Acute stressor exposure both suppresses acquired immunity and potentiates innate immunity

MONIKA FLESHNER,1 KIEN T. NGUYEN,2 CRYSTAL S. COTTER,2 LINDA R. WATKINS,2 AND STEVEN F. MAIER2
1Department of Kinesiology and Applied Physiology and 2Department of Psychology, University of Colorado, Boulder, Colorado 80309

Fleshner, Monika, Kien T. Nguyen, Crystal S. Cotter, Linda R. Watkins, and Steven F. Maier. Acute stressor exposure both suppresses acquired immunity and potentiates innate immunity. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R870–R878, 1998.—Acute stressor exposure alters immune function. Rats exposed to inescapable tail shock stress (IS) generate less antibody to a benign, antigenic protein, keyhole limpet hemocyanin (KLH). The following studies examined the effect of IS on peritoneal cavity, spleen, and mesenteric lymph node cell number, interferon-γ (IFN-γ) production, and nitrite production. Rats were injected intraperitoneally with KLH (200 μg) or saline immediately before IS exposure and killed 0, 48, and 96 h after IS termination. KLH immunization resulted in elevated cell numbers and IFN-γ levels 2–4 days later in nonstressed control rats. In contrast, rats exposed to IS failed to increase cell number and IFN-γ levels in response to KLH. The T cell subpopulations affected were CD4 T cells, specifically the Th1-like subset. In addition, in rats exposed to IS + KLH, nitrite production was potentiated 2–4 days after stressor termination. IS had little effect on these measures in saline-injected rats. These data support the conclusion that exposure to IS suppresses the expansion of anti-KLH lymphocytes, possibly anti-KLH Th1 cells. In addition, stressor exposure potentiates the production of nitrite. Importantly, this potentiated response occurred only in KLH-immunized animals, suggesting that macrophages may be primed by stressor exposure and thus respond more vigorously to antigen. The potential links between these changes are discussed.

stress; Th1 cells; CD4 T cells; nitric oxide; keyhole limpet hemocyanin; interferon-γ

EXPOSURE TO ACUTE psychological or physical stressors can result in modulation of the immune system (see Ref. 3 for review). The changes in immune function reported depend on many factors, including the type of immunologic response measured, the cellular compartment examined, and the time between stressor termination and immune function assessment (see Refs. 19, 22, and 27 for reviews). Some commonly reported immunologic changes produced by acute stressor exposure include suppression of mitogenic and allogenic proliferative responses (7, 20), serum antibody responses (8, 11, 28, 31), natural killer cell (NK) cytotoxicity responses (1), Th1 cytokine responses (2, 10, 12, 28), and macrophage phagocytic function (32). In addition to numerous reports of immunosuppression after exposure to acute stressors, there also have been reports of immunopotentiation. Acute stressor exposure, for example, can result in increased Th2 cytokine responses (28), delayed-type hypersensitivity responses (6), and release of macrophage products, such as prostaglandin E2 (18, 25), interleukin (IL)-6 (34), and nitric oxide (NO) (4). It is possible that the increases and decreases in immune responses reported are related. Th1 and Th2 cells, for example, regulate each other in a reciprocal fashion (30), such that an increase in Th2 cytokines (primarily IL-4 and IL-10) can result in a decrease in Th1 cytokines (primarily IL-2 and interferon-γ (IFN-γ)). In addition, the macrophage products prostaglandin E2 and NO can suppress general cellular proliferation, including lymphocyte proliferation (24, 33). Thus some factors elevated by stressor exposure could be responsible, in part, for stress-induced immunosuppression. This possibility makes it particularly important to assess different aspects of immune system function after exposure to the same stressor.

