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Antibody Response to Bovine Serum Albumin in Mice: The Effects of Psychosocial Environmental Change (40899)[1]

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Abstract. The effects of psychosocial environmental change upon circulating antibody response to antigenic challenge was investigated in CBA/USC mice. Mice were reared in isolation and selected groups were subsequently exposed to psychosocial stimulation. Antibody titers of mice that remained in isolation were significantly higher than the titers of mice exposed to psychosocial stimulation. One group of mice exposed to psychosocial stimulation and then returned to isolation showed titers significantly below those of mice exposed to psychosocial stimulation only. These data indicate that psychosocial environmental changes can be productive of significant suppression of antibody formation in mice.

Stress and its effects on immunological function, in both animal and human studies, has been a recent focus of psychosomatic medicine (1–4). Significant modifications of host resistance of mice and rats has been observed in housing studies (5), population sizes (6), pre- and postnatal handling (7), and light and electrical shock stimulation (5, 8). Most of these stresses studied were "physical" ones. The effect of psychosocial stress on immunological function remains undetermined. While the mechanism(s) of increased susceptibility to disease resulting from psychosocial stress has not been fully illucidated, the data suggests that such stress plays a key role in precipitating infectious diseases in a number of situations (5).

In the present study, we wished to explore whether psychosocial environmental change (resulting in "cognitively-mediated" stress) has an adverse effect upon antibody formation in the mouse. We chose as our research paradigm residential change from isolation to a competitive social environment. Our interest in a mouse model lies in the possible explanation it may offer for the known increased susceptibility to infection (acute respiratory disease) in young men abruptly introduced into the Armed Services (9, 10).

Materials and methods. The research design was to form three groups of approximately equal numbers of mice, each group receiving a different set of psychosocial environmental manipulations. The first group, Group A, would serve as control animals and would remain in an isolation cage throughout the experiment. Isolated animals were raised in individual bottle chambers out of view of other mice. They were provided unlimited access to food and water but were deprived of normal social interaction. The second group, Group B, were moved from isolation to a population cage at 16 weeks of age and remained there until the end of the experiment (2 weeks). The third group, Group C, experienced two psychosocial environmental changes, one at 16 weeks of age where they were moved from isolation to a population cage, and a second change at 17 weeks of age, where they were moved back into isolation. (All mice experienced, at weaning at 3 weeks of age, a move to isolation cages.) This design allowed us to test the hypothesis that psychosocial environmental change adversely modifies antibody response to antigen stimulation.

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Figure 1 schematically presents the procedure we followed with 55 mice. Two separate runs were conducted, one with 24 mice and the other with 31 mice. During the first experiment 3 mice died and one had a very high titer to BSA and were not used in the analysis. All mice were CBA/USC males. At 16 weeks of age 0.2 cc blood was drawn by retro-orbital puncture from each mouse to determine baseline antibody titer. Each mouse was then subcutaneously immunized with 4 mg of bovine serum albumin in Freund's adjuvant (BSAF). Sixteen of the twenty-four males were then transferred to a population cage containing 16 normally socialized females, the remaining mice were kept isolated. The purpose of the addition of the 16 normally socialized female mice was to increase the interaction between members as they competed for social goals including food and water. Although both males and females interacted in the cage, only the males were studied. The mice had access to six perpheral cages connected to a central hexagonal cage containing food and water. Tubes connected one peripheral cage to another, as well as all peripheral cages to the central cage. As the tubes were narrow, confrontations and aggressive encounters were inevitable. The majority of the interactions occurred in the central cage (11).

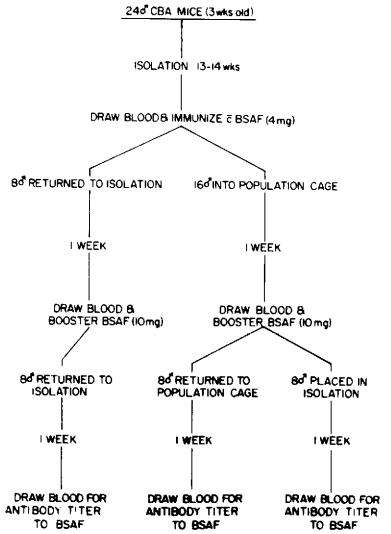

FIG. 1. A schematic representation of the research paradigm to create groups of mice with zero, one, and two psychosocial environmental changes.

After a week had elapsed, blood samples were again drawn and all study mice were given a 10-mg additional challenge dose of BSAF. The mice in isolation were returned to isolation, eight males were retained in the population cage, and eight were put back into isolation. To keep the social setting constant eight normally socialized males were added to the population cage for the final week of the experiment.

At the end of the second experimental week, blood was drawn from all animals and the experiment was ended. Antibody titers to BSAF were measured on all blood samples obtained when the animals were 16, 17, and 18 weeks of age. The results indicated a suppression effect of environmental change on antibody titer. The entire experiment was then repeated with a new group of 31 male mice. Fifteen (15) mice were assigned to Group A, 8 to Group B, and 8 to Group C. The increase in number of control group mice was to provide a population of suitable size for data analysis purposes.

