Gibco). To expedite the processing of the immunological specimens, the experiment was split into two replications ( $n = 12$ ).

### Mitogen Stimulation Assay

A mitogen stimulation assay was performed with leukocytes from the spleen, blood, and lymph nodes. The mitogens Con-A (Difco) and PHA (Burroughs-Wellcome) were used to assess T-lymphocyte responsiveness, and lipopolysaccharide (LPS, Difco) was used to assess B-lymphocyte responsiveness.

For the spleen and lymph nodes, a single-cell suspension of each tissue was prepared for each subject by gently pressing the tissue between the ends of sterile frosted microscope slides in supplemented RPMI enriched with 10% fetal calf serum. Leukocyte counts were determined using a Coulter counter (Model ZBI). Each subject's leukocytes were plated at  $5 \times 10^5$ /well into 96-well plates containing the media, Con-A, PHA, or LPS.

The concentrations of Con-A and PHA were 0, 0.1, 0.5, and 1.0 µg/well, and the concentrations of LPS were 0.1 and 1.0 µg/well. One hundred microliters of the mitogen preparations were added in triplicate to the wells of a 96-well, flat-bottom microtiter plate (Costar No. 3596). Next, 100 µl of the adjusted cell suspension was added to each well and the plates were incubated at 37°C in a humidified incubator, with 5% carbon dioxide (CO<sub>2</sub>). The cultures were pulsed with 1 µCi <sup>3</sup>H-thymidine (specific activity = 6.7 Ci mMole, ICN) in 50 µl of RPMI-1640 during the last 5 h of a 48-h incubation. The cultures were harvested onto glass fiber filter paper using a Skatron harvester. The incorporation of <sup>3</sup>H-thymidine was determined with a liquid scintillation counter (Packard Model 1500) and expressed as counts per minute.

A standard whole-blood assay was used to assess the mitogenic responsiveness of blood lymphocytes. The blood was diluted 1:10 with supplemented RPMI-1640, and leukocyte counts were determined using a hemocytometer and Unopette (Test 5856). Next, 100 µl of the diluted blood were added in triplicate to the wells of a 96-well, flat-bottom microtiter plate (Costar No. 3596) containing 100 µl of the prepared Con-A, PHA, or LPS (0.5, 1.0, or 2.0 µg/well), and to background wells with just media. The incubation and harvest conditions were the same as above, except that the total incubation time was extended to 96 h, with the cultures being pulsed during the last 18 h. To account for variations in the number of leukocytes plated, the counts per minute were adjusted to counts per minute per  $5 \times 10^5$  leukocytes.

### Natural Killer Cell Assay

A natural killer cell assay was performed using splenic leukocytes. A murine T-cell lymphoma, YAC-1, was maintained in sup-

plemented RPMI plus 10% FCS in a CO<sub>2</sub> incubator. On the day of the assay, YAC-1 targets were prepared by labeling them for 70 min with 200  $\mu$ Ci of sodium chromate-51 (<sup>51</sup>Cr, Dupont-New England Nuclear) and the YAC-1 cells were then washed three times to remove exogenous <sup>51</sup>Cr. Splenic leukocytes, prepared as above, were used as effectors and were plated in triplicate at 10, 5, 2.5, and 1.25  $\times 10^6$  cells/well. The labeled targets were diluted and plated at 1  $\times 10^4$  cells/well. Thus, effector:target ratios of 100:1, 50:1, 25:1, and 12:1 were obtained. Control wells containing only labeled targets were also plated to determine the spontaneous release and maximum release possible. The plates were centrifuged at 600 rpm for 4 min and incubated 4 h at 37°C in a humidified CO<sub>2</sub> incubator. Immediately prior to harvest, the targets in one set of control wells were lysed with 100  $\mu$ l of 10% trichloroacetic acid and the microtiter plates were centrifuged. The supernates were harvested using a Skatron supernatant collection system. The amount of <sup>51</sup>Cr released in the supernatant was counted using an LKB gamma counter (Mingamma Model 1275).

