Cytokines Mediate the Adverse Effects of Social Stress in an Animal Model of Multiple Sclerosis

by Mary W. Meagher and C. Jane R. Welsh

Mary W. Meagher is Professor and Cornerstone Faculty Fellow in the Department of Psychology at Texas A&M University. Meagher received her doctorate in Psychology from the University of North Carolina at Chapel Hill in Experimental and Biological Psychology and completed postdoctoral training in Clinical Psychology at Texas A&M University followed by a clinical internship at the San Antonio VA. Her research focuses on the role of stress and emotion in health, with an emphasis on pain and immune-related diseases. Meagher collaborates with immunologist Jane Welsh on research investigating the impact of psychosocial stressors on vulnerability to an animal model of multiple sclerosis. This work is funded by grants from NIH, NSF, and the National Multiple Sclerosis Society.

C. Jane R. Welsh is Professor and Associate Department Head in the Department of Veterinary Integrative Biosciences at Texas A&M University. Dr. Welsh has a B.Sc. in Microbiology and a Ph.D. in Biochemistry/Immunology from London University, UK. She received postdoctoral training in autoimmune diseases at the Liver Unit King’s College Hospital, UK and the Department of Pathology at the Cambridge University, UK. Her current research focuses on understanding the mechanisms by which viruses cause autoimmunity, therapies for multiple sclerosis and the role of stress in autoimmune diseases, in collaboration with Dr. Meagher.

Introduction

When Charcot first described multiple sclerosis (MS) in the 1800s he suggested that social stress was related to the onset of MS (Charcot, 1877), but only in recent decades have scientists provided support for this hypothesis. Although human clinical studies have provided evidence that stress is correlated with disease onset and subsequent disease exacerbations (e.g., Mohr et al., 2000 and Mohr, Hart, Julian, Cox, and Pelletier, 2004), surprisingly little is known about the underlying mechanisms. Here we discuss research from
our laboratory suggesting that stress-induced increases in central nervous system inflammation mediate the adverse effects of stress in an animal model of MS. These studies not only provide evidence that stress-induced increases in proinflammatory cytokines exacerbate disease; they also show that these adverse effects can be prevented by blocking stress-induced increases in cytokine activity. These findings are important because they suggest ways to prevent, and possibly reverse, the negative effects of social stress in humans.

Multiple Sclerosis

MS is a chronic inflammatory demyelinating disease of the central nervous system (CNS) that affects approximately 2.5 million individuals throughout the world. Demyelination is caused by the inflammatory and autoimmune responses that damage the myelin sheath surrounding the axons of neurons within the CNS. The clinical symptoms vary depending on the location of the inflammatory lesions, but can include muscle weakness, numbness, paralysis, vision problems, fatigue, depression, pain, and loss of bowel or bladder control. Although the etiology of MS remains uncertain, evidence suggests that environmental factors interact with genetic factors to cause disease (Sospedra & Martin, 2005). Family and twin studies indicate that genetic susceptibility is necessary, but not sufficient, for disease vulnerability. Although rates of disease are higher among relatives of MS patients, concordance rates for identical twins are modest (approximately 25%), suggesting that environmental factors must be involved. Potential environmental risk factors include viral infection and stress. Both human and animal studies indicate that adolescent exposure to certain viruses is associated with the later development of MS. Research from our animal laboratory suggests that stress may be an important co-factor that interacts with viral infection to determine vulnerability to MS.
selected to be socially dominant. During a typical session, a dominant intruder is placed into the home cage of the three adolescent mice for a two-hour session and the intruder repeatedly chases and pins the resident mice. In response to these aggressive encounters, the resident mice exhibit submissive responses indicative of social defeat. This procedure is repeated for three consecutive nightly sessions with one night off, followed by an additional three nightly sessions. Prior research indicates that social disruption stress induces profound effects on neuroendocrine and immune function. Unlike other commonly used laboratory stressors, it induces a phenomenon known as glucocorticoid resistance, which refers to a decrease in the immune system’s capacity to respond to the inhibitory effects of corticosterone (cortisol in humans) in terminating inflammatory responses. A reduction in tissue sensitivity to glucocorticoids induced by chronic social stress may be one mechanism that increases vulnerability to inflammatory diseases such as MS.

We have previously shown that exposure to social disruption stress one week prior to infection exacerbates both the early viral infection and the later autoimmune demyelinating MS-like phase of the disease (Johnson et al., 2004 and Johnson et al., 2006). Social disruption increases inflammation in the spinal cord and brain of mice infected with Theiler’s virus, which is associated with increasing circulating levels of the proinflammatory cytokine interleukin-6 (IL-6) and the development of glucocorticoid resistance. These stress-induced increases in inflammation are associated with exacerbation of motor impairment, sickness behaviors, and disruption of viral clearance from CNS. During late disease, social stress increases circulating levels of antibodies to Theiler’s virus and myelin suggesting that social stress alters the immune response and the autoimmune response to infection. Recently, we have shown that the deleterious effects of social stress can be prevented by blocking stress-induced increases in the proinflammatory cytokine IL-6 (Meagher et al., 2007; Meagher & Welsh, in press). IL-6 is released during stress and plays an important role in regulating the immune system’s response to infection. Our findings suggest that the stress-induced release of IL-6 may make socially stressed mice more vulnerable to infection.

