PSYCHOLOGICAL STRESS AND IMMUNE FUNCTION AMONG MILD ASTHMATICS

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PSYCHOLOGICAL STRESS AND IMMUNE FUNCTION AMONG MILD

ASTHMATICS

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Asthma is a chronic inflammatory condition characterized by acute bronchoconstriction and a protracted inflammatory response marked by elevated levels of T-helper (Th)-2 cytokine production. Common precipitants of asthma exacerbation include allergens (e.g. pollen), viruses (e.g. URI); however, recent evidence has demonstrated that psychological stress can also be a trigger. While the pathophysiological mechanism linking stress and asthma exacerbation is unknown, modulation of the immune system has been proposed as one potential pathway. The primary aim of this study was to examine impact of examination stress (i.e. Pennsylvania bar exam) on Th2 and Th1 cytokine production and other asthma-relevant immune parameters among asthmatics and healthy controls. To this end, five mild asthmatics and 10 healthy controls completed a battery of psychosocial measures and underwent venipuncture for immunological assessment one week prior and one month following the Pennsylvania bar exam. Overall, participants reported greater levels of distress during the exam period when compared to the post-exam period. With respect to immunological changes, a group (asthmatic vs non-asthmatic) by period (exam vs. post-exam) interaction was observed on Th1 cytokine production, with non-asthmatic participants showing a stress-related decrease in Th1 cytokine production, i.e. IFN-gamma production. In addition, basal differences in IFN-gamma production were observed with asthmatics producing lower levels of IFN-gamma relative to non-asthmatics, potentially rendering asthmatics more susceptible to viral infections. Exploratory analyses of

health behaviors revealed an intriguing relationship between alcohol consumption and IFN-gamma production that warrants further investigation. Future studies employing larger sample sizes are needed to better interpret these findings.

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PREFACE

1.0   INTRODUCTION

Asthma is a common chronic disease marked by intermittent episodes of airflow obstruction and hypersensitivity to normally benign proteins. During an episode, asthmatics experience acute bronchoconstriction, mucus hypersecretion, and a protracted inflammatory response (Busse & Lemanske, 2001). Currently, approximately 16 million people within the United States are affected by this disease, which has a lifetime prevalence of approximately 25 million individuals, 12% of the U.S. population (CDC, 2002). Despite a concerted effort by national health institutions to design more effective asthma treatments, the prevalence of asthma has consistently risen over the past two decades (CDC, 2002).

Primarily considered a disorder of immune function, asthmatics display stable immunological differences when compared with non-asthmatics, including elevated T helper (Th) 2 cytokine production and increased numbers of eosinophils in both the bronchial mucosa and in peripheral circulation (Busse & Lemanske, 2001). Within asthmatics, higher Th2 cytokine production has been positively associated with asthma severity and may confer increased risk for asthma exacerbation (Humbert et al., 1997; Sandford et al., 2000). In addition, Th2 cytokines have the capacity to down regulate Th1 cytokine activity, which may render asthmatics more vulnerable to upper respiratory infection (URI), a known trigger of asthma episodes (Jung et al., 1995; Marshall & Agarwal, 2000).

Known precipitants of asthma exacerbation include allergens, cold air, exercise and, in some cases, psychological stress. In regard to the latter, a large body of evidence links psychological stress and emotional arousal to increased subjective report of asthma symptoms as well as objective declines in pulmonary functioning (Lehrer, Feldman, Giardino, Song & Schmaling, 2002; Schmaling, Lehrer, Feldman & Giardino, 2003). Furthermore, recent prospective evidence reveals that stressful life events precipitate asthma exacerbations (Sandberg, Jarvenpaa, Penttinen, Paton & McCann, 2004).

One possible mediator of stress-related exacerbation of asthma is modulation of immune function, influencing host responses to allergens and susceptibility to viral infections that often precipitate asthma episodes. In this regard, it is well established that psychological stress is associated with modulation of immune function (Herbert & Cohen, 1993; Marsland, Bachen, Cohen & Manuck, 2001), including an elevation in Th2 cytokine production (Segerstrom & Miller, 2004) that may increase risk of asthma exacerbation. To date, however, this literature has focused on the stress-related immune responses of healthy individuals, with only a handful of studies examining these relationships among asthmatics. Thus, the primary purpose of the current investigation was to explore this possible pathway by examining stress-related changes in Th2 and Th1 cytokine production, circulating numbers of eosinophils, and serum IgE levels among asthmatics and non-asthmatics. For this purpose, a quasi-experimental study examining immune responses to examination stress was conducted.

Before turning to a presentation of the study methods, a brief overview of the pathophysiology of asthma is offered to orient the reader. This is followed by an overview of existing evidence for associations between stress, immune function, and the exacerbation of asthma.

2.0   WHAT IS ASTHMA?

Asthma, as defined by National Heart, Lung and Blood Institute (NHLBI, 1997), is a chronic inflammatory disorder of the airways characterized by episodes of widespread and variable airflow obstruction that are reversible spontaneously or with treatment. In susceptible individuals, inflammation of the airways causes recurrent episodes of wheezing, breathlessness and chest tightness. This can be accompanied by autonomic bronchial hyperresponsiveness to various external stimuli, further contributing to reduced airflow. Asthma symptoms tend to worsen during the night and in the early morning. Treatment involves a prophylactic medication regimen that can include short and long acting beta-agonists, inhaled corticosteroids and leukotriene antagonists.

Typically, the onset of asthma is in childhood, with a variable course resolving for many prior to adolescence, but continuing for others throughout adulthood (Sears et al., 2003). The majority of individuals predisposed to asthma have an atopic phenotype, which is the tendency to respond to normally benign proteins with inflammatory processes characterized by elevated Th2 cell activity and the production of immunoglobin E (IgE) (Novak & Bieber, 2003). IgE is the antibody responsible for classic allergic reaction. While the specific factors that promote the development of an atopic phenotype are unclear, genetic and environmental factors (e.g. early exposure to allergens and viruses) are thought to play a role (Busse & Lemanske, 2001).

2.1   THE IMMUNOLOGY OF ASTHMA

The elevated Th2 activity that is characteristic of asthma results in greater production of the Th-2 cytokines, interleukin (IL)-4 and 5 (Bettiol et al., 2000; Rodriguez et al., 1998; Shiota et al., 2002). These cytokines initiate allergic inflammation, contribute to asthma exacerbations (Busse & Lemanske, 2001), and are related to greater asthma severity (Humbert et al., 1997). Th1 and Th2 cytokines are mutually inhibitory (Jung et al., 1995; Maggi et al., 1992), which explains findings that asthmatics also produce lower levels of Th1 cytokines following stimulation by a nonspecific mitogen than non-asthmatics (Rodriguez et al., 1998). Th1 cells secrete cytokines, including interferon (IFN)-gamma and IL-2, that are involved in cellular immune responses to invading intracellular pathogens (e.g. viruses)(Liu, 2000). Thus, it is proposed that patterns of Th1:Th2 activation that accompany asthma promote a down-regulation of cellular immune function and an up-regulation of inflammatory pathways (Marshall & Agarwal, 2000), rendering the individual more susceptible to asthma exacerbation via a decrease in host resistance to viral infection and/or an increase in allergic response.

Asthma is an inflammatory disorder of complex and integrated immune processes. In addition to T-helper cells and their cytokines, immune components known to play a major role include B cells, eosinophils, mast cells, and chemical mediators, such as chemokines, which are known to be instrumental in promoting bronchial autonomic hyperresponsiveness, mucus hypersecretion and the influx of immune cells into the bronchial mucosa. Accumulation of immune cells, particularly eosinophils, in the bronchial tract, and related inflammation contribute to the impediment of airflow that is a cardinal symptom of an asthma episode (Krishna, Salvi & Holgate, 2001). Even between acute asthma episodes, asthmatics have higher numbers of eosinophils in peripheral circulation than non asthmatics (Lewis et al., 2001; Ulrik, 1998).

Studies examining the immunopathology of allergen-induced asthma exacerbation demonstrate a clear 2 phase process (Krishna et al., 2001), with an early phase that starts within 15 minutes of exposure to the allergen, followed by a late phase occurring 2-6 hours later. During the early phase, allergen proteins are taken up by dendritic cells and macrophages that line the airway. These antigen-presenting cells (APCs) travel to lymph nodes where they present the processed antigen to T and B cells, stimulating the activation of Th2 cells and the release of IL-4 and IL-5. These cytokines activate the humoral immune system, resulting in isotype switching of B cells to produce IgE. Once released from B cells, IgE cross-links with mast cells, resulting in the release of preformed mediators such as histamine and leukotrienes that cause constriction of the bronchial smooth muscle, and the onset of asthma symptoms (Krishna, Salvi & Holgate, 2001).

The late phase of an asthma exacerbation is marked by excessive inflammation as well as narrowing of the bronchial tract secondary to smooth muscle constriction. During this phase, elevated levels of IL-5 secreted by Th2 cells into the circulatory system promote the differentiation and migration of eosinophils from the bone marrow to the airway (Shi et al., 1998) contributing to airflow obstruction. Peripheral blood levels of both eosinophils and IL-5 have been shown to correlate highly with levels in the bronchial mucosa (Liu et al., 2002). Furthermore, in a recent study, mild asthmatics treated with monoclonal antibodies to IL-5 displayed reduced levels of blood and sputum eosinophils following an inhaled allergen challenge (Leckie et al., 2000).

Triggers of asthma episodes vary widely across individuals and include allergens (e.g. pollen, cockroaches, pet dander), smoking, cold air, exercise and URI. In addition, evidence

suggests that psychological stress and/or emotional arousal may play a role (Lehrer, Isenberg & Hochron, 1993; Schmaling et al., 2003). This is the primary focus of the current study.

2.2   INFLUENCE OF STRESS ON ASTHMA EXACERBATION

There is no one single, widely accepted definition of stress. For the purposes of this study stress is conceptualized as a psychological process that elicits an adaptive physiological response. Often associated with a negative emotional state, an individual experiences stress when he or she determines that the environmental demands exceed their ability to access coping resources (Lazarus & Folkman, 1984). The ability to access adaptive coping resources in the presence of a stressor is thought to be a product of past experience, personality and presence or absence of social resources (e.g. social support) (Cohen, Doyle, Skoner, Rabin & Gwaltney, 1997; Cohen & Herbert, 1996).

A controversial issue in asthma research concerns the extent to which psychological stress contributes to asthma exacerbations. Many asthmatics report that stress and emotional arousal play a significant role in precipitating their asthma attacks (Rees, 1980). Moreover, documented case studies have shown increased susceptibility to asthma episodes following stressful life events (Levitan, 1985). However, it is only recently that prospective studies have provided empirical support for this popular notion.

Early studies were largely cross-sectional and while not all findings are consistent, they generally support a positive relationship between psychological stress and the exacerbation of asthma. Indeed, in a review of this literature, Lehrer and colleagues (1993) concluded that negative emotion, thought to be elicited by stressful experience, was correlated consistently with

a higher frequency of subjective asthma symptoms (e.g. feelings of breathlessness) and often with a decline in pulmonary function. More recent cross-sectional studies provide further evidence for an association between stress and asthma. Numerous stressors including family conflict, financial burden and the presence of depressive symptoms have been associated with more frequent asthma exacerbations (Sturdy et al., 2002), increased hospitalization (Chen, Bloomberg, Fisher & Strunk, 2003), and increased asthma mortality (Strunk, Mrazek, Fuhrmann & LeBrecque, 1985). Naturalistic field studies have found proximal relationships between daily stress and negative emotional states and poorer lung functioning among asthmatics (Apter et al., 1997; Ritz & Steptoe, 2000; Steptoe & Holmes, 1985). In these studies, daily diaries are used to determine levels of perceived stress, current mood and the occurrence of stressful life events. Each diary entry generally corresponds with an objective measure of lung function (e.g. peak expiratory flow rate (PEFR) or forced expiratory volume in one second (FEV1). For example, Ritz and Steptoe (2000) followed 20 asthmatics and 20 non-asthmatics over 21 days and found that an average decrease in lung function of 7.9% accompanied negative emotions relative to neutral experiences among only the asthmatics; in fact, five asthmatics showed decreases in FEV1 of greater than 15%.

In sum, a large literature supports a positive relationship between stress and asthma; however, the cross-sectional nature of the studies makes it impossible to determine the direction of these effects. Indeed, asthma is characterized by unpredictable and uncontrollable exacerbations, making it likely that more severe disease results in heightened stress and negative emotional reactions and influences retrospective recall of precipitating events. To better examine causal relationships, experimental studies have been conducted. In these studies, asthmatics are presented with an acute emotional stimulus and their pulmonary functioning is

measured in response to a bronchoconstricting chemical or saline challenge. In a review of this literature, Isenberg, Lehrer, and Hochron (1992) reported that approximately 20% of asthmatics responded with a significant decline in pulmonary functioning in response to emotional arousal. However, small samples sizes, difficulties with replication, and a lack of standard demographic and background controls (e.g. measures of asthma severity, medication usage) make it difficult to draw firm conclusions about the role of stress and emotional arousal in precipitating asthma exacerbation (Isenberg et al., 1992).

