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Adrenal Involvement in the Expression of Delayed-Type Hypersensitivity to SRBC and Contact Sensitivity to DNFB in Stressed Mice[1] (41339)

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Abstract. The stress of immobilization has previously been demonstrated to suppress delayed-type hypersensitivity (DTH) responses to an iv injection of sheep erythrocytes (SRBC). However, immobilization enhances contact sensitivity reactions to the cutaneous application of 2,4-dinitro-1-fluorobenzene (DNFB). In the following experiments, both adrenalectomy and the corticosteroid inhibitor, metyrapone, were used to evaluate the influence of corticosterone on the expression of these cell-mediated immune events in stressed mice. Adrenalectomy and metyrapone after induction of the immune response abolished the suppression of DTH to SRBC that was observed in immobilized, control animals. In contrast, the immobilization-induced increase in contact sensitivity to DNFB was still observed after adrenalectomy or metyrapone treatment. Similar results were observed when mice were given metyrapone or adrenalectomized before sensitization. These data indicate that adrenal corticosteroids are involved in the stress-induced suppression of DTH to SRBC, but do not account for the stress-induced enhancement in contact sensitivity to DNFB.

Environmental stressors alter the susceptibility of animals to infectious and noninfectious diseases. It has been suggested that these stress-induced changes in host resistance are mediated by alterations in regulatory cells or molecules that control immune events (1). Data from our laboratory have shown that mice stressed by immobilization or exposed to cold or hot air temperatures express characteristic alterations in cell-mediated immune responses (2). A striking feature of those data was that 2.5 hr of immobilization either immediately before sensitization or challenge with sheep erythrocytes (SRBC) suppressed the subsequent delayed-type hypersensitivity (DTH) response. Conversely, the same stress regimen enhanced contact sensitivity to 2,4-dinitro-1-fluorobenzene (DNFB).

Acute, adverse environmental stimuli typically cause an increase in plasma concentrations of adrenal corticosteroids. Corticosterone, the major adrenal corticosteroid in the mouse (3), has been shown to increase in the plasma of rodents by approximately threefold after an immobilization stressor (4). Adrenal corticosteroids are known to influence the expression of DTH to SRBC (5, 6) and contact sensitivity to DNFB (7). These findings led to the hypothesis that the stress-induced release of adrenal corticoids may be responsible for the characteristic changes in cell-mediated immune events in stressed mice. In the following experiments, we report that adrenal corticosteroids are responsible for the stress-induced suppression in DTH to SRBC, but not the enhancement in contact sensitivity reactions to DNFB observed in stressed mice.

Materials and Methods. *Animals.* Five- to six-week-old Swiss Webster male mice were obtained from the Laboratory Animal Resource Center (Washington State University, Pullman, Wash.). All mice were maintained in the same animal room on a 12-hr light:dark cycle (7:00 AM–7:00 PM) and fed the same diet (Purina Mouse Chow, Ralston Purina, St. Louis, Mo.).

Contact sensitivity and DTH. Induction and elicitation of contact sensitivity to DNFB (Eastman Kodak Co., Rochester,

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N.Y.) and DTH to SRBC were produced as previously described (2). Briefly, mice were sensitized to DNFB by two consecutive daily applications of one drop (20 μl) of 0.5% DNFB in 4:1 acetone:olive oil on the clipped abdomen. Expression of DNFB contact sensitivity was evaluated 5 days after the last sensitizing application by applying one drop of 0.25% DNFB in the same vehicle on the dorsal aspect of the right ear. Delayed-type hypersensitivity to SRBC was induced by an iv injection of 200 μl of a 0.01% suspension of three times washed SRBC. Four days later, mice were challenged with 30 μl of a 25% suspension of SRBC into the right rear footpad.

Ear and footpad thickness for the contact sensitivity and DTH assays, respectively, were measured at 24, 48, and 72 hr after challenge and compared to the prechallenge ear and footpad size. Previous experiments with over 100 mice (2) indicated that nonspecific ear and footpad swelling was minimal ($\leq$0.04 mm) at 48 and 72 hr in immobilized, nonsensitized mice. Therefore, this control was not included in the present study.

Depletion of adrenal steroids. a. Inhibition of 11β-hydroxylation. Metyrapone (2- methyl- 1,2- di- 3- pyridyl- 1- propanone, Sigma Chemical Co., St. Louis, Mo.) competes with deoxycorticosterone for the same binding site on cytochrome *P*-450, thus inhibiting the biogenesis of corticosterone (8). Metyrapone also permitted us to study the effects of adrenal corticosteroid blockage in the presence of an intact adrenal gland. Therefore, adrenal catecholamine secretion remained intact.

