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Original Article

Differential expression of carcinoembryonic antigen-related cell adhesion molecule-5 (CEACAM5) and dipeptidyl peptidase-4 (DPP4) with detection of Middle East respiratory syndrome-coronavirus in peripheral blood

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Abstract

Background

Middle East respiratory syndrome-coronavirus (MERS-CoV) utilizes CD26 (dipeptidyl peptidase-4) and CD66e or CEACAM5 (carcinoembryonic antigen-related cell adhesion molecule 5) receptors for cell infection. Peripheral blood mononuclear cells (PBMCs) play a critical role in mounting adaptive immune response against the virus. This study was performed to assess the expression of CD26 and CD66e on PBMCs and their susceptibility to MERS-CoV infection.

Methods

Surface expression of CD26 and CD66e receptors on PBMCs from MERS-CoV patients (n=20) and healthy controls (n=20) was assessed by flow cytometry and the soluble forms were determined by enzyme-linked immunosorbent assay (ELISA). MERS-CoV *UpE* and *Orf1a* genes in PBMCs were detected by using Altona diagnostics reverse transcription polymerase chain reaction (RT-PCR) kit.

Mean fluorescent intensity (MFI) of CD66e was significantly higher on CD4+ lymphocytes (462.4±64.35 vs 325.1±19.69; p<0.05) and CD8+ lymphocytes (533.8±55.32 vs 392.4±37.73; p<0.04) from patients with MERS-CoV infection compared to the normal controls. No difference in MFI for CD66e was observed on monocytes (381.8±40.34 vs 266.8±20.6; p=0.3) between the patients and controls. Soluble form of CD66e among MERS-CoV patients was also higher than the normal controls (mean= 338.7±58.75 vs 160.7±29.49 ng/mL; p<0.01). Surface expression of CD26 on PBMCs and its soluble form were no different between the groups. MERS-CoV was detected by RT-PCR in 16/20 (80%) patients from whole blood, among them 8 patients were tested in PBMCs, 4/8 (50%) patients were positive.

Conclusion

Increased expression levels of CD66e (CEACAM5) may contribute to increased susceptibility of PBMCs to MERS-CoV infection and disease progression.

Keywords

CD66e; CD26; CEACAM5; DPP4; PBMCs; MERS-CoV; Saudi Arabia

The Middle East Respiratory Syndrome coronavirus (MERS-CoV) belongs to a group of Beta-coronaviruses that first emerged in the Middle East, especially Saudi Arabia in 2012 [1]. The virus causes severe lower respiratory tract infection with up to 30% mortality, particularly in patients with co-morbidities [2], and has led to numerous hospital outbreaks [3], [4], [5], [6], [7], [8], [9], [10]. MERS-CoV is a zoonotic infection that originated from bats [11]. Later, the virus was transmitted to humans through dromedary camels, that are believed be the intermediate hosts [12]. To date, the precise mechanism of animal to human and/or human to human transmission is not clear. With MERS-CoV circulating around the Arabian Peninsula and from there spread worldwide to 27 countries, there are currently no authorized vaccines or therapeutics presently available for clinical use [13]. The clinical presentation of MERS-CoV infection ranges from asymptomatic or mild disease, to critical illness resulting in acute respiratory distress syndrome (ARDS) and multiorgan failure, the mechanisms involved in disease pathogenesis is still not fully understood [14]. A recent *in-vitro* study has shown that the spike protein of MERS-CoV mediates infection by binding to CD26 (dipeptidyl peptidase-4 (DPP4) as well as CD66e (carcinoembryonic antigen-related cell adhesion molecule-5 (CEACAM-5)) receptors on host cells surfaces [15], [16], [17].

