REVIEW ARTICLE

Neuroendocrine and cytokines-induced responses to minutes, hours, and days of mental stress

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Abstract

OBJECTIVES: Previously, a large number of studies reported that psychological stress and psychiatric illness reduces immune responsiveness. However, it turned out that stress reduces immune responsiveness is an oversimplified statement because the interactions between central nervous system, endocrine system and the immune system are undoubtedly complex. Therefore, this study aims in reviewing mental stress models (e.g. brief and written examination stress as subacute and acute type of stressor) that have been utilized to understand the effect of stress on the neuroendocrine and immune systems.

METHODS: The published findings from human mental stress models on catecholamines, cortisol, prolactin levels and on T helper (Th) 1 and 2-induced cytokines are presented and discussed with respect to the *in vitro* and *in vivo* effects of glucocorticoids, catecholamines, and prolactin on the induction of cytokines.

RESULTS: This review shows evidence that short-time (minutes) or preparation to a written examination, in those students who are stressed, induces the production of proinflammatory cytokines which may be related to Th1 response. However, longer mental stress (days) causes dysregulation in the immune function by shifting the cytokine response to Th2 response.

CONCLUSIONS: The outcome from neuroendocrine and immune function prior to, following and after mental stress depends on multiple variables most importantly on the amount of stress, exposure time, coping behavior and adjustment of the individual. A few minutes of stress may improve immune performance but longer times of mental stress have detrimental effects that may lead to loss of immune integrity. Furthermore, studies on stress and common heath problems are necessary to increase our knowledge and understanding of the mechanisms responsible for producing neuroendocrine-induced immune changes in health and common diseases.

1. Introduction

Stress is the reaction of the body to stimuli that disturb its homeostasis. These stimuli could be physical, chemical, or psychological which commonly stimulate the hypothalamic-pituitary-adrenal (HPA) axis, leading to increasing levels of serum glucocorticoids (and other hormones), and peripheral sympathetic/adrenomedullary (SAM) system followed by release of catecholamines [1]. Glucocorticoids and catecholamines and other hormones (e.g. prolactin) were shown to have detrimental effects on the immune system via their expressed receptors on the immune cells. Most of these detrimental affects are on cytokines produced from immune cells.

The immune response is composed of cell-mediated and humoral components. The balance between cell mediated and humoral responses is basically maintained by the release of cytokines from the T helper (Th) lymphocytes. Th lymphocytes are divided into two subpopulations, Th1 and Th2 cells, based on their ability to produce specific pattern of cytokines [2,3]. Th1 cells induce cell-mediated immunity via their release of cytokines such as interleukin (IL)-2 and interferon (IFN)-γ while Th2 cells induce humoral immunity via their release of cytokines such as IL-4, IL-5, and IL-10. However, naive T helper cells (Th0) serve as precursors to either Th1 or Th2 cells depending on the signal of activation. Cytokine such as IL-12, produced by activated monocytes/macrophages or other antigen presenting cells, is a major inducer of Th1 cell and its cytokines. Monocytes/macrophages-derived IL-12 and tumor necrosis factor-α (TNF-α) with natural killer (NK) cells and Th1-derived IFN-γ, stimulate the function of T cytotoxic cells, NK cells, and activated macrophages. The cytokines such as, IL-12, IFN-γ and TNF-α, are considered major inflammatory cytokines because they stimulate the synthesis of nitric oxide and other inflammatory mediators that derive chronic delayed hypersensitivity reactions [2,3]. While IL-12 and IFN-γ can inhibit Th2 response, Th2 cytokines such as, IL-10 and IL-4, inhibit Th1 activity and macrophage activation. In addition, they stimulate differentiation of B cells to antibody-producing cells (especially class switching to IgE) and stimulate the growth and activation of eosinophils and mast cells, but inhibit macrophage activation. Therefore, Th1 and Th2 responses are mutually inhibitory [4]. Additionally, T cytotoxic cells can secrete Th1 as well as Th2 cytokines and were referred as Tc1 and Tc2.