More recently a prospective study has provided further support for the role of stress as a trigger of asthma exacerbation. In this study, Sandberg and colleagues (2004) examined whether negative stressful life events precipitated asthma exacerbations among asthmatic children. For this purpose, 60 children with chronic asthma were followed for 18 months and completed daily diaries monitoring asthma symptoms and negative life events. Acute exacerbations were defined as a documented fall in PEFR below 70% of the child's normal value combined with an increase in reported symptoms. Children who reported the presence of a severely negative life event (e.g. moving, traumatic events, family problems) were significantly more likely (OR= 4.69) to have an asthma attack within the next 2 days. This finding provides persuasive initial evidence that stress may play a clinically relevant role in promoting asthma exacerbations.

In conclusion, it is well established that psychological stress is associated with increased subjective report of asthma symptoms as well as objective declines in pulmonary functioning, with recent prospective evidence showing that stressful life events precipitate asthma exacerbations in children (Sandberg et al., 2004). These empirical findings support anecdotal evidence from asthma sufferers who report that stress increases their susceptibility to exacerbation. Although evidence is mounting that stress can exacerbate asthma, at least for a

subgroup of asthmatics, the mechanism of this effect remains to be determined (Wright, Rodriguez and Cohen, 1998).   The purpose of this investigation was to begin to examine a potential pathway of this relationship by examining whether life event stress was associated with inflammatory mechanisms known to play a role in the pathophysiology of asthma.

3.0   PATHWAYS LINKING STRESS AND IMMUNE FUNCTION

Stress sets in motion a cascade of biological processes including activation of the hypothalamic-pituitary adrenal (HPA) axis and the autonomic nervous system (ANS).   In addition, stress is associated with changes in health behaviors.   In the following sections, the possibility that stress-related changes in these biological and behavioral processes act as potential pathways by which stress influences immune parameters relevant to asthma is considered.
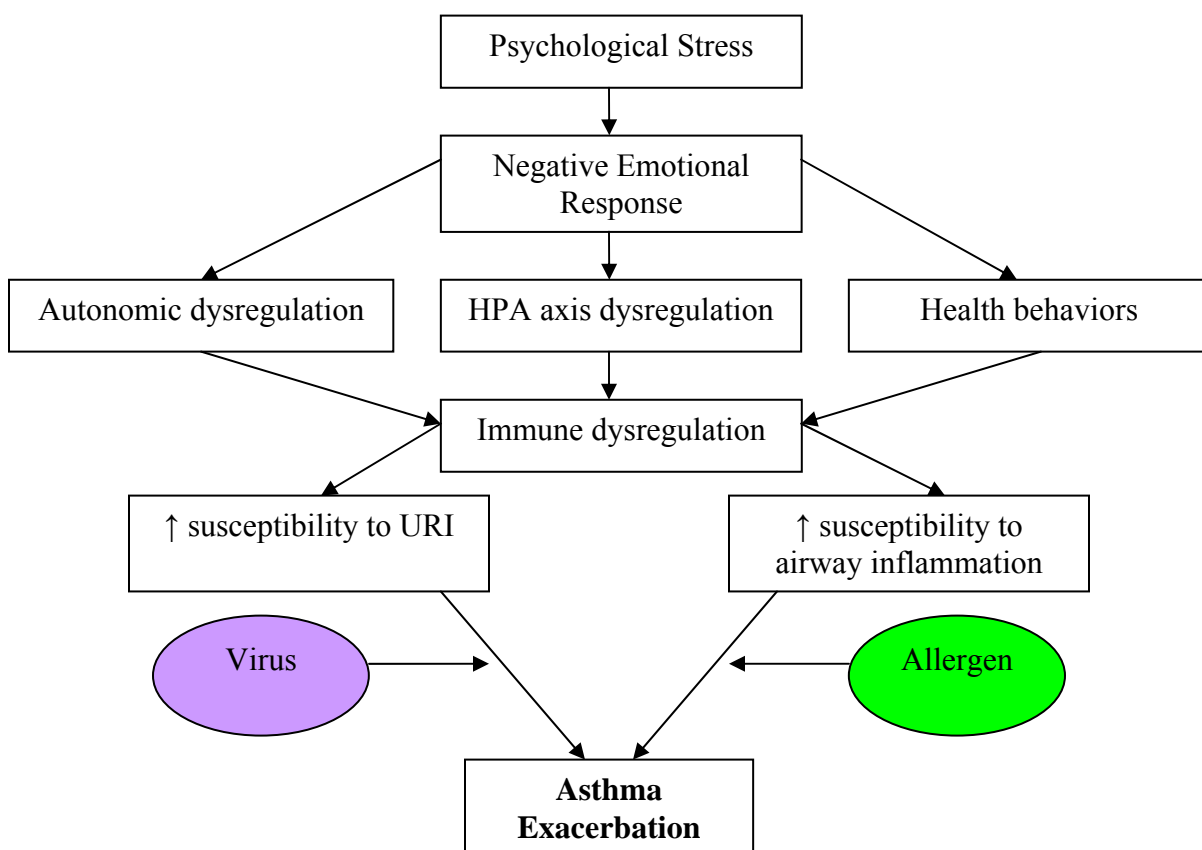
Figure 1: Proposed model linking stress and asthma exacerbation

3.1 NEUROENDOCRINE AND AUTONOMIC PATHWAYS

Stress-induced activation of the HPA axis and the ANS leads to the systemic release of a number of neurochemicals, including corticosteroids and catecholamines, which can bind to receptors on immune cells that migrate between lymphoid organs and the peripheral blood stream. It is known that these neurochemicals have the capacity to modulate immune function, including immune parameters relevant to asthma pathophysiology (Rabin, 1999). With respect to the HPA axis, it is widely accepted that elevated levels of the corticosteroids accompany psychological stress (Dickerson & Kemeny, 2004; Wolkowitz, Epel & Reus, 2001). In general, cortisol has anti-inflammatory properties. Indeed, corticosteroids are widely prescribed in the treatment of asthma-related inflammation. Paradoxically, however, a number of pathways have been proposed that also link cortisol to the promotion of asthma-related inflammatory processes (Wright, Cohen & Cohen, 2005)

Cortisol plays an important role in the modulation of the production and release of cytokines. In general, cortisol acts to "shut off" the release of proinflammatory cytokines. However, in vitro studies show that cortisol can also stimulate the production of the Th2 cytokines, IL-4 and IL-5 (Agarwal & Marshall, 1998; Chrousos, 1995), possibly increasing allergic response. Moreover, cortisol can downregulate Th1 cytokine production (e.g. IFN-gamma) potentially increasing URI susceptibility (Agarwal & Marshall, 2001). Other studies suggest that prolonged elevation of cortisol levels, possibly as a result of chronic stress or corticosteroid treatment, can result in adaptation of the HPA axis and the development of

glucocorticoid resistance (Miller, Cohen, and Ritchey, 2002). In this case, the glucocorticoid receptors become less sensitive to cortisol, allowing the production of pro-inflammatory cytokines to go unchecked. Indeed, approximately 5% of asthmatics develop glucocorticoid resistance in response to corticosteroid treatment (Leung, 1995).

Stress is also associated with activation of the sympathetic branch of the ANS and the associated release of catecholamines into peripheral circulation. Catecholamines, such as epinephrine and norepinephrine, released during times of stress have the capacity to modulate immune function. For instance, in vitro and in vivo studies have demonstrated that epinephrine and norepinephrine can act on peripheral blood cells to produce elevated levels of Th2 cytokines (Agarwal & Marshall, 2000) as well as increase the circulating numbers of T cells (Bachen et al., 1995).

Another pathway by which stress-induced ANS activation may influence asthma exacerbation is through direct innervation of the smooth muscle of the airways. Although direct sympathetic innervation of the bronchus is sparse, the parasympathetic arm of the ANS innervates airway smooth muscle via efferent fibers of the vagus nerve and directly influences contraction of the airways through cholinergic activation. It has been suggested that vagal nerve activity may contribute to bronchoconstriction, bronchial reactivity and mucus hypersecretion (Jartti, 2001). To date, studies examining whether stress results in exacerbation of asthma via autonomic regulation of airways are inconclusive (Isenberg et al., 1992; Lehrer et al., 1996; Lehrer et al., 1993). However, eosinophils have been shown to interact with cholinergic nerves to promote increased release of acetylcholine in the airway (Sawatzky, Kingham, Durcan, McLean & Costello, 2003), providing a potential immunologic pathway to bronchoconstriction.

Taken together, findings suggest that stress-induced activation of the HPA axis and ANS may promote asthma exacerbation via modulation of immune function salient to asthma pathophysiology. While a large literature supports the role of neurochemicals, such as catecholamines and cortisol, in modulating immune function, limited research has examined these changes in the context of asthma.

3.2   BEHAVIORAL PATHWAYS

In addition to direct activation of biological pathways stress may influence immunity via the indirect influence of health behaviors. For example, poor nutritional status, smoking, lack of physical activity, alcohol use, poor sleep quality and poor social connectedness are more likely at times of high stress and have been related to modulation of immune function (e.g., Kiecolt-Glaser & Glaser, 1988). In the current study, the potential roles of alcohol consumption, physical activity and sleep are considered. Alcohol consumption is often used as a method of coping with elevated levels of stress (Johnson & Pandina, 2000). Although alcohol use is not commonly considered a trigger of asthma exacerbation, recent evidence suggests that moderate alcohol consumption may influence inflammatory processes of relevance to asthma. For instance, moderate alcohol consumption has been associated with a down-regulation of host resistance to viral and bacterial infection (Szabo, 1999); however, not all findings are consistent and other studies suggest that alcohol use may increase resistance to URI (Cohen et al., 1997; Cohen, Tyrrell, Russell, Jarvis & Smith, 1993). Alcohol use has also been associated with increased activation of Th2-related immune pathways, including the promotion of IgE hypersensitivity to various allergens (Gonzalez-Quintela, Vidal & Gude, 2004), as well as a shift towards IL-4

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production (Szabo, 1999). Thus, it is possible that alcohol consumption at times of stress could impact immune processes that are involved in inflammatory diseases, such as asthma.

Sleep is another health behavior that is subject to change at times of stress. Decrements in sleep quality and quantity have been associated with elevated levels of inflammation and pro-inflammatory cytokine production (Motivala, Sarfatti, Olmos & Irwin, 2005; Pollmacher et al., 2000). Moreover, poor sleep efficiency has also been associated with decreased host resistance to URI (Cohen et al., 1997). Interestingly, recent evidence suggests that there is a diurnal pattern of cytokine release across a typical night's sleep, with a shift towards Th2 cytokine production during late night sleep (Dimitrov, Lange, Tieken, Fehm & Born, 2004), a time when asthma symptoms are more prevalent. It is possible that stress-related changes in sleep may disrupt this pattern and modulate risk of asthma exacerbation.

A final health practice of interest in the current study is physical activity. Higher levels of perceived stress and negative affect have been associated with decreased energy and physical activity (Allgower, Wardle & Steptoe, 2001; Baum & Posluszny, 1999). As lack of physical activity has also been associated with higher levels of pro-inflammatory cytokines (Nicklas, You & Pahor, 2005), this provides another potential pathway linking stress to the exacerbation of inflammatory disease.

In sum, there is extensive evidence for direct anatomical and functional links as well as indirect behavioral links between the central nervous and immune system, providing potential pathways for the influence of stress on immune function. In turn, this raises the possibility that stress-related changes in immune function link stress to asthma exacerbation.

3.3   UPPER RESPIRATORY INFECTION AND ASTHMA

Another pathway by which stress could exacerbate asthma is via its association with increased susceptibility to URI. Experimental viral challenge studies, inoculating healthy individuals with common cold virus or placebo, have clearly shown that stressful life events predict the probability of developing a biologically-verified cold, with greater stress related linearly to increased susceptibility (Cohen, Tyrrell & Smith, 1991; Cohen et al., 1998). To date, however, the biological mechanisms of stress-related susceptibility to viral infection remain unknown. It has been speculated that the production of cytokines may play a significant role (Cohen & Rodriguez, 2000).   In this regard, stress is associated with the decreased production of Th1 cytokines that are involved in mounting a cellular immune response to viral challenge and this may increase susceptibility to URI.

The capacity for URI to promote asthma exacerbation is well documented. As many as 50% of asthma exacerbations experienced among adults are precipitated by respiratory infections (Busse, 1990; Busse & Gern, 1997; Nicholson, Kent & Ireland, 1993). Furthermore, viral respiratory infections have been shown to promote allergic inflammation and increase airway autonomic reactivity (Folkerts, Busse, Nijkamp, Sorkness & Gern, 1998). For example, Lemanske and colleagues (1989) showed that inoculation with a common cold rhinovirus resulted in heightened bronchoconstriction to inhaled allergens among allergic rhinitis patients. Furthermore, these individuals continued to show increased frequency of late allergic reactions for as long as 4 weeks following inoculation.

