

REVIEW

Role of the hypothalamic–pituitary–adrenal axis, glucocorticoids and glucocorticoid receptors in toxic sequelae of exposure to bacterial and viral products

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Abstract

The hypothalamic–pituitary–adrenal (HPA) axis is activated during many bacterial and viral infections, resulting in an increase in circulating glucocorticoid levels. This HPA axis activation and glucocorticoid response are critical for the survival of the host, as demonstrated by the fact that removal of the HPA axis (by adrenalectomy or hypophysectomy) or glucocorticoid receptor (GR) blockade enhances the severity of the infection and in some

cases enhances the mortality rate. Replacement with a synthetic glucocorticoid reverses these effects by reducing the severity of the infection and provides protection against lethal effects. In addition, some bacteria and viral infections have been shown to affect the GR directly. These have been described and the implications of such an effect discussed.

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Introduction

Upon bacterial or viral infection, a cascade of events, termed the ‘acute phase response’, is initiated. These include inflammation at the local site and, systemically, fever, leukocytosis, hormone release and other pathways, which all function to restore the host homeostasis that has been disturbed by the infection. These pathways elicit signaling mechanisms, most notably cytokines, which mediate these responses. Macrophages at the site of infection are one of the most important cell types in initiating this acute phase response. For more in-depth reviews on the mechanisms and pathways induced by infections leading to the acute phase response refer to the reviews by Biron (1994), Koj (1996) and Van Amersfoort *et al.* (2003).

Undoubtedly, the release of glucocorticoids has many effects on bacterial and viral infection and on the immunity induced by these infectious agents. It is also well known that hypothalamic–pituitary–adrenal (HPA) axis activation – for example, by stress – can enhance the susceptibility to infectious disease. For example, HPA axis activation enhances *Mycobacterium bovis* growth in susceptible mice (Brown *et al.* 1993, Brown & Zwillig 1994) and increases susceptibility to viral disease (Glaser & Kiecolt-Glaser 1998, Rozlog *et al.* 1999). Moreover, glucocorticoids enhance the replication of some viruses.

However, these effects are not the focus of this review; we will concentrate purely on the effects of these infections on the HPA axis and glucocorticoid responses. For more information on the effect of HPA axis activation and glucocorticoids on bacterial and viral infections and the acute phase response, see the reviews by Berczi (1993, 1998), Pearce *et al.* (2001) and Bailey *et al.* (2003).

HPA axis

A bidirectional communication exists between the brain and immune systems, in which the immune system signals the brain via cytokines (Mulla & Buckingham 1999), and the brain responds by regulating the immune system, in part through the action of the HPA axis with resultant release of glucocorticoids (Webster *et al.* 2002). Other central nervous system (CNS) response pathways, including the sympathetic and parasympathetic and peripheral nervous systems, also play a role; however, these are not the focus of this review (for a detailed review, see Elenkov *et al.* (2000)). Upon either inflammatory, physical or psychosocial stimulation, the cells of the paraventricular nucleus of the hypothalamus express corticotropin-releasing hormone (CRH) in the hypophyseal blood supply. This, in turn, stimulates the anterior pituitary

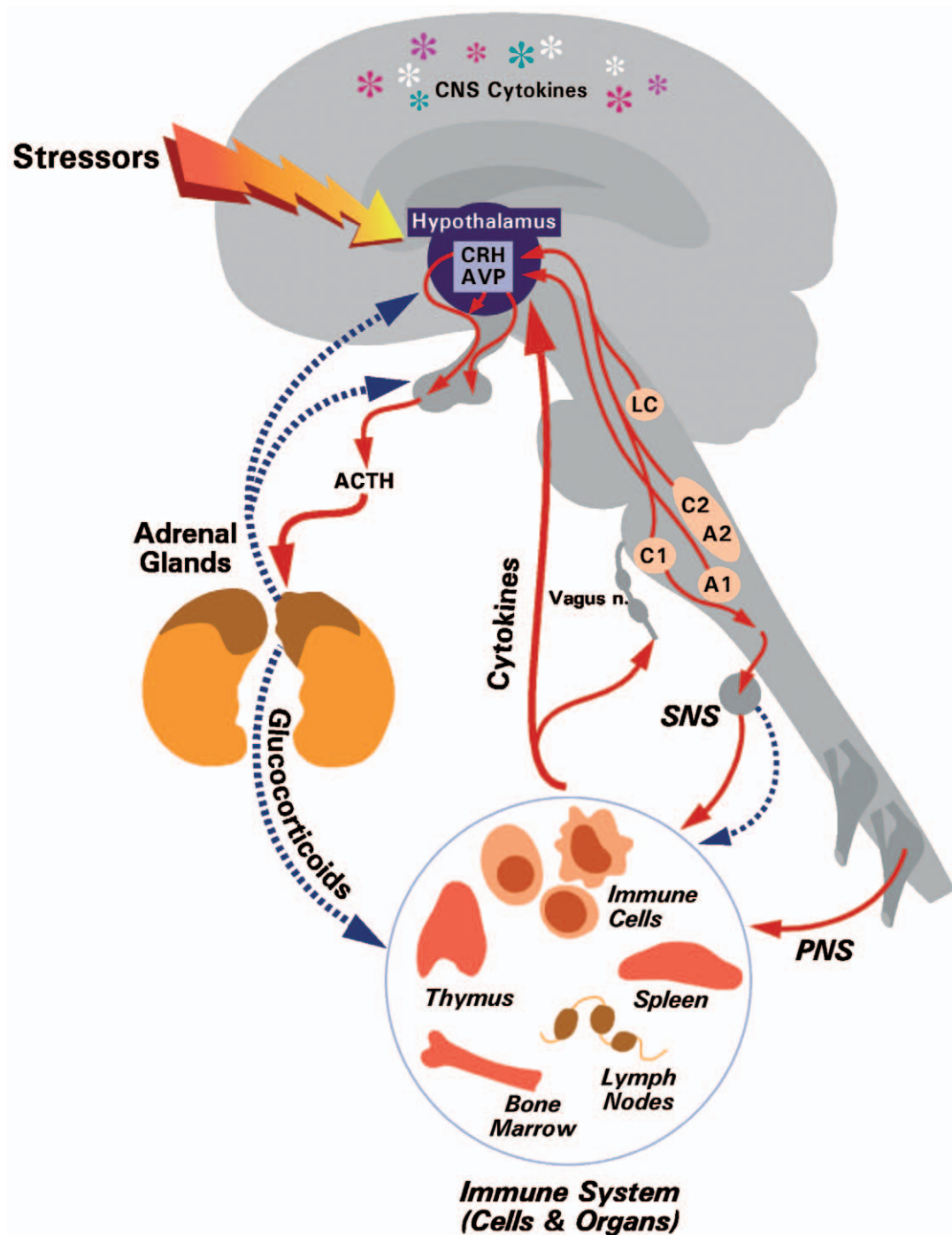


Figure 1 The hypothalamic–pituitary–adrenal (HPA) axis. Dotted lines represent negative regulatory pathways, solid lines represent positive regulatory pathways. Reprinted with permission from *Annual Review of Immunology*, Volume 20 © 2002 by Annual Reviews (www.annualreviews.org). CRH, corticotrophin releasing hormone; AVN, arginine vasopressin; ACTH, adrenocorticotrophin hormone; SNS, sympathetic nervous system; PNS parasympathetic nervous system.

