

# Psychoneuroimmunology: Animal Models of Disease

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**Objective:** Psychoneuroimmunology, which investigates the bidirectional communication between the central nervous system and the immune system, has been greatly advanced by the use of animal models. The objective of this paper is to describe animal models of disease that can or might be utilized to elucidate neural-immune interactions that alter pathogenesis.

**Methods:** This paper reviews animal studies that have demonstrated a link among the brain, behavior, immunity, and disease, highlighting models in which the potential contribution of CNS-immune interactions has not yet been explored.

**Results:** Animal studies allow for careful control of environmental stimuli, genetic background, and immunological challenge. As such, they are an important component of psychoneuroimmunology research. Models in which one might study the role of psychosocial factors in immunologically mediated disease processes, as in the case of other pathophysiologic processes, profit from an ability to manipulate both stressful events and the magnitude of the challenge to the immune system.

**Conclusions:** Animal studies in psychoneuroimmunology highlight the complexity of the interactions among behavior, the brain, the immune system, and pathogen. The genetic background of the animal (both in terms of central nervous and immune system responses), its previous history, the nature of the stressor, the nature of the pathogen and the type of immune response generated are some of the interacting factors that determine the magnitude and direction of stress-induced changes in disease outcome.

**Key words:** psychoneuroimmunology, animal models, stress, infectious disease, T helper cell subsets, behavioral conditioning.

## INTRODUCTION

In reacting against the invasion of foreign material, the immune system is critical for maintenance of health. Although once treated as a self-regulating, autonomous defense system, it is now evident that immune responses can be modulated by the central nervous system (CNS) (1). Thus, it can be hypothesized that the immune system mediates the effects of psychosocial factors on the susceptibility to and/or the progression of infectious and other immunologically mediated disease processes. Human studies provide evidence that behavioral and psychosocial factors, including stressful life experiences, are associated with altered immune function (2–4) and altered susceptibility to infection (5–8). They also document that psychological well-being (eg, social support) is associated with changes in immune function, eg, increased natural killer cell function (9, 10)

and increased survival in cancer patients (11–13). However, can we yet conclude or assume that psychosocially induced changes in the susceptibility to or progression of disease are a direct effect of the psychosocially induced changes in immune function?

As in other areas, animal studies, which enable the control of environmental stimuli, genetic background, and immunological challenge, provide important data for understanding the complex mechanisms involved in brain, behavior, immune system interactions that influence pathogenic processes. Animal studies in psychoneuroimmunology do not derive from a need to better understand a particular disease process. They are driven by a need to better understand the integrated nature of adaptive processes in general, which presumably, have implications for a variety of pathophysiological processes. Therefore, in addition to highlighting studies demonstrating a link among brain, behavior, immunity, and disease, we make note of intriguing animal models in which CNS-immune system interactions have not been explored. Early studies investigated the effects of stressful life experiences on the progression of infectious diseases, but contained no measures of immune function. We will mention some of this work but emphasize those studies that included measures of immunity—especially measures that are *biologically relevant* to the particular disease model under study.

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Animal research on brain-behavior-immune system interactions and disease have examined both cell-mediated immunity (mediated by T lymphocytes) and humoral immunity (mediated by B lymphocyte secretion of soluble antibody). In addition, many studies have examined innate, nonspecific effector functions mediated by natural killer (NK) cells and monocyte/macrophages. Also, much of the literature has focused on the role of leukocyte-derived cytokines, soluble proteins that are pivotal for inducing the growth, differentiation, and function of both antigen-specific and nonspecific effector cells.

T lymphocytes express either the cell surface antigen CD8 (a marker for T cytotoxic/suppressor cells) or CD4 (which delineates T helper cells). Recent advances in immunology include the identification of two subsets of mature T helper (Th) cells: Th1 and Th2, found in rodents and humans, differentiated on the basis of the cytokines they produce after antigenic stimulation (14–18). Th1 cells produce interleukin (IL)-2 and interferon (IFN)- $\gamma$ , cytokines that are important for the generation of cell-mediated immunity. Th2 cells produce IL-4, IL-5, IL-6, and IL-10, and these cytokines promote humoral immune responses. Presently, there are no known surface antigens that discriminate between the two subsets of T cells in mice.

Preferential skewing toward a Th1-dominant or Th2-dominant response is important for the generation of an adaptive immune response to antigens (including parasites, as well as a number of viruses). A number of stimulus factors such as antigen concentration (14, 17, 19) and host factors such as the strain of the experimental subject (20–23) influence the activation of these two subsets. C57Bl/6 mice respond to a number of intracellular pathogens, eg, with a strong Th1 cell response, resulting in a protective cell-mediated immune response (15, 20). The same antigens elicit from BALB/c mice a response that is largely Th2 cell-driven, with production of IL-4 and IL-10, resulting in a humoral immune response that is not very effective against that pathogen (20, 21). BALB/c mice are relatively sensitive to infection with the parasite *Leishmania major*, for example, but can be made resistant by treatment with anti-IL-4 antibody (20); conversely, resistant mice can be made susceptible by treatment with anti-IFN- $\gamma$  antibody (23).