4.0   STRESS, IMMUNE FUNCTION AND ASTHMA

Several review articles (Glaser & Kiecolt-Glaser, 2005; Marsland et al., 2001) and meta-analyses (Herbert & Cohen, 1993; Segerstrom & Miller, 2004) of the literature on stress and immunity in humans conclude that naturalistic stress (as measured by both self-report and chronic or brief life events) is reliably associated with modulation of functional and enumerative immune measures.   Examples of naturally occurring stressors associated with immunomodulation include loss of a loved one (Kemeny et al., 1995), marital conflict (Robles & Kiecolt-Glaser, 2003), caring for a relative with Alzheimer's disease (Kiecolt-Glaser, Glaser, Gravenstein, Malarkey & Sheridan, 1996), and living near a damaged nuclear power plant (McKinnon, Weisse, Reynolds, Bowles & Baum, 1989). These stressors are consistently associated with functional alterations in immunity, including lower natural killer (NK) cell function, decreased T cell proliferation, and increased antibody production to latent viruses when compared with levels among non-stressed control groups. In terms of enumerative parameters, stress is associated with reliable decreases in circulating numbers of B cells, T helper cells, T cytotoxic cells and NK cells. Additionally, there is evidence that immune changes may persist with protracted stressor exposure (Baum, 1990).

Further evidence for an association between stress and immune function comes from studies that employ a quasi-experimental design, examining immune function within individuals at times of high and low naturalistic stress. Probably best known in this literature are the series of

studies by Kiecolt-Glaser, Glaser and colleagues (Glaser et al., 1987; Glaser, Pearl, Kiecolt-Glaser & Malarkey, 1994; Glaser et al., 1991; Glaser, Rice, Speicher, Stout & Kiecolt-Glaser, 1986; Kiecolt-Glaser et al., 1986) examining immune responses of medical students to examination stress. Of particular relevance to asthma, examination stress has been associated with reliable elevations in the production of the Th2 cytokines, IL-6 and IL-10 and concomitant decreases in the Th1 cytokines, IFN-gamma and IL-2, among healthy participants (Kang & Fox, 2001; Marshall & Agarwal, 2000; Marshall et al., 1998; Paik, Toh, Lee, Kim & Lee, 2000). For instance, Marshall and colleagues (1998) assessed immune function among 16 healthy first- year medical students 4 weeks prior to and 48 hours following medical school exams. During the exam period, medical students showed relative increases in Th2 cytokine production as well as a decrease in the Th1:Th2 ratio when compared to cytokine production 4 weeks earlier.   In addition, number of daily hassles during the pre-exam period was inversely associated with Th1:Th2 cytokine production pre- and post-exam.

To date, only a limited number of studies have examined the association between stress and immune function among an asthmatic population. Overall, studies provide initial support for an increase in Th2 cytokine production among chronically stressed asthmatics. For example, Chen, Fisher, Bacharier, and Strunk (2003) found that among asthmatics lower socioeconomic status (SES) was associated with a significantly higher level of IL-5 production when compared with higher SES.   Regression analysis showed that when stress and control beliefs were controlled SES no longer predicted IL-5 production suggesting that psychological stress may be driving this relationship. Similarly, studies examining immune responses to examinations show a stress-related increase in the production of Th-2 cytokine production among asthmatics. For example, Kang and colleagues (1997) examined cytokine production among 21 adolescent

asthmatics and 13 healthy controls a month prior to final exams, during final exams and 2-3 weeks following the exam period. Although there were no significant changes in cytokine production across the three time periods, asthmatics produced significantly more mitogen stimulated IL-5 during the exam period and lower IL-2/IL-5 (Th1:Th2) ratios across all 3 periods than healthy controls.. The same finding was reported for IFN-gamma/IL-5 (Th1:Th2) during the exam and post-exam periods providing initial support for differential cytokine production at times of stress among asthmatics and healthy controls. Unfortunately, this study is limited by a failure to assess levels of perceived stress and thus to validate the examination stress paradigm.

In a more recent study, the same group of investigators examined 24 college students (13 with asthma and 11 without asthma) during the middle of the semester and again during the final exam week (Kang & Fox, 2001). In contrast to their previous study, Kang and colleagues reported a significant exam-related increase in stress using a single item measure of stress, but not when assessed with the Perceived Stress Scale (PSS; Cohen, Kamarck & Mermelstein, 1983). In this study, both groups displayed a significant increase in Th2 cytokine (IL-6) production as well as a decrease in Th1 cytokine (IFN-gamma and IL-2) production from before to during the exam period. However, there were no stress-related changes in the production of IL-4 or IL-5 among asthmatics or controls. Asthmatics did show higher basal IL-5 production than controls. It is possible that their failure to find more significant effects is the consequence of the level of stress, with no detectable differences between exam and mid-semester periods on the PSS, a widely used and reliable measure of stress. In addition, the asthmatics in this sample were heterogeneous with respect to asthma medication usage. As a consequence, the immunomodulatory effects of asthma medication may have obscured further differences in cytokine production among asthmatic participants.

In contrast to studies that examine the impact of psychological stress on cytokine production within peripheral blood, Liu and colleagues (2002) utilized a more proximal model assessing changes in cytokine levels in sputum in response to the inhalation of a nonspecific mitogen. In this study, 20 mild asthmatic college students underwent the inhaled antigen challenge during a low stress period and again during final exams. In addition to the cytokine assessment, percentages of eosinophils were evaluated both in sputum and in peripheral blood. Liu and colleagues reported a significant stress-related increase in IL-5 production in sputum following the inhaled antigen challenge. Additionally, the percentage of eosinophils, assessed both in sputum and peripheral blood, were significantly elevated during the high versus low stress period with the percentage in sputum at 24 hours inversely associated with pulmonary function, as assessed by FEV1.

Taken together, studies examining the impact of examinations on cytokine production among asthmatics and healthy individuals provide initial evidence for a stress-induced shift from Th1 to Th2 cytokine production. This shift may have clinical implications for asthmatic individuals by (1) increasing their risk of airway inflammation in response to benign antigens, and (2) increasing their susceptibility to URI a known trigger of asthma episodes. Unfortunately methodological shortfalls in the existing literature, including inconsistent measurement of psychological stress and variability in asthma medication usage, make it difficult to conclude that psychological stress is reliably associated with greater Th2 activity among asthmatics. Additionally, in light of the limited number of studies examining the impact of stress among asthmatics and available evidence linking stress to changes in cytokine production patterns that may confer increased susceptibility to asthma, further research examining the impact of psychological stress on cytokine production among asthmatics is warranted.

To this end, the primary aim of this study was to examine changes in Th1 and Th2 cytokine production during periods of high and low stress among an asthmatic population and to determine whether these changes in cytokine production differ from those observed in healthy controls. For this purpose, a quasi-experimental study was conducted examining immune responses to the stress of taking the Pennsylvania bar exam among asthmatics and healthy controls.

4.1   HYPOTHESES

Hypothesis 1: Asthmatic and healthy participants will report higher levels of perceived stress, anxiety and negative affect prior to the bar exam relative to a lower stress period (1 month following the exam).

Hypothesis 2: Asthmatics will show higher basal levels of IL-4 and IL-5 and greater stress-related increases in stimulated levels of IL-5 and IL-4 production and concomitant decreases in IFN-gamma production when compared to healthy controls

Hypothesis 3: Asthmatics will show higher basal numbers of eosinophils and levels of IgE in peripheral circulation than non-asthmatics. Asthmatics will also show greater stress-related increases in circulating numbers of eosinophils than controls. In addition, IL-5 production will be associated with the levels of circulating eosinophils in peripheral blood during both high and low stress period.

5.0   METHODS

The primary goal of this study was to examine the impact of examination stress on Th1 and Th2 cytokine production in response to whole blood stimulation by phytohemagglutinin (PHA), a nonspecific mitogen, among mild asthmatics and healthy controls. To this end, we employed a quasi-experimental design in which participants were assessed approximately 1 week prior to taking the Pennsylvania bar exam (high stress period) and 1 month following the exam (low stress period). This design allowed for the examination of differences in cytokine production both within and between asthmatic and non-asthmatic participants.

5.1   PARTICIPANTS

Sixteen recently graduated law students who were scheduled to take the Pennsylvania bar exam in July 2005 were recruited to take part in this study. Individuals were eligible to participate if they were 1) healthy or had been diagnosed with mild asthma by a physician sometime in their lifetime, 2) non-smokers, 3) free from systemic disorders or medications affecting the immune, nervous or endocrine systems, with the exception of asthma and asthma medications 4) not currently being treated for a psychiatric disorder 5) consumed less than 15 alcoholic beverages per week, and 6) not experiencing an acute respiratory infection on days of data collection. The study protocol was approved by the Institutional Review Board at the

University of Pittsburgh and informed consent was obtained from each student prior to participation.

5.2   PROCEDURES

The study consisted of two separate data collection periods. The first study session took place at the Behavioral Immunology Laboratory, within one week of the Pennsylvania bar exam; participants returned for a second visit approximately 1 month later. Laboratory sessions were generally scheduled in the morning, and great effort was made to ensure that time of day was kept consistent within subject. Upon arrival, the study procedures were reviewed, any questions were answered and consent to participate was obtained. Prior to any data collection, participants were screened for the presence of acute upper respiratory infections using modified Jackson criteria. No participant met this criterion at either the exam or post-exam data collection period. Height and weight were obtained using a standard medical-grade balance scale. Participants then completed a battery of psychological questionnaires and underwent a venipuncture to collect 12 mls of blood for later assessment of immune functioning. Participants were compensated $50 for taking part in both study sessions.

5.3   PSYCHOLOGICAL MEASURES

The following psychological questionnaires were completed at both study visits. The Perceived Stress Scale *(PSS-10; Cohen, et al. 1983)* was used to assess the degree to which

participants' perceived their daily lives as unpredictable, uncontrollable, and overloading. The PSS is a reliable measure of perceived stress (Cronbach's coefficient alpha = .85; Cohen & Williamson, 1988). Moreover, the PSS has been shown to be a reliable measure of the stress associated with taking professional examinations (Malarkey, Pearl, Demers, Kiecolt-Glaser & Glaser, 1995). It was expected that this measure would confirm that high levels of stress were associated with preparing for the Pennsylvania bar exam.

Negative affect was measured using *Positive and Negative Affect Schedule- expanded form (PANAS-X; Watson & Clark, 1994)*. The PANAS-X is considered to be a reliable measure of general positive and negative affect (Cronbach's coefficient alpha for positive affect= .88, negative affect= .85; Watson & Clark, 1994). Negative affect is often experienced during periods of stress. Thus, it was expected that higher scores on the negative affect subscale of this measure would be observed prior to the examination when compared with a lower stress period.

Anxiety was measured using the *Speilberger State Anxiety Inventory* (STAI; *Spielberger, Gorsuch, Lushene, Vagg, and Jacobs, 1983*). The STAI is considered a reliable measure of state anxiety (Cronbach's coefficient alpha= .92, Spielberger et al., 1983). Anxiety is often experienced during periods of stress and thus was expected to increase during the examination period.

The *Beck Depression Inventory-2 (BDI-2; Beck, Steer, & Brown, 1996)* was used to measure depression at both time points. Research has found the BDI-2 to be a valid measure of depressive symptomatology (Cronbach alphas for the BDI = .92; Beck et al., 1996). This measure was employed to rule out individuals who met criteria for clinical depression (scores > 19).

5.4   HEALTH BEHAVIORS

A number of health behaviors known to be associated with both stress and immune parameters relevant to asthma exacerbation were measured, including sleep quality, physical activity and alcohol consumption. Sleep quality was assessed using the *Pittsburgh Sleep Quality Index (PSQI; Buysse, Reynolds, Monk, Berman & Kupfer, 1989).* The PSQI is a well accepted and reliable measure of sleep quantity and quality over the past month. (Cronbach coefficient alpha= .83; Buysse et al., 1989). Physical activity was measured using the *Paffenbarger Activity Index (PAI; Paffenbarger, Blair, Lee & Hyde, 1993).* The PAI is a well accepted and reliable measure of the average amount of physical activity per week (Cronbach coefficient alpha= .66-.83; Paffenbarger et al., 1993). Finally, at both time points, participants were asked to report their average consumption of alcohol (number of alcoholic drinks/day), for both weekdays and weekends.

5.5   BIOLOGICAL MEASURES

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6.0   DATA ANALYSIS AND RESULTS

Data was analyzed with SPSS version 14.0.   Initially, the distributions, descriptive statistics, and intercorrelations of all variables were examined to determine normality and multicollinearity. To correct for non-normally distributed data, natural log transformations were employed.   Pearson product-moment and point-biserial correlations were used to examine associations between demographic, psychosocial measures, self-report health behaviors and immune outcomes. Based on these preliminary analyses, demographic and background variables found to be significantly associated with primary outcomes were treated as covariates in further analyses.

Repeated measures analysis of variance (ANOVA) was employed to analyze the significance of overall group differences, cell responses across the two time points (exam and post-exam), and group x time interactions.   When appropriate, between and within group differences were examined using independent and paired t-tests.

Finally, exploratory analyses were conducted to assess whether health behaviors were associated with both stress and cytokine production and thus could be a possible mediator of the effect of stress on cytokine production. This study did not have the statistical power to test mediating effects. Thus relationships were examined using Pearson product-moment correlations. Health behaviors found to correlate with immune outcomes were then treated as covariates.

