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Stress-Induced Alterations in Delayed-Type Hypersensitivity to SRBC and Contact Sensitivity to DNFB in Mice[1,2] (41338)

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Abstract. Several experiments were conducted to evaluate the influence of environmental stressors on *in vivo* cell-mediated immune events in mice. Three stressors were studied: immobilization, heat, and cold. Contact sensitivity reactions to 2,4-dinitro-1-fluorobenzene were enhanced by stress, regardless of the type of stressor that was employed. Enhanced responses occurred when stress was administered at either the induction or expression of contact reactions. The effect of stress on delayed-type hypersensitivity reactions to sheep erythrocytes was more complex. Footpad swelling that was induced by sheep red blood cells was enhanced by heat stress and suppressed by immobilization. Cold stress was shown to either enhance or suppress this delayed-type hypersensitivity response, an effect which depended on the timing of stress relative to the induction and expression of the cell-mediated immune reaction. These data demonstrate that environmental stressors alter regulatory events that control the induction and expression of cell-mediated immune reactions in mice. These results also show that a single stressor can either enhance or suppress cell-mediated immune events, an effect which probably depends on the type of regulatory cell that controls a given T-cell response.

Adverse environmental stimuli affect the susceptibility of animals to infectious and neoplastic diseases. It has been recently postulated that stress alters physiological systems that regulate immunological function (1, 2). This, in turn, affects resistance of the host to microbial insults, the uncontrolled proliferation of neoplastic cells and recrudescence of viral infections. Many stressors clearly alter antibody-mediated immunity (3–8), but the effect of environmental stressors on cell-mediated immune responses has not been adequately investigated. Data from our laboratory indicate that cellular immune reactions are deficient in both chickens (9) and calves (10) that have been stressed. It is likely that alterations in the susceptibility of stressed animals to infectious diseases are related to stress-induced changes in cellular immune function. *In vivo* experiments with rat spleen cells have shown that stress alters membrane characteristics of regulatory lymphocytes (11). This finding implies that environmental stressors alter events that regulate cell-mediated immunity.

Stress does not always lead to immunosuppression. Heat stress has been shown to increase (12) and decrease (13–15) the susceptibility of animals to disease, an effect which depends on the particular pathogenic microbe. For instance, social stress increases the susceptibility of birds to mycoplasmal and viral diseases, but reduces susceptibility to bacterial diseases (16). Similarly, heat stress has been suggested to reduce contact sensitivity reactions while cold stress may enhance this response (17). Furthermore, stress induced via electric shock has been shown to both augment and inhibit tumor growth in mice (18). This effect was attributed to

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either acclimation of the mice to the stressor or to differential sensitivity of tumor cells relative to the time of stress. In the following studies, we provide evidence which demonstrates that stress can either enhance or suppress cell-mediated immune events in mice. It is suggested that these stress-induced changes depend on the type of regulatory T cells or T-cell products that are involved in the cellular event.

Materials and Methods. *Animals.* Four to five-week-old-male Swiss Webster mice from the Laboratory Animal Resource Center (Washington State University, Pullman, Wash.) were maintained in our colony one week before use. All mice were maintained in the same animal room and fed the same diet (Purina Mouse Chow, Ralston Purina, St. Louis, Mo.). Different mice were used for immobilization, heat stress, and cold stress experiments, as well as for contact sensitivity and DTH assays.

Induction and elicitation of contact sensitivity. Contact sensitivity (CS) to 2,4-dinitro-1-fluorobenzene (DNFB, Eastman Kodak Co., Rochester, N.Y.) was induced by the method of Phanuphak *et al.* (19). Briefly, mice were sensitized with 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. Five days after the last sensitizing application of DNFB, ear thickness of all mice was measured with a constant-tension dial micrometer (Mitutyo, Tokyo, Japan). Each measurement was made under light ether anesthesia. Following measurement, all mice were challenged with one drop of 0.25% DNFB in the same vehicle on the dorsal aspect of the right ear. Ear thickness was again measured at 24, 48, and 72 hr after challenge and results expressed as a change in ear thickness (24, 48, and 72 hr measurement minus initial measurement).

Delayed-type hypersensitivity (DTH). Low sensitizing doses of sheep erythrocytes (SRBC) have been shown to result in a subsequent cell-mediated immune response (20). In these experiments, mice were immunized iv with 200 μl of a 0.01% suspension of three times washed SRBC. Four days later, 30 μl of a 25% suspension of SRBC was injected into the right rear footpad. Footpad thickness was measured at 24, 48, and 72 hr after challenge and compared to the prechallenge footpad size.

