

## Stress, immune reactivity and susceptibility to infectious disease

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### Abstract

Psychological stress is known to affect immune function and to predict infectious disease susceptibility. However, not all individuals who are stressed develop disease. In the present article, we report on a series of studies from our laboratory describing interindividual variability of immune responses to psychological stress. In our initial series of experimental investigations, we demonstrated that acute laboratory stress alters both quantitative and functional components of cellular immunity. An examination of response variability revealed that individuals differ substantially in the magnitude of these immune responses. These differences were found to parallel (and be predicted by) interindividual variability in stress-induced sympathetic nervous system activation. Further investigation revealed that individuals vary consistently in the magnitude of their immune responses to stress, making it conceivable that individual differences in immune reactivity provide a vulnerability factor mediating relationships between stress and disease. In support of this possibility, we have recently reported initial evidence that individual differences in the magnitude of stress-induced reduction of immune function may be of clinical significance, being related to an immune response relevant for protection against infection, antibody response to hepatitis B vaccination.

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Psychological stress is known to affect immune function and to predict infectious disease susceptibility, as seen in both humans and animals [1–5]. Because not all similarly stressed individuals are equally likely to develop disease (e.g., Refs. [2,6]), some variability must exist either in behavior or in biological vulnerability. Here, we report a series of studies describing interindividual variability of immune responses to stress.

### 1. The impact of acute psychological stress on immune function

Our initial series of five experimental studies examined the effects of acute laboratory stress on immune functioning

in healthy young adults (Study 1: [7], Study 2: [8], Study 3: [9], Study 4: [10], Study 5: [11]). In these studies, subjects were exposed to standardized, short-term laboratory stressors designed to characterize the transient stresses of daily life. All of the studies recruited healthy, young volunteers (18–30 years) who were nonsmokers, reported no history or symptoms of diseases known to affect immunity and denied taking medications (other than oral contraceptives). Subjects were randomly assigned to a stress-exposed condition (experimental group) or to an unstressed, control condition. The experimental group had blood samples drawn for the determination of cellular immune parameters at the end of a baseline adaptation period and again after performing one of three laboratory stressors: a mental arithmetic task, an evaluative speech task or the Stroop Color–Word Interference Test. The mental arithmetic task consisted of 10 min of consecutive one-to-three-digit addition/subtraction problems. The speech task involved 2 min of preparation for a speech in which the subject was asked to defend him/herself

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against an alleged transgression (shoplifting or traffic violation), followed by 3 min of videotaped speech delivery. The Stroop task was a 21-min computerized version of the Stroop Color–Word Interference Test. In this task, the subject is presented with one of four-color names, appearing in an incongruent color. The subject is required to identify, from a response selection of four-color names (also in incongruent colors), the color name corresponding to the color of the target stimulus. This task was performed under pressure of time and against a distractor (random test responses) generated by computer voice synthesis. The control group rested quietly during the experimental period and had measures taken at corresponding intervals. A battery of laboratory tests was employed to provide a general index of immune function. Tests included measures of the numbers and functional abilities of various subgroups of leukocytes in peripheral blood. In the enumerative assays, the circulating numbers of T lymphocytes (and their subtypes), B lymphocytes and NK cells were assessed using flow cytometry. Functional immune measures included mitogen-stimulated T lymphocyte proliferation using the nonspecific mitogens, phytohemagglutinin (PHA) and concanavalin A (Con A). These laboratory measures of immune function measure the rate at which T cells proliferate when exposed to experimental antigens. Greater cell division is taken as a measure of a more effective immune response.