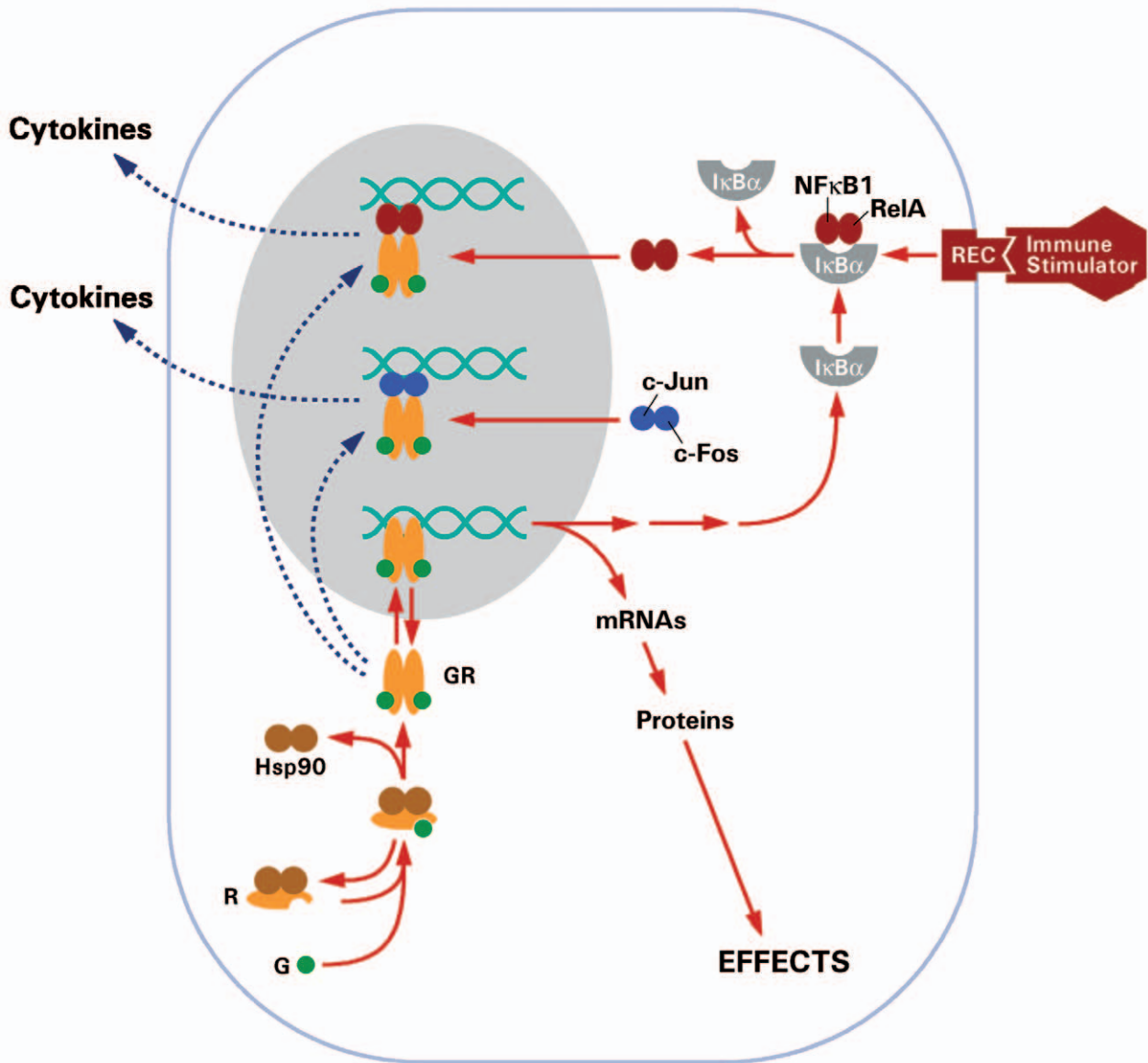


Figure 2 Schematic diagram illustrating the mechanism of action of the glucocorticoid receptor (GR). Dotted lines represent negative regulatory pathways. Reprinted with permission from *Annual Review of Immunology*, Volume 20 © 2002 by Annual Reviews (www.annualreviews.org).

gland to release adrenocorticotropin hormone (ACTH) into the bloodstream. At the adrenals, ACTH stimulates the synthesis and release of glucocorticoids. This axis is self-regulated, with glucocorticoids feeding back to the hypothalamus and pituitary to downregulate the HPA axis (Fig. 1). Cortisol is the endogenous glucocorticoid in man; in rodents, it is corticosterone. Regulation of the immune system is not the only function of glucocorticoids. They are also essential for the regulation of several homeostatic mechanisms in the body, including the CNS, cardiovascular system and metabolic homeostasis. The precise

mechanism by which glucocorticoids regulate the immune system will not be discussed here in detail, but it has been the subject of another recent review (Webster *et al.* 2002).

