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Brief Communication

Productive replication of Middle East respiratory syndrome coronavirus in monocyte-derived dendritic cells modulates innate immune response

Hin Chu [a,1], Jie Zhou [a,b,c,1], Bosco Ho-Yin Wong [a], Cun Li [a], Zhong-Shan Cheng [a], Xiang Lin [e], Vincent Kwok-Man Poon [a], Tianhao Sun [a], Candy Choi-Yi Lau [a], Jasper Fuk-Woo Chan [a,b,c,d], Kelvin Kai-Wang To [a,b,c,d], Kwok-Hung Chan [a], Liwei Lu [e], Bo-Jian Zheng [a,b,c,d], Kwok-Yung Yuen [a,b,c,d,*]

[a] Department of Microbiology, The University of Hong Kong, Hong Kong Special Administrative Region, China
[b] State Key Laboratory of Emerging Infectious Diseases, The University of Hong Kong, Hong Kong Special Administrative Region, China
[c] Research Centre of Infection and Immunology, The University of Hong Kong, Hong Kong Special Administrative Region, China
[d] Carol Yu Centre for Infection, The University of Hong Kong, Hong Kong Special Administrative Region, China
[e] Department of Pathology, The University of Hong Kong, Hong Kong Special Administrative Region, China

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ABSTRACT

The Middle East respiratory syndrome coronavirus (MERS-CoV) closely resembled severe acute respiratory syndrome coronavirus (SARS-CoV) in disease manifestation as rapidly progressive acute pneumonia with multi-organ dysfunction. Using monocyte-derived-dendritic cells (Mo-DCs), we discovered fundamental discrepancies in the outcome of MERS-CoV- and SARS-CoV-infection. First, MERS-CoV productively infected Mo-DCs while SARS-CoV-infection was abortive. Second, MERS-CoV induced significantly higher levels of IFN-γ, IP-10, IL-12, and RANTES expression than SARS-CoV. Third, MERS-CoV-infection induced higher surface expression of MHC class II (HLA-DR) and the co-stimulatory molecule CD86 than SARS-CoV-infection. Overall, our data suggests that the dendritic cell can serve as an important target of viral replication and a vehicle for dissemination. MERS-CoV-infection in DCs results in the production of a rich combination of cytokines and chemokines, and modulates innate immune response differently from that of SARS-CoV-infection. Our findings may help to explain the apparent discrepancy in the pathogenicity between MERS-CoV and SARS-CoV.

Introduction

The novel Middle East respiratory syndrome coronavirus (MERS-CoV), previously also known as human coronavirus (HCoV) EMC, was first identified in a patient who died from severe acute pneumonia and renal failure in Bisha, Saudi Arabia, in September 2012 (Chan et al., 2013b; Chan et al., 2012; de Groot et al., 2013; Zaki et al., 2012). Although a zoonotic source has been suspected in bats and camels, the definitive and intermediate animal reservoirs have so far not been confirmed, which may have partly contributed to the continuing spread of the epidemic (Annan et al., 2013; Chan et al., 2013c; Chan, K.H. et al., 2013; Lau et al., 2013; Reusken et al., 2013; Woo et al., 2007, 2012). As of February 7th, 2014, a total of 182 laboratory-confirmed cases of MERS-CoV infection with 79 fatalities have been reported in the Middle East, Europe, and Africa (WHO, 2014). The evolving outbreak has raised global concern of a SARS-like epidemic particularly because of their comparably protean clinical manifestations involving both the respiratory tract and extrapulmonary tissues, and the unusually high crude fatality rate among infected patients (Assiri et al., 2013b; Chan et al., 2012; Guery et al., 2013; Memish et al., 2013). These clinical observations corroborated with several key experimental findings on the high pathogenicity of MERS-CoV. First, MERS-CoV replicated more rapidly and showed a much broader tissue tropism in-vitro than any other coronaviruses associated with human infections including SARS-CoV (Chan et al., 2013a). Second, using human lung epithelial cell line as a model, it was shown that MERS-CoV induced a massive dysregulation of the host transcriptome, which may prevent the host from mounting

* Corresponding author at: Carol Yu Centre for Infection, Department of Microbiology, The University of Hong Kong, Queen Mary Hospital, 102 Pokfulam Road, Pokfulam, Hong Kong Special Administrative Region, China. Tel.: +852 225 548 97; fax: +852 2855 1241.
E-mail address: kyyuen@hkucc.hku.hk (K.-Y. Yuen).
[1] These authors contributed equally to the study.