Findings revealed that when compared with control subjects who were not exposed to stress, individuals who were challenged showed reliable changes in immunity from baseline-to-task measures across the five studies. These changes included a statistically significant increase in the number of circulating NK (CD56+) and cytotoxic T (CD8+) cells (see Fig. 1), and a decrease in the ratio of helper-to-cytotoxic T (CD4+:CD8+) lymphocytes and in proliferative responses to PHA and Con A ( $P$ 's < .05). In contrast, there were no consistent changes in the number of circulating B (CD19+) or helper T (CD4+) cells. Further examination of findings demonstrated that immune changes occurred rapidly, being present within 5 min of stressor onset, and was maintained throughout the task period [9]. Others have

shown that, while changes in cell subset number return to baseline within 15 min of the end of the task [12], changes in proliferative response last longer, with reduction in lymphocyte proliferation remaining for at least 90 min after challenge [13]. In sum, our findings and those of others demonstrate that acute stress induces reliable changes in both enumerative and functional aspects of immunity (see review, Ref. [14]).

## 2. Sympathetic mediation of interindividual variability in the magnitude of stress-induced immune responses

Although we found main effects of stress on immune function, an examination of response variability revealed that individuals differ substantially in the magnitude of their immunologic reactivity to stress, with many individuals exhibiting little or no response. Findings from one of our initial studies [15] revealed that immunologic responses to acute laboratory challenge are observed only among individuals who also show heightened sympathetic responses to stress, as measured by a composite index of their cardiovascular and catecholamine reactions to the Stroop task. High sympathetic responders showed stress-induced increases in cytotoxic T cells numbers and a diminished mitogenic response to PHA, whereas low sympathetic reactors showed no stress-related change in immunity (see Fig. 2). These results suggest that much of the variability of subjects' immune reactions to acute stress reflect individual differences in behaviorally evoked sympathetic nervous system activation. To further assess this possibility, Bachen and colleagues administered labetalol, a nonselective  $\alpha$ - and  $\beta$ -adrenergic antagonist, to subjects before they were exposed to two cognitive tasks and a public speaking stressor [8]. As expected, adrenergic receptor inhibition prevented stress-related elevations in NK cell number and activity, reductions in the ratio of helper to cytotoxic T cells, and decreases in proliferative responses to PHA and Con A, providing more direct evidence for sympathetic mediation of acute stress-immune reactions. Recent evidence from another laboratory

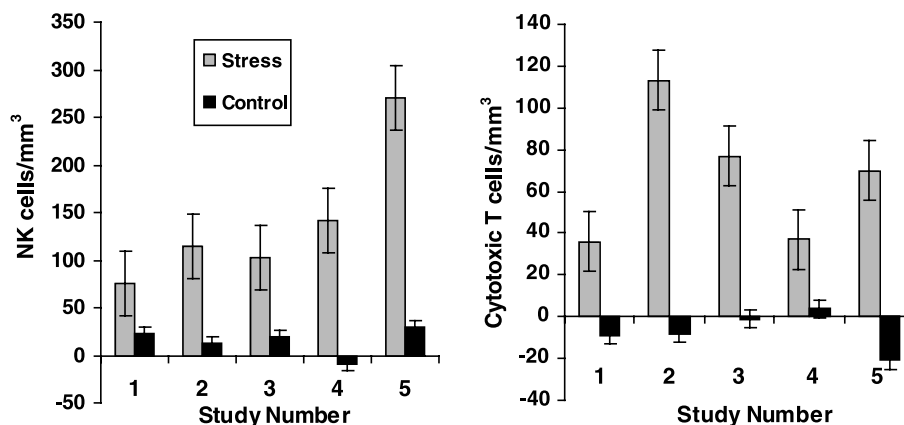


Fig. 1. Stress-related changes in the numbers of circulating NK and cytotoxic T cells in five studies.