Previously, a large number of studies reported that psychological stress and psychiatric illness reduces immune responsiveness. However, it turned out that stress reduces immune responsiveness is an oversimplified statement. Many studies have shown the impact of different types of stressors on CNS, endocrine and the immune system. For instance, surgery [5], depression [6,7], bereavement [8], exercise [9], marital conflict [10–12] and academic stress [13–23] were some of the stress models used. However, each of the above stress model differs basically in duration and intensity, thus different responses from the CNS, endocrine, and the immune systems would develop. Also, within the same type of stressor used, time of testing or challenge, subjects and their health status, coping-behavior, age, sex, lymphoid compartment examined and seasonal variations all could play a major influence on the outcome measured and therefore the data in the literature seem conflicting.

In this review, the effects of different models of mental and academic stress (as subacute to acute type of stress) on the levels of catecholamines, cortisol, prolactin, Th1 and Th2-induced cytokines are presented and discussed. The discussion is performed with respect to the *in vitro* and *in vivo* effects of glucocorticoids, catecholamines, and prolactin on the induction of cytokines. This review shows evidence that short-time (minutes) or preparation to a written examination, in those subjects who are stressed, induces the production of proinflammatory cytokines and maybe related to Th1 response. The brief mental stress or preparation to exam (depending on the type of exam and duration of preparation) is more likely to induce a challenge (i.e. potential for growth [24,25]) to the individual and causes a mild and transient SAM and HPA activation, which is correlated with self-confidence and anxiety. This mild and transient increase in catecholamines, glucocorticoids and prolactin were found to induce the production of proinflammatory cytokines probably via the induction of transcription nuclear factor (NF-kB). However, longer mental stress (days) causes dysregulation in the immune function through shifting towards Th2 mediated response. During repeated examinations, for instance, the stressed individuals are exposed to repeated stressors without recovery period and the threat feeling or losing is eventually higher and it is more likely to induce a moderate and consistent HPA activation, which follows SAM activation, and thus consistently glucocorticoids are increased [24,25]. The latter form is more likely to induce an immune dysregulation by shifting the cytokine response into Th2 rather than Th1 response. However, this dysregulation might be limited if other hormonal levels like prolactin are increased. Moreover, the aim is to provide a resource for helping in the formulation of strong rationales for the design of future stress studies which will help understanding the mechanisms responsible for producing neuroendocrine-induced immune changes in health and different disease conditions.

2. Effect of Glucocorticoids, Catecholamines and Prolactin on Cytokines-Induced Levels

It is well known that glucocorticoids and their analogs down regulate the immune system via binding glucocorticoid receptors on lymphocytes and monocytes/macrophages [26]. This down regulation consisted mainly of inhibiting mitogen or antigen-induced levels of IL-1 [27], IL-12 from monocytes/macrophages [28,29], and IL-2, IFN- γ from T lymphocytes [30,31]. In addition, glucocorticoids down regulate the expres-

sion of IL-12 receptors on Th1 and NK cells [28]. However, glucocorticoids showed different effects on IL-4 and IL-10 induction levels. In phytohemeagglutinin (PHA)+ lipopolysaccharide (LPS)- stimulated whole blood or PHA-stimulated peripheral blood mononuclear cells (PBMC), glucocorticoids inhibited the production of IL-10 induction less than IFN-γ causing a sharp decrease in IFN-γ/IL-10 ratio [32,33,34]. However, when the same cells were further stimulated with IL-2, IL-4 and IL-10 levels increased while IFN-γ levels decreased [34]. Furthermore, the addition of glucocorticoid-treated monocytes/macrophages to antigen-primed CD4+ T cells was associated with increased IL-4 production levels [35,36]. In addition, glucocorticoids *in vitro* have no effect on the production of IL-10 from LPS-stimulated monocytes [29]. In endotoxemia and multiple sclerosis patients treated with glucocorticoids there was increasing plasma levels of IL-10 [37,38]. These results indicate that glucocorticoids inhibit directly the production of IL-12 from monocytes/macrophages and thereby inhibiting production from IFN-γ from Th1/NK cells, but have less effect on IL-10 productions. The IL-10 productions might increase, however, if concomitant with another stimulatory signal to T cells. In this case, the levels of Th1 and Th2 cytokines are decreased and increased respectively, and thus down regulation of Th1 persists. The stimulatory signals could be cytokines, such as IL-2, or other hormones e.g. estradiol which results in increase in IL-10 levels [33,34]. On the other hand and following brief *in vitro* exposure to glucocorticoids, mitogen-stimulated PBMC increased IFN-γ and IFN-γ/IL-10 ratio [34]. In addition, brief exposure to cortisol (2 h) in rats increased delayed type hypersensitivity reaction (DTH) [39] and found to be mediated by local increase in IFN-γ production [40].

