

Review article

Dysregulation of the stress response in asthmatic children

The stress system co-ordinates the adaptive responses of the organism to stressors of any kind. Inappropriate responsiveness may account for increased susceptibility to a variety of disorders, including asthma. Accumulated evidence from animal models suggests that exogenously applied stress enhances airway reactivity and increases allergen-induced airway inflammation. This is in agreement with the clinical observation that stressful life events increase the risk of a new asthma attack. Activation of the hypothalamic–pituitary–adrenal (HPA) axis by specific cytokines increases the release of cortisol, which in turn feeds back and suppresses the immune reaction. Data from animal models suggest that inability to increase glucocorticoid production in response to stress is associated with increased airway inflammation with mechanical dysfunction of the lungs. Recently, a growing body of evidence shows that asthmatic subjects who are not treated with inhaled corticosteroids (ICS) are likely to have an attenuated activity and/or responsiveness of their HPA axis. In line with this concept, most asthmatic children demonstrate improved HPA axis responsiveness on conventional doses of ICS, as their airway inflammation subsides. Few patients may experience further deterioration of adrenal function, a phenomenon which may be genetically determined.

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Although the clinical spectrum of asthma is highly variable, the dominant pathological feature is airway inflammation (1). The characteristic pattern of inflammation in the airways appears to be similar in all clinical forms of the disease, with activation of mast cells, and an increase in the number of activated eosinophils and T lymphocytes, which release inflammatory mediators that contribute to the manifestations of the disease (1, 2).

Importantly, these mediators cause activation of the stress system, which co-ordinates the adaptive responses of the organism to stressors, maintaining basal and stress-related homeostasis. The stress system influences the activity of many other body systems, including the central nervous, cardiorespiratory, metabolic, endocrine, and immune systems the functions of which are closely intertwined (3). A major component of the stress system is the hypothalamic–pituitary–adrenal (HPA). Stimulation of this axis by inflammatory cytokines [e.g. tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), IL-6] results in an increase in systemic glucocorticoids (corticosterone or cortisol in rodents and primates, respectively), which, in turn, feed back and suppress the immune and inflammatory reaction (4). This suppressive activity includes the anti-inflammatory effects of glucocorticoids on the airways.

Recent evidence indicates that the balance between systemic and/or local airway anti- and pro-inflammatory

actions might be dysregulated in asthmatics (5, 6). This review focuses on current knowledge regarding the effects of the stress system on airway inflammation, obtained from animal models and clinical experience in humans. It also provides a brief overview on the role of the HPA axis in asthmatic subjects not treated with inhaled corticosteroids (ICS) and on long-term ICS therapy.

Stress system–immune reaction interactions

The stress and immune systems play crucial roles in maintaining homeostasis (3, 4). The stress response is co-ordinated and mediated by the centers of the stress system in the brain, along with their respective peripheral limbs. The centers in the brain consist of the corticotropin-releasing hormone (CRH), the arginine-vasopressin (AVP) neurons of the paraventricular nuclei of the hypothalamus, and the brainstem noradrenergic neurons of the locus caeruleus/norepinephrine (LC/NE)–central sympathetic systems. The peripheral limbs include the HPA axis and the systemic sympathetic, and adrenomedullary nervous systems. Activation of the central stress system leads to the secretion of CRH and AVP into the hypophyseal portal circulation, hence, to the stimulation of pituitary adrenocorticotrophic hormone (ACTH) and adrenocortical glucocorticoid secretion. Stress system

activation also leads to the stimulation of the systemic sympathetic and adrenomedullary nervous systems, thus, to the peripheral secretion of NE, epinephrine, and several neuropeptides (7) (Fig. 1).

The stress system receives information neurally and humorally through the systemic circulation, from injurious agents or signal-carrying molecules, such as hormones, growth factors, neurotransmitters, neuropeptides, cytokines, and other mediators of inflammation (3).

The immune system is responsible for the defence against different injurious agents and the immune or inflammatory response is activated by such agents

through several classes of recognition molecules or receptors, such as the toll-like receptors (TLR). Once the magnitude of the immune response exceeds a certain threshold, activation of the stress response also occurs, with effects that antagonize or potentiate those of the immune response. Whether stress activates or inhibits these immune responses is often dependent on the duration and quality of the stress stimulus (5, 8).

The principal peripheral stress hormones glucocorticoids and catecholamines affect major immune functions, such as antigen presentation, lymphocyte proliferation and trafficking, secretion of cytokines and antibodies, and selection of the T helper-1 (Th1) vs Th2 response. In the 1970s and 1980s, stress was often regarded as merely immunosuppressive. Recent evidence, however, indicates that stress hormones influence the immune response in a multi-dimensional way; both glucocorticoids and catecholamines systemically mediate a Th2 shift by suppressing antigen presentation, Th1 production, and by upregulating Th2 cytokine production. On the other hand, in certain local responses and under certain conditions, stress hormones may actually facilitate inflammation, through redeployment of immune cells, induction of TNF- α , IL-1, IL-6, IL-8, and C-reactive protein production or through activation of the CRH-substance P-histamine axis (3). It is thus becoming increasingly clear that stress hormone-induced inhibition or upregulation of the systemic or local pro- and anti-inflammatory mediator production and the Th1/Th2 balance may affect disease susceptibility and outcome (3, 9, 10).

Allergy is a hypersensitivity reaction initiated by immune mechanisms, humoral, or cell-mediated; allergic mechanisms are thus of paramount importance in asthma (11). As Th2 cells have a primary role in the development of asthma, stress mechanisms may be linked to asthma.

