Loneliness, dysphoria, stress and immunity: A role for cytokines

Louise C. Hawkley[1,2], Jos A. Bosch[3,4], Christopher G. Engeland[3],

Phillip T. Marucha[3] & John T. Cacioppo[1,2]

[1] Chicago Center for Cognitive and Social Neuroscience, University of Chicago, 5848 S. University Ave., Chicago, IL 60637

[2] Department of Psychology, University of Chicago, 5848 S. University Ave., Chicago, IL 60637

[3] Department of Periodontics, University of Illinois at Chicago, 801 S. Paulina St., Chicago, IL 60612

[4] School of Sports and Exercise Sciences, University of Birmingham, United Kingdom

Running Head: Loneliness, stress & cytokines

Corresponding author: Louise C. Hawkley, University of Chicago, 940 E. 57th St., Chicago, IL 60637. Phone: (773) 834-9152; Fax: (773) 702-6898; Email: hawkley@uchicago.edu.

This research was supported by National Institute on Aging Program Grants PO1 AG-18911 and P01 AG-16321, and NIH Grant P50 DE-13749.

Loneliness, dysphoria, stress and immunity: A role for cytokines

1. Why study immunity and cytokines in loneliness?

Human existence is founded on social bedrock, so it is not surprising that the most stressful experiences people endure are typically those that strain or break social connections. The oft-reported health benefits of social integration and conversely, the health risks of social isolation (e.g., House et al.[1, 2]), are not limited to the presence or absence of social ties but include satisfaction with social relationships. When personal relationships are perceived to be inadequate to meet one's intimate and social needs, loneliness may ensue. Although social isolation contributes to loneliness by depriving individuals of opportunities to have their social needs met,[3-5] lonely individuals can feel as though they live an isolated existence even when with others.[6, 7] For this reason, loneliness is characterized as *feelings* of social isolation, absence of companionship, and rejection by peer groups,[8, 9] with feelings of an isolated life in a social world forming the dominant experience.[10, 11]

Negative and dissatisfying personal relationships have been shown to be powerful modulators of immune processes,[12, 13] and loneliness is no exception. For instance, loneliness has been associated with lower natural killer (NK) cell activity, poorer blastogenic response to PHA, and higher levels of circulating EBV antibodies.[14-16] The important question for the purpose of this chapter, however, is, "Why study immunity in loneliness?"

One answer to this question follows on the well-known relationship between social isolation and health outcomes.[1] Objective social isolation is not linked to any one disease pathway but is a risk factor for a broad array of illnesses and causes of death including cancer, cardiovascular disease, and diabetes. An understanding of the processes that link social isolation with broad-based morbidity and mortality will therefore benefit from greater specificity in identifying intervening factors to help

explain how social isolation contributes to diverse disease states. Research at the psychological level of analysis, namely the study of loneliness, may help bridge the gap between the epidemiological (i.e., objective social isolation) and biological (i.e., disease) levels of analysis.

Secondly, the United States is experiencing rapidly growing numbers of older individuals who are also increasingly likely to find themselves living alone and possibly lonely in their old age. The aging of America is occurring at a stunning rate: in 1950, adults over the age of 65 comprised 8% of the United States population; in 2000 they represented 12%, and by 2030 they are projected to represent 20% of the population.[17, 18] Moreover, among the more than 27 million people who are living alone, a full 36% are over the age of 65,[19] and this proportion is projected to increase in the future.[20] Objective social isolation carries the risk of engendering loneliness, and this risk is particularly high in older individuals. Indeed, although loneliness is relatively constant throughout adult life, older individuals (i.e., over 75 yrs) report significantly higher levels of loneliness than do younger adults.[21]

Notably, an increasingly large number of elderly individuals are finding themselves socially isolated at the same time that their immune functioning is exhibiting signs of decline. It is widely known that older individuals are more vulnerable to immune challenges than are young adults. Prime examples of their vulnerability include their heightened susceptibility to bacterial (e.g., pneumonia) and viral (e.g., influenza) disease.[22] Social isolation and loneliness could serve to further compromise immune functioning of already immune-compromised older adults.[23, 24] In fact, because younger adults possess a resilient physiology, loneliness may not exert a demonstrable influence on immune functioning until older age. Elucidation of the pathways by which immunity is influenced by loneliness could be informative in the search for appropriate treatment targets in a growing population of older and isolated adults.

A final reason to study immunity in loneliness derives from the dual facts that the effects of social relationships on health outcomes seem to unfold over long periods of time (i.e., years) and that loneliness tends to be self-perpetuating.[25] Lonely individuals tend to be more anxious, pessimistic, and fearful of negative evaluation than nonlonely individuals, and consequently, they are more likely to act and relate to others in an anxious, self-protective fashion. Moreover, lonely individuals perceive that they have little control over their ability to fulfill their social needs.[26] Not only are lonely individuals less accepting of nonlonely others than are nonlonely individuals,[27] but lonely people are also recognized as lonely by others and are viewed more negatively than are nonlonely people.[27, 28] Once others form the impression that a person is lonely, their behaviors toward that individual can reinforce the lonely individual's negative social expectancies[29, 30] and sustain his or her isolated existence. The continual social deficit felt by lonely individuals and the caustic nature of their social cognition provide a neural basis for the chronic activation of physiological pathways that, over time, could have deleterious effects on health.

2. Stress, dysphoria, and loneliness

One set of physiological pathways potentially linking loneliness to health consequences involves the stress-responsive hypothalamic-pituitary-adrenocortical (HPA) and sympathetic-adrenomedullary (SAM) endocrine systems, and the sympathetic (SNS) and parasympathetic (PNS) nervous systems. Lonely and nonlonely individuals may differ in stress exposure, stress reactivity, or stress buffering, as well as in restorative processes that enhance stress resistance, each of which could independently or synergistically contribute to loneliness-related differences in physiological activity.[31] For instance, to the extent lonely individuals experience more frequent and/or more intense stress than do nonlonely individuals, they may exhibit more frequent, more prolonged, and/or greater activation of the HPA and SAM systems. Consistent

with increased HPA and SAM activation, loneliness has been linked with a greater post-awakening rise in cortisol[32] and increased urinary excretion of cortisol and epinephrine.[15, 33] The immunosuppressive effects of HPA and SAM activation may place lonely individuals at an immune disadvantage relative to more socially connected individuals.

Loneliness is itself a stressor that produces negative affect (e.g., anxiety, depression), negative reactivity (e.g., irritability, hostility, mistrust), and lowered feelings of self-worth (see review by Ernst et al.[34]). Indeed, loneliness and dysphoria exhibit considerable experiential overlap, and this is reflected in significant correlations (r's > .4) between scores on the R-UCLA Loneliness Scale (R-UCLA[10]) and the Beck Depression Inventory (BDI[35]), or Center for Epidemiologic Studies Depression Scale (CESD[36]). However, results of factor analyses of items from this loneliness scale and either depression scale support the notion that loneliness and depressed affect are distinct constructs on theoretical and statistical grounds.[37] Moreover, loneliness appears to have stronger effects on depressive symptomatology than depression has on loneliness. For instance, loneliness was a stronger predictor of depression in older men than women despite greater susceptibility to, and higher levels of, depression in women.[38]

On the other hand, depression and dysphoria may, at least in some instances, account for the effects of loneliness on physiological functioning. Depression, for instance, has been associated with alterations of the HPA system,[39] down-regulation of the cellular immune response,[40] and stimulation of the production of pro-inflammatory cytokines associated with cardiovascular disease, diabetes, and other age-related chronic conditions.[41, 42] Indeed, the cytokine hypothesis of depression is based on the assumption that a chronic stressor (e.g., as might be produced by loneliness) can lead to increased cytokine levels which, in turn, elicit depressed affect.[43]

Stress and dysphoria have also been directly related to plasma markers of inflammation (e.g., IL-6, C-reactive protein, gp130). Examination stress, for example, has been associated with local increases in IL-1β levels in crevicular exudate (the plasma fluid between the gums and the teeth) after accumulation of oral bacteria.[44] Glaser et al.[45] found that influenza vaccination increased plasma IL-6 levels in elderly individuals who reported a high number of depressive symptoms, and Cohen et al.[46] found that higher levels of perceived stress were positively associated with nasal IL-6 secretion (in conjunction with more severe clinical symptoms) in adults infected with influenza. Even without obvious sources of antigenic stimulation, consistent associations can be found between depressive and stress-related symptoms and elevated plasma markers of inflammation.[47-50] Thus, psychological distress appears to have an intrinsic capacity to activate various components of the immune system.