Catecholamines, the end products of SAM system also regulate immune system response through specific α (α1 and α2) and β (β1 and β2) receptors, which are categorized, based on their different sensitivity to certain agonists. β2 receptors, for example, have been identified only on Th1 cells, but not on Th2 cells [41]. *In vitro*, catecholamines or β and β2 adrenoceptor agonists induced a decrease in IL-12 and IFN-γ and an increase in IL-10, IL-4 and IL-5 levels [29,42,43]. These effects were prevented by β -adrenoceptor antagonist and IL-12. In one of these studies [43], salbutamol, a β2 adrenoceptor agonist, inhibited IL-12 production but not IL-1α and β, IL-6, or IL-10 in IFN-γ-primed LPS-stimulated monocytes or whole blood cultures. In addition, β2 adrenoceptor agonists seemed to inhibit neonatal T cells to differentiate to Th1 cells but promote Th2 differentiation [43]. Moreover, administration of β2-adrenoreceptor agonists in healthy volunteers elevated plasma IL-6 [44], suppressed the production of IL-12 from IFN-γ-primed LPS-stimulated whole blood [43] and a massive release of catecholamines following acute brain trauma increased IL-10 levels [45]. Furthermore, the α adrenoceptors are also involved in cytokines alteration. *In vitro* α2 adrenoceptor agonists decreased IFN-γ and IFN-γ/IL-10 ratio in PHA+LPS stimulated whole blood cultures [46]. However, administration of α2 versus β-adreceptor agonists in LPS-treated mice gave contrasting patterns on IL-10 plasma levels. β-adreceptor agonists or α2 adrenoceptor antagonists increased IL-10 plasma whereas α2 adrenoceptor agonists or β-adrenoceptor antagonists decreased IL-10 plasma levels [47]. These results could be explained by the fact that LPS does not induce the production of IFN-γ, and the only source of IL-10 in LPS-treated whole blood or PBMC is monocytes [31,32]. Therefore α and β- adrenoceptors expressed on monocytes/macrophages play different roles on IL-10 induction [47]. However, brief exposure to epinephrine (2 h) in rats increased DTH [39] and found to be mediated by local increase in IFN-γ production [40].

Prolactin is another hormone, which has an important role in immune regulation [48,49]. A great deal of evidence suggests that lymphocytes and immunocompetent cells from thymus, spleen and peripheral lymphocytes contain prolactin mRNA and these cells release a bioactive prolactin which is similar to pituitary prolactin [49–51]. Furthermore, it has been shown that lymphocytes contain dopamine receptors, such as D4 and D5, which may be involved in regulation of prolactin release from lymphocytes [52,53]. It has been shown that lymphocyte proliferation and macrophage activation is reduced by either antibodies against prolactin or suppression of prolactin release from the pituitary [48,54]. However, the proliferation of lymphocytes and IL-2 production from hyperprolactinemia patients was decreased [56]. *In vitro*, prolactin enhanced IFN-γ activity from PBMCs and NK cells [55], and increased IL-12 and IFN-γ productions from PHA+LPS -stimulated whole blood [32]. The latter was not seen in LPS-stimulated whole blood, however, it demonstrated an increase in IL-10 levels, which indicates that prolactin effects on cytokines induction is stimulus specific [32].

