Published in final edited form as:

Ann N Y Acad Sci. 1998 May 1; 840: 803–808.

Stress-Induced Neuroendocrine Modulation of Viral Pathogenesis and Immunity^a

JOHN F. SHERIDAN^{b,c,d,e}, CATHLEEN DOBBS^c, JAEHO JUNG^c, XIAOHONG CHU^b,
ALEXANDRIA KONSTANTINOS^c, DAVID PADGETT^{b,c}, and RONALD GLASER^{c,d}

^bDepartment of Oral Biology, College of Dentistry,

^cDepartment of Medical Microbiology and Immunology, College of Medicine,

^dInstitute for Behavioral Medicine Research, The Ohio State University Health Sciences Center, Columbus, Ohio 43210 USA

Abstract

Physical restraint (RST) was used to examine the interactions among the hypothalamic-pituitary-adrenal (HPA) axis, sympathetic nervous system, and the immune response to infection. In these studies, mice were infected with either herpes simplex virus (HSV) or influenza A/PR8 virus so that the impact of neuroendocrine activation could be assessed on disease pathophysiology and anti-viral immunity. RST suppressed lymphadenopathy in draining lymph nodes, reduced mononuclear cellular infiltration in the lungs, and suppressed virus-specific cytokine and cytolytic T-cell responses. Blockade of type II glucocorticoid receptors (by RU486) restored cellularity and cytokine responses to both organs in restraint-stressed, infected mice. Thus, the HPA axis modulated cell trafficking and T-cell cytokine responses. However, RU486 treatment failed to restore cytolytic T-cell responses. Blockade of β -adrenergic receptors (by nadolol), in combination with RU486 treatment, fully restored cytolytic T-cell responses, suggesting that catecholamines were involved in suppressing the virus-specific CD8⁺ cytolytic T-cell response. RST also modulated the local development or expression of antibody-secreting cells (ASC) in the lungs draining lymph nodes, and spleen following infection of restrained mice. RST significantly suppressed the number of virus-specific ASC (IgM, IgG and subclasses IgG1 and IgG2a) in the lungs, mediastinal (MLN) lymph nodes and spleen, while it enhanced the responses in the superficial cervical (SCV) lymph nodes. This observation of differential modulation of ASC responses in the MLN and SCV lymph nodes supports the concept of tissue-specific immunoregulation in response to stress.

The host's response to a viral infection is designed to limit the initial spread of the pathogen and then terminate its replication. Innate host mechanisms, such as natural killer (NK) cell activity and proinflammatory cytokines (IL-1, IL-6, and TNF), along with the α and β interferons, function in the early hours of infection to limit the spread of virus. These noncognate responses, however, usually are not sufficient to end viral replication. Therefore, adaptive host responses occur that take days to weeks to mature. These adaptive responses promote the entry of antigen-specific lymphocytes into the cell cycle and result in clonal selection, proliferation, and maturation to the effector stage. Because many viruses are tropic to and replicate in nonlymphoid tissues, effector cells must traffic to the site of virus replication to effectively eliminate the pathogen.

^aThis research was supported in part by grants to J.F.S. from the National Institute of Mental Health (RO1-MH46801 & T32-MH18831), the National Institute on Aging (PO1-AG11585), and the John D. and Catherine T. MacArthur Foundation.

^eAddress for correspondence: John F. Sheridan, Department of Oral Biology, Box 192 Postle Hall, The Ohio State University, 305 W. 12th Ave., Columbus, Ohio 43210 or e-mail at sheridan.1@osu.edu.

Two major themes underlie the work that we have done on anti-viral immunity, namely, mononuclear cell trafficking and lymphocyte activation. Whereas trafficking and activation are highly regulated by the cognate cellular interactions (resulting in the release of regulatory cytokines and adhesion molecule expression), modulation by other physiological systems also occurs. Our goal has been to understand how neuroendocrine responses, induced by a stressor, play an immunomodulatory role. Furthermore, a key question concerning the relevance of integrated physiological responses has been whether stress-induced activation of neuroendocrine pathways impacts on the pathophysiology of an infectious process by downregulating immune responsiveness. In other words, can stress increase the susceptibility or severity of a viral infection by suppressing immunity?