Lytic units (LUs) were calculated using a computer program from the Pittsburgh Cancer Institute, based on the equations of Pross and Maroun (1984). The percentage of cytotoxicity at all effector:target ratios was utilized to determine LUs. LUs were based on the number of leukocytes per 10<sup>7</sup> effectors necessary to lyse 20% of the targets.

#### IL-2 Assay

IL-2 is a T-cell growth factor produced by stimulated T lymphocytes. To test for the functional ability of T cells to produce IL-2, 5  $\times 10^6$  splenocytes in 1 ml of media were incubated with 5  $\mu$ g/ml Con-A for 24 or 48 h in a 12-well microculture plate. The supernatants were then harvested and tested for the presence of IL-2. The supernatants and a positive IL-2 control were serially diluted 1:2 (total of six dilutions) in duplicate in a 96-well microtiter plate using a 100- $\mu$ l octapet (Costar). The IL-2-dependent cell line, CTLL-2, was washed to remove any exogenous IL-2 and was then diluted in RPMI-1640 plus 10% FCS to 1  $\times 10^5$ /ml. Then 100  $\mu$ l of the CTLL-2 cell suspension was plated in each well and in four control wells containing no IL-2. The plates were incubated for 24 h at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. Each well was pulsed with 1  $\mu$ Ci of <sup>3</sup>H-thymidine (50  $\mu$ l) for the last 5 h of the incubation. The cultures were then harvested using a Skatron harvester and the thymidine incorporation was determined using a scintillation counter. The data were expressed as half-max units, as calculated using a computer program (Lilly Research Laboratories).

#### Plasma Corticosterone Assay

A sample of the heparinized blood obtained from the abdominal aorta was centrifuged at 12,000 rpm for 3 min. Then 0.5 ml of plasma was obtained and frozen at -70°C. Plasma corticosterone was determined in the laboratory of S. Antelman (Department of Psychiatry, University of Pittsburgh) by a competitive protein-binding radioassay (Murphy, 1967). This assay requires only 25  $\mu$ l of plasma and is sensitive to 0.2  $\mu$ g/dl.