Antibody titer was determined by the passive hemagglutination test described by Katz and Kohn (12). Briefly, sheep erythrocytes were collected in Alsever's solution and washed three times with 0.01 *M* phosphate-buffered saline (PBS) at pH 7.2. Ten milliliters of a 10% suspension of washed erythrocytes were mixed with 20 mg of BSA. One milliliter of 2.5% glutaraldehyde in PBS was added slowly and gently stirred for 1 hr at room temperature. The suspension was then washed three times in PBS and resuspended in 5 ml of PBS. When determinations were made, the cells were diluted to a 1% suspension in PBS; tests were performed in microtiter trays using 0.025-ml volumes. Twofold dilutions (0.025 ml) of inactivated mouse sera were made in PBS. Following this, 0.025 ml of sensitized sheep erythrocytes were added, thoroughly mixed, and allowed to stand at room temperature until the negative cell control had formed a "button." Titers were the reciprocal of the highest dilution causing discernible agglutination. All sera for a single mouse were tested using the same reagents

and performed in a single test run. Sera samples remained coded until all tests were performed; results were then decoded and categorized by test groups of animals.

Results. BSA antibody titers (log_2) at 16, 17, and 18 weeks are shown for Group A through C in Table I and Fig. 2. It was apparent that in all groups of mice, antibody titers 1 week following the initial dose of antigen showed no significant increases from baseline titers. Following the second inoculation, however, large titer increases were seen in Groups A and B by the following week. The mice in Group C, which had to adapt to two psychosocial changes, produced relatively little antibody even after the second inoculation.

A three-way analyses of variance of log titers (experiment × group × time period) indicated a significant difference among the three groups ($F(1,45) = 11.14$, $P < 0.001$); between the three time periods ($F(1,45) = 156.26$, $P < 0.001$); and a significant interaction of groups × time periods ($F(1,45) = 9.82$, $P < 0.001$). There were no significant differences between the two experiments.

Analyses of the total data by Tukey's method (13) (Experiments 1 plus 2) showed that the 18-week titer increase seen for Group A was significantly greater than that seen for Group B ($P < 0.001$), and also to that seen for Group C ($P < 0.001$). Additionally, Group B's 18-week titer was significantly greater than that for Group C ($P < 0.05$).

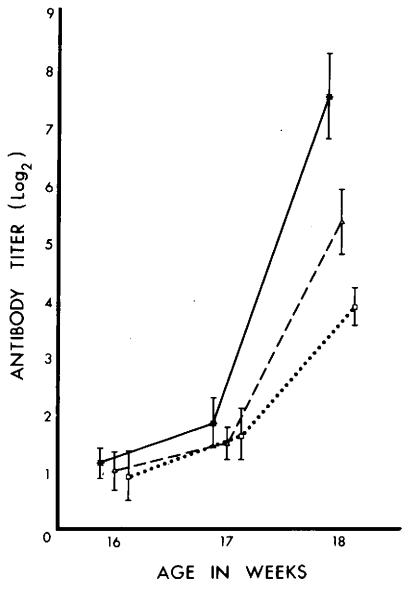

FIG. 2. Mean antibody levels attained by groups of mice experiencing psychosocial stress. (*) Group A mice maintained in isolation; (△) group B mice (one environmental change); (□) group C mice (two environmental changes). (I) Standard error.

Discussion. Although the immunological design did not permit determining the maximum antibody response which would have been seen in 3–5 weeks after inoculation, it did permit an estimate of the effect

TABLE I. MEAN HEMAGGLUTINATING TITERS (Log_2) OF MICE IN TWO SEPARATE EXPERIMENTS

		Weeks		
	n	16	17	18
Experiment 1				
Group A	5	1.20 ± .84	3.20 ± 1.92	9.20 ± 3.90
Group B	8	1.38 ± 1.06	1.25 ± .89	6.00 ± 1.69
Group C	7	.72 ± .76	1.57 ± .79	4.14 ± 1.07
Experiment 2				
Group A	15	1.07 ± .89	1.33 ± .98	7.07 ± 2.40
Group B	8	.75 ± .89	1.75 ± .89	4.75 ± 1.98
Group C	8	1.13 ± 1.36	1.75 ± 1.67	3.75 ± 1.17

Note. Animals in group A were in isolation throughout the experiment. Group B were raised in isolation and transferred to a social environment at 16 weeks. Group C were raised in isolation, transferred to a social environment at 16 weeks, and back to isolation at 17 weeks.

[a] CBA/USC mice were immunized at 16 weeks, of age with Freund's bovine serum albumin.

of psychosocial adaptation on the early antibody response to the antigenic stimuli. From these data, we conclude that psychosocial stress in mice having to cope with one or two environmental changes was sufficient to significantly reduce the early level of circulating antibody response to BSA. In the model described here, the greater the number of environmental changes, the greater the reduction in antibody production.

In previous reports (14, 15) it was found that overcrowding of mice was a psychosocial stress which resulted in a significant reduction of antibody response. Thus, these data support the hypothesis that alterations in immunological functions secondary to cognitively mediated environmental stress are at least as potent as previously studied physical stresses. Such a mechanism might help explain the relationship of psychological stress to acute infectious disease onset in humans (1, 2, 16).

Current studies are underway to determine whether psychosocial stress causes immunodepression in both B- and T-cell functions as well as the relationship between B and T response and cortisol levels. These studies include mice raised in isolation and mice in a normal social environment.

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