Glucocorticoid receptor (GR)

Glucocorticoids exert their many effects through a cytosolic receptor, the glucocorticoid receptor (GR), a member of the nuclear hormone receptor superfamily, which also includes the thyroid hormone, mineralocorti-

Table 1 Bacterial and viral infections that activate the HPA axis

Bacterial	Viral
LPS	Murine cytomegalovirus (MCMV)
Endotoxin	Lymphocytic choriomeningitis virus (LCMV)
<i>Mycoplasma fermentans</i>	Poly (I:C)
<i>Clostridium difficile</i> toxin A	HIV
Shiga toxin 2	Herpes simplex virus type 1 (HSV-1)
Tetanus toxoid	Sindbis virus
Staphylococcal enterotoxin A	Newcastle disease virus (NDV)
Staphylococcal enterotoxin B	Influenza virus

coid, estrogen and progesterone receptors (Evans 1988). In the absence of the ligand, GR is located in the cytoplasm in a protein complex that includes Hsp90 and Hsp70. Upon activation by binding of its ligand, GR is released from the protein complex, dimerizes, and translocates to the nucleus, where it binds to specific DNA sequences called glucocorticoid response elements (GRE) (Fig. 2). Thus, GR functions as a ligand-dependent transcription factor (Aranda & Pascual 2001). GR is able to upregulate gene expression through direct DNA binding; for example, it binds to the gluconeogenic enzyme tyrosine aminotransferase (TAT), whose promoter contains a consensus GRE sequence (Jantzen *et al.* 1987). GR can also bind to negative GREs (nGRE) to repress gene activation, such as for the pro-opiomelanocortin (POMC) gene (Drouin *et al.* 1989). However, GR can also repress gene transcription by interfering with the action of other signaling pathways, such as nuclear factor kappa B (NFκB) and activator protein-1 (AP-1) (Fig. 2), and it is through this mechanism that glucocorticoids exert many of their anti-inflammatory actions (McKay & Cidlowski 1999, Adcock 2000). The importance of GR is emphasized by the fact that lack of GR is incompatible with life, and GR knockout mice die shortly after birth due to a defect in lung maturation (Cole *et al.* 1995). However, it appears that the anti-inflammatory actions of GR associated with its ability to interfere with other signaling mechanisms may be the most critical for survival. Dimerization knockout mice (GR^{dim/dim}), in which GRE-mediated gene activation, which is entirely dependent on GR dimerization, is removed but GR interactions with NFκB and AP-1, which are independent of dimerization, are still possible, are viable (Reichardt *et al.* 1998).

Activation of the HPA axis during bacterial or viral infection

Many bacterial and viral infections result in an activation of the HPA axis and increased glucocorticoid release (Table 1). This activation of the HPA axis is probably the result of the action of inflammatory mediators such as cytokines. In some cases, the inflammatory mediators have

been identified. However, it is also possible, if the infections are located in the hypothalamus, that these infections could have a direct effect on the HPA axis. Some of these are described below.

Bacterial HPA axis activation

Endotoxins, lipopolysaccharides (LPS) from the outer membrane of Gram-negative bacteria, mediate many of the peripheral and brain responses caused by the bacteria (Van Amersfoort *et al.* 2003). In animals, injection of endotoxin or LPS activates the HPA axis and increases plasma ACTH and corticosterone levels (Butler *et al.* 1989, Feuerstein *et al.* 1990, Ramachandra *et al.* 1992, Schotanus *et al.* 1994, Ma *et al.* 2000). Similarly, in humans, HPA axis responses are increased during endotoxemia (Vedder *et al.* 1999). This increase is influenced by the diurnal glucocorticoid cycle, enhanced HPA axis responses to endotoxin occurs when endogenous glucocorticoid levels are lowest (Pollmacher *et al.* 1996). The bacterium *Mycoplasma fermentans* also causes an increase in serum ACTH and corticosterone (Weidenfeld *et al.* 1995).

Bacterial proteins have also been shown to activate the HPA axis. *Clostridium difficile* toxin A increases plasma corticosterone levels in Wistar rats and in C57BL/6J mice (Castagliuolo *et al.* 2001, Mykoniatis *et al.* 2003). It is thought that this toxin activates the HPA axis through inflammatory mediators, such as tumor necrosis factor α (TNF α) and prostaglandin E₂ (PGE₂), which are released prior to corticosterone induction following injection into rodent intestine (Castagliuolo *et al.* 1997). Shiga toxin 2 also induces increased plasma corticosterone levels in BALB/c mice (Gomez *et al.* 2003), and the tetanus toxoid increases cortisol and ACTH levels following vaccination in volunteers (Catania *et al.* 1990, Oken *et al.* 1998).

The bacterial superantigen *Staphylococcus aureus* enterotoxin B (SEB), but not SEA, stimulates the HPA axis with increased plasma corticosterone and ACTH, and even increased c-fos expression (a marker for neuronal activation) and increased CRH mRNA in the hypothalamus in BALB/c mice (Shurin *et al.* 1997, Kusnecov *et al.* 1999,

Goehler *et al.* 2001). This SEB activation of the HPA axis is dependent on T cells but independent of macrophages (Shurin *et al.* 1997). However, SEA, but not SEB, activates the HPA axis in C57BL/6 mice with increases in corticosterone and ACTH. This differential HPA axis activation by these superantigens is dependent on the type of T cells in these mice strains (Kawashima & Kusnecov 2002). A repertoire of T cells exists due to gene splicing. The V β region of the T-cell receptor is coded by up to 20 different genes, and the V β gene utilized can be used to differentiate T cells. SEA preferentially activates V β 3⁺ T cells whereas SEB activates T cells with the V β 8 motif. C57BL/6 mice have a prominent V β 3⁺ T-cell repertoire whereas BALB/c mice lack V β 3⁺ T cells (Takimoto *et al.* 1990, Shurin *et al.* 1997).

Viral HPA axis activation

Some viral infections activate the HPA axis, inducing an increase in glucocorticoid release. The kinetics of glucocorticoid release is both virus-specific and specific to the phase of the response, and is also influenced by the route of exposure. The murine cytomegalovirus (MCMV) (Price *et al.* 1996, Ruzek *et al.* 1997, Pearce *et al.* 2001), lymphocytic choriomeningitis virus (LCMV) (Miller *et al.* 1997, Pearce *et al.* 2001) and poly (I:C) (a synthetic viral analog) induced increases in glucocorticoid levels (Ruzek *et al.* 1997, Pearce *et al.* 2001). Furthermore, MCMV virus and poly (I:C) have been shown to induce glucocorticoid release through an interleukin (IL)-6-dependent pathway (Ruzek *et al.* 1997).

Patients with HIV infection are known to exhibit a glucocorticoid hypersensitive state with increased plasma glucocorticoid levels (Christeff *et al.* 1997). In an animal model for retrovirus-induced immunodeficiency, an increase in ACTH and corticosterone was observed, beginning only 3 months after infection (Espey & Basile 1999). Expression of the HIV protein pg120 in the brain, as is often seen in AIDS patients, increases CRH, ACTH and cortisol levels (Raber *et al.* 1996, Costa *et al.* 2000).