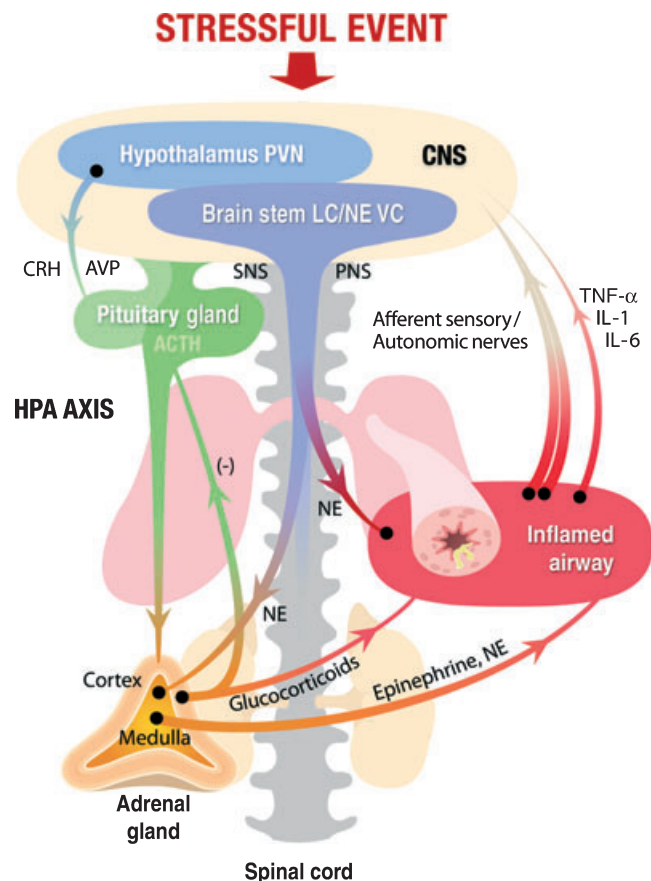


Figure 1. Interactions between the stress system composed of the hypothalamic–pituitary–adrenal (HPA) axis and the LC/NE sympathetic and parasympathetic systems and an immune and inflammatory response of the airways during a stressful event. The stress response via the brain activates the HPA axis, which produces glucocorticoids as the end-hormone. Additionally, stress can activate the sympathetic nervous system and primary sensory nerves. Whether stress activates or inhibits the immune response is often dependent on the duration, timing and quality of the stressor. ACTH, adrenocorticotrophic hormone; AVP, arginine vasopressin; CNS, central nervous system; CRH, corticotropin-releasing hormone; IL, interleukin; LC, locus caeruleus; NE, norepinephrine; PNS, parasympathetic nervous system; PVN, paraventricular nucleus; SNS, sympathetic nervous system; TNF- α , tumor necrosis factor alpha; VC, vagal complex.

Stress increases airway inflammation

Animal models, human relevance

Despite the long-standing clinical assumption of an association between stress and asthma morbidity, evidence on its pathophysiological mechanism was available only fairly recently. Few groups have worked on this area and have provided interesting data.

Joachim et al. (12–14) in Germany used mice sensitized by intraperitoneal injection of ovalbumin (OVA) and challenged with OVA aerosol via the airways. In addition, some mice were stressed by exposure to an ultrasonic stressor. Airway hyperreactivity (AHR) was measured, bronchoalveolar lavage fluid (BAL) was obtained, and cell numbers determined. Their findings demonstrated that exogenously applied stress dramatically enhances airway reactivity in OVA-sensitized and challenged mice. Furthermore, stress significantly increases allergen-induced airway inflammation

identified by increased leukocyte (i.e. eosinophil) numbers in BAL.

Silverman et al. (15) subjected CRH-knockout mice to an OVA-induced airway inflammation protocol that mimics many features of asthma. They found that these mice showed increased airway inflammation and goblet cell hyperplasia. In contrast, IgE induction remained unaffected by CRH deficiency. The increase in inflammation in CRH-knockout mice was associated with an increase in tissue resistance, elastance, and hysteresivity. Levels of IL-4, IL-5, IL-13, RANTES, interferon gamma (IFN- γ), and eotaxin were all increased, whereas serum corticosterone levels were decreased. The authors concluded that inherited or acquired CRH deficiency could increase asthma severity in human subjects. At the clinical level, it is of interest that genetic variants of CRH receptor 1 (CRHR1), the predominant CRHR in the pituitary gland, demonstrated also in the lung, have been associated with enhanced response to ICS (16). Thus, given the pleiotropic actions of CRH in the immune system, any modifications in its gene expression, as well as changes in its mechanism of action, could be related to the pathogenesis and treatment of asthma.

To understand the role of duration of stress and its effects on asthma, another series of studies were undertaken. Thus, in line with the above-mentioned findings in animal models, other researchers reported on the effects of acute vs chronic stress on allergic airway inflammation. Vliagoftis' group (17) investigated the ability of psychological stress to modulate airway inflammation, and AHR to methacholine in a murine model of asthma. Animals were exposed to a stressor daily for 3 (short-term stress) or 7 (long-term stress) days. After allergen challenge, AHR was assessed through plethysmography, and BAL cytology was performed as a measure of inflammation. After short-term stress, inflammatory cell number in BAL was decreased compared with unstressed animals, whereas BAL levels of IL-6, IL-9, and IL-13 were increased. Administration of a glucocorticoid receptor antagonist before stress, prevented the decrease in inflammatory cell numbers. In contrast, animals stressed for 7 consecutive days showed a significant increase in inflammatory cell numbers that was independent of the glucocorticoid response, with no concomitant change in BAL cytokine levels. Airway hyperreactivity was not altered in either the short-term or the long-term stressed animals. Their results indicate that repeated exposure to stress over the long-term engages stress mechanisms different from those applied in the short-term, and can exacerbate the chronic inflammatory responses of the airway (18).