3. Acute stress, immunity, and cytokines

The study of physiological stress responses has benefited from a distinction between acute (short-lasting) and chronic stress, and the same distinction may be useful when considering the means by which loneliness might affect physiological functioning. Loneliness is not only a source of chronic stress, but has also been associated with greater acute stress. For example, in our comprehensive study of undergraduate students,[6] lonely individuals reported more daily "hassles" (in both frequency and severity) and fewer and less intense "uplifts" than did nonlonely individuals. In addition, the routine activities that these students engaged in every day were rated as more stressful by lonely than by nonlonely individuals.[51] We next consider the differential impact of acute and chronic stress on immunological functioning to introduce mechanisms by which stress experienced by lonely individuals might influence health.

The immune effects of acute stress have been well-documented in animal studies. For instance, placing a rodent in an unfamiliar open-field causes a rise in both core body temperature (CBT) and circulating levels of IL-6.[52] Other acute stressors (e.g., foot shock, restraint) have been shown to cause fever and to increase quantities of circulating leukocytes, acute phase proteins (APPs) and IL-6, and these effects may last for days (reviewed by Maier et al.[53]). In addition, very intense stressors (e.g., inescapable tail shock) produce the same set of sickness behaviors that are induced by LPS (i.e., increases in sleep; decreases in food/water intake, activity and social interactions). Thus, stress appears able to make animals genuinely sick, even without antigenic stimulation.

Indeed, many of the immune alterations induced by acute stress also occur during systemic inflammation. These include increases in both circulating leukocytes and plasma levels of IL-6, and activation of the acute phase response which causes increases of APPs such as protease inhibitors and haptoglobin.[54, 55] Importantly, activation of these immune components alone does not necessarily cause sickness or inflammation. Rather, when induced by acute stress, these changes serve largely to limit pathogen growth, buffer inflammation and minimize damage to the organism in the event of infection. For instance, APPs function to remove cellular debris, inhibit pathogen growth, promote bacterial destruction by the activation of complement, and stimulate IL-1ra synthesis.[56, 57] In addition, protease inhibitors (e.g., C reactive protein, serum amyloid A) limit the tissue damage that occurs from excess inflammation.[56] Enzymatic activity in blood is also shifted to a state that is less conducive for bacterial growth/replication.[58] Lastly, the systemic actions of IL-6 during infection appear to be largely anti-inflammatory in nature (see Section 5). Thus, stating that acute stress causes immune activation (or suppresses immune activity) is misleading. Rather, acute stress seems to prime the immune system, placing it into a

state of readiness to combat potential injury and infection. This is logical from an evolutionary standpoint, as in a fight or flight situation an organism might be injured and would then have to deal with subsequent inflammation, tissue repair and infection. If the immune system is placed in a state of readiness prior to such an injury, this will conceivably increase survival rates.

In support of this idea of priming the immune response, Dhabhar[59] has repeatedly shown that acute stress (2h restraint) in mice enhances delayed type hypersensitivity (DTH) reactions, which represent cell-mediated immune responses, and this effect is reversed by adrenalectomy indicating HPA involvement. This enhancement is likely due to a mobilization of leukocytes from blood to skin, which is induced by stress and is hypothesized to increase immune surveillance in the skin.[60] This enhancement also occurs due to increased migration of skin dendritic cells, mediated via norepinephrine, which results in greater priming of CD8(+) T cells in draining lymph nodes and increases the recruitment of these effector cells to the skin upon challenge.[61] Thus, stress appears to prime the DTH response by inducing the migration of immune cells prior to antigen challenge.

Using a model of oral wound healing in humans, we have recently observed that individuals with higher anticipatory stress (of the wounding procedure) healed significantly faster than individuals who were less stressed at the time of wounding. This appeared related to higher circulating levels of glucocorticoids (GCs) at the time of wounding and decreased inflammation in the wound tissue 24h post-wounding.[62] Other studies have shown that animals, previously stressed with inescapable tail shock and then challenged with LPS, displayed an enhanced induction of pro-inflammatory cytokines,[63] and an augmentation of both fever and sickness behaviors.[64] Similar stressors also activate the acute phase response[65] and enhance recovery from bacterial challenge.[66] Moreover, the macrophages from stressed rats exhibited

increased production of nitrite (*in vitro*) when challenged with keyhole limpet hemocyanin (KLH). However, in the absence of KLH, macrophage nitrite production was similar between stressed and non-stressed animals. Thus, stress primed but did not activate nitrite production.[67] Taken together, these findings indicate that acute stress can both prime and increase the effectiveness of the immune response against antigenic challenge and injury.

How stress is able to influence cytokine levels and sickness may stem back to basic energy production in the body. GCs catalyze the conversion of glycogen to glucose and muscle protein to amino acids, antagonize the actions of insulin (resulting in decreased uptake of glucose to fat and muscle) and liberate fatty acids from fat reserves. The overall effect is that GCs liberate stored energy by increasing glucose availability to peripheral tissues, muscles and the brain. The immune system requires vast amounts of energy for tissue repair, increased metabolism and fever maintenance. Evidence suggests that the immune system has made use of GCs as an energy production system from a very early time point in evolution and, indeed, even primitive organisms (e.g., mollusks) exhibit activation of such a system upon immune challenge (see Maier et al.[53] for review). In vertebrates, peripheral immune activation (e.g., infection) leads to the release of pro-inflammatory cytokines (e.g., IL-1β, TNF-α) which signal the brain through a variety of mechanisms. This, in turn, induces the release of a variety of substances centrally (e.g., IL-1β, prostaglandins) which signal the hypothalamus, raising the set-point for core body temperature and activating the HPA axis. This results in GC release which liberates energy for immune activation such as fever. Fever is an adaptive response to infection as it bolsters the immune system and inhibits bacterial growth and reproduction.[58] Thus, one of the original purposes of GC release may have been to liberate stored energy for fever maintenance and tissue repair.

During a fight/flight situation (i.e., a situation of acute stress requiring complex, integrated responses), energy demands are high and immediate, and are largely met by GC release. However, the fight/flight response is evolutionarily younger than the immune system (very primitive creatures incapable of fight/flight possess an immune mechanism for dealing with infection). Based on this, Maier and Watkins[54] have proposed that as organisms evolved, GC release which was already used for quick energy production by the immune system also became utilized when a fight/flight response was needed. As a result, the stress response began to stimulate the same circuitry that is stimulated following infection. In fact, stress appears to activate the same cascade of events as LPS does (i.e., central activation causing fever and HPA activation), although this stems from a neural pathway rather than from peripheral cytokine release.[53] This circuitry is illustrated in Figure 1.

Indeed, it has been shown that tail shock and social isolation each cause central increases in brain IL-1β, immobilization stress increases brain IL-1β mRNA, and IL-1ra (icv) blocks endocrine and behavioral responses to some stressors.[53] Furthermore, following intense stress (e.g., tail shock) the central release of IL-1β is related to increases in plasma cytokine levels (e.g., IL-1β, IL-6) and sickness behaviors,[68] all of which can be blocked by the pre-administration of IL-1β antagonists (e.g., IL-1ra, α-MSH) centrally but not peripherally.[53] Taken together, this suggests that central increases in IL-1β influence peripheral inflammatory responses that occur following psychological stress.

