What caused lymphopenia in SARS and how reliable is the lymphokine status in glucocorticoid-treated patients?

N.S. Panesar *

Department of Chemical Pathology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

Summary   Severe Acute Respiratory Syndrome (SARS) outbreak in 2002–03 caused morbidity in over 8000 individuals and mortality in 744 in 29 countries. Lymphopenia along with neutrophilia was a feature of SARS, as it is in respiratory syncytial virus (RSV) and Ebola infections, to name a few. Direct infestation of lymphocytes, neutrophils and macrophages by SARS coronavirus (CoV) has been debated as a cause of lymphopenia, but there is no convincing data. Lymphopenia can be caused by glucocorticoids, and thus any debilitating condition has the potential to induce lymphopenia via stress mechanism involving the hypothalamic–pituitary–adrenal axis. Cortisol levels are elevated in patients with RSV and Ebola, and cortisol was higher in SARS patients with lymphopenia before any steroid therapy. Glucocorticoids also down-regulate the production of proinflammatory lymphokines. Because of the insidious presentation, SARS was treated with antibacterial, antiviral and supra-physiological doses of glucocorticoids. Treatment with glucocorticoids complicated the issue regarding lymphopenia, and certainly calls into question the status of lymphokines and their prognostic implications in SARS.

Introduction

The outbreak of severe acute respiratory syndrome (SARS) in November 2002 led to infection in over 8000 individuals in 29 countries, and by August 2003 had resulted in 774 deaths worldwide. In Hong Kong 1755 individuals were diagnosed with SARS of whom 299 died. The agent causing SARS was found to be a RNA coronavirus (CoV), aptly named SARS-CoV [1], a member of coronaviridei which causes gastrointestinal, respiratory and systemic diseases in animals. Some members, OC43 (HCoV-OC43) and HCoV-229E have long been known to cause common cold in human beings. SARS-CoV caused an influenza-like syndrome of malaise, rigors, fatigue and high fevers, which progressed to atypical pneumonia, with shortness of breath and poor oxygen exchange in the alveoli. Respiratory insufficiency led to respiratory failure, which was the most common cause of death. The condition was empirically treated with a combination of antibacterial, antiviral and supra-physiological doses of glucocorticoids [2,3]. Lymphopenia along with

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* Tel.: +852 2632 2333; fax: +852 2636 5090.
  E-mail address: nspanesar@cuhk.edu.hk

neutrophilia were common features in SARS patients [4,5].

Respiratory syncytial virus (RSV), a human paramyxovirus, is the most important cause of viral lower respiratory tract disease in infants and children world-wide. The disease too is characterized by lymphopenia (and neutrophilia), which is more severe in children admitted to intensive care unit [6,7]. Lymphopenia (and neutrophilia) has also been reported in Ebola [8]. Thus there seems to be a connection between viral infections and lymphopenia, and the question is whether the viruses directly cause it. With regard to SARS, a review in Nature Reviews Immunology [9] highlighted a study of Gu et al. [10] as the one which ''indicates that SARS-CoV infection of lymphocytes is a crucial determinant of disease outcome''. However of the 22 SARS patients whose white blood corpuscles were studied for SARS-CoV, the virus was detected in only six patients and in only 51.5% of the lymphocytes [10]. The prevalence rate of 27.3% statistically does not support the contention that SARS-CoV infection of lymphocytes determines the disease outcome, let alone cause lymphopenia as authors claimed. Moreover, whilst the SARS patients who had not received exogenous glucocorticoids exhibited lymphopenia, the lymphocyte count decreased further in patients who were given steroids [10]. This certainly suggests glucocorticoids had a role in the development of lymphopenia. The lymphopenia in the former group may just have been a prognosticator of the stress response, involving the hypothalamic—pituitary—adrenal (HPA) axis [11]. An intact HPA axis is capable of secreting 225—440 mg of cortisol under extreme stress, and achieving blood levels in the range 830—7220 nmol/L [12].

Viral infections and the stress response

Simply put, an invasion of a mammalian body by a foreign organism such as a virus activates a host's immune system [9]. The initial invasion triggers a pro-inflammatory response involving cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1α, IL-1β, interferon-γ, IL-2, IL-3, IL-5, IL-6, IL-8, IL-12, and granulocyte-macrophage colony-stimulating factor, which attract neutrophils and macrophages to the site of infection. This is subsequently countered by the anti-inflammatory response, involving cytokines IL-4, IL-10, and IL-13, naturally circulating antagonists such as the IL-1 receptor antagonist (IL-1RA) and two soluble TNF receptors, and cortisol [8—12].

Viral infections such as SARS, RSV and Ebola are debilitating diseases, and would inevitably lead to the activation of the HPA axis and the secretion of cortisol [12]. In children with RSV infection, blood cortisol was significantly higher during the active phase (550—650 nmol/L) than during convalescent phase (300 nmol/L) a few months later [13]. Cortisol ranged from 555 to 1110 nmol/L in seven asymptomatic patients with Ebola infection compared to 133—377 nmol/L in 10 endemic control subjects [8]. In SARS, lymphopenia (and neutrophilia) was more prevalent in patients with higher prevailing cortisol, before any steroid therapy had been commenced [14], which probably reflected the integrity of HPA axis and stress response [11,15]. However, the insidious presentation of SARS led to its empirical treatment with antibacterial, antiviral and supra-physiological doses of glucocorticoids [2,3]. The latter would thus have had a profound effect on circulatory lymphocytes and lymphokines in SARS patients.

