Cytokines and chemokines in mild/asymptomatic cases infected with avian influenza A (H7N9) virus

In March 2013, human infections with a novel avian-origin influenza A (H7N9) virus were first identified in South Eastern China (Gao et al., 2013b; National Health and Family Planning Commission, 2013). The current findings indicated that H7N9 virus was able to cause a wide spectrum of diseases in humans. Most reported patients of H7N9 virus infection presented with severe lower respiratory illness, with about one-third being fatal (Gao et al., 2013a; Yu et al., 2013), and mild and asymptomatic human infections were also found (Yang et al., 2013; Yu et al., 2013; Wang et al., 2014a).

The pathogenesis of H7N9 virus resulting in severe outcomes in humans remains unclear. Previous studies with highly pathogenic avian influenza A (H5N1) virus as well as pandemic (H1N1) 2009 virus suggested that poor prognosis in humans with these viruses might be attributed to a cytokine storm or hypercytokinaemia in the host (Peiris et al., 2004; de Jong et al., 2006; To et al., 2010). Some studies with H7N9 virus also showed the presence of high levels of a series of cytokines and chemokines, such as IP-10, IL-6, IL-8, IL-10, MCP-1, MIG and MIP-1β, in human infections with severe disease without or without fatal outcomes (Chi et al., 2013; Zhou et al., 2013; Shen et al., 2014; Wang et al., 2014b; Wu et al., 2014). It should be noted that investigation on the profiles of cytokines and chemokines induced in mild/asymptomatic cases will aid in assessing their role in mild/asymptomatic versus severe/fatal outcomes of infection with H7N9 and the role of innate immunity and provides a new perspective on the evaluation of prognosis and the innovation of treatment. In the present study, we assessed the profiles of cytokines and chemokines in patients who either had a mild illness or were asymptomatic but infected with H7N9 virus in Beijing, China.

In this study, we included four mild or asymptomatic cases infected with H7N9 virus, confirmed by real-time reverse transcriptase PCR assay, and four household close contacts as controls. The four H7N9 cases included one with mild pneumonia, one with upper respiratory symptoms and two asymptomatic cases (Yang et al., 2013). Clinical profiles of these cases and controls are presented in Table S1 (available in the online Supplementary Material). As the H7N9 case with pneumonia in this study did not fit the criteria of H7N9 severe cases according to the Diagnostic and Treatment Guidance for Human Infections with Avian Influenza A (H7N9) of China (National Health and Family Planning Commission, 2014), this case was defined as mild pneumonia.

Paired serum samples were harvested from the H7N9 cases and their close contacts. For the two asymptomatic cases, serum samples were harvested within 1 week of illness onset (acute phase), as well as between 3 and 4 weeks after illness onset (convalescent phase). For the two asymptomatic cases, serum samples were harvested within 1 week of the time of testing positive for H7N9 virus, as well as between 3 and 4 weeks after the detection. For close contacts, serum samples were harvested within 1 week of completion of medical observation as well as between 3 and 4 weeks after the completion of medical observation.

The profiles of cytokines/chemokines of sera from cases and close contacts were analysed with a commercial kit that detects 40 cytokines and chemokines (EOTAXIN, EOTAXIN-2, GCSF, GM-CSF, ICAM-1, IFN-γ, IL-2, IL-6, IL-10, MCP-1, MIG, MIP-1α, TNF-α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-6sR, IL-7, IL-8, IL-10, IL-11, IL-12 p40, IL-12 p70, IL-13, IL-15, IL-16, IL-17, IP-10, MCP-1, MCP-2, M-CSF, MIG, MIP-1α, MIP-1β, MIP-16, RANTES, TGFB1, TNF-α, TNF-β, sTNF RI, sTNF RII, PDGF-BB, TIMP-2; RayBio Human Inflammation Antibody Array G-Series 3; Raybiotech) according to the manufacturer’s instructions. Each serum sample was detected in two replicated spots in an experiment, and the results were represented as intensity of fluorescence signals. The consistency of two results for all serum samples was good (intra-class correlation coefficient, 0.996), and the average of two replicated intensities of fluorescence signals of each cytokine/chemokine for each serum sample was used for further analysis. The intensities of fluorescence signals were normalized using negative, positive and internal controls included in the array.