Another potential variable in determining the balance between Th1 and Th2 cells is the neuroendocrine state of the animal. Studies by Daynes and others (24–26) indicate that Th1 and Th2 cell activation can be affected by at least two adrenal hormones: glucocorticoids, the classic “stress” hormones, and the weak

androgen dehydroepiandrosterone. Glucocorticoids decrease IL-2 and increase IL-4 production by antigen-stimulated lymphocytes both in vivo and in vitro. Dehydroepiandrosterone has opposite effects: enhancing IL-2 production and suppressing IL-4 production.

## AUTOIMMUNE DISEASE

### Experimental Allergic Encephalomyelitis

From a psychoneuroimmunological perspective, the best studied animal model of autoimmune disease is experimental autoimmune encephalomyelitis (EAE). EAE is a demyelinating, paralytic disease that is widely used as a model for multiple sclerosis (18, 27–42). Approximately 10 to 12 days after injection of adjuvants and myelin basic protein (MBP), emulsified guinea pig spinal cord, or the relevant MBP peptide, susceptible rats develop a progressive ascending but transient paralysis (27). EAE is an autoimmune, CNS inflammatory response, and rats that have been adoptively transferred with MBP-specific CD4+ T cells alone develop the disease (28).

In the early 1960s Levine et al. (29) and then others (30, 31) reported that multiple, prolonged sessions of physical restraint beginning before and after the induction of EAE decreased the incidence and clinical severity of disease in male and female rats. Furthermore, restraint could prevent relapses of disease that had been observed in female Lewis rats (32). Daily sessions of electric shock also decreased the incidence and severity of histological lesions in EAE-susceptible DA rats when shock was administered after, but not before, injection of guinea pig spinal cord (33). Most of these studies focused on disease onset, severity, and recovery as outcome measures, and did not contain immunological outcomes. However, other data (27, 30, 31, 33) suggest that stressful experiences affect EAE in susceptible strains of rats via direct effects on the immune system.

The effects of stressful experiences on EAE seem to be mediated in part by glucocorticoids. Exogenous glucocorticoid administration was associated with decreases in clinical severity of the disease (eg, 34). Lewis rats with signs of EAE had elevated serum corticosterone levels, and recovery from disease began when corticosterone levels were at their peak (35). Also, adrenalectomy inhibited the spontaneous recovery of Lewis rats from EAE. EAE-resistant PVG rats developed EAE after adrenalectomy (36). A high dose of the opioid methionine-enkephalin also inhibited clinical symptoms of EAE and reduced histological lesions; a low dose, however, yielded op-

posite effects (37). Glucocorticoid receptors are present in lymphocytes (42) and opioid receptors have been found on some lymphoid cells (43), but it is not clear whether these neurochemical changes in response to stressful experiences influence the development of EAE via direct effects on lymphoid cells.

Recent studies suggest that EAE is mediated by Th1 cells. MBP-specific Th1 cells can induce the disease, whereas Th2 cells specific for the same peptide-MHC complexes can not (38). During the peak of the disease, IL-2, IFN- $\gamma$ , and tumor necrosis factor (TNF)- $\alpha$  (Th1 cytokines) are present in CNS tissues, but IL-4 or IL-10 are not (39). During the recovery phase, IFN- $\gamma$  messenger RNA (mRNA) is absent and IL-10 mRNA expression becomes dominant (28). In rats made tolerant to MBP, there is high expression of IL-4, but not IL-2 or IFN- $\gamma$ , in the CNS (28). In addition, spontaneous recovery from the disease is associated with production of MBP-specific antibody (40). Serum from rats recovering from EAE can inhibit disease when given to rats at the time of immunization with MBP, and there is an inverse relationship between anti-MBP antibody and the severity of EAE (40, 41).

Thus, there is good evidence that the onset of and recovery from EAE is orchestrated by Th1 and Th2 cytokine production. There is substantial evidence that stressful life experiences, acting via glucocorticoids and/or opioids, can decrease the clinical severity of the disease, as well as histopathological lesions and production of at least one Th1-derived cytokine, IL-2 (30). Future studies should determine if and how stressful life experiences influence the Th1/Th2 balance in autoimmune disease.

### Arthritis

Rheumatoid arthritis (RA) is the most common inflammatory polyarthritis and the best recognized of the rheumatic diseases. The etiology and pathogenesis of RA is largely unknown but there is considerable evidence of CNS involvement in this disorder. Consistent with its susceptibility to EAE and the relative dominance of Th1 compared with Th2 responses to the immunologic challenge, the Lewis rat is also highly susceptible to an arthritis induced by subcutaneous injection of adjuvant containing either *Mycobacterium tuberculosis*, streptococcal cell wall (SCW), or collagen (44–50), inducing an inflammatory response that mimics many features of human RA.

