Stress and the immune system

Article *in* Pathophysiology · May 2003

DOI: 10.1016/S0928-4680(03)00003-8 · Source: PubMed

1 author:

Stephen Pruett
Mississippi State University
156 PUBLICATIONS **3,375** CITATIONS

SEE PROFILE

ELSEVIER

Pathophysiology 9 (2003) 133–153

ISP PATHOPHYSIOLOGY

www.elsevier.com/locate/pathophys

Review

Stress and the immune system

Stephen B. Pruett *

Department of Cellular Biology and Anatomy, Louisiana Health Sciences Center-Shreveport, 1501 Kings Hwy, Shreveport, LA 71130, USA

Received 26 November 2002; received in revised form 2 December 2002; accepted 8 January 2003

Abstract

Stressors can positively or adversely affect immune and inflammatory responses. However, the current understanding of these effects at the cellular and molecular levels is not sufficient to allow prediction of the effects of a particular stressor on a particular immune or inflammatory function. Three complementary conceptual frameworks are presented that may prove useful in developing such an understanding. In addition, specific examples of the action of particular stress mediators on particular immune or inflammatory end points are discussed, and the relationship of these observations to the conceptual frameworks is indicated. Several of the effects discussed are relevant clinically, and the prospects for pharmacological intervention to prevent adverse effects of stressors on the immune system are discussed. Finally, some of the factors that can (sometimes unexpectedly) influence the outcome of stress-immunology studies and some of the pitfalls that continue to make this area of research controversial in some circles are discussed.

Keywords: Stress; Immune system; HPA axis

1. Introduction

Most investigators in the field of neuroendocrine–immune interactions focus more of their attention on end points in one system than the other. In keeping with this pattern, this review will focus more on the effects of the neuroendocrine system on immune system end points than the effects of the immune system on neuroendocrine end points. Some of the latter effects will be noted, but for more thorough discussions several excellent reviews are available [1,2].

There seems to be no single definition that adequately describes stress. In the present discussion, the term is used to describe the physiological response of the whole animal, in contrast to cellular stress responses, the induction and regulation of which are distinct from responses of the intact animal. Stimuli that induce stress responses are stressors, and common stressors include psychological, physical, and drug or chemical stimuli. Activation of this physiological response almost always is associated with activation of the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system, leading to changes in the concentrations of several stress-related mediators [3]. These responses are tightly regulated by complex feedback mechanisms [4]. Although most stressors activate the HPA axis and the sympathetic nervous system leading to changes in the concentrations of mediators in the periphery, there are indications that stressors are not functionally equivalent and that different patterns of mediators may be induced by different stressors [5].

There is now compelling evidence that stress responses can cause clinically relevant immunosuppression [6,7] as well as other types of immune system dysfunction [8–12]. In cases in which the stressor cannot be quickly alleviated, it would be beneficial to block the production or action of those stress mediators that are primarily responsible for adverse immunological effects. However, physiological stress responses can have beneficial effects, such as down regulating cytokine production during infections to prevent systemic inflammatory response syndrome (SIRS) [13] or preventing lethal effects of a variety of toxic chemicals [14]. The differential role for various stress-induced neuroendocrine mediators in protective versus harmful effects is almost entirely unknown. In this review, a framework is

* Tel.: +1-318-675-4386; fax: +1-318-675-5889.
E-mail address: spruet@lsuhsc.edu (S.B. Pruett).

0928-4680/03/$ - see front matter
doi:10.1016/S0928-4680(03)00003-8

proposed that incorporates previously proposed conceptual frameworks as well as quantitative aspects of the induction of various stress mediators. This framework could permit a more comprehensive, unified view of the mechanisms by which stress adversely affects the immune system. Such a view seems necessary before proposing a rational approach for pharmacological intervention to minimize the adverse effects of stress while avoiding substantial inhibition of the beneficial effects of the physiological stress response.

1.1. The immune system

Cellular elements of the immune system include lymphocytes, macrophages, monocytes, eosinophils, neutrophils, basophils, mast cells, and dendritic cells. Most of these cell types do not reside permanently at any particular anatomical location. Rather, they recirculate via the blood and, in the case of lymphocytes, via the lymphatic circulation. Even the most sessile of these cells, the dendritic cells and macrophages, can be induced to migrate to sites of inflammation or to regional lymph nodes to function as antigen presenting cells. In addition to routine recirculation of immune system cells in their 'patrol' function, most cells of the immune system can be attracted to sites of infection or inflammation and accumulate there to participate in events that lead to the elimination or isolation of microbes and to tissue repair [15–17]. Thus, the immune system is inherently dynamic.

In addition to the dynamics of cellular trafficking and anatomical distribution, cellular population size is dynamic and carefully regulated. The life span of most cell types in the immune system is relatively brief, so these cells must constantly be replaced in a controlled manner [18]. Cellular proliferation and differentiation in the bone marrow compensates for the loss of cells caused by senescence or by apoptosis, which acts to eliminate potentially auto-reactive lymphocytes and to prevent excessive cellular proliferation during immune responses [19,20].

The immune system is also dynamic with regard to interactions between the different cell types that comprise it and with regard to interactions between the immune system and other systems. Immunity to microbes or tumor cells is typically categorized as innate or acquired. Innate immunity is generally non-specific, requires little or no induction period, and does not respond more quickly or more vigorously upon the second encounter with a particular microbe (i.e. it does not exhibit a memory response). It is mediated mostly by neutrophils, eosinophils, basophils, macrophages, and natural killer cells (which are mostly large granular lymphocytes). These cells work by direct anti-microbial action, by killing virus-infected cells or tumor cells, and by secreting cytokines, which orchestrate inflammation and other defense mechanisms. Acquired immunity is specific, requires an induction period, and exhibits a memory response upon subsequent encounters with the initiating microbe or substance. Acquired immunity requires interactions between three different cells types (antigen presenting cell, Th cells, and B cells or Tc cells) and involves several cytokines. Acquired immune responses can be categorized as cell-mediated or humoral. The major effector cells in cell-mediated responses are Th1 cells, which produce cytokines that activate macrophages for increased microbicidal activity, and Tc cells which produce cytokines and are directly cytotoxic to cells infected with viruses or other intracellular parasites. The major effector cells in the humoral response are B lymphocytes, which are stimulated to mature into antibody-secreting plasma cells. This maturation process requires primarily Th2 cells, but some contribution of Th1 cells is also needed. The relative amount of Th1 cytokines (e.g. IFN-γ, IL-2, TNF-α) and Th2 cytokines (e.g. IL-4, IL-5, IL-10) strongly influence B cell maturation in terms of the magnitude of the response and in terms of the immunoglobulin isotype that the mature plasma cell will eventually secrete. Thus, Th1/Th2 balance has become a popular concept, and a response in which Th1 cells are prevalent favors the IgG2a isotype in the mouse, whereas a predominant Th2 response favors IgG1, IgA, or IgE [21].

The innate and acquired immune systems interact at several points. Cytokines induced by microbes as part of the innate response and microbial components per se can activate the most effective antigen presenting cells, dendritic cells [22]. Some of these cytokines can also influence the predominance of Th1 or Th2 cells [23], which determine if the response will be primarily cell-mediated or primarily antibody-mediated [21]. Although the immune system has a number of redundant components and mechanisms and can tolerate considerable suppression or alteration of some parameters without substantial changes in host resistance [24], it has been argued that significant changes in major immunological functions would lead to decreased resistance to some pathogen under some circumstance. For example, a relatively high but normally not infectious challenge dose might become infectious. Although there is not as much experimental support for this idea as one might expect, there are indications that varying the challenge dose of microbe or cancer cell often reveals immunosuppression that may be missed in an experiment using a single, relatively low challenge dose [25]. Thus, it could be argued that any significant alteration in an important immunological parameter has the potential to affect resistance to infection, even though it is clear that this would not apply to all pathogens or all cancer cells under all circumstances [24].

The importance of appropriate regulation of the immune system and its interaction with the neuroendo-

crine system is perhaps best illustrated by the host response to bacterial septicemia. This condition, as well as some other types of infection can induce such an overwhelming innate immune response that the blood levels of pro-inflammatory cytokines such as IL-1, TNF-α, and IL-6 produce Systemic Inflammatory Response Syndrome (SIRC) [26]. In severe cases, this causes shock, multiple organ failure, and death. The neuroendocrine response to pro-inflammatory cytokines may be an important feedback control mechanism (which will be discussed further in the next section) to prevent excessive cytokine production in most cases. In addition, inflammation can induce anti-inflammatory cytokines that exert negative feedback regulation (e.g. TGF-β). This can probably also be affected by stressors. These relationships are best considered in the context that immune or inflammatory responses that are either insufficient or excessive can be detrimental to the organism.

1.2. Stress responses

A wide variety of physical, chemical, and psychological stimuli induce a complex, physiological response in mammals. Such stress responses are characterized by activation of the HPA axis leading to increased levels of adrenal hormones such as glucocorticoid, epinephrine, and norepinephrine [4]. In addition, the sympathetic nervous system is activated leading to release of norepinephrine from adrenergic nerve terminals, including those in the spleen, thymus, and other lymphoid tissues [27]. Many other neuroendocrine mediators are also increased or decreased in the peripheral circulation during most stress responses, thereby exposing cells of the immune system to increased or decreased concentrations. For example concentrations of glucocorticoids (predominantly cortisol in humans and corticosterone in rodents), catecholamines (epinephrine and norepinephrine), endogenous opiates, ACTH, bombesin [28], and prolactin (PRL) increase in response to a wide variety of stressor. In contrast, concentrations of GH, melatonin, and testosterone decrease in response to stress. One or more cell types in the immune system have receptors for each of the mediators listed here, and this is only a partial list of stress-related mediators [27]. Clearly the potential for modification of immune system function by the neuroendocrine system exists, and the interactions are likely to be complex.

An additional level of complexity is added by the ability of the immune system to exert a powerful influence on the neuroendocrine system. Several cytokines, most prominently the pro-inflammatory cytokines IL-1, TNF-α, and IL-6, can activate the HPA axis and produce other effects on the nervous system (e.g. the induction of sleep and elevated body temperature). These cytokines are often produced most abundantly by the immune system in response to immunological stimuli (i.e. microbes or microbial components). In fact, the constellation of signs and symptoms that have recently been termed 'sickness behavior' are apparently mediated by cytokines that are produced mostly by the immune system [29]. The interactions between the nervous and immune systems have received considerable attention in recent years, as indicated by a recent, thorough review article on the subject in which more than 1000 references are cited [2].

Two recently proposed frameworks for understanding stress responses are particularly pertinent with regard to the effects of stressors on the immune system. McEwen and colleagues have proposed the concept of allostasis as more descriptive of the physiological response to stressors than homeostasis [30]. This concept incorporates the idea that major and minor internal stressors are a routine part of life and that physiological systems may remain in a responsive state for a long period of time as the host adapts to these stressors. Thus, the traditional concept of homeostasis seems inadequate in describing day-to-day responses of organisms to stressors. An additional concept within this framework is allostatic load. Allostatic load is a term that expresses the cumulative damaging impact of maintaining the allostatic response to stressors. Over time it is proposed that this reduces the ability of the organism to maintain normal physiological functions, including immunological functions (illustrated in Fig. 1). As will be noted in a later section, chronic exposure to stressors can unquestionably have detrimental effects on immune function. Another useful framework has been proposed for the role of glucocorticoids in response to stressors. It notes that glucocorticoids have distinct categories of functions, which depend in part on glucocorticoid concentration and on the duration of exposure [31]. These categories include: permissive, suppressive, stimulatory, and preparative functions. In some cases, more than one of these functions impinge to produce a particular set of physiological responses, including immunological responses. For example, low concentrations of glucocorticoids may be necessary for expression of some cytokine receptors (a permissive function), whereas production of the cytokines that act through those receptors may be decreased by higher concentrations of glucocorticoids (illustrated in Fig. 1) [31]. Of course, this concept could be expanded to incorporate other stress mediators, many of which exert comparable categories of functions.

An additional conceptual framework is proposed here and presented in Table 1. This framework focuses on the idea that differences in the effects of different stressors on the immune system or differences between acute and chronic exposure to the same stressor are caused at least in part by different patterns of production of various neuroendocrine mediators. This idea is developed more extensively in Section 4. This is not intended as a

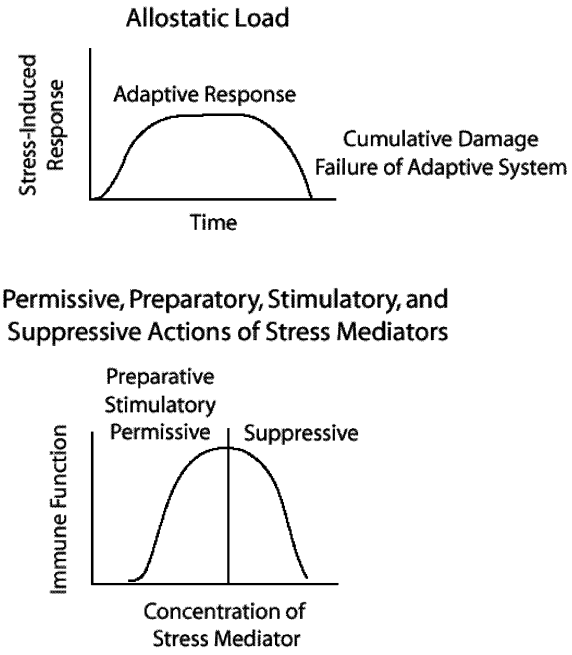

Fig. 1. Conceptual frameworks for interpreting stress-immunology studies. The concept of allostatic load suggests that chronic stressors can induce prolonged responses that lead to damage and eventual dysfunction [30]. This is consistent with many reports in stress-immunology research suggesting that chronic stress can be more immunosuppressive than acute stress. Sapolsky et al. proposed that glucocorticoids have several different functions and that the beneficial ones may predominate at normal or even at moderate stress-induced levels, whereas a harmful one (generally suppression) may predominate at higher concentrations [31]. It seems likely that parts of this framework are also applicable to other stress-related mediators.

substitute for either framework noted in Fig. 1, but as an adjunct to address an issue not directly addressed in them.