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6.1   PARTICIPANT STATISTICS

Sixteen participants were recruited to take part in this study, 5 asthmatics and 11 non-asthmatics. One healthy participant was unable to be contacted for the post-exam period; therefore, the final sample consisted of 5 asthmatic and 10 non-asthmatic participants. Demographic information describing the sample is provided in Table 1. All participants recently graduated from law school and were taking the Pennsylvania bar exam for the first time in July of 2005. Participants ranged in age from 24-35 with a mean of 27.3 (SD= 3.17), they were primarily Caucasian (87%) and a little more than half were female (60%). Asthma diagnosis was based on participants self-report of physician diagnosis. Asthma severity was determined by NHBLI guidelines with all asthmatic participants meeting criteria for mild asthma. No study participants endorsed taking corticosteroids. With the exception of having mild asthma, all participants reported being in good physical health. No participant reported being currently treated for a psychiatric disorder. The presence of depressive symptoms was measured at both study time points using the Beck Depression Inventory. While the scores ranged from 6-15 with a mean of 9.3 (SD= 4.4) during the exam and 3.3 (SD= 2.7) during the post-exam period, no participant exceeded the established cutoff score of 19 indicating moderate depression. However, twenty-five percent of the sample endorsed taking antidepressants.

In order to determine whether any systematic differences in demographic information existed between asthmatic and healthy participants, chi-squared and independent t-tests were performed. Analyses revealed that asthmatic and healthy participants did not differ statistically (p>.05) on any of the demographic variables (gender, age, BMI, ethnicity), nor were any differences detected among other background variables (antidepressant usage or upper respiratory infection scores).

Table 1: Means and standard deviations for Demographics and Background Variables

	Entire Sample N= 15	Asthmatic N= 5	Non-Asthmatic N= 10
Gender (% female)	9/15 (60%)	3/5 (60%)	6/10 (60%)
Age	27.3 (3.17)	26.2 (2.28)	27.9 (3.51)
BMI	25.2 (4.62)	24.4 (3.10)	25.6 (5.26)
Ethnicity (% Caucasian)	13/15 (87%)	4/5 (80%)	9/10 (90%)
Modified Jackson infection score T1	1.30 (2.19)	1.00 (1.00)	1.45 (2.63)
Modified Jackson infection score T2	1.47 (2.59)	2.40 (3.29)	1.00 (2.21)
Antidepressant use T1	4/15 (27%)	1/5 (20%)	3/10 (30%)
Antidepressant use T2	4/15 (27%)	1/5 (20%)	3/10 (30%)

To identify potential co-variates correlations were computed between demographic variables and psychosocial and immune outcomes (Tables 2 and 3). Demographic variables that were significantly correlated with psychosocial or immune outcomes at both time points were included as covariates in all analyses. Age and ethnicity were not associated with any psychosocial or immune variables at either study time point. Body mass index (BMI) correlated with eosinophil count ($r=.64$, $p=.01$) and state anxiety ($r=.78$, $p=.001$) during the exam period but was not associated with these variables during the low stress period. Gender was associated with eosinophil count during the both the exam ($r_{pb}=.70$, $p=.004$) and the post-exam session ($r_{pb}=.71$, $p=.003$) with males showing a higher level of circulating eosinophils. As a consequence, gender was treated as a covariate in analyses involving numbers of circulating eosinophils. In addition, given the high correlation between BMI and eosinophil count during post-exam and the relationship between BMI and inflammatory mediators (Tilg & Moschen, 2006), BMI was also treated as a covariate.

Table 2: Correlations among immune demographic and background variables

	Age	Ethnicity	Gender	BMI	Psych Medication	Infection Score
Stimulated IFN-gamma (pg/ml)						
EXAM	-.24	.16	.46	.45	.11	.28
POST-EXAM	-.09	.11	.34	.17	.25	-.07
Serum IgE (IU/ml)						
EXAM	-.29	-.02	.21	-.42	-.39	.21
POST-EXAM	-.31	-.01	.18	-.41	-.32	.46
Eosinophil Count (x 10^6 cells/ml)						
EXAM	-.15	.23	.70**	.64**	.01	.46
POST-EXAM	-.13	.26	.71**	-.01	.12	.49

**p<.01

Correlations between background variables, such as the use of antidepressant medication and participants' Modified Jackson criteria infection scores, and psychosocial and immune outcomes revealed no association between the use of antidepressant medication and any psychological or immune outcome. In contrast, infection scores during the post-exam period were found to be associated with levels of state anxiety (r=.52, p=.045), negative affect (r=.57, p=.028) and perceived stress (r=.68, p=.005) during that time point. Based on these preliminary analyses, post-exam infection scores were used as a covariate in analyses involving psychological measures.

Table 3: Correlations among psychosocial, demographic and background variables

	Age	Ethnicity	Gender	BMI	Psych Medication	Infection Score
Perceived Stress Scale						
EXAM	-.36	-.13	-.06	.26	.05	.38
POST-EXAM	-.51	.32	.39	.41	.40	.68**
State Negative Affect (PANAS-X)						
EXAM	-.10	-.29	-.15	.39	.03	.35
POST-EXAM	-.41	.22	.38	.23	.11	.57*
State Anxiety (STAI)						
EXAM	-.12	-.01	.10	.78**	.08	.47
POST-EXAM	-.26	.40	.30	.38	.39	.52*
Beck Depression Inventory						
EXAM	-.37	-.58*	-.19	.27	-.30	.32
POST-EXAM	-.50	.36	.46	.43	.11	.68**

*p<.05, **p<.01

6.2   HYPOTHESIS 1: ANALYSES AND RESULTS

It was hypothesized that asthmatic and healthy participants would report higher levels of perceived stress, state anxiety and state negative affect during the week prior to the bar exam than 1 month following the examination, with no group differences in the magnitude of this response.   To examine this hypothesis, 2x2 repeated measure Analysis of Covariance (ANCOVA) were conducted, with 2 between group factors (asthmatic vs. non-asthmatic) and 2 within group factors (examination period vs. post-exam period), to examine differences in levels of perceived stress, state anxiety, and negative affect.

Only participants who completed psychosocial measures at both time points were included in these analyses. As noted earlier, the infection score from the post-exam period was significantly correlated with the psychological measures and thus was entered as a covariate in

these analyses.   Means and standard deviations for the psychosocial measures are presented in

Table 4 and Figure 2 depicts changes in perceived stress. Similar changes were observed for

state negative affect and state anxiety over time. Indeed, we observed a main effect of time on

perceived stress $(F(1,13)= 22.98, p=.001,$ partial eta$^2 = .657)$, negative affect $(F(1,13)= 14.86,$

$p=.003,$ partial eta$^2 =.547)$, and state anxiety $(F(1, 13)= 16.38, p= .002,$ partial eta$^2 = .577)$. Both

asthmatics and non-asthmatics reported significantly more perceived stress, negative affect, and

state anxiety during the exam period relative to the post-exam period. As predicted, there was no

main effect of group or group x time interaction on analysis of any of these parameters,

indicating that both asthmatic and healthy participants experienced similar levels of distress.

Table 4: Means and standard deviations for psychosocial measures

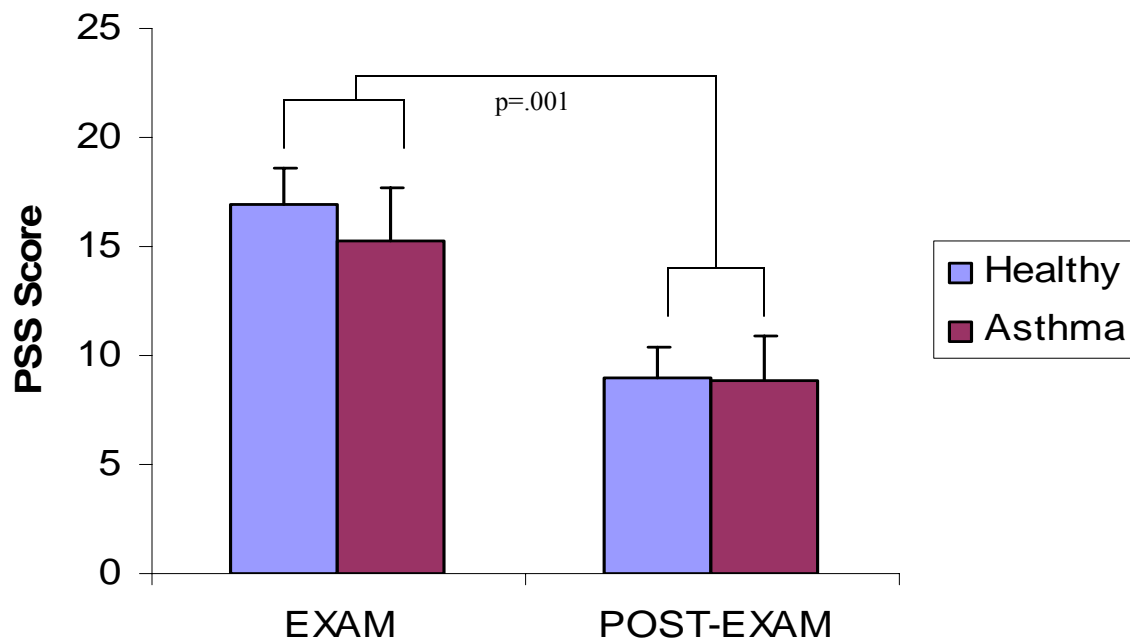|	Asthmatic N= 5		Non-Asthmatic N= 10		Contrasts (asthmatic vs. non-asthmatic) p-value
	<u>M</u>	<u>SD</u>	<u>M</u>	<u>SD</u>	
Perceived Stress Scale					
EXAM	16.0	4.4	16.5	5.8	n.s.
POST-EXAM	10.2	5.6	8.3	5.8	n.s.
State Negative Affect (PANAS-X)					
EXAM	14.8	6.6	16.1	7.2	n.s.
POST-EXAM	6.4	4.7	8.0	4.7	
State Anxiety (STAI)					
EXAM	44.4	9.3	47.0	11.7	n.s.
POST-EXAM	30.8	7.0	33.7	9.2	n.s.
Beck Depression Inventory					
EXAM	9.3	6.4	9.2	3.3	n.s.
POST-EXAM	3.2	2.7	3.3	2.8	n.s.

Figure 2: Changes in perceived stress from exam to post-exam period after controlling for post-exam URI infection score.

6.3   HYPOTHESIS 2: ANALYSES AND RESULTS

The second set of hypotheses concerned stress-related changes in immune function, specifically cytokine production, among asthmatic and non-asthmatic participants. Here, it was hypothesized that there would be baseline differences, with greater stimulated production of the Th2 cytokines IL-4 and IL-5. Additionally, it was predicted that asthmatics would show a more substantial increase in IL-5, IL-4 and a more substantial decrease in IFN-gamma production in response to examination stress when compared with their non-asthmatic counterparts. Unfortunately, the assay employed in this study did not reliably stimulate levels of IL-5 and IL-4 that were detectable using commercial ELISAs and thus IL-4 and IL-5 data could not be

included in these analyses. Levels of IFN-gamma production were quantifiable and results are presented below.

The means and standard deviations for all available immune parameters are presented in Table 5. Stimulated IFN-gamma levels were adjusted for total lymphocyte cell number and were natural log transformed to better approximate a normal distribution prior to analysis. As expected, unstimulated IFN-gamma levels were undetectable. A group (asthmatic versus non-asthmatic) x period (exam versus post-exam) repeated measures ANOVA was conducted to assess stress-related changes in IFN-gamma production. These analyses revealed a significant interaction ($F(1,13)$= 14.611, p=.002, partial eta^2 squared= .529), which is plotted in Figure 3. No main effect of group or time was observed. Paired and independent t-tests revealed that non-asthmatics experienced a significant increase in IFN-gamma production from the exam to the post-exam period ($t(9)$= 2.85, p=.019, eta^2 = .475). Change in IFN-gamma production over time among asthmatics tended to decrease, but did not achieve statistical significance ($t(4)$= -2.38, p=.076, eta^2 =.585). However, the large size of the effect of time on IFN-gamma production among asthmatics suggests that if there was greater power to detect effects this decrease in IFN-gamma production may have met statistical significance.
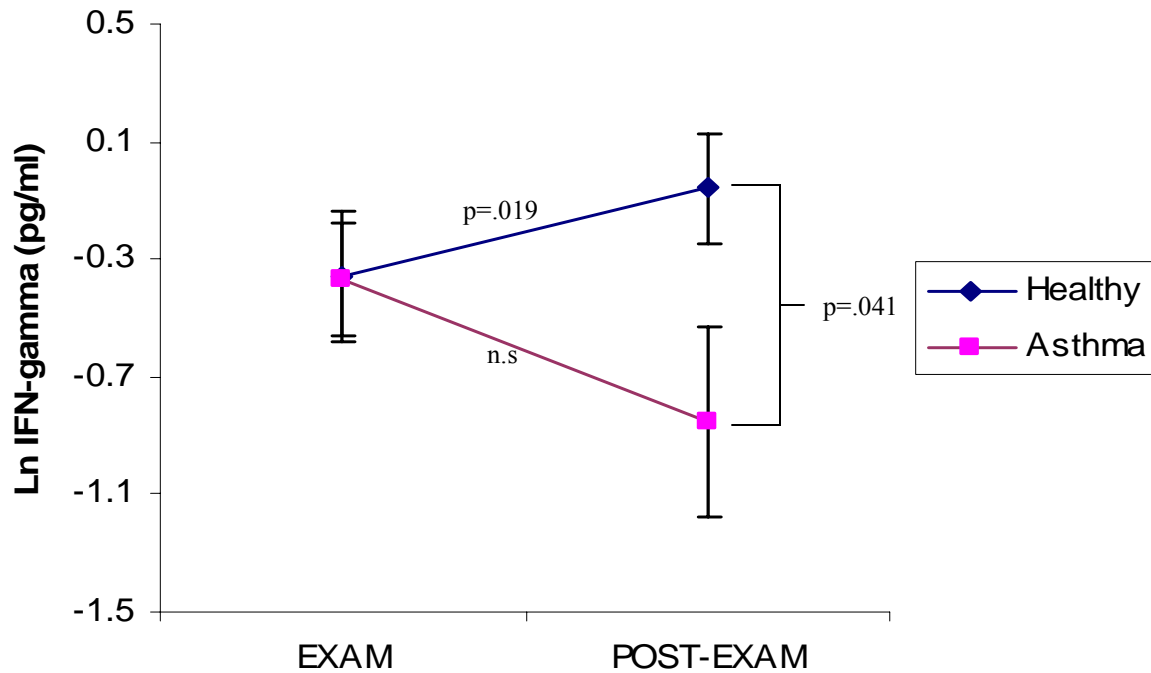
Figure 3: Changes in stimulated IFN-gamma from exam to the post-exam period