The Role of IL-6 in Mediating the Adverse Effects of Social Stress

Proinflammatory cytokines help to orchestrate the immune responses involved in viral clearance during early infection and in demyelination during late disease (Oleszak et al., 2004). During acute Theiler’s virus infection, IL-6 and other proinflammatory cytokines are elevated in all strains of mice, but higher levels are observed in mice that develop severe demyelination. Elevated levels of IL-6 have also been found in the lesions and cerebral spinal fluid of MS. Other research indicates that stressors can increase circulating and central levels of IL-6 and other proinflammatory cytokines. These observations are consistent with reports that circulating levels of IL-6 are elevated in humans suffering from major depression and chronic stress (Maes et al., 1997; Kiecolt-Glaser et al., 2003). Prior exposure to a stressor can potentiate or prolong the release of proinflammatory cytokines following immune challenge. This phenomenon of cross-sensitization may explain how exposure to social disruption stress alters immune cell function and promotes sustained increases in inflammation following Theiler’s virus infection.

To test the hypothesis that stress-induced increases in central IL-6 mediate the adverse effects of social conflict, we conducted two experiments. Our first study was designed to determine whether social stress increased IL-6 levels and whether these stress-induced increases in IL-6 could be blocked by central administration of a neutralizing antibody to IL-6. As predicted, we found that mice exposed to social stress had elevated central and circulating blood levels of IL-6. However, infusions of a neutralizing antibody to IL-6 into the brain prevented this stress-induced increase in IL-6 in brain and in circulating blood.

Our second experiment was designed to determine whether intracranial administration of the neutralizing antibody to IL-6 could prevent the adverse effects of social stress on Theiler’s virus infection. Before each social disruption session, mice in the social stress and no stress groups received either an intracranial injection of a neutralizing antibody to IL-6 or the vehicle. Following the last social disruption session, the mice were infected with Theiler’s virus and monitored for the development of sickness behaviors, motor impairment, and physiological indicators of disease course. As expected, social stress exacerbated a range of virus-induced sickness behaviors. For example, the sucrose preference task was used to measure anhedonia, a loss of pleasure seeking behavior that occurs following stress or infection. In this test, mice are allowed drink from two containers, one with tap water and the other with 2% sucrose water. Although healthy mice prefer to drink sucrose water, social stress decreased sucrose preference following infection and this effect was blocked by the
IL-6 neutralizing antibody treatment infused during the stress exposure period. Similarly, social stress increased virus-induced motor impairment, including increased hind limb impairment, reduced stride length, and decreased locomotor activity. On all of these behavioral measures, the deleterious effects of social stress were prevented by the infusion of the IL-6 neutralizing antibody during the stress exposure period.

Furthermore, the IL-6 neutralizing antibody reversed the adverse effects of social stress on several physiological measures of disease. Consistent with prior studies, social stress disrupted the normal process of viral clearance in spinal cord and brain, which occurs over the first month of infection. As expected, the non-stressed mice cleared the virus from the CNS to low levels by day 21 post-infection, whereas the socially stressed vehicle treated mice failed to clear the virus. Increased levels of virus during early disease have been shown to lead to more severe later demyelinating disease. Again, this negative effect of social stress on viral clearance was reversed by IL-6 neutralizing antibody treatment during the stress exposure period. As expected, social stress increased infection-related inflammation in spinal cord and brain at both 7 and 21 days after infection. Once more, this stress-induced increase in CNS inflammation was prevented by IL-6 neutralizing antibody treatment. Taken together, these findings suggest that stress-induced increases in central IL-6 contribute to the adverse effects of social stress during acute Theiler’s virus infection.

Based on these findings, we propose that the adverse effects of stress-induced IL-6 on Theiler’s virus infection play an important role in inducing a pro-inflammatory environment that interferes with the immune response to infection. Because the early immune response shapes the later specific immune response to infection, impairment of the early response could account for the increased viral level, prolonged viral infection, increased CNS inflammation, and the subsequent exacerbation of the chronic autoimmune disease.

**Implications for Human Disease Vulnerability**

These findings may have implications for understanding the mechanisms mediating the adverse effects of social stress on a broad range of diseases affecting humans. While acute inflammation is beneficial when it is tightly regulated, chronic inflammation can seriously damage host tissue when sustained at high levels or inappropriately regulated. Chronic inflammation plays a major role in mediating the damage caused by autoimmune diseases (e.g., MS, rheumatoid arthritis), while also greatly influencing the pathogenesis of other diseases where inflammation plays a modulatory role (e.g., cancer, diabetes, cardiovascular disease). Our research suggests that prolonged social stress is likely to amplify chronic inflammatory diseases by inducing glucocorticoid resistance and overproduction of the proinflammatory cytokine IL-6. Recent evidence indicates that humans exposed to chronic stress also develop glucocorticoid resistance (e.g., Miller, Cohen, & Ritchey, 2002; Miller & Chen, 2006) and overproductions of IL-6 (Kiecolt-Glaser et al., 2003), suggesting that similar mechanisms may increase disease risk in humans.

Our work indicates that the adverse effects of social stress on disease vulnerability in humans may be prevented by interventions capable of blocking stress-induced increases in proinflammatory cytokine expression. Potential interventions include certain anti-inflammatory drugs, exercise, antidepressant medication, omega-3 fatty acids, and mindfulness relaxation training. While the implications for human health are intriguing, additional animal studies and human clinical trials are needed to fully evaluate this issue.

**Acknowledgements**

This research is supported by the NIH’s National Institute for Neurological Disorders (R01-NS060822, R01-NS39566; F31-NS050476-2) and National Multiple Sclerosis Foundation (RG 3128). Additional support for this project has been provided by two NSF graduate fellowships to Robin Johnson and Elisabeth Good Vichaya and by a Texas A&M postdoctoral fellowship to Erin Young. Tom Welsh, Ralph Storts, Colin Young, Wentao Mi, Andrew Steelman, Mallory Frazier, Jessica Harrison, Patrick Bridegam, Elisabeth Harden, Marilyn Connor, and an army of undergraduate research assistants made important contributions to this research program.

**References**


infection. *Journal of Neuroimmunology*, 175, 39-51.