A consideration of the temporal relationship between stressor exposure and the immune functions measured is also important. The neuroendocrine changes induced by exposure to an acute stressor persist from minutes to days after stressor termination. For example, exposure to an acute stressor can result in elevated serum corticosterone, altered serum proteins, and fever that persist for several days after stressor cessation (5, 9). In addition, if the immune response being measured involves in vivo antigenic challenge, this response can extend over days after antigen administration and can vary in cellular makeup (T cell, B cell, and antigen-presenting cells). Thus the following experiments were designed to examine the effect of acute stressor exposure at multiple time points and for several measures of immune function.

We previously reported a reduction in serum levels of antibody specific to keyhole limpet hemocyanin (KLH), a benign soluble protein, in rats immunized with KLH and exposed to a single session of intermittent inescapable tail shock (1; 100.6 mA, 5 s) (8, 10). This effect of IS only occurs if KLH is administered within 24 h of stressor exposure (11). The reduction persists for 3–6 wk and is caused by fewer KLH-specific B cells being formed in the spleen (19). We have proposed that the reduction in B cell formation could be due to reduced T cell help provided by CD4 T cells. KLH is a T cell-dependent antigen; thus T cell help is required to generate an antibody response. We previously reported that IS exposure suppresses the KLH-specific expansion of the CD4 T cell population. Specifically, we have reported that acutely stressed rats initially fail to expand, in vivo, the Th1-like subset (10) and, subsequently, the Th2-like subset (22). A reduction in T cell help could result in fewer KLH-specific B cells and, consequently, reduced antibody.
The mechanism(s) responsible for the stress-induced reduction in CD4 T cell help and the manner in which that reduction could contribute to fewer KLH-specific B cells remain unknown. IS exposure (4 days after KLH + IS) also reduces levels of IFN-γ, a cytokine produced by Th1 cells. The current study, therefore, examines the time course of the changes in IFN-γ after acute stressor exposure. IFN-γ is secreted by Th1 and CD8 T cells. It is involved in a variety of immunologic functions, such as upregulation of major histocompatibility molecules, IgG2a class switching, and potentiation of macrophage, NK, and CD8 T cell function (17).

In addition to suppressed T and B cell function, we have also reported that IS modulates macrophage function (7). Rats exposed to IS have a suppressed mixed lymphocyte reaction (MLR). The MLR in these experiments measured primarily CD4 T cell proliferation. If adherent macrophages were removed from culture, then IS no longer suppressed the MLR. Importantly, if adherent macrophages from a stressed rat were transferred to the MLR of a nonstressed rat, then the nonstressed rat MLR was also suppressed. Thus IS exposure stimulated adherent macrophages to secrete a factor that suppressed the proliferation of CD4 T cells (7). One possible macrophage product that could be responsible for these changes is NO. NO is a free radical gas derived from l-arginine and molecular oxygen in a reaction catalyzed by NO synthase. It has been reported to increase after stressor exposure (4) and to suppress T cell proliferation (4, 24). NO produces many different biologic effects and has been established as a major molecule involved in the regulation of immune function. One immunologic function of NO is the dissipation of cellular proliferation through several mechanisms, including disruption of mitochondrial respiration (29). This is advantageous when macrophages are stimulated by bacteria or virally infected cells, since NO will reduce the rate at which pathogens will replicate (13, 16). Unfortunately, NO is an indiscriminate effector and can suppress lymphocyte proliferation as well (4, 24, 29). This suggests that NO could be, in part, responsible for the failure to proliferate in vivo anti-KLH CD4 T cells after IS.

Thus the following experiments examined 1) the time course of IS-induced reduction in antigen-stimulated lymphocyte proliferation and IFN-γ production and 2) the specific antigen-stimulated T cell subsets affected by IS. In addition, the following experiments determined whether exposure to IS alters NO production and, if so, the time course and antigen dependency of that effect.