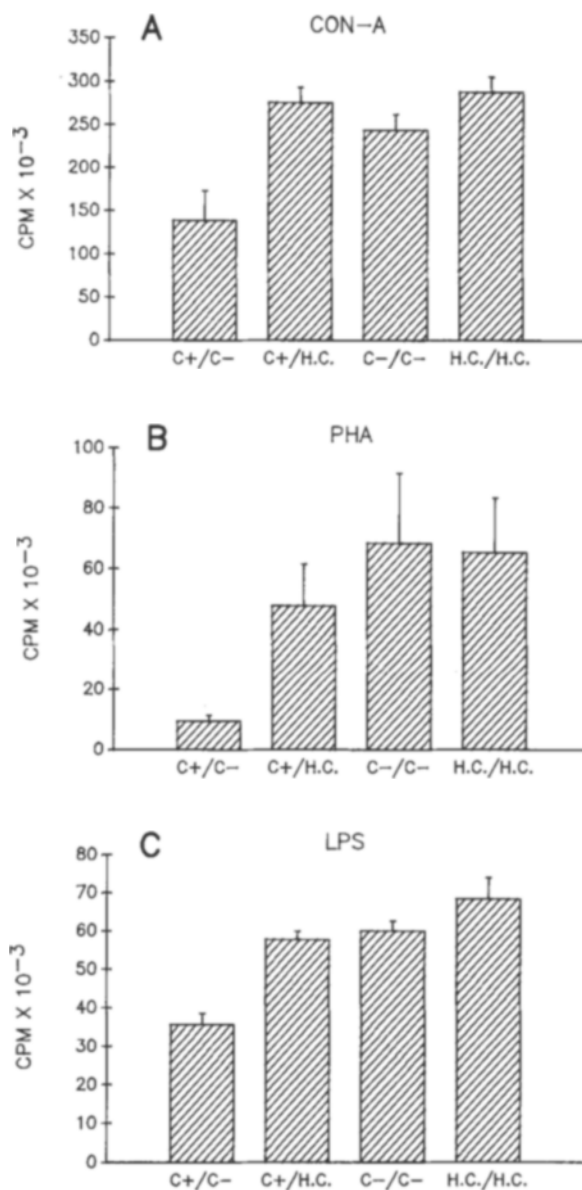
#### Statistical Treatment of Data

Statistical assessments were made via a computerized program for analysis of variance (Statistix, NH Analytical Software). A two-way analysis of variance was used to assess the total leukocyte counts from each tissue, the mean of the triplicate counts per minute for the different concentrations of Con-A, PHA, and LPS, the lytic units from the natural killer cell assay, the half-max values from the IL-2 assay, and the plasma corticosterone levels. In the analysis, the first factor represented the four group treatments and the second factor represented the two replications. Planned contrasts were performed to determine whether the treatment groups were different from the home-cage control group (HC/HC). The level of significance for the *F* test was set at a probability of .05.

## RESULTS

### Mitogen Stimulation Assay

**Spleen assay.** The mitogen stimulation assay showed comparable effects across all concentrations of Con-A, PHA, and LPS. Figure 1 displays the mean counts per minute for the optimal concentration of Con-A (1.0  $\mu$ g/well), PHA (1.0  $\mu$ g/well), and LPS (1.0  $\mu$ g/well). Analysis of variance of these optimal concentrations showed a significant treatment effect for Con-A [ $F(3,16) = 9.00$ ,  $p < .01$ ], PHA [ $F(3,16) = 12.37$ ,  $p < .001$ ], and LPS



**Figure 1.** Effect of group treatments on the mitogenic responsiveness of splenic lymphocytes induced by the optimal concentration of (A) concanavalin-A (Con-A), (B) phytohemagglutinin (PHA), and (C) lipopolysaccharide (LPS), expressed as the mean  $\pm$  SE of the averaged triplicate counts per minute. The optimal concentration of the mitogens was 1.0  $\mu$ g/well.

[ $F(3,16) = 14.39, p < .001$ ]. Contrasts showed that the conditioned group exposed to the conditioned stimulus on the test day (Group C+/C-) had significantly reduced mitogenic responsiveness to Con-A, PHA, and LPS relative to the home-cage control group ( $F_s > 21.0, p_s < .001$ ). The two control groups (C+/HC and C-/C-) were not significantly different from the home-cage control group (HC/HC).

There was no significant main effect or interaction of replication for the Con-A, PHA, and LPS data.

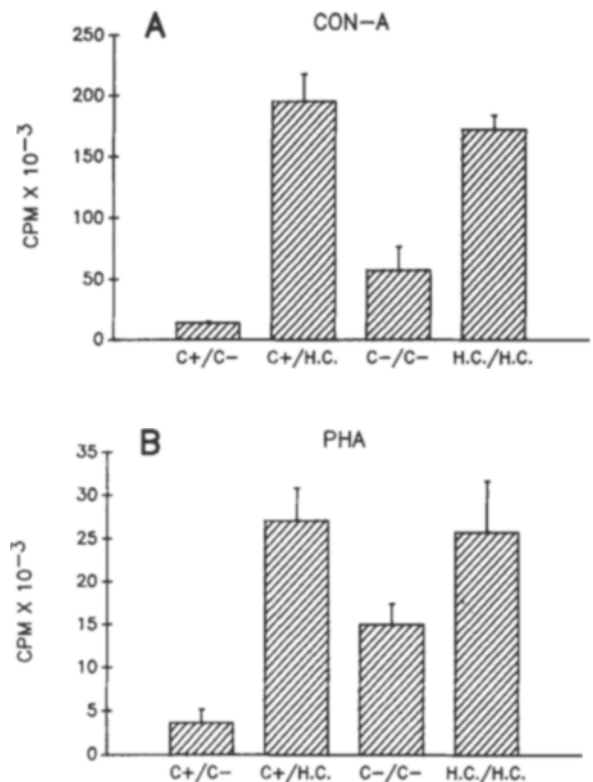
There is evidence that electric shock can produce a decrease in the number of blood leukocytes (Keller et al., 1981). However, in the present study, analysis of variance of the number of spleen leukocytes showed no significant treatment, replication, or interaction effect. The mean leukocyte count of the spleen was  $929.64 \times 10^6$ .