An independent t-test revealed that during the lower stress post-exam period, asthmatics produced significantly less IFN-gamma than healthy participants (t(13)= 2.26, p=.041, eta^2 = .28. Taken together, these findings suggest there are baseline differences in IFN-gamma production between asthmatics and healthy participants. Moreover, during periods of naturalistic stress healthy participants appear to respond with a significant decrease in IFN-gamma production while asthmatics show a marginal increase.

Table 5: Means and standard deviations for immune outcomes

	Asthmatic N= 5		Non-asthmatic N=10		Contrasts (asthmatic vs. non-asthmatic) p-value
	<u>M</u>	<u>SD</u>	<u>M</u>	<u>SD</u>	
Stimulated IFN-gamma (pg/ml)*					
EXAM	0.74	0.31	0.87	0.61	n.s.
POST-EXAM	0.52	0.36	1.10	0.57	.041
Eosinophil count (x 10^6 cells/ml)					
EXAM	0.26	0.25	0.28	0.29	n.s.
POST-EXAM	0.29	0.18	0.18	0.18	n.s.
Serum IgE (IU/ml)					
EXAM	173.6	145.6	62.6	95.6	.031
POST-EXAM	187.5	125.8	61.2	90.0	.038

* adjusted for cell number

6.4   HYPOTHESIS 3: ANALYSES AND RESULTS

The third hypothesis examined circulating levels of IgE and numbers of eosinophils. Here, it was hypothesized that asthmatics would show greater stress-related increases in circulating eosinophils count than non-asthmatics. Moreover, given that both IgE and eosinophil number have been associated with asthma severity, it was predicted that asthmatics would show greater basal levels of both circulating eosinophils and serum IgE than non-asthmatics.

Prior to these analyses, serum IgE levels were natural log transformed to better approximate a normal distribution. On analysis of serum IgE, an ANOVA revealed no main effect of time or group x time interaction. As predicted, however, there was a main effect of group (F(1, 13)= 5.148, p=.040, partial eta^2 = .285) (see Figure 4), indicating that asthmatics had higher levels of serum IgE relative to healthy participants across both periods. These findings

are consistent with the literature showing higher levels of serum IgE in asthma, which is largely

an atopic disease (Novak & Bieber, 2003).
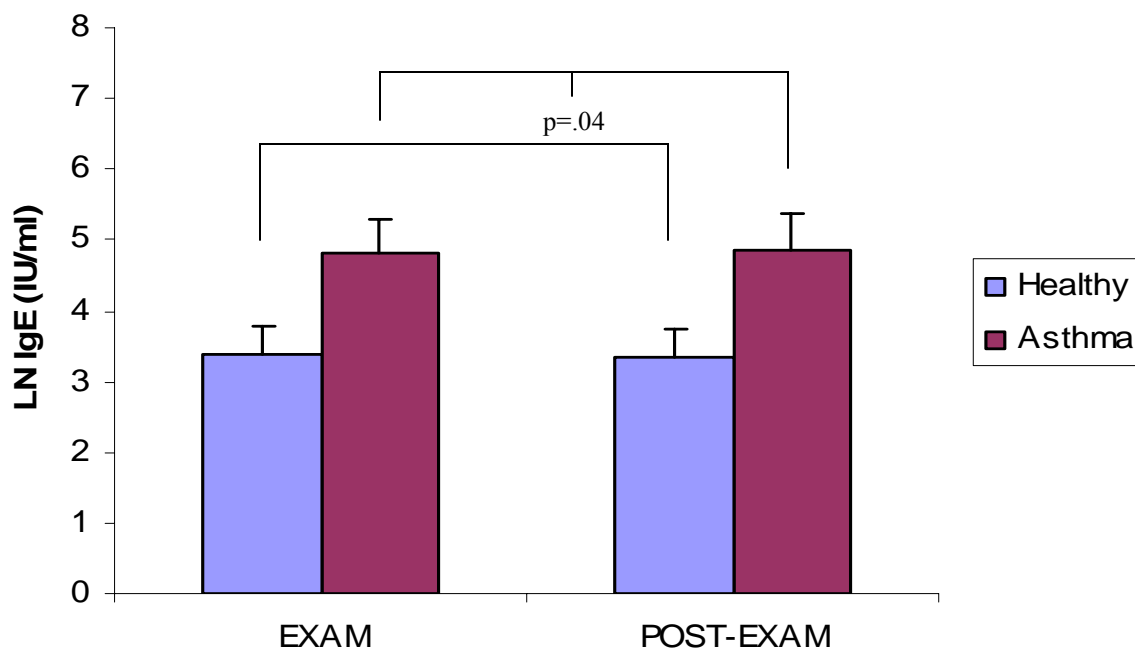
Figure 4: Changes in serum IgE from exam to the post-exam period

Absolute eosinophil numbers were also natural log transformed prior to analysis. Here a

2x2 ANCOVA was employed with gender and BMI as covariates. Results showed no significant

change in eosinophil number in response to stress ($F_{(1, 11)}$= .115 p=.702, partial eta^2 = .014). In

addition, there was no effect of group or group x time interaction.

In sum, these findings suggest that circulating numbers of eosinophils and levels of IgE

are not responsive to brief, naturalistic stress. As expected, asthmatic participants displayed

higher levels of circulating serum IgE relative to non-asthmatics across both sessions. In

contrast, asthmatics and healthy controls showed no difference in circulating eosinophil number.

The small size of the sample employed in this study limits our ability to draw firm conclusions and as a result findings must be considered preliminary and interpreted with caution.

6.5   ROLE OF HEALTH BEHAVIORS

Stress-related changes in health behaviors have been posited as potential pathways linking psychological stress to immune function (Kiecolt-glaser & Glaser, 1988). While our small sample size limited our statistical power to test the mediating effects of health behaviors, exploratory analyses were conducted in the hope that they would help guide future research.

At both study time points, participants reported on the quality and quantity of their sleep, their amount of physical activity and the amount of alcohol consumed over the last week. In addition to recording total alcohol consumption, participants were asked to report the amount of alcohol consumed during the week and on the weekends. Means and standard deviations for self-reported health behaviors are provided in Table 6. Due to missing data, sleep scores were only available for 11 participants during the exam period and 13 participants at post-exam. Data was collected from all 15 participants for the remaining health behaviors. To determine whether systematic differences in health behaviors existed between asthmatic and non-asthmatic participants, independent t-tests were employed; no significant differences were observed. Next, changes in health behaviors across the exam and post-exam periods were examined. A series of 2x2 repeated measures ANOVAs revealed no statistically significant group x time interactions, or main effects.

Table 6: Means and standard deviations for health behaviors

	Asthmatic N= 5		Non-Asthmatic N= 10		Contrasts (asthmatic vs. non-asthmatic) p-value
	M	SD	M	SD	
PSQI Global Sleep Score[a]					
EXAM	4.8	1.3	6.1	2.0	n.s.
POST-EXAM	4.0	1.6	4.2	2.0	n.s.
Physical Activity (kcal/week)					
EXAM	1275.5	1355.9	1847.0	1302.6	n.s.
POST-EXAM	1929.2	1508.3	2413.8	1893.7	n.s.
Total alcohol consumption (# drinks/week)					
EXAM	5.1	2.7	2.9	2.7	n.s.
POST-EXAM	5.3	2.5	3.1	2.6	n.s.
Alcohol consumption- weekdays (# drinks)					
EXAM	1.5	1.5	1.0	1.3	n.s.
POST-EXAM	1.5	1.3	0.6	0.8	n.s.
Alcohol consumption- weekends (# drinks)					
EXAM	3.6	1.6	1.9	2.2	n.s.
POST-EXAM	3.8	1.3	2.5	2.3	n.s.

[a] missing data, exam period (asthmatic n= 4, non-asthmatic n= 7); post-exam (asthmatic n=4, non-asthmatic n=9)

In order to explore the association between health behaviors and immune function, pearson product-moment correlations were conducted (see Table 7). The only significant result was an association between stimulated IFN-gamma production during the post-exam period and alcohol consumption on the weekdays (r=-.66, p=.008) across all participants. This result suggests that during low stress periods individuals who consume more alcohol during the week are less able to mount an IFN-gamma response to mitogen stimulation, suggesting a down-regulation of cellular immune response. It is worth noting that, although failing to achieve statistical significance, total alcohol consumption and weekend alcohol consumption were moderately correlated with IFN-gamma production during the post-exam period (r=-.47 and r=-

.27, respectively), providing further support for a possible relationship between alcohol use and down-regulation of cellular immune function.

Table 7: Correlations among immune outcomes and health behaviors

	Sleep Score	Physical Activity	Total alcohol consumption	Alcohol consumption-weekdays	Alcohol consumption-weekends
Stimulated IFN-gamma (pg/ml)					
EXAM	.05	-.19	.01	-.23	.15
POST-EXAM	-.38	.08	-.47	-.66**	-.27
Serum IgE (IU/ml)					
EXAM	-.53	.06	.27	.13	.28
POST-EXAM	.28	.51	.26	.19	.24
Eosinophil count					
EXAM	.06	-.37	-.04	-.09	.00
POST-EXAM	.23	.04	.43	.18	.46

**p<.01

Given the high correlation between weekday alcohol consumption and post-exam IFN-gamma production as well as the existing literature suggesting that alcohol can modulate immune function, we reanalyzed stress-related changes IFN-gamma production, including post-exam weekday alcohol consumption as a covariate. The group x time interaction, though marginally reduced, remained significant on analysis of IFN-gamma production (p=.009; change in partial eta^2 from .53 to .45), suggesting that while alcohol consumption is significantly correlated with IFN-gamma production it does not account for the group differences seen during the low stress period. The main effect of time and group remained nonsignificant (p=.456 and p=.631).

7.0   DISCUSSION

Growing evidence supports the role of psychological stress in asthma pathogenesis and exacerbation (Wright, et al., 1998), with stress-related modulation of Th2 and Th1 cytokine production proposed as one potential mediating mechanism. The aim of the present study was to assess the impact of naturalistic stress on Th1:Th2 cytokine production, as well as other asthma-relevant immune parameters, among mild asthmatics and healthy participants. For this purpose, immune responses to the stress of preparing for the Pennsylvania bar examination were evaluated. As expected, this stressor was associated with a significant increase in perceived stress, anxiety and negative affect when compared with a follow-up period 1 month later. There were no differences among asthmatics and healthy controls in the level of this exam-related distress. These emotional changes validate the use of the bar examination as a naturalistic stressor.

7.1   EXAM STRESS AND IFN-GAMMA PRODUCTION

Immune function was assessed at both study time points. On analysis of stress-related changes in stimulated of IFN-gamma production, interesting differences were observed between healthy controls and mild asthmatics. Specifically, healthy controls showed a significant stress-related decrease in IFN-gamma production. In contrast, asthmatics showed a tendency to respond

in the opposite direction with an increase in IFN-gamma production from periods of low-to-high stress. The stress-related decrease in IFN-gamma that was observed among healthy controls is consistent with existing literature demonstrating a down-regulation of Th1 function at times of naturalistic stress (Glaser et al., 1986; Marshall et al., 1998; Paik et al., 2000; Segerstrom and Miller, 2004), supporting a stress-related down regulation of cellular immune function. Findings from the few studies that have examined how stress influences Th-1 cytokine production among asthmatics have been less consistent. The present findings do not corroborate earlier observations that IFN-gamma production also decreases in response to naturalistic stress among asthmatics (Kang and Fox, 2001; Hoglund et al., 2006). However, other studies report no stress-related changes in IFN-gamma production among asthmatics (Kang et al., 1997; Liu et al., 2002). Furthermore, a recent cross-sectional study showed elevated levels of IFN-gamma production among chronically stress asthmatics when compared to their less stressed healthy counterparts (Chen et al., 2003).