STRESS AND IMMUNITY IN HUMANS

We have demonstrated in humans that both acute (“academic” stress) and chronic (caregivers to Alzheimer’s patients) stress responses can impact innate and adaptive immunity and slow the healing of experimentally induced wounds.^{1–3} Other laboratories have demonstrated that stress alters the susceptibility to several different strains of respiratory viruses.⁴ Taken together, these data support a role for stress-induced neuroendocrine modulation of inflammatory and immune responses.

Many studies of human responses have examined the links among plasma glucocorticoids [GC], catecholamines, and immunity. However, as peripheral blood is generally the only “window” available through which the human immune response can be studied, it is difficult to assess the mechanisms by which neuroendocrine responses affect either the inductive or effector phases of immunity, as both generally occur in tissues and not in the blood. Lymph nodes draining an inflammatory site and the spleen sampling the circulation are the recipients of viral antigen, which is presented to antigen-specific lymphocytes. As secondary lymphoid tissues (lymph nodes and spleen) have been shown to be innervated by the sympathetic nervous system,⁵ it is probable that catecholamine and/or neuropeptide-induced modulation of lymphocyte responses occurs initially in the lymph nodes and spleen. To see this level of immunomodulation, the responses must be studied in these tissues. Thus, we have established a number of experimental animal models of viral infection and stress in which to study tissue responses of the neuroendocrine and immune systems.

ANIMAL MODELS OF VIRAL INFECTION, STRESS, AND IMMUNITY

We have used experimental viral infections of mice to examine the effects of physical restraint on viral pathogenesis and anti-viral immunity. This work has included infections with either a DNA virus (herpes simplex virus [HSV])⁷ or an RNA virus (influenza A/PR8 virus [A/PR8])⁶ with different tissue tropisms (HSV to the skin and A/PR8 to the respiratory tract).

Physical restraint (RST) restricts an animal’s movement and access to food and water. If multiple cycles of RST are applied during experimental viral infections with HSV or A/PR8, a significant alteration in mononuclear cell trafficking occurs that is accompanied by changes in the pathophysiology of the infection. During RST, lymphadenopathy in the lymph nodes draining the sites of virus replication and cellularity at the inflammatory site in the lungs were diminished during an influenza infection.⁶ Further, functional measures of innate immunity such as proinflammatory cytokine responses⁸ and NK activity⁷ were reduced, as were adaptive responses such as T-cell cytokine gene expression,¹⁷ cytokine secretion,^{6,12} and the development of cytolytic T cells.^{7,15} Suppression of T-cell responses also had an impact on B-cell activation, leading to altered kinetics of the anti-viral antibody responses^{6,18} and diminished antigen-specific plasma cell responses in RST mice.¹⁹ Following the initial description of the modulation of these responses, we examined which elements of the host’s response to stress were modulating innate and adaptive immunity.

NEUROENDOCRINE PATHWAYS AND MODULATION OF IMMUNITY

Although there was evidence that stress influences the inflammatory responses to influenza virus infection in the lung,^{6,9} an assessment of the tissue proinflammatory cytokine concentrations under RST conditions was necessary to link the host's response to stress to modulation of innate immunity in the target organ. Infection of C57B1/6 mice with influenza virus A/PR8 elicited a proinflammatory cytokine response (IL-1 α and IL-6) detectable in lung homogenates.⁸ IL-1 α was significantly elevated within 24 hours p.i. with a peak response at 48 hours p.i. Restrained mice demonstrated an inability to mount an IL-1 α response throughout the course of infection. However, unlike the IL-1 α response, the IL-6 response to A/PR8 infection was not suppressed by restraint.⁸