3. Mental Stress Models

In this review, the stress models are categorized as a brief mental stressor, one written exam and multiple exams and the time of testing the stressed individuals. For a brief mental stress (i.e. minutes of mental stress), a speaking stressor or laboratory (Stroop test) or solving a difficult puzzle stress tests were used. For a written examination (i.e. hours of mental stress), some researchers used the time 24h prior to one exam to test the stressed individual, others used 0.5–1h (just) before examination, immediately after examination or 24–48 h following an exam. The last two models described in the literature were during examinations period and 48-h post examinations period (i.e. days of mental stress).

All the studies presented here were performed on college students (or otherwise stated). The parameters tested were compared between blood samples drawn at the "stressed" time (stressed samples) to pre- and/or post levels (baseline samples). For written examination, the pre-stress samples were drawn at the

beginning of a semester or 2–4 weeks before examination. The post-stress samples were drawn 10–30 days following examination.

3.1 Effect of Mental Stress on Cortisol Levels

Minutes after beginning a laboratory stressor test or oral exam, adrenocorticotropic hormone (ACTH) and cortisol levels rose significantly when compared to levels just before examination. However these values went back to baseline after the exam was finished [57,58]. It has to be mentioned that the increase in cortisol was delayed few minutes after increase in ACTH, which indicates a descending activation of HPA. Furthermore, twenty-four hours prior to examination, cortisol levels were either not changed (also in students who have high stress scores) [59] or significantly higher [60]. In this respect, the release of cortisol depends on the time of testing and magnitude level of the stressor [61]. Also, there are students' variations regarding exam preparation and time of anxiety, therefore, in some cases drawing blood 24 h prior to exam may be early to see an endocrine effect because the elevation of cortisol is transient and might be missed in non-kinetic studies. This was clearly observed when blood was drawn immediately before examination where cortisol levels significantly increased [61–63] and became even higher immediately after the exam [63]. In addition, urine excretion of cortisol was increased during and immediately after a 6-h exam and was more in males than females [64,65]. During examinations period, the average ACTH levels from male students were significantly increased (38%, 1 pmol/L) during the day of the fall time but not the spring-time [66]. This increase, however, was not sufficient to increase cortisol levels. The adrenal sensitivity to ACTH in dogs was found that a 2 pmol/L change in ACTH increased cortisol level by 55 nmol/L [67] and ACTH stimulation (1 mg) in humans significantly increased cortisol levels (~30 nmol/L) after one hour of injection [68]. In Malarkey et al [66] study, however, when students were categorized according to their stress scores, the average cortisol level significantly increased (~28 nmol/L) during examination periods which was accompanied with ~1.4 pmol/L increase in ACTH. This study suggests that a strong stimulation and or the less coping behavior of the responder is of critical importance in determining the endocrine profile to stress. Furthermore, during examinations period, also a significant increase of cortisol levels was observed in female students [22]. These results indicate that minutes, hours or days of mental stress stimulate HPA axes and resulted in increasing cortisol level. This increase in cortisol levels, however, was not seen days after ending examinations period [21,69]. The latter suggest that cortisol levels subsides when the stressor ends (half life of cortisol 1–1.5 h) or such studies are in need of collecting urine for period of time (6–12 h) to detect differences in cortisol values.

3.2 Effect of Mental stress on Catecholamines Levels

Minutes after beginning a laboratory stressor test or oral presentation, levels of catecholamines (epinephrine and norepinephrine) increased significantly when compared to levels just before examination [58,70,71]. However these values went back to baseline after the exam was finished [58,70,71]. Furthermore, during an examination plasma and urine catecholamine levels were also increased [64,65,72,73]. In addition, Powlak et al [71] demonstrated an increase in β2-adrenoceptors on PBMC following a 10-min stress. These studies also showed that epinephrine excretion was higher in males more than females [64,65,72]. Overall, these results showed that minutes or hours of mental stress are able to stimulate SAM and thus catecholamine levels are increased. Feeling of success and confidence, however, were more common in males than females and high discomfort was correlated with poor performance in males but with good performance in females [64,65,72]. These results suggest that coping behavior adopted by males and females during stressful situations are different. Males seemed to be more dependent on SAM as well as HPA activation when confronted with challenging situations. However, females tend to be more sensitive to sympathetic stimulation than men [64].