Corneal inoculation of minute amounts of herpes simplex virus type 1 (HSV-1) results in nervous system and brainstem replication of the virus without disease symptoms. During this asymptomatic infection, an acute increase in ACTH and corticosterone levels is observed over 14 days, but it returns to basal by 4 weeks after inoculation (Ben-Hur *et al.* 1995). Similarly, purified viral proteins inoculated intracerebroventricularly (ICV) also induce an acute increase in ACTH and corticosterone expression. This HSV-1 induction of the HPA axis is dependent on brain IL-1 and circulating glucocorticoids, since either ICV injected IL-1 receptor antagonist or adrenalectomy completely abolished this HSV-1 induction of ACTH and corticosterone (Ben-Hur *et al.* 2001b). Other viruses that have been shown to activate the HPA axis include the

Sindbis virus (Trgovcich *et al.* 1997), Newcastle disease virus (NDV) (Dunn *et al.* 1987a,b, Besedovsky & del Rey 1989, Dunn & Vickers 1994) and the influenza virus (Dunn *et al.* 1989, Hermann *et al.* 1994).

The mechanism by which bacterial and viral infections activate the HPA axis will not be fully discussed in this review. However, it should be mentioned that cytokines are considered to be the mediators of such viral and bacterial-induced glucocorticoid release. Cytokine release and HPA axis activation during a bacterial or viral infection are far more complicated than during administration of a single cytokine or purified bacterial product such as LPS (Biron 1994). IL-6 has been shown to be the primary mediator of glucocorticoid release following MCMV infection (Ruzek *et al.* 1997). Some viruses, including poly (I:C), are IL-6 specific (Pearce *et al.* 2001) whereas NDV glucocorticoid release is IL-1 dependent (Dunn & Vickers 1994). Additional cytokine interactions are probably also involved. For example, IL-2 and TNF synergize to enhance glucocorticoid release following LCMV E350 infection in IL-12-treated mice (Orange *et al.* 1995). The role of cytokines in activation of the HPA axis by bacterial endotoxin (Berczi 1993, Tilders *et al.* 1994, Beishuizen & Thijs 2003) and by viral infection (Pearce *et al.* 2001, Bailey *et al.* 2003) has been extensively reviewed by others and will not be further discussed here.

It is worth remembering that, although bacterial and viral infections do have effects on the HPA axis and glucocorticoid release, pain, psychological stress and the debilitating effects of infection also activate the HPA axis and cause glucocorticoid release, thereby complicating the pattern and causes of glucocorticoid release during infection.

Direct adrenal glucocorticoid release following bacterial or viral infection

Glucocorticoid release from the adrenal glands is typically thought to be under the control of the HPA axis. However, glucocorticoid release from the adrenals is also controlled by neural (catecholaminergic and peptidergic) pathways and directly by the immune system through cytokine stimulation (for a review, see Bornstein & Chrousos (1999)). The increased glucocorticoid level induced during MCMV virus infection is not mediated only through the HPA axis, since MCMV infection in CRH-deficient mice has no effect on ACTH but is still able to induce glucocorticoid release, whereas in wild-type mice this infection induces both ACTH and CRH. This suggests that although MCMV infection stimulates the HPA axis, it may also have secondary direct effects on the adrenal glands to stimulate glucocorticoid release (Pearce *et al.* 2001). LPS was also shown to increase plasma corticosterone in hypophysectomized rats (Suzuki *et al.* 1986, Mazzocchi *et al.* 1995). In one study, endotoxin effects on ACTH appeared to be independent of CRH

Table 2 Interruption of HPA axis and glucocorticoid responses in increased mortality or severity of bacterial and viral infections and the role of exogenous glucocorticoid in protection

Organism	Effect of HPA axis blockade	Effect of glucocorticoid treatment	Reference
Endotoxin/LPS	Increased mortality or fever	Reduced mortality or fever	Jeffries & Wilkins 1973, McCallum & Stith 1982, Lazar & Agarwal 1986, Nakano <i>et al.</i> 1987, Butler <i>et al.</i> 1989, Sternberg <i>et al.</i> 1989, Edwards <i>et al.</i> 1991, Coelho <i>et al.</i> 1992, Hawes <i>et al.</i> 1992, Lazar <i>et al.</i> 1992b, 1995, Ramachandra <i>et al.</i> 1992, Morrow <i>et al.</i> 1993, Silverstein <i>et al.</i> 1993, McClellan <i>et al.</i> 1994
<i>E. coli</i>	Increased mortality	Reduced mortality	Silverstein & Johnson 2003
<i>Clostridium difficile</i> toxin A	Increased inflammation	Reduced inflammation	Castagliuolo <i>et al.</i> 2001, Mykoniatis <i>et al.</i> 2003
Shiga toxin	Increased mortality and disease symptoms	Reduced mortality	Palermo <i>et al.</i> 2000, Gomez <i>et al.</i> 2003
<i>Staphylococcus aureus</i> enterotoxin B (SEB)	Increased mortality	Reduced mortality	Gonzalo <i>et al.</i> 1993
MCMV virus	Increased mortality	Reduced mortality	Price <i>et al.</i> 1996, Ruzek <i>et al.</i> 1999

expression, as paraventricular nucleus lesions did not greatly affect endotoxin effects on ACTH or corticosterone, suggesting a direct effect on the pituitary (Elenkov *et al.* 1992). In another study, high-dose endotoxin appeared to have direct effects on the adrenals, since an anti-CRH antibody had minimal effect on corticosterone but abolished ACTH responses, whereas low-dose endotoxin functioned purely through the classical HPA axis (Schotanus *et al.* 1994). In addition, LPS has been shown to stimulate cortisol release directly from the human adrenocortical cell line H295R (Vakharia *et al.* 2002). NDV also induced corticosterone release in hypophysectomized mice, suggesting a direct induction of corticosterone from immune mediators, the so-called lymphoid-adrenal axis (Smith *et al.* 1982). However, these results could not be repeated by others (Dunn *et al.* 1987a,b, Dunn & Vickers 1994), and another study found no evidence of an ACTH-like immunoreactive substance (Besedovsky & del Rey 1989). The reason for this discrepancy is unclear.

Role of an intact HPA axis and glucocorticoid responses in protecting against bacterial and viral insults

Natural and synthetic glucocorticoids protect against the lethal effects of many bacterial and viral components. These are summarized in Table 2.

Bacterial endotoxin

It has long been known that for glucocorticoids to protect against endotoxin lethality they must be administered

either before or concurrent with the endotoxin challenge (Geller *et al.* 1954). After endotoxin, they are ineffective (Geller *et al.* 1954). Agents that activate the HPA axis and cause concurrent glucocorticoid release, such as hydrazine sulfate, have also been shown to protect against endotoxin lethality (Silverstein *et al.* 1989, 1991).