Both the human disease and the rat arthritis are

affected by stressful life experiences. Clinical studies in humans indicate that stressful events are associated with the onset and/or an increased severity of RA (52, 53); in rats, stressful experiences are associated with decreases and increases in disease severity. Rogers et al. (44) reported that exposure of outbred Wistar rats to a cat, or just transportation and handling decreased the incidence and severity of collagen-induced arthritis. In contrast, exposure to 90 to 95 decibels of white noise before and after injection of collagen increased the severity of arthritic symptoms. Amkraut et al. (54) found that the severity of adjuvant-induced arthritis was increased in group-housed compared with individually housed male Fischer rats and that handling had no effect on the course of the disease. In neither case were there any treatment effects on serum protein, corticosteroid, or fibrinogen levels. Thus, although psychosocial factors can affect arthritis in susceptible subjects, the nature of the experiences and the nature of the experimental subject are important factors for predicting the direction of such effects.

In addition to stress-induced alteration in arthritis, there is evidence that behavioral conditioning can modify the response to adjuvant-induced arthritis. Klosterhalfen and Klosterhalfen (55, 56) found that a conditioned stimulus (CS) previously paired with immunosuppressive drugs could inhibit the development of arthritic swelling in Wistar rats. Lysle and his colleagues (45) observed that male Lewis rats previously exposed to electric footshock had an aversive response to the CS–reexposure to the shock chambers. Reexposure to the CS 12 to 16 days after induction of arthritis reduced the severity of arthritis compared with nonconditioned rats and rats reexposed to the CS soon after the injection of adjuvant. This conditioned effect was abrogated by injection of the  $\beta$ -adrenergic receptor antagonist propranolol before to reexposure to the CS.

Glucocorticoids are the most conspicuous of the neuroendocrine responses that influence inflammatory responses. Sternberg et al. (46, 47), Aksentijevich et al. (48), and Zelazowski et al. (49, 50) have focused on the role of abnormal behavioral and neuroendocrine responses in the susceptibility of female Lewis rats to SCW-induced arthritis. In addition to an attenuation of corticosterone responses to the injection of SCW or a variety of physical/psychosocial stressors, Lewis female rats had lower levels of plasma adrenocorticotrophic hormone, as well as corticotropin-releasing hormone mRNA in the hypothalamic paraventricular nucleus compared with histocompatible Fischer rats which show only a mild and transient form of the disease (46, 47). These investi-

gators hypothesized that the decreased HPA axis reactivity of the Lewis rat results in their susceptibility to inflammatory stimuli, whereas the intact HPA response of the Fischer rats is protective against the same inflammatory challenge. Fischer rats treated with a corticosterone receptor antagonist develop the disease; and Lewis rats treated with glucocorticoids become resistant to SCW-induced arthritis. Adrenal responses differentially affect the release of Th1 and Th2 cytokines (24–26), but it is not yet clear what the precise immunological target(s) for the HPA response are in this model of arthritis. In addition to the effects of adrenal responses in autoimmune inflammatory disease in both humans and rodents, greater involvement of distal joints is correlated with the density of substance P-containing afferent nerve fibers to these areas, focal neural lesions alter the bilateral symmetry of the disease, and arthritic inflammation does not occur in the limb paralyzed by a prior CNS lesion (51). Thus, neurotransmitter release in joints provides another pathway through which the nervous system contributes to the pathogenesis and severity of inflammatory diseases.

### Systemic Lupus Erythematosus

Although there are several animal models of systemic lupus erythematosus, none have been used to any great extent in assessing the role of behavioral or psychosocial factors in the development or progression of this autoimmune disease. The majority of the behavioral research in humans and in animals has concentrated on the emotional and cognitive deficits that are reported to accompany the disorder. The work of Forster and Lal (57), for example, has addressed the role of brain reactive antibodies in the learning behavior of lupus-prone mice. Because of the immunological changes that occur during the relatively brief life span of these animals, lupus-prone mice are also used as an experimental model of the aging process.

Some notable but incomplete exceptions are the studies of Ader and his colleagues in which conditioning operations were used in the pharmacotherapy of female New Zealand hybrid mice (58–60). Saccharin consumption was paired with cyclophosphamide and, in experimental animals, only saccharin plus intraperitoneal injections of saline (the CS) were presented on half of the weekly pharmacotherapy (conditioning) trials. As hypothesized, the development of lupus and mortality were delayed by a cumulative dose of drug that was not, by itself,

sufficient to alter the progression of autoimmune disease. When chemotherapy was discontinued, mice that received neither CS or UCS presentations died relatively rapidly. However, mortality rate in conditioned mice that continued to be exposed to the CS approximated that of mice that continued to receive active drug. Lupus-prone Mrl-lpr/lpr mice with manifest symptoms of disease were observed, over time, to voluntarily consume more of a distinctively flavored drinking solution containing cyclophosphamide than asymptomatic congenic control animals (61), suggesting that the lpr mice learn that the distinctively flavored drinking solution is alleviating peripheral manifestations of the disease or correcting their immunologic dysregulation. The signals to which these animals are responding and the measurement of immune responses that are biologically relevant to the onset and progression of autoimmune disease need to be explored in these genetically susceptible animals.