3.3 Effect of Mental stress on Prolactin Levels

Prolactin plasma levels were also studied following mental stress. Just before examination, male students showed a significant increase in prolactin levels but no change was seen in female students when compared to baseline levels [14]. However, immediately after an exam and in males only prolactin levels were significantly less than baseline levels. In addition, Meyerhoff *et al.* [57] have shown that minutes after beginning an oral examination, prolactin levels were increased in young males when compared to values just before examination. This increase, however, went back to baseline levels after the exam was finished. During and after examinations period, prolactin levels in males did not change when compared to baseline levels [69,74], however, in female students prolactin levels showed a tendency to increase [22]. These results indicate two things: first, males and females behaves differently in prolactin secretion upon response to stress which could be due to differences in prolactin regulatory secretions though tuberoinfundibular dopaminergic system such as dopamine, norepinephrine, serotonin, endorphins, estrogen and prolactin itself [75]. Second, the differences in stress duration (one exam versus examinations period) may enable other hormones or peptides, like estradiol, to cause significant change in prolactin level especially in female students [75]. More studies regarding the effect of mental stress (short versus long times of mental stress) on prolactin in both sexes are mandatory to establish a link between student behavior during such stress and prolactin levels.

3.4 Effect of Mental Stress on the Induced-Cytokines Level

The effect of mental stress on cytokines production from mitogen-stimulated blood cells either using PBMCs or whole blood was studied. Twenty-four hours prior to examination, Maes et. al. [76] reported that mitogen-stimulated whole blood at 24 h prior to examination significantly produced higher levels of IFN-γ, TNF-α, IL-6, and IL-10 than baseline. However, when students were divided according to stress perception and anxiety, subjects with higher stress perception and anxiety showed even higher production of IFN-γ, TNF-α and IL-6. However, mitogen-stimulated blood from students with low anxiety scores produced significantly higher levels of IL-10, IL-5 and IL-4 than from students with high anxiety. In other words, the results of Maes et al [76] suggest that students who are responding to stress and anxiety show a proinflammatory response and those students who are capable to cope with such stress or less anxious show a Th2 response. In a similar model, Guidi et. al. [60], reported a significant reduction in lymphocyte proliferation and IL-2 production and an increase in cortisol levels. Just before examination, however, it was found that production of IL-1α, IL-10 and IL-6 from mitogen stimulated whole blood was increased, IFN-γ level was decreased, and no change in TNF-α production [77]. A similar effect immediately after examination was seen when stimulated monocytes produced significantly higher levels of IL-1β when compared to baseline samples but stimulated PBMCs produced significantly less IFN-γ. These changes in IL-1α and IFN-γ returned to baseline values within 10 days [78]. Twenty four hours after an exam, Uchakin *et al.* [79] reported that the percentage of IL-2 producing cells (CD4+ and CD8+) and CD8+ IFN-γ cells and IL-2 production levels was significantly lower than in non-stressed samples but no change in IFN-γ and IL-10 productions were observed.

During examinations period, IFN-γ mRNA, IFN-γ [16,17], IFN-γ (only from PBMC but not whole blood) and IL-2 [80] productions were less in stressed samples, IL-4 and IL-5 did not change [80], but higher IL-6 levels were observed when compared to baseline values [80]. From adolescence students (~16 y), examinations period reduced IL-4 and IL-5 productions in healthy but not in asthmatic subjects [81]. Furthermore, Marshall *et al.* [21] studied synthesis and release of IFN γ and IL-10 form stimulated PBMCs 48 h post examination periods and found a significant increase (87%) in IL-10 production but insignificant decrease in IFN-γ and was correlated positively to number of hassles. Subjects who reported more hassles and greater subjective adjustment to hassles at pre-exam had higher IL-10 levels and lower IFN-γ/IL-10 ratio at both pre exam and 48 h post examinations period [21]. The above results may indicate that the coping behavior or health status (e.g. asthmatic) of the individual at baseline has a major effect on immune changes observed during the stress period [21,76,81]. Individuals who were at baseline (pre-stress) shifting toward Th2 response (high hassle group or asthmatic) will be less responsive to stress than those individuals who have a predominant Th1 response (low hassle group or healthy individuals). In addition, students who are less anxious before examination produce more of a TH2 response [76].