In summary, acute stress activates many peripheral components of innate and even some components of acquired (e.g., DTH) immunity which, in turn, are protective in nature or serve to prime the immune system for antigenic challenge. However, IL-1β may also be released centrally, and under intense stress this can lead to peripheral IL-1β and IL-6 release, along with

increased inflammation, fever, and sickness behaviors. These data suggest that the repeated and more intense bouts of acute stress experienced by lonely compared to nonlonely individuals represents one mechanism by which loneliness may have a negative impact on immune activity and health.

Proper HPA functioning largely prevents the peripheral release of pro-inflammatory cytokines following acute stress.[69] Chronic stress, however, involves a lasting dysregulation of the HPA axis, and also a resistance to the immunosuppressant effects of GCs.[70, 71] Thus, the higher degree of peripheral inflammation and sickness that is reported in chronically stressed individuals may stem from a decreased ability to prevent peripheral inflammation following the stress-induced release of IL-1β centrally.

4. Chronic stress, dysphoria, glucocorticoid sensitivity, and macrophage migratory inhibitory factor

Loneliness is aversive, and when social needs go unmet for extended periods of time, the stress of perceived isolation may pervade every other aspect of life. The chronic stress of loneliness is further exacerbated by the repeated and intense bouts of acute stress which are common to lonely individuals. Indeed, stress responses to even relatively minor recurring daily stressors can accumulate and have long-term consequences on health.[72] Chronic stress therefore represents a second mechanism that may lead to greater peripheral inflammation and sickness in lonely rather than nonlonely individuals. Moreover, loneliness has been shown to be a strong predictor of depressive symptomatology,[37, 38] and depression offers another venue for loneliness to make inroads on immune functioning and health. GCs play a key role in determining the physiological consequences of chronic stress and depression and thus provide a useful avenue to examine how loneliness might affect health.

The observation that chronic stress can potentiate inflammatory processes appears at odds with the potent anti-inflammatory effects of GCs and other stress hormones. One possible explanation for this discrepancy, discussed in the previous section, is that the systemic signs of inflammation may have been misinterpreted, and actually reflect a response that helps to contain inflammatory processes (cf.,[73]). Thus, as inflammation is primarily a localized event, many of the systemic "pro-inflammatory" mediators that are elevated in distressed individuals may function in conjunction with GCs to keep this process localized.

An additional possibility is that protracted stress diminishes the immune system's sensitivity to GCs, which normally control the inflammatory cascade. Strong support for this hypothesis comes from the work of Sheridan et al., who have employed social disruption (SDR) as a stress model in animals.[74-76] In this experimental model, male mice are housed in groups of five until a stable hierarchy develops. Next, an aggressive intruder is introduced, upon which excessive fighting re-establishes the social hierarchy. In a series of studies, it was found that SDR stress induces a strong HPA activation in the defeated animals,[76, 77] and simultaneously leads to glucocorticoid resistance in splenocytes that have been activated with LPS.[77] It is relevant to add that chronic restraint stress does not result in glucocorticoid resistance, and may even increase sensitivity to GCs, despite the fact that this stressor induces similar HPA activation to that of SDR.[76, 78, 79] Hence, the induction of glucocorticoid sensitivity appears stressor-specific. Consistent with the glucocorticoid sensitivity hypothesis, the SDR-induced reduction in glucocorticoid sensitivity results in greater proneness to hyperinflammation, leading to increased mortality from experimental influenza infection and septic shock.[76, 78]

Studies in humans confirm and extend the results of animal experiments. In human studies, glucocorticoid sensitivity is measured ex vivo, whereby immune cells are incubated with

bacterial endotoxin (e.g., LPS) or a mitogen (e.g., PHA) in combination with varying concentrations of GCs (e.g., dexamethasone). Using this approach, studies have found a reduced glucocorticoid sensitivity of immune cells (e.g., greater *in vitro* production of IL-6 and TNF-α in the presence of dexamethasone) in spousal caregivers of dementia patients,[80] in parents of children undergoing cancer treatment,[81] and in stress-related syndromes such as vital exhaustion and depression.[82-84]

Thus, animal and human research each provide good support for the hypothesis that psychological stressors can down-regulate GC sensitivity of immune cells. As shown in Figure 2, this can result in an increased release of pro-inflammatory cytokines (e.g., IL-1β, TNF-α), and suggests that the consequences of GC resistance may include elevated pro-inflammatory cytokine responses to inflammatory stimuli. Although it is still unclear how stress-induced GC resistance is mediated, macrophage migratory inhibitory factor (MIF) is one candidate. In two studies, described below, we therefore explored whether MIF is up-regulated by psychological stress.

MIF is one of the earliest identified cytokines, discovered nearly 40 years ago, although its exact functions remained elusive for many years.[85] Studies using pure recombinant MIF and specific neutralizing antibodies have shown MIF to be a potent pro-inflammatory cytokine, and a key modulator of immune and inflammatory processes.[86, 87] Moreover, in the early nineties, it was discovered that MIF overrides the anti-inflammatory actions of GCs, and restores macrophage cytokine production and T cell activation during treatment with immunosuppressive levels of GCs.[88, 89] Subsequent studies demonstrated a critical role of MIF in various inflammatory processes and syndromes, including atherosclerosis, wound repair, rheumatoid

arthritis, inflammatory lung diseases, and sepsis, whereas MIF-neutralizing antibodies reduced disease severity and mortality in models of arthritis, glomerular nephritis, and sepsis.[86, 89-105]

Monocytes/macrophages and T cells are probably the main immune cells to produce MIF.[87] Interestingly, MIF secretion by leukocytes is induced, rather than suppressed, by low concentrations of synthetic GCs.[88, 93] Animal studies have revealed other remarkable associations between MIF and HPA-axis activities:[106] MIF is a significant pituitary protein (0.05% of total protein; as a comparison, ACTH forms 0.2% and prolactin 0.08% of pituitary protein[101]) secreted by the same cells of the anterior pituitary that secrete ACTH, and pituitary MIF partly derives from ACTH-containing secretory granules.[89, 107, 108] Moreover, *in vitro* studies showed that CRH is a potent MIF secretagogue, although the signaling pathway appears distinct from that controlling ACTH release.[108, 109] MIF is also expressed in the adrenals,[110, 111] and adrenal MIF protein expression is reduced in animals that have the pituitary surgically removed, indicating that adrenal MIF production is dependent on stimulation by pituitary hormones.[110] Animal studies further show that plasma levels of MIF increase in response to various HPA-activating signals, including endotoxin and handling stress.[88] The latter finding provided the first indication that psychological stressors may upregulate plasma MIF levels.

Although extensively studied in rodents, data on the regulation of MIF in humans is somewhat sparser. In healthy human subjects, MIF is at relatively high plasma concentrations (2-8 ng/ml), and much higher concentrations are found in inflammatory disease and sepsis.[97, 101, 112, 113] Consistent with the MIF-HPA association in animals, MIF shows a circadian cycle that parallels that of cortisol.[114] In contrast with the finding of animal studies, however, plasma levels of MIF are not affected by various HPA stimulants or inhibitors, including injections of CRH or ACTH, the insulin tolerance test, and the dexamethasone suppression test.[113] Likewise, in our

studies we did not observe an effect of acute stress (public speaking) on plasma MIF levels, in spite of the fact that this laboratory stressor had a strong effect on plasma ACTH and cortisol levels.[48]

Our data did indicate, however, that protracted forms of distress can affect MIF levels in both humans and animals. For our human studies we selected young undergraduate students (mean age 20.4) that scored in the upper or lower quintile of their peer group on the Beck Depression Inventory (BDI).[48] The BDI is one of the most frequently used self-report questionnaires for assessing depression and dysphoria, and high scores on this questionnaire are predictive of the presence of clinical depression.[115] Approximately 4 to 6 weeks after this initial screening, subjects were invited to the laboratory, where they underwent a 15-minute public speaking task (i.e., giving a presentation in front of a camera and an audience). Participants who at the time of this laboratory visit had BDI scores indicative of mild to moderate depression (i.e., ≥13) were denoted dysphoric (N=36), whereas subjects with a BDI score ≤ 5 were denoted non-dysphoric (N=39). Although the 15-minute speaking stressor had no effect on plasma MIF levels, the participants in the dysphoric group had higher average levels of MIF. Dysphoria was also associated with marginally increased numbers of peripheral blood lymphocytes (p <.05), which is consistent with the idea that increased MIF levels indicate the presence of low-grade immune activation. Subsequent analyses to detect potential confounders showed that the two groups did not differ in gender or ethnic composition, age, body mass index, smoking, alcohol consumption, or average hours of exercise or sleep, and the use of these variables as covariates in the analyses did not attenuate the observed group differences.[48]

We also investigated MIF expression in the SDR animal model (described above). In this model, it has been shown that glucocorticoid resistance does not develop until after 4-5 days of

SDR. Quantitative polymerase chain reaction (PCR) tests of the spleens and pituitaries of mice that were exposed to 6 days of SDR showed a 50% increase in MIF gene expression in both tissues (J.F. Sheridan, personal communication). Additional experiments in which spleens were removed after 1, 3 or 6 days of SDR demonstrated that MIF expression did not significantly increase until day 6. Hence, MIF expression shows a pattern that appears to parallel the kinetics of the development of glucocorticoid resistance (J.F. Sheridan, personal communication).