Table 1
A conceptual framework for the effects of stress on the immune system based on differing concentrations of stress mediators

Neuroendorine mediator	Stressor 1	Stressor 2 or chronic stressor 1
1	+++	+
2	+++	+
3	+	+++
4	+	+++
5	+	+

Although direct evidence is relatively limited, there seem to be differences in the concentrations of particular stress mediators induced by different stressors, and it also seems likely that this may apply to acute vs. chronic stressors. In the figure the relative concentration of each mediator is indicated by +++ (high) or + (low). The pattern of concentrations noted for Stressor 1 may not affect a particular immune parameter, whereas the pattern noted for Stressor 2 (or chronic exposure to Stressor 1) may affect that immunological parameter.

2. Evidence for meaningful stress-related interactions between the immune and neuroendocrine systems

One of the criticisms that many immunologists have expressed about the effects of stressors on the immune system and about psychoneuroimmunology in general has been that alterations in immune functions caused by neuroendocrine mediators are generally too small to indicate a meaningful impact on human health. However, results from several recent studies sharply and convincingly contradict this conclusion. For example, two independent groups (one in the US and one in the UK) reported substantial decreases in the efficacy of influenza vaccines in persons who are primary caregivers for a patient with dementia [6,7]. In one of these studies there was an excellent correlation between the area under the cortisol concentration versus time curve (an excellent indicator of the magnitude of the stress response) and suppression of the antibody response to the vaccine [7]. In an intriguing experimental study with 55 human subjects, severity of disease following laboratory exposure to influenza virus was strongly correlated with scores on a written instrument designed to measure psychological stress [32]. Similar results have been reported in a study involving over 200 human subjects with laboratory exposure to a common cold virus. The probability of developing disease was closely associated with chronic, but not short-term psychological stress [33]. However, this study also illustrates the complexity of the relationship between psychological stress and immune function, because there was no correlation between pre-exposure endocrine measures and subsequent risk of infection. Similar effects have been noted with regard to immunological end points other than host resistance to infection. In fact a meta-analysis of many studies conducted up to 1993 indicated a clear, detrimental effects of psychological stress on a variety of immunological measures in humans [34].

Such findings are even more interesting when one considers that several major health problems associated with aging now seem to be mediated at least in part by immune system-related mechanisms. For example, increases in certain inflammatory cytokines (e.g. IL-6) have been reported in association with exposure to psychological stressors [35], even though these cytokines seem to be down-regulated by glucocorticoids induced by infection [36]. These psychological stress-induced increases in cytokine production may contribute to cardiovascular disease, type 2 diabetes, osteoporosis, and frailty and functional decline [37]. There is a clear association between exacerbation of various autoimmune conditions and psychological stress [8–12]. The mechanisms remain obscure and represent one of the more interesting, health-related issues of stress-immunology research. However, stress-induced exacerbation of autoimmune disease probably should not be surpris-

ing in view of reports discussed above, which show that stress responses can enhance as well as suppress some immune and inflammatory functions.

Thus, psychological stress can suppress immune function sufficiently to increase the risk of infectious disease or diminish the efficacy of vaccines, and it can also contribute to conditions that are characterized by increased inflammatory or immune activity. Although this may seem paradoxical, it is actually consistent with elements of the frameworks noted previously by which stress responses can be understood. Although the type, duration, and intensity of psychological stress has not been documented or evaluated extensively, in general, chronic or especially intense stressors are associated with immunosuppression, whereas acute and less intense stressors may actually be associated with enhancement of some immunological or inflammatory conditions. This would be consistent with the idea that allostatic load resulting from chronic stress eventually leads to immunosuppression, whereas relatively mild acute stress may lead to the production of stimulatory or preparative levels of neuroendocrine mediators. There is considerable support for the importance of the intensity, duration, and type of stressor in data from animal models (discussed further in Section 4).

3. Molecular and cellular basis of stress effects on the immune system

Cells of the immune system have receptors for most of the neurotransmitters and hormones associated with stress responses [38], and these receptors are generally linked to signaling systems similar to those of other cell types that express these receptors [39,40]. Immunomodulation by these neuroendocrine mediators has been discussed in a number of excellent reviews [3,27,41–43]. Therefore, the present discussion will focus on a few mediators for which recent results either clarify their role in immunomodulation or raise new questions.

3.1. Growth hormone, prolactin, insulin-like growth factor-1, and thyroid hormone

Stress-induced neuroendocrine mediators are generally thought to act primarily by up or down regulating immune responses. However, it is also clear that some mediators are required at normal levels to maintain normal immune function or normal development of cells of the immune system. In particular, there is evidence that this is the case for growth hormone (GH), PRL, insulin like growth factor 1 (IGF-1) and thyroid hormone [44]. These hormones increase in response to stressors, and can thus be categorized as stress hormones. There are reports that increased levels of these mediators can be immunosuppressive [45,46]. However, more attention has been directed to reported decreases in normal immune function when these hormones are decreased in concentration in experimental models such as mutant mice that have abnormal pituitary function [47] or with hypophysectomized mice or rats [48,49]. Various immunological deficits have been reported in such animals, and in some cases these deficits can be corrected by administration of one of these hormones [50,48]. However, it is very interesting that recent results from mutant and knockout mice deficient in one these hormones (or its receptor) have failed to reveal immune deficiencies, with the exception of decreased B cell development as a consequence of thyroid hormone deficiency [44]. In a review article from the same group who reported these findings, it was noted that contradictory results have been common in this area of research for several years and that some investigators have failed to detect immune deficiencies in the same mutant mouse strains in which other groups have reported deficiencies [51].

The hypothesis proposed to reconcile these contradictory results is that these hormones act in a general anabolic manner or act in specific ways to counteract the immunosuppressive actions of other stress-related mediators [51]. Thus, many of the studies in which immune deficiencies have been detected in mice deficient in GH, PRL, IGF-1, or thyroid hormones were done several years ago when animal care conditions varied widely and may have included stress-inducing stimuli (e.g. group housing of male mice, uncontrolled noise, improper ventilation, infrequent changing of bedding or cleaning of cages, etc.). The actual immunosuppressive mediators in these animals may have been corticosterone or other immunosuppressive hormones or neurotransmitters, which would have been at least partially counteracted by increases in GH, PRL, IGF-1, or thyroid hormone in normal animals. Results from other groups provide some support for this hypothesis. For example, GH activates the PI-3/Akt/NF-κB pathway to prevent glucocorticoid-induced apoptosis and promote progression of lymphoid cells through the cell cycle [52]. Similarly, up-regulation of Bcl-2 by GH and subsequent prevention of the Fas-mediated apoptosis has been reported in human monocytic cells [53]. Thus, protection of immune system cells from other stress-induced mediators may play an important role in the action of GH, PRL, and IGF-1. However, it also remains possible that results from hypophysectomy studies reflect indirect effects of lack of pituitary hormones or effects of hormones that are not yet known to regulate the immune system. Collectively, results from studies of the immunological effects of GH, PRL, IGF-1, and thyroid hormones illustrate the complexity of the interactions between the neuroendocrine and immune systems and the importance of rigorous control of experimental conditions an maintaining a broad per-

spective in interpreting results from studies designed to examine these interactions.

3.2. Epinephrine and norepinephrine

Catecholamines (especially epinephrine and norepinephrine) have a unique and complex role in modifying immune functions. Norepinephrine is one of the few neuroendocrine mediators that meet the criteria outlined by Ader and colleagues for neurotransmitters that regulate the immune system [27]. Several cell types in the immune system have adrenergic receptors, the spleen and other lymphoid organs are innervated by adrenergic nerve fibers, norepinephrine can be released from terminals of these fibers directly into the extracellular fluid of the spleen, and changes in the concentration of norepinephrine can cause meaningful changes in immune system function. Using a variety of complementary methods, several investigators have demonstrated that catecholamines mediate significant functional changes in the immune system [54–59]. There is also evidence that the immunological effects of agents that act through other receptors (e.g. morphine) also involve adrenergic receptors [60,61]. However, research on the role of catecholamines in stress-induced immunomodulation is also characterized by some findings on which there is general agreement and others about which there are still unexplained contradictory findings.

Rapid stress-induced changes in leukocyte numbers and percentages in the blood seem to be mediated mostly by norepinephrine. Exercise, particularly intense exercise, induces a stress response, and this is associated with a rapid, transient increase in the concentration of neutrophils, monocytes and lymphocytes (particularly NK cells) [62–64]. In a recent meta-analysis involving over 900 subjects described in 94 reports, a significant increase in NK cell number was noted at the end of acute moderate to intense exercise, with levels decreasing to values significantly below normal within about 2 h after exercise followed by a return to normal values within 24 h. In most studies, the initial increase in NK cell concentration in the blood was attributed to catecholamine-induced demargination [65]. Evidence has also been presented suggesting that the spleen is an important source for these rapidly mobilized NK cells [66]. However, direct administration of epinephrine or norepinephrine to normal and splenectomized human subjects revealed generally similar increases in NK cells (as well as T cells) [58]. The effect was greater for epinephrine than for norepinephrine and was not associated with changes in some of the adhesion molecules of NK cells (e.g. CD11b, CD43, or CD44). However, since only one mediator was given in each trial, these results do not necessarily represent the mechanisms involved in stress responses. However, they do provide convincing, direct evidence that epinephrine could be responsible for some of the effects of exercise stress. Thus, it is not surprising that NK cells taken from human subjects during and shortly after exercise stress had decreased levels of the adhesion molecules CD18 and CD44 [67], even though epinephrine or norepinephrine alone did not decrease the expression of CD 44 [58].

Catecholamines have also been implicated in stress-related modulation of cell-mediated and humoral immune responses, particularly in the spleen. Whereas the catecholamines influencing altered trafficking of blood leukocytes probably originate mostly from the adrenal glands, it seems likely that norepinephrine released directly from adrenergic nerve terminals [27] plays a more important role in secondary lymphoid organs. In a review on the subject, it was noted that chemical sympathectomy (using 6-hydroxydopamine) generally suppresses cell-mediated (Th1) responses and may enhance humoral (Th2) responses [40]. However, separate studies conducted later by two of the authors of this review indicate that the situation is more complex. The primary IgG1 and IgG2a antibody responses to the T-dependent antigen keyhole limpet hemocyanin (KLH) were significantly increased in sympathectomized C57Bl/6 mice, but the increase was significant only at a later time point for IgG1 in Balb/c mice [68]. This suggests that normal norepinephrine levels in the spleen provide a strain selective inhibition of IgG1 and IgG2a responses. However, using an adoptive transfer system to examine the effects of sympathectomy on the antibody response per se rather than development and maturation of the requisite cells types (which might also be influenced by peripheral catecholamines), it was noted that lack of norepinephrine suppressed the IgG1 response [69]. This suggests that normal levels of norepinephrine are a positive regulator of IgG1 responses. However, it remains possible that elevated (stress-induced) concentrations are suppressive.

The molecular and cellular mechanisms by which norepinephrine affects the antibody response have been investigated, and the results are instructive with regard to themes that should probably be considered in future studies of this and other neuroendocrine-immune interactions. An unanticipated finding was that β_2-adrenergic receptors are preferentially expressed on Th1 cells but not Th2 cells and that stimulation of these receptors suppressed subsequent generation of IgG2a antibody responses in vitro [70]. The signaling mechanism in this system involves an increase in cAMP, just as in other cell types with β_2-adrenergic receptors. These results are actually consistent with the findings noted above indicating increased IgG2a responses to KLH in C57Bl/6 mice that had been chemically sympathectomized (thus removing the down-regulatory effect of norepinephrine) [68]. Further studies have revealed that suppressed IgG1 levels reported in chemically sym-

phathectomized mice [69] reflect direct action of catecholamines on B lymphocytes (which also express β_2-adrenergic receptors) leading to up regulation of the co-stimulatory molecule B7-2 [71]. This is mediated through activation of several well-characterized kinases and leads to an increase in B7-2 mRNA stability. Thus, lack of β_2-adrenergic receptor stimulation in chemically symphathectomized mice apparently suppresses B7-2 expression, whereas experimental stimulation of β_2-adrenergic receptors enhances B7-2 expression. These treatments cause increased or decreased IgG1 responses, respectively. Although the impact of stress-induced levels of catecholamines on these humoral responses has not yet received much attention, the data now available on the molecular and cellular mechanisms of catecholamine action in this system will facilitate studies of this type by identifying parameters and mechanisms that are relevant to catecholamine-mediated modulation of humoral responses.

3.3. Opioids

Immune system cells apparently have receptors for opioids, and both endogenous and exogenous opioids can affect immune functions. This topic has been reviewed recently [72], and only selected issues will be examined here. Recent evidence suggests that activated T lymphocytes, which had previously been thought to possess classic opioid receptors, actually do not have opioid binding sites that meet all the criteria usually used to define these receptors [73]. Thus, direct effects of morphine on these cells may not be mediated trough classical opiate receptors. Knockout mice lacking μ-opiate receptors did not have dysfunctions in the immune parameters initially evaluated [74], suggesting that basal levels of endogenous opiates acting through these receptors are not critical for the development of at least some of the functions of immune system cells. However, contradictory results have been obtained with regard to the effects of morphine on the immune system in normal and opioid receptor knockout mice. For example, twice daily escalating doses of morphine for 6 days caused significant cell loss in the spleen and thymus, but cell loss was not significant in identically treated μ-opiate receptor knockout mice [74]. In contrast, continuous morphine administration by implantation of a 75 mg timed-release morphine pellet yielded a greater loss of cells from the thymus and spleen than noted with twice daily escalating doses [74], and these decreases in spleen and thymus cell number were essentially the same in wild-type and μ-opiate receptor knockout mice [75]. The basis for this difference is not clear, but an earlier study indicated that the loss of thymocytes in mice treated with a 75 mg timed-release morphine pellet are mediated entirely by the morphine-induced increase in serum corticosterone [76], which is remarkable and prolonged in wild-type mice [77]. Although a much smaller single dose of morphine (25 mg/kg) did not cause a significant increase in corticosterone in μ-receptor knockout mice [78], it remains possible that the pellet does increase corticosterone in knockout mice sufficiently to produce thymic atrophy by the induction of apoptosis [76]. It should also be noted that treatment of thymocytes in culture with morphine did not induce apoptosis [76]. This is consistent with the idea that the effects of morphine on the thymus are indirect. Similar results were noted with regard to the effects of opioids on antibody responses by spleen cell cultures [79]. However, others did find direct effects of opiates on splenocytes responses in vitro [80], and subsequent work suggested that the apparent contradiction was caused by mouse strains that bore the same designation, but had been maintained separately for several decades. This matter is discussed further in Section 6.