### Insulin-Dependent Diabetes Mellitus

Insulin-dependent diabetes mellitus (IDDM, Type I diabetes) is an autoimmune disease targeting the insulin-producing  $\beta$  cells of the Islets of Langerhans, resulting in loss of insulin production and excessive accumulation of glucose and fatty acids (62). It has been accepted for many years that stressful life experiences can influence the onset or exacerbation of human Type I and Type II diabetes (63, 64). Such studies have been conducted with animals, but there are virtually no data on immune responses relevant to the development or progression of IDDM. Multiple, concurrent stressors (restraint, rotation, crowding, and random rehousing in group cages) result in an earlier onset of IDDM in the susceptible BB Wistar rat compared with control animals (65). Lehman et al. (66) investigated the effects of three randomly administered stressors on the age of onset and percentage of BB rats developing IDDM. Eighty percent of the males and 70% of the females that were stimulated over a period of 6 weeks developed disease compared with 50% of either control group; no differences were found in the age of onset of symptoms.

Using female nonobese diabetic (NOD) mice, Durrant et al. (67) reported that chronic restraint or "overcrowding" (20 vs 10 mice/cage) delayed the onset of disease. One session of restraint each week decreased insulinitis and islet destruction; in contrast, "overcrowding" was associated with increased insulinitis and destructive islet lesions. This study in-

cluded measurement of spleen and thymus weights, as well as the response to T and B cell mitogens. No differences in these immune measures were noted. It is not clear, however, that changes in T or B cell responses to mitogens are of any predictive value for autoimmune processes in the pancreas. It should be noted that other investigators have not found an effect of housing density (one, five, or eight mice/cage) on disease parameters in NOD mice, but have noted that both high emotionality and housing in a cage on the top of the rack in the animal colony are associated with delays in the onset of disease (67).

Animal studies of IDDM have not established a clear-cut relationship between stressful experiences, changes in immune function and disease outcome measures. Nevertheless, IDDM may be an appropriate model for evaluating the immunologic mediation of the effects of psychosocial factors on diabetes. There is good evidence that Th1 cells are involved in IDDM in humans and in animals. First, a large number of IFN- $\gamma$ -secreting cells are found in the islets in autopsies of human pancreas (18). Second, spontaneous diabetes can be prevented in adult thymectomized and irradiated BB rats by transfer of Th2 cells (18). Third, glutamic acid decarboxylase, a key  $\beta$  cell antigen that is recognized by T and B cells in NOD mice, stimulates production of large amounts of IFN- $\gamma$  by T cells from NOD mice (38). Finally, anti-IFN- $\gamma$  antibodies or systemic administration of IL-4 can prevent the development of diabetes in NOD mice (38). Thus, studies aimed at determining the effects of stressful life experiences on IDDM could profitably address changes in Th1 and Th2 cells and the cytokines that they produce in diabetes-susceptible strains of mice and rats.

### INFECTIOUS DISEASE

There is ample literature describing the effects of a variety of stressors on the course of or resistance to a variety of infectious diseases (69). Most of the early studies, however, did not include measures of pathogen-specific immune function. In one of the first studies of stress-induced changes in anti-viral immunity, Yamada et al. (70) subjected female SW mice to avoidance conditioning in a shuttlebox apparatus for 2 or 15 days before inoculation with vesicular stomatitis virus. There were no experimentally induced changes in antibody titers, but muscles from the stimulated mice contained significantly more virus than the muscles of control mice. In a subsequent study interferon activity was depressed in the brains of the animals subjected to avoidance

conditioning (71). The cellular derivation of this brain-derived interferon was not specified, and cell-mediated responses, which are critical for recovery from intracellular viral and other pathogenic infections, were not measured. In an earlier experiment (72), this same avoidance conditioning experience was associated with prolonged skin allograft survival (suggesting decreased cell-mediated immunity) in SW mice.

In another early study (73), adult BALB/cJ mice subjected to periodic electric footshock plus inoculation with Coxsackie virus showed symptoms of disease that did not occur in animals that experienced the same stressful stimulation or the same virus inoculation alone. Forced swimming increased mortality in Coxsackie-virus infected rats (74), and, in Coxsackie-infected mice, Reyes and Lerner (75) found that forced swimming resulted in a 75-fold increase in virus titers in sera and a 1000-fold increase in the heart (Coxsackie virus causes myocarditis). In both groups, however, virus was absent by day 13 postinfection. The kinetics of the antibody response to the virus were similar in both groups, but the magnitude of the response was lower in the exercised compared with the control rats. Interferon titers in unmanipulated animals peaked on day 2 postinfection and waned quickly thereafter, whereas interferon titers in the exercised rats peaked on Day 4 and remained high until 9 days postinfection. Thus, there seemed to be a relationship among the delay in the interferon response, a decrease in antibody responses, and increased infectious virus.