In previous studies it had been demonstrated that plasma GC levels were elevated following restraint of A/PR8 infected mice,^{10,11} and further study revealed that the elevated levels of GC-modulated mononuclear cell trafficking to the draining lymph nodes and lungs.⁹ Therefore, we suspected that elevated GC in restrained mice might be downregulating IL-1 α production in the lung. However, pretreatment and daily administration of RU486 (a type II steroid receptor antagonist that restored cellularity at the inflammatory site) did not significantly restore the lung IL-1 α response to infection, suggesting that GC was not involved in the regulation of IL-1 α in this model.⁸ Thus, in response to restraint, the proinflammatory cytokine responses in the lung were differentially regulated, and suppression of IL-1 α was not mediated by GC.

Because numerous studies have described the immunomodulatory effects of GC on lymphocyte trafficking and cytokine production, studies were performed to examine the role of the stress-induced plasma GC in regulating the accumulation and function of mononuclear cells in the lungs and lymph nodes during infection. Restraint suppressed lymphadenopathy and cellular accumulation in the lungs; functionally, restraint suppressed A/PR8 virus-specific production of Th1-type cytokines (IL-2 and IFN- γ) and Th2-type cytokines (IL-10), but enhanced IL-6 production by cells from draining mediastinal lymph nodes (MLN).¹² Blockade of the type II GC receptor by treatment with RU486 restored lymphadenopathy to draining lymph nodes and cellularity to the lungs of virus-infected animals.⁹ MLN cells from the RST animals treated with RU486 produced cytokine responses similar to A/PR8 virus-infected mice; that is, IL-2, IFN- γ , and IL-10 levels were enhanced, and the IL-6 response diminished to control levels.¹² Thus, it appeared that the RST-induced alterations in cytokine responses were mediated by GC.

Recently, in an effort to move closer to an *in vivo* assessment of cellular immune responses, we examined the pattern of cytokine gene expression that occurred in the lungs and MLN of A/PR8 virus-infected mice and then determined whether restraint stress modulated gene expression. Analysis by quantitative PCR revealed that mRNAs for IL-2, IFN- γ , IL-4, and IL-10 increased in response to infection and peak levels occurred at 5–7 days p.i. Restraint stress was found to delay cytokine mRNA expression for all four cytokines, thus suggesting that stress may interfere with antiviral immune responses by altering the pattern of cytokine gene expression.¹³

Resolution of an experimental influenza viral infection has been associated with the generation of virus-specific cytolytic T cells.¹⁴ The optimal generation of CD8⁺ CTL responses during a primary viral infection requires cytokines produced by activated CD4⁺T helper cells.¹⁵ Since we had shown that RST suppressed virus-specific cytokine responses through a GC-dependent mechanism, we examined the effects of RST on the generation of virus-specific CD8⁺ CTLs. The initial studies were done in the HSV model of infection and showed that restraint stress suppressed virus-specific CD8⁺ CTL activation.⁷ However, blockade of the type II steroid

receptor (by treatment with RU486) failed to restore CD8⁺ CTL activation despite the accumulation of lymphocytes in the draining lymph nodes.¹⁶ Thus, restoring trafficking was necessary but not sufficient to abrogate stress-induced suppression of CTL activation. To restore activation, treatment with a peripherally acting β -adrenergic receptor antagonist (nadolol) in combination with RU486 was required. Therefore, while the data supported roles for GC in modulating trafficking and cytokine responses, a GC-independent mechanism mediated by catecholamine blocked the actual activation of HSV-specific CTL.¹⁶ These observations on the role of GC and catecholamines in modulating trafficking and T cell activation have been confirmed and extended in the influenza viral pneumonitis model with restraint stress.^{12,18}