Immobilization. Mice were placed in wire-mesh cones for 2.5 hr. Wires were inserted through the cones at the posterior of the mice to prevent their escape. The mice were maintained in an upright horizontal position during the entire period of immobilization.

Experiments were conducted to evaluate the effect of immobilization on the induction and expression of CS to DNFB and DTH to SRBC. Induction of the response was studied by maintaining the mice in immobilization cones for 2.5 hr immediately prior to the sensitizing doses of DNFB or the intravenous injection of SRBC. Expression of the response was evaluated by immobilizing the mice for 2.5 hr immediately prior to challenge with the respective immunogen.

Thermal stress. Mice were housed individually in wire cages and placed within environmental chambers (Scientific Systems Corp., Baton Rouge, La.) that were specifically designed for animal research. Feed and water were provided *ad libitum.* Cold stress consisted of exposure to a dry bulb (DB) air temperature of 5° with a dewpoint (DP) of 0°. Mice exposed to the hot environment were housed at DB = 35°, DP = 15°. Control mice were maintained at a DB and DP of 25° and 15°, respectively.

Experiments were conducted to determine the influence of cold or hot exposure on the induction of CS to DNFB and DTH to SRBC by housing the animals in the environmental chambers for 3 and 2 days, respectively, from the time of sensitization or immunization. All mice were then maintained at environmental conditions that were similar to the controls. Expression of the response was evaluated 3 days later for the SRBC immunogen and 4 days later for DNFB. The effect of exposing the mice to cold or hot air temperature throughout the induction and expression of the immune response was also studied. In these experiments, mice were both sensitized and challenged during exposure to the hot and cold

TABLE I. EFFECT OF DIFFERENT STRESSORS ON DTH RESPONSES TO LOW DOSES OF SRBC

Period of stress		Sensitization status		Immune response[a]								Standard deviation
				Control				Treatment				
Induction	Expression	Immunized	Nonimmunized	*N*	24	48	72	*N*	24	48	72	
Immobile	—	Immobile	—	30	0.38***	0.26***	0.11	26	0.26	0.16	0.09	0.064
Immobile	—	—	Immobile	30	0.05**	0.02	0	24	0.10	0.01	0	0.064
—	Immobile	Immobile	—	31	0.51***	0.35***	0.19***	29	0.36	0.26	0.12	0.067
—	Immobile	—	Immobile	30	0.10	0.04	0.02	23	0.08	0.02	0	0.067
Cold	—	Cold	—	25	0.39*	0.26**	0.12	24	0.36	0.21	0.10	0.054
Cold	—	—	Cold	24	0.18**	0.09	0.04	23	0.23	0.08	0.02	0.054
Cold	Cold	Cold	—	24	0.29*	0.22***	0.10***	23	0.33	0.46	0.43	0.055
Cold	Cold	—	Cold	23	0.14	0.07	0.05	10	0.17	0.09	0.02	0.055
Heat	—	Heat	—	25	0.39**	0.26***	0.12**	25	0.44	0.35	0.16	0.054
Heat	—	—	Heat	24	0.18***	0.09*	0.04	23	0.30	0.13	0.05	0.054
Heat	Heat	Heat	—	24	0.19	0.12**	0.05	24	0.19	0.17	0.07	0.063
Heat	Heat	—	Heat	24	0.12	0.07	0.04	12	0.10	0.06	0.02	0.063

[a] Mean of the change in footpad thickness (mm).
* Control different than treatment, $P < 0.05$.
** Control different than treatment, $P < 0.01$.
*** Control different than treatment, $P < 0.001$.

ambient conditions (10 days for CS to DNFB; 8 days for DTH to SRBC).

Experimental design and statistical analysis. All experiments were analyzed as completely randomized, split-plot designs with immobilization, cold or heat stress as main plots and time of measurement as subplots (21). Most experiments were conducted at least twice with a minimum of 10 mice per treatment group. The data were subjected to analysis of variance procedures. Mean differences between treatments within measurement times were determined by Student's *t*-test.

Results. *Immobilization immediately prior to immunization.* Mice were immobilized immediately prior to immunization and returned to their home cages. Expression of the DTH response to SRBC (footpad swelling) was measured 4 days later. In these experiments, DTH to SRBC was significantly reduced at 24 and 48 hr postchallenge (Table I). As expected, mice that had been previously immunized with SRBC showed significantly more footpad swelling than nonimmunized mice. In this experiment and a few others, the stressor that was employed sometimes altered footpad or ear swelling at 24 hr postchallenge relative to the nonimmunized, nonstressed control mice. This swelling was sometimes enhanced and sometimes depressed, and probably related to nonspecific clearance of the injected immunogen.