METHODS

Subjects. Adult male Harlan Sprague Dawley rats (250–275 g, n = 5–9/group) were maintained on a 12:12-h light-dark schedule (lights on from 0600 to 1800) in a virus-free environment. Virus-free conditions were maintained using a BioBubble air filtration system (model M501A) in the colony room as well as the treatment room. Animals were allowed 2 wk to acclimate to the colony room before experimentation, and they were handled briefly each day for 2 days before the start of the study. All animals were individually housed in metal hanging cages with standard rat chow and water freely available. Colony room temperature was maintained at 23°C. The care and treatment of the animals were in accordance with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

Stress protocol. Animals remained in their home cages as controls (HCC) or were exposed to the acute stress of IS. The IS procedure involved placing the rats in a Plexiglas restraining tube (23.4 cm long, 7 cm diameter). Electrodes were attached to the tail, and rats were exposed to 100 5-s, 1.6-mA tail shocks. The average intertrial interval was 60 s. All animals were shocked between 0800 and 1000. At the end of the IS session, animals were returned to their home cages, which were moved to the opposite side of the colony room (14 × 12 ft) to minimize stress odor exposure to the HCC animals. IS as well as HCC animals were injected intraperitoneally with KLH (200 µg) or saline immediately before the stress session.

KLH immunization. Rats were injected intraperitoneally with 200 µg of soluble KLH (lot no. 790193 in 50% glycerol, Calbiochem) in 0.5 ml of 0.9% sterile pyrogen-free saline. Saline injection. To determine the potential role of antigen in the IS-induced changes in cell numbers, nitrite, or IFN-γ, rats were injected intraperitoneally with sterile pyrogen-free saline before IS (see above) or HCC.

Cell culture procedures. Animals were briefly anesthetized with ether and killed via cervical dislocation. Rats immunized with KLH were killed 0, 2, and 4 days after stressor exposure and immunization. Saline-injected rats were killed 0 and 4 days after stressor exposure and saline injection. Peritoneal cells were removed by a lavage. Cold dissection medium [30 ml of I scove's medium (GIBCO) with 1% penicillin-streptomycin (GIBCO)] was sprayed into the peritoneal cavity, and the abdomen was briefly massaged. Peritoneal lavage fluid (20 ml) was removed, spun down (10 min, 2,000 rpm), and resuspended in 3 ml of culture medium [Iscove's medium supplemented with 1% penicillin-streptomycin, 2 nM L-glutamine (GIBCO), and 10% FCS (GIBCO)]. Mesenteric lymph node tissue and spleen were aseptically dissected from each animal and placed in 7 and 10 ml of cold sterile dissection medium, respectively. Cells were kept on ice during the entire enumeration procedure, then added to culture plates. Lymph nodes and spleen were dissociated using sterile modified glass-tissue homogenizers. Total cell numbers of all three compartments were assessed using a Coulter counter. Peritoneal cells, mesenteric lymphocytes, and splenocytes were resuspended in culture medium and added in duplicate to sterile 24-well flat-bottom culture plates (Falcon) at the following concentrations: 2.0 × 10^6 cells/well for peritoneal cells and 5.0 and 10.0 × 10^6 cells/well for mesenteric and spleen cells, respectively. Peritoneal cells were stimulated with 100 µg of lipopolysaccharide (LPS 0111:B4, lot no. 114H4063, Sigma Chemical). Mesenteric lymphocytes and splenocytes were stimulated with 5.0 µg of concanavalin A (ConA, lot no. 12H9408, Sigma Chemical). Stimulation with LPS and ConA were necessary, because nitrite is undetectable in cultures from unstimulated cells. Plates were incubated in a humidified atmosphere with 5% CO_2 at 37°C. After 48 h, culture supernatants were collected and stored at −20°C until time of assay.

NO assessment. NO production was assessed by measuring nitrite levels in each culture. Nitrite is a stable nitrogen intermediate formed from the degradation of NO in an aerobic environment. Nitrite concentration was assessed using the Griess reagent, which was prepared immediately before use. The Griess reagent consisted of a 1:1 (vol/vol)
solution of a 1% sulfanilamide (Sigma Chemical) in a 4.25% phosphoric acid solution (Mallinckrodt) and 0.1% N-(1-naphthyl)ethylenediamine dichloride (Sigma Chemical) in distilled-deionized water. A standard curve was generated with a starting concentration of 2.5 mM sodium nitrite (Sigma Chemical) in culture medium. Standards (100 µl) were serially diluted 1:2, 10 times, to obtain a standard curve. All standards were added to 96-well flat-bottom plates (Immuno- lon 4, Dynatech Labs) in duplicates. Griess reagent (100 µl) was added to each well and allowed to incubate at room temperature for 10 min. Absorbances were read at 540-nm wavelength using a microplate reader (model MRX, Dynatech Labs).