These results suggest that cytokines production pattern is altered prior to, after one exam, during and post examinations period. Proinflammatory cytokines levels such as IL-1β and IL-6 are increased in almost all the mental stress conditions [76–78,80]. IL-1β and IL-6 are produced from immune and non-immune cells and in the studies mentioned here, their increasing levels are mainly from mitogen-stimulated monocytes. For TNF-α, the inconsistent results could be due to TNF genes polymorphism between individuals. However, the pattern of Th1 versus Th2-induced cytokines seemed to be different depending on when the blood sample was drawn, age of the students examined and in the students who reacted with such type of stress (high perceived stress scores, anxiety or hassles). The discussion below will provide an explanation of how transient activation of SAM and/or HPA resulted from brief stress will lead to increase in proinflammatory cytokines, which may be related to Th1 response. On the other hand, days of mental stress results in consistent activation of SAM and HPA which will lead to Th2 shift.

3.5 Minutes Versus Days of Mental Stress

Many important issues have emerged while writing this review. First, does brief mental stress (minutes) or 24 h prior to one major exam induce a challenge to the individual and more likely be a preparation of the stressed or the challenged individual to cope with the stressor? This is suggested because minutes of stress or preparation to exam caused transient increase in NK cells [73, 82–85], increase in CD8+, CD2+CD26+, CD2+HLADR+ cells [62] increase in IFN-γ, TNF-α, IL-6, IL-1 and IL-10 production from stimulated immune cells [76,77], and increase in S-IgA [63,86,87] (Table I). Similarly, a brief restrained stress (2h) in animal skin hypersensitivity (DTH) model enhanced skin immune function by increasing drainage of T lymphocytes from lymph nodes and increased local IFN-γ, which was abrogated by adrenalectomy [39,40]. In the latter condition, a similar pattern was seen when rats were exposed to low levels of cortisol or epinephrine. In addition, brief exposure of PBMC to glucocorticoids caused an initial increase in IFN-γand IFN-γ/IL-10 ratio [34]. A very recent work has shown that a 15-min of laboratory stressor test increased ACTH, cortisol, epinephrine and norepinephrine [58]. This was paralleled with an increase in redox-sensitive NF-kB induction from PBMC. This increase in NF-kB activity went back to normal within 60 minutes as well as the hormones. Subjects who did not show any stress-dependent increase in stress hormones did not induce NF-kB binding activity indicating that the latter response depends on the response to psychological stress [58]. Thus, timing and duration of stress may significantly affect the nature, enhancing or suppressing, of stress influence

Table I. The immune parameters outcome following short mental stressors (minutes) or during preparation to an exam:

Immune parameter	Outcome	References
Antibodies:		
S-IgA (Saliva)	Increased	**63, 86, 87**
Immune Cells:		
Total leukocytes	Increased	**59, 83**
Total lymphocytes	Increased	**59**
Monocytes	Increased	**59**
NK cells	Increased	**71, 82, 83, 84, 85**
CD8 +	Increased	**59, 83, 84, 85**
CD4/CD8 ratio	Decreased	**59, 83 84, 85**
CD2CD26+	Increased	**59**
CD2+	Increased	**59**
CD2+HLADR+	Increased	**59**
Transcription Factors		
NF-kB	Increased	**58**
Cytokines:		
IFN-γ	Increased	**76**
IL-6	Increased	**76**
TNF-α	Increased	**76**
IL-10	No change*	**76**
IL-4	No change*	**76**
IL-5	No change*	**76**

* See text for more details

Table II. The immune parameters during or after days of mental examination.