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an optimal immune response (Josset et al., 2013). Third, the utilization of dipeptidyl peptidase 4 (DPP4) instead of angiotensin-converting enzyme 2 (ACE2; SARS-CoV and HCoV-NL63), aminopeptidase N (HCoV-229E), and 9-O-acetylated sialic acid (HCoV-OC43) as a functional receptor by MERS-CoV may also account for the differences in the spectrum of organ involvement between MERS and other human coronavirus infections (Du et al., 2013; Raj et al., 2013). Finally, there is currently no specific anti-MERS-CoV treatment proven to be effective in clinical trials, though mycophenolic acid, interferons, ribavirin, cyclosporin A, and an HR2 peptide have demonstrated in-vitro activities (Chan et al., 2013d; de Wilde et al., 2013; Falzarano et al., 2013; Lu et al., 2014).

Despite these initial clinical and laboratory correlations, the virulence of MERS-CoV has remained controversial especially after a recent study which reported that most of the patients who required hospitalization or died from MERS were elderlies with comorbidities, specifically diabetes mellitus, hypertension, and chronic renal, heart and lung diseases (Assiri et al., 2013b). In contrast, healthy children and adults might be asymptomatic or develop mild infection detected only during contact tracing (Assiri et al., 2013b; Chan et al., 2013b). Furthermore, many MERS patients developed extrapulmonary manifestations including renal impairment, hepatic dysfunction, gastrointestinal symptoms, coagulopathy, cytopenias, and pericarditis (Assiri et al., 2013a; Chan et al., 2013b). The mechanisms by which these extrapulmonary tissues are involved in MERS are incompletely understood. MERS-CoV RNA was detected in mononuclear cells and stellate cells in the mediastinal lymph nodes of infected rhesus macaques, hinting the possibility of macrophage or dendritic cell involvement (de Wit et al., 2013). More in-depth studies on the pathogenesis of MERS-CoV are urgently needed to ascertain its virulence and pathogenetic mechanisms for more accurate prediction of its clinical behavior and better design of therapeutic options. Given the ability of MERS-CoV to infect different human immune cell lines including monocytes and T lymphocytes, we postulated that it might also be able to infect dendritic cells (DCs), which are potent antigen presenting cells (APCs) with instrumental roles in linking the innate and adaptive immune systems. Although previous literatures suggested that SARS-CoV was unable to establish a productive infection in DCs, we suspected that MERS-CoV might be able to infect DCs in a more efficient manner due to the wide distribution of its cellular receptor DPP4 in human cells including activated leukocytes (Raj et al., 2013).

DCs are key players of the innate immune system. As perhaps the most potent APCs, DCs are capable of entering peripheral tissues, taking up antigens, migrating to lymphoid tissues, and activating helper T cells (Banchereau and Steinman, 1998; Steinman and Banchereau, 2007). In this capacity, DCs play unique roles in bridging the innate and the adaptive immunity and therefore become important targets for microbial invasion. For instance, HIV has evolved a number of mechanisms to exploit the function of DCs for its own benefit, including escaping from the immune surveillance and facilitating cell-to-cell dissemination (Wu and KewalRamani, 2006).

