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MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile

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ABSTRACT

The Middle East respiratory syndrome coronavirus (MERS-CoV) has been recognized as a highly pathogenic virus to humans that infects the respiratory tract and is associated with high morbidity and mortality. Studies in animal models suggest that MERS-CoV infection induces a strong inflammatory response, which may be related to the severity of disease. Data showing the cytokine profiles in humans during the acute phase of MERS-CoV infection are limited. In this study, we have analyzed the profile of cytokine responses in plasma samples from patients with confirmed MERS-CoV infections (n = 7) compared to healthy controls (n = 13). The cytokine profiles, including Th helper (Th) 1, Th2 and Th17 responses, were analyzed using cytometric bead array (CBA). A prominent pro-inflammatory Th1 and Th17 response was clearly seen in patients with MERS-CoV infection, with markedly increased concentrations of IFN-γ, TNF-α, IL-15 and IL-17 compared to controls. IL-12 expression levels showed no difference between patients with MERS-CoV infection and the healthy controls despite the significantly increased levels of IFN-α2 and IFN-γ (P < .01). No changes were observed in the levels of IL-2, IL-4, IL-5, IL-13, and TGF-α (P > .05). Our results demonstrate a marked pro-inflammatory cytokine response during the acute phase of MERS-CoV infection in humans.

1. Introduction

The Middle East respiratory syndrome (MERS) was first discovered in 2012 in the Kingdom of Saudi Arabia [1,2]. MERS is caused by a single-stranded RNA virus named MERS-corona virus (MERS-CoV) that belongs to the genus Beta coronavirus [3]. It is believed that the virus enters the cells through fusion with its plasma membrane. This is achieved when the viral envelop protein S (S protein) binds to dipeptidyl peptidase 4 (DPP4, also known as CD26) as the host receptor, which is abundantly expressed in the lung tissue of mammals [4–6].

According to January of 2018 data from the European Centre for Disease Prevention and Control, 2122 cases of MERS infection leading to 740 fatalities have been documented worldwide. This indicated a mortality rate of about 39% that was associated with patients who have medical co-morbidities [7–9]. The majority of cases were reported in the Middle East countries, particularly Saudi Arabia [3,7]. More recently, a MERS outbreak in the Republic of Korea in 2015 caused a total of 186 confirmed cases with a mortality rate of about 20% [10]. Cases have also been documented in other Asian countries (e.g. Iran, Bangladesh and Malaysia), North Africa (e.g. Egypt, Tunisia, and Algeria), Europe (e.g. United Kingdom, France and Germany) and USA [11–17].

An earlier study in Saudi Arabia by Briese et al. [18] showed that the whole genome sequence of viruses obtained from humans and camels were almost identical. It was suggested that the virus is transmitted to humans via direct contact with animals, especially dromedary camels in the Middle East [14,19–22]. Human-to-human transmission is usually via direct contact with affected individuals and is higher among household and in healthcare settings [23–25]. Commonly reported symptoms of MERS-CoV infection included cough, fever, breathing difficulty, sore throat, headache, vomiting and diarrhea [7,26]. Some patients may develop acute respiratory failure and acute kidney injury, which are considered as the major complications of the disease [27,28].

Laboratory data associated with MERS included leucopaenia, lymphopaenia, disseminated intravascular coagulation, elevated blood creatinine, elevated lactate dehydrogenase and disturbed liver enzyme levels [29–31]. Viral particles are enriched in respiratory samples while...
low levels were detected in other body fluids, including blood, urine and stool samples [32]. In this study, we have analyzed the profile of cytokine responses in plasma samples from patients infected with MERS-CoV and show a prominent Th1 and Th17 cytokine profile consistent with a pro-inflammatory response during the acute phase of MERS-CoV infection.

2. Material and methods

2.1. Patients and samples

Blood samples were collected from seven patients (all males, age range between 24 and 49 years) that were admitted to MOH hospitals with an acute onset of “flu-like” symptoms in 2014. Approximately, all patients were presented with fever, chills, cough, and dyspnea. Three patients were suffering from myalgia and two were having gastrointestinal pain. One patient was admitted to the intensive care unit, however, ventilation therapy was not required in his case. Age and gender matched healthy individuals were recruited from the blood bank to serve as the healthy control group.