To conclude, both animal and human studies show that chronic stress is associated with reduced immune cell sensitivity to the anti-inflammatory actions of GCs, and stress-induced elevations in MIF could be a potential mediator of this effect. However, correlation is not causation, and additional experiments are clearly needed to confirm a role of MIF in stress-related glucocorticoid resistance. For the SDR paradigm, further experiments with MIF knock-out animals appear the most obvious way to proceed. Correlational studies in humans could examine statistical associations between MIF expression, glucocorticoid sensitivity, and indices of distress. Such research might help explain the link between stress and inflammatory conditions such as atherosclerosis, autoimmunity, and allergic diseases, and also help to solve the paradox of how stressors can act as both immunosuppressants and immunostimulants.

5. IL-6: Pro-inflammatory or anti-inflammatory?

A hallmark of immune activation in major depression is increased production of IL-6.[116] Indeed, many studies have linked IL-6 with immune activation during aging, stress and disease (e.g.,[50, 117]) and cite this cytokine as an important marker for inflammatory events, which it is. Taken a step further, the most common interpretation of IL-6 release is that it is pro-inflammatory, although the majority of studies that relate increased inflammation or fever to elevated levels of IL-6 are correlational in nature. For instance, of the pro-inflammatory

cytokines released during infection, IL-6 correlates most closely with fever and HPA activation (both temporally and quantitatively).[118-120] It has been clearly shown that IL-6 rises in concordance with both IL-1β and TNF-α, as each stimulates the synthesis and release of IL-6.[121] However, is IL-6 truly a pro-inflammatory cytokine? We propose that a misinterpretation exists in the literature, as many of the reports that IL-6 acts in a pro-inflammatory manner during systemic infection have been largely inferred on the basis of its stronger correlation with fever, HPA activity and other indices of inflammation than either IL-1β or TNF-α.

IL-6 certainly does have pro-inflammatory qualities during infection, such as being a strong progenitor of myeloid cell differentiation (e.g., macrophages, neutrophils) and being involved in cell maturation and maintenance (e.g., NK cells, CD8(+) T cells). However, IL-6 also has many anti-inflammatory properties during infection. For instance, IL-6 is released in response to rising levels of IL-1β and TNF-α,[121] and dampens the inflammation caused by these pro-inflammatory cytokines through a variety of mechanisms: 1) once released, IL-6 directly inhibits the further synthesis of both IL-1β and TNF-α;[122, 123] 2) the administration of IL-6 in humans causes the induction of the soluble receptors IL-1ra and p55[57] which inhibit IL-1β and TNF-α activity, respectively; and 3) IL-6 acts at both the pituitary gland and the adrenals to activate the HPA axis and is the main modulator of the release of GCs during immune activation[124] which, in turn, suppress inflammation. Thus, a main brake on the inflammatory response seems to stem from IL-6.

Whereas in the literature IL-6 is routinely mentioned in the same context as the cytokines IL-1β and TNF-α, it should be noted that the biological effects of IL-6 are very different from these two pro-inflammatory cytokines. For example, although systemic administration of either IL-1β and TNF-α causes high fever and even septic shock at relatively low doses, fairly high

doses of IL-6 are tolerated and do not cause shock in mice, dogs or primates.[56] Furthermore, unlike IL-1β and TNF-α, IL-6 does not cause: 1) the upregulation of major inflammatory mediators (e.g., chemokines, prostaglandins, nitric oxide, matrix metalloproteinases); 2) the induction of cyclooxygenase activity; 3) tissue damage by proteases; or 4) the synthesis of adhesion molecules (e.g., intracellular adhesion molecule, ICAM-1).[56, 57, 122] Rather, IL-6 is a chief mediator of the acute phase response,[125] which serves to limit pathogen growth, inflammation and tissue damage. Also, T-cell mediated inflammation, such as delayed type hypersensitivity and adjuvant arthritis, or if triggered by superantigen, is inhibited by IL-6.[56, 123]

Finally, in a murine model of toxic shock, it has been shown that IL-6 pre-treatment decreases mortality rates in a dose-dependent fashion and treatment with IL-6 antibodies increases mortality rates, likely due to the inhibitory effect IL-6 has on TNF-α release.[123] Similarly, treatment with IL-6 antibodies increases mortality in a septic shock model.[126] Thus, although IL-6 is one of the main cytokines released during bacterial infection and inflammation, its principal systemic actions may be anti-inflammatory. In summary, the authors encourage readers to allow for this possibility when interpreting results that involve correlations between immune activation or disease severity and levels of IL-6.

Conclusion

Sociodemographic changes in the United States are finding increasing numbers of people living alone, and a growing percentage of these isolated individuals are elderly. Given the risk for morbidity and mortality associated with social isolation and loneliness, one challenge for researchers is to identify mechanisms by which social factors take a toll on physiological functioning and health. Relatively recent developments in the field of immunology have

introduced cytokines as potential players in the physiological processes that link psychosocial factors with increased risk for disease.

Investigations of the numerous actions of cytokines involved in psycho-neuro-immune interactions are ongoing, and examinations of the role of cytokines in the pathways that lead from loneliness to morbidity and mortality have only begun. Because cytokine research is in its infancy, special care needs to be taken to ensure that empirical evidence supports inferences regarding the role of cytokines in physiological and health outcomes. A role for IL-6 is a case in point. Although typically referred to as a pro-inflammatory cytokine, an anti-inflammatory interpretation of IL-6 actions is consistent with data from many studies. For example, although greater age-related increases in IL-6 among current and former caregivers (relative to controls) may mark inflammatory processes,[127] IL-6 increases may reflect an attempt to contain inflammation. Indeed, decreases in IL-6 under conditions of chronic stress may signal failure in self-regulation of the inflammatory process.

The recently discovered role of MIF in immune processes is another reminder that much has yet to be learned regarding the interplay among psychological, endocrine (i.e., HPA), and immune systems. The pro-inflammatory actions of MIF are particularly interesting in their potential to explain the development of glucocorticoid resistance, an especially troubling consequence of chronic stress and depression. Given that loneliness may operate through distress and dysphoria to affect health, MIF may play an important role in increasing the risk for morbidity and mortality in lonely individuals.

To sum, it appears obvious that loneliness can contribute to disease processes through its influence on acute and chronic stress. Figure 1 illustrates the main cascade of physiological changes that occur following acute stress. Figure 2 illustrates how chronic stress impacts on this

same cascade of events, and how it promotes peripheral inflammation to a greater extent than does acute stress. Overall, acute stress typically elicits an adaptive response, as it primes the immune system, placing it into a state of readiness to combat potential injury and infection. Conversely, chronic stress, an overshoot of this response, is generally maladaptive to the organism. Observing Figure 2, it appears that a chronic stressor such as loneliness can cause low-grade peripheral inflammation which, in turn, has been linked to inflammatory diseases such as diabetes, cardiovascular disease (e.g., atherosclerosis), and autoimmune disorders (e.g., rheumatoid arthritis, lupus). Whether chronic stress works causally or synergistically with underlying disease mechanisms remains unresolved. Nevertheless, the outcomes of these processes can be influenced by the stress and depression associated with loneliness. Indeed, the known centrality of social relationships to well-being implies that loneliness may extract a great cost on human health, the mechanisms for which have only begun to be explored.