Even though the effects of morphine on thymic atrophy seem to be indirect, there is evidence for the involvement of endogenous opiates in restraint stress-induced loss of cells (by apoptosis) from the spleen [81,82]. Two groups of researchers using complementary methods (μ-opiate receptor knockout mice or opiate antagonists) concluded that restraint stress (two sessions of 12 h each) caused an opiate receptor-mediated up regulation of CD95 in the spleen and that this caused an increased rate of apoptosis leading to decreased numbers of splenic lymphocytes [81,82]. However, it is still not clear if the opiate receptors involved are located on splenocytes or if this is another indirect effect. The latter interpretation is favored by the observation that direct addition of opiates to spleen cell cultures does not decrease their response to antigen [79] and thus, presumably, does not directly cause substantial apoptosis. Nevertheless, these findings indicate an important role for stress-induced endogenous opiates in the induction of apoptosis in the spleen in vivo.

One of the earlier reports on the effects of endogenous opiates on the immune system demonstrated an important role for these mediators in suppression of NK cell activity in mice subjected to a particular footshock stress paradigm [83]. In another study, exogenous Met-enkephalin suppressed NK cell activity to a similar degree as overnight restraint stress [84]. However, it is clear that several factors influence the impact of endogenous opiates on NK cells. Exogenous Met-enkephalin treatment for 8 or 28 days had little effect on NK cells in athymic mice (which lack T lymphocytes) [85]. A forced swimming stress paradigm revealed a stress-induced suppression of NK cell activity that was not blocked by naltrexone, suggesting that endogenous opiates are not always essential for this effect [86]. In fact, some investigators have found that endogenous opiates can enhance NK cell activity [87,88]. Chronic

stress may deplete endogenous opiates, and this decrease has been implicated in the suppression of NK cell activity by chronic administration of ethanol [87]. Interestingly, there is even a report indicating that endogenous opioids may suppress the function of NK cells from human subjects who normally have high levels of NK cell activity, whereas the same opioids enhance the activity of NK cells from subjects whose NK cells normally have low levels of lytic function [89]. In some of these experiments, effects were noted in vitro, suggesting direct action of endogenous opioids through receptors on cells of the immune system. However, there is also evidence suggesting that endogenous opioids may not be necessary for suppression of NK cell activity by some stressors. Trauma and burn injury are potent stressors, and they cause substantial suppression of NK cell activity [90]. Administration of a mixture of cortisol, epinephrine, and glucagon to produce blood levels similar to those measured in trauma patients produced similar suppression of NK cell activity [90].

Weber and colleagues have shown that the brain region responsible for morphine-induced suppression of NK cell and macrophage function is the periaqueductal gray matter [91,92]. However, the mechanism by which morphine stimulation of this brain region affects NK cells or macrophages in the periphery is not known. In vitro exposure of macrophages to morphine suggests that in addition the central nervous system-mediated effects, morphine acts directly on these cells through activation of p38 MAPK and NF-κB resulting in apoptosis [93]. Morphine delivered by subcutaneous implantation of a timed-release pellet profoundly suppresses NK cell activity, but this effect seems to be mediated entirely by morphine-induced increases in corticosterone [94]. Thus, there is evidence for both direct and indirect effects of exogenous opioids on the immune system and for a role for endogenous opioids in some of the immunological effects of stressors.

3.4. Glucocorticoids

The immunosuppressive and anti-inflammatory characteristics of endogenous glucocorticoids have been known for many years, and this observation prompted the development of more potent synthetic analogs that are widely used in the treatment of allograft rejection, autoimmune disease, and allergy [95]. More recently, it has been appreciated that mild or brief elevations of corticosterone are not always immunosuppressive [96,97] and may even enhance some types of immune responses in some anatomical locations [98–100]. The effects of glucocorticoids on cells of the immune system are thought to be mediated primarily through the cytoplasmic glucocorticoid receptor. Binding of glucocorticoids (which enter the cell by passive diffusion) to the receptor causes dissociation of hsp90 and other proteins from the glucocorticoid receptor (GR) and allows migration of the GR to the nucleus [101,102]. The promoter regions of numerous genes have glucocorticoid response elements, and binding of the GR to these sites can either induce or suppress transcription [101]. In addition, there are a number of other mechanisms whereby glucocorticoids can alter gene expression, most of which occur rapidly [103–106]. For example, glucocorticoids can interfere with TCR signaling by a mechanism that does not require prior changes in transcription, but involves rapid inhibition of kinases [107]. However, synthetic glucocorticoids are often used in these studies, and the results may not be applicable to stress-induced natural glucocorticoids [108,109]. There are even indications that rapid signaling through membrane bound glucocorticoid receptors may complement slightly slower signaling mediated by cytoplasmic glucocorticoid receptors [103]. A number of investigators have noted rapid effects of glucocorticoids that for a variety of reasons are not entirely consistent with classical signaling through cytoplasmic receptors [103–106]. Thus, it will be interesting if additional studies definitively establish the presence of cytoplasmic GR and meaningful physiological functions for them.

It should also be noted that simplistic models of signaling through the cytoplasmic GR that have been widely accepted in the past should probably be reconsidered. The generally accepted model has been that glucocorticoid binding to the cytoplasmic GR complex leads to dissociation of the GR protein from the complex, rapid translocation of the GR to the nucleus, and binding to GR response elements leading to increased or decreased transcription [101]. However, immune system cells in vivo always have some amount of GR in the nucleus [110] (Fig. 2). Thus, an all-or-none signaling scheme as demonstrated with cell lines in culture probably does not represent glucocorticoid signaling during stress responses in vivo [111]. In addition, trafficking of GR into and from the nucleus

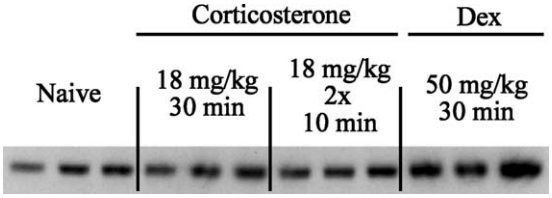

Fig. 2. Western blot illustrating glucocorticoid receptors (GR) in nuclear extracts of T cells isolated from mouse spleen. Each lane represents nuclear extract from T cells isolated from the spleen of one mouse. Thus, the results shown are for groups of three mice for each treatment. Mice were either undisturbed (naive) or treated with one dose of corticosterone at 18 mg/kg (subcutaneously) and sampled 30 min later, treated with two doses of corticosterone at 18 mg/kg (2 h apart) and sampled 10 min after the second dose, or treated with dexamethasone (Dex) at a pharmacological dose (50 mg/kg) as a positive control. Densitometry indicated that only the increase in nuclear GR caused by Dex was significantly different from naive.

is a dynamic, regulated process [112]. In a recent study of GR signaling in T lymphocytes in vivo, we noted that stress-inducible levels of corticosterone did not cause significant increases in nuclear GR protein (by Western blot) (Fig. 2), but a high dose of dexamethasone (the positive control) did noticeably increase nuclear GR levels. Interestingly, corticosterone did significantly increase nuclear proteins able to bind a GR response element (determined by electrophoretic mobility shift assay, not shown) [111]. The same dosages of corticosterone caused profound suppression of anti-CD3-induced cytokine production, demonstrating that functionally relevant signaling was induced in this system [111]. This suggests changes in the state of the GR in the nucleus can be as important as the amount of GR in the nucleus in modulating gene expression.

Virtually all aspects of immune and inflammatory responses can be modified (usually suppressed) by glucocorticoids. In addition, several approaches have been used to demonstrate that, for many types of stressors and many immunological end points, glucocorticoids are the major mediators of stress-induced immunosuppression [113–115,94,116–120], of course, there are exceptions. Elevated corticosterone is not always associated with immunosuppression, and preventing the action of corticosterone does not always prevent stress-induced immunosuppression [121–124]. It is also interesting that both endogenous and exogenous (synthetic) glucocorticoids cause a substantial increase in the neutrophil concentration in the blood at the same time as the number and function of virtually all lymphocyte populations are dramatically decreased [24,77]. Thus, it is not surprising that a vigorous drug-induced stress response or treatment with pharmacological dosages of a synthetic glucocorticoid do not suppress host resistance to *Listeria monocytogenes*, which depends on neutrophils [24,77]. It seems likely that the increased number of neutrophils is sufficient to compensate for the loss of lymphocyte functions, which are also normally important in resistance to this organism. This precarious balance may explain some of the contradictory results regarding the importance of glucocorticoids in stress-induced decreases in resistance to this bacterium [121,120].

Quantitative comparisons of the effects of exogenous corticosterone, restraint stress, and a chemical stressor indicate that the effects of other mediators can substantially alter the action of corticosterone on some immunological parameters [125]. Although quantitative measurements of corticosterone can be used to accurately predict stress-induced changes in some immunological parameters, effects on other parameters are clearly stressor specific [126,125]. Thus, glucocorticoids are often major mediators of stress-induced immunosuppression, but other mediators can either modify the effects of corticosterone or can have direct effects on some immunological parameters.

The mechanisms by which neuroendocrine mediators interact to affect the immune system have only rarely been investigated. Infusion of cortisol (at stress inducible levels) for 3 days decreased NK cell activity less effectively than a combination of cortisol, epinephrine, and glucagon designed to mimic stress hormone levels in burn patients [90]. In this case, immunosuppression may simply reflect the additive immunosuppressive effects of each mediator. Evaluations of this type are complicated by factors in addition to the amount of glucocorticoid exposure that can substantially modify the immunological effects. For example, the amount of corticosterone binding globulin, the amount and affinity of the intracellular GR, and the amount of 11 β-hydroxysteroid hydroxylase (which breaks down corticosterone) also play a role in determining the effects of glucocorticoids in vivo [127–130]. These factors may be up or down regulated by exposure to stressors, and they probably should be considered when interpreting results from studies of this type [131,129].

Treatment of animals with bacterial lipopolysaccharide can induce SIRS [132]. This is often used as a model for the induction of SIRS during bacterial septicemia, a condition that can lead to cytokine-induced multiple organ failure and death [133,134]. Corticosterone and possibly other stress mediators induced by the cytokines act as negative regulators of this cytokine production. Thus, adrenalectomized mice are more sensitive to virus-induced SIRS, and survival is improved by administration of exogenous corticosterone [13]. A key mediator in cytokine-induced stress responses (and probably in responses to other stressors as well) is macrophage migration inhibitory factor (MIF). As the name indicates, this mediator was initially described on the basis of its ability to inhibit macrophage migration and thus retain macrophages at sites of injury or infection. However, a recent study demonstrates an important role in the pathogenesis of sepsis. Inhibiting MIF by injection of an antibody markedly increases survival in a mouse model of SIRS caused by peritonitis [26]. Administration of recombinant MIF in this model of SIRS substantially increased mortality. One of the mechanisms by which MIF seems to work is by preventing the glucocorticoid-mediated down regulation of cytokine production that normally serves to diminish the severity SIRS [135]. Importantly, elevated MIF has also been reported in humans with septicemia [26]. However, the complexity of this system is illustrated by a recent report demonstrating that MIF knockout mice are more susceptible to mortality caused by *Salmonella typhimurium* infection [136]. Levels of corticosterone and nitric oxide were higher than in normal mice infected with these bacteria, and this apparently contributed to decreased cell-mediated immune re-

sponses that normally lead to clearance of the bacteria. The complementary results obtained with septicemia-induced SIRS and a more routine bacterial infection provide an excellent illustration of regulatory interactions between microbes, stress responses, and MIF on one hand and clearance of harmful bacteria or pathological SIRS on the other.

Another interesting example of important glucocorticoid effects on the immune system serves to illustrate how hazardous it can be to generalize from specific results to broad principles. Several investigators reported that increased glucocorticoid concentrations in vivo or in vitro are associated with decreased Th1 responses and increased Th2 responses, which were mostly assessed by the production of Th1 or Th2 cytokines or the relative levels of Th1 or Th2-dependent antibody responses [137,127,138,105]. However, other investigators have reported no evidence for this association [139–143]. None of these studies was done in precisely the same way, but there are no obvious common factors that would explain the different outcomes. Thus, the generality of this phenomenon remains unclear, and the use of glucocorticoids to purposely shift the Th1/Th2 balance (for example, to decrease IgE-mediated allergies) may not be effective.

3.5. Composite view of mediators involved in immunological effects of one model stressor (restraint) in rodents

Restraint is a potent stressor for rats and mice. Although it induces a stress response that is often considered an analog of psychological stress in humans, there are actually substantial differences between the effects of restraint and a natural psychological stressor (the presence of a predator) [144]. Nevertheless, restraint is one of the most commonly used stressors, and the role of several neuroendocrine mediators in restraint-induced immunomodulation has been determined. Studies in my lab have demonstrated that some immunological parameters (for example, suppression of the expression of MHC class II proteins) are affected to the same extent by restraint as by exogenous corticosterone, at equivalent values for the area under the corticosterone concentration versus time curve [145]. However, other parameters (e.g. the percentages of the major cellular subpopulations in the thymus) are affected very differently by restraint and exogenous corticosterone [126]. These results strongly suggest that the action of restraint on some immunological parameters is mediated solely by glucocorticoids, whereas other mediators are involved (perhaps in conjunction with glucocorticoids) in the effects of restraint on other immunological parameters [146,145,126,125].