Immune parameter	Outcome	References
Antibodies:		
S-IgA (Saliva)	Decreased	**94, 95, 96, 97, 98, 99**
EBV IgG	Increased	**15, 18, 19, 23, 100**
EBV IgG (Saliva)	Increased	**101**
EBV IgA (Saliva)	Increased	**101**
Immune Cells:		
Total leukocytes	No Change	**22**
Total lymphocytes	Decreased	**22, 62**
Monocytes	No Change	**22, 62**
NK cells	Decreased	**94**
Cytokines:		
IFN-γ	Decreased	**16, 17, 21, 77, 78**
IL-2	Decreased	**80**
IL-6	Increased	**77, 78, 80, 81**
IL-10	Increased	**21**
IL-1β	Increased	**78**
TNF-α	No Change	**77**
IL-4	No Change	**80, 81**
IL-5	No Change	**80, 81**
IL-5 (sputum cells)	Increased	**109**

on the immune system. The low concentration and or exposure time of stress hormones (catecholamines and cortisol) may enhance immune function by informing the immune system about the impending challenges such as infection [88]. Further studies are needed to prove if brief mental stress protects individuals from infections, e.g. respiratory.

Second, if catecholamines, cortisol and prolactin increasing levels during different times of mental examinations are concomitant then the effect on leukocytes, leukocyte subsets, Th1 and Th2 mediated responses would be different. Each of the mentioned hormones has different effects on leukocytes distribution, lymphocyte responses upon challenge and Th1 and Th2 cytokine release. In addition, catecholamines may enhance the intensity of the cortisol signal by increasing either cortisol receptor activity [89] or the transfer of occupied glucocorticoid receptor or transcription factors to the nucleus [90,91], and cortisol might either induce an effect on β2 adrenergic receptor expression on the immune cells surface to increase the number of receptors available for stimulation [92]. Prolactin, on the other hand, reverses suppression of IFN-γ and IL-12 production induced by cortisol [32]. Therefore, the ratio of catecholamine/cortisol, catecholamines/prolactin, cortisol/prolactin would be an important factor to measure in mental stress.

Third, the stressed individual during days of examinations period is more likely to feel more threatened and the potential of loss is higher [24,25]. Furthermore, stress from examinations period seemed to induce a Th2 mediated response upon immune cells challenge (Table II). It may be proposed that these immune effects reported in these studies are attributable to increased and/or prolonged exposure to endogenous cortisol and catecholamines. As it has been shown that prolonged stress (days) shifts the cytokine response towards Th2 mediated response and thus cell-mediated immunity is dysregulated. This dysregulation increased susceptibility to viral infection as it was found that the rate of respiratory infection (e.g. common cold), increased in a dose-response manner with increases in the degree of psychological stress in individuals who had been intentionally exposed to rhinoviruses [93]. During examinations period, NK cell activity was reduced [94] and S-IgA secretions and concentrations were lowered [95–99]. This reduction of S-IgA remained for 6–14 days following examination period [97,98]. The above studies correlated lower S-IgA secretion rate and concentration to high stress [95,96] and strong power motives especially with prolonged increase in salivary norepinephrine [86]. In addition, students with social support or characterized to maintain warm personal relationship had consistently higher S-IgA at baseline (pre stress) and also at stress periods than those with less social support indicating that social support might enhances health outcomes irrespective to stressful experiences [95,96].

Furthermore, latent herpesvirus reactivation also indicates dysregulation of Th1 mediated response. Several studies showed that specific IgG concentrations against EBV viral capsid antigen (VCA) were increased during examinations period [15,18,19,23,100]. Similarly, a recent study has shown a significant increase in salivary IgG and IgA against EBV during examination period [101]. In addition, the effect of mental stress on specific IgG concentrations against CMV and HSV1 and HHV-6, was also studied [15,99,23] but failed to

show a significant increase in viral-specific antibody concentration. The increase in IgG specific antibodies against latent herpes viruses indicates latent viral reactivation. Even though this might not be a complete viral reactivation [18], it is still seen more in EBV and not other latent herpesviruses. It has to be mentioned that *in vitro* studies were almost always successful in showing reactivation of herpesviruses following exposure with pharmacological doses of cortisol [102–106], catecholamines [107], and both cortisol and catecholamines [108], but many *in vivo* studies, such as stress studies, failed to show such reactivation. It is evident that where the virus becomes latent (cell type), hormonal influence (e.g. ↑cortisol, ↑catecholamines, ↓ prolactin), cytokines (e.g. ↓IFN-γ and ↑IL-6), mechanism of replication, immune control and other factors play an important role in latent viral reactivation. For example, even though not statistically significant, the percent change in EBV VCA IgG levels showed a negative correlation ($r = -0.457$) with the percent increase in prolactin levels during examination stress [23]. The latter means that stress-induced prolactin might help in controlling viral reactivation. Further studies are needed to understand the mechanisms of stress on latent viral reactivation.