The peripheral limbs of the stress system are modulated in stressful conditions by opioid peptides through the binding of ligands to μ -opioid receptors (19); asthmatic exacerbations therefore caused by psychological stress might be influenced by the activation of μ -opioid receptors. Okuyama et al. (20) studied wild and μ -opioid

receptor deficient mice, with a different genetic background (BALB_c and C57BL_6) sensitized and exposed to OVA, in conditions of acute or chronic restraint stress. Airway inflammation was evaluated by measuring the number of inflammatory cells and cytokine content in BAL. In BALB_c, but not in C57BL_6 mice the total cell count, number of eosinophils, and lymphocytes in the acute stress group were significantly decreased compared with those in the chronic stress group. In contrast, chronic stress significantly increased the BAL cell numbers and contents of IL-4 and IL-5 in both mouse strains. Furthermore, these exacerbations were abolished in μ -opioid receptor-deficient mice. These results suggest that acute stress modifies the allergic airway responses selectively, depending on the genetic background; activation of μ -opioid receptors, and consequent release of stress hormones might therefore be involved in the chronic stress-induced exacerbation of allergic airway inflammation.

In their effort to investigate the long-lasting effect of early life stress on adult asthma in mice, another Japanese group (21) exposed mice sensitized to OVA to either psychological or physical stress every other day for three times during their 4th week of life; the mice were sensitized to OVA at 8 and 10 weeks; and an OVA airway challenge was conducted at the age of 11 weeks. Twenty-four hours after OVA challenge, stress-exposed mice exhibited a significant acceleration in the number of total mononuclear cells, eosinophils, and in AHR as compared with controls not exposed to stress. In the psychologically but not in the physically stressed group, an elevation of serum corticosterone levels during OVA challenge was significantly attenuated as compared with the control group. The authors conclude that early psychological and physical stress accelerated airway inflammation and AHR in their mouse model of adult asthma. This stress-induced exacerbation of asthma was critically involved in antigen challenge, but not in antigen sensitization. Interestingly, this study also provided evidence that distinct physiological mechanisms that depend on the type of stress, psychological or physical, are involved in stress-induced asthma exacerbation; early psychological stress exacerbated adult asthma via HPA axis hyporesponsiveness during antigen challenge, whereas physical stress did so through a pathway(s) distinct from the HPA axis or natural killer (NK)-1 receptors.

As outlined above, data from animal models clearly indicate that stress may produce a marked increase in allergen-induced airway inflammation. It appears that different mechanisms in acute vs chronic stress influence the inflammatory responses of the airway. In acute stress, activation of the HPA axis and consequent cortisol release lead to reduction of airway inflammation. However, after continuous prolonged or intermittent stimulation, as in chronic stress, HPA axis activity is suppressed and its anti-inflammatory effect is reduced.

Clinical and epidemiologic studies

A significant role of stress in asthma exacerbation in every day clinical practice was proposed in the past (22, 23). However, it is based on the patient's and physician's experience rather than documented evidence. In recent years, there are a few interesting and indicative reports on the subject (24, 25).

A group of asthmatic children were prospectively followed up for 18 months. Key measures included asthma exacerbations, high threat life events, and chronic stressors. Using statistical methods capable of investigating short-time lags between stressful life events and asthma exacerbations, the authors found a significant increase in the risk of a new asthma attack immediately after a stressful event; a delayed increase in the risk, 5–7 weeks later, was also evident. The risk was increased further and brought earlier in time, if multiple chronic stressors were present in the child's life (23, 26).

In a recent meta-analysis by Chida et al. (27) on prospective cohort studies investigating the influence of psychosocial factors on atopic disorders, among 34 studies the major atopic disease assessed was asthma (90.7%), allergic rhinitis 4.7%, atopic dermatitis 2.3%, and food allergies 2.3%. The overall meta-analysis exhibited a positive association between psychosocial factors and future atopic disorder. More notably, the subgroup meta-analysis on the healthy and atopic disorder populations showed psychosocial factors had both an etiologic and prognostic effect on atopic disorders. In their meta-analysis conclusions, the authors suggested the use of psychologic interventions in addition to the conventional physical and pharmacologic interventions, in the successful prevention and management of atopic disorders.

In a series of studies, a link between low socioeconomic status and immunologic markers of asthma was demonstrated (28, 29). Asthmatic adolescents of low socioeconomic status had significantly higher eosinophil counts, and increased levels of inflammatory cytokines associated with asthma (IL-5, IL-13); they also demonstrated marginally lower morning serum cortisol values than those of the high socioeconomic status group, a possible indication of a less active HPA axis in the former. Lower socioeconomic status was also associated with higher emotional stress and perceived threat. Higher levels of stress and perception of threat were associated with heightened production of IL-5 and IL-13 and higher eosinophil counts in the children with asthma compared with healthy controls. Statistical tests revealed that chronic emotional stress and threat perception represented significant mediation pathways between socioeconomic status and immune processes in children with asthma.

A pilot study (30), which indicated that sustained stressful life events such as academic examinations alter the pattern of cytokine release in asthmatic individuals,

was followed by another study by the same group; their goal was to investigate the effect of a relatively chronic stressful life event on allergic inflammatory response to allergens. An airway antigen challenge was performed in 20 college students with asthma during a low-stress phase (mid-semester or 2 weeks postfinal examination) and a high-stress phase (final examination week). Sputum eosinophils and eosinophil-derived neurotoxin levels significantly increased 6- and 24-h postchallenge and were enhanced during the high-stress period. Production of IL-5 by sputum cells was also increased 24-h postchallenge; this resulted in a greater decrease in the IFN- γ /IL-5 ratio during the week of examinations, and correlated with airway eosinophils (31). These data raise the possibility that allergen-induced airway eosinophilia can be significantly augmented during a period of stress. The association between stress and decreased ability of peripheral blood lymphocytes to synthesize IFN- γ when stimulated *in vitro* has been reported (32, 33). However, the observation of an increase in IL-5 and a shift towards a Th2 phenotype in airway cells in asthmatic patients during stress is novel, and may indicate an alteration in airway inflammation.