Other investigators have reported results that are consistent with these findings. However, it should be emphasized (as discussed in the next section of this review) that the duration of restraint and the mode of exposure (single or repeated) are critical variables. In general, we have noted no immunosuppression with brief duration of restraint (up to 2 h) [146,145,126,125], and others have reported that some immunological parameters can be enhanced [98,100]. As with most stress-immunology studies, there are important technical issues that must be considered in interpreting results from these studies. Although some investigators have noted minimal effects on some parameters with daily exposure to restraint, it should be noted that some rat strains become habituated to restraint during a 4 h session or following repeated daily sessions [147]. However, other strains do not become habituated and continue to exhibit full activation of the HPA axis during each exposure [147]. We have noted that B6C3F1 mice do not become habituated during exposures as long as 8 h.

Most of the neuroendocrine mediators discussed in this review seem to be involved in the effects of restraint on at least some immunological parameters. Thymocyte apoptosis and subsequent thymic atrophy are mediated by glucocorticoids [118]. However, apoptosis and loss of lymphocytes in the spleen of mice exposed to 12 h restraint sessions is apparently mediated by endogenous opiates, because these effects are eliminated in μ-opiate receptor knockout mice [81]. Repeated daily restraint suppresses resistance of Fisher 344 rats (which do not become habituated to restraint) to dimethylbenz[*a*]anthrcene-induced tumors [148]. Experiments using antagonists suggest that endogenous opiates and PRL are involved in the suppression of resistance to these tumors. Resistance to lethality in mice challenged with Theiler's virus is decreased by restraint, and corticosterone seems to be a major mediator in this decreased resistance [113]. Catecholamines and corticosterone are both important in restraint stress-induced suppression of host resistance to herpes simplex virus in mice [115]. In contrast, restraint stress increases survival in mice treated with influenza virus, which is lethal primarily because of the induction of a systemic inflammatory response [149]. In that model, decreased changes in leukocyte trafficking and initial inflammation were mediated primarily by corticosterone, whereas decreased activation of specific immunity was mediated by catecholamines. In addition to adrenergic receptors, dopamine receptors seem to be involved in the restraint stress-induced suppression of NK cell activity and the balance of Th1 and Th2 cytokine production [150].

A reasonably consistent composite picture of the role of various stress mediators in restraint-induced immunomodulation can be derived from the available literature on this topic. It is apparent that the sensitivity of particular immune parameters to different stress-induced mediators varies considerably and that the effects

of restraint on a particular immunological parameter are often mediated by the combined action of more than one mediator.

4. Quantitative aspects of stress–immune interaction and stressor-specific effects

We have noted in a recent review that one of the more interesting and important aspects of stress–immune interactions is only just beginning to receive detailed attention. The work of Dhabhar and colleagues has dramatically demonstrated that depending on the duration and intensity of the stressor, stress responses can suppress or enhance certain immune responses [99,151,100]. The basis for this has not been completely determined, but altered trafficking of lymphocytes to the skin and possibly other locations may serve to enhance immune responses in those locations [152,153].

We have noted that measurements of stress-related mediators at a single time point in the course of an experiment cannot reveal the extent to which stress is likely to alter immune functions. However, if the exposure of the animal to even one mediator (corticosterone) is quantified by measuring the area under the concentration versus time curve, this can be used to accurately predict the effects of restraint and chemical stressors on some (but not all) immunological parameters [146,145,142,126,125]. Although area under the curve determinations are labor intensive and require large numbers of animals, they are necessary to relate the actual quantitative increase in exposure to stress-related mediators and immunological effects. In fact, linear models have been derived using mice exposed to different periods of restraint stress, and these models allow accurate prediction of the effects of a chemical stressor [125].

In studies that have not yet been published we also noted that for a few immunological parameters there were significant differences in the effects of three chemical stressors or restraint stress, at the same corticosterone AUC value. This is consistent with results from a few studies that suggest different stressors yield different relative amounts of stress mediators [154,155,5]. There are also indications that psychological stressors produce different profiles of neuroendocrine mediators in persons who are better able to cope with these stressors than in persons who are not [10]. Of course, there is substantial evidence for differences in the immunological effects of different stressors, and this constitutes indirect evidence that different mediators or at least different concentrations of a common set of mediators are induced. For example, foot shock stress suppresses NK cell activity by an endogenous opioid-dependent mechanism [83]. However, forced swimming stress leads to an endogenous opiate-independent suppression of NK cell activity [86]. Differences have also been reported with regard to the types of stressors that suppress resistance to upper respiratory infections in humans [33]. However, partly because of the substantial technical challenges associated with accurately measuring the rapidly responding stress mediators (e.g. epinephrine and norepinephrine released by direct activation of the sympathetic nervous system, which occurs almost instantaneously), studies that precisely quantitate the exposure of animals to several major stress mediators in response to several different stressors are lacking. Such studies are needed before substantial progress can be made in this area and in order to rationally develop specific pharmacological interventions to prevent stress-related immunosuppression.

5. Therapeutic intervention for stress-mediated immune dysfunction

One of the ultimate goals of stress-immunology research is to devise approaches that will abrogate the adverse immunological effects of stress while not eliminating the homeostatic mechanisms, which may be advantageous to the host. Some recent studies suggest promising possibilities in this regard. For example, blocking the action of MIF by administration of a specific antibody improves survival in models of sepsis, even when administered 8 h after initiation of sepsis [26]. Such protection after the initiation of SIRS is not usually observed with traditional treatments such as administration of glucocorticoids. However, in many cases chronic stress is associated with immunosuppression as the adverse effect. Unfortunately, it is not usually known which mediators are responsible for suppression of particular immunological functions. However, there is accumulating evidence suggesting that chronic elevations in glucocorticoids may contribute to degenerative changes in the brain [31]. In addition, at least one study notes a strong relationship between the area under the cortisol concentration versus time curve and suppression of the antibody response in persons subjected to chronic stress [7]. Therefore, decreasing the up regulation of glucocorticoids may be therapeutically desirable.

Pharmacological agents that will decrease stress-induced elevation of glucocorticoids are already available [95], and the prevention of some of the immunosuppressive effects of stress may be feasible. However, such treatments have the potential at least of exacerbating autoimmune disease [156] or allergy. Psychotherapy and biofeedback approaches would seem particularly suitable, because they can diminish the underlying stress response to some psychological stimuli [157–160]. However, short-term pharmacological intervention to diminish glucocorticoid responses may be

desirable in persons exposed to severe psychological stress [158] or to other stressors such as surgery or burn injury, which are known to be immunosuppressive [90,161,162].

A particularly interesting aspect of immunosuppression associated with stress is decreased expression of major histocompatibility class II proteins on antigen presenting cells [163–167]. Substantially decreased expression of MHC class II proteins in trauma patients correlates remarkably well with the risk of infection [168–170]. Although it has been assumed by some investigators that decreased expression of MHC class II proteins and consequent decreases in antigen presentation are responsible for the inverse relationship between MHC II expression and infection, restoration of MHC class II expression by administration of Interferon-γ to trauma patients did not significantly decrease the rate of infection [171,172]. In addition, the neuroendocrine mediator responsible for decreased MHC class II expression is not entirely clear. Studies with animal models and studies using human cells indicate that glucocorticoids can decrease MHC class II expression [173,174]. At least one stressor acts exclusively through increased levels of endogenous glucocorticoids to suppress basal expression of MHC class II in the spleen [119]. However, increased catecholamines leading to induction of IL-10 (which can suppress MHC class II expression and suppress other immune parameters as well) [175] have also been implicated, at least in persons with brain injury [168,170]. In any case, the association between stress, stress-induced immunomodulation and infection are strong.

Although pharmacological intervention to prevent some of the adverse effects of stress mediators seems feasible [176,13,119], issues such as the identity of the predominant immunosuppressive mediator and potential untoward effects such as exacerbation of autoimmunity or allergy and failure to appropriately regulate systemic inflammatory responses are still unresolved.

6. Pitfalls in stress-immunology research

One of the criticisms of stress-immunology research often voiced by immunologists is that the literature on the subject is full of contradictory reports. A quick review of the literature on almost any aspect of stress immunology seems to confirm this. In fact, several examples of such reports have been cited in this review. The assumption that sometimes follows from this observation is that the overall quality of research in this field is poor. As in any field of research, examples of stress-immunology studies of questionable quality can be identified. However, it is unlikely that poor quality studies explain the frequent occurrence of contradictory observations in this field.

Most of the contradictory observations that have been published seem to reflect the complexity of the physiological systems under investigation. The immune and neuroendocrine systems considered separately are among the most complex systems in mammals. Studying the interactions of these two dynamic, complex systems is necessarily more difficult than studying either separately. Careful evaluation of the literature suggests that many studies that seem to have yielded contradictory results actually involved sufficient differences in procedure, animal species or strain, or other substantive matters to account for differences in results. Table 2 lists some conditions or variables that can affect the outcome of immunological and stress-related end points. Because it is rare for two labs to use precisely the same stress paradigm, the same methodology for evaluating immunological end points, and animals of the same species, strain, age, and sex, it should not be

Table 2
Factors that can influence immune and stress-related end points

	References
Factors that can influence immune end points	
Age of animal	[190,191]
Sex of animal	[192]
Strain of animal	[193]
Housing conditions	[194]
Nutritional status (varies with different lab chow formulations)	[195]
Timing of stress relative to immune challenge	[196]
Timing of immune end point assessment (peak vs. early or late)	[126]
Time of day	[197,198]
Anatomical location of the immune assessment	[100]
Season of the year	[199]
Brain asymmetry (e.g. right or left preferring mice)	[200]
Factors that can influence stress-related end points	
Age of animal	[190]
Sex of animal	[201]
Strain of animal	[128]
Time of day	[202]
Housing conditions	
Presence of males and females in same room	[203]
Number of animals per cage	[204]
Exposure of mother to stressors during gestation	[201]
Noise	[205]
Frequent entry/exit from animal room	Probably related to noise or odor
Length/timing of light/dark cycle	[206]
Social interactions	[207]
Time allowed for recovery from shipping stress	[208]
Anesthesia	[209]

surprising that results differ. Perhaps the only solution to this problem is a more deliberate attempt by investigators to precisely replicate the conditions of a previous study, when their work depends heavily on the methods or conclusions of that study.

Validation studies sponsored several years ago by the National Toxicology Program for methods to assess immunotoxicity provide an excellent illustration of how difficult it can be to obtain consistent results in studies designed to evaluate immunosuppressive treatments [177]. Studies were conducted in three independent labs, which used mice of the same strain, age, and sex (from the same supplier). These studies were conducted using the same standard operating procedures, and in most cases reagents from the same suppliers and the same strains of challenge microbes or tumor cells were used. Under these conditions, there was generally good agreement between labs with regard to the dosages of particular immunotoxic compounds that significantly affected particular immunological end points. However, even under these stringently controlled conditions, there were several differences that would have seemed contradictory had they been viewed in isolation in separate studies. For example, one lab reported that diethylstilbesterol at 0.1 mg/kg increased mortality following challenge with *L. monocytogenes* from 34 to 53% (not significant), whereas another lab reported an increase from 20 to 95% ($P < 0.05$). Had these studies been published separately, without a full dose–response experiment and without repeating the experiment, they would have seemed contradictory, even though the two labs used the same protocols and materials. Thus, measures such as repeating experiments and conducting dose–response experiments as a routine part of stress-immunology studies might serve to diminish the discrepancies in reported results. However, it should be noted that differences observed in the National Toxicology Program validation studies may simply reflect the fact that the effects of environmental stimuli on some immunological and neuroendocrine processes can be described best by non-linear models [178]. It seems likely that elements of chaos theory, such as sensitive dependence on initial conditions and attractor equations, which predict outcomes that seem almost random, but actually fall within rather well-defined boundaries, apply to immunological systems as well [179]. If so, some apparently contradictory data are probably unavoidable. Perhaps at the present stage of development in this field of research, more attention should be given to studies designed to test the generality and factors affecting the expression of previously reported phenomena than to studies designed to identify new phenomena.

Although the complexity of the immune and neuroendocrine systems is likely responsible for many of the contradictory reports in this field, there are also a few errors or less than optimum experimental approaches that seem to occur more frequently than one would hope and probably contribute to discrepancies in results. For example, it is becoming clear that over-reliance on in vitro approaches, particularly when studying cellular signaling, can provide misleading results. For example, signaling through the T cell receptor complex induced by the same stimulus activates signaling cascades with fundamental differences, depending on whether the signaling is induced in vitro or in vivo [180].

The use of non-physiological concentrations of neuroendocrine mediators in experiments with cultured immune system cells is still fairly common. Although it is reasonable to use higher than physiological concentrations as part of a complete concentration–response experiment, it is not appropriate to interpret as meaningful changes induced only when the concentration is orders of magnitude greater than expected in vivo. In some cases, the exact concentration of a mediator in a particular location in vivo is not known. In these cases, the K_d for the receptor in question provides a useful reference point. If the concentration of the mediator required to cause a significant effect in vitro is more than 10-fold greater than the K_d, this effect may not be physiologically relevant. Attempts to identify receptors for neurotransmitters and hormones in the immune system have also produced a number of questionable results. For example, cholinergic receptors have been reported on various cell of the immune system, but the K_d values reported vary by more than 1000-fold, and binding curves with no evidence of saturation have nevertheless been interpreted as indicating specific receptors [181–185]. Finally, differences in mouse or rat strain between experiments are often regarded to be inconsequential when comparing results from different labs. It should be emphasized that there are fundamental differences in the stress axis in different rat strains [128] and probably in different mouse strains as well.

In fact, there are indications that sub-strains that have the same basic strain designation may differ in important ways, if they have been maintained separately by suppliers. We found that direct addition of morphine or other opiate agonists to spleen cells in Mishell–Dutton cultures did not affect the antibody response in these cultures [79]. However, another group used virtually identical methodology and found direct effects that were blocked by appropriate antagonists [80]. In a follow-up study, this group determined that there are substantial strain differences in the direct effects of opiates on antibody responses in vitro, with cells from some strains not being affected at all [186]. However, their results with B6C3F1 mice were still not identical to those we had reported, in that they still observed suppression of antibody responses with a κ-opiate receptor agonist (though not with morphine). However, upon further investigation these authors noted that the B6C3F1 mice

we had used (from the National Cancer Institute's strains maintained by Charles River Labs) and the ones they had used (from Jackson Labs) had been maintained separately for 45 years and were derived from different C3H sublines, one of which was susceptible to LPS and the other of which was not [186]. It is a little disconcerting that something as fundamental as the expression or function of opioid receptors on immune system cells varies in different mouse strains, but this seems to have been solidly established [187,186,79]. It would seem appropriate to consider strain difference as a factor whenever differences in results are reported for experiments that otherwise seem quite similar. In addition, caution is indicated in the degree to which any conclusion obtained in a single strain can be generalized.