In a study of the effects of differential housing on responses to vaccination with thyphoid-paratyphoid vaccine or challenge with the LD<sub>50</sub> of the bacterium *Salmonella thyphimurium*, a higher percentage of mice caged 2 or 10 per cage compared with mice housed either 30 or 60 per cage showed an antibody response to the vaccine (76). Among the "responders," however, levels of antibody were similar regardless of caging condition. Also, significantly more of the mice caged 30 or 60 per cage died after infection with *S. thyphimurium* compared with mice grouped 2 or 10 per cage. Thus, "crowding" decreased the percentage of mice producing protective antibody responses to a bacterial vaccine preparation and increased mortality after infection with live bacterial challenge. These data suggest that mortality was inversely related to antibody titer, but the correlation between antibody titers and mortality was not calculated.

Rabin et al. (77, 78) investigated the effects of housing on resistance to an intravenous injection of the yeast *Candida albicans*. Male C3H/HeJ mice

housed five per cage for 10 days had lower resistance to the organism than individually housed mice. Although *Candida*-specific immune responses were not measured in these mice, antibody titers to sheep red blood cells were suppressed in group-housed mice. These effects were not observed in mice that were differentially housed for 25 days, suggesting that there was habituation to the housing conditions. Differential housing did not alter responses to either *C. albicans* or sheep erythrocytes in female C3H/HeJ mice and male and female C57Bl/6 mice (78)—emphasizing, again, the role of host factors such as gender and strain in determining the response to potentially pathogenic stimuli.

#### Mycobacterial infection

Recent reports (79–81) suggest that the incidence of *Mycobacterium tuberculosis* and *M. avium* in humans is rising, due in part to increased infection in individuals infected with human immunodeficiency virus. However, other environmental and psychosocial factors are also important. To determine the contribution of stressful life experiences to infection with mycobacteria, the effects of physical restraint and HPA axis activation on the response to infection with *M. avium* was examined in resistant and susceptible congenic strains of BALB/c mice (79–81).

Restraint (18 hour/day for 1, 5, or 10 days) resulted in a significant increase in mycobacterial growth in both the spleen and lungs of BALB/c.*Bcg*<sup>s</sup> (disease sensitive) male mice, but not in the organs of congenic BALB/c.*Bcg*<sup>r</sup> (disease resistant) animals (79). There were no differences in corticosterone levels after a single restraint session, but a single session of restraint could suppress the expression of major histocompatibility complex antigens by macrophages from sensitive, but not resistant, mice (80). Mycobacteria are ingested by phagocytes during the course of infection, and splenic macrophages obtained from restrained sensitive (but not resistant) mice were less protective against the *in vitro* growth of *M. avium*. These investigators also demonstrated that adrenalectomy abolished the effects of restraint on mycobacterial growth: replacement of basal concentrations of *d*-aldosterone and epinephrine plus high levels of corticosterone partially restored the increase in mycobacterial growth. High levels of corticosterone did not affect the growth of *M. avium* in resistant mice. Daily administration of the glucocorticoid receptor antagonist RU-486, beginning 2 days before restraint, fully abrogated the effects of

restraint on mycobacterium growth (79). Thus, it is clear that stressful life experiences, mediated at least in part by corticosterone, can contribute to the growth of mycobacterium in sensitive mice. Interestingly, in both resistant and susceptible mice, restraint suppressed production of TNF- $\alpha$  and nitric oxide. These are two potent microbicidal substances produced by macrophages in response to stimulation of splenic macrophages with IFN- $\gamma$  and lipopolysaccharide, suggesting that these parameters of immunologic reactivity are independent of the gene that determines sensitivity to mycobacterial infection. Thus, some of the functions of macrophages from both substrains of mice are responsive to stressful life experiences and HPA axis activation.

#### Influenza Infection

Influenza epidemics cause millions of people to be ill each year, and the flu is especially pathogenic in the elderly. A number of studies of the effects of stressful life experiences have been performed in mouse models of influenza, a particularly well-characterized model for upper respiratory infections. Chetverikova et al. (82) examined the effects of 6 hour of physical restraint on the response to influenza in male CBA or (CBA  $\times$  C57Bl/6) F<sub>1</sub> mice. Immobilization 1 day, but not 5 days, before virus inoculation suppressed *in vivo* production of interferon. In addition, restraint 1 day before infection with a lethal concentration of virus significantly accelerated the mortality rate.