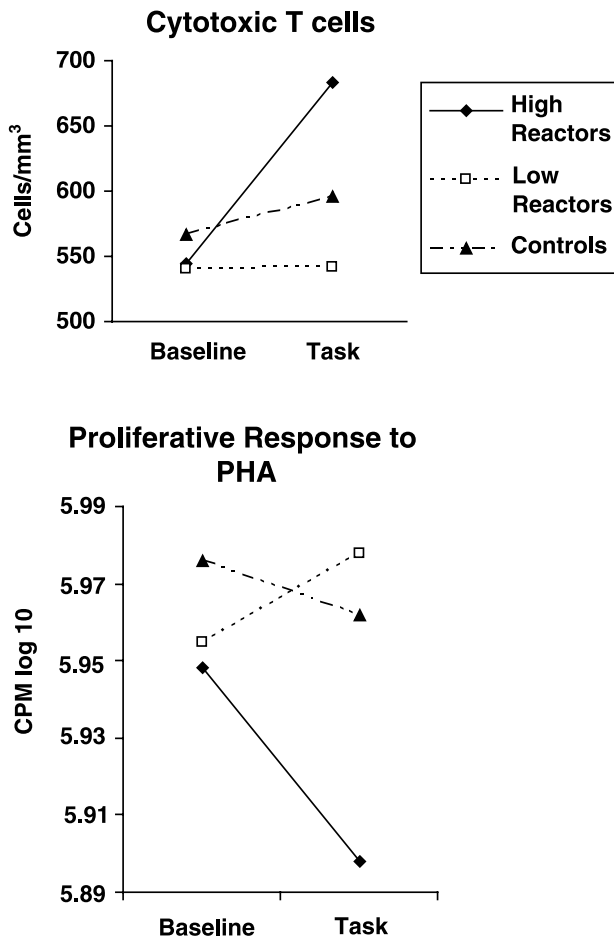


Fig. 2. Change in number of cytotoxic T cells and proliferative response to PHA among low and high sympathetic reactors in response to the Stroop Color-Word Interference Task.

suggests that activation of the hypothalamic-pituitary-adrenocortical system may also play a role. Indeed, individuals who show high sympathetic responses to acute stress also show a stress-induced increase in plasma cortisol levels, when compared with low sympathetic responders who show no change in cortisol following stress [16,17]. This is worthy of note given extensive evidence that cortisol is associated with longer-term down regulation of cellular immune function [18], which may render biologically reactive individuals more susceptible to immune-mediated disease.

The exact mechanism of acute sympathetic-immune mediation remains unclear. A meta-analysis of the data from four of our studies suggests that activation of the sympathetic nervous system may influence the immune system by both active and passive processes [19]. Under conditions of stress, an increase in arterial blood pressure driven by activation of the sympathetic nervous system causes fluid to filter out of circulation into extravascular spaces, leading to a passive increase in the concentration of all nondiffusible constituents of blood, termed hemoconcentration. By arithmetically correcting changes in lymphocyte number for this

reduction in plasma volume, we have demonstrated that increases in the numbers of circulating cytotoxic T and NK cells following acute stress are partly, but not wholly, attributable to hemoconcentration [19]. Interestingly, a similar adjustment of helper T and B cell numbers for concomitant reduction in plasma volume revealed an active decrease in the circulating number of these cell populations during acute stress. This raises the possibility that there is a decrease in these cell subtypes attributable to the experimental stress that is normally masked by a simultaneous reduction in plasma volume. Hence, it is possible that there are more pervasive effects of acute stress than previously thought, being related to active increases in some cell types and decreases in others. The observation that passive hemoconcentration only partly accounts for acute rises in cytotoxic T and NK cell numbers, and the presence of stress-related changes in functional measures of immunity suggests that the sympathetic nervous system must also affect the immune system via more active mechanisms, such as the regulation of receptors and cytokines necessary for immune function [20]. It has also been demonstrated that activation of the sympathetic nervous system alters the expression of adhesion molecules on the surface of lymphocytes, leading to their release from the marginal pools of blood vessels into general circulation [21]. Overall, there is extensive evidence for direct anatomical and functional links between central nervous and immune systems, providing a biological pathway for the influence of stress on immunity (e.g., Refs. [22,23]).