In contrast, agents that block the HPA axis, as in pretreatment with GR antagonists (pregnenolone 16 α -carbonitrile (PCN) or mifepristone (RU486)) or metyrapone (an inhibitor of glucocorticoid synthesis), enhance LPS and endotoxin lethality and LPS-induced fever (Jeffries & Wilkins 1973, Lazar & Agarwal 1986, Sternberg *et al.* 1989, Hawes *et al.* 1992, Lazar *et al.* 1992b, 1995, Ramachandra *et al.* 1992, Silverstein *et al.* 1993, McClellan *et al.* 1994). Even the normally endotoxin-nonresponsive C3H/HeJ mice could be made endotoxin sensitive by RU486 (Lazar & Agarwal 1986, Lazar *et al.* 1992b, 1995). This effect, including the C3H/HeJ mice, can be reversed by concurrent treatment with glucocorticoids (cortisone or dexamethasone) (Jeffries & Wilkins 1973, Lazar & Agarwal 1986, Hawes *et al.* 1992, Lazar *et al.* 1992b, 1995, Ramachandra *et al.* 1992). Likewise, removal of endogenous glucocorticoids by adrenalectomy significantly enhances the lethality of endotoxin or LPS (McCallum & Stith 1982, Nakano *et al.* 1987, Butler *et al.* 1989, Coelho *et al.* 1992, Morrow *et al.* 1993, Silverstein *et al.* 1993). This mortality can be reversed by administration of dexamethasone, but not by the natural glucocorticoids, corticosterone and deoxycorticosterone, suggesting that synthetic glucocorticoids are more effective than the endogenous glucocorticoids in protecting against endotoxin/LPS lethality in adrenalectomized animals (Butler *et al.* 1989, Silverstein *et al.* 1993). In one study, 10 mg/kg dexamethasone was effective in preventing

50 µg LPS toxicity in adrenalectomized BALB/c mice, whereas 10 mg/kg corticosterone was ineffective (Butler *et al.* 1989). In another study, 500 µg dexamethasone prevented 50 µg LPS toxicity in adrenalectomized CF1 mice, whereas a combination of 400 µg corticosterone and 200 µg deoxycorticosterone was ineffective. However, this combination of corticosterone and deoxycorticosterone was able partially to prevent toxicity from 10–20 µg LPS (Silverstein *et al.* 1993). Surgical interruption of the HPA axis at other levels, such as hypophysectomy, also increases mortality from shock. Thus, mortality rates from *Salmonella typhimurium* endotoxin or LPS are increased in hypophysectomized rodents (Butler *et al.* 1989, Edwards *et al.* 1991, Silverstein *et al.* 1993). This LPS/endotoxin-induced mortality upon removal of endogenous glucocorticoids or HPA axis blockade most likely occurs as a result of increased cytokine production, particularly TNF α (Butler *et al.* 1989, Lazar *et al.* 1992a). Such increases in cytokine levels (TNF α and IL-6) following LPS/endotoxin administration are also seen in HPA axis blockade (adrenalectomy or RU486), and these can be reversed by glucocorticoid treatment (Hawes *et al.* 1992, Morrow *et al.* 1993).

The importance of glucocorticoids in modulating endotoxin effects has also been shown in humans by IV injection of endotoxin to healthy men at different time points during the circadian cycle. Those receiving the endotoxin in the evening, when endogenous glucocorticoids are low, showed more severe temperature and plasma ACTH and cortisol changes than those receiving the endotoxin in the morning when glucocorticoid levels are the highest (Pollmacher *et al.* 1996). Finally, the requirement for an intact glucocorticoid response for survival from endotoxin is further demonstrated by the fact that GR overexpression in mice renders them resistant to LPS-induced endotoxin shock (Reichardt *et al.* 2000).

Live bacteria

Although much research has focused on bacterial endotoxins or purified products such as LPS, some research has focused on live bacteria. In these experiments, the effects of adrenalectomy in enhancing mortality and the protective efficacy of dexamethasone in preventing this effect vary with bacterial strain and type. Thus, as in those studies described above, adrenalectomy enhanced lethality from Gram-negative bacteria, such as *E. coli*, and this could be reversed by dexamethasone treatment (Silverstein & Johnson 2003). However, dexamethasone treatment did not prevent the mildly enhanced lethality from the Gram-positive bacterium *S. aureus* in adrenalectomized animals (Silverstein & Johnson 2003). In a D-galactosamine mouse model of septic shock, dexamethasone prevented lethality in all 10 Gram-negative strains tested but in only three (*Enterococcus faecalis*) of the 11 Gram-positive strains tested (Papasian *et al.* 2002).

Antibiotic treatment concomitant with dexamethasone treatment is more effective than dexamethasone treatment alone for treatment of shock resulting from exposure to live bacteria (Pitcairn *et al.* 1975, Hinshaw *et al.* 1981, 1985). For example, antibiotics in combination with dexamethasone protect against *S. aureus* whereas dexamethasone alone was ineffective (Silverstein *et al.* 2000, Silverstein & Johnson 2003). This difference between dexamethasone alone and dexamethasone in conjunction with antibiotic treatment is probably due to the release of bacterial products during antibiotic bacterial death, which in some cases may induce a different cytokine pattern (Cui *et al.* 2000, 2003, Silverstein *et al.* 2000).

Bacterial toxins

HPA axis blockade shows effects on shock responses similar to those of bacterial toxins. Thus, removal of endogenous glucocorticoid responses by the GR antagonist RU486 or by adrenalectomy results in enhanced *C. difficile* toxin A-induced fluid secretion and inflammation (Castagliuolo *et al.* 2001, Mykoniatis *et al.* 2003). Similarly, adrenalectomy enhances time to lethality in Shiga toxin 2-injected BALB/c mice and also increases the pathologic effects of this toxin, as seen by increased serum urea levels and histopathology (Gomez *et al.* 2003). Likewise, adrenalectomy or RU486 increases the lethality of normally nonlethal doses of the bacterial superantigen SEB in BALB/c mice (Gonzalo *et al.* 1993).