Another troublesome issue in stress-immunology research is the use of antagonists in vivo to identify the role of particular mediators in stress-induced immunomodulation. Although this is a common approach and can certainly yield valid information, one aspect of these methods does not get as much attention as it probably deserves. Neuroendocrine and immune mediators are components in complex regulatory networks, and suppressing the action of one mediator often affects the concentration of other mediators. For example, the widely used glucocorticoid and progesterone antagonist RU 486 (mifepristone) not only blocks the action of these two hormones in the immune system, it blocks the normal feedback control in the hypothalamus and pituitary leading to massive up regulation of ACTH [188]. Because cells of the immune system have receptors for ACTH [27], effects of RU 486 on the immune system could be caused by blockade of glucocorticoid action or by increased ACTH action. Some investigators have investigated the role of such effects in their experimental system. For example, in one study, 6-hydroxydopamine was used to chemically sympathectomize mice, but the authors were aware that this compound activates the HPA axis, leading to increased glucocorticoid levels [55]. To determine if the increased glucocorticoid concentration was responsible for some of the effects that had been attributed to sympathectomy, the mice were also treated with RU 486. The results suggested that glucocorticoids did not have a role in the observed effects [55]. Of course, the most rigorous possible consideration of this experiment suggests that this process could continue ad infinitum (next ACTH would need to be blocked, then CRH/AVP, etc.). Instead, a useful practice has been to use alternate approaches to block a particular mediator. For example, if a glucocorticoid antagonist produces a particular effect and similar results are obtained with a glucocorticoid synthesis inhibitor, this provides greater confidence in the conclusions of the study. Unfortunately such options are not always available. Thus, due caution should be exercised in drawing conclusions from studies based on the effect of a single antagonist in vivo.

It should also be emphasized that knockout mice and pharmacological antagonists are the major tools that have been used to determine the role of particular mediators in stress-induced immunomodulation. These approaches do not always permit determination of whether these mediators act directly on the immune system or whether they are part of a cascade leading to up regulation of the ultimate immunomodulatory mediator. For example, it has been demonstrated that β-endorphin and other opioids in the brain are involved in restraint-induced activation of the HPA axis and the sympathetic nervous system [189]. Thus, antagonists that can cross the blood–brain barrier (including the most commonly used ones, naltrexone and naloxone) may act indirectly in the brain rather than by blocking direct action of opioids on cells of the immune system. Similar situations likely occur with other mediators as well.

7. Conclusion

Stressors can have profoundly positive or negative effects on the immune system, depending on the duration, intensity, and type of stressor, the immunological function evaluated, the timing of the stressor, the timing of evaluation of the immunological end points, and many other factors. Even so, it has been possible to identify a number of immunological effects that are consistently associated with certain stressors or stress-related mediators. Considerable progress has been made in discerning the cellular and molecular mechanisms of these effects. Three theoretical frameworks have been described that should facilitate interpretation of existing data and the design of future studies. Within these or other frameworks, it may be possible to understand the complex interactions between the neuroendocrine and immune systems to a sufficient degree that changes measured at the molecular or cellular level could be used to predict the immunological consequences of stressor exposure. Such a detailed understanding would facilitate therapeutic intervention designed to prevent deleterious immunological consequences of stressors without eliminating beneficial aspects of the neuroendocrine response induced by the stressor. The complexity of these systems and experimental pitfalls that are evident in the literature may have delayed progress in this field of research. The experimental pitfalls generally have simple remedies, and the complexity of these systems should become less problematic as more data are accumulated. This should eventually support increasingly broad generalizations.

Acknowledgements

The results described in Fig. 2 were obtained in the author's lab as part of studies funded by National Institutes of Health Grant ES09158. The assistance of Dr Pamela Hébert in preparing Table 2 is greatly appreciated.

References

[1] A.J. Dunn, Cytokine activation of the HPA axis, Ann. NY Acad. Sci. 917 (2000) 608–617.
[2] A.V. Turnbull, C.L. Rivier, Regulation of the hypothalamic–pituitary–adrenal axis by cytokines: actions and mechanisms of action, Physiol. Rev. 79 (1999) 1–71.
[3] R. Ader, N. Cohen, D. Felten, Psychoneuroimmunology: interactions between the nervous system and the immune system, Lancet 345 (1995) 99–103.
[4] M.H. Whitnall, Regulation of the hypothalamic corticotropin-releasing hormone neurosecretory system, Prog. Neurobiol. 40 (1993) 573–629.
[5] K. Pacák, J.S. Baffi, R. Kvetnansky, D.S. Goldstein, M. Palkovits, Stressor-specific activation of catecholaminergic systems: implications for stress-related hypothalamic–pituitary–adrenocortical responses, Adv. Pharmacol. 42 (1998) 561–564.
[6] J.K. Kiecolt-Glaser, R. Glaser, S. Gravenstein, W.B. Malarkey, J. Sheridan, Chronic stress alters the immune response to influenza virus vaccine in older adults, Proc. Natl. Acad. Sci. USA 93 (1996) 3043–3047.
[7] K. Vedhara, N.K. Cox, G.K. Wilcock, P. Perks, M. Hunt, S. Anderson, S.L. Lightman, N.M. Shanks, Chronic stress in elderly carers of dementia patients and antibody response to influenza vaccination [see comments], Lancet 353 (1999) 627–631.
[8] E.M. Farber, L. Nall, Psoriasis: a stress-related disease, Cutis 51 (1993) 322–326.
[9] R. Jacobs, C.R. Pawlak, E. Mikeska, D. Meyer-Olson, M. Martin, C.J. Heijnen, M. Schedlowski, R.E. Schmidt, Systemic lupus erythematosus and rheumatoid arthritis patients differ from healthy controls in their cytokine pattern after stress exposure, Rheumatology (Oxford) 40 (2001) 868–875.
[10] F. Lechin, B. van der Dijs, A. Lechin, B. Orozco, M. Lechin, S. Baez, I. Rada, G. Leon, E. Acosta, Plasma neurotransmitters and cortisol in chronic illness: role of stress, J. Med. 25 (1994) 181–192.
[11] R.R. Leker, A. Karni, O. Abramsky, Exacerbation of myasthenia gravis during the menstrual period, J. Neurol. Sci. 156 (1998) 107–111.
[12] V. Vasquez, R.A. Barzaga, B.A. Cunha, Cytomegalovirus-induced flare of systemic lupus erythematosus, Heart Lung 21 (1992) 407–408.
[13] M.C. Ruzek, B.D. Pearce, A.H. Miller, C.A. Biron, Endogenous glucocorticoids protect against cytokine-mediated lethality during viral infection, J. Immunol. 162 (1999) 3527–3533.
[14] H. Selye, Resistance to various pesticides, Arch. Environ. Health 21 (1970) 706–710.
[15] M. Baggiolini, Chemokines and leukocyte traffic, Nature 392 (1998) 565–568.
[16] R.F. Guo, N.C. Riedemann, I.J. Laudes, V.J. Sarma, R.G. Kunkel, K.A. Dilley, J.D. Paulauskis, P.A. Ward, Altered neutrophil trafficking during sepsis, J. Immunol. 169 (2002) 307–314.
[17] T.P. Salazar-Mather, R. Ishikawa, C.A. Biron, NK cell trafficking and cytokine expression in splenic compartments after IFN induction and viral infection, J. Immunol. 157 (1996) 3054–3064.
[18] A.W. Goldrath, Maintaining the status quo: T-cell homeostasis, Microbes Infect. 4 (2002) 539–545.
[19] D.A.M. Grillot, R. Merino, J.C. Pena, W.C. Fanslow, F.D. Finkelman, C.B. Thompson, G. Nunez, *bcl-x* exhibits regulated expression during B cell development and activation and modulates lymphocyte survival in transgenic mice, J. Exp. Med. 183 (1996) 381–391.
[20] C.G. Park, S.Y. Lee, G. Kandala, S.Y. Lee, Y. Choi, A novel gene product that couples TCR signaling to Fas(CD95) expression in activation-induced cell death, Immunity 4 (1996) 583–591.
[21] T.R. Mosmann, S. Sad, The expanding universe of T-cell subsets: Th1, Th2 and more, Immunol. Today 17 (1996) 138–146.
[22] N. Kadowaki, S. Ho, S. Antonenko, R.W. Malefyt, R.A. Kastelein, F. Bazan, Y.J. Liu, Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens, J. Exp. Med. 194 (2001) 863–869.
[23] M. Schnare, G.M. Barton, A.C. Holt, K. Takeda, S. Akira, R. Medzhitov, Toll-like receptors control activation of adaptive immune responses, Nat. Immunol. 2 (2001) 947–950.
[24] D. Keil, R.W. Luebke, S.B. Pruett, Quantifying the relationship between multiple immunological parameters and host resistance: probing the limits of reductionism, J. Immunol. 167 (2001) 4543–4552.
[25] S.D. Wilson, J.A. McCay, L.F. Butterworth, A.E. Munson, K.L. White, Jr, Correlation of suppressed natural killer cell activity with altered host resistance models in B6C3F1 mice, Toxicol. Appl. Pharmacol. 177 (2001) 208–218.
[26] T. Calandra, B. Echtenacher, D.L. Roy, J. Pugin, C.N. Metz, L. Hultner, D. Heumann, D. Mannel, R. Bucala, M.P. Glauser, Protection from septic shock by neutralization of macrophage migration inhibitory factor, Nat. Med. 6 (2000) 164–170.
[27] R. Ader, D. Felten, N. Cohen, Interactions between the brain and the immune system, Annu. Rev. Pharmacol. Toxicol. 30 (1990) 561–602.
[28] H. Anisman, S. Lacosta, P. Kent, D.C. McIntyre, Z. Merali, Stressor-induced corticotropin-releasing hormone, bombesin, ACTH and corticosterone variations in strains of mice differentially responsive to stressors, Stress 2 (1998) 209–220.
[29] A. Inui, Cytokines and sickness behavior: implications from knockout animal models, Trends Immunol. 22 (2001) 469–473.
[30] B.S. McEwen, Protective and damaging effects of stress mediators, New Engl. J. Med. 338 (1998) 171–179.
[31] R.M. Sapolsky, L.M. Romero, A.U. Munck, How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions, Endocr. Rev. 21 (2000) 55–89.
[32] S. Cohen, W.J. Doyle, D.P. Skoner, Psychological stress, cytokine production, and severity of upper respiratory illness, Psychosom. Med. 61 (1999) 175–180.
[33] S. Cohen, E. Frank, W.J. Doyle, D.P. Skoner, B.S. Rabin, J.M. Gwaltney, Types of stressors that increase susceptibility to the common cold in healthy adults, Health Psychol. 17 (1998) 214–223.
[34] T.B. Herbert, S. Cohen, Stress and immunity in humans: a meta-analytic review, Psychosom. Med. 55 (1993) 364–379.
[35] T.J. Connor, B.E. Leonard, Depression, stress and immunological activation: the role of cytokines in depressive disorders, Life Sci. 62 (1998) 583–606.
[36] J.B. van Woensel, M.H. Biezeveld, A.M. Alders, A.J. Eerenberg, E. Endert, E.C. Hack, I.A. von Rosenstiel, T.W. Kuijpers, Adrenocorticotropic hormone and cortisol levels in relation to inflammatory response and disease severity in children with meningococcal disease, J. Infect. Dis. 184 (2001) 1532–1537.