In these experiments, metyrapone was mixed with Purina Mouse Chow at a rate of 20 mg per 100 g of ground chow and provided *ad libitum* to test animals (9–11). Control mice were fed ground mouse chow without metyrapone. Mice were offered the metyrapone diet at either 4 days before or 24 hr after induction with DNFB or SRBC and maintained on the metyrapone-treated diet throughout the experiments.

b. Adrenalectomy. All surgical procedures were done under anesthesia induced by an ip injection of a tribromoethanol solution with amylene hydrate (Avertin, Winthrop Laboratories, New York, N.Y.). Bilateral adrenalectomies were performed using a dorsal approach (12). A single incision was made through the cutis and a small incision was made through the abdominal musculature on each side of the vertebral column with iridectomy scissors. After blunt dissection, adrenals were located and grasped with a Pasteur pipet attached to an aspirator. Forceps were attached to the perirenal adipose tissue at a point below the tip of the pipet. The adrenals were excised by abrading the pipet tip (containing the gland) against the forceps. After removal of the glands, the two abdominal musculature incisions were closed with 5-O chromic gut sutures while 5-O silk suture (Ethicon, Inc., Somerville, N.J.) was used to close the cutis incision. Saline (1%) was provided for the adrenalectomized mice. Sham-adrenalectomies were performed on control mice.

Adrenalectomies were conducted 6 days before or 24 hr after the induction with DNFB or SRBC. Mice that were adrenalectomized 24 hr after SRBC immunization were challenged 4 days later.

Immobilization. Mice were immobilized in wire mesh cones for 2.5 hr immediately prior to elicitation of contact sensitivity to DNFB or DTH to SRBC. Wires were placed through the cones at the posterior of the mice to restrain their movement. Mice remained in an upright, horizontal position for the duration of immobilization.

Collection of plasma for corticosterone assay. An experiment using nonsensitized mice was conducted to determine the influence of immobilization in adrenalectomized or sham-operated mice on plasma corticosterone. Deoxycorticosterone, which accumulates in metyrapone-fed mice (13), is a major cross-reacting contaminant (40%) in the corticosterone radioimmunoassay assay used in this study (14). Therefore, corticosterone concentrations in plasma of metyrapone-fed animals were not considered. Both immobilized and nonimmobilized mice were bled at 4:00 PM. Approximately 500 μl of blood was collected from anesthesized mice by retroorbital puncture with heparinized capillary tubes immediately after 2.5 hr of immobilization and at 48 hr postimmobilization. Blood was centrifuged

at 600*g* for 15 min. Plasma was collected and stored at −20° until analyzed for plasma corticosterone.

Corticosterone assay. Plasma corticosterone concentrations were determined by modification of a radioimmunoassay developed for use with avian plasma (14). Procedural changes occurred only during Phase 1 (sample preparation) of the assay. Specifically, 25 μl of mouse plasma was initially extracted as described with 2,2,4-trimethylpentane. Air-dried dichloromethane extracts were subsequently reconstituted in 2 ml of borate buffer. These changes yielded an expanded assay standard curve range of 0 to 240 ng·μl^{-1}, with an assay sensitivity ($P < 0.01$) by t test statistic of at least 8 ng·ml^{-1}. Coefficients of determination (r^2) for the logit-log standard curves from the two assays were 0.998 and 0.999, respectively. Intra- and interassay coefficients of variation obtained by repeated measurement of the corticosterone concentration of a pooled mouse plasma source were 8.2 and 21.3%, respectively. Extraction efficiency testing yielded results similar to those previously reported (14), except removal of [^{3}H]corticosterone from plasma into the dichloromethane washing was slightly higher (88%).

Experimental design and statistical analysis. The SRBC and DNFB data were analyzed as completely randomized, split plot designs, with treatments as main plots and measurement times as subplots. All data were subjected to analysis of variance procedures (15). Treatment differences in SRBC DTH and DNFB contact sensitivity within measurement times were detected by Student's t test. The corticosterone variances between sham-adrenalectomized and adrenalectomized mice were different ($P < 0.05$). Therefore, corticosterone values were transformed logarithmically before analysis of variance. Corticosterone differences between treatments within measurement times were determined by Duncan's new multiple range test (16).

Results. *Immobilization, adrenal steroids, and SRBC DTH.* Similar to earlier results from our laboratory (2), immobilization stress significantly decreased the SRBC DTH response relative to nonimmobilized, sham controls at all measurement times in all four experiments (Table I). Furthermore, this suppression was abrogated by both adrenalectomy and metyrapone. This abrogation occurred whether the blocker was administered before (Expts 3 and 4) or after (Expts 1 and 2) sensitization.