These enhanced inflammatory responses in adrenalectomized animals can be reversed by dexamethasone administration or by corticosterone replacement. Replacement with a physiological corticosterone dose results in an inflammatory response equivalent to sham-operated animals while replacement of high pharmacologic corticosterone dose results in a reduction of the inflammatory response (Castagliuolo *et al.* 2001). Similarly, endogenous induction of glucocorticoid by HPA axis stimulation also reduces the mortality from shock. Survival rates of BALB/c mice from Shiga toxin 2 were enhanced by 18-h pretreatment of either LPS or dexamethasone whereas only 1 h of LPS pretreatment decreased survival rates. This protection afforded by the 18 h LPS pretreatment condition was shown to be due to the increased endogenous corticosterone production secondary to LPS-induced IL-1 β activation of the HPA axis (Palermo *et al.* 2000). In these studies, the role of endogenous glucocorticoids in protection against Shiga toxin lethality was further supported by the decreased survival of adrenalectomized animals or those treated with the GR antagonist RU486. Furthermore, dexamethasone treatment reversed the increased mortality in adrenalectomized animals (Gomez *et al.* 2003). Likewise, administration of exogenous dexamethasone protected adrenalectomized BALB/c mice

Table 3 Bacteria that interact with the glucocorticoid receptor (GR)

Bacteria/bacterial product	Effect	Reference
Endotoxin	GR downregulation	McCallum & Stith 1982, Stith & McCallum 1983
	Downregulation of leukocyte GR number	Li & Xu 1988
	Inhibition of Dex binding (rat liver nuclei)	Vaptzarova <i>et al.</i> 1989
	Inhibition of Dex binding (peritoneal macrophages)	Jiayi & Chen 1992
	Increased GR number, reduced affinity (bronchial epithelial cells)	Verheggen <i>et al.</i> 1996
<i>Chlamydia pneumoniae</i>	Activation of GR	Gencay <i>et al.</i> 2003
Superantigen SEB	Induction of GR β	Hauk <i>et al.</i> 2000
Shiga toxin 2	Increased GR expression	Gomez <i>et al.</i> 2003
Anthrax lethal toxin	Repression of GR activity	Webster <i>et al.</i> 2003

Dex, dexamethasone.

from bacterial superantigen SEB lethality (Gonzalo *et al.* 1993).

Viral infections

Interruption of the HPA axis and glucocorticoid replacement has also been shown to alter mortality from viral infection. These effects are both host- and virus strain-dependent. Adrenalectomy has been shown to increase the mortality from MCMV infection in C57BL/6 mice, and this mortality could be reversed by glucocorticoid treatment. This increased mortality following adrenalectomy was correlated with increases in levels of the cytokines IFN γ , TNF and IL-6 (Ruzek *et al.* 1999). In another study, adrenalectomy of BALB/c, but not C57BL/6, mice increased the mortality rates from MCMV virus (Price *et al.* 1996). In the study by Price *et al.* 4×10^4 pfu (plaque-forming units) MCMV virus was used, and 85% of the adrenalectomized C57BL/6 mice survived (Price *et al.* 1996). However, in the study by Ruzek *et al.* (1999), when a viral load similar to that used by Price *et al.* (5×10^4 pfu MCMV virus) was used, approximately 70% of the C57BL/6 adrenalectomized mice survived, while a higher viral load (1×10^5 pfu MCMV virus) was fatal. Thus, there appears to be a differential sensitivity in these two strains, independent of corticosterone production, to the lethality of MCMV virus.

In some cases, data indicate that circulating glucocorticoids are essential for expression of symptoms of viral infection. Thus, intracerebral inoculation of HSV-1 causes symptoms of fever, motor hyperactivity and aggressive behavior in rats. Adrenalectomy prevented HSV-1 induction of all these symptoms, and hypophysectomy or RU486 prevented HSV-1 induced fever. However, neither adrenalectomy, hypophysectomy nor RU486 affected mortality rates. Dexamethasone restored these HSV-1-induced symptoms in these adrenalectomized animals (Ben-Hur *et al.* 2001a).

In summary, while the effect is dependent on the specific pathogen, its dose and the strain of animal infected, in most cases, interruption of the HPA axis by adrenalectomy, hypophysectomy, inhibition of glucocorticoid synthesis or use of a GR antagonist enhances bacterial or viral toxicity or severity of disease. These effects can be reversed by glucocorticoid administration (Table 2). These data emphasize the critical role the HPA axis and glucocorticoid responses play in modulating host responses to infection and the necessity of such responses for survival.

Direct effects of bacterial or viral proteins on the GR

Some bacteria and viruses or their proteins have a direct effect on the GR, either on the activity or the number of receptor molecules. A list of the interactions by bacteria is shown in Table 3 and by viruses in Table 4.

Effect of endotoxin on GR

Glucocorticoids play an important role in glucose metabolism and, in particular, affect some of the key enzymes in gluconeogenesis. Endotoxins induce changes in glucose metabolism, with an initial hyperglycemic state with increased plasma glucose levels being followed by a hypoglycemic state with decreased plasma glucose levels before death (McCallum *et al.* 1983, Stith & McCallum 1988). Adrenalectomized mice do not exhibit the initial hyperglycemia but show a more pronounced hypoglycemic state (McCallum *et al.* 1983) and a reduced endotoxin LD₅₀ (McCallum & Stith 1982). Endotoxins also reduce liver glycogen levels (McCallum & Berry 1973) and the activity of several gluconeogenic enzymes, including the rate-limiting enzyme phosphoenolpyruvate kinase (PEPCK) (McCallum *et al.* 1983, Stith & McCallum

Table 4 Viruses that interact with the glucocorticoid receptor (GR)

Virus/viral product	Effect	Reference
HIV (Vpr)	Enhances GR activity (acts as cofactor)	Kino <i>et al.</i> 1999, 2002 Sherman <i>et al.</i> 2000
Poxvirus MC protein MC013L	Inhibits GR activity	Chen <i>et al.</i> 2000
Influenza virus	Increases GR activity	Ghoshal <i>et al.</i> 2001
HSV-1	Increases GR number and activity	Erlandsson <i>et al.</i> 2002
Epstein–Barr virus	Increases GR number	Tomita <i>et al.</i> 1985
MCMV	Decreases GR binding in spleen	Miller <i>et al.</i> 1997

1988), glycogen synthase (Giger & McCallum 1976), fructose-1,6-diphosphatase and glucose-6-phosphatase (McCallum & Berry 1972). Neither dexamethasone nor glucagon increased plasma glucose levels in endotoxin-treated mice (Stith & McCallum 1983, 1988).