[37] J.K. Kiecolt-Glaser, L. McGuire, T.F. Robles, R. Glaser, Psychoneuroimmunology and psychosomatic medicine: back to the future, Psychosom. Med. 64 (2002) 15–28.
[38] D.A. Weigent, J.E. Blalock, Associations between the neuroendocrine and immune systems, J. Leuk. Biol. 57 (1995) 137–150.
[39] N.E. Kaminski, Immune regulation by cannabinoid compounds through the inhibition of cyclic AMP signaling cascade and altered gene expression, Biochem. Pharmacol. 52 (1996) 1133–1140.
[40] K.S. Madden, V.M. Sanders, D.L. Felten, Catecholamine influences and sympathetic neural modulation of immune responsiveness, Annu. Rev. Pharmacol. Toxicol. 35 (1995) 417–448.
[41] E.M. Friedman, D.A. Lawrence, Environmental stress mediates changes in neuroimmunological interactions, Toxicol. Sci. 67 (2002) 4–10.
[42] G. Hasko, Receptor-mediated interaction between the sympathetic nervous system and immune system in inflammation, Neurochem. Res. 26 (2001) 1039–1044.
[43] J.I. Webster, L. Tonelli, E.M. Sternberg, Neuroendocrine regulation of immunity, Annu. Rev. Immunol. 20 (2002) 152–163.
[44] M.P. Foster, E.R. Jensen, E. Montecino-Rodriguez, H. Leathers, N. Horseman, K. Dorshkind, Humoral and cell-mediated immunity in mice with genetic deficiencies of prolactin, growth hormone, insulin-like growth factor-I, and thyroid hormone, Clin. Immunol. 96 (2000) 140–149.
[45] R. Gerli, P. Rambotti, I. Nicoletti, S. Orlandi, G. Migliorati, C. Riccardi, Reduced number of natural killer cells in patients with pathological hyperprolactinemia, Clin. Exp. Immunol. 64 (1986) 399–406.
[46] A. Vidaller, L. Llorente, F. Larrea, J.P. Mendez, J. Alcocer-Varela, D. Alarcon-Segovia, T-cell dysregulation in patients with hyperprolactinemia: effect of bromocriptine treatment, Clin. Immunol. Immunopathol. 38 (1986) 337–343.
[47] E.W. Bernton, M.T. Meltzer, J.W. Holaday, Suppression of macrophage activation and T-lymphocyte function in hypoprolactinemic mice, Science 239 (1988) 401–404.
[48] J.H. Exon, J.L. Bussiere, J.R. Williams, Hypophysectomy and growth hormone replacement effects on multiple immune responses in rats, Brain Behav. Immun. 4 (1990) 118–128.
[49] Q.B. Saxena, R.K. Saxena, W.H. Adler, Regulation of natural killer activity in vivo. III. Effect of hypophysectomy and growth hormone treatment on the natural killer activity of the mouse spleen cell population, Int. Arch. Allergy Appl. Immun. 67 (1982) 169–174.
[50] C.K. Edwards, R.M. Lorence, D.M. Dunham, S. Arkins, L.M. Yunger, J.A. Greager, R.J. Walter, R. Dantzer, K.W. Kelley, Hypophysectomy inhibits the synthesis of tumor necrosis factor α by rat macrophages: partial restoration by exogenous growth hormone or interferon γ, Endocrinology 128 (1991) 989–996.
[51] K. Dorshkind, N.D. Horseman, The roles of prolactin, growth hormone, insulin-like growth factor-I, and thyroid hormones in lymphocyte development and function: insights from genetic models of hormone and hormone receptor deficiency, Endocr. Rev. 21 (2000) 292–312.
[52] S. Jeay, G.E. Sonenshein, M.C. Postel-Vinay, P.A. Kelly, E. Baixeras, Growth hormone can act as a cytokine controlling survival and proliferation of immune cells: new insights into signaling pathways, Mol. Cell. Endocrinol. 188 (2002) 1–7.
[53] A. Haeffner, O. Deas, B. Mollereau, J. Estaquier, A. Mignon, N. Haeffner-Cavaillon, B. Charpentier, A. Senik, F. Hirsch, Growth hormone prevents human monocytic cells from Fas-mediated apoptosis by up-regulating Bcl-2 expression, Eur. J. Immunol. 29 (1999) 334–344.
[54] M. Irwin, R.L. Hauger, L. Jones, M. Provencio, K.T. Britton, Sympathetic nervous system mediates central corticotropin-releasing factor induced suppression of natural killer cytotoxicity, J. Pharmacol. Exp. Ther. 255 (1990) 101–106.
[55] B. Kruszewska, D.L. Felten, S.Y. Stevens, J.A. Moynihan, Sympathectomy-induced immune changes are not abrogated by the glucocorticoid receptor blocker RU-486, Brain Behav. Immunol. 12 (1998) 181–200.
[56] D.R. Murray, M. Irwin, A. Rearden, M. Ziegler, H. Motulsky, A.S. Maisel, Sympathetic and immune interactions during dynamic exercise. Mediation via a β_2-adrenergic-dependent mechanism, Circulation 86 (1992) 203–213.
[57] V.M. Sanders, F.E. Powell-Oliver, β_2-Adrenoceptor stimulation increases the number of antigen-specific precursor B lymphocytes that differentiate into IgM-secreting cells without affecting burst size, J. Immunol. 148 (1992) 1822–1828.
[58] M. Schedlowski, W. Hosch, R. Oberbeck, R.J. Benschop, R. Jacobs, H.-R. Raab, R.E. Schmidt, Catecholamines modulate human NK cell circulation and function via spleen-independent β_2-adrenergic mechanisms, J. Immunol. 156 (1996) 93–99.
[59] M.A. Swanson, W.T. Lee, V.M. Sanders, IFN-gamma production by Th1 cells generated from naive CD4(+) T cells exposed to norepinephrine (In Process Citation), J. Immunol. 166 (2001) 232–240.
[60] D.J.J. Carr, B.M. Gebhardt, D. Paul, Alpha adrenergic and mu-2 opioid receptors are involved in morphine-induced suppression of splenocyte natural killer activity, J. Pharmacol. Exp. Ther. 264 (1993) 1179–1186.
[61] K. Fecho, L.A. Dykstra, D.T. Lysle, Evidence for beta adrenergic receptor involvement in the immunomodulatory effects of morphine, J. Pharmacol. Exp. Ther. 265 (1993) 1079–1087.
[62] L. Hoffman-Goetz, B.K. Pedersen, Exercise and the immune system: a model of the stress response?, Immunol. Today 15 (1994) 382–387.
[63] S. Shinkai, S. Watanabe, H. Asai, P.N. Shek, Cortisol response to exercise and post-exercise suppression of blood lymphocyte subset counts, Int. J. Sports Med. 17 (1996) 597–603.
[64] J.R. Simpson, L. Hoffman-Goetz, Exercise stress and murine natural killer cell function, Proc. Soc. Exp. Biol. Med. 195 (1990) 129–135.
[65] R.J. Shephard, P.N. Shek, Effects of exercise and training on natural killer cell counts and cytolytic activity: a meta-analysis, Sports Med. 28 (1999) 177–195.
[66] L.J.H. Van Tits, M.C. Michel, H. Grosse-Wilde, M. Happel, F.-W. Eigler, A. Soliman, O.-E. Brodde, Catecholamines increase lymphocyte β_2-adrenergic receptors via a β_2-adrenergic, spleen-dependent process, Am. J. Physiol. 258 (1990) E191–E202.
[67] F. Nagao, M. Suzui, K. Takeda, H. Yagita, K. Okumura, Mobilization of NK cells by exercise: downmodulation of adhesion molecules on NK cells by catecholamines, Am. J. Physiol. Regul. Integr. Comp. Physiol. 279 (2000) R1251–1256.
[68] B. Kruszewska, S.Y. Felten, J.A. Moynihan, Alterations in cytokine and antibody production following chemical sympathectomy in two strains of mice, J. Immunol. 155 (1995) 4613–4620.
[69] A.P. Kohm, V.M. Sanders, Suppression of antigen-specific Th2 cell-dependent IgM and IgG1 production following norepinephrine depletion in vivo, J. Immunol. 162 (1999) 5299–5308.
[70] V.M. Sanders, R.A. Baker, D.S. Ramer-Quinn, D.J. Kasprowicz, B.A. Fuchs, N.E. Street, Differential expression of the β_2-adrenergic receptor by Th1 and Th2 clones. Implications for cytokine production and B cell help, J. Immunol. 158 (1997) 4200–4210.
[71] A.P. Kohm, A. Mozaffarian, V.M. Sanders, B cell receptor- and beta 2-adrenergic receptor-induced regulation of B7-2 (CD86) expression in B cells, J. Immunol. 168 (2002) 6314–6322.

[72] L. McCarthy, M. Wetzel, J.K. Sliker, T.K. Eisenstein, T.J. Rogers, Opioids, opioid receptors, and the immune response, Drug Alcohol Depend. 62 (2001) 111–123.
[73] J.J. Madden, W.L. Whaley, D. Ketelsen, R.M. Donahoe, The morphine-binding site on human activated T-cells is not related to the mu opioid receptor, Drug Alcohol Depend. 62 (2001) 131–139.
[74] C. Gaveriaux-Ruff, H.W.D. Matthes, J. Peluso, B.L. Kieffer, Abolition of morphine-immunosuppression in mice lacking the μ-opioid receptor gene, Proc. Natl. Acad. Sci. 95 (1998) 6326–6330.
[75] S. Roy, R.A. Barke, H.H. Loh, Mu-opioid receptor knockout mice: role of mu-opioid receptor in morphine mediated immune functions, Brain Res. Mol. Brain Res. 61 (1998) 190–194.
[76] B.A. Fuchs, S.B. Pruett, Morphine induces apoptosis in murine thymocytes in vivo but not in vitro: involvement of both opiate and glucocorticoid receptors, J. Pharmacol. Exp. Ther. 266 (1993) 417–423.
[77] D.G. LeVier, J.A. McCay, M.L. Stern, L.S. Harris, D. Page, R.D. Brown, D.L. Musgrove, L.F. Butterworth, K.L. White, A.E. Munson, Immunotoxicological profile of morphine sulfate in B6C3F1 female mice, Fundam. Appl. Toxicol. 22 (1994) 525–542.
[78] RoyS., J.H. Wang, S. Balasubramanian, R. Sumandeep, Charboneau, R. Barke, H.H. Loh, Role of hypothalamic-pituitary axis in morphine-induced alteration in thymic cell distribution using mu-opioid receptor knockout mice, J. Neuroimmunol. 116 (2001) 147–155.
[79] S.B. Pruett, Y.C. Han, B.A. Fuchs, Morphine suppresses primary humoral immune responses by a predominantly indirect mechanism, J. Pharmacol. Exp. Ther. 262 (1992) 923–928.
[80] D.D. Taub, T.K. Eisenstein, E.B. Geller, M.W. Adler, T.J. Rogers, Immumomodulatory activity of μ-and κ-selective opioid agonists, Proc. Natl. Acad. Sci. USA 88 (1991) 360–364.
[81] J. Wang, R. Charboneau, R.A. Barke, H.H. Loh, S. Roy, Opioid receptor mediates chronic restraint stress-induced lymphocyte apoptosis, J. Immunol. 169 (2002) 3630–3636.
[82] D. Yin, D. Tuthill, R.A. Mufson, Y. Shi, Chronic restraint stress promotes lymphocyte apoptosis by modulating CD95 expression, J. Exp. Med. 191 (2000) 1423–1428.
[83] Y. Shavit, J.W. Lewis, G.W. Terman, Opioid peptides mediate the suppressive effect of stress on natural killer cell cytotoxicity, Science 223 (1983) 188–190.
[84] T. Marotti, J. Gabrilovac, S. Rabatic, L. Smejkal-Jagar, B. Rocic, H. Haberstock, Met-enkephalin modulates stress-induced alterations of the immune response in mice, Pharmacol. Biochem. Behav. 54 (1996) 277–284.
[85] R. Zalys, I.S. Zagon, R.H. Bonneau, C.M. Lang, P.J. McLaughlin, In vivo effects of chronic treatment with [MET5]-enkephalin on hematological values and natural killer cell activity in athymic mice, Life Sci. 66 (2000) 829–834.
[86] S. Ben-Eliyahu, R. Yirmiya, Y. Shavit, J.C. Liebeskind, Stress-induced suppression of natural killer cell cytotoxicity in the rat: a naltrexone-insensitive paradigm, Behav. Neurosci. 104 (1990) 235–238.
[87] N. Boyadjieva, M. Dokur, J.P. Advis, G.G. Meadows, D.K. Sarkar, Chronic ethanol inhibits NK cell cytolytic activity: role of opioid peptide beta-endorphin, J. Immunol. 167 (2001) 5645–5652.
[88] P.M. Mathews, C.J. Froelich, W.L. Sibbitt, Jr, A.D. Bankhurst, Enhancement of natural cytotoxicity by beta-endorphin, J. Immunol. 130 (1983) 1658–1662.
[89] D.R. Oleson, D.R. Johnson, Regulation of human natural cytotoxicity by enkephalins and selective opiate agonists, Brain Behav. Immun. 2 (1988) 171–186.
[90] B.A. Blazar, M.L. Rodrick, J.B. O'Mahony, J.J. Wood, P.Q. Bessey, D.W. Wilmore, J.A. Mannick, Suppression of natural killer-cell function in humans following thermal and traumatic injury, J. Clin. Immunol. 6 (1986) 26–36.
[91] R. Gomez-Flores, J.-L. Suo, R.J. Weber, Suppression of splenic macrophage functions following acute morphine action in the rat mesencephalon periaqueductal gray, Brain Behav. Immun. 13 (1999) 212–224.
[92] R.J. Weber, A. Pert, The periaqueductal gray matter mediates opiate-induced immunosuppression, Science 245 (1989) 188–190.
[93] P.C. Singhal, M. Bhaskaran, J. Patel, K. Patel, B.S. Kasinath, S. Duraisamy, N. Franki, K. Reddy, A.A. Kapasi, Role of p38 mitogen-activated protein kinase phosphorylation and Fas–Fas ligand interaction in morphine-induced macrophage apoptosis, J. Immunol. 168 (2002) 4025–4033.
[94] D.O. Freier, B.A. Fuchs, A mechanism of action for morphine-induced immunosuppression: corticosterone mediates morphine-induced suppression of natural killer cell activity, J. Pharmacol. Exp. Ther. 270 (1994) 1127–1133.
[95] B.P. Schimmer, K.L. Parker, Adrenocorticotropic hormone; adrencocortical steroids and their synthetic analogs; inhibitors of the synthesis and actions of adrenocortical hormones, in: J.G. Hardman, L.E. Limbird, P.B. Molinoff, R.W. Ruddon, A.G. Gilman (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, 1996, pp. 1459–1486.
[96] C.M. Flores, M.C. Hernandez, K.M. Hargreaves, B.M. Bayer, Restraint stress-induced elevations in plasma corticosterone and beta-endorphin are not accompanied by alterations in immune function, J. Neuroimmunol. 28 (1990) 219–225.
[97] R.R.R. Rowland, E. Reyes, R. Chukwuocha, S. Tokuda, Corticosteroid and immune responses of mice following mini-osmotic pump implantation, Immunopharmacology 20 (1990) 187–190.
[98] F. Berkenbosch, D.A.W. Wolvers, R. Derijk, Neuroendocrine and immunological mechanisms in stress-induced immunomodulation, J. Steroid Biochem. Mol. Biol. 40 (1991) 639–647.
[99] F.S. Dhabhar, Stress-induced enhancement of cell-mediated immunity, Ann. NY Acad. Sci. 840 (1998) 359–372.
[100] F.S. Dhabhar, B.S. McEwen, Enhancing versus suppressive effects of stress hormones on skin immune function, Proc. Natl. Acad. Sci. USA 96 (1999) 1059–1064.
[101] W.Y. Almawi, O.K. Melemedjian, Molecular mechanisms of glucocorticoid antiproliferative effects: antagonism of transcription factor activity by glucocorticoid receptor, J. Leukoc. Biol. 71 (2002) 9–15.
[102] C.M. Bamberger, H.M. Schulte, G.P. Chrousos, Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids, Endocrinol. Rev. 17 (1996) 245–261.
[103] F. Buttgereit, A. Scheffold, Rapid glucocorticoid effects on immune cells, Steroids 67 (2002) 529–534.
[104] V. Doucas, Y. Shi, S. Miyamoto, A. West, I. Verma, R.M. Evans, Cytoplasmic catalytic subunit of protein kinase A mediates cross-repression by NF-κB and the glucocorticoid receptor, Proc. Natl. Acad. Sci. USA 97 (2000) 11893–11898.
[105] D. Franchimont, J. Galon, M. Gadina, R. Visconti, Y. Zhou, M. Aringer, D.M. Frucht, G.P. Chrousos, J.J. O'Shea, Inhibition of Th1 immune response by glucocorticoids: dexamethasone selectively inhibits IL-12-induced Stat4 phosphorylation in T lymphocytes, J. Immunol. 164 (2000) 1768–1774.
[106] F. Paliogianni, N. Hama, J.E. Balow, M.A. Valentine, D.T. Boumpas, Glucocorticoid-mediated regulation of protein phosphorylation in primary human T cells. Evidence for induction of phosphatase activity, J. Immunol. 155 (1995) 1809–1817.
[107] F. Van Laethem, E. Baus, L.A. Smyth, F. Andris, F. Bex, J. Urbain, D. Kioussis, O. Leo, Glucocorticoids attenuate T cell receptor signaling, J. Exp. Med. 193 (2001) 803–814.