Reasons for different findings across studies remain unclear. To date, available findings, including those reported here, derive from small studies and it is possible that multiple factors contribute to observed inconsistencies in the IFN-gamma responses of asthmatics to stress, including differences in the magnitude and chronicity of the stress, in the severity of the asthma, in medication use, and in multiple health behaviors that could influence immune competence. Further research is warranted to explore these possibilities and determine whether asthmatics show reliable differences from healthy controls in their IFN-gamma responses to naturalistic stress.

In addition to stress effects, the current findings reveal lower baseline levels of IFN-gamma production among asthmatics than healthy controls. This finding is consistent with

previous reports (Rodriguez et al., 1998) and with studies showing that higher ratios of Th2:Th1 cytokine production are associated with asthma (Bettiol et al., 2000). Indeed, a growing literature suggests that asthma is characterized by higher than normal Th2 cytokine production (Liu, 2000). In light of evidence that Th2 cytokines inhibit the production of Th1 cytokines, it is likely that the immune cells of asthmatics are primed by the inhibitory properties of the Th2 cytokines to produce lower levels of Th1 cytokines, possibly accounting for the lower levels of IFN-gamma observed in the current study.

IFN-gamma plays an integral role in cell-mediated immunity which is, in part, responsible for protecting the body from viral infections. Prior findings have shown that asthmatics are more susceptible to URI than non-asthmatics (Busse, 1990), suggesting that diminished resistance to viral infection may accompany this disease. As a major trigger of asthma episodes, increased susceptibility to URIs may play a role in the pathophysiology of this disease. Thus, dampened IFN-gamma production following mitogen challenge may be a biomarker of a downregulated, or impaired, cellular immune pathway which results in increased risk of asthma. Future studies would benefit from the inclusion of other Th1 cytokines, such as IL-2, that also play an important role in host resistance and from an examination of how Th1 cytokine production may contribute asthma pathogenesis.

7.2   EXAM STRESS, IgE, AND EOSINOPHIL COUNT

In addition to an examination of T-helper derived cytokines, the current study examined how stress impacted two other parameters of relevance to allergic pathology, circulating numbers of eosinophils and serum levels of IgE. In regard to eosinophil number, the current findings did

not corroborate an earlier observation that the percentage of eosinophils in peripheral circulation increases in response to naturalistic stress among asthmatics (Liu et al., 2002). Indeed, no stress-related changes in eosinophil number were observed for mild asthmatics or controls. The present findings did provide some support for existing findings that asthmatics have higher circulating numbers of eosinophils than non-asthmatics (Lewis et al., 2001; Ulrik, 1998), with a tendency for asthmatics to show higher eosinophil numbers across both measurement periods than controls. It is possible that the failure of this effect to achieve significance denotes a lack of robust association between asthma and eosinophil number. A second and more likely explanation is that the present study lacks the statistical power necessary to detect effects. Previous research reporting group differences had a larger subject numbers and hence more power than the present investigation.

A final possible explanation for our failure to find more convincing group differences in eosinophil counts is the inclusion of only mild asthmatics. Recent research suggests that the elevation in circulating numbers of eosinophils observed among asthmatics is secondary to an increase in circulating levels of IL-5, which occurs during the late phase of an asthma attack (Shi et al., 1998). The mild asthmatics recruited for the current study rarely experience asthma attacks and do not require asthma control medications. Previous research demonstrating differences in eosinophil counts between asthmatics and controls has included individuals with more moderate asthma who experience more frequent exacerbation of their disease (Krishna et al., 2001). Further attempts to determine whether eosinophil counts vary by asthma severity and differentiate asthmatics from healthy controls is warranted if the interpretation of these results is to be elucidated.

On analysis of serum IgE levels, our findings revealed an expected group difference, with asthmatics demonstrating higher levels across both measurement periods than healthy controls. This is consistent with existing literature (Novak & Bieber, 2003; Krishna et al., 2001) and supports the atopic nature of asthma. There were no stress-related changes in serum IgE levels among asthmatics or controls.

7.3   EXAM STRESS AND CHANGES IN HEALTH BEHAVIORS

Stress-related changes in health behaviors are considered a possible pathway linking psychological stress to increased disease susceptibility and exacerbation (Kiecolt-Glaser & Glaser, 1988). A growing body of evidence suggests that stress is associated with decreased physical activity (Ng & Jeffery, 2003; Heslop et al., 2001), decreased sleep quality (Hall et al., 2000), and increased alcohol consumption (Heslop et al., 2001; Steptoe, Wardle, Pollard, Canaan, and Davies, 1996). In contrast, the current study does not reveal a consistent relationship between examination stress and any of these health behaviors.   Given the existing evidence supporting these relationships, it seems unlikely that this failure to find effects reflects the lack of a robust association. As before, it is more likely that this reflects the lack of statistical power to detect effects.   Indeed, an examination of the observed relationships demonstrates that most of these relationships are in the expected directions, with effect sizes in the range to achieve significance with more subjects. The exception to this is alcohol consumption, which decreased slightly during the exam period. It is possible the absence of stress-related changes in health behaviors reflect the role of moderating variables, such as social support. In this regard, perceived availability of social resources has been demonstrated to buffer the appraisal of a

situation as stressful and impact health behavioral choices (Cohen, 2004). Consistent with the current findings, Steptoe and colleagues (1996) found no main effect of examination stress on alcohol consumption among 115 students. However, they did find a significant social support x time interaction with individuals high in social support reporting a decrease in alcohol consumption during examinations and those with low support endorsing an increase in alcohol consumption, supporting a moderating role of social support in stress-related alcohol use. Future work assessing social support as well as other potential moderators, e.g. self-esteem, perceived coping ability, and social network size, may aid in the interpretation of relationships between stress and health behaviors.

7.4   IFN-GAMMA PRODUCTION AND ALCOHOL CONSUMPTION

Interestingly, weekday alcohol consumption during the low stress period was inversely correlated with stimulated IFN-gamma production across both asthma and control groups. These findings corroborate a growing literature demonstrating an association between alcohol use and the decreased ability of Th1 cells to secrete IFN-gamma (Shellito, 1998; Waltenbaugh, Vasquez and Peterson, 1998). Indeed, decreased IFN-gamma production has been observed in response to both chronic and acute alcohol administration (Szabo, 1999).

To date, findings with respect to the impact of alcohol on asthma exacerbation have been mixed. While some beneficial effects of alcohol on asthma have been reported, such as the capacity for alcohol to reduce airway sensitivity (Cuddy, 2001), much of literature focuses on alcohol-related downregulation of immune function (Szabo, 1999). There were no group differences in alcohol consumption during the low stress period; thus, it is unlikely that alcohol

use account for differences across asthmatics and controls in IFN-gamma production. This was confirmed by multivariate analyses showing that alcohol use does not account for much of the between group variance in IFN-gamma production. Nevertheless associations between alcohol use and a down-regulation of cellular immune pathways warrant further investigation and may be relevant to susceptibility to viral infections.

7.5   BIOLOGICAL PATHWAYS

Activation of the HPA axis and associated release of cortisol is one potential mechanism of stress-related changes in IFN-gamma production among healthy participants. Indeed, evidence supports stress-related activation of the HPA axis, as measured by increased levels of cortisol in peripheral circulation (Wolkowitz et al., 2001). Cortisol plays an important role in the modulation of immune function. Of relevance to the current findings, cortisol can act on T-cells to potentiate Th2 cytokine production and downregulate Th1 cytokines responses. For example, Argawal and Marshall (1998) treated lymphocytes taken from healthy individuals with varying concentrations of the synthetic corticosteroid, dexamethasone and observed significant decreases in IFN-gamma production and a concomitant increase in the production of Th2 cytokines, including IL-4 and IL-10. Thus, it is possible that stress-related cortisol release accounted for the decrease in IFN-gamma production observed among the control group in the current study. Interestingly, however, the asthmatic subjects tended to show the opposite effect, with stress related increases in IFN-gamma production. Reasons for this are less clear. One possible explanation is a decrease in the sensitivity of immune cells to cortisol that has been reported among asthmatics (Leung, 1995), which could result in an increase in IFN-gamma production. It

remains to be determined whether this decline in glucocorticoid sensitivity is an individual difference associated with asthma pathogenesis or is an adaptation to the chronic use of corticosteroids in the treatment of asthma or to prolonged elevation of cortisol resulting from chronic stress. It is unlikely that chronic steroid use accounts for any glucocorticoid resistance in the current sample of mild asthmatics; however further research examining the possible role of glucocorticoid resistance is warranted.

Activation of the sympathetic and parasympathetic branches of the ANS are other mechanisms by which naturalistic stress may influence IFN-gamma production among asthmatic and healthy participants. Indeed, stress-induced activation of the sympathetic nervous system has been shown to modulate lymphocyte distribution (Bachen et al., 1995; Kin & Sanders, 2006) and to promote Th2 cytokine production. In vitro findings demonstrate that immune cells exposed to the sympathetic neurotransmitter epinephrine show a significant increase in Th2 production and a concomitant decrease in IFN-gamma (Agarwal & Marshall, 2000). Parasympathetic activation, often associated with bronchoconstriction, also has immunomodulatory effects. For instance, experimental studies show that eosinophils interact with cholinergic nerve fibers to induce the release of acetylcholine, a chemical mediator of bronchoconstriction (Sawatzky et al., 2003). In addition, emerging evidence suggests that vagal activity can directly influence cytokine production. While not yet applied to asthma-relevant immune parameters vagal activation has been shown to downregulate the inflammatory response (Tracey, 2002). Future studies examining role of vagal activity on asthma relevant cytokine production may shed further light on the multiple pathways between the central nervous and immune systems.

7.6   LIMITATIONS

There are a number of limitations of the present study. First, interpretation of results is hindered by the small sample size. Thus, findings should be considered as preliminary and interpreted with caution. Although other studies demonstrating relationships between stress and asthma-relevant immune parameters have also employed small samples sizes, e.g. 13 asthmatics in the study by Kang and Fox (2001), the current study only included 5 individuals with mild asthma. Nevertheless, the current findings provide some initial evidence that mild asthmatics differ from matched healthy controls in their ability to produce IFN-gamma, an important Th1 cytokine that is involved in cellular immune function. Further research to determine whether this is a robust difference is warranted, especially given the possibility that a dysregulation of Th1 pathways may render individuals more susceptibly to viral infections, which are a common trigger of asthma exacerbation.

Another limitation of the current study was a failure of the stimulation assay to successfully activate T-helper cells and upregulate the expression of Th2 cytokines. Despite pilot testing designed to ensure that the concentration of PHA (10 ug/ml) employed in the current assay was adequate to produce a quantifiable Th2 response, and prior research demonstrating that 10 ug/ml of PHA reliably stimulates T cell activation, we were unable to detect reliable levels of Th2 cytokines when the stimulated samples stored from the current study were analyzed by ELISA. Reasons for this technical problem remain unclear. One possible explanation is that the potency of the PHA was lower than originally anticipated. Findings from other assays conducted in the laboratory around the same time and using the same batch of PHA suggest that this may have been the case. Indeed, when employed in another study to stimulated lymphocyte proliferation, this batch of PHA resulted in lower than expected cell counts. IFN-gamma is

typically produced at higher concentrations than IL-4 or IL-5. As a consequence, it appears that IFN-gamma levels fell in the detectable range while the Th2 cytokines did not. In light of these results, further preliminary testing is indicated before the current study is replicated to determine whether stress influences the production of Th-2 cytokines among asthmatics

Finally, this study was limited by the exclusive recruitment of mild asthmatics. Previous studies utilizing more severe asthmatics have found more significant stress-related immune effects (Chen et al, 2003). Indeed, authors of previous studies examining stress-related immune changes among only mild asthmatics have suggested that utilizing individuals with mild disease makes it difficult to discern asthmatics from healthy controls (Kang et al., 1997). That said, however, more severe asthmatics often rely on medications known to affect the immune and endocrine system thus confounding studies aimed at examining the stress-immune link.

7.7   FUTURE DIRECTIONS

The present findings contribute to a growing literature demonstrating immune changes in response to naturalistic stress. Moreover, intriguing basal differences in IFN-gamma production provide initial evidence for possible diminished host resistance among asthmatics when compared with matched health controls. Taken together, these findings suggest that future studies examining the relationship between psychological stress, susceptibility to viral infection and asthma exacerbation should be conducted. Further examination of whether stress influences the production of Th2 cytokines and other immune parameters known to be related to the exacerbation of asthma is also indicated. Emerging research indicates that stress impacts a variety of biological processes relevant to asthma. For instance, oxidative stress, shown to be

exacerbated by psychological stress (Forlenza & Miller, 2006), has been postulated to enhance inflammation among asthmatics (Wright et al., 2005). Additionally, stress-induced break down in negative feedback loops, e.g. glucocorticoid resistance, may contribute to prolonged inflammatory processes in asthma (Wright et al., 2005).