References

1. House, J.S., Landis, K.R., and Umberson, D., Social relationships and health, *Science*, 241, 540, 1988.
2. Seeman, T.E., Health promoting effects of friends and family on health outcomes in older adults, *Am. J. Hlth. Promot.,* 14, 362, 2000.
3. de Jong-Gierveld, J., Developing and testing a model of loneliness, *J. Pers. Soc. Psychol.*, 53, 119, 1987.
4. Fees, B.S., Martin, P., and Poon, L.W., A model of loneliness in older adults, *J. Gerontol.: Psychol. Sci.*, 54, P231, 1999.
5. Henderson, A.S., Scott, R., and Kay, D.W., The elderly who live alone: Their mental health and social relationships, *Austr. N.Z. J. Psychiat.*, 20, 202, 1986.
6. Cacioppo, J.T. et al., Lonely traits and concomitant physiological processes: The MacArthur Social Neuroscience Studies, *Int. J. Psychophys.*, 35, 143, 2000.
7. van Baarsen, B. et al., Lonely but not alone: Emotional isolation and social isolation as two distinct dimensions of loneliness in older people, *Educat. Psychol. Measur.*, 61, 119, 2001.
8. Austin, B.A., Factorial structure of the UCLA Loneliness Scale, *Psychol. Rep.*, 53, 883, 1983.
9. Hawkley, L.C., Browne, M.W., and Cacioppo, J.T., How can I connect with thee? Let me count the ways, *Psychol. Sci.*, In press, 2005.
10. Russell, D., Peplau, L.A., and Cutrona, C.E., The revised UCLA Loneliness Scale: Concurrent and discriminant validity evidence, *J. Pers. Soc. Psychol.*, 39, 472, 1980.

11. Hays, R.D. and DiMatteo, M.R., A short-form measure of loneliness, *J. Person. Assess.*, 51, 69, 1987.

12. Kiecolt-Glaser, J.K., Stress, personal relationships, and immune function: Health implications, *Brain Behav. Immun.*, 13, 61, 1999.

13. Uchino, B.N., Cacioppo, J.T., and Kiecolt-Glaser, J.K., The relationship between social support and physiological processes: a review with emphasis on underlying mechanisms and implications for health, *Psychol. Bull.*, 119, 488, 1996.

14. Glaser, R. et al., Stress, loneliness, and changes in herpesvirus latency, *J. Beh. Med.*, 8, 249, 1985.

15. Kiecolt-Glaser, J.K. et al., Urinary cortisol levels, cellular immunocompetency,and loneliness in psychiatric inpatients, *Psychosom. Med.*, 46, 15, 1984.

16. Kiecolt-Glaser, J.K. et al., Stress and the transformation of lymphocytes by Epstein-Barr virus, *J. Beh. Med.*, 7, 1, 1984.

17. Meyer, J., Age: 2000, In Census 2000 Brief, U.S. Census Bureau, Government Printing Office, Washington, D.C., 2001.

18. U.S. Census Bureau, Projections of the Total Resident Population by 5-year Age Groups, and Sex with Special Age Categories, Middle Series, 2025-2045 (NP-T3-F), Population Projections Program, Population Division, Government Printing Office, Washington, D.C., 2000.

19. Hobbs, F. and Stoops, N., Demographic Trends in the 20th Century, Census 2000 Special Reports, Series CENSR-4, U.S. Census Bureau, Government Printing Office, Washington, D.C., 2002.

20. U.S. Census Bureau, Projections of the Number of Persons Living Alone by Age and Sex: 1995 to 2010, Series 1, 2, and 3, Population Projections Program, Population Division, Government Printing Office, Washington, D.C., 1996.

21. Andersson, L., Loneliness research and interventions: A review of the literature, *Aging Ment. Hlth.*, 2, 264, 1998.

22. Yoshikawa, T.T., Clinical relevance of age-related immune dysfunction, *Clin. Infect. Dis.*, 31, 578, 2000.

23. Hawkley, L.C. and Cacioppo, J.T., Stress and the aging immune system, *Brain Behav. Immun.*, 18, 114, 2004.

24. Kiecolt-Glaser, J.K. and Glaser, R., Stress and immunity: Age enhances the risks, *Curr. Dir. Psychol. Sci.*, 10, 18, 2001.

25. Cacioppo, J.T. and Hawkley, L.C., People thinking about people: The vicious cycle of being a social outcast in one's own mind, in *The social outcast: Ostracism, social exclusion, rejection, and bullying*, Williams K.D., Forgas J.P., and von Hippel W., Eds., Psychology Press, New York, In press, 2004.

26. Solano, C.H., Loneliness and perceptions of control: General traits versus specific attributions, *J. Soc. Beh. Pers.*, 2, 201, 1987.

27. Rotenberg, K.J. and Kmill, J., Perception of lonely and non-lonely persons as a function of individual differences in loneliness, *J. Soc. Pers. Relat.*, 9, 325, 1992.

28. Lau, S. and Gruen, G.E., The social stigma of loneliness: Effect of target person's and perceiver's sex, *Pers. Soc. Psychol. Bull.*, 18, 182, 1992.

29. Rotenberg, K., Loneliness and interpersonal trust, *J. Soc. Clin. Psychol.*, 13, 152, 1994.

30. Rotenberg, K.J., Gruman, J.A., and Ariganello, M., Behavioral confirmation of the loneliness stereotype, *Basic Appl. Soc. Psychol.*, 24, 81, 2002.

31. Hawkley, L.C. and Cacioppo, J.T., Loneliness and pathways to disease, *Brain Behav. Immun.*, 17, S98, 2003.

32. Steptoe, A. et al., Loneliness and neuroendocrine, cardiovascular, and inflammatory stress responses in middle-aged men and women, *Psychoneuroendocrinol.*, 29, 593, 2004.

33. Hawkley, L.C. et al., Cardiovascular & endocrine functioning in an aging population: Unique and combined contributions of loneliness, depression, perceived stress, social support, and hostility, In preparation, 2004.

34. Ernst, J.M. and Cacioppo, J.T., Lonely hearts: Psychological perspectives on loneliness, *Appl. Prev. Psychol.*, 8, 1, 1999.

35. Beck, A.T. and Beck, R.W., Screening depressed patients in a family practice: A rapid technique, *Postgrad. Med.*, 52, 81, 1972.

36. Radloff, L.S., The CES-D Scale: A self-report depression scale for research in the general population, *Appl. Psychol. Measur.*, 1, 385, 1977.

37. Cacioppo, J.T. et al., Loneliness within a nomological net: Is social connectedness central?, Under review, 2004.

38. Cacioppo, J.T. et al., Loneliness and depressive symptoms, self-rated health, and chronic health conditions: Evidence from two population-based studies, Under review, 2004.

39. Tsigos, C. and Chrousos, G.P., Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress, *J. Psychosom. Res.*, 53, 865, 2002.

40. Miller, A.H., Neuroendocrine and immune system interactions in stress and depression, *Psychiat. Clin. N. Am.*, 21, 443, 1998.

41. Dantzer, R. et al., Cytokines and depression: Fortuitous or causative association? *Molec. Psych.*, 4, 328, 1999.

42. Kiecolt-Glaser, J.K. and Glaser, R., Depression and immune function: Central pathways to morbidity and mortality, *J. Psychosom. Res.*, 53, 873, 2002.

43. Leonard, B.E. and Song, C., Stress, depression, and the role of cytokines, in *Cytokines, stress, and depression*, Dantzer R., Wollman E.E., and Yirmiya R., Eds., Kluwer Academic Publishers, New York, 1999, 251.