In a study of children predisposed to atopy on the basis of their family history, Wright et al. (34) examined the influence of reported parental caregiver stress in the first 6 months of their child's life on the lymphocyte allergen-specific proliferative response, cytokine production, and total IgE expression when the children were 2–3 years of age. They demonstrated that higher early life chronic caregiver stress was associated with an enhanced allergen-specific proliferative response, a respectively increased and decreased production of TNF- α and IFN- γ by stimulated peripheral blood mononuclear cells and increased total IgE expression.

According to the above mentioned clinical data, exposure to stress in early development might result in functional changes in immune reactivity in susceptible children potentiating the inflammatory response. Therefore, this could be considered as the *allostatic* or, more correctly, *cacostatic load*, i.e. the disease burden or cost the body has to pay for maintenance of stability outside the normal homeostatic range (*allostasis* or, more correctly, *cacostasis*) (35). Figure 2 depicts the conceptual model through which the stress response to various stressors may influence the HPA axis, and exacerbate the disease in asthmatic children.

There is also evidence that stressful life experiences diminish expression of genes encoding the glucocorticoid and the beta₂-adrenergic receptors in children with asthma (36). In 77 children (39 asthmatics, 38 healthy controls), chronic stress was associated with reduced expression of the mRNA of the beta₂-adrenergic receptor among children with asthma. In the sample of healthy children, however, the direction of this effect was the opposite. The occurrence of a major life event in the 6 months preceding the study was not sufficient to

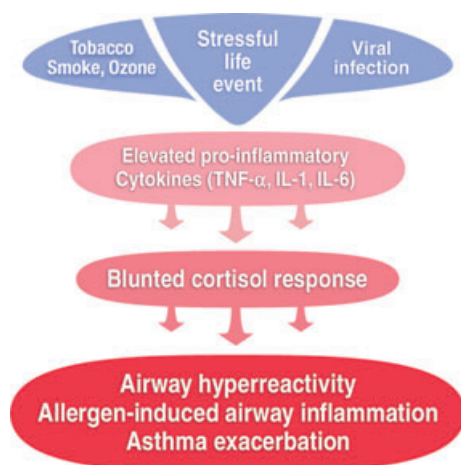


Figure 2. Conceptual model highlighting the pathway through which the stress response influences the manifestations of asthma in children. The elevated pro-inflammatory cytokines, such as TNF- α , IL-1, and IL-6 are the principal conduits between the stress and immune system; they may induce a state of hyporesponsiveness of the HPA axis, whereby attenuated cortisol secretion and exacerbation of allergic airway inflammation ensue. The probability that other systems and/or other effector cells may be implicated in a biologic cascade resulting in similar endpoints cannot be excluded at the present time.

influence patterns of gene expression. When such events occurred in the context of a chronic stressor, however, their association with patterns of gene expression was accentuated. Children with asthma, who simultaneously experienced acute superimposed on chronic stress, exhibited a 5.5-fold reduction in glucocorticoid receptor mRNA, and a 9.5-fold reduction in beta₂-adrenergic receptor mRNA compared to children with asthma without similar stressor exposure.

The findings that chronic stressors facilitate childhood asthma exacerbations are supported by more recent observational studies on the role of the social environment in children and adolescents with asthma (37); in addition, victimization and missed school because of feeling unsafe are important but poorly understood risk factors for asthma morbidity that pertain to a large population of children and adolescents (38).

In line with the above-mentioned clinical observations, a series of recent findings have formed the basis for the fetal programming of asthma hypothesis, which proposes that stress experienced during pregnancy may lead to vulnerability of the immune system towards atopic diseases and asthma (39, 40). Pincus-Knackstedt et al. (41) in an experimental setting showed that stress during pregnancy aggravates asthma in the offspring in later life by severe alteration in the immune response to allergens, and likely by impairment of lung organogenesis. In a birth cohort study in Manitoba, Canada, using health care and prescription databases, the association between maternal distress during the first year of life and onward

and asthma at age 7 was assessed. An increased risk of childhood asthma among children exposed to continued maternal distress from birth until age 7 was demonstrated. Exposure to maternal depression and anxiety limited to the first year of life did not have a demonstrable association with subsequent asthma (42).

Stress response to viral infection

Viral infection of the respiratory tract induces a series of defence responses designed to terminate viral replication. The first is a rapid-onset innate response which involves the synthesis of IFNs and the stimulation of NK lymphocytes. If the infection progresses, the adaptive immune response with its humoral, and cell-mediated arms ensues (43). During the early, innate immune response to viral infections, the pro-inflammatory cytokines TNF- α , IL-1, and IL-6, are induced to aid in the process of anti-viral immunity. However, cytokine receptors have been detected at all HPA axis levels, and therefore, each level can serve as an integration point for immune and neuroendocrine signals; the effects of innate pro-inflammatory cytokines – TNF- α , IL-1, and IL-6 – on HPA axis function have been studied the most (44–46).

In an experimental murine infection with influenza A/PR8 virus, Sheridan's group examined the effects of stress-induced activation of the nervous and endocrine systems on components of innate immunity (47). Pro-inflammatory cytokine responses (TNF- α , IL-1 α , and IL-6) were measured in the lungs during an influenza A/PR8 viral infection. Restraint stress was applied to activate the nervous and endocrine systems prior to and during infection. Following infection, IL-1 α increased transiently, while elevated IL-6 persisted; TNF- α was not detected. Restraint stress suppressed virally-induced IL-1 α , while IL-6 remained unaffected. These data demonstrate differential regulation of pro-inflammatory cytokines by stress. The downregulation of the lung IL-1 α in stressed mice may contribute to increased viral pulmonary pathogenesis in stressed individuals.