In more recent studies, Sheridan et al. (69, 83) and Hermann et al. (84) have shown that restraint can modulate the influenza-specific immune response in mice. C57Bl/6 mice were infected intranasally with the PR8 strain of influenza (83). Restraint imposed for 16 hours/day for 1 day before and 7 days after infection decreased pulmonary inflammation compared with controls, and the restrained mice had increases in plasma corticosterone measured during the restraint period on day 7 postinfection. Restraint for 1 day before and 14 days after infection decreased virus-specific *in vitro* IL-2 production for at least 25 days after the last cycle of restraint. The effects of restraint on IL-2 production by spleen or mediastinal lymph node cells could be produced by four or more sessions of restraint, but not fewer. No effect of restraint was observed on the magnitude or class of the anti-influenza antibody response, but the kinetics of the response seemed to lag in restrained animals (69). The restraint-induced suppression of cell-mediated antiviral processes was associated

with reduced inflammatory responses and with increased levels of corticosterone that could have mediated the reduced inflammatory response directly via its effects on lymphocyte proliferation and trafficking (83).

In a subsequent study (84), C57Bl/6, DBA/2, and C3H/HeN male mice were infected intranasally with the LD<sub>50</sub> of the PR8 strain of influenza. Experimental mice were restrained for 12 hours/day for 1 day before and 10 days after infection. This protocol was associated with increased corticosterone levels in all three strains of mice on day 10 postinfection, and, overall, DBA/2 mice had higher corticosterone levels than the other two strains. Restraint decreased survival only in the DBA/2 strain and was not associated with changes in virus titer in lung tissue or with pulmonary inflammation on day 10 in any of the mice. Restraint did suppress virus-specific IL-2 production in all three strains on day 10 postinfection, but IgG anti-influenza titers were unaffected. These studies highlight, again, the role of genetic factors in determining the effects of stressful life experiences on pathophysiologic processes and the value of examining strain differences in behavioral and neurochemical responses to stressful experiences in attempting to understand the possible immunological mediation of such effects.

#### Herpes Simplex Virus Infection

The same experimental manipulation that Bonneau, Sheridan and colleagues (85, 86) used in studying the response to influenza has also been used in a model of herpes simplex virus (HSV)-type 1 infection (87). Immobilization for 16 hr/day for a varying number of days reduced anti-HSV cytotoxic T lymphocyte (CTL) responses in C57Bl/6 male mice, diminished natural killer cell activity, increased infectious virus in the hind footpads (the site of infection), reduced popliteal lymph node cellularity, and suppressed the ability to activate CTL memory cells (85). In a subsequent study (87), mice were implanted with time-release pellets containing the nonselective  $\beta$ -adrenergic receptor antagonist nadolol 7 or 9 days before infection. Nadolol did not restore the suppression in lymph node cell number in restrained mice; it did, however, partially restore CTL activation in lymph nodes from restrained mice, suggesting a role for catecholamines in the restraint-induced suppression. To determine the contribution of the restraint-induced corticosterone elevation to the observed immunosuppression, restrained and control mice were injected daily with the glucocor-

ticoid receptor antagonist RU-486. RU-486 abrogated the restraint stress-induced suppression of lymph node cellularity but not the suppression of the CTL response. Combined  $\beta$ -receptor and glucocorticoid receptor blockade fully restored both the lymph node cell numbers and the CTL activity of popliteal lymph node cells, indicating that both catecholamines and corticosterone play a role in regulating the cell-mediated immune response to HSV.

Our laboratory had established the generality of stressor-induced suppression of both cell-mediated and humoral immunity to HSV by exposing C57Bl/6 female mice to mild foot shock (88). Mice were shocked once every 30 minutes during the dark portion of the light/dark cycle beginning 1 day before and continuing for 7 days after infection with HSV. The numbers of leukocytes in spleen and draining popliteal lymph nodes of shocked mice were suppressed compared with either "apparatus" or "home cage" (unmanipulated) control mice. In addition, CTL responses in both organs were depressed, as was serum IgM anti-HSV antibody titer in the foot shocked group. These changes were associated with significantly increased viral titers at the site of infection (the hind footpads). The diminished primary immune response to HSV might lead to a greater degree of latent virus in the sensory neurons. Although reactivation of latent HSV in mice is problematic, HSV remains an attractive model for studying the impact of psychosocial factors on the reactivation of latent infectious diseases.

#### Acquired Immunodeficiency Syndrome

Considerable attention is being paid to the psychosocial factors that may affect the progression to acquired immunodeficiency syndrome (AIDS) in human immunodeficiency virus-infected individuals. A few papers have noted correlations between psychosocial factors and relevant immune measures (89, 90), but it has been difficult to establish a reliable association among psychosocial factors, immune status, and AIDS progression (91).

Again, the progression of this disease may be related to the relative balance of Th1 and Th2 cells that may be regulated to some extent by psychosocial factors. Clerici and Shearer (92) and their colleagues (93, 94) suggest that dominant Th1-like responses are associated with maintenance of robust cell-mediated immunity and a lack of seroconversion to the antibody-positive state in infected individuals. These investigators hypothesize, and there is some evidence to suggest, that at some (highly variable)

time after infection, there is a shift in the balance of cytokine production by Th cells: high levels of IL-4 and IL-10 are produced and there is a corresponding suppression of Th1 cell activity and antiviral cell-mediated immunity. Unknown cofactors are presumed to potentiate the switch from Th1 to Th2 dominance.