The effects of endotoxin on GR ligand binding has been studied, since PEPCK and other gluconeogenic enzymes are enhanced by dexamethasone through induction of gene transcription by the GR. Stith & McCallum (1982, 1983) showed that endotoxin downregulated liver GR numbers. In ligand-binding studies, endotoxin reduced the B_{max} (maximum number of binding sites) 3–6 h after injection of 100 µg LPS but had no effect on association or dissociation rates, or on the equilibrium dissociation constant (K_d). They showed that endotoxin reduced GR numbers in other tissues, including liver, kidney, skeletal muscle, spleen, lung and heart, but had no effect on PEPCK activity in kidney or glycogen content in skeletal muscle (Stith & McCallum 1986). In radioligand competition studies, endotoxin did not directly compete with [³H]dexamethasone for binding and so did not directly affect GR (Stith & McCallum 1983). Additional studies have also shown an effect of endotoxin on GR. In dogs treated with endotoxin, an endotoxin-induced downregulation of leukocyte GR numbers, as assayed by [³H]dexamethasone binding, was also observed (Li & Xu 1988). Endotoxin inhibition of [³H]dexamethasone binding was also seen in rat liver nuclei (Vaptzarova *et al.* 1989) and peritoneal macrophages (Jiayi & Chen 1992). In bronchial epithelial cells, LPS caused an increase in GR number but also reduced the ligand affinity, as seen by an increase in the K_d (Verheggen *et al.* 1996). However, others could find no effect of endotoxin on GR numbers (Shackleford *et al.* 1986).

Some studies have attempted to determine how endotoxin downregulates GR numbers. A soluble factor released from macrophages following endotoxin treatment has been reported to account for endotoxin downregulation of GRs (Hill *et al.* 1987). This factor was termed ‘glucocorticoid antagonizing factor’ (GAF) and was shown

to reduce liver glycogen levels (Sakaguchi *et al.* 1990) and inhibit PEPCK activity (Goodrum & Berry 1978, 1979). Purification showed it to be a 90 kDa glycoprotein (Sakaguchi & Yokota 1987). Although the exact components of GAF have never been fully identified, it does show some similarities to TNF α (Moore *et al.* 1978), and cytokines, including IL-1 and TNF α , are also released from macrophages following endotoxin (Koj 1996, Van Amersfoort *et al.* 2003). IL-1 and TNF α have also been shown to repress glucocorticoid induction of the gluconeogenic enzyme PEPCK (Hill *et al.* 1986, 1988, Hill & McCallum 1992), and IL-1 and IL-6 downregulate GR numbers (Hill *et al.* 1986, 1988, Stith *et al.* 1989) in a similar way to endotoxin. Whether GAF is a cytokine or not has not been determined, and, interestingly, there has been no mention of GAF in the scientific literature since 1990. More recently, interest has focused on the glucocorticoid counter-regulator, macrophage migration inhibitor factor (MIF) (Baugh & Bucala 2002). A monoclonal antibody against MIF has been shown to protect against septic shock induced by cecal ligation and puncture (CLP) and *E. coli* peritonitis (Calandra *et al.* 2000, Roger *et al.* 2001), whereas IP injection of recombinant MIF enhanced the lethality of *E. coli* peritonitis (Calandra *et al.* 2000).

Effect of other bacteria and bacterial proteins on GR

Chlamydia pneumoniae infection of epithelial cells leads to activation of GR, as determined by nuclear translocation, DNA binding and induction of the GR-inducible gene p21^(Waf1/Cip1), and this activation can be inhibited by RU486. GR activation leads to increased host cell proliferation, which again can be inhibited by RU486. Thus, in this system, activation of GR ensures the survival and propagation of the infected host cells (Gencay *et al.* 2003). In human peripheral blood mononuclear cells (PBMCs), the bacterial superantigen SEB induces glucocorticoid resistance in T cells by increasing the expression of the proposed dominant negative GR isoform, GR β (Hauk *et al.* 2000).

Shiga toxin 2 has been shown to increase GR expression, as determined by fluorescence activated cell sorting (FACS) analysis, 24 h after injection, and this is sustained through 48 h. However, the implications of this are not clear, particularly as dexamethasone is not protective at 24 h after injection (Gomez *et al.* 2003). We have recently shown that the anthrax lethal toxin represses GR activity as well as the activity of other nuclear hormone receptors and inhibits the glucocorticoid increase in the liver gluconeogenic enzyme, tyrosine aminotransferase. Although the precise mechanism of this repression is not yet fully understood, it does not occur by direct competition with ligand or DNA binding, and may involve one of the many cofactors necessary for GR effects on transcription (Webster *et al.* 2003).

Effect of viral proteins on GR

As in the case of bacterial products, viral proteins also interact with GR, and the specific effects of viruses are strain dependent. It has long been realized that many of the symptoms associated with AIDS are similar to those associated with excess glucocorticoid production. In fact, AIDS patients do exhibit a glucocorticoid-hypersensitive state. Recently, it has been shown that one of the HIV-1 accessory proteins, Vpr, can function as a GR coactivator (Kino *et al.* 1999, Sherman *et al.* 2000). This protein contains the consensus LXXLL nuclear receptor signature motif and can bind directly to GR (Kino *et al.* 1999) to function as an adaptor protein enabling interaction between GR and cofactors such as p300 (Kino *et al.* 2002). This interaction has been shown to affect GR functionally, as extracellularly administered Vpr can enhance dexamethasone suppression of IL-12 in PMBCs (Mirani *et al.* 2002). In addition, Vpr has been shown to interact with the viral protein, R interacting protein 1 (Rip-1), and both Rip-1 and Vpr can be co-immunoprecipitated with GR as part of the activated receptor complex (Refaeli *et al.* 1995). Vpr action as a GR cofactor requires an intact GRE and is dependent on the ligand and the presence of the receptor, but is independent of cell-cycle arrest (Sherman *et al.* 2000), as has been suggested by others (Forget *et al.* 1998). In addition, GR affinity can vary in patients with AIDS, with some expressing normal affinity GR and others low-affinity receptors (Norbiato *et al.* 1997).

MC013 L, a protein from the poxvirus molluscum contagiosum (MC), has recently been shown to inhibit GR- and vitamin D (VDR)-mediated gene transactivation, but not retinoid- or estrogen-mediated gene transactivation. Direct interaction between the MC013 L protein and GR and VDR has been shown. This GR and VDR repression has been suggested to promote virus replication by preventing the differentiation of the infected keratinocytes (Chen *et al.* 2000). The influenza virus also increases GR activity. The expression of the GR-regulated genes, metallothionein I (MT-I) and II (MT-II),

is induced by the influenza virus. This effect can be repressed by RU486, suggesting a direct role for GR in viral induction of these proteins. In addition, influenza virus has been shown to enhance GR binding to the promoter of these genes (Ghoshal *et al.* 2001).

HSV-1 virus upregulates GR number and increases GR nuclear translocation of GR in human primary gingival fibroblasts. This increase in GR translocation is accompanied by an increase in GR transactivation, as indicated by the fact that HSV-1 virus can induce gene transcription from a GR-controlled reporter construct in HEK 293 cells (Erlandsson *et al.* 2002). Transformation of human lymphocytes with Epstein-Barr virus also increases GR number without affecting K_d (Tomita *et al.* 1985). In contrast, MCMV virus infection has also been shown to decrease GR binding in the spleen (Miller *et al.* 1997).