44. Deinzer, R. et al., Acute stress effects on local Il-1β responses to pathogens in a human in vivo model, *Brain Behav. Immun.*, 18, 458, 2004.

45. Glaser, R. et al., Mild depressive symptoms are associated with amplified and prolonged inflammatory responses after influenza virus vaccination in older adults, *Arch. Gen. Psychiatry*, 60, 1009, 2003.

46. Cohen, S., Doyle, W.J., and Skoner, D.P., Psychological stress, cytokine production, and severity of upper respiratory illness, *Psychosom. Med.*, 61, 175, 1999.

47. Miller, G.E. et al., Clinical depression and inflammatory risk markers for coronary heart disease, *Am. J. Cardiol.*, 90, 1279, 2002.

48. Bosch, J.A. et al., Elevated Macrophage Migration Inhibitory Factor in dysphoric young adults, In preparation.

49. Penninx, B.W. et al., Inflammatory markers and depressed mood in older persons: results from the Health, Aging and Body Composition study, *Biol. Psychiatry*, 54, 566, 2003.

50. Kiecolt-Glaser, J.K. et al., Chronic stress and age-related increases in the proinflammatory cytokine IL-6, *Proc. Natl. Acad. Sci. USA*, 100, 9090, 2003.

51. Hawkley, L.C. et al., Loneliness in everyday life: Cardiovascular activity, psychosocial context, and health behaviors, *J. Pers. Soc. Psychol.*, 85, 105, 2003.

52. LeMay, L.G., Vander, A.J., and Kluger, M.J., The effects of psychological stress on plasma interleukin-6 activity in rats, *Physiol. Behav.*, 47, 957, 1990.

53. Maier, S.F., Bi-directional immune-brain communication: Implications for understanding stress, pain, and cognition, *Brain Behav. Immun.*, 17, 69, 2003.

54. Maier, S.F. and Watkins, L.R., Bidirectional communication between the brain and the immune system: implications for behaviour, *Animal Behav.*, 57, 741, 1999.

55. Yeager, M.P., Guyre, P.M., and Munck, A.U., Glucocorticoid regulation of the inflammatory response to injury, *Acta Anaesthiol. Scand.*, 48, 799, 2004.

56. Barton, B., The biological effects of interleukin 6, *Med. Res. Rev.*, 16, 87, 1996.

57. Tilg, H., Dinarello, C., and Mier, J., IL-6 and APPs: Anti-inflammatory and immunosuppressive mediators, *Immunol. Today*, 18, 428, 1997.

58. Hart, B.L., Biological basis of the behavior of sick animals, *Neurosci. Biobehav. Rev.*, 12, 123, 1988.

59. Dhabhar, F.S., Stress-induced augmentation of immune function--the role of stress hormones, leukocyte trafficking, and cytokines, *Brain Behav. Immun.*, 16, 785, 2002.

60. Dhabhar, F.S. and McEwen, B.S., Stress-induced enhancement of antigen-specific cell-mediated immunity, *J. Immunol.*, 156, 2608, 1996.

61. Saint-Mezard P. et al., Psychological stress exerts an adjuvant effect on skin dendritic cell functions in vivo, *J. Immunol.*, 171, 4073, 2003.

62. Engeland, C.G., Cacioppo, J.T., and Marucha, P.T., Stress hormones modulate the healing rates of oral wounds, In preparation.

63. Johnson, J.D. et al., Prior stressor exposure sensitizes LPS-induced cytokine production, *Brain Behav. Immun.*, 16, 461, 2002.

64. Johnson, J.D. et al., Effects of prior stress on LPS-induced cytokine and sickness responses, *Am. J. Physiol.*, 284, R422, 2003.

65. Deak, T. et al., Evidence that brief stress may induce the acute phase response in rats, *Am. J. Physiol.*, 273, R1998, 1997.

66. Deak, T. et al., Acute stress may facilitate recovery from a subcutaneous bacterial challenge, *Neuroimmunomodulation*, 6, 344, 1999.

67. Fleshner, M. et al., Acute stressor exposure both suppresses acquired immunity and potentiates innate immunity, *Am. J. Physiol.*, 275, R870, 1998.

68. Johnson, J.D. et al., The role of IL-1beta in stress-induced sensitization of proinflammatory cytokine and corticosterone responses, *Neurosci.*, 127, 569, 2004.

69. Nguyen, K.T. et al., Timecourse and corticosterone sensitivity of the brain, pituitary, and serum interleukin-1beta protein response to acute stress, *Brain Res.*, 859, 193, 2000.

70. Avitsur, R., Stark, J.L., and Sheridan, J.F., Social stress induces glucocorticoid resistance in subordinate animals, *Horm. Behav.*, 39, 247, 2001.

71. O'Connor, K.A. et al., Inescapable shock induces resistance to the effects of dexamethasone, *Psychoneuroendocrinol.*, 28, 481, 2003.

72. McEwen, B.S., Protective and damaging effects of stress mediators, *N. Eng. J. Med.*, 338, 171, 1998.

73. Tracey, K.J., The inflammatory reflex, *Nature*, 420, 853, 2002.

74. Bailey, M.T. et al., Physical defeat reduces the sensitivity of murine splenocytes to the suppressive effects of corticosterone, *Brain Behav. Immun.*, 18, 416, 2004.

75. Engler, H. et al., Effects of repeated social stress on leukocyte distribution in bone marrow, peripheral blood and spleen, *J. Neuroimmunol.*, 148, 106, 2004.

76. Padgett, D.A. et al., Social stress and the reactivation of latent herpes simplex virus type 1, *Proc. Natl. Acad. Sci. U.S.A.*, 95, 7231, 1998.

77. Stark, J.L. et al., Social stress induces glucocorticoid resistance in macrophages, *Am. J. Physiol.: Regul. Integr. Comp. Physiol.*, 280, R1799, 2001.

78. Quan, N. et al., Social stress increases the susceptibility to endotoxic shock, *J. Neuroimmunol.*, 115, 36, 2001.

79. Bauer, M.E. et al., Restraint stress is associated with changes in glucocorticoid immunoregulation, *Physiol. Behav.*, 73, 525, 2001.

80. Bauer, M.E. et al., Chronic stress in caregivers of dementia patients is associated with reduced lymphocyte sensitivity to glucocorticoids, *J. Neuroimmunol.*, 103, 84, 2000.

81. Miller, G.E., Cohen, S., and Ritchey, A.K., Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoid-resistance model, *Hlth. Psychol.*, 21, 531, 2002.

82. Miller, A.H., Pariante, C.M., and Pearce, B.D., Effects of cytokines on glucocorticoid receptor expression and function. Glucocorticoid resistance and relevance to depression, *Adv. Exp. Med. Biol.*, 461, 107, 1999.

83. Wirtz, P.H. et al., Reduced glucocorticoid sensitivity of monocyte interleukin-6 production in male industrial employees who are vitally exhausted, *Psychosom. Med.*, 65, 672, 2003.

84. Bauer, M.E. et al., Altered glucocorticoid immunoregulation in treatment resistant depression, *Psychoneuroendocrinol.*, 28, 49, 2003.

85. Bucala, R., Neuroimmunomodulation by macrophage migration inhibitory factor (MIF), *Ann. N.Y. Acad. Sci.*, 840, 74, 1998.

86. Donn, R.P. and Ray, D.W., Macrophage migration inhibitory factor: molecular, cellular and genetic aspects of a key neuroendocrine molecule, *J. Endocrinol.*, 182, 1, 2004.

87. Calandra, T. and Roger, T., Macrophage migration inhibitory factor: a regulator of innate immunity, *Nat. Rev. Immunol.*, 3, 791, 2003.

88. Calandra, T. et al., MIF as a glucocorticoid-induced modulator of cytokine production, *Nature*, 377, 68, 1995.

89. Bernhagen, J. et al., MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia, *Nature*, 365, 756, 1993.

90. Bernhagen, J. et al., An essential role for macrophage migration inhibitory factor in the tuberculin delayed-type hypersensitivity reaction, *J. Exp. Med.*, 183, 277, 1996.