Immobilization, adrenal steroids, and DNFB contact sensitivity. Immobilization stress significantly enhanced DNFB contact reactions relative to sham controls at all but one measurement times in all four experiments (Table II). This immunoenhancement was observed after adrenal gland blockage by both metyrapone and adrenalectomy (Table II, Expts 1 and 2). Similarly, when metyrapone treatment was initiated prior to sensitization (Expt 3), the stress-induced increase in DNFB contact reactions remained. However, when mice were adrenalectomized prior to sensitization (Expt 4), a spurious result occurred: immobilization did not increase the DNFB response in adrenalectomized mice. This finding occurred because the sham control, adrenalectomized mice displayed nearly maximal swelling even in the absence of immobilization. This result may have been due to a lack of glucocorticoid control when mice initially encountered DNFB.

Plasma corticosterone. Plasma corticosterone concentrations in nonimmobilized and immobilized mice that were either sham- adrenalectomized or adrenalectomized are shown in Table III. Immediately after 2.5 hr of immobilization, mice with intact adrenals had approximately three times ($P < 0.01$) more plasma corticosterone than nonimmobilized animals. Two days after the immobilization stress, plasma corticosterone in sham-adenalectomized mice was lower ($P < 0.05$) in stressed than in nonstressed animals. Adrenalectomy significantly reduced plasma corticosterone in the nonimmobilized mice and also prevented the stress-induced increase in plasma corticosterone in the immobilized mice.

Discussion. Mice subjected to 2.5 hr of immobilization immediately prior to elicitation of DNFB-induced contact sensitiv-

TABLE I. LOSS OF ADRENAL GLAND FUNCTION ABOLISHES THE IMMOBILIZATION-INDUCED SUPPRESSION OF SRBC DELAYED-TYPE HYPERSENSITIVITY

	Type of blocker			Administration of blocker relative to sensitization		Immune response: Control, Hour postchallenge				Immune response: Immobile[a], Hour postchallenge				
Expt.	Sham	Metyrapone	Ax	Before	After	*n*	24	48	72	*n*	24	48	72	Standard deviation
1	+	−	−	−	+	10	0.36[b],***	0.32***	0.16**	10	0.14	0.12	0.08	0.055
	−	+	−	−	+	10	0.32	0.34	0.17	10	0.37	0.29	0.18	0.055
2	+	−	−	−	+	9	0.19***	0.16***	0.10***	9	0.12	0.09	0.05	0.020
	−	−	+	−	+	9	0.21	0.18	0.13	9	0.19	0.18	0.13	0.020
3	+	−	−	+	−	10	0.28***	0.27***	0.14**	10	0.19	0.16	0.07	0.049
	−	+	−	+	−	10	0.31	0.29	0.14*	10	0.32	0.33	0.20	0.049
4	+	−	−	+	−	7	0.31***	0.34***	0.18**	7	0.16	0.17	0.08	0.048
	−	−	+	+	−	7	0.27	0.32	0.15	7	0.28	0.29	0.20	0.048

[a] Mice were restrained for 2.5 hr immediately prior to challenge.
[b] Mean (mm) of change in footpad thickness.
* Control different than immobile, $P < 0.05$.
** Control different than immobile, $P < 0.01$.
*** Control different than immobile, $P < 0.001$.

ity or DTH to SRBC display characteristic changes in cell-mediated immune reactions. Specifically, immobilization facilitates the expression of contact sensitivity to DNFB and suppresses DTH to SRBC. These data are in agreement with earlier results evaluating the same stress regimen (2).

The elevation of plasma corticosterone in stressed mice and the elimination of the stress-induced decrease in DTH to SRBC in adrenalectomized or metyrapone-fed animals suggests that plasma corticosterone may be the physiological mediator of this immune suppression. However, adrenal corticosteroids did not mediate the enhancement in contact sensitivity to DNFB that was observed in immobilized mice. Why the same stressor has opposite effects on two models of cellular immunity is unclear. However, some of the immunological

TABLE II. LOSS OF ADRENAL GLAND FUNCTION DOES NOT ALTER THE IMMOBILIZATION-INDUCED INCREASE IN CONTACT SENSITIVITY TO DNFB