The same group of researchers (48) examined the life-long effects of neonatal stress on the response to a viral influenza infection. Mouse pups were repeatedly separated from their dams between postnatal days 1 and 14 (maternal separation). When these mice reached adulthood, they were infected with influenza A/PR8 virus and lung cytokine, and plasma corticosterone responses to the viral infection were measured. The results of the study indicated that maternal separation augments several aspects of the response to infection. First, infection-induced lung pro-inflammatory cytokine (IL-1, IL-6, TNF- α) mRNA expression was higher in maternally separated mice than in controls. In addition, maternal separation augmented infection-induced lung IL-12 and IFN- γ , but had no effect on IL-18 mRNA. Interestingly, the maternal separation-induced increase in IL-1, TNF- α , and IFN- γ mRNA

expression was evident in females, but not in males. These findings suggest that maternal separation disrupted the regulation of innate resistance resulting in enhanced cytokine responses in the lungs during an infectious challenge. These changes in host response to the viral infection were accompanied by an increase in viral replication in the lungs of maternally separated mice. It is also of interest that influenza-induced corticosterone secretion was blunted in maternally separated mice, suggesting that the increase in immune reactivity to the virus was because of lack of glucocorticoid feedback control.

Early life stress, such as maternal separation, has been associated with a decrease of glucocorticoid receptor binding in the hippocampus and hypothalamus, an increase in hypothalamic CRH, and eventually an augmented activity of the HPA axis through disruption of glucocorticoid negative feedback control (49). Similarly, in a recent report, early postnatal stimulative, or adverse experiences in rats exerted long-lasting changes of the 'neuroendocrinoimmune' interface in adulthood resulting in either protective or aggravating mechanisms in allergic airway disease (50). Consequently, long lasting effects of negative life events have implications for the host immune status, susceptibility to infections throughout life, and may be the basis for the individual differences in host susceptibility to infection. Moreover, differences in the effects of stress on the HPA axis during an infectious challenge may exist between asthmatic and other populations augmenting the allergic inflammatory response of the former.

Certain cytokines activate the HPA axis causing increased glucocorticoid secretion, resulting in protection from cytokine-mediated pathology. Even in the case of acute or chronic CRH deficiency there are alternative neuroendocrine pathways through which virus-induced immune responses influence a glucocorticoid response (51). It has been demonstrated that during the acute period of respiratory syncytial virus infection, there is an increase in the level of plasma cortisol (52). Although this increase was observed in infants with mild and severe disease, an imbalance of the T helper 1 (Th1)/Th2 response with a deficient type 1 response was present only in those cases with severe bronchiolitis, and higher levels of plasma cortisol. A natural Th1/Th2 switch with a shift to a Th2 profile of cytokine production is further evidenced by the increased endogenous cortisol production under stress conditions.

A glucocorticoid receptor antagonist was used in mice to confirm that the effects of stress on cell trafficking and cytokine production were mediated by elevated corticosterone (53). In this experimental model, the induction of elevated glucocorticoids by an acute psychological stressor was associated with a reduction in lung pathology, and increased survival following a respiratory viral infection (54).

In clinical practice, the concept that chronic stress is associated with acute respiratory episodes has been demonstrated in epidemiologic observations in adults

(55); a similar association between maternal stress and more frequent and severe children's respiratory symptoms has also been reported (56). Higher psychological stress before viral challenge (influenza A virus) was associated with greater symptom scores, greater mucus weights, and higher IL-6 lavage concentrations in response to infection (57), whereas low childhood socioeconomic status, possibly associated with chronic stress, was pointed out as a risk factor for developing a common cold when exposed to a rhinovirus (58).

Viral infection of the respiratory tract not only is the most common precipitant of acute asthma exacerbations but may also induce nonspecific AHR in allergic or even nonallergic children (59–62). Consequently, the attenuated host defense responses to viral infection under stress conditions may facilitate airway reactivity, therefore enhancing childhood asthma exacerbations (Fig. 2).

The hypothalamic–pituitary–adrenal axis in asthmatic children

In addition to evidence from animal experiments suggesting that early psychologic and physical stress may aggravate asthma later in life by inducing hyporesponsiveness of the HPA axis (21, 63), human studies have shown that various stressors during the early part of a child's life may affect his or her HPA axis, and thus cause dysregulation of immune system function with implications for the development of asthma (64).

Blunted cortisol response to stress in children with allergies

A low HPA axis activity in allergic patients has been reported in a large number of clinical studies. Initially, research was focused on the HPA axis of asthmatics who were on long-term treatment with ICS; however, a growing number of studies subsequently recognized that allergic/asthmatic patients not treated with ICS were also likely to have an attenuated activity and/or responsiveness of their HPA axis.

Buske-Kirschbaum et al. (64, 65) demonstrated reduced cortisol levels in response to psychosocial stress in children and adults with atopic dermatitis, pointing to a dysfunction of the HPA axis in patients with this disorder. The same group also found that children with allergic asthma showed significantly attenuated cortisol responses to psychosocial stress when compared with matched healthy controls (66). On the same line of thought, Wamboldt et al. (67), in a large community sample of adolescents, of whom approximately one-third had an atopic disorder, found a lower cortisol response to the stressor of laboratory procedures. Furthermore, a reverse association between adrenal and bronchial responsiveness in asthmatic children was reported, showing that children with more severe disease may have relative adrenal insufficiency compared with those with milder disease (68).

There are two recent interesting reports on HPA axis function in allergic subjects. In the first, the circadian rhythm of salivary cortisol was evaluated in infants with an increased risk for allergic disease (69). Thus, infants of mothers with allergy or asthma or an asthmatic father exhibited flattening of the circadian cortisol rhythm because of diminution of the expected morning surge of cortisol. In the second study, the basal and synacthen-stimulated morning plasma cortisol concentrations of wheezing infants aged 5–9 and 9–12 months were evaluated by high performance liquid chromatography (70). In general, mean basal plasma cortisol concentrations were similar in the two age groups, and increased to comparable levels 60 min after synacthen administration. However, both groups had a wider range of basal and stimulated values than older children, with approximately 15% of these infants below the normal range of basal or stimulated plasma cortisol concentrations.