91. Nishihira, J., Novel pathophysiological aspects of macrophage migration inhibitory factor (review), *Int. J. Mol. Med.*, 2, 17, 1998.

92. Rossi, A.G. et al., Human circulating eosinophils secrete macrophage migration inhibitory factor (MIF). Potential role in asthma, *J. Clin. Invest.*, 101, 2869, 1998.

93. Leech, M. et al., Macrophage migration inhibitory factor in rheumatoid arthritis: Evidence of proinflammatory function and regulation by glucocorticoids, *Arthritis Rheum.*, 42, 1601, 1999.

94. Calandra, T. et al., Protection from septic shock by neutralization of macrophage migration inhibitory factor, *Nat. Med.*, 6, 164, 2000.

95. Leech, M. et al., Regulation of macrophage migration inhibitory factor by endogenous glucocorticoids in rat adjuvant-induced arthritis, *Arthritis Rheum.*, 43, 827, 2000.

96. Abe, R. et al., Regulation of the CTL response by macrophage migration inhibitory factor, *J. Immunol.*, 166, 747, 2001.

97. Beishuizen, A. et al., Macrophage migration inhibitory factor and hypothalamo-pituitary-adrenal function during critical illness, *J. Clin. Endocrinol. Metab.*, 86, 2811, 2001.

98. Lehmann, L.E. et al., Plasma levels of macrophage migration inhibitory factor are elevated in patients with severe sepsis, *Intensive Care Med.*, 27, 1412, 2001.

99. Burger-Kentischer, A. et al., Expression of macrophage migration inhibitory factor in different stages of human Atherosclerosis, *Circulation*, 105, 1561, 2002.

100. Ashcroft, G.S. et al., Estrogen modulates cutaneous wound healing by downregulating macrophage migration inhibitory factor, *J. Clin. Invest.*, 111, 1309, 2003.

101. Baugh, J.A. and Donnelly, S.C., Macrophage migration inhibitory factor: a neuroendocrine modulator of chronic inflammation, *J. Endocrinol.*, 179, 15, 2003.

102. Lai, K.N. et al., Role for macrophage migration inhibitory factor in acute respiratory distress syndrome, *J. Pathol.*, 199, 496, 2003.

103. Chen, Z. et al., Evidence for a role of macrophage migration inhibitory factor in vascular disease, *Arterioscler. Thromb. Vasc. Biol.*, 24, 709, 2004.

104. Ichiyama, H. et al., Inhibition of joint inflammation and destruction induced by anti-type II collagen antibody/lipopolysaccharide (LPS)-induced arthritis in mice due to deletion of macrophage migration inhibitory factor (MIF), *Cytokine*, 26, 187, 2004.

105. Nakamaru, Y. et al., Macrophage migration inhibitory factor in allergic rhinitis: its identification in eosinophils at the site of inflammation, *Ann. Otol. Rhinol. Laryngol.*, 113, 205, 2004.

106. Petrovsky, N. and Bucala, R., Macrophage migration inhibitory factor (MIF). A critical neurohumoral mediator, *Ann. N.Y. Acad. Sci.*, 917, 665, 2000.

107. Nishino, T. et al., Localization of macrophage migration inhibitory factor (MIF) to secretory granules within the corticotrophic and thyrotrophic cells of the pituitary gland, *Mol. Med.*, 1, 781, 1995.

108. Waeber, G. et al., Transcriptional activation of the macrophage migration-inhibitory factor gene by the corticotropin-releasing factor is mediated by the cyclic adenosine 3',5'-monophosphate responsive element-binding protein CREB in pituitary cells, *Mol. Endocrinol.*, 12, 698, 1998.

109. Tierney, T. et al., Macrophage migration inhibitory factor is released from pituitary folliculo-stellate-like cells by endotoxin and dexamethasone and attenuates the steroid-induced inhibition of interleukin 6 release, *Endocrinology*, In press, 2004.

110. Fingerle-Rowson, G. et al., Regulation of macrophage migration inhibitory factor expression by glucocorticoids in vivo, *Am. J. Pathol.*, 162, 47, 2003.

111. Imamura, K. et al., Identification and immunohistochemical localization of macrophage migration inhibitory factor in human kidney, *Biochem. Mol. Biol. Int.*, 40, 1233, 1996.

112. Fingerle-Rowson, G.R. and Bucala, R., Neuroendocrine properties of macrophage migration inhibitory factor (MIF), *Immunol. Cell. Biol.*, 79, 368, 2001.

113. Isidori, A.M. et al., Response of serum macrophage migration inhibitory factor levels to stimulation or suppression of the hypothalamo-pituitary-adrenal axis in normal subjects and patients with Cushing's disease, *J. Clin. Endocrinol. Metab.*, 87, 1834, 2002.

114. Petrovsky, N. et al., Macrophage migration inhibitory factor exhibits a pronounced circadian rhythm relevant to its role as a glucocorticoid counter-regulator, *Immunol. Cell Biol.*, 81, 137, 2003.

115. Beck, A.T., Steer, R.A., and Garbin, M.G., Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation, *Clin. Psychol. Rev.*, 8, 77, 1988.

116. van West, D. and Maes, M., Activation of the inflammatory response system: A new look at the etiopathogenesis of major depression, *Neuroendocrinol. Lett.*, 20, 11, 1999.

117. Yudkin, J.S. et al., Inflammation, obesity, stress and coronary heart disease: Is interleukin-6 the link?, *Atherosclerosis*, 148, 209, 2000.

118. Engel, A. et al., Kinetics and correlation with body temperature of circulating interleukin-6, interleukin-8, tumor necrosis factor alpha and interleukin-1 beta in patients with fever and neutropenia, *Infect.*, 22, 160, 1994.

119. Lenczowski, M. et al., Individual variation in hypothalamus-pituitary-adrenal responsiveness of rats to endotoxin and interleukin-1b, *Ann. N.Y. Acad. Sci.*, 856, 139, 1998.

120. Roth, J. et al., Kinetics of systemic and intrahypothalamic IL-6 and tumor necrosis factor during endotoxin fever in guinea pigs, *Am. J. Physiol.*, 265, R653, 1993.

121. Luheshi, G.N. et al., Febrile response to tissue inflammation involves both peripheral and brain IL-1 and TNF-a in the rat, *Am. J. Physiol.*, 272, R862, 1997.

122. Barton, B., IL-6: Insights into novel biological activities, *Clin. Immunol. Immunopathol.*, 85, 16, 1997.

123. Barton, B., Shortall, J., and Jackson, J., Interleukins 6 and 11 protect mice from mortality in a staphylococcal enterotoxin-induced toxic shock model, *Infect. Immun.*, 64, 714, 1996.

124. Bethin, K., Vogt, S., and Muglia, L., Interleukin-6 is an essential, corticotropin-releasing hormone-independent stimulator of the adrenal axis during immune system activation, *Proc. Natl. Acad. Sci. U.S.A.*, 97, 9317, 2000.

125. Streetz, K.L. et al., Mediators of inflammation and acute phase response in the liver, *Cell Mol. Biol.*, 47, 661, 2001.

126. Barton, B.E. and Jackson, J.V., Protective role of interleukin 6 in the lipopolysaccharide-galactosamine septic shock model, *Infect. Immun.*, 61, 1496, 1993.

127. Steptoe, A. et al., Inflammatory cytokines, socioeconomic status, and acute stress responsivity, *Brain Behav. Immun.*, 16, 774, 2002.