	Type of blocker			Administration of blocker relative to sensitization		Immune response: Control, Hour postchallenge				Immune response: Immobile[a], Hour postchallenge				
Expt.	Sham	Metyrapone	Ax	Before	After	*n*	24	48	72	*n*	24	48	72	Standard deviation
1	+	−	−	−	+	10	0.20[b],***	0.21***	0.10***	10	0.30	0.30	0.18	0.032
	−	+	−	−	+	10	0.21***	0.22***	0.11***	10	0.30	0.30	0.19	0.032
2	+	−	−	−	+	8	0.12***	0.14***	0.09***	10	0.16	0.21	0.15	0.024
	−	−	+	−	+	7	0.14*	0.17***	0.11**	8	0.17	0.21	0.15	0.024
3	+	−	−	+	−	20	0.12*	0.08***	0.03***	20	0.14	0.17	0.10	0.024
	−	+	−	+	−	20	0.10***	0.14***	0.08***	14	0.14	0.18	0.13	0.024
4	+	−	−	+	−	9	0.22	0.19***	0.14***	10	0.23	0.27	0.23	0.023
	−	−	+	+	−	10	0.29	0.27	0.22	10	0.29	0.28	0.22	0.023

[a] See footnote *a*, Table I.
[b] Mean (mm) of change in ear thickness.
*** See footnote *a*, Table I, for *P* values.

TABLE III. PLASMA CORTICOSTERONE ($ng \cdot ml^{-1}$) OF SHAM-ADRENALECTOMIZED (SHAM-AX) OR ADRENALECTOMIZED (AX) NONIMMOBILIZED OR IMMOBILIZED MICE

	Sampling hour			
	Nonimmobilized[a]		Immobilized[b]	
	0	48	0	48
Sham-Ax	$66.4 \pm 8.1^{c,d}$	83.8 ± 7.1^{c}	194.2 ± 9.7^{e}	59.9 ± 10.7^{d}
Number of mice	9	10	9	8
Ax	4.5 ± 1.4^{f}	7.9 ± 3.8^{f}	5.4 ± 1.8^{f}	7.9 ± 2.5^{f}
Number of mice	8	7	7	8

Note. Results are least-square means ± SE.
[a] Mice were not immobilized but blood was collected at the same times as immobilized mice.
[b] Mice were immobilized for 2.5 hr prior to the time zero blood collection.
[c,d,e,f] Means within the same row and column with different superscripts are different ($P < 0.01$).

characteristics of these two cell-mediated immune events may explain the differential influence of immobilization on the expression of DTH to SRBC versus DNFB-induced contact sensitivity.

The DTH response to SRBC in mice is evanescent (17). The short length of this DTH response has been reported to be caused by suppressor cells (18, 19). However, Askenase *et al.* (17) have shown that evanescent DTH reactions in mice are not due to suppressor cell influence, but rather to the activation of a T-cell subset with an inherently short life span. Conversely, contact sensitivity to immunogens painted on the skin leads to a relatively long-lived form of immunity (20). Although the mouse is a corticosteroid-sensitive species (21), variations in sensitivity to cortisol have been shown: long-lived lymphocytes are cortisol resistant and short-lived lymphocytes are cortisol sensitive (22, 23). Thus, the suppressive effect of immobilization on SRBC DTH may reflect the influence of corticosterone on a corticosterone-sensitive, short-lived T cell. Reduction in plasma corticosterone should then eliminate the suppressive effect of immobilization on SRBC DTH. This was observed in mice fed metyrapone or adrenalectomized.

This hypothesis would also explain the inability of corticosterone to suppress the enhanced contact sensitivity reactions in stressed mice. For instance, if the T-cell subset mediating contact sensitivity to DNFB is a long-lived, corticosterone-resistant lymphocyte, then an elevation in corticosterone predictably would not inhibit this response. The stress-induced enhancement in contact sensitivity to DNFB supports this view and implies that corticosterone does not affect T cells, T-cell products, or mononuclear cells that regulate the contact sensitivity response. However, the mechanism for immobilization-induced enhancement in contact sensitivity to DNFB remains unknown. Interestingly, contact sensitivity has also been shown to exist in an evanescent form (20). Whether a stress-induced increase in plasma corticosterone would yield a decreased response in evanescent contact sensitivity, similar to evanescent DTH to SRBC, is unknown.

The route of antigen administration has also been shown to influence the induction of T cells (18). Antigen-sensitive lymphocytes in the SRBC DTH reactions probably localize in the spleen, whereas antigen-sensitive lymphocytes in DNFB contact sensitivity reactions localize in regional lymph nodes. Furthermore, Gershon *et al.* (24) have shown that spleen-localizing T cells are more likely to induce or function as suppressor cells than T cells that localize in lymph nodes. Therefore, since the route of antigen administration can preferentially induce different T-cell subsets, it is conceivable that injecting SRBC iv versus skin painting DNFB could induce T lymphocytes with differential sensitivities to corticosterone or other hormones. A differential hormonal sensitivity of lymphoid cells in-

volved in the cellular immune response may ultimately explain the characteristic response of stress on the expression SRBC DTH and DNFB contact sensitivity.

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