Protection against reinfection by a viral pathogen is generally associated with the production of a virus-specific neutralizing antibody response. Such antibody responses are usually T-cell dependent. Because restraint stress had been shown to suppress T-cell-mediated immunity during viral infection, we examined the effect of restraint on antibody responses at the local tissue level. The numbers of antibody secreting cells (ASC) were estimated in the lung, draining lymph nodes, and spleen using an ELISPOT assay. A/PR8 virus-specific IgM, IgG, and IgA (as well as IgG1 and IgG2a) ASC were studied following infection of restrained mice. Restraint significantly suppressed IgM and IgG ASC responses in the lung, MLN, and spleen, while it enhanced the response in the superficial cervical lymph node (SCV). IgG antibody subclass responses (both IgG1 and IgG2a) were also suppressed in the MLN but enhanced in the SCV; however, the dominant response in the SCV during stress was the IgG1 subclass.¹⁹

Suppression of both IgG1 and IgG2a in MLN and their enhancement in SCV suggests that a GC-induced shift from Th1 to Th2 helper cytokine responses during the response to the stressor did not occur, instead both populations (or Th0 responses) were simultaneously modulated by restraint stress. This pattern of responsiveness, at least for the simultaneous suppression of both antibody subclasses as occurred in the MLN, was supported by the previous observation of restraint-induced suppression of both Th1- and Th2-type cytokines in the lung and MLN.^{12,17,19} The observation that restraint stress differentially modulated ASC responses in the MLN and SCV lymph nodes supports the concept of tissue-specific manifestations of the host's response to stress and may indicate that the effects of stress, when viewed from an immunological perspective, may be compartmentalized.²⁰

CONCLUSION

Highly regulated, virus-specific immune responses, as well as innate immunity, can be modulated by products of the HPA axis and sympathetic nervous system. Stress-induced elevation in plasma GC alters mononuclear cell trafficking to draining lymph nodes and inflammatory sites, while activation of virus-specific T cells is suppressed by catecholamines. As a consequence of HPA axis and SNS modulation of immunity, the pathophysiology of experimental HSV and influenza viral infections was altered. Higher virus titers were found in the tissues of HSV infected mice subjected to RST, suggesting that stressed animals were less effective in terminating virus replication than nonstressed controls. In the influenza viral pneumonitis model, pulmonary infiltration by mononuclear cells was significantly diminished in stressed mice, thereby reducing the host local immune response to infection. Thus, in response to an experimental stressor, each viral infection was more severe. Clearly, there are other neuroendocrine products that modulate immunity and need to be examined in the context of an infectious challenge. A short list of factors for which previous studies support an immunomodulatory role includes various neuropeptides, the opioids, and nerve growth factor. Much work remains to be done in elucidating the role of individual responses to stress as they impact disease pathogenesis and immunity.