Glucocorticoids and lymphocytes

The anti-inflammatory actions of glucocorticoids have been known since the 1940s, and in mid 1950s they found use as cytotoxic agents in the treatment of haematological malignancies, long before the term apoptosis became associated with them [16]. Around this time it was also shown that glucocorticoids changed the circulatory behaviour of lymphocytes, particularly T lymphocytes [17]. Glucocorticoids induced lymphopenia within 4 h of steroid administration [18]. Even under normal situation lymphocytes exhibited a pattern that was inversely correlated with the cortisol diurnal rhythm [19], a pattern that was absent in patients with adrenal insufficiency and duly restored with physiological doses of exogenous steroids [20]. In depressed patients who generally have hypercortisolaemia, lymphopenia and neutrophilia are common [21]. Although glucocorticoids acutely drive the T lymphocytes out of the peripheral circulation [22], they ultimately initiate apoptosis in these cells [23]. Intriguingly, while glucocorticoids cause apoptosis in lymphocytes, they inhibit the process in neutrophils [24], hence the probable reason for simultaneous occurrence of lymphopenia and neutrophilia.

Glucocorticoids and lymphokines

Several hypotheses have been proposed regarding the role of cytokines and chemokines in the aetiol-

ogy and prognosis of SARS [25–28]. Essentially all studies reported the up-regulation of same chemokines, namely macrophage inflammatory protein 1α (MIP-1α), regulated on activation normal T cell expressed and secreted (RANTES), interferon-inducible protein 10 (IP-10), and monocyte chemoattractant protein 1 (MCP-1). However, these studies were performed in SARS patients who were treated with glucocorticoids at supra-physiological doses, e.g., 0.1–1 mg/kg/day oral prednisolone. More severe patients with dyspnoea were further given intravenous infusions of 100 mg hydrocortisone every 8 h and 500 mg methylprednisolone (MP) pulses [3] (see [2] for other glucocorticoid dosage regimen). One study debated the T helper 1 (Th1) cytokine and chemokine status in SARS patients subdivided into glucocorticoid ''not treated'' or ''treated'' groups with regard to pulsed steroid only, and claimed corticosteroid treatment lowered IL-8, MCP-1 and IP-10 concentrations after 5–8 days [25]. However the authors failed to mention that the ''not treated'' patients had also received the initial steroid therapy. Zhang et al. [29] reported increased IL-6 and decreased IL-8 and TGF-β in SARS patients, and further concluded that steroid treatment had no effect on the levels of eight cytokines in both less and more severe SARS patients. However, this conclusion was based on wrong statistical analysis used. Closer examination of their IL-6 data in untreated more severely sick SARS patients, presented as mean ± SEM, clearly was non-Gaussian, and yet the authors used a parametric unpaired ''t'' test for the statistical comparison between steroid treated and untreated SARS patients. Despite their erroneous conclusions, both less and more severely sick SARS patients treated with steroids had lower IL-6 levels. Another study which used corticosteroids primarily to dampen the local proinflammatory cytokine production believed to cause lung immunopathology [27], erroneously hypothesized up-regulation of chemokines in the SARS-CoV infected dendritic monocytes. It must be appreciated that systemically administered glucocorticoids do not just act locally, but have wider reach.

Not only do supra-physiological doses of glucocorticoids inhibit cytokine production [30,31], even the daily physiological variation in blood cortisol impacts inversely on the levels of IFN-γ, TNF-α, IL-1α and IL-12 during the day [19]. The administration of 25 mg cortisone acetate (equivalent to the daily adrenal cortisol output) at 21 h that raised blood cortisol to 450–676 nmol/L physiological range, promptly depressed all four cytokines within an hour, followed by a decrease in CD3+, CD4+, CD8+ and CD56+ lymphocytes by 2–3 h [19]. Given that physiological levels of cortisol had such profound effect in down-regulating the proinflammatory cytokines (and affecting the trafficking of lymphocytes), it is not difficult to envisage the effects of supra-physiological doses of exogenous glucocorticoids on lymphokine production.

The use of exogenous glucocorticoids in SARS patients with acute respiratory distress syndrome (ARDS) was criticised [32] and they were certainly implicated in a patient developing a fatal secondary aspergillosis infection [33]. Jiang et al. while promulgating the elevations of proinflammatory IL-6, IL-8, and MCP-1 as the sign of superinfection and increased risk of death in SARS [26], overlooked the fact that patients in their study, with or without secondary infections had received 162.5 ± 116.3 or 105.8 ± 78.7 mg MP per day, respectively. MP is five times more potent than cortisol and would have left the patients immunocompromised, vulnerable to secondary infections. The same treatment should also have down-regulated the proinflammatory cytokines [19,30,31], but mysteriously did not. Lymphokines can be produced by any nucleated cell in the body [34], and moreover the dogma confining their production to any particular immune cell, e.g., Th1 vs. Th2 has been challenged [35].

Conclusion

A serious condition such as SARS should have triggered a stress response involving the HPA axis and the secretion of cortisol. Cortisol would have caused lymphocytes to migrate out of the peripheral circulation initially, and later caused their demise via apoptosis. However, the administration of exogenous glucocorticoids complicated the whole issue regarding the cause of lymphopenia (and neutrophilia) in SARS. But all indications are that glucocorticoids, whether endogenous or exogenous were the main arbiters of lymphopenia (and neutrophilia). Secondly, in a setting where the immune system must have been severely compromised by exogenous glucocorticoids, the up- and down-regulation of various lymphokines and their prognostic implications for SARS, should be interpreted with caution [36].

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