2.2. Inclusion and exclusion criteria for MERS-CoV patients

Initial diagnosis of MERS-CoV infections was established based on clinical presentation and examination according to the criteria set by World Health Organization recommendations (WHO, 2015). Nasopharyngeal swabs were obtained from patients and healthy controls using viral transport media kits obtained from Hardy Diagnostics (Santa Maria, CA, USA). The diagnosis of MERS-CoV infection was confirmed by real-time polymerase chain reaction (qRT-PCR) using swab specimens as previously described [24]. Patients with other acute or chronic diseases, or infections with other agents were excluded from the study.

2.3. Ethical approvals

The study was approved by the ethical and research committee of the College of Applied Medical Sciences (MLT 2017001), and written informed consents were obtained from all participants.

2.4. Blood samples

Patients with confirmed diagnosis of MERS-CoV infection and who fit the inclusion criteria were included in the study. Venous blood samples from patients were collected directly when the diagnosis was confirmed (approximately 24–48 h after hospital admission) into the vacutainer EDTA tube, and were centrifuged at 4000 g for 5 min at 4 °C. The time of sampling relative to the onset of symptoms was estimated to be approximately 4 days based on the information reported by patients to physicians. Plasma samples were collected and stored at −80 °C until used.

2.5. Cytometric Bead Array (CBA) for cytokine measurements

The Cytometric Bead Array (CBA) allows for the simultaneous measurement of several analytes from a single biological sample [33]. CBA array was used in this study to measure multiple cytokines from plasma samples following the manufacturer’s instructions (BD Bioscience). The analyzed cytokines included IL-2, IL-4, IL-5, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IFN-α2, IFN-γ, TNF-α and TGF-α. Following flow cytometry (FACScalibur, Becton Dickinson), cytokine concentrations were calculated based on the standard curves using the BD CBA Analysis Software.

Fig. 1. Changes in the plasma levels of interferons in patients with MERS-CoV infection. Significant increases in the plasma levels of (A) IFN-α2 and (B) IFN-γ. Data are expressed as Mean ± SEM. * indicates significant difference.

2.6. Statistical analysis

All the calculations and statistical analysis were performed using GraphPad Prism statistical software (version 5, USA). Data were expressed as mean ± standard deviation (SD). Two-group comparisons were performed using Mann and Whitney test. P values of < .05 were considered statistically significant.

3. Results

3.1. Infection with MERS-CoV elicits a type-I and type-II interferon response

Significant increases in the levels of interferon gamma (IFN-γ) were observed in the infected patients compared to healthy controls (Fig. 1, P < .001). IFN-γ is a type-II interferon that is produced by several cell types including NK, NKT and CD4 Th1 cells. IFN-γ interferes with viral replication and plays an important role in both innate and acquired immunity [34]. Notably, there was also a marked increase in IFN-α2 concentration in MERS-CoV infected patients, compared to the healthy controls (Fig. 1, p < .01). All the 7 infected patients had a significant increase in IFN-α2 ranging from 26 to 71-fold increase when compared to healthy controls. IFN-α2 is a type-I interferon that serves as a first line of defense against viral infections, which is usually secreted by virally-infected cells to protect neighboring and adjacent cells [35].

3.2. Infection with MERS-CoV is associated with a Th1 and Th17 cytokine profile

Cytokine profiles, including Th1, Th2 and Th17 cytokines were analyzed in plasma samples from patients diagnosed with an acute phase of MERS-CoV infection as well as from healthy controls. IFN-γ (Fig. 1), TNF-α (Fig. 3) and IL-17 (Fig. 3) concentrations were markedly increased in MERS-CoV infected patients compared to healthy controls.
Interleukin 17 is a pro-inflammatory cytokine produced by T-helper cells in response to STAT3 and NF-κB signaling pathways [36]. All the patients with MERS-CoV infection had a marked increase in IL-17, IFN-γ and TNFα but not IL-12 (IL-12p40 and IL-12p70) when compared to the healthy controls. This finding suggests that MERS-CoV infection in humans activates a Th1 and Th17 pro-inflammatory cytokine profile. On the other hand, there was no significant increase in IL-4 (Fig. 2B), IL-5 (Fig. 3A), and IL-13 (Fig. 3C) concentration in MERS-CoV infected patients compared to healthy controls (p > .05). Moreover, significant increase in the levels of TNF-α was detected in plasma samples of patients (P < .01, Fig. 3E) while no change was detected with respect to TGF-α level (P > .05, Fig. 3F). TNF-α is produced by many cells, including macrophages, CD4+ lymphocytes, NK cells, lung epithelial cells and others [37]. Upon binding to its receptor, TNF-α activates NF-κB, MAPK and apoptotic pathways that interfere with viral replication and assistance viral clearance [37].