Stress-related changes in health behaviors remain a possible pathway linking stress to asthma exacerbation, either directly or via behaviorally-related modulation of immune function. In this regard, future investigations would benefit from the exploration of the role of a range of health behaviors. Given the inverse relationship between IFN-gamma production and alcohol consumption reported in the current study, future work measuring alcohol consumption in a more rigorous fashion is indicated. For instance, the use of daily diaries to more reliably track alcohol use would be beneficial. Additionally, manipulation of alcohol consumption or the recruitment of asthmatics that chronically consume alcohol may help to elucidate the role of alcohol in the exacerbation of asthma.

In addition to determining the underlying mechanisms linking stress and asthma exacerbation, it is a hope that knowledge about the impact of stress or the course of asthma will translate from the benchtop to the bedside in the form of clinical interventions. To date, asthma interventions have focused primarily on pulmonary function with mixed success (Lehrer et al., 2002). Others have examined the benefit of teaching relaxation as a form of stress management. Here, the results have been mixed. Among healthy individuals, relaxation training has been associated with both a shift towards (Carlson, Speca, Patel and Goodey, 2003) and away from (Jones, 2001) Th2 cytokine production. Future studies measuring both pulmonary function and asthma relevant cytokine production in response to psychosocial interventions is warranted.

8.0   CONCLUSION

In conclusion, this study provides initial evidence of stress-related changes in asthma relevant immune parameters among healthy and mild asthmatic participants. Indeed, consistent with previous research, findings suggest a stress-related decrease in IFN-gamma production among healthy participants while asthmatics responded with a marginal increase. In addition, and consistent with a tendency for asthmatics to have poorer host resistance, basal differences in IFN-gamma were observed with asthmatics producing lower levels when compared to healthy individuals. No other stress-related differences in asthma relevant immune parameters were observed; however, as expected, asthmatics did show higher levels of circulating IgE across both study time points. Finally, exploratory analyses of health behaviors revealed an intriguing relationship between alcohol consumption and IFN-gamma production that warrants further investigation. Future studies employing larger sample sizes are needed to better interpret these findings. Moreover, an accurate assessment of Th2 cytokine production, as well as other biological processes emerging in the asthma literature, e.g. glucocorticoid sensitivity and oxidative stress, will help to elucidate the various pathways linking stress and asthma and inform the development of appropriate clinical interventions. Together with an understanding of the underlying pathophysiology, the role of health behaviors and the impact of naturalistic stress, it is a hope that, over time, interventions will be designed to make asthma a more manageable disease.

BIBLIOGRAPHY

Agarwal, S. K., & Marshall, G. D., Jr. (1998). Glucocorticoid-induced type 1/type 2 cytokine alterations in humans: A model for stress-related immune dysfunction. *Journal of Interferon & Cytokine Research, 18*(12), 1059-1068.

Agarwal, S. K., & Marshall, G. D., Jr. (2000). Beta-adrenergic modulation of human type-1/type-2 cytokine balance. *Journal of Allergy & Clinical Immunology, 105*(1 Pt 1), 91-98.

Agarwal, S. K., & Marshall, G. D., Jr. (2001). Dexamethasone promotes type 2 cytokine production primarily through inhibition of type 1 cytokines. *Journal of Interferon & Cytokine Research, 21*(3), 147-155.

Allgower, A., Wardle, J., & Steptoe, A. (2001). Depressive symptoms, social support, and personal health behaviors in young men and women. *Health Psychology, 20*(3), 223-227.

Apter, A., Affleck, G., Reisine, S. T., Tennen, H. A., Barrows, E., Wells, M., et al. (1997). Perception of airway obstruction in asthma: Sequential daily analyses of symptoms, peak expiratory flow rate, and mood. *The Journal of Allergy and Clinical Immunology, 99*(5), 605-612.

Bachen, E. A., Manuck, S. B., Cohen, S., Muldoon, M. F., Raible, R., Herbert, T. B., et al. (1995). Adrenergic blockade ameliorates cellular immune responses to mental stress in humans. *Psychosomatic Medicine, 57*(4), 366-372.

Baum, A. (1990). Stress, intrusive imagery, and chronic distress. *Health Psychology, 9*, 653-675.

Baum, A., & Posluszny, D. M. (1999). Health psychology: Mapping biobehavioral contributions to health and illness. *Annual Review of Psychology, 50*, 137-163.

Beck, A. T., Steer, R. A., & Brown, G. (1996). *Beck depression inventory manual* (2nd. ed.). San Antonio, TX: Psychological Corporation.

Bettiol, J., Bartsch, P., Louis, R., De Groote, D., Gevaerts, Y., Louis, E., et al. (2000). Cytokine production from peripheral whole blood in atopic and nonatopic asthmatics: Relationship with blood and sputum eosinophilia and serum ige levels. *Allergy, 55*(12), 1134-1141.

Busse, W. W. (1990). Respiratory infections: Their role in airway responsiveness and the pathogenesis of asthma. *Journal of Allergy & Clinical Immunology, 85*(4), 671-683.

Busse, W. W., & Gern, J. E. (1997). Viruses in asthma. *Journal of Allergy & Clinical Immunology, 100*(2), 147-150.

Busse, W. W., & Lemanske, R. F., Jr. (2001). Asthma. *New England Journal of Medicine, 344*(5), 350-362.

Buysse, D. J., Reynolds, C. F., 3rd, Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry Research, 28*(2), 193-213.

Carlson, L.E, Speca, M., Patel, K.D., & Goodey, E. (2003). Mindfulness-based stress reduction in relation to quality of life, mood, symptoms of stress, and immune parameters in breast and prostate cancer outpatients. *Psychosomatic Medicine, 65,* 571-581.

CDC. (2002). Fast stats a-z, vital and health statistics. from http://www.cdc.gov/nchs/fastats/asthma.htm

Chen, E., Bloomberg, G. R., Fisher, E. B., Jr., & Strunk, R. C. (2003). Predictors of repeat hospitalizations in children with asthma: The role of psychosocial and socioenvironmental factors. *Health Psychology, 22*(1), 12-18.

Chen, E., Fisher, E. B., Bacharier, L. B., & Strunk, R. C. (2003). Socioeconomic status, stress, and immune markers in adolescents with asthma. *Psychosomatic Medicine, 65*(6), 984-992.

Chrousos, G. P. (1995). The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *New England Journal of Medicine, 332*(20), 1351-1362.

Cohen, S. (2004). Social relationships and health. *American Psychologist, 59,* 676-684.

Cohen, S., Doyle, W. J., Skoner, D. P., Rabin, B. S., & Gwaltney, J. M., Jr. (1997). Social ties and susceptibility to the common cold. *Journal of American Medical Association, 277*(24), 1940-1944.

Cohen, S., Frank, E., Doyle, W. J., Skoner, D. P., Rabin, B. S., & Gwaltney, J. M., Jr. (1998). Types of stressors that increase susceptibility to the common cold in healthy adults. *Health Psychology, 17*(3), 214-223.

Cohen, S., & Herbert, T. B. (1996). Health psychology: Psychological factors and physical disease from the perspective of human psychoneuroimmunology. *Annual Review of Psychology, 47,* 113-142.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior, 24*(4), 385-396.

Cohen, S., & Rodriguez, M. (2000). Stress, viral respiratory infections, and asthma. In D. P. Skoner (Ed.), *Asthma and respiratory infections*. New York: Marcel Dekker.

Cohen, S., Tyrrell, D. A., Russell, M. A., Jarvis, M. J., & Smith, A. P. (1993). Smoking, alcohol consumption, and susceptibility to the common cold. *American Journal of Public Health, 83*(9), 1277-1283.

Cohen, S., Tyrrell, D. A., & Smith, A. P. (1991). Psychological stress and susceptibility to the common cold. *New England Journal of Medicine, 325*(9), 606-612.

Cohen, S., & Williamson, G. (1988). Perceived stress in the probability sample of the united states. In S. Spacapan & S. Oskamp (Eds.), *The social psychology of health.* Newbury Park, CA: Sage.

Cuddy, R. & Li, G. (2001). The role of alcohol in asthma: a review of clinical and experimental studies. *American Journal of Emergency Medicine, 19,* 501-503.

Dickerson, S. S., & Kemeny, M. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130*(3), 355-391.

Dimitrov, S., Lange, T., Tieken, S., Fehm, H. L., & Born, J. (2004). Sleep associated regulation of t helper 1/t helper 2 cytokine balance in humans. *Brain Behavior & Immunity, 18*(4), 341-348.

Folkerts, G., Busse, W. W., Nijkamp, F. P., Sorkness, R., & Gern, J. E. (1998). Virus-induced airway hyperresponsiveness and asthma. *American Journal of Respiratory and Critical Care Medicine, 157*(6 Pt 1), 1708-1720.

Forlenza, M.J. & Miller, G.E. (2006). Increased serum levels of 8-hydroxy-2'-deoxyguanosine in clinical depression. *Psychosomatic Medicine, 68,* 1-7.

Glaser, R., & Kiecolt-Glaser, J. K. (2005). Stress-induced immune dysfunction: Implications for health. *Nature Review of Immunology, 5*(3), 243-251.

Glaser, R., Pearl, D. K., Kiecolt-Glaser, J. K., & Malarkey, W. B. (1994). Plasma cortisol levels and reactivation of latent epstein-barr virus in response to examination stress. *Psychoneuroendocrinology, 19*(8), 765-772.

Glaser, R., Pearson, G. R., Jones, J. F., Hillhouse, J., Kennedy, S., Mao, H. Y., et al. (1991). Stress-related activation of epstein-barr virus. *Brain Behavior & Immunity, 5*(2), 219-232.

Glaser, R., Rice, J., Sheridan, J., Fertel, R., Stout, J., Speicher, C., et al. (1987). Stress-related immune suppression: Health implications. *Brain Behavior & Immunity, 1*(1), 7-20.

Glaser, R., Rice, J., Speicher, C. E., Stout, J. C., & Kiecolt-Glaser, J. K. (1986). Stress depresses interferon production by leukocytes concomitant with a decrease in natural killer cell activity. *Behavioral Neuroscience, 100*(5), 675-678.

Gonzalez-Quintela, A., Vidal, C., & Gude, F. (2004). Alcohol, ige and allergy. *Addiction Biology, 9*(3-4), 195-204.

Hall, M., Buysse, D.J., Nowell, P.D., Nofzinger, E.A., Houck, P., Reynolds, C.F., et al.,(2000). Symptoms of stress and depression as correlates of sleep in primary insomnia. *Psychosomatic Medicine, 62,* 227-230.

Herbert, T. B., & Cohen, S. (1993). Stress and immunity in humans: A meta-analytic review. *Psychosomatic Medicine, 55*(4), 364-379.

Heslop, P. Smith, G.D., Carroll, D., Macleod, J., Hyland, F. & Hart, C. (2001). Perceived stress and coronary heart disease risk factors: the contribution of socio-economic position. *British Journal of Health Psychology, 6,* 167-178.

Hoglund, C.O., Axen, J., Kemi, S., Jernelov, Grunewald, J., Muller-Suur, C.et al., (2006). Changes in immune regulation in response to examination stress in atopic and healthy individuals. *Clinical and Experimental Allergy, 36,* 982-992.

Humbert, M., Corrigan, C. J., Kimmitt, P., Till, S. J., Kay, A. B., & Durham, S. R. (1997). Relationship between il-4 and il-5 mrna expression and disease severity in atopic asthma. *American Journal of Respiratory and Critical Care Medicine, 156*(3 Pt 1), 704-708.

Isenberg, S. A., Lehrer, P. M., & Hochron, S. (1992). The effects of suggestion and emotional arousal on pulmonary function in asthma: A review and a hypothesis regarding vagal mediation. *Psychosomatic Medicine, 54*(2), 192-216.

Jartti, T. (2001). Asthma, asthma medication and autonomic nervous system dysfunction. *Clinical Physiology, 21*(2), 260-269.

Johnson, V., & Pandina, R. J. (2000). Alcohol problems among a community sample: Longitudinal influences of stress, coping, and gender. *Substance Use & Misuse, 35*(5), 669-686.

Jones, B.M. (2001). Changes in cytokine production in healthy subjects practicing Guolin Qigong : a pilot study. *BMC complementary and alternative medicine,8,* 1-7.

Jung, T., Lack, G., Schauer, U., Uberuck, W., Renz, H., Gelfand, E. W., et al. (1995). Decreased frequency of interferon-gamma- and interleukin-2-producing cells in patients with atopic diseases measured at the single cell level. *Journal of Allergy & Clinical Immunology, 96*(4), 515-527.

Kang, D. H., Coe, C. L., McCarthy, D. O., Jarjour, N. N., Kelly, E. A., Rodriguez, R. R., et al. (1997). Cytokine profiles of stimulated blood lymphocytes in asthmatic and healthy adolescents across the school year. *Journal of Interferon & Cytokine Research, 17*(8), 481-487.

Kang, D. H., & Fox, C. (2001). Th1 and th2 cytokine responses to academic stress. *Research in Nursing & Health, 24,* 245-257.