There are data to indicate that the neuroendocrine changes associated with stressful experiences can influence the production of Th1- and Th2-derived cytokines. BALB/c mice that are exposed to pheromones emitted by foot-shocked conspecifics over a 24-hour period have depressed cell-mediated immune function and IL-2 production in vitro, indicating that Th1 cell function is decreased (95). Consistent with the purported effects of Th1 and Th2 function, "stress odor" exposure is also associated with enhanced antibody production in response to immunization with the protein antigen keyhole limpet hemocyanin (KLH), as well as enhanced antigen-stimulated production of the Th2-derived cytokine IL-4 (96). Different environmental conditions, however, may not affect the same changes (or any changes) in cytokine production. It is of some interest and importance, perhaps, that compared with group-housed animals, there is increased production of IL-2 in singly housed C57Bl/6 mice, and an increased production of IL-4 in singly housed BALB/c mice (97). Thus, this manipulation results in up-regulation of the reported dominant cytokine produced by each strain (14–17). However, contrary to predictions that would follow from the hypothesized relationship between Th1 and Th2 functions, IgM antibody responses to KLH were higher in the group than the individually housed mice of both strains. IgG anti-KLH antibody responses were higher in individually housed C57Bl/6 mice compared with group-housed C57Bl/6 mice. The pattern of neural and endocrine changes associated with stressful life experiences may influence changes in Th1 and Th2 cell activity and several other components of immune effector function. It is not unreasonable, however, to hypothesize that stressful experiences may be an important factor in the Th1-Th2 switch that occurs in AIDS.

One of the best murine AIDS (MAIDS) models is induced by infection with the LP-BM5 retrovirus mixture (98–100). Infection is associated with a switch from the Th1 to Th2 dominant responsive state during the course of the ultimately fatal disease (98, 99). IL-4-deficient ("knock-out"), virally-infected mice survive infection with LP-BM5, indicating that production of this Th2 cell-derived cytokine is of primary importance for disease progression (100).

Some stressful life experiences should decrease survival time in LP-BM5 infected mice, and that decreased survival should be associated with more rapid transition from Th1- to Th2-like cytokine production.

#### Peptic Ulcers and *Helicobacter pylori*

It has been established that the bacterium *Helicobacter pylori* is associated with virtually 100% of duodenal ulcers in humans, and with 58 to 100% of gastric ulcers (101). Infection with *H. pylori* and development of chronic gastritis is extremely common; 50% of humans have been infected by the age of 65. Most infected individuals are asymptomatic, but a small percentage subsequently develop peptic ulcers. Several factors have been proposed to affect the development of ulcers in the infected population, including age, sex, family history, and smoking. Stressful life experiences have long been considered to negatively affect ulcer formation, principally via gastric secretions. Perhaps, stressful life experiences affect *H. pylori* infection via alterations of immune function.

Clearly, infection with *H. pylori* is necessary but not sufficient for ulcer formation. Research into ulcer development and assessment of risk factors has been hampered by the lack of appropriate animal models since laboratory strains of the bacterium do not infect rodents. Recently, however, mice were infected with fresh clinical isolates of type I *H. pylori* (which is correlated with ulcer formation), resulting in gastric pathology that resembled the findings in humans (102). This mouse model of infection might provide a controlled paradigm in which to examine the contribution of psychosocial factors to the variable pathogenicity of *H. pylori* in ulcer formation.

#### TUMOR DEVELOPMENT AND METASTASES

Numerous studies have examined psychosocial factors in relation to the susceptibility to naturally occurring and experimentally induced tumors (eg, 103–105); but only a few of these have examined tumor growth or metastases in relation to immune changes. Ben-Eliyahu et al. (106) found that forced swimming increased lung metastases and decreased NK cell lysis in male Fischer rats injected with the NK cell-sensitive MADB106 mammary adenocarcinoma line. Lung metastases were affected only when rats were forced to swim 1 hour before tumor injection, not 24 hours before or after. The opioid antag-



onist naltrexone did not alter the effects of swimming. Using surgery as the stressor (107), similar increases in metastases were observed; analgesic doses of morphine administered postsurgery attenuated the effect.

Brenner et al. (108) examined the effects of simply handling mice on lung metastases and NK cell activity. BALB/c.ByJ female mice that were simply handled for 2 min/day for 2 weeks before injection with the alveolar carcinoma line 1 (another NK cell-sensitive tumor) had increased numbers of lung metastases compared with control mice. There were no changes in NK cell activity *in vitro* or *in vivo* and handling did not affect CTLs generated after priming of mice with irradiated tumor cells. Thus, the increase in tumor metastases is not associated with an observed change in specific or nonspecific cytotoxic responses.