A low adrenocortical response during a pretreatment evaluation of poorly controlled asthmatics has been previously reported, but was not commented upon as an important finding. Indeed, Volovitz et al. (71) found that 60 min after ACTH administration during the standard synacthen stimulation test, serum cortisol concentrations in four out of 15 asthmatic children were lower than 496.6 nmol/l, while cortisol suppression was no longer present when these children were clinically controlled by budesonide therapy. In another study by Kannisto et al. (72), cortisol values were obtained during the low-dose synacthen test (LDST) before the patients were started on ICS treatment. The authors recognized that asthmatic patients had peak poststimulation serum cortisol levels that were lower (330 nmol/l) than normally expected (i.e. 500 nmol/l). Similarly, Ozbek et al. (73), using the same diagnostic protocol, found that their patients also had lower peak cortisol values (389 and 438 nmol/l) than the lower limit of normal cortisol response. Finally, Bacharier et al. (74) reported that four out of 45 children receiving long-term treatment with nedocromil or placebo, but not ICS, demonstrated abnormally low serum cortisol levels during the ACTH stimulation test.

In line with these observations, we recently reported the results of a prospective 12-month study of a cohort of 41 preadolescent asthmatic children that were placed on long-term treatment with inhaled budesonide and followed up by serial LDSTs. Approximately 10% of our cohort had a low adrenal reserve before starting any ICS treatment. These patients, as well as more than half of the remaining cohort, showed improved adrenal responses while receiving long-term ICS (75) (Fig. 3). These findings support the concept that chronic allergic disease, regardless of the organ affected, may be associated with reduced activity and/or responsiveness of the HPA axis. Production of certain allergic inflammation-related cytokines may blunt the response of the HPA axis to both inflammation and acute stress, contributing to the

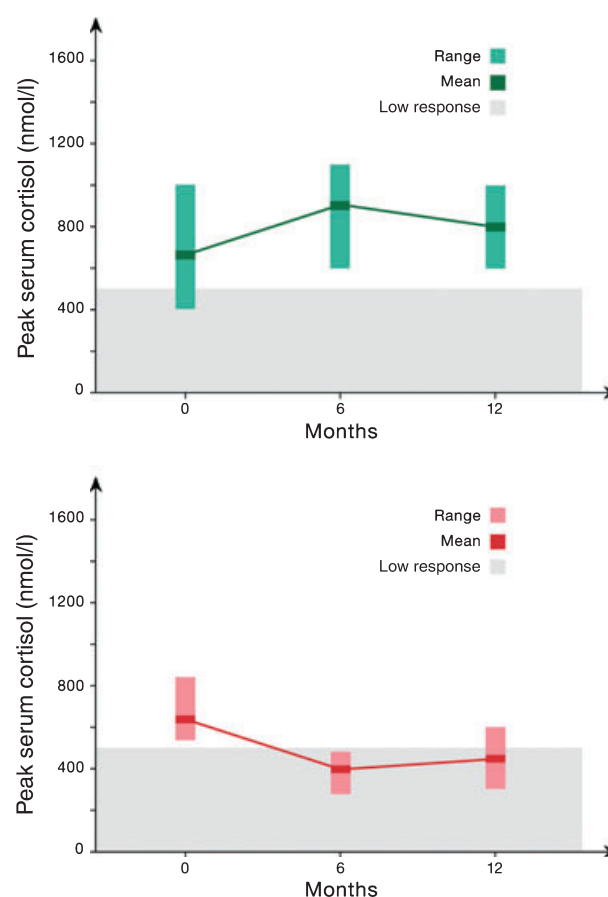


Figure 3. Adrenal response to a low-dose synacthen test in asthmatic children before and on maintenance of ICS treatment. Most asthmatics have an improvement of their hypothalamic–pituitary–adrenal axis responsiveness while on conventional doses of ICS (green), whereas, adrenal suppression is evident in a subgroup of patients (red). A low adrenal response in poorly controlled asthmatics may be observed during pretreatment evaluation (green), as cytokines involved in the pathophysiology of asthma appear to be inversely related to cortisol production. ICS, inhaled corticosteroids (6).

aggravation of allergic inflammation because of insufficient anti-inflammatory restraint. The heterogeneity in glucocorticoid responsiveness may reflect the variety of mechanisms involved in HPA axis regulation, and the involvement of multiple cytokines with stimulatory or inhibitory actions in the regulation of the HPA axis (10, 76, 77). The mediators involved in stress-induced airway inflammation that have been shown in various studies are summarized in Table 1.

Adrenal function improves in patients with asthma while on inhaled corticosteroids

There is a large amount of data regarding the safety profile of ICS on the HPA axis (78, 79). However, the clinical importance of studies of HPA axis function lies in

Table 1. Immune mediators potentially involved in stress-induced airway inflammation in animal models or clinical studies