References

1. Glaser R, Kiecolt-Glaser JK, Bonneau RH, Kennedy S, Hughes J. Stress-induced modulation of the immune response to hepatitis B vaccine. *Psychosom Med* 1992;54:22–29. [PubMed: 1553399]
2. Kiecolt-Glaser JK, Glaser R, Gravenstein S, Malarkey WB, Sheridan JF. Chronic stress alters the immune response to influenza virus vaccine in older adults. *Proc Natl Acad Sci USA* 1996;93:3043–3047. [PubMed: 8610165]
3. Kiecolt-Glaser JK, Marucha PT, Malarkey WB, Mercado AM, Glaser R. Slowing of wound healing by psychological stress. *Lancet* 1995;346:1194–1196. [PubMed: 7475659]
4. Cohen S, Tyrrell DAJ, Smith AP. Psychological stress and susceptibility to the common cold. *N Engl J Med* 1991;325:606–612. [PubMed: 1713648]
5. Felten DL, Ackerman KD, Wiegand SJ, Felten SY. Noradrenergic sympathetic innervation of the spleen. I Nerve fibers associated with lymphocytes and macrophages in specific compartments of the splenic white pulp. *J Neurosci Res* 1987;18:28–36. [PubMed: 3316680]
6. Sheridan JF, Feng N, Bonneau RH, Allen CM, Huneycutt BS, Glaser R. Restraint-induced stress differentially affects anti-viral cellular and humoral immune responses. *J Neuroimmunol* 1991;31:245–255. [PubMed: 1847396]
7. Bonneau RH, Sheridan JF, Feng N, Glaser R. Stress-induced suppression of Herpes simplex virus (HSV)-specific cytotoxic T lymphocyte and natural killer cell activity and enhancement of acute pathogenesis following local HSV infection. *Brain Behav Immun* 1991;5:170–192. [PubMed: 1654165]
8. KONSTANTINOS, A. P. 1995. The early pro-inflammatory cytokine response to influenza A/PR8 viral infection in the murine lung and its modulation by restraint stress. Ph.D. thesis, The Ohio State University, Columbus, Ohio.
9. Hermann G, Beck FM, Sheridan JF. Stress-induced glucocorticoid response modulates mononuclear cell trafficking during an experimental influenza viral infection. *J Neuroimmunol* 1995;56:179–186. [PubMed: 7860713]
10. Dunn AJ, Powell ML, Meitin C, Small PA. Virus infection as a stressor: Influenza virus elevates plasma concentrations of corticosterone, and brain concentration of MHPG and tryptophan. *Physiol Behav* 1989;145:591–594. [PubMed: 2756050]
11. Hermann G, Tovar CA, Beck FM, Sheridan JF. Kinetics of glucocorticoid response to restraint stress and/or experimental influenza viral infection in two inbred strains of mice. *J Neuroimmunol* 1994;49:25–33. [PubMed: 8294561]
12. Dobbs CM, Feng N, Beck M, Sheridan JF. Neuroendocrine regulation of cytokine production during experimental influenza viral infection. *J Immunol* 1996;157:1870–1877. [PubMed: 8757304]
13. JUNG, J. 1996. Cytokine and chemokine gene expression during influenza virus infection, and the effects of restraint stress. Ph.D dissertation, The Ohio State University, Columbus, Ohio.
14. Lukacher AE, Braciale VL, Braciale TJ. In vivo effector function of influenza virus specific cytotoxic T lymphocytes is highly specific. *J Exp Med* 1984;160:814–826. [PubMed: 6206190]
15. Jennings SR, Bonneau RH, Smith PM, Wolcott RM, Chervenak R. CD4-positive T lymphocytes are required for the generation of the primary but not the secondary CDS-positive cytolytic lymphocyte responses to herpes simplex virus in C57BL/6 mice. *Cell Immunol* 1991;133:234–252. [PubMed: 1671342]
16. Dobbs CM, Vasquez M, Glaser R, Sheridan JF. Mechanisms of stress-induced modulation of viral pathogenesis and immunity. *J Neuroimmunol* 1993;48:151–160. [PubMed: 8227313]
17. DOBBS, C. M. 1996. Neuroendocrine modulation of anti-viral cell-mediated immunity. Ph.D dissertation, The Ohio State University, Columbus, Ohio.
18. Feng N, Pagniano R, Tovar CA, Bonneau RH, Glaser R, Sheridan JF. The effect of restraint stress on the kinetics, magnitude, and isotype of the humoral immune response to influenza virus infection. *Brain Behav Immun* 1991;5:370–382. [PubMed: 1777731]
19. CHU, X. 1996. Restraint stress modulation of the murine humoral response to influenza A virus. Ph.D dissertation, The Ohio State University. Columbus, Ohio.

20. Pezzone MA, Dohanics J, Rabin BS. Effects of footshock stress upon spleen and peripheral blood lymphocyte mitogenic responses in rats with lesions of the paraventricular nuclei. *J Neuroimmunol* 1994;53:39–46. [PubMed: 8051296]