DCs are susceptible to SARS-CoV infection. However, as suggested by a number of studies, the infection was either abortive (Law et al., 2005; Tseng et al., 2005; Ziegler et al., 2005) or at low level (Spiegel et al., 2006) and caused no adverse effect to cell viability. Infection of DCs by SARS-CoV failed to trigger a strong type I interferon (IFN) response but resulted in an up-regulation of inflammatory cytokines and chemokines (Law et al., 2005; Tseng et al., 2005). Functional activation of DCs by SARS-CoV has been analyzed but the result was inconclusive as both activation (Spiegel et al., 2006; Tseng et al., 2005) and absence of activation (Ziegler et al., 2005) have been proposed. In the current study, we used monocyte-derived DCs (Mo-DCs) as a system to recapitulate MERS-CoV infection in DCs and we compared the results with that of SARS-CoV-infected Mo-DCs. Our results revealed fundamental differences in the replication kinetics of MERS-CoV and SARS-CoV in infected Mo-DCs. In addition, we also demonstrated characteristic changes in the pattern of cytokine/chemokine expression as well as antigen-presenting function of infected Mo-DCs.

Results

Monocyte-derived-dendritic cells (Mo-DCs) were susceptible to MERS-CoV infection

To determine whether primary human DCs are susceptible to MERS-CoV infection, we infected Mo-DCs with MERS-CoV and examined the expression of MERS-CoV nucleoprotein (NP) at different time points post inoculation. Our data revealed that MERS-CoV NP expression could be detected at 12 hours post infection (hpi) (Fig. 1A). The signal for NP appeared to be puncta-like and distributed in the cytoplasm of infected cells. At 48 hpi, the signal for NP was dramatically enhanced and was detected throughout the cytoplasm of infected cells (Fig. 1B). Mock-infected cells (Fig. 1C) as well as cells treated with pre-immune sera (data not shown) both failed to display any signal for NP. Our immunostaining study revealed that Mo-DCs were susceptible to MERS-CoV infection. The enhanced expression of NP at 48 hpi versus 12 hpi suggested that MERS-CoV not only infected Mo-DCs but also continued viral transcription and translation in these infected cells. In addition, the percent of Mo-DCs infected by MERS-CoV was assessed by flow cytometry and was determined to be $12.6\% \pm 1.2\%$ at 48 hpi (data not shown).

MERS-CoV infection in Mo-DCs was productive

With the immunofluorescence study described above, we illustrated that MERS-CoV was capable of infecting DCs with efficient transcription and translation of the viral genome, which was supported by the substantial increase in cellular NP expression (Fig. 1). To obtain a more comprehensive picture of the kinetics of MERS-CoV infection in Mo-DCs, we infected Mo-DCs with MERS-CoV and compared the results with that of SARS-CoV-infected cells. Importantly, a 2-to-4 log increase in viral RNA in both cell lysates (Fig. 2A) and supernatants (Fig. 2B) of MERS-CoV-infected Mo-DCs was detected in samples from all donors. In stark contrast, little or no increase in viral RNA was detected in the cell lysates or supernatants of SARS-CoV-infected Mo-DCs, which agreed with previous reports that SARS-CoV could infect but was unable to propagate in Mo-DCs. We further assessed the infectivity of viral particles released from infected Mo-DCs with $TCID_{50}$ assays. Our results demonstrated that while low levels of infectious particles were detected from the supernatants of SARS-CoV-infected Mo-DCs, MERS-CoV-infected Mo-DCs consistently released a considerable amount of infectious particles with a peak at 24 hpi (Fig. 2C). Overall, our data suggested that MERS-CoV could establish a productive infection in Mo-DCs.

MERS-CoV triggered stronger cytokine and chemokine response than SARS-CoV in Mo-DCs

DCs are among the first line of innate immune response and are capable of producing a large number of cytokines and chemokines upon microbial challenge (Banchereau and Steinman, 1998). We examined the ability of MERS-CoV to trigger cytokine and chemokine response in Mo-DCs and compared the results with that of SARS-CoV-infected Mo-DCs. Mo-DCs were infected with MERS-CoV or SARS-CoV at 2 $TCID_{50}$ per cell for one hour and cell lysates