IFN-γ, IFN-γ levels were measured from ConA-stimulated cell culture supernatants using a rat-specific ELISA kit (catalog no. ASY-18, Biosource). Samples were diluted 1:200 for IFN-γ and assayed according to the manufacturer’s instructions.

**RESULTS**

Effect of IS on total cell number in KLH-immunized rats. The basic result found consistently in every compartment examined (peritoneal cells, mesenteric cells, and splenocytes) was a greater increase in total lymphocyte numbers in HCC than in IS rats 2 and 4 days after KLH (Fig. 1). As shown in Fig. 1A, the total number of cells collected from the peritoneal lavage increased after immunization with KLH \( F(2,34) = 4.9, P = 0.01 \). In rats exposed to IS on the day of immunization, there was no increase in the number of peritoneal cells \( F(1, 24) = 11.5, P = 0.002 \) in response to KLH compared with HCC animals. The same pattern of results was
found in the mesenteric lymph nodes and spleen. The total number of mesenteric (Fig. 1B) lymphocytes and splenocytes (Fig. 1C) increased after immunization, and IS prevented that increase. These observations in the mesenteric lymph node are supported by a reliable main effect of group [IS vs. HCC: F(1,36) = 10.6, P = 0.002] and a group (IS vs. HCC) × time (0, 2, and 4 days) interaction [F(2,36) = 3.2, P = 0.05]. These observations in the spleen are also supported statistically by reliable main effects of group [IS vs. HCC: F(1,36) = 13.8, P = 0.0007] and time [0, 2, and 4 days: F(2,36) = 5.7, P = 0.0072].

Effect of IS on nitrite production in KLH-immunized rats. Nitrite concentrations were measured from stimulated (LPS or ConA) cell culture supernatants, as described above. Unstimulated cultures produced very low levels of nitrite that could not be reliably measured using the Griess reagent assay. The basic result (Fig. 2) found consistently in every compartment examined (peritoneal cells, mesenteric cells, and splenocytes) was that stimulated nitrite levels were higher 2–4 days after stress than in nonstressed HCC animals. Nitrite levels from LPS-stimulated peritoneal macrophages were not overall statistically reliably increased (Fig. 2A). ConA-stimulated mesenteric lymphocytes [F(1,34) = 13.5, P = 0.0008; Fig. 2B] and splenocytes [F(1,34) = 13.5, P = 0.0008; Fig. 2C] from IS animals produced more nitrite. In mesenteric cell cultures the IS-induced elevation was greatest at 4 days of IS exposure, resulting in a reliable group (IS vs. HCC) × time (0, 2, and 4 days) interaction [F(2,34) = 6.5, P = 0.004].

Effect of IS on IFN-γ in KLH-immunized rats. ConA-stimulated mesenteric lymphocyte [Fig. 3A; F(2,29) = 4.0, P = 0.02] and splenocyte [Fig. 3B; F(2,36) = 14.5, P = 0.0001] IFN-γ levels increased after KLH immunization. Rats exposed to IS at the time of KLH immunization had overall lower IFN-γ levels produced by mesenteric lymphocytes [F(1,29) = 13.5, P = 0.001] and splenocytes [F(1,36) = 3.9, P = 0.05]. This reduction appears to be due to a failure to increase IFN-γ after KLH immunization. This is true primarily in the mesenteric lymph node cultures.