[108] A.H. Miller, R.L. Spencer, A. Husain, R. Rhee, B.S. McEwen, M. Stein, Differential expression of type I adrenal steroid receptors in immune tissues is associated with tissue-specific regulation of type II receptors by aldosterone, Endocrinology 133 (1993) 2133–2140.
[109] R.L. Spencer, A.H. Miller, H. Moday, M. Stein, B.S. McEwen, Diurnal differences in basal and acute stress levels of type I and type II adrenal steroid receptor activation in neural and immune tissues, Endo 133 (1993) 1941–1950.
[110] J.A. Brewer, B.P. Sleckman, W. Swat, L.J. Muglia, Green fluorescent protein-glucocorticoid receptor knockin mice reveal dynamic receptor modulation during thymocyte development, J. Immunol. 169 (2002) 1309–1318.
[111] S.B. Pruett, R. Fan, Q. Zhang, Characterization of glucocorticoid receptor translocation, cytoplasmic IkB, nuclear NFkB, and activation of NFkB in T lymphocytes exposed to stress-inducible concentrations of corticosterone in vivo, Int. Immunopharmacol. 3 (2003) 1–16.
[112] J. Liu, D.B. DeFranco, Chromatin recycling of glucocorticoid receptors: implications for multiple roles of heat shock protein 90, Mol. Endocrinol. 13 (1999) 355–365.
[113] T. Campbell, M.W. Meagher, A. Sieve, B. Scott, R. Storts, T.H. Welsh, C.J. Welsh, The effects of restraint stress on the neuropathogenesis of Theiler's virus infection: I. Acute disease, Brain Behav. Immun. 15 (2001) 235–254.
[114] S.W. Cha, H.J. Lee, M.H. Cho, M.H. Lee, W.S. Koh, S. Han, J. Kim, E. Lee, D. Nam, T.C. Jeong, Role of corticosterone in ethyl carbamate-induced immunosuppression in female BALB/c mice, Toxicol. Lett. 119 (2001) 173–181.
[115] C.M. Dobbs, N. Feng, F.M. Beck, J.F. Sheridan, Neuroendocrine regulation of cytokine production during experimental influenza viral infection. Effects of restraint stress-induced elevation in endogenous corticosterone, J. Immunol. 157 (1996) 1870–1877.
[116] D. Kim, A. Reilly, D.A. Lawrence, Relationships between IFNgamma, IL-6, corticosterone, and *Listeria monocytogenes* pathogenesis in BALB/c mice, Cell. Immunol. 207 (2001) 13–18.
[117] T. Shimizu, T. Kawamura, C. Miyaji, H. Oya, M. Bannai, S. Yamamoto, A. Weerasinghe, R.C. Halder, H. Watanabe, K. Hatakeyama, T. Abo, Resistance of extrathymic T cells to stress and the role of endogenous glucocorticoids in stress associated immunosuppression, Scand. J. Immunol. 51 (2000) 285–292.
[118] N. Tarcic, H. Ovadia, D.W. Weiss, J. Weidenfeld, Restraint stress-induced thymic involution and cell apoptosis are dependent on endogenous glucocorticoids, J. Neuroimmunol. 82 (1998) 40–46.
[119] P.A. Weiss, S.D. Collier, S.B. Pruett, Role of glucocorticoids in ethanol-induced decreases in expression of MHC class II molecules on B cells and selective decreases in spleen cell number, Toxicol. Appl. Pharmacol. 139 (1996) 153–162.
[120] D. Zhang, K. Kishihara, B. Wang, K. Mizobe, C. Kubo, K. Nomoto, Restraint stress-induced immunosuppression by inhibiting leukocyte migration and Th1 cytokine expression during the intraperitoneal infection of *Listeria monocytogenes*, J. Neuroimmunol. 92 (1998) 139–151.
[121] L. Cao, N.M. Filipov, D.A. Lawrence, Sympathetic nervous system plays a major role in acute cold/restraint stress inhibition of host resistance to *Listeria monocytogenes*, J. Neuroimmunol. 125 (2002) 94–102.
[122] G.K. De Krey, L. Baecher-Steppan, J.A. Deyo, B. Smith, N.I. Kerkvliet, Polychlorinated biphenyl-induced immune suppression: castration but not adrenalectomy or RU 38486 treatment partially restores the suppressed cytotoxic T lymphocyte response to alloantigen, J. Pharmacol. Exp. Ther. 267 (1993) 308–315.
[123] S.E. Keller, J.M. Weiss, S. Schleifer, N.E. Miller, M. Stein, Stress-induced suppression of immunity in adrenalectomized rats, Science 221 (1983) 1301–1304.
[124] R.B. Vinson, J.L. Carroll, S.B. Pruett, Mechanism of suppressed neutrophil mobilization in a mouse model for binge drinking: role of glucocorticoids, Am. J. Physiol. 275 (1998) R1049–R1057.
[125] S.B. Pruett, R. Fan, Q. Zheng, L.P. Myers, P. Hebért, Modeling and predicting selected immunological effects of a chemical stressor (3,4 dichloropropionanalide) using the area under the corticosterone concentration vs. time curve, Toxicol. Sci. 58 (2000) 77–87.
[126] S.B. Pruett, R. Fan, L.P. Myers, W.-J. Wu, S.D. Collier, Quantitative analysis of the neuroendocrine-immune axis: linear modeling of the effects of exogenous corticosterone and restraint stress on lymphocyte subpopulations in the spleen and thymus in female B6C3F1 mice, Brain Behav. Immun. 14 (2000) 270–287.
[127] R.A. Daynes, B.A. Araneo, T.A. Dowell, K. Huang, D. Dudley, Regulation of murine lymphokine production in vivo. III. The lymphoid tissue microenvironment exerts regulatory influences over T helper cell function, J. Exp. Med. 171 (1990) 979–996.
[128] F.S. Dhabhar, B.S. McEwen, R.L. Spencer, Stress response, adrenal steroid receptor levels and corticosteroid-binding globulin levels-a comparison between Sprague–Dawley, Fischer 344 and Lewis rats, Brain Res. 616 (1993) 89–98.
[129] M. Fleshner, T. Deak, R.L. Spencer, M.L. Laudenslager, L.R. Watkins, S.F. Maier, A long term increase in basal levels of corticosterone and a decrease in corticosterone binding globulin after acute stressor exposure, Endocrinology 136 (1995) 5336–5342.
[130] A.H. Miller, R.L. Spencer, B.D. Pearce, T.L. Pisell, Y. Azrieli, P. Tanapat, H. Moday, R. Rhee, B.S. McEwen, Glucocorticoid receptors are differentially expressed in the cells and tissues of the immune system, Cell. Immunol. 186 (1998) 45–54.
[131] A. Beishuizen, L.G. Thijs, I. Vermes, Patterns of corticosteroid-binding globulin and the free cortisol index during septic shock and multitrauma, Intensive Care Med. 27 (2001) 1584–1591.
[132] P. Lauzurica, S. Martinez-Martinez, M. Marazuela, P. Gomez del Arco, C. Martinez, F. Sanchez-Madrid, J.M. Redondo, Pyrrolidine dithiocarbamate protects mice from lethal shock induced by LPS or TNF-alpha, Eur. J. Immunol. 29 (1999) 1890–1900.
[133] M.E. Astiz, E.C. Rackow, Septic shock, Lancet 351 (1998) 1503–1505.
[134] J.C. Gutierrez-Ramos, H. Bluethmann, Molecules and mechanisms operating in septic shock: lessons from knockout mice, Immunol. Today 18 (1997) 329–334.
[135] M. Bacher, C.N. Metz, T. Calandra, K. Mayer, J. Chesney, M. Lohoff, D. Gemsa, T. Donnelly, R. Bucala, An essential regulatory role for macrophage migration inhibitory factor in T-cell activation, Proc. Natl. Acad. Sci. USA 93 (1996) 7849–7854.
[136] H. Koebernick, L. Grode, J.R. David, W. Rohde, M.S. Rolph, H.W. Mittrucker, S.H. Kaufmann, Macrophage migration inhibitory factor (MIF) plays a pivotal role in immunity against *Salmonella typhimurium*, Proc. Natl. Acad. Sci. USA 23 (2002) 23.
[137] R.A. Daynes, B.A. Araneo, Contrasting effects of glucocorticoids on the capacity of T cells to produce the growth factors interleukin 2 and interleukin 4, Eur. J. Immunol. 19 (1989) 2319–2325.
[138] M. Fleshner, F.X. Brennan, K. Nguyen, L.R. Watkins, S.F. Maier, RU-486 blocks differentially suppressive effect of stress on in vivo anti-KLH immunoglobulin response, Am. J. Physiol. 271 (1996) R1344–R1352.
[139] C.M. Braun, S.K. Huang, G.G. Bashian, A. Kagey-Sobotka, L.M. Lichtenstein, D.M. Essayan, Corticosteroid modulation of