In allergic and asthma responses, the inflammatory response is mediated by Th2 cytokines, particularly, IL-4 and IL-5. Recent studies suggest that catecholamines directly affect Th1 and not Th2 cells cytokine production and function through β2 adrenergic receptor that are expressed on Th1 cells [41]. Catecholamines and β2 adrenergic receptor agonists inhibited IFN-γ production from Th1 cells and increased IL-10, IL-4 and IL-5 production from Th2 cells [42,43]. Also, cortisol suppresses Th1 cytokines production and may even induce an increase in Th2 cytokines depending on stimulatory signals to T cells [31–36]. Therefore, if mental stress (examinations period) can constantly elevates catecholamine and cortisol levels and these hormonal concentrations can decrease IFN-γ, increase IL-4 production (in low stressed individuals) and induce B cell responsiveness to produce IgE, then does long-term effect of stress increase vulnerability to allergies? It has been shown that examination period did not exhibit an exam–related drop in lung function in healthy or patients with asthma [94]. However, when sputum samples were collected during hours of examination stress, eosinophils and eosinophils-derived neurotoxin and IL-5 levels increased significantly [109]. Thus, examination stress may act as cofactor to increase the airway inflammation and a local Th2 mediated response especially in those individual who become exposed to a virulent respiratory pathogen at the time of stress, or in individuals with poor managed asthmatic or under chronic type of stress. This opens a challenge for future studies to resolve such common abnormality under also common psychological challenge.

4. Conclusions

The above review describes the modulations in the immune system following minutes, hours and days of mental stress, which is considered as subacute to acute type of stressor. A chronic type of stressors, as caregiving of persons with dementia, also caused a shift from Th1 to Th2 response by demonstrating an increase in IL-10+ CD4 and CD8 T cells [110]. This shift was also negatively related to age i.e. the pattern of change was higher with younger than older caregivers, which indicates an age related influence on Th1 to Th2 shift. In addition, parents of cancer patients were found to be resistant to glucocorticoids to suppress *in vitro* production of IL-6, one of the proinflammatory cytokines [111].

In subacute or acute mental stress, the following points are concluded: 1) minutes of mental stress induces the production of proinflammatory cytokines (a Th1 like response) via a mild and transient increase in catecholamines and cortisol, 2) a strong stimulation, coping behavior and adjustment of the responder is of critical importance in determining the endocrine profile to stress, 3) males seemed to behave differently than females during or following mental stress and thus SAM as well as HPA activation to induce catecholamines, cortisol or prolactin, respectively, are different when confronted with challenging situations especially with long term stress. 4) coping behavior (high versus low anxiety or hassle and health status) of the individual at baseline (prior to stress) have a major effect on immune changes observed during the stress period, 5) hours of mental stress may cause shift in Th1/Th2 ratio however, this depends on multiple variables such as the amount of successful coping and adjustments to the stress, 6) the dysregulation to Th2 mediated response is more evident in days or longer of stress.

The effects of stress on neuroendocrine and immunity are undoubtedly complex. Evidence from mental stress models suggests that such stress can induce changes in the SAM, endocrine and immune systems. However, whether these changes are only unidirectional (SAM-Immune or HPA-Immune), or bi-directional is still far from clear. Further studies in mental stress should provide a rational on how individual state of mind/SAM/HPA/immune system interacts. Furthermore, studies on stress and common heath problems are necessary to increase our knowledge and understanding of the mechanisms responsible for producing neuroendocrine-induced immune changes in health and common diseases.

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