Effect of IS on total cell number in saline-injected rats. As shown in Fig. 4, no increase in total cell number was found 4 days after saline (P > 0.05), as had been found 4 days after KLH. In addition, IS exposure had
no effect on peritoneal (Fig. 4A) and splenic (Fig. 4C) total cell numbers (P > 0.05) but had a small effect on mesenteric cell number (Fig. 4B). Total mesenteric cell number was increased by KLH immediately, but not 4 days after saline [F(1,26) = 5.6, P = 0.01].

Effect of IS on nitrite production in saline-injected rats. IS exposure had no effect on peritoneal (Fig. 5A) and splenic (Fig. 5C) nitrite levels immediately or 4 days after saline (P > 0.05). Mesenteric cell (Fig. 5B) nitrite levels were reduced in saline-injected rats immediately but not 4 days after IS + saline compared with HCC [F(1,25) = 5.5, P = 0.02].

Effect of IS on IFN-γ in saline-injected rats. ConA-stimulated mesenteric lymphocyte (Fig. 6A) and splenocyte (Fig. 6B) IFN-γ levels did not change 4 days after saline injection. IS exposure did not alter IFN-γ levels in saline-injected rats (P > 0.05).

Effect of IS on T cell phenotypes. Four days after IS + KLH, IFN-γ levels produced by mesenteric lymphocytes were once again reliably reduced [F(1,9) = 10.6, P < 0.01] in stressed rats compared with nonstressed HCC animals: 6.2 ± 0.5 and 8.5 ± 0.48 ng/ml, respectively. The cellular source of the reduction in IFN-γ was CD4 T cells, specifically Th1-like cells. Phenotypic analysis (Fig. 7) of the same cultures that produced the reduction in IFN-γ supported this conclusion. In rats immunized with KLH and exposed to IS, the number of CD4 T cells (TCR+CD4+) increased immediately, but not 4 days after IS + saline compared with HCC [F(1,26) = 10.6, P = 0.01].
CD4 T cell blasts (CD134^+CD4^+) was reduced. This reduction in the cell number was specific to the CD4 T cell subset; i.e., CD8 T cells (TCR^+CD4^-) and Th2-like cells (CD45RC^+CD4^-) were relatively unaffected by IS. ANOVA revealed a reliable main effect of stress for CD4 T cells \( F(1,16) = 8.7, P < 0.01 \) as well as the Th1 subset \( F(1,16) = 4.5, P < 0.05 \). There was also a stress \( \times \) cell type interaction for the T4 and T8 phenotypes \( F(1,16) = 9.5, P < 0.01 \). There was not a reliable stress \( \times \) cell type interaction for Th1 and Th2 subsets \( P = 0.17 \). These results suggest that although stress had a much greater suppressive effect on the Th1 subset, the Th2 subset was also slightly affected. CD4 T cell activation or blast formation was also suppressed by IS \( T(8) = 2.5, P < 0.05 \).