3.3. MERS-CoV infection induces the expression of IL-10

IL-10 is an important cytokine that is critical in regulating pro-inflammatory response. In this study, we showed significant increase in IL-10 concentrations in MERS-CoV infected patients compared to healthy controls (Fig. 3). IL-10 belongs to class-II cytokines and has a major anti-inflammatory effect mediated via JAK-STAT pathway [38]. Its production by monocytes and lymphocytes is triggered by pathogenic agents and involve activation of MAPK and NF-κB signaling, and post-transcriptional mechanisms such as control of mRNA stability and microRNAs [39,40].

4. Discussion

The Middle East respiratory syndrome coronavirus MERS-CoV was first described as an emerging virus in 2012. Since then, the virus has caused a number of outbreaks in many regions of the Middle East. Despite intensive efforts of many researchers, the World Health Organization (WHO) has yet to reach a common consensus on the best treatment approach [41]. This is in part due to the high fatality of the viral infection which causes an acute onset of respiratory symptoms including pneumonia and alveolar destruction [42] leading, in many cases, to death. In this study, we analyzed a panel of 13 inflammatory
markers in plasma samples obtained from seven patients diagnosed with MERS-CoV infection. Our results showed a significant elevation in IFN-α2, IFN-γ, TNF-α, IL-15, IL-17 and IL-10 when compared to the healthy group.

In our cytokine array, we examined two interferons (IFN-α2 and IFN-γ) that were significantly elevated in MERS-CoV infected patients. Type-1 interferons are produced by the interaction between certain cellular receptors with viral components including viral genome. Binding of IFN-α2 to its receptor initiates a signaling cascade that leads to an increase in the expression of many downstream signaling targets called interferon-stimulated genes [43]. The products of these genes may play a critical role in inhibition of viral spread into non-infected cells and interference of viral replication in the infected cells [44]. IFN-γ, on the other hand, is known to mediate its effect via the JAK-STAT signaling pathway leading to the subsequent activation of macrophages and NK cells, and expression of specific major histocompatibility complex proteins [45,46]. A study by Zhou et al. [47] showed that infection of human monocyte-derived macrophages with MERS-CoV induced the expression of IFN-γ and the subsequent expression of major histocompatibility complex class-I genes [47]. In addition, infection of airway epithelial cell lines with MERS-CoV induced the expression of IFN-α2, IFN-γ and TNF-α, possibly through the activation of STAT3 pathway [48]. Elevation of IFN-α2 and IFN-γ was reported from patients infected with MERS-CoV [41]. Since IFN-α2 and IFN-γ were known to promote antigen presentation and the development of a robust antiviral adaptive Th-1 immune response, the elevation of these interferons may be essential to develop immunity against MERS-CoV infection [49]. Consistent with previous findings [47], our results showed a significant elevation in TNF-α levels in response to MERS-CoV infection. Previous studies showed that TNF-α exerts a strong antiviral activity against avian, swine, and human influenza viruses [50].

Our results showed a significant elevation in IL-17A plasma levels in response to MERS-CoV infection, which is in agreement with previous findings from both in vivo [41] and in vitro studies [51]. IL-17 is known to mediate airway remodeling during respiratory infections and allergy by recruiting monocytes and neutrophils to the site of inflammation and enhancing the production of other cytokines and chemokines such as IL-6, IL-1β, TGF-β, TNF-α, IL-8 and MCP-1 [52].

Our results also showed a significant increase in the plasma levels of IL-10 in infected patients. The IL-10 expression has been shown to be associated with persistent viral infections such as HIV, HCV and HBV [53]. Elevation of IL-10 was reported in patients infected with MERS-CoV.
CoV [41]. The increase in levels of IL-10 after infection with MERS-CoV may play a role in host immune regulation in inhibiting the proinflammatory response induced by the infection.

The notion that STAT3 may antagonize the expression of certain NF-κB activated genes is not without precedence. It has been previously shown that IL-10 and IL-17 not only act as STAT3 activators, but are also upregulated by its expression [54,55]. When induced, STAT3 selectively inhibits NF-κB activated genes, such as IL-12, required for the successful activation of Th1 immune response [56]. The exact role played by STAT3 in viral infections is still to be elucidated. Some viral infections, such as in SARS CoV [41]. The increase in levels of IL-10 after infection with MERS-CoV and its receptor CD26, Nature 500 (2013) 227–231.

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