Kemeny, M., Weiner, H., Duran, R., Taylor, S., Visscher, B., & Fahey, J. (1995). Immune system changes after the death of a partner in hiv-positive gay men. *Psychosomatic Medicine, 57*(6), 547-554.

Kiecolt-Glaser, J. K., & Glaser, R. (1988). Methodological issues in behavioral immunology research with humans. *Brain Behavior & Immunity, 2*(1), 67-78.

Kiecolt-Glaser, J. K., Glaser, R., Gravenstein, S., Malarkey, W. B., & Sheridan, J. (1996). Chronic stress alters the immune response to influenza virus vaccine in older adults. *Proceedings of the National Academy of Sciences of the U S A, 93*(7), 3043-3047.

Kiecolt-Glaser, J. K., Glaser, R., Strain, E. C., Stout, J. C., Tarr, K. L., Holliday, J. E., et al. (1986). Modulation of cellular immunity in medical students. *Journal of Behavioral Medicine, 9*(1), 5-21.

Kin, N.W. & Sanders, V.M. (2006). It takes nerve to tell T and B cells what to do. *Journal of Leukocyte Biology, 79,* 1093-1104.

Krishna, M. T., Salvi, S. S., & Holgate, S. T. (2001). Pathogenesis of asthma. In R. R. Rich, T. A. Fleisher, W. T. Shearer, B. L. Kotzin & H. W. J. Schroeder (Eds.), *Clinical immunology: Principles and practice* (Second ed.). London: Mosby.

Lazarus, R. S., & Folkman, S. (1984). *Stress, appraisal, and coping*. New York: Springer Pub. Co.

Leckie, M. J., ten Brinke, A., Khan, J., Diamant, Z., O'Connor, B. J., Walls, C. M., et al. (2000). Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet, 356*(9248), 2144-2148.

Lehrer, P., Feldman, J., Giardino, N., Song, H. S., & Schmaling, K. (2002). Psychological aspects of asthma. *Journal of Consulting and Clinical Psychology, 70*(3), 691-711.

Lehrer, P. M., Hochron, S., Carr, R., Edelberg, R., Hamer, R., Jackson, A., et al. (1996). Behavioral task-induced bronchodilation in asthma during active and passive tasks: A possible cholinergic link to psychologically induced airway changes. *Psychosomatic Medicine, 58*(5), 413-422.

Lehrer, P. M., Isenberg, S., & Hochron, S. M. (1993). Asthma and emotion: A review. *Journal of Asthma, 30*(1), 5-21.

Lemanske, R. F., Jr., Dick, E. C., Swenson, C. A., Vrtis, R. F., & Busse, W. W. (1989). Rhinovirus upper respiratory infection increases airway hyperreactivity and late asthmatic reactions. *Journal of Clinical Investigation, 83*(1), 1-10.

Leung, D. Y. (1995). Steroid-resistant asthma. *Western Journal of Medicine, 163*(4), 367-368.

Levitan, H. (1985). Onset of asthma during intense mourning. *Psychosomatics, 26*(12), 939-941.

Lewis, S. A., Pavord, I. D., Stringer, J. R., Knox, A. J., Weiss, S. T., & Britton, J. R. (2001). The relation between peripheral blood leukocyte counts and respiratory symptoms, atopy, lung function, and airway responsiveness in adults. *Chest, 119*(1), 105-114.

Liu, A. H. (2000). Allergy and asthma: Classic th2 diseases. *Allergy and Asthma Proceedings, 21*(4), 227-230.

Liu, L. Y., Coe, C. L., Swenson, C. A., Kelly, E. A., Kita, H., & Busse, W. W. (2002). School examinations enhance airway inflammation to antigen challenge. *American Journal of Respiratory and Critical Care Medicine, 165*(8), 1062-1067.

Maggi, E., Parronchi, P., Manetti, R., Simonelli, C., Piccinni, M. P., Rugiu, F. S., et al. (1992). Reciprocal regulatory effects of ifn-gamma and il-4 on the in vitro development of human th1 and th2 clones. *Journal of Immunology, 148*(7), 2142-2147.

Malarkey, W. B., Pearl, D. K., Demers, L., Kiecolt-Glaser, J. K., & Glaser, R. (1995). Influence of academic stress and season on 24-hour mean concentrations of acth, cortisol, and b-endorphin. *Psychoneuroendocrinology, 20*(5), 499-508.

Marshall, G. D., Jr., & Agarwal, S. K. (2000). Stress, immune regulation, and immunity: Applications for asthma. *Allergy & Asthma Proceedings, 21*(4), 241-246.

Marshall, G. D., Jr., Agarwal, S. K., Lloyd, C., Cohen, L., Henninger, E. M., & Morris, G. J. (1998). Cytokine dysregulation associated with exam stress in healthy medical students. *Brain Behavior & Immunity, 12*(4), 297-307.

Marsland, A. L., Bachen, E. A., Cohen, S., & Manuck, S. B. (2001). Stress, immunity, and susceptibility to infectious disease. In A. Baum, T. A. Revenson & J. E. Singer (Eds.), *Handbook of health psychology*. Mahwah, New Jersey: Lawrence Erlbaum Associates.

McKinnon, W., Weisse, C. S., Reynolds, C. P., Bowles, C. A., & Baum, A. (1989). Chronic stress, leukocyte subpopulations, and humoral responses to latent viruses. *Health Psychology, 8*(4), 389-402.

Miller, G. E., Cohen, S., & Ritchey, A. K. (2002). Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoid-resistance model. *Health Psychology, 21*(6), 531-541.

Motivala, S. J., Sarfatti, A., Olmos, L., & Irwin, M. R. (2005). Inflammatory markers and sleep disturbances in major depression. *Psychosomatic Medicine, 67*(2), 187-194.

National Heart, Lung and Blood Institute.(1997). Guidelines for the diagnosis and management of asthma. from www.nhlbi.nih.gov/nhlbi/lung/asthma/prof/asthhc.htm

Ng, D.M. & Jeffery, R.W. (2003). Relationships between perceived stress and health behaviors in a sample of working adults. *Health Psychology, 22,* 638-642.

Nicholson, K. G., Kent, J., & Ireland, D. C. (1993). Respiratory viruses and exacerbations of asthma in adults. *British Medical Journal, 307*(6910), 982-986.

Nicklas, B. J., You, T., & Pahor, M. (2005). Behavioural treatments for chronic systemic inflammation: Effects of dietary weight loss and exercise training. *CMAJ, 172*(9), 1199-1209.

Novak, N., & Bieber, T. (2003). Allergic and nonallergic forms of atopic diseases. *Journal of Allergy & Clinical Immunology, 112*(2), 252-262.

Paffenbarger, R. S., Jr., Blair, S. N., Lee, I. M., & Hyde, R. T. (1993). Measurement of physical activity to assess health effects in free-living populations. *Medical Science & Sports Exercise, 25*(1), 60-70.

Paik, I. H., Toh, K. Y., Lee, C., Kim, J. J., & Lee, S. J. (2000). Psychological stress may induce increased humoral and decreased cellular immunity. *Behavioral Medicine, 26*(3), 139-141.

Pollmacher, T., Schuld, A., Kraus, T., Haack, M., Hinze-Selch, D., & Mullington, J. (2000). Experimental immunomodulation, sleep, and sleepiness in humans. *Annals of N Y Academy of Sciences, 917*, 488-499.

Rabin, B. S. (1999). *Stress, immune function, and health: The connection*: Wiley-Liss Inc.

Rees, W. L. (1980). Etiological factors in asthma. *Psychiatry Journal of the University of Ottawa, 5*, 250-254.

Ritz, T., & Steptoe, A. (2000). Emotion and pulmonary function in asthma: Reactivity in the field and relationship with laboratory induction of emotion. *Psychosomatic Medicine, 62*(6), 808-815.

Robles, T. F., & Kiecolt-Glaser, J. K. (2003). The physiology of marriage: Pathways to health. *Physiology and Behavior, 79*(3), 409-416.

Rodriguez, M. F., Patino, P. J., Montoya, F., Montoya, C. J., Sorensen, R. U., & Garcia de Olarte, D. (1998). Interleukin 4 and interferon-gamma secretion by antigen and mitogen-stimulated mononuclear cells in the hyper-ige syndrome: No th-2 cytokine pattern. *Annals of Allergy Asthma & Immunology, 81*(5 Pt 1), 443-447.

Sandberg, S., Jarvenpaa, S., Penttinen, A., Paton, J. Y., & McCann, D. C. (2004). Asthma exacerbations in children immediately following stressful life events: A cox's hierarchical regression. *Thorax, 59*(12), 1046-1051.

Sandford, A. J., Chagani, T., Zhu, S., Weir, T. D., Bai, T. R., Spinelli, J. J., et al. (2000). Polymorphisms in the il4, il4ra, and fcerib genes and asthma severity. *Journal of Allergy & Clinical Immunology, 106*(1 Pt 1), 135-140.

Sawatzky, D. A., Kingham, P. J., Durcan, N., McLean, W. G., & Costello, R. W. (2003). Eosinophil-induced release of acetylcholine from differentiated cholinergic nerve cells. *Am J Physiol Lung Cell & Molecular Physiology, 285*(6), L1296-1304.

Schmaling, K., Lehrer, P., Feldman, J., & Giardino, N. (2003). Asthma. In A. M. Nezu & C. M. Nezu (Eds.), *Handbook of psychology: Health psychology*. New York, NY: John Wiley & Sons, Inc.

Sears, M. R., Green, J. M., Willan, A. R., Wiecek, E. M., Taylor, D. R., Flannery, E. M., et al. (2003). A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. *New England Journal of Medicine, 349*(15), 1414-1422.

Segerstrom, S. C., & Miller, G. E. (2004). Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. *Psychological Bulletin, 130*(4), 601-630.

Shellito, J.E. (1998). Alcohol and host defense against pulmonary infection with pneumocystis carinii. *Alcoholism: clinical and experimental research, 22,* 208-211.

Shi, H. Z., Xiao, C. Q., Zhong, D., Qin, S. M., Liu, Y., Liang, G. R., et al. (1998b). Effect of inhaled interleukin-5 on airway hyperreactivity and eosinophilia in asthmatics. *American Journal of Respiratory and Critical Care Medicine, 157*(1), 204-209.

Shiota, Y., Arikita, H., Horita, N., Hiyama, J., Ono, T., & Yamakido, M. (2002). Intracellular il-5 and t-lymphocyte subsets in atopic and nonatopic bronchial asthma. *Journal of Allergy & Clinical Immunology, 109*(2), 294-298.

Spielberger, C.D., Gorsuch, R.L., Lushene, P.R., Vagg, P.R., Jacobs, A.G. (1983). Manual for the State-Trait Anxiety Inventory, Form Y. Consulting Psychologists Press, Inc.: Palo Alto.

Steptoe, A., & Holmes, R. (1985). Mood and pulmonary function in adult asthmatics: A pilot self-monitoring study. *British Journal of Medical Psychology, 58 (Pt 1),* 87-94.

Steptoe, A., Wardle, J., Pollard, T.M., Canaan, L., Davies, G.J. (1996). Stress, social support and health-related behavior: a study of smoking, alcohol consumption and physical exercise. *Journal of Psychosomatic Research, 41,* 171-180.

Strunk, R. C., Mrazek, D. A., Fuhrmann, G. S., & LaBrecque, J. F. (1985). Physiologic and psychological characteristics associated with deaths due to asthma in childhood. A case-controlled study. *Journal of the American Medical Association, 254*(9), 1193-1198.

Sturdy, P. M., Victor, C. R., Anderson, H. R., Bland, J. M., Butland, B. K., Harrison, B. D., et al. (2002). Psychological, social and health behaviour risk factors for deaths certified as asthma: A national case-control study. *Thorax, 57*(12), 1034-1039.

Szabo, G. (1999). Consequences of alcohol consumption on host defence. *Alcohol Alcohol, 36*(6), 830-841.

Tilg H. & Moschen, A.R. (2006). Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nature reviews. Immunology, 6,* 772-783.

Tracey, K.J. (2002). The inflammatory reflex. *Nature, 420,* 853-859.

Ulrik, C. S. (1998). Eosinophils and pulmonary function: An epidemiologic study of adolescents and young adults. *Annals of Allergy Asthma & Immunology, 80*(6), 487-493.

Waltenbaugh, C., Vasquez, K, & Peterson, J.D. (1998). Alcohol consumption alters antigen-specific Th1 responses: mechanisms of deficit and repair. *Alcoholism: clinical and experimental research, 22,* 220-223.

Watson, D., & Clark, L. A. (1994). *The panas-x: Manual for the positive and negative affect schedule- expanded form.* The University of Iowa.

Wolkowitz, O. M., Epel, E. S., & Reus, V. I. (2001). Stress hormone-related psychopathology: Pathophysiological and treatment implications. *World Journal of Biological Psychiatry, 2,* 113-141.

Wright, R. J., Cohen, R. T., & Cohen, S. (2005). The impact of stress on the development and expression of atopy. *Current Opinions Allergy & Clinical Immunology, 5*(1), 23-29.

Wright, R. J., Rodriguez, M., & Cohen, S. (1998). Review of psychosocial stress and asthma: An integrated biopsychosocial approach. *Thorax, 53*(12), 1066-1074.