**DISCUSSION**

Exposure to an acute, 90-min, intermittent, uncontrolled, and unpredictable stressor produced long-term changes in immune function. Development of specific immune responses to KLH was suppressed, whereas nonspecific NO levels were potentiated by acute stressor exposure. Four days after IS + KLH, stressed rats failed to increase total cell number and IFN-\( \gamma \) levels in response to antigen. Phenotypic analysis of T cell subsets revealed that the CD4 T cells, specifically the Th1-like subset, were affected by IS. In contrast, 2–4 days after IS + KLH, nitrite levels were higher in stressed rats than in nonstressed controls. Importantly, none of these changes occurred in stressed rats injected with saline. Thus in vivo antigenic challenge was necessary to produce the changes found. These data support the hypothesis that exposure to stress potentiates innate and suppresses acquired immune function (22). This supposition that stress potentiates and suppresses immune function is supported by the following observations. First, total cell number was elevated 2–4 days after KLH in the peritoneal cavity, draining lymph nodes (mesenteric) and spleen of nonstressed HCC rats (Fig. 1). Second, we previously reported that the in vivo elevation in cell number measured 4 days after KLH is primarily CD4 T cells, specifically of the Th1-like subtype (CD45RC^-CD4^+) (10). Rats that were exposed to IS immediately after KLH failed to increase total cell number (Fig. 1) and, specifically, Th1-like cell numbers 2–4 days after antigen (10). This suggests that stress disrupts in vivo proliferation of T cells in response to antigen. Third, the same pattern was found for IFN-\( \gamma \) levels (Fig. 3), thus replicating and extending our previous findings (10). The suppression in IFN-\( \gamma \) levels also lends support to the possibility that the CD4 T cells that failed to expand 4 days after antigen include the Th1 subtype, inasmuch as IFN-\( \gamma \) is a Th1 cytokine. Additional support for the hypothesis that reduced IFN-\( \gamma \) levels are due to a reduction in Th1 cell numbers can be found in Fig. 7. T cell subset analysis revealed a selective effect of IS on CD4 T cells. Specifically, the number of Th1-like cells and CD4 T cell blasts was reduced 4 days after IS + KLH, whereas T8 and Th2 cell numbers were relatively unaffected. IFN-\( \gamma \) levels were measured in supernatant of the same cells that were phenotyped and were once again reduced. In addition, no changes in total cell numbers (Fig. 4) and IFN-\( \gamma \) levels (Fig. 6) were found in saline-injected HCC rats, and, importantly, rats that were stressed immediately after saline injection also showed no changes in total cell numbers or IFN-\( \gamma \) levels. These data, therefore, support the hypothesis that stress is disrupting Th1 cell clonal expansion in response to in vivo antigen.
The effect of stress on nitrite levels was opposite to the effect on cell numbers and IFN-γ levels. Exposure to acute IS resulted in an elevation in nitrite 2–4 days after IS (Fig. 2). Interestingly, IS had no effect on nitrite levels in saline-injected rats (Fig. 5). The role of antigen in stress-induced nitrite elevation is unclear. In contrast to cell numbers and IFN-γ levels, nitrite levels remained constant 0, 2, and 4 days after KLH in nonstressed HCC rats. These data suggest that nitrite levels are not directly affected by KLH immunization. Antigen is, nonetheless, playing some role in this effect, because nitrite levels are not elevated in saline-injected stressed rats. NO is derived from several different cell types. The primary in vitro cell type, however, is the macrophage (29). The percentage of Sprague-Dawley peritoneal cells, mesenteric cells, and splenocytes that are macrophages is ~75–85, 1–2, and 2–5%, respectively (15). The nitrite levels reported here nicely reflect these relative differences, with peritoneal cells producing the most NO, mesenteric cells the least, and splenic cells an intermediate amount, producing two to five times more nitrite than mesenteric cells.

One role antigen could play in the stress-induced elevation of nitrite is that the macrophage may need to be in a primed state of activation to be receptive to potentiating stress effects. For example, in vivo KLH could stimulate the macrophage, such that stress hormones released by exposure to IS now stimulate macrophage nitrite release. There is evidence of such a mechanism. It has been reported that stressor exposure can suppress unprimed macrophage function (36) but potentiate antigen-primed macrophage function (23). The studies reported here support these findings. NO release is one indicator of macrophage function. In rats injected with only saline, NO levels were slightly suppressed or unchanged immediately after IS termination (Fig. 5), whereas in rats injected with KLH, nitrite levels were elevated (Fig. 2) when cells were taken 2–4 days after IS termination. Thus, in the presence of antigen, stress potentiated nitrite levels, whereas in the absence of antigen, stress had a negligible effect on nitrite levels. The importance of macrophage state of activation could help explain the myriad effects of stress on macrophage function.