human, antigen-specific Th1 and Th2 responses, J. Allergy Clin. Immunol. 100 (1997) 400–407.
[140] I.A. MacPhee, D.R. Turner, D.B. Oliveira, The role of endogenous steroid hormones in the generation of T helper 2-mediated autoimmunity in mercuric chloride-treated Brown-Norway rats, Immunology 99 (2000) 141–146.
[141] J.A. Moynihan, T.A. Callahan, S.P. Kelley, L.M. Campbell, Adrenal hormone modulation of type 1 and type 2 cytokine production by spleen cells: dexamethasone and dehydroepiandrosterone suppress interleukin-2, interleukin-4, and interferon-γ production in vitro, Cell. Immunol. 184 (1998) 58–64.
[142] S.B. Pruett, R. Fan, Quantitative modeling of suppression of IgG1, IgG2a, IL-2, and IL-4 responses to antigen in mice treated with exogenous corticosterone or restraint stress, J. Toxicol. Environ. Health 62 (2001) 175–189.
[143] J. Schmidt, S. Fleissner, I. Heimann-Weitschat, R. Lindstaedt, I. Szelenyi, The effect of different corticosteroids and cyclosporin A on interleukin-4 and interleukin-5 release from murine TH2-type T cells, Eur. J. Pharmacol. 260 (1994) 247–250.
[144] Z.W. Lu, C. Song, A.V. Ravindran, Z. Merali, H. Anisman, Influence of a psychogenic and a neurogenic stressor on several indices of immune functioning in different strains of mice, Brain Behav. Immun. 12 (1998) 7–22.
[145] S.B. Pruett, S. Collier, W.-J. Wu, R. Fan, Quantitative relationships between the suppression of selected immunological parameters and the area under the corticosterone concentration vs. time curve in B6C3F1 mice subjected to exogenous corticosterone or to restraint stress, Toxicol. Sci. 49 (1999) 272–280.
[146] S.B. Pruett, Quantitative aspects of stress-induced immunomodulation, Int. Immunopharmacol. 1 (2001) 507–520.
[147] F.S. Dhabhar, B.S. McEwen, R.L. Spencer, Adaptation to prolonged or repeated stress—comparison between rat strains showing intrinsic differences in reactivity to acute stress, Neuroendocrinology 65 (1997) 360–368.
[148] G.A. Tejwani, K.P. Gudehithlu, S.H. Hanissian, I.E. Gienapp, C.C. Whitacre, W.B. Malarkey, Facilitation of dimethylbenz[*a*]anthracene-induced rat mammary tumorigenesis by restraint stress: role of beta-endorphin, prolactin and naltrexone, Carcinogenesis 12 (1991) 637–641.
[149] G. Hermann, F.M. Beck, C.A. Tovar, W.B. Malarkey, C. Allen, J.F. Sheridan, Stress-induced changes attributable to the sympathetic nervous system during experimental influenza viral infection in DBA/2 inbred mouse strain, J. Neuroimmunol. 53 (1994) 173–180.
[150] A. Fiserova, M. Starec, M. Kuldova, H. Kovaru, M. Pav, L. Vannucci, M. Pospisil, Effects of D2-dopamine and alpha-adrenoceptor antagonists in stress induced changes on immune responsiveness of mice, J. Neuroimmunol. 130 (2002) 55–65.
[151] F.S. Dhabhar, B.S. McEwen, Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking, Brain Behav. Immun. 11 (1997) 286–306.
[152] F.S. Dhabhar, A.H. Miller, B.S. McEwen, R.L. Spencer, Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms, J. Immunol. 154 (1995) 5511–5527.
[153] F.S. Dhabhar, A.H. Miller, B.S. McEwen, R.L. Spencer, Stress-induced changes in blood leukocyte distribution. Role of adrenal steroid hormones, J. Immunol. 157 (1996) 1638–1644.
[154] R. Kvetnansky, K. Pacak, E.L. Sabban, I.J. Kopin, D.S. Goldstein, Stressor specificity of peripheral catecholaminergic activation, Adv. Pharmacol. 42 (1998) 556–560.
[155] H. Osada, A. Matsui, D. Men, S. Sekiya, Y. Matsui, Plasma norepinephrine and epinephrine responses of SHR and WKY rats to foot shock stress under different anesthetic conditions, in: R. Kvetnansky, R. McCarty, J. Axelrod (Eds.), Stress: Neuroendocrine and Molecular Approaches, vol. 2, Gordon and Breach Science Publishers, New York, 1992, pp. 537–544.
[156] R.L. Wilder, Neuroendocrine–immune system interactions and autoimmunity, Annu. Rev. Immunol. 13 (1995) 307–338.
[157] M.H. Antoni, S. Cruess, D.G. Cruess, M. Kumar, S. Lutgendorf, G. Ironson, E. Dettmer, J. Williams, N. Klimas, M.A. Fletcher, N. Schneiderman, Cognitive-behavioral stress management reduces distress and 24-hour urinary free cortisol output among symptomatic HIV-infected gay men, Ann. Behav. Med. 22 (2000) 29–37.
[158] D.G. Cruess, M.H. Antoni, B.A. McGregor, K.M. Kilbourn, A.E. Boyers, S.M. Alferi, C.S. Carver, M. Kumar, Cognitive-behavioral stress management reduces serum cortisol by enhancing benefit finding among women being treated for early stage breast cancer, Psychosom. Med. 62 (2000) 304–308.
[159] A.V. McGrady, R. Yonker, S.Y. Tan, T.H. Fine, M. Woerner, The effect of biofeedback-assisted relaxation training on blood pressure and selected biochemical parameters in patients with essential hypertension, Biofeedback Self Regul. 6 (1981) 343–353.
[160] L.A. Pawlow, G.E. Jones, The impact of abbreviated progressive muscle relaxation on salivary cortisol, Biol. Psychol. 60 (2002) 1–16.
[161] K. Ogawa, M. Hirai, T. Katsube, M. Murayama, K. Hamaguchi, T. Shimakawa, Y. Naritake, T. Hosokawa, T. Kajiwara, Suppression of cellular immunity by surgical stress, Surgery 127 (2000) 329–336.
[162] R.E. Pollock, E. Lotzova, Surgical-stress-related suppression of natural killer cell activity: a possible role in tumor metastasis, Nat. Immun. Cell. Growth Regul. 6 (1987) 269–278.
[163] P.V. Giannoudis, R.M. Smith, A.C. Windsor, M.C. Bellamy, P.J. Guillou, Monocyte human leukocyte antigen-DR expression correlates with intrapulmonary shunting after major trauma, Am. J. Surg. 177 (1999) 454–459.
[164] R.A. Gibbons, O.M. Martinez, R.C. Lim, J.K. Horn, M.R. Garovoy, Reduction in HLA-DR, HLA-DQ and HLA-DP expression by Leu-M3+ cells from the peripheral blood of patients with thermal injury, Clin. Exp. Immunol. 75 (1989) 371–375.
[165] P.J. Guillou, Biological variation in the development of sepsis after surgery or trauma, Lancet 342 (1993) 217–220.
[166] R.M. Smith, P.V. Giannoudis, M.C. Bellamy, S.L. Perry, R.A. Dickson, P.J. Guillou, Interleukin-10 release and monocyte human leukocyte antigen-DR expression during femoral nailing, Clin. Orthop. 373 (2000) 233–240.
[167] C.H. Wakefield, P.D. Carey, S. Foulds, J.R. Monson, P.J. Guillou, Changes in major histocompatibility complex class II expression in monocytes and T cells of patients developing infection after surgery, Br. J. Surg. 80 (1993) 205–209.
[168] K. Asadullah, C. Woiciechowsky, W.D. Docke, K. Egerer, W.J. Kox, S. Vogel, W. Sterry, H.S. Volk, Very low monocytic HLA-DR expression indicates high risk of infection-immunomonitoring for patients after neurosurgery and patients during high dose steroid therapy, Eur. J. Emerg. Med. 2 (1995) 184–190.
[169] W.G. Cheadle, The human leukocyte antigens and their relationship to infection, Am. J. Surg. 165 (1993) 75S–81S.
[170] C. Woiciechowsky, K. Asadulla, D. Nestler, B. Eberhardt, C. Platzer, B. Schöning, F. Glöckner, W.R. Lanksch, H.-D. Volk, W.-D. Döcke, Sympathetic activation triggers systemic interleukin-10 release in immunodepression induced by brain injury, Nat. Med. 4 (1998) 808–813.
[171] D.H. Livingston, S.H. Appel, S.R. Wellhausen, G. Sonnenfeld, H.C. Polk, Jr, Depressed interferon gamma production and monocyte HLA-DR expression after severe injury, Arch. Surg. 123 (1988) 1309–1312.
[172] D.H. Livingston, P.A. Loder, S.M. Kramer, U.E. Gibson, H.C. Polk, Jr, Interferon gamma administration increases monocyte HLA-DR antigen expression but not endogenous interferon production, Arch. Surg. 129 (1994) 172–178.

[173] A. Celada, S. McKercher, R.A. Maki, Repression of major histocompatibility complex IA expression by glucocorticoids: the glucocorticoid receptor inhibits the DNA binding of the X box DNA binding protein, J. Exp. Med. 177 (1993) 691–698.
[174] B.S. Zwilling, D. Brown, N. Feng, J. Sheridan, D. Pearl, The effect of adrenalectomy on the restraint stressed induced suppression of MHC class II expression by murine peritoneal macrophages, Brain Behav. Immun. 7 (1993) 29–35.
[175] S. Redpath, A. Angulo, N.R. Gascoigne, P. Ghazal, Murine cytomegalovirus infection down-regulates MHC class II expression on macrophages by induction of IL-10, J. Immunol. 162 (1999) 6701–6707.
[176] Z. Islam, Y.S. Moon, H.R. Zhou, L.E. King, P.J. Fraker, J.J. Pestka, Endotoxin potentiation of trichothecene-induced lymphocyte apoptosis is mediated by up-regulation of glucocorticoids, Toxicol. Appl. Pharmacol. 180 (2002) 43–55.
[177] M.I. Luster, A.E. Munson, P.T. Thomas, M.P. Holsapple, J.D. Fenters, K.L. White, L.D. Lauer, D.R. Germolec, G.J. Rosenthal, J.H. Dean, Methods evaluation, development of a testing battery to assess chemical-induced immunotoxicity: National Toxicology Program's guidelines for immunotoxicity evaluation in mice, Fundam. Appl. Toxicol. 10 (1988) 2–19.
[178] A.A. Marino, R.M. Wolcott, R. Chervenak, F. Jourd'heuil, E. Nilsen, C. Frilot, 2nd, S.B. Pruett, Coincident nonlinear changes in the endocrine and immune systems due to low-frequency magnetic fields, Neuroimmunomodulation 9 (2001) 65–77.
[179] A. Dalgleish, The relevance of non-linear mathematics (chaos theory) to the treatment of cancer, the role of the immune response and the potential for vaccines, Q. J. Med. 92 (1999) 347–359.
[180] T. Zell, A. Khoruts, E. Ingulli, J.L. Bonnevier, D.L. Mueller, M.K. Jenkins, Single-cell analysis of signal transduction in CD4 T cells stimulated by antigen in vivo, Proc. Natl. Acad. Sci. USA 98 (2001) 10805–10810.
[181] S.F. Atweh, J.J. Grayhack, D.P. Richman, A cholinergic receptor site on murine lymphocytes with novel binding characteristics, Life Sci. 35 (1984) 2459–2469.
[182] L. Costa, G. Kaylor, S.D. Murphy, Muscarinic cholinergic binding sites on rat lymphocytes, Immunopharmacology 16 (1988) 139–149.
[183] M.A. Gordon, J.J. Cohen, I.B. Wilson, Muscarinic cholinergic receptors in murine lymphocytes: demonstration by direct binding, Proc. Natl. Acad. Sci. USA 75 (1978) 2902–2904.
[184] T.B. Strom, M.A. Lane, K. George, The parallel, time-dependent, bimodal change in lymphocyte cholinergic binding activity and cholinergic influence upon lymphocyte-mediated cytotoxicity after lymphocyte activation, J. Immunol. 127 (1981) 705–710.
[185] S.J. Zalcman, L.M. Neckers, O. Kaayalp, R.J. Wyatt, Muscarinic cholinergic binding sites on intact human lymphocytes, Life Sci. 29 (1981) 69–73.
[186] T.K. Eisenstein, J. Meissler, T.J. Rogers, E.B. Geller, M.W. Adler, Mouse strain differences in immunosuppression by opioids in vitro, J. Pharm. Exp. Ther. 275 (1995) 1484–1489.
[187] J.L. Bussiere, M.W. Adler, T.J. Rogers, T.K. Eisenstein, Differential effects of morphine and naltrexone on the antibody response in various mouse strains, Immunopharmacol. Immunotoxicol. 14 (1992) 657–673.
[188] L. Laue, M.T. Lotze, G.P. Chrousos, K. Barnes, D.L. Loriaux, T.A. Fleisher, Effect of chronic treatment with the glucocorticoid antagonist RU 486 in man: toxicity, immunological, and hormonal aspects, J. Clin. Endocrinol. Metab. 71 (1990) 1474–1480.
[189] N. Yamauchi, T. Shibasaki, I. Wakabayashi, H. Demura, Brain beta-endorphin and other opioids are involved in restraint stress-induced stimulation of the hypothalamic–pituitary–adrenal axis, the sympathetic nervous system, and the adrenal medulla, Brain Res. 777 (1997) 140–146.
[190] J. Kanno, A. Wakikawa, M. Utsuyama, K. Hirokawa, Effect of restraint stress on immune system and experimental B16 melanoma metastasis in aged mice, Mech. Ageing Dev. 93 (1997) 107–117.
[191] P.A. Plett, E.M. Gardner, D.M. Murasko, Age-related changes in interferon-alpha/beta receptor expression, binding, and induction of apoptosis in natural killer cells from C57BL/6 mice, Mech. Ageing Dev. 118 (2000) 129–144.
[192] C.J. Grossman, G.A. Roselle, C.L. Mendenhall, Sex steroid regulation of autoimmunity, J. Steroid Biochem. Mol. Biol. 40 (1991) 649–659.
[193] M.S. Flint, S.S. Tinkle, C57BL/6 mice are resistant to acute restraint modulation of cutaneous hypersensitivity, Toxicol. Sci. 62 (2001) 250–256.
[194] V. Riley, Psychoneuroendocrine influences on immunocompetence and neoplasia, Science 212 (1981) 1100–1109.
[195] S.K. Bhatia, N.R. Rose, B. Schofield, A. Lafond-Walker, R.C. Kuppers, Influence of diet on the induction of experimental autoimmune thyroid disease, Proc. Soc. Exp. Biol. Med. 213 (1996) 294–300.
[196] S.B. Pruett, D.K. Ensley, P. Crittenden, The role of chemical-induced stress responses in immunosuppression: a review of quantitative associations and cause-effect relationships between chemical-induced stress responses and immunosuppression, J. Toxicol. Environ. Health 39 (1993) 163–192.
[197] J. Born, T. Lange, K. Hansen, M. Molle, H.-L. Fehm, Effects of sleep and circadian rhythm on human circulating immune cells, J. Immunol. 158 (1997) 4454–4464.
[198] G. Magnus, M. Cavallini, F. Halberg, G. Cornelissen, D.E.R. Sutherland, J.A. Najarian, W.J.M. Hrushesky, Circadian toxicology of cyclosporin, Toxicol. Appl. Pharmacol. 77 (1985) 181–185.
[199] M.M. Dozier, H.V. Ratajczak, R.B. Sothern, P.T. Thomas, The influence of vehicle gavage on seasonality of immune system parameters in the B6C3F1 mouse, Fundam. Appl. Toxicol. 38 (1997) 116–122.
[200] D. Kim, J.N. Carlson, R.F. Seegal, D.A. Lawrence, Differential immune responses in mice with left- and right-turning preference, J. Neuroimmunol. 93 (1999) 164–171.
[201] J. Weinberg, A.N. Taylor, C. Gianoulakis, Fetal ethanol exposure: hypothalamic–pituitary–adrenal and beta-endorphin responses to repeated stress, Alcohol. Clin. Exp. Res. 20 (1996) 122–131.
[202] E. Peloso, M. Wachulec, E. Satinoff, Stress-induced hyperthermia depends on both time of day and light condition, J. Biol. Rhythms 17 (2002) 164–170.
[203] D.J. Nichols, P.F. Chevins, Adrenocortical responses and changes during the oestrous cycle in mice: effects of male presence, male urine and housing conditions, J. Endocrinol. 91 (1981) 263–269.
[204] P. Clausing, T. Bocker, J. Diekgerdes, K. Gartner, J. Guttner, A. Haemisch, A. Veckenstedt, A. Weimer, Social isolation modifies the response of mice to experimental Mengo virus infection, J. Exp. Anim. Sci. 36 (1994) 37–54.
[205] J. Kugler, K.T. Kalveram, K.W. Lange, Acute, not chronic, exposure to unpredictable noise periods affects splenic lymphocytes and plasma corticosterone in the mouse, Int. J. Neurosci. 51 (1990) 233–234.
[206] M.A. Ventura, C. Gardey, P. d'Athis, Rapid reset of the corticosterone rhythm by food presentation in rats under acircadian restricted feeding schedule, Chronobiol. Int. 1 (1984) 287–295.
[207] C.A. Hardy, J. Quay, S. Livnat, R. Ader, Altered T-lymphocyte response following aggressive encounters in mice, Physiol. Behav. 47 (1990) 1245–1251.

[208] M.S. Landi, J.W. Kreider, C.M. Lang, L.P. Bullock, Effects of shipping on immune function in mice, Am. J. Vet. Res. 43 (1982) 1654–1657.

[209] A.N. Rowan, Refinement of animal research technique and validity of research data, Fundam. Appl. Toxicol. 15 (1990) 25–32.