Study (reference no.)	Mediators	Animal model, clinical study	Comments
Silverman et al. (15)	IL-4, IL-5, IL-13, RANTES, IFN- γ , eotaxin (in BAL)	CRH-knockout mice	Increased inflammation in CRH-knockout mice was associated with increased levels of IL-4, IL-5, IL-13, RANTES, IFN- γ , and eotaxin in BAL and impaired adrenal responses to stress
Joachim et al. (14)	Tachykinins-like substance P	Mice sensitized to ovalbumin	Sound stress led to the stimulation of SP expression in airway-specific neurons
Forsythe et al. (17)	IL-6, IL-9, IL-13 (in BAL)	Adult male mice were exposed to a stressor daily for 3 (short-term stress) or 7 (long-term stress) days	After short-term stress, BAL inflammatory cell number was decreased, after long-term stress increased; cytokines were increased in both exposures. Neither short-term stress nor long-term stress resulted in changes in airway hyperresponsiveness
Okuyama et al. (20)	IL-4, IL-5	Female wild and μ -opioid receptor-deficient mice sensitized and subsequently exposed to ovalbumin during either acute or chronic restraint stress	Acute stress modified the allergic airway responses distinctively depending on the genetic background; chronic stress significantly increased the cell numbers and BAL content of IL-4 and IL-5
Chen et al. (28)	IL-5, IL-13, IFN- γ	Adolescents with persistent asthma from either low or high socioeconomic status groups	Low socioeconomic status was associated with elevations in certain immune responses (IL-5/IFN- γ)
Chen et al. (29)	IL-5, IL-13, IFN- γ	Asthmatic children 9–18 years were interviewed about chronic life stress, perceptions of threat, socioeconomic status	Higher levels of emotional stress and threat perception were associated with heightened production of IL-5 and IL-13, and higher eosinophil counts in children with asthma
Kang et al. (30)	IL-2, IL-4, IL-5, IFN- γ	Asthmatic adolescents were tested three times over a semester: mid-semester, during the week of final examinations, 2–3 weeks after examinations	Different IL-4 and IL-5 production responses in asthmatics that may become pronounced during stressful periods of the academic semester
Liu et al. (31)	Eosinophil-derived neurotoxin, IL-5, IFN- γ (in sputum supernates)	Asthmatic students evaluated during both a low-stress phase (mid-semester or 2-week postfinal examination) and a stress phase (final examination week)	Stress associated with final examinations can act as a cofactor to increase eosinophilic airway inflammation in response to antigen challenge
Wright et al. (34)	IFN- γ , TNF- α , IL-10, IL-13	Infants whose families have a history of asthma or allergy	High-level early-life chronic caregiver stress might influence the immune response of the infants, associated with increased TNF- α and reduced IFN- γ levels
Pincus-Knackstedt et al. (41)	TNF- α , IFN- γ , IL-5, IL-4, IL-2 (in BAL)	Pregnant female mice exposed to stress during gestation; female offspring were sensitized and subsequently exposed to ovalbumin	Increased vulnerability towards airway hyperresponsiveness and inflammation in prenatally stressed adult offspring
Kruschinski et al. (50)	IL-13	Rats were subjected to either repeated handling stimulation or maternal separation in early life; later on, experimental asthma was induced	After induction of asthma, IL-13 levels increased in handling stimulated animals compared with controls

their ability to identify which children placed on ICS therapy will not be able to respond properly to stress.

Basal state HPA axis tests are generally inferior in diagnosing HPA suppression, while dynamic testing has the advantage of providing an assessment of stress reserve (80, 81). Although the insulin tolerance (ITT) and metyrapone tests have been considered by some physicians as the gold standard of adrenal function tests, both include risks and are practically of limited use; ITT has been linked to deaths in children, while metyrapone is usually unavailable (82). As first line alternatives, the CRH stimulation test and the standard Symachtem test (SST) have been proposed and are usually employed (83). The conventional high dose of intravenous injection of 250 μ g is primarily useful in determining severe and clinically important adrenocortical insufficiency. This test produces supraphysiologic ACTH levels in the circulation, and might occasionally provide false-negative

results, even in patients with a clinically impaired adrenal reserve. A variation of the SST is the LDST (0.5–1.0 μ g) that is considered more accurate in assessing physiological cortisol secretion, and more sensitive in detecting evolving or mild adrenal suppression (81, 82). The salivary low-dose ACTH test yields results that parallel the response of circulating cortisol, and may provide an alternative to blood testing (84, 85).

The recent Global Strategy for Asthma Management and Prevention (GINA) report correctly concludes that although differences exist between the various ICSs and the inhalation devices employed, treatment with the recommended doses of an ICS is usually not associated with any clinically significant suppression of the HPA axis in children. With higher doses, small changes in adrenal function can be detected with sensitive methods (2).

We reported that 20% of asthmatic children on long-term treatment with low-to-moderate doses of inhaled

budesonide had mild biochemical adrenal suppression (LDST) that was not related to the ICS dosage or duration of treatment (86). Adrenal suppression in asthmatic children on even moderate doses of ICS has also been detected by LDST by other researchers (87). Also, a flat adrenal response in the LDST was reported in 2.8% of asthmatic children receiving maintenance fluticasone propionate, whereas impaired responses were observed in 39.6% of children receiving more than 1000 µg of fluticasone per day (88).

In a 12-month observational study of 35 prepubertal asthmatic children requiring at least 1000 µg/day of budesonide or equal potency of fluticasone propionate, 46% of the subjects had evidence of biochemical adrenal suppression demonstrated with the LDST (87).

In a survey of adrenal crises associated with ICS in the United Kingdom, 33 patients (28 children, five adults) met the diagnostic, clinical, and biochemical (abnormal SST or glucagon stimulation test) criteria for an adrenal crisis (89). Most of these patients were on fluticasone metered dose inhaler with a spacer and on very high doses ranging between 500 and 2000 µg/day. Twenty-three children had acute hypoglycaemia, one associated with coma, convulsions, and death. In 65% of the patients, there was no obvious precipitating cause; in the rest, there was evidence of a stressful event or a reduction/discontinuation of ICS. A systematic review of the literature was recently performed by Pedersen focusing on randomized, controlled studies of 12 months or more duration, to identify studies examining each of the following three areas: growth, bone mineral density, and cortisol levels (90). Ten studies met the inclusion criteria for cortisol levels. It was determined that recommended doses of ICS generally had little or no effect on plasma- or urinary-cortisol levels *vs* nonsteroidal therapies.

From the aforementioned studies, it is obvious that a dose-dependent adrenal suppression in asthmatic children on ICS does exist, and may be detected even when small-to-moderate doses of ICS are employed. We do not know whether these children would develop symptomatic adrenal insufficiency if they were treated with larger doses, but it is certainly possible. Sometimes, the results of various studies appear contradictory. This could be because they may have been derived by various testing methods with different abilities to detect HPA axis impairment, for instance, morning serum cortisol or urinary free cortisol concentrations are generally poor discriminators of adrenal hypo-activity.