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multiorgan failure, the mechanisms involved in disease pathogenesis is still not fully understood [14]. A recent *in-vitro* study has shown that the spike protein of MERS-CoV mediates infection by binding to CD26 (dipeptidyl peptidase-4 (DPP4) as well as CD66e (carcinoembryonic antigen-related cell adhesion molecule-5 (CEACAM-5)) receptors on host cells surfaces [15], [16], [17]. Additionally, blocking the interaction between the viral spike protein and the cell surface CD26 or CD66e receptors with specific antibodies, recombinant proteins, or small interfering RNA (siRNA) could effectively block viral cell entry [18], [19]. CD26 is a co-stimulatory molecule involved in T cell activation, it is expressed on many cell types including epithelial cells of many organs including lungs, kidneys, thymus, intestine, liver, and activated T lymphocytes in bone marrow [16], [17], [20]. On contrary, CD66e is a member of CD66 adhesion molecules family which has been shown to be involve in cell differentiation cell survival and apoptosis, and expressed on epithelial cells as well as on leukocytes including T cells and monocytes [17], [21], [22], [23]. peripheral blood mononuclear cells (PBMCs) including lymphocytes (CD4 T cells, CD8 T cells) and monocytes play a vital role in controlling and clearing pathogens by their anti-microbial properties. They act as phagocytic and cytolytic cells. In addition, upon activation they produce various cytokines such as IL-1-α and β, IL-6, IL-2, IL-12, IL-10, and IL-18, which serve to propagate the adaptive immune responses. Moreover, they function as antigen presenting cells [24],

This study for the first time ever demonstrated that patients with MERS-CoV infection had increased expression of CD66e receptors on CD4 and CD8 lymphocytes compared to the normal healthy controls. Elevated expression CD66e on CD4+ and CD+ lymphocytes was associated with increased levels of plasma soluble forms of CD66e in MERS-CoV patients as well. The increased expression of CD66e could be due to direct effect of viral antigens on different cell types including PBMCs or indirectly by the induction of cytokines. Studies have shown that the expression CD66e could be increased on the epithelial cells of the lung by IFN and bacterial respiratory infections [31] or by cytokines such as IL-6 [32]. We and others have previously reported increased levels of several cytokines including IL-10, IL-13, IL-4, IL-5, IL-6, and IL-1 and Th-2 differentiation [33], [34]. Moreover, overexpression of CD66e in permissive cells has clearly been shown to enhance MERS-CoV attachment and entry in BHK21 cells [17]. It is possible that CD66e may facilitate MERS-CoV entry in cells in conjunction with CD26.

No difference in CD26 expression on PBMCs or the soluble forms of CD26 was observed between the patients and controls. A recent study has shown decreased levels of soluble CD26 receptors in severe MERS-CoV patients [35]. Another study has reported that persistent infection with MERS-CoV was associated with down-regulation of CD26 expression in bat cells [20].

Following influenza vaccination as an example of resolved successful immune response high level of CD26 expression was induced on memory CD8+ lymphocytes whereas chronic infection with persistent antigen such as cytomegalovirus (CMV), Epstein–Barr virus (EBV) or human immunodeficiency virus (HIV) lead to defective T-cell memory with low expression of CD26 [36]. High expression of CD26 is considered as a characteristic feature of memory cells. The low expression of CD26 observed in the present study could possibly be due to absence of MERS-CoV specific memory lymphocytes due to lack of previous exposure to the virus.

In the current study, we detected MERS-CoV genes in patients whole blood and isolated PBMCs. Although, MERS-CoV is a respiratory virus which targets primarily epithelial cells lining the respiratory system and alveoli, it was detected in 50% of the patients tested for PBMCs confirming its capability of infecting PBMCs through, at least in part, CD66e receptors. Recent studies have suggested that MERS-CoV could also utilize other cell surface proteins such as 78-kDa glucose–regulated protein (GRP78) and the cell surface glycoprotein CD9 to infect human cells [37], [38]. These molecules however, were not invested in the present study. In agreement with our result, it has been reported that MERS-CoV infection could be detected and diagnosed from patient serum [39],

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