The signal responsible for stimulating macrophage NO release has not been determined. One type of signal could be circulating hormones. For example, there is evidence that stress-induced circulating catecholamines (23) and substance P (35) could be involved. A second type of signal could involve T cell-macrophage cognate interactions. For example, there is evidence that T cells expressing CD40 ligand can help activate proinflammatory macrophage responses by binding to CD40 on the macrophage cell surface (17). The data presented here do not address this mechanism; however, the fact that elevated nitrite was greatest in the T cell-containing compartments (mesenteric lymph node and spleen) and that the percentage of CD4 T cells increases immediately after IS in an antigen-nonspecific fashion (11) may support a T cell-macrophage-signaling mechanism.

Thus the studies reported here support the conclusions that acute stressor exposure simultaneously suppresses and potentiates immune function and emphasizes the importance of examining several different time points and components of immune processes. Stressor exposure can potentiate NO release and simultaneously suppress the development of specific immune processes (anti-KLH T cell formation). Additional in vivo work needs to be done to test whether elevated nitrite levels are responsible for suppressed KLH cellular expansion. Although there is convincing in vitro evidence that elevated nitrite can suppress T cell proliferation (4, 7), the data presented here cannot support the claim that elevated nitrite was responsible for the failure to expand the in vivo anti-KLH T cell population.

Stress-induced elevation of antigen-stimulated NO could be beneficial as well as detrimental to the stressed organism. One scenario whereby antigen-stimulated NO could be beneficial is after an aggressive encounter. The stress response is triggered in response to an aggressive attack. If a skin wound were inflicted during the attack, increased NO levels in response to bacteria infecting the wound would aid in preventing infection at the site and promoting wound healing. Thus, in acutely stressful situations, elevated antigen-induced NO would be beneficial to the organism.

The detrimental effects of stress-induced elevations in NO could occur in several different ways. If, for example, an acutely stressed organism is exposed to a pathogen at the time of stress and that pathogen requires a T cell-dependent response, then elevated NO could suppress the antigen-induced proliferation of T cells, leading to diminished immunologic protection. This mechanism could be responsible for IS-induced suppression of the antibody response to KLH (8, 11). This diminished response is long lasting (3–6 wk) and reflects a reduction in a functionally important aspect of in vivo immune function. It is important to note, however, that for this detrimental aspect of NO elevation to occur, the timing between acute stressor and antigen exposure is critical. The stress-induced suppression of anti-KLH antibody responses occurs only if KLH is administered within 48 h of IS exposure (11). Thus there is a window of opportunity whereby stress-induced elevations in NO could be immunosuppressive. In the case of the anti-KLH antibody response, KLH-specific T cell proliferation occurs 2–4 days after antigenic challenge (10, 21). The elevation in nitrite is present 2–4 days (Fig. 2) and is absent 7 days (unpublished observation) after IS + KLH. Thus, if KLH is administered 24–48 h before IS, then the KLH-specific T cells would have 48–72 h to proliferate in the absence of elevated nitrite. If, in contrast, KLH is administered 24–48 h after IS, then the elevated nitrite response would be gone by the time peak KLH-specific T cell proliferation would occur (2–4 days after KLH).

A second possible detrimental effect of elevated NO is that certain disease states could be exacerbated. For
example, psoriasis is a skin disease that is exacerbated by stress (14). Recently, Morhenn (26) proposed that NO production by skin macrophages (Langerhans cells) may trigger psoriatic disease. The "antigen" responsible for inducing NO release from Langerhans cells is unclear. If, however, stressor exposure potentiates antigen-induced NO release, then this could be one mechanism whereby stress exacerbates psoriatic disease.

The results of the present series of studies add to others indicating the complex nature of stress responses and their immunologic impact. The present work contributes to mounting evidence that changes in the immune system produced by acute stressor exposure can be beneficial as well as detrimental and depend on the aspect of immune function that is assessed.

This research was supported by National Institute of Mental Health Grants MH-55283 and MH-45045. Address for reprint requests: M. Fleshner, Dept. of Kinesiology and Applied Physiology, Campus Box 354, University of Colorado, Boulder, CO 80309-0354.

Received 26 November 1997; accepted in final form 29 April 1998.

REFERENCES