Collectively, the above results establish that a conditioned aversive stimulus can have a dramatic suppressive effect on the mitogenic responsiveness of splenic T and B lymphocytes independent of the effect of either shock or exposure to the experimental chamber alone.

**Whole-blood assay.** The results of the whole-blood assay showed comparable effects across all concentrations of Con-A and PHA. LPS did not induce stimulation of the blood lymphocytes above the level of the background wells. Therefore, the results for LPS are not reported. Figure 2 shows the mean counts per minute per  $5 \times 10^5$  leukocytes for the optimal concentration of Con-A ( $2.0 \mu\text{g}/\text{well}$ ) and PHA ( $1.0 \mu\text{g}/\text{well}$ ). Analysis of variance of these optimal concentrations showed a significant treatment effect for Con-A [ $F(3,16) = 36.69, p < .0001$ ] and PHA [ $F(3,16) = 12.08, p < .001$ ]. Contrasts indicated that the group trained and tested with the conditioned stimulus (Group C+/C-) had a significantly reduced mitogenic response to both Con-A and PHA relative to the home-cage control group ( $F_s > 24.0, p_s < .001$ ). The C+/HC group, which was conditioned but not re-exposed to the conditioning chamber, was not significantly different from the home-cage control group in response to Con-A or PHA. However, the C-/C- group, which was exposed to the conditioning chamber without any shock experience, showed a significant reduction in mitogenic responsiveness to Con-A and PHA relative to the home-cage control group [ $F(1,16) = 31.25, p < .0001$  for Con-A, and  $F(1,16) = 5.90, p < .05$  for PHA]. Nonetheless, an ancillary contrast showed that the suppression of lymphocyte responsiveness to both Con-A and PHA was greater for the C+/C- group than for the C-/C- control group [ $F(1,16) = 4.50, p < .05$  for Con-A, and  $F(1,16) = 6.90, p < .05$  for PHA].

Collectively, the results for the whole-blood assay indicate that exposure to the conditioning chamber alone can alter the mitogenic responsiveness of lymphocytes in the blood, but that pairing the chamber stimulus with electric shock induces an even greater suppression of responsiveness by the blood lymphocytes.

There was no significant main effect or interaction of replication for the Con-A and PHA assays of the whole



**Figure 2.** Effect of group treatments on the mitogenic responsiveness of whole-blood lymphocytes induced by the optimal concentration of (A) concanavalin-A (Con-A) and (B) phytohemagglutinin (PHA), expressed as the mean  $\pm$  SE of the averaged triplicate counts per minute per  $5 \times 10^5$  leukocytes. The optimal concentration of Con-A and PHA was  $2.0$  and  $1.0 \mu\text{g}/\text{well}$ , respectively.

blood. The analysis of the blood leukocyte counts also showed no significant effects. The mean leukocyte count was  $5.16 \times 10^6/\text{ml}$ .

**Mesentery lymph-node assay.** The mitogen stimulation assay of the lymph nodes showed comparable effects across all concentrations of Con-A, PHA, and LPS. Analysis of variance indicated that there were no significant main effects or interactions for any of the concentrations of Con-A, PHA, and LPS. The mean counts per minute for the optimal concentration of Con-A ( $1.0 \mu\text{g}/\text{well}$ ) was  $1.94 \times 10^4$  ( $SE = 1.6 \times 10^3$ ),  $2.74 \times 10^4$  ( $SE = 9.6 \times 10^2$ ) for PHA, and  $1.03 \times 10^3$  ( $SE = 1.1 \times 10^2$ ) for LPS. These results indicate that the mitogenic reactivity of lymphocytes residing in the lymph nodes is unaffected by exposure to a conditioned aversive stimulus.

Analysis of variance of the lymph-node leukocyte counts also showed no significant effects. The mean leukocyte count was  $140.9 \times 10^6$  per set of lymph nodes.