### 3. Stability of individual differences in immune reactivity

Observations that interindividual variability of immune reactivity to acute stress reflect variability among individuals in the magnitude of sympathetic responsivity to stress, a response that is relatively stable across both time and task [17,24–26], led to our interest in whether immune reactivity is also an enduring characteristic of individuals that is trait-like in nature and may have implications for susceptibility to disease. Are some individuals' "immune reactors" who are predisposed to large immune responses to the stresses of everyday life, rendering them more susceptible to disease? To begin to determine the extent to which individual differences in the magnitude of immune reactivity reflect stable characteristics of individuals, our next study compared individual's immune responses to an evaluative speech task on two occasions of testing scheduled 2 weeks apart [11]. Test-retest correlations were significant for the magnitude of change in proliferative response to PHA ( $r=.50$ ,  $P<.005$ ), and in numbers of circulating cytotoxic T and NK cells ( $r=.53$ ,  $P<.005$ ;  $r=.42$ ,  $P<.05$ , respectively), indicating that variability in subjects' cellular immune responsivity to acute stress is moderately reproducible on retesting. Further evidence for the stability of immune reactivity comes from a second study exploring whether individuals mount similar

responses to different acute stressors [27]. In this study, subjects were exposed to a speech task and a mental arithmetic task on the same occasion of testing. Intertask correlations were significant for the magnitude of decrease in proliferative response to PHA ( $r=.76$ ,  $P<.0001$ ) and increase in the number of circulating NK cells ( $r=.46$ ,  $P<.005$ ). Taken together, these findings and those of others suggest that individuals vary consistently in the magnitude of their cellular immune reactivity to acute stress [17,28].

#### 4. Individual differences in immune reactivity and vulnerability to disease

The existence of such enduring characteristics makes it conceivable that individual differences in immune reactivity moderate associations between psychological stress and susceptibility to infectious disease. In this regard, we have hypothesized that individuals who show exaggerated immune responses to laboratory stressors exhibit similarly exaggerated reactions to everyday hassles, e.g., work demands and time pressures, rendering them more or less susceptible to infectious disease. To begin to explore this possibility, we examined whether immune reactivity predicts antibody response to hepatitis B vaccination, a real-life measure of host resistance [29]. In the initial study, 84 healthy, male and female graduate students (ages 20–35) who tested negative for prior exposure to hepatitis B virus were administered the standard series of three hepatitis B vaccinations. The first two vaccinations were given 6 weeks apart, with a follow up booster dose administered 6 months following the first shot. Five months after the first dose, each subject completed a battery of psychosocial measures, assessing levels of stress during the past 12 months, and a blood sample was drawn to assess hepatitis B surface antibody levels. Four to six weeks following completion of the vaccination series, subjects returned to the laboratory to perform an acute laboratory stress protocol, measuring immunologic responses to an evaluative speech task.

Consistent with prior findings, acute laboratory stress was associated with a significant increase in numbers of circulating cytotoxic T and NK cells, and a significant decrease in proliferative responses to PHA, Con A and pokeweed mitogen (PWM). The primary question of interest in this study was whether individual differences in the magnitude of these immune responses to acute stress were related to subjects' ability to mount an antibody response to the vaccine. In this regard, we found that, when compared with high antibody responders, subjects who mounted lower antibody responses to hepatitis B vaccination following the first two doses displayed greater stress-induced suppression of immune function, as measured by proliferative response to PHA ( $b=.000001$ ,  $P<.04$ ) (see Fig. 3). A similar pattern was observed for relationships between antibody response to the vaccination and Con-A induced proliferation; how-

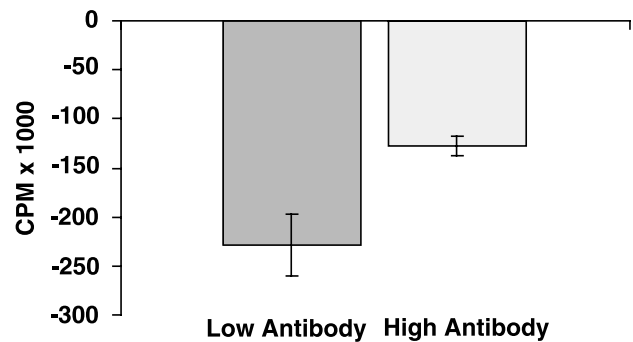


Fig. 3. Change in proliferative response to PHA from before to after an evaluative speech task among low and high antibody responders.