The database was set up using Microsoft Excel Software (version 2003; Microsoft Corporation) and was analysed using SAS® University Edition (SAS Institute). Comparison of fluorescence intensities of cytokines and chemokines at two time points between H7N9 cases and close contacts was made by a one-way repeated-measures ANOVA. All statistical tests were two sided, and P<0.05 was considered statistically significant. This study was subject to approval by the institutional review boards of Beijing Center for Disease Prevention and Control.

In this study, we found that the fluorescence intensities of IL-1α, IL-3, IL-6sR, IL-11, IL-12 p40, IL-17, IP-10 and RANTES were statistically significantly elevated in the mild/asymptomatic H7N9 cases when compared with the controls (Fig. 1), and that of M-CSF was statistically significantly lower in the mild/asymptomatic H7N9 cases. Other cytokines and chemokines, including those dramatically elevated in severe H7N9 cases as reported in previous studies, such as IL-6, IL-8, IL-10, MCP-1, MIG and MIP-1β, did not differ between the mild/asymptomatic H7N9 cases and the
Fig. 1. Significantly elevated cytokines and chemokines in mild/asymptomatic cases infected with avian influenza A (H7N9) virus compared to close contacts. Paired serum samples were harvested from four mild/asymptomatic H7N9 cases and four close contacts in this study. For the symptomatic cases, serum samples were harvested within 1 week of illness onset (acute phase) as well as between 3 and 4 weeks after illness onset (convalescent phase). For the asymptomatic cases, serum samples were harvested within 1 week of the time of being tested positive for H7N9 virus as well as between 3 and 4 weeks after detection. For the close contacts, serum samples were harvested within 1 week of completion of medical observation as well as between 3 and 4 weeks after the completion of medical observation. The cytokines and chemokines in sera from cases and close contacts were analysed with human inflammation antibody arrays that detect 40 cytokines and chemokines and were compared using a one-way repeated-measures ANOVA.
controls. Moreover, the cytokine profile in the H7N9 case with mild pneumonia was similar to that of the three mild/asymptomatic H7N9 cases but was different from that in severe H7N9 cases as reported in previous studies. Our findings suggested that mild/asymptomatic H7N9 cases might have a distinct profile of cytokines and chemokines as compared to severe H7N9 cases. Comparisons between mild/asymptomatic H7N9 cases and controls for all 40 cytokines/chemokines are presented in Fig. S1.

Previous studies showed that sIL-6R exhibited extensive antiviral activity against numerous viruses, including hepatitis B virus, influenza virus and human enterovirus 71 (Zhang et al., 2011; Wang et al., 2013). The elevation of IL-6sR in mild/asymptomatic H7N9 cases might play a role in inhibiting the replication of H7N9 virus at the early stage of infection, resulting in averting severe/fatal outcomes. Previous studies showed that IL-12 was able to confer an early inhibitory effect on influenza virus replication (Monteiro et al., 1998; Biron et al., 1999), so the higher level of IL-12 p40 in mild/asymptomatic H7N9 cases might be beneficial to their outcomes.

Earlier studies showed that IP-10, IL-6, IL-8, IL-10, MCP-1, MIF and MIP-1β were dramatically increased in severe H7N9 cases and H5N1 cases compared to controls (Peiris et al., 2004; de Jong et al., 2006; Chi et al., 2013; Zhou et al., 2013; Shen et al., 2014; Wang et al., 2014b; Wu et al., 2014). However, these cytokines in mild/asymptomatic H7N9 cases were similar to those in controls except that the level of IP-10 increased very slightly. Our findings suggest that normal or low levels of these cytokines and chemokines predict a favourable outcome of H7N9 infection. However, the sample size of this study was small, which might have an effect on making substantial interpretations and limit the validity and generalizability of our findings.

This study gives a new insight into the response of cytokines and chemokines in mild/asymptomatic H7N9 cases. The molecules that we found in this study might be taken into account as targets for predicting outcomes and developing new therapeutic strategies to modulate the disease process of H7N9 cases.

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Peng Yang,† Xiaomin Lou,‡ Yang Zheng,§ Weixian Shi,† Yang Li,¶ Yushuo Dong,† Yuning Han,† Xinghuo Pang,† Siqi Liu‡ and Quanyi Wang†

†Beijing Center for Disease Prevention and Control, Beijing Research Center for Preventive Medicine, School of Public Health, Capital Medical University, Beijing, PR China

‡Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing, PR China

§Beijing Protein Innovation, Beijing, PR China

Correspondence: Quanyi Wang

bjcdcxm@126.com

†These authors contributed equally to this work.

References