Immobilization stress caused an opposite effect on the induction of CS reactions. At all time periods tested, immobilization at the time of sensitization enhanced the expression of DNFB-induced contact sensitivity (Table II). There was no ear swelling in the nonsensitized mice that were challenged with the 0.25% DNFB solution.

Immobilization immediately prior to expression. When mice were immobilized immediately prior to challenge instead of prior to induction of the response, reactions were identical to those described above: immobilization caused a significant decrease in footpad swelling with SRBC (Table I) and a significant increase in ear swelling with DNFB (Table II). Throughout these latter studies, maximal ear swelling in nonstressed, nonimmunized mice generally occurred at 24 hr after the eliciting dose. However, immobilized, DNFB-sensitized mice demonstrated maximal ear swelling at 48 hr postchallenge and maintained a significant response even at 72 hr postchallenge.

Cold stress during induction. Exposure of mice to 5° for 2 days following immunization with SRBC decreased footpad swelling at 24 and 48 hours (Table I) when mice were subsequently challenged at 25°. Contact sensitivity to DNFB, as influenced by cold exposure during only the inductive phase of the immune responses, was significantly enhanced at all three measurement periods (Table II). These results with cold stress are similar to the results with immobilization stress for both immunogens.

Cold stress throughout induction and expression. Mice that were maintained in the cold environment from the time of immunization with SRBC throughout measurement of the expression of the response (i.e., 8 days) demonstrated significantly greater footpad swelling at all three measurement times (Table I). The same regimen of cold exposure also increased the contact sensitivity response to DNFB (Table II). The nonsensitized, DNFB challenged, cold-stressed mice exhibited a substantial degree of nonspecific ear swelling at all three measurement periods. However, DNFB-induced ear swelling in the cold-exposed sensitized mice was significantly greater than in the cold-exposed nonsensitized mice at both the 24- and 72-hr measurements.

Heat stress during induction. As in previous experiments with the inductive phase only, mice were exposed to 35° for 2 days following immunization with SRBC. They were then returned to an air temperature of 25° before they were challenged with SRBC 3 days later or with DNFB 4 days later. When mice were heat stressed for 2 days following immunization with SRBC and tested at thermoneutral conditions, increased footpad swelling was recorded at all three measurement periods (Table I). A similar increase in ear swelling was observed at 48 and 72 hr in the CS response to

TABLE II. CONTACT SENSITIVITY RESPONSES TO DNFB IN STRESSED MICE

Period of stress		Sensitization status		Immune response[a]								Standard
				Control				Treatment				
Induction	Expression	Immunized	Nonimmunized	*N*	24	48	72	*N*	24	48	72	deviation
Immobile	—	Immobile	—	10	0.21***	0.14***	0.08***	10	0.29	0.25	0.19	0.027
Immobile	—	—	Immobile	10	0	0	0	10	0	0	0	0.027
—	Immobile	Immobile	—	21	0.21	0.10***	0.07***	22	0.20	0.24	0.20	0.024
—	Immobile	—	Immobile	23	0.01***	0**	0	23	0.04	0.02	0.01	0.024
Cold	—	Cold	—	13	0.16***	0.09***	0.07***	12	0.20	0.14	0.11	0.020
Cold	—	—	Cold	12	0.01*	0.01	0.01	12	0.03	0.02	0.01	0.020
Cold	Cold	Cold	—	10	0.09***	0.07***	0.06***	7	0.27	0.18	0.14	0.033
Cold	Cold	—	Cold	10	0***	0.02***	0.01***	7	0.19	0.16	0.10	0.033
Heat	—	Heat	—	13	0.16	0.09***	0.07**	17	0.17	0.12	0.09	0.020
Heat	—	—	Heat	12	0.01	0.01	0.01	8	0.02	0.02	0.02	0.020
Heat	Heat	Heat	—	10	0.09	0.07	0.06	10	0.09	0.05	0.05	0.033
Heat	Heat	—	Heat	10	0	0.02	0.01	8	0.01	0.01	0.01	0.033

[a] Mean of the change in footpad thickness (mm).
* Control different than treatment, $P < 0.05$.
** Control different than treatment, $P < 0.01$.
*** Control different than treatment, $P < 0.001$.

TABLE III. SUMMARY OF THE STRESS-INDUCED FACILITATION (↑) OR SUPPRESSION (↓) OF CS TO DNFB OR DTH TO SRBC IN MICE[a]

	Stressor					
	Immobilization		Cold		Heat	
Assay	Induction	Expression	Induction	Induction and expression	Induction	Induction and expression
CS to DNFB	↑79[b]	↑140	↑56	↑157	↑33	No Δ
DTH to SRBC	↓38	↓26	↓19	↑109	↑35	↑42

[a] Details of stress regimens are described under Materials and Methods.
[b] Results are percentages expressed relative to sensitized, control, nonstressed mice at 48 hr.