Septic shock

An animal model of septic shock has been developed with a murine model of septic peritonitis using cecal ligation just below the ileocecal valve followed by puncture with a 20–21 gauge needle (CLP). The rate of survival was dependant on the gauge of the needle used, survival rates being greater with the smaller needle. Plasma corticosterone levels are similarly elevated in both CLP and sham operated rats following polymicrobial sepsis, but the CLP operated animals showed enhanced ACTH levels compared with sham operated animals, suggesting adrenal insufficiency (Koo *et al.* 2001). This model has been used to support the role of endotoxins in septic shock, since LPS-tolerant mice showed higher survival rates than LPS-sensitive mice (Lazar *et al.* 1992b, 1995). Administration of RU486 decreased the survival rates following cecal puncture (Lazar *et al.* 1992b).

Since it is generally older patients who develop lethal endotoxic shock, the effect of endotoxin in older animals has been investigated. Older mice have a reduced LD_{50} to LPS even though they have a large increase in plasma corticosterone levels following a low dose of LPS (1 mg/kg) compared with younger mice, which have only a small increase in plasma corticosterone following this dose of LPS. Thus, it appears that their increase in corticosterone is insufficient to protect against a large increase in TNF α and nitric oxide (NO) levels (Chorinchath *et al.* 1996). Age-related differences in GR signaling following endotoxin challenge have also been reported in older mice in which plasma corticosterone levels were similar to younger mice. However, increased [3 H]dexamethasone binding without increased gluconeogenic enzyme activity, such as that of PEPCK, was shown in these older mice, suggesting some degree of glucocorticoid resistance (Stith & McCallum 1985).

From the animal data presented above and the effectiveness of glucocorticoids in inflammation, one might

expect that glucocorticoids would be an effective treatment for septic shock. However, the use of glucocorticoids in the treatment of septic shock has been a matter of controversy since the 1950s. In some instances they have been shown to enhance survival rates, but in others they have been shown to enhance mortality. The pros and cons of glucocorticoid therapy have recently been reviewed in detail (Balk 2003, Sessler 2003), and will not be reviewed here. However, it is generally now accepted that high doses of glucocorticoids are not effective in the treatment of septic shock while controversy still exists about the effectiveness of physiological doses.

It is worth noting that glucocorticoid levels are increased in humans during critical illness and septic shock (Vermes *et al.* 1995). During the acute phase of critical illness, the increased glucocorticoid release is correlated with increased ACTH levels (Vermes *et al.* 1995). However, during the chronic phase of sepsis, there is a dissociation between ACTH and cortisol levels, ACTH being low while cortisol levels are still high (Siegel *et al.* 1994, Vermes *et al.* 1995); this suggests that the sustained disease state can have a non-ACTH-mediated effect on cortisol release (Bornstein & Chrousos 1999). During a prolonged critical illness or sepsis, there is evidence that there is, in fact, adrenal insufficiency despite the patient's exhibiting normal or elevated glucocorticoid levels (Sibbald *et al.* 1977, Rothwell *et al.* 1991, Williamson & Lapointe 2003). It has been reported that 25–40% of septic shock patients have adrenal insufficiency (Soni *et al.* 1995, Koo *et al.* 2001, Marik & Zaloga 2003). It is also possible that there are differences in glucocorticoid sensitivity at the level of the receptor during septic shock. In one study, enhanced sensitivity of peripheral leukocytes to glucocorticoids has been noted (Molijn *et al.* 1995*b*). In another study, a decreased affinity was noted (Molijn *et al.* 1995*a*). Therefore the use of glucocorticoids in the treatment of septic shock may be dependent on the stage of the sepsis, the reactivity of the HPA axis, particularly the adrenals, and the sensitivity of GR to the ligand. Taken together these variables make the effects of the therapeutic use of glucocorticoids in septic shock difficult to predict.

Conclusions

Many bacterial and viral infections result in an activation of the HPA axis and resultant release of glucocorticoids. This can either occur centrally at the level of the hypothalamus or pituitary or through direct activation of the adrenal glands. The HPA axis activation and resultant glucocorticoid release following bacterial or viral infections are advantageous to both the infectious agent and the host. HPA axis activation can be advantageous to the invading organism, as it serves to minimize the host immune responses, thereby enhancing the organism's ability to survive. However, enhanced glucocorticoid release that

generally suppresses immune system responses prevents overreactivity of the immune system with associated release of dangerous levels of cytokines, such as TNF α . In this manner, HPA axis activation is involved in regulatory feedback control of the acute phase response to an infection, which serves, through mechanisms not discussed here, to restore the homeostasis of the host.

Indeed, an intact HPA axis and resultant glucocorticoid release are necessary for host survival after exposure to an infectious agent. It has now been shown in numerous viral and bacterial infections that interruption of the HPA axis, by hypophysectomy, adrenalectomy, inhibition of glucocorticoid synthesis or the use of the GR antagonist RU486, can enhance the severity or lethality of the infection, and that replacement with glucocorticoids can prevent these effects.

Some bacteria and viral products also have direct effects on the GR. In the case of those that upregulate either GR numbers or GR activity, there is an obvious evolutionary advantage to the pathogen, since increased GR functioning would be expected to have a greater suppressive effect on the host immune system thereby promoting survival of the bacteria or virus. For example, *C. pneumoniae* increases GR activation, resulting in survival of host infected cells, and the MC poxvirus protein MC013 L inhibits GR, thereby preventing differentiation of the infected keratinocytes, and allowing virus replication. Bacterial superantigens increase the expression of GR β , the inactive form of GR. For those bacteria and viruses that cause a decrease in GR number or activity, as anthrax lethal toxin does, it is more difficult to see an evolutionary advantage to the invading organism. However, inhibition of GR would be predicted to be detrimental to host survival.

In summary, bacteria and viruses modulate host responses by activating the HPA axis and increasing glucocorticoid levels; in turn, an intact HPA axis and glucocorticoid response are critical to the host survival from inflammatory sequelae of exposure to infectious agents. Such agents can also modulate host responses by directly affecting GR functioning. These effects are organism and host strain specific, and are also dependent on dose. The ultimate lethality of a pathogen thus depends on a complex interplay between the specific organism's induction of an appropriate level of the host's HPA axis response, the intactness of that response and possible direct pathogen interactions at the level of the GR. Precise definitions of these interactions in an individual host exposed to a particular pathogen will be essential to designing effective therapy for shock targeted at HPA axis components.

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