## ATHEROSCLEROSIS

The effects of psychosocial factors on the development of coronary heart disease are, presumably, mediated by sympathetic activation (109). The mechanisms by which sympathetic activity influences atherosclerotic lesions might involve the immune system. Within atherosclerotic lesions there is evidence of immunoglobulin and complement, activated T cells, mononuclear phagocytes, and production of numerous proinflammatory cytokines such as IL-1 and IL-6 (110). Wick et al. (110) speculate that atherosclerosis may result from cellular and humoral autoimmune responses that are triggered by the expression of heat shock proteins (HSPs) by endothelial cells in response to stressful stimuli, infection, and other insults that stimulate sympathetic activity. Injection of rabbits with Hsp65 result in atherosclerotic lesions that are exacerbated by a high cholesterol diet. T cell lines derived from human atherosclerotic lesions have higher reactivity to HSPs than T cells from peripheral blood, further strengthening the link between an immune response to HSPs and atherosclerosis. In addition, reactivation of cytomegalovirus and expression of the p53 protein may be important in restenosis after bypass surgery and/or angioplasty (111). These may be interesting models in which to examine behavior-neuroendocrine-immune system interactions.

There is a clear involvement of psychosocial factors in coronary heart disease—and some speculation that some aspects of heart disease may be immunologically mediated. It remains to be determined if psychosocial factors and sympathetic activation

have an effect on the reactivation of latent virus or the hypothesized autoimmune response leading to atherosclerosis. Rodent models have not yet been developed to study these issues, however, when they are available, cardiovascular disease could become another avenue of research in psychoneuroimmunology.

## CONCLUDING REMARKS

This paper is intended to provide a general overview of animal models in psychoneuroimmunology and disease pathogenesis, with some speculation about potential areas for future research. Most of the data on psychosocial factors and immunologically mediated disease processes come from studies of the effects of stressful life experiences and, like all such research, the effects are complex. The genetic background of the animal (both in terms of central nervous and immune system responses), its previous history, the nature of the stressor, the nature of the pathogen and the type of immune response generated are some of the interacting factors that determine the magnitude and direction of stress-induced changes in disease outcome.

Given the critical nature of the immune system in providing defense against pathogens, it is highly adaptive for an animal to have evolved redundant mechanisms for dealing with such invasion. Considering its primary functions, it is also adaptive that the changes in immunity elicited by psychosocial circumstances or stressful experiences do not exceed the range of normal values. Animal models in which one might study the role of psychosocial factors in immunologically mediated disease processes, as in the case of other pathophysiologic processes, profit from an ability to manipulate both stressful events and the magnitude of the challenge to the immune system. This enables one to study the interaction between these variables which, in all likelihood, is the way psychosocial factors influence disease susceptibility in the real world. Animal studies also enable one to model the hypothesis that the effects of stressful experiences on immune function may be especially negative for people who are immunologically compromised—including the elderly, patients undergoing chemotherapy, and HIV-infected individuals.

Finally, studies of behavioral conditioning using rodent models suggest that behavioral tools may be of clinical use (58–61). For one example, conditioning might be used to decrease the amount of immunosuppressive drugs administered to transplant pa-

tients (112, 113) or to patients with autoimmune diseases (114). More generally, the clinical implications of psychoneuroimmunology will unfold when more is known about the interrelationship between the brain and the immune system. Accepting the notion that the immune system is part of an integrated homeostatic network that influences and is influenced by neural and endocrine functions removes what could be viewed as a limitation of a disciplinary approach to the pathogenesis and treatment of a variety of disease states. Animal models will be a critical component in the development of interdisciplinary research.

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## ANNOUNCEMENT

### Behavioral Medicine Postdoctoral Research Fellowships

Behavioral medicine postdoctoral research fellowships available at the University of Pittsburgh. Program includes didactic training in physiology/psychophysiology, cardiovascular disease/pathophysiology, principles of behavior and behavior change, and research methods and statistics. The program runs for 1-3 years with stipends at current NIH levels of support. Must be a U.S. citizen or noncitizen national in accordance with NIH regulations for a NRSA fellowship award. Majority of training is in the laboratory with training faculty, including Drs. Karen Matthews (Training Director), Matthew Muldoon (Co-Director), Sarah Berga, Jacqueline Dunbar-Jacob, Rolf Jacob, J. Richard Jennings, Thomas Kamarck, Stephen Manuck, Marsha Marcus, Robert McDonald, Kenneth Perkins, Robert Robertson, Michael Scheier, Saul Shiffman, Alvin Shapiro, Thomas Smitherman, Paul Thompson, and Rena Wing. Send statement of objectives, curriculum vitae and three letters of recommendation to Karen Matthews, PhD, Department of Psychiatry, University of Pittsburgh, 3811 O'Hara Street, Pittsburgh, PA 15213 or call (412) 624-2041.