ever, these findings did not achieve significance. Enumerative measures and proliferative response to PWM were unrelated to antibody response. These findings lend some support to the hypothesis that individual differences in the magnitude of acute stress-induced modulation of immune function may have clinical significance, being related to an *in vivo* immune response relevant for protection against infection.

A relationship was also observed between trait negative affect, also known as neuroticism, and antibody response to the vaccine. Subjects who described themselves as having higher levels of negative affect than their peers mounted lower antibody responses to the vaccine, as measured 5 months after the initial vaccination ( $b = -.65$ ,  $P < .02$ ). These data provide an important extension of past research on psychosocial factors and immunity. To date, research has focused on demonstrating associations between state psychological measures and laboratory assays of immunity. Relations between trait characteristics and immunity have received little attention, even though there is a large literature relating trait negative affect to health (see Ref. [30] for a review). Results of this study extend previous findings to demonstrate a relationship between trait negative affect and a measure of immune function of health significance. The relationship between trait negative affect and antibody response in this study was not explained by individual differences in immune reactivity to stress. Thus, lower antibody response to hepatitis B vaccine was predicted independently by (1) high levels of trait negative affect and (2) stress-induced suppression of T cell proliferation in response to the nonspecific mitogen, PHA.

At present, the clinical significance of the observed differences in magnitude of antibody response among the high and low responders is unknown. This study was conducted using young, healthy subjects and a vaccination protocol designed to produce maximal immunity to hepatitis B in greater than 90% of individuals. Although our findings show that the majority of subjects mounted a response that is considered protective by the end of the vaccination series, subjects who mounted a low antibody response after the second vaccination had a significantly

lower final antibody response (as measured 1–2 months after the final vaccination) than subjects who mounted a high response after the second dose (mean = 92 vs. 150 mIU/ml, respectively). Given evidence that the magnitude of final antibody response is the main determinant of the duration of hepatitis B-vaccine induced immunity [31,32], subjects who showed greater immune reactivity following acute stress might be expected to have a decreased duration of immunity to hepatitis B than individuals who are less immunoreactive. To date, psychosocial factors associated with maintenance of hepatitis B antibody level have not been investigated. Stress has been associated with loss of antibody levels over time following other vaccines [33]. Thus, maintenance of antibody levels may be of greater clinical relevance than the absolute antibody level immediately following the vaccination series. It is also likely that individual differences in reactivity would have an even greater impact on vaccination response among populations who are more stressed and/or who have more vulnerable immune systems, e.g., the elderly or those with compromised immune function [34].

To summarize, it is now well established that acute laboratory stress, designed to simulate daily hassles, alters both quantitative and functional aspects of cellular immunity, although the clinical significance of these stress-induced changes has not been established. An examination of response variability reveals that individuals vary markedly in the magnitude of these cellular immune reactions to stress and there is growing evidence that this variability is relatively stable across both time and task and hence may represent a stable trait of the individual. The existence of such dispositional characteristics makes it conceivable that there is a meaningful distribution of differences in immune reactivity that may form a physiological basis for observed differences in susceptibility to infection at times of naturalistic stress. Individuals who by disposition mount large immune responses to the stresses of everyday life may be more or less susceptible to immune-related disease. Evidence from our laboratory demonstrates that the magnitude of stress-induced suppression of immune function, as measured by decreases in proliferative response to PHA, is related to an immune response relevant for protection against infection and to an antibody response to hepatitis B vaccination. However, before it can be concluded that dispositional attributes such as immune reactivity are vulnerability factors for increased susceptibility to infectious disease at times of high natural stress, prospective studies are required, employing measures of individual difference to predict disease outcome.

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