DNFB when mice were maintained at 35° during only the inductive phase of the immune response (Table II).

Heat stress throughout induction and expression. Exposure of mice to 35° throughout the induction and expression of the DTH response to SRBC increased footpad swelling at 48 hr postchallenge (Table I). The same heat stress regimen did not alter the contact sensitivity response to DNFB (Table II).

Discussion. An overall summary of these experiments is given in Table III. These data clearly demonstrate that several diverse type of environmental stressors alter the induction and expression of cell-mediated immunity in mice. Moreover, these data show that (a) contact sensitivity to DNFB is consistently enhanced by stress, regardless of the type of stressor, (b) different types of cell-mediated immune responses may yield opposite results when evaluating the same stressor, and (c) the stressor effect may be contigent upon the timing of the stress relative to the phase of the immune response.

Stressed mice exhibit an enhanced contact sensitivity response to DNFB, regardless of the type of stressor employed. A single, common, physiological response to several kinds of adverse environmental stimuli is the commonly accepted dogma in stress physiology. However, other investigators (17, 22) have suggested that a single stressor may differentially affect the host's cellular immune response. Our data are also consistent with this conclusion, because the stress of immobilization suppressed cellular reactions to sheep erythrocytes even though contact sensitivity responses were significantly enhanced by this same stressor. These results suggest that the two T-cell-mediated immune assays elicit populations of effector or regulatory T lymphocytes that have a differential sensitivity to environmental stressors.

The opposing results obtained with two separate assays within the same stress regimen emphasize the complexity of cell-mediated immunity. It has been suggested that the differential effect of a single stressor on host resistance is related to the type of leukocyte that is involved in host defense against the invading pathogen (23). However, it is also possible that stress may differentially affect subsets of T lymphocytes that control cellular immune events. Folch and Waksman (11) have shown that stress can change the membrane characteristics of a suppressor cell population in rat spleen cultures. Furthermore, the DTH effector cell to SRBC has been shown to express the Ly 1^+ antigenic phenotype (24), but the DNFB effector cell may not solely exhibit Ly 1^+ surface markers and an interaction between Ly 1^+ and Ly 23^+ cell types has been suggested (25). Therefore, these two assays probably involve different regulatory T cells. Since stress can alter suppressor cell populations, a differential effect of the same stressor on two different cellular subsets would seem conceivable.

The phase of the immune response in which animals are exposed to stress may be

an important determinant in the expression of antibody and cell-mediated immunity (26). Mice infected with *Plasmodium berghei* exhibit an increased resistance to the parasite if subjected to stress after inoculation (27). However, if the animals are inoculated after a period of stress, the enhancing affect of stress on the hosts' resistance to the parasite is eliminated. The data of Ipsen (28, 29) also showed that the resistance of mice to tetanus toxin could be enhanced or suppressed by thermal stress, depending on the immune status of the animal. Our data support these earlier conclusions (i.e., effect of cold stress on DTH to SRBC, Table I). These results indicate that the timing of the stress episode in relation to the phase of the immune event may be a critical factor in determining whether the expression of cell-mediated immunity is facilitated or suppressed by stress.

The physiological mechanism whereby stress may facilitate or suppress cell-mediated immunity in mice is unknown. As discussed in earlier reports, heat, cold, and immobilization stressors cause characteristic alterations in neuroendocrine components, as well as eliciting typical changes in the thymus and peripheral blood leukocytes (2, 30, 31). We have also demonstrated that the immobilization stressor used in the present study causes a threefold increase in plasma corticosterone (32). This elevation of glucocorticoids in stressed animals (33–35) and the immunoregulatory actions of glucocorticoids (36–38) would suggest that these hormones may be involved in stress-induced alterations of cell-mediated immunity. However, both a suppressed and facilitated cell-mediated immune response induced by stress is difficult to explain only by an increase in serum glucocorticoids. This conclusion implies that other hormones or factors may also be responsible for the results observed in this study.

Many questions about the role of stress in the modulation of cellular immunity have been raised by these experiments. However, the data clearly demonstrate that stress is involved in the manifestation of *in vivo* cellular immunity in mice. Furthermore, these findings emphasize the need for researchers that evaluate cellular immunity in mice to be cognizant of stress-induced alterations that may occur in experimental animals. Further studies as to cell phenotypes, hormonal mechanisms, and other factors involved in stress-induced alterations of *in vivo* cell-mediated immunity are warranted.

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