The role of genetics in impaired stress response in asthma

Recent research has disclosed interesting data regarding the role of genetics in modifying the risk of impaired stress response in asthma. A number of pathways through which stress may impact asthma expression could potentially be associated with genetic factors. The most

important of these pathways include the ones that influence immune development and airway inflammation, including HPA axis, adrenergic system, and cytokine pathway genes (91).

Early-life experiences interact with a child's genotype to influence the developing immune and stress systems in a fashion that would predispose to or protect from asthma and other allergic diseases (92–94). The Childhood Origins of Asthma project (95) evaluated children from birth to 3 years, who were at high risk for asthma, to study the relationships among environmental, genetic factors, and the development of atopic diseases. For the entire cohort, cytokine responses did not develop according to a strict Th1 or Th2 polarization pattern during infancy. First year wheezing illnesses caused by respiratory viral infection were the strongest predictor of subsequent 3rd year wheezing. Also, genotypic variation interacted with environmental factors, including day care and was associated with clinical and immunologic phenotypes that may precede the development of asthma.

Researchers have reported on various associations between asthma and genes related to HPA axis activity. Indeed, single nucleotide polymorphisms of such genes determined an individual's ability to respond favorably to ICS therapy regarding severity of asthma symptoms or a faster improvement of lung function. Thus, Tantisira et al. (16) suggested a relationship between an individual's lung function improvement in response to 8 weeks of ICS therapy and a polymorphism of the CRHR1. Slawik et al. (96) described an ACTH receptor promoter polymorphism (CCC/CCC) that results in a lower promoter activity *in vitro* and is associated with a lower cortisol secretion to prolonged ACTH stimulation *in vivo*. This polymorphism might influence cortisol homeostasis under stress conditions. Stevens et al. (97) performed haplotype analysis of the glucocorticoid receptor gene and found a three-marker haplotype across intron B that includes a single nucleotide polymorphism altering a *BclII* site, associated with enhanced sensitivity to glucocorticoids, which also might be predisposing to a better response to ICSs.

In another interesting study, a significant difference in salivary cortisol responses to psychosocial stress between three polymorphisms of the glucocorticoid receptor gene (*BclII* RFLP, N363S, ER22/23EK) was detected (98). Compared with subjects with two wild-type alleles, N363S carriers showed a markedly greater cortisol response during a standardized stress test (Trier test), whereas the mean cortisol response in *BclII* homozygotes and heterozygotes was attenuated.

Variations in the TLR-2 were shown to confer susceptibility to severe infection and severe atopic dermatitis, but were also associated with reduced susceptibility to asthma and allergies in children (99–101). Furthermore, in TLR-2^{-/-} mice, the release of inflammatory cytokines – including TNF-α, IL-1, and IL-6 – from immune cells or fibroblasts after exposure to inflammatory stimuli was

impaired (102). Absence of TLR-2 in mice was also associated with marked cellular alterations in adrenocortical tissue, reduced plasma corticosterone levels, and elevated plasma ACTH levels suggesting a possible impairment of the HPA axis at the level of the adrenal gland, even under basal conditions (103, 104).

Oxidative stress is a host defence mechanism and glutathione-S-transferase (GST) is one of the fundamental antioxidant systems in oxidant-induced lung injury and inflammation (105). The maternal GST P1 Val105/Val105 genotype was associated with offspring lung function (106). Children with asthma who are homozygous for the GSTP1 Val105 allele have substantially larger deficits in forced vital capacity, forced expiratory volume in 1 s, and maximal mid-expiratory flow than children without asthma (107); an association between the number of GST M1 mutant alleles in the genotype; and risk for atopy has been observed (108).

Polymorphisms in the proinflammatory cytokine TNF genes have been associated with asthma and atopy (109). Genetic variation in TNF- α and TNF- β was associated with respiratory effects of ozone in humans (110) and might influence the lung inflammatory response to tobacco smoke (111). Recently, in a Mexican population with high ozone exposure, an association of TNF polymorphisms (TNF-308) with asthma, predominantly in children without smoking parents was reported; it is suspected that the combination of exposure to second-hand smoke and ozone, both of which increase TNF production, overwhelms the smaller impact of TNF polymorphisms on TNF expression or action (112).

Therefore, there is good evidence that genes are involved in altering the risk of asthma and allergies in children; various pathways through which stress may affect asthma expression are possibly genetically determined. Moreover, recent data suggest that a mechanism linking the social environment early in life, and long-term epigenetic programming of behavioural and physical responsiveness to

stress and health status later in life does exist (113). Experimental studies provide substantial *in vitro* data indicating that DNA methylation of genes critical to T helper cell differentiation may induce polarization towards or away from an allergic phenotype. Thus, asthma risk may be modified by epigenetic regulation (114).

Concluding comments

The stress system coordinates adaptive responses of the organism to stressors of any kind; inappropriate responsiveness may account for a variety of disorders. Asthma and allergy are characterized by a dysregulation of the pro-inflammatory vs anti-inflammatory and Th1 vs Th2 cytokine balance. The development of these conditions primarily depends on the genetic and epigenetic vulnerability of the individual and the duration and timing of the stressful events.

A number of factors including psychosocial stress, viral infection, environmental pollutants, and allergy may influence the stress response resulting in immune response dysregulation causing asthma. There is also good evidence that genes involved in the stress and inflammatory response may affect asthma expression.

Pro- and anti-inflammatory cytokines involved in the pathophysiology of allergic disease, regardless of the target organ affected, appear to be inversely associated with cortisol production. In line with this concept, the anti-inflammatory properties of ICS may have favorable effects on the HPA axis of asthmatics with a subnormal adrenal response at baseline improving during successful long-term treatment. On the other hand, some patients may experience further deterioration of adrenal function, a phenomenon which may be genetically determined. As a rule, when ICS are administered at higher than conventional doses, they may be associated with secondary adrenal insufficiency.

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