#### Natural Killer Cell Assay

The results of the natural killer cell assay are displayed in Figure 3. Analysis of LUs showed a significant treatment effect [ $F(3,16) = 14.53, p < .001$ ]. Contrasts showed that the conditioned group exposed to the condi-

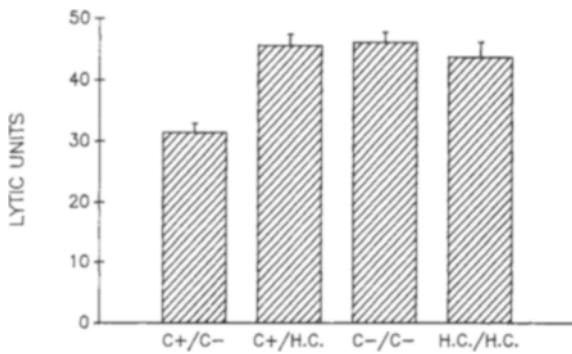


Figure 3. Effect of group treatments on splenic natural killer cell activity expressed as lytic units (mean  $\pm$  SE).

tioned stimulus on the test day (C+/C-) was suppressed in its natural killer cell activity relative to the home-cage control group (HC/HC) [ $F(1,16) = 22.85, p < .001$ ]. However, the other two groups (C+/HC and C-/C-) did not show a level of natural killer cell activity significantly different from that of the home-cage control group. There was no significant main effect or interaction of replication. Thus, these results indicate that a conditioned aversive stimulus can induce substantial suppression of natural killer cell activity.

#### IL-2 Assay

The bioassay for IL-2, using the dependent cell line CTLL-2, did not show any significant alteration in IL-2 production. Analysis of variance of the data from the 24- and 48-h cultures did not show any significant effects of treatment or replication. The mean half-max values for the 24- and 48-h cultures were 15.54 and 23.50, respectively. These results indicate not only that the suppression of mitogenic responsiveness was not the result of a decrease in the production of IL-2, but that presentation of the conditioned stimulus did not alter all T-lymphocyte functions.

#### Plasma Corticosterone

Figure 4 displays the results of the radioassay for plasma corticosterone. Analysis of these results showed a signifi-

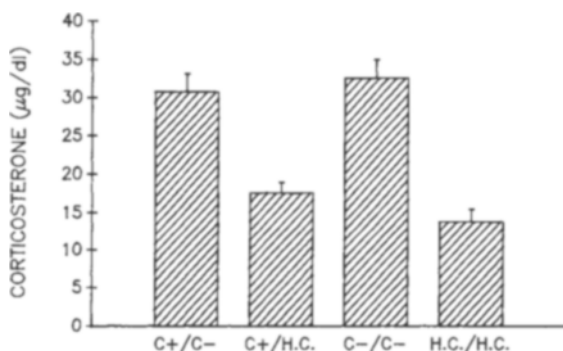


Figure 4. Effect of group treatments on plasma corticosterone level expressed as micrograms per deciliter (mean  $\pm$  SE).

cant treatment effect [ $F(3,16) = 19.70, p < .0001$ ]. Contrasts indicated that the group exposed to the conditioned stimulus (C+/C-) had a significantly elevated level of plasma corticosterone relative to the home-cage control group [ $F(1,16) = 32.38, p < .0001$ ]. The C+/HC control group, which was conditioned but not re-exposed to the conditioning chamber, was not significantly different from the home-cage control group. However, the C-/C- control group, which was exposed to the conditioning chamber without any shock experience, also showed a significant elevation of plasma corticosterone relative to the home-cage control group [ $F(1,16) = 39.44, p < .0001$ ]. An ancillary contrast indicated that the elevation of corticosterone for Group C+/C- was not significantly different from that for the C-/C- chamber control group. These findings indicate that exposure to the conditioning chamber, whether or not paired with the shock, induces a secretion of corticosterone.

It is noteworthy that the groups that had a significantly suppressed blood mitogenic response (see Figure 2) were the same ones that had significantly elevated corticosterone levels. A post hoc correlational analysis indicated a strong negative relationship between corticosterone levels and the blood mitogenic response to the optimal concentrations of Con-A ( $r = -.6883, p < .001$ ) and PHA ( $r = -.5430, p < .01$ ). This relationship was not significant for any of the spleen or lymph-node immune measures. Thus, this finding suggests that glucocorticoids may be responsible for the suppression of mitogenic reactivity of the blood lymphocytes, but not for the spleen lymphocytes.