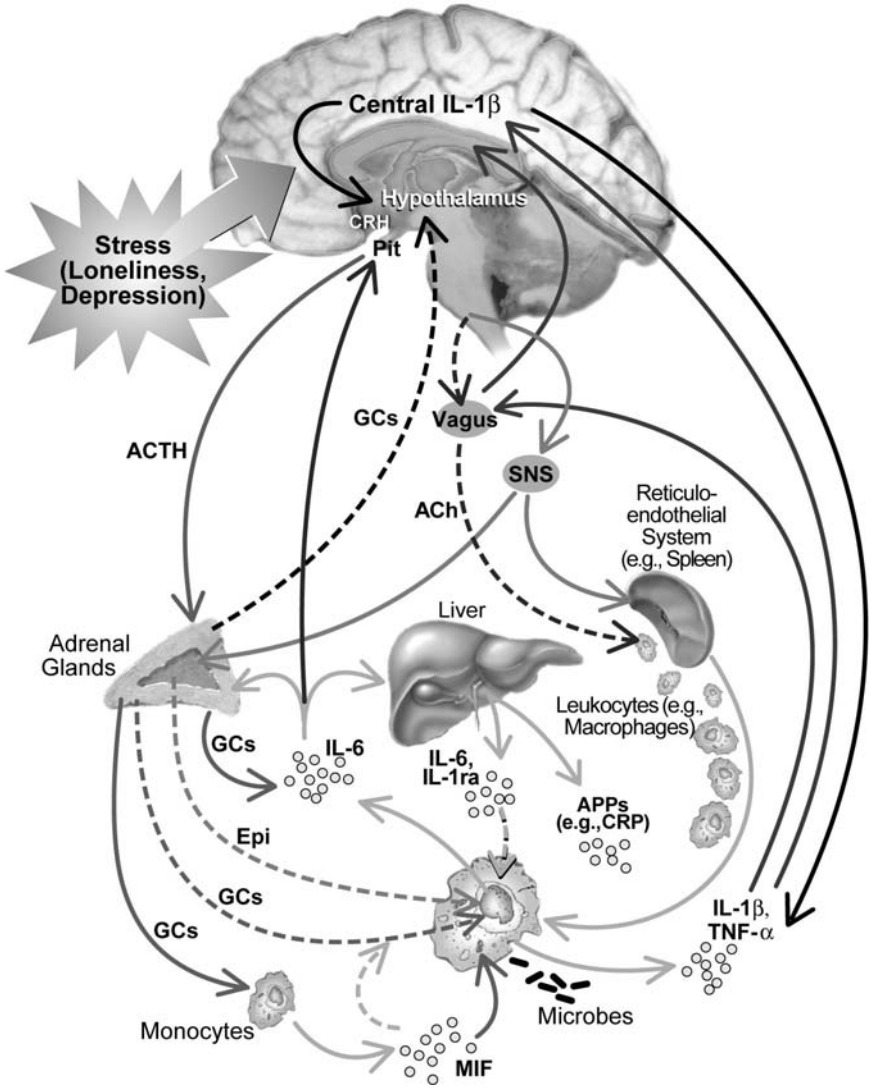

Figure 1. *The Acute Stress Cascade*. Perceived stress triggers a physiological cascade that influences inflammatory processes. Activation of the SNS primes the immune system by mobilizing leukocytes from the spleen (as well as lungs, marginal pools, and bone marrow pools) into the blood. Stress-induced vagal withdrawal results in reduced ACh release and permits inflammatory activity of tissue macrophages in the vagally innervated reticulendothelial system (liver, heart, spleen, GI tract). Acute psychological stressors also stimulate the release of IL-1β in the brain and initiate direct activation of the HPA axis, which culminates in the secretion of GCs (e.g., cortisol). Under severe stress, brain IL-1β appears capable of causing signs of low-grade peripheral inflammation (e.g., fever, elevated plasma cytokine levels). Circulating GCs reduce/contain this inflammation by inhibiting the release of these cytokines. GCs also stimulate IL-6 production which, in turn, induces hepatic APPs (e.g., CRP) that help to minimize cellular damage. However, GCs induce macrophages to release MIF, which reduces immune cell sensitivity to the anti-inflammatory actions of GCs and promotes TNF-α release. This may help to sustain low-grade inflammation during conditions of chronic stress (see Fig. 2). *Solid arrows represent stimulatory effects and dashed arrows represent inhibitory effects.*
(Illustrated by Karen Dirr, M.A.M.S, University of Chicago)

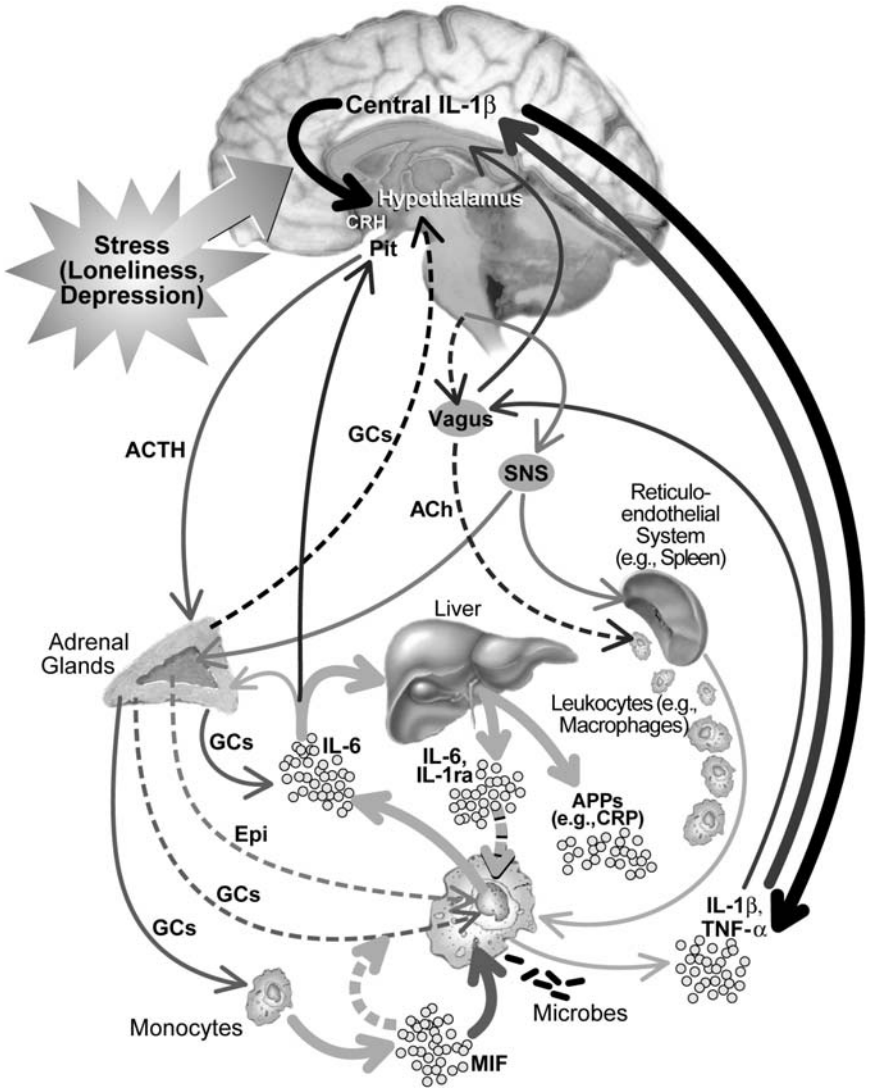

Figure 2. *Impact of Chronic Stress*. Despite higher circulating levels of GCs under conditions of chronic stress, the anti-inflammatory effects of GCs appear to be substantially lessened. This is due to the formation of GC insensitivity by many immune cells, and may be mediated by MIF production. The end result is that susceptibility to inflammation and its associated morbidities (e.g., sickness, disease) may be greater in chronically stressed (e.g., lonely, depressed) individuals. *Solid arrows represent stimulatory effects and dashed arrows represent inhibitory effects. Thicker arrows represent effects made stronger due to chronic stress.*
(Illustrated by Karen Dirr, M.A.M.S, University of Chicago)

Abbreviations: ACh, acetylcholine; APPs, acute phase proteins; CRH, corticotropin releasing hormone; CRP, C-reactive protein; GCs, glucocorticoids; Epi, epinephrine; HPA axis; hypothalamic-pituitary-adrenocortical axis; MIF, macrophage migratory inhibitory factor; Pit, pituitary gland; SNS, sympathetic nervous system