#### DISCUSSION

The present research demonstrates that the effect of a conditioned aversive stimulus on immune function is dependent on the particular compartment of the immune system studied. The conditioned aversive stimulus induced a pronounced suppression of the mitogenic responsiveness of splenic and blood lymphocytes and a reduction in splenic natural killer cell activity. In contrast, the conditioned stimulus did not induce any alteration in the mitogenic responsiveness of lymphocytes from the mesenteric lymph nodes. These results are consistent with other work from our laboratory in showing that unconditioned aversive stimuli, such as electric shock and 2-deoxy-D-glucose, have differential effects on immune compartments (Lysle, Cunnick, Wu, et al., 1988; Lysle et al., 1987).

The present results provide some insight into the mechanism responsible for immune alterations induced by the conditioned aversive stimulus. The results of the assay for plasma corticosterone indicate that exposure to the conditioning chamber, whether paired or not paired with shock, induces a secretion of corticosterone. There is substantial evidence to corroborate the finding that exposure to a relatively unfamiliar experimental chamber results in an increase in adrenocortical activity (Friedman & Ader, 1967; Friedman, Ader, Grota, & Larson, 1967;

Levine & Treiman, 1964). There is also evidence that the addition of aversive stimulation to the experimental context does not produce a further elevation in corticosterone level (Bassett, Cairncross, & King, 1973). In the present study, plasma corticosterone levels were correlated with the suppression of the mitogenic responsiveness of blood lymphocytes. This finding supports the evidence that suggests that cortical steroids can induce immunosuppression (Crabtree et al., 1980; Johnson et al., 1982; Onsrud & Thorsby, 1981; Robbins & Gershwin, 1978). However, the results of the present study further indicate that glucocorticoids are not completely responsible for the immune alterations, because there was no significant correlation between plasma corticosterone levels and the mitogenic responsiveness of lymphocytes in the spleen or lymph nodes. There was also no significant correlation between plasma corticosterone levels and splenic natural killer cell activity, or between plasma corticosterone levels and IL-2 production by splenic lymphocytes.

The present study indicates that a reduction in the level of IL-2 is not responsible for the suppression of the mitogenic response induced by a conditioned aversive stimulus, as splenic lymphocytes from subjects exposed to the conditioned stimulus produced normal levels of IL-2. Moreover, the suppression of mitogenic responsiveness concurrent with the absence of an alteration of IL-2 production indicates that only certain lymphocyte functions are altered by the conditioned stimulus.

There are several theoretical accounts as to how a conditioned aversive stimulus acquires the ability to modulate immunoresponsiveness. One account is that the conditioned stimulus becomes directly associated with the immunosuppressive reaction induced by electric shock, and therefore automatically elicits that reaction on its own. Other accounts emphasize the role of some mediational process. For example, the conditioned stimulus for shock elicits an emotional state—fear—and it is this fear that induces the alteration of immune function. There is substantial evidence that indicates that a conditioned stimulus paired with electric shock acquires the ability to induce a conditioned emotional reaction, such as fear (Estes & Skinner, 1941; Miller, 1948; Rescorla & LoLordo, 1965). There is parallel evidence that suggests that drugs that induce fear or anxiety can suppress immunological function (Arora, Hanna, Paul, & Skolnick, 1987; Petitto, Skolnick, & Arora, 1989).

What is required to distinguish between these accounts are studies that manipulate the emotional response or immunological state of the subject during training or testing of the conditioned stimulus. For example, pharmacological or physiological procedures could be used to render the animal incapable of making an emotional response to the conditioned stimulus during the test. The presence or absence of the alteration of immune function during that test would lend support to the direct conditioning account or the mediational account, respectively. Conversely, pharmacological or physiological procedures could be used to render the animal incapable of altering immune

function during the training of the conditioned stimulus. The presence or absence of the conditioned immune alteration to that conditioned stimulus during a test would lend support to the mediational account or the direct conditioning account, respectively.

In conclusion, the investigation of immune alterations induced by a conditioned stimulus has implications not only for understanding the relationship between psychological processes and immune function, but also for understanding the conditioning process itself.

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