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Original article

Longevity of seropositivity and neutralizing antibodies in recovered MERS patients: a 5-year follow-up study

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Abstract

Objectives: We aimed to assess the longevity of spike-specific antibody responses and neutralizing activity in the plasma of recovered Middle East respiratory syndrome (MERS) patients.

Methods: We traced the antibody responses and neutralizing activity against MERS coronavirus (MERS-CoV) in peripheral blood samples collected from 70 recovered MERS patients for 5 years after the 2015 MERS outbreak in South Korea. We also measured the half-life of neutralizing antibody titres in the longitudinal specimens.

Results: The seropositivity rate persisted for up to 4 years (50.7–56.1%), especially in MERS patients who suffered from severe pneumonia, and then decreased (35.9%) in the fifth year. Although the spike-specific antibody responses decreased gradually, the neutralizing antibody titres decreased more rapidly (half-life: 20 months) in 19 participants without showing negative seroconversion during the study period. Only five (26.3%) participants had neutralizing antibody titres greater than 1/1000 of PRNT50, and a high neutralizing antibody titre over 1/5000 was not detected in the participants at the fifth year, whereas the neutralizing antibody titres against MERS-CoV decreased more rapidly and were significantly reduced at 4 years after infection. Shinhye Cheon, Clin Microbiol Infect 2022;28:292

Introduction

Newly emerging zoonotic coronaviruses, which cause acute respiratory syndrome, have posed enormous threats to global public health within just two decades [1]. Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in China in 2002 and the Middle East in 2012, respectively; these viruses have resulted in
11% and 35% of deaths, respectively [1,2]. The third zoonotic coronavirus, SARS-CoV-2, has been spreading at an alarming rate worldwide since December 2019, with a mortality rate of approximately 2.0% (https://covid19.who.int). Despite the catastrophic impact of coronaviruses on public health worldwide, few effective therapeutic options are available for the diseases caused by these viruses [3]. Immune serum or plasma samples collected from recovered patients have been found to retain neutralizing activity in clinical therapeutic applications [4–7]. However, knowledge of the long-term dynamics and durability of specific antibody responses in recovered MERS and COVID-19 patients is limited. Moreover, the nature of humoral immunity as a form of protective therapy remains unclear [8]. We previously found that anti-MERS-CoV spike antigen-specific IgG responses, including the neutralizing activity, and the presence of antibody-secreting memory B cells lasted up to 3 years after infection and were significantly correlated with disease severity [7]. In addition, sera with high neutralizing antibody titres suppressed viral replication but could not reduce excessive pulmonary inflammation during fatal infection in a transgenic mouse model [7]. In the present study, we further assessed antibody titres against the MERS-CoV spike antigen in plasma samples collected from recovered Korean MERS patients for 5 years after the 2015 MERS outbreak and assessed the longevity of spike-specific antibody responses and neutralizing activity in order to determine their half-lives.

Neutralizing antibody assays

For assessing the neutralizing antibody titres in the collected serum samples against MERS-CoV, a plaque reduction neutralization titre (PRNT) assay was performed as described previously [7]. In brief, serially diluted sera were incubated with wild-type MERS-CoV (0.0004 m.o.i. [multiplicity of infection]) isolated from Korean patients (NCBI genome sequence: KTO29139.1) for 1 hr at 37°C; the mixtures were then added to a 24-well plate containing a monolayer of Vero E6 cells in duplicates. After incubation for 1 hr at 37°C, each well was washed and overlaid with overlay media containing 1% methylcellulose and 10% fetal bovine serum. After 3 days of incubation, the cells were fixed with 4% paraformaldehyde. MERS-CoV plaques were immunostained with rabbit anti-MERS-CoV N protein antibody (Sino Biological Inc., Beijing, China) and goat anti-rabbit IgG secondary antibody conjugated with alkaline phosphatase (Invitrogen) and then visualized using nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) (Merck). The plaque reduction percentage was calculated using the following formula: [(number of plaques without antibody) – (number of plaques with antibody)]/(number of plaques without antibody) × 100. The ppNT50 and PRNT50 titres were determined by nonlinear regression analysis [using the log(inhibitor) vs. normalized response method] embedded in GraphPad Prism Software v8.0 (GraphPad Software Inc., San Diego, CA, USA).

Material and methods

Study design and participants

In total, 70 recovered MERS patients were recruited in the present study; their baseline characteristics are summarized in Table S1 and can also be found in our previous report [7]. All the participants were confirmed to be positive for MERS-CoV infection by a real-time RT-PCR assay targeting the uPE and orf1a sequences of the virus at a diagnostic laboratory in the Korean Center for Disease Control. During the study period, 31 participants were lost to follow-up, leaving 39 participants at 60 months. Serum samples were collected from the participants at 12-month intervals after symptom onset. Data for the first 3 years were assessed in our previous study [7] and have been included in this research. Ethics approval for using the participants' clinical data and specimens was obtained from the institutional review boards of Chunchang National University Hospital (CNUH2017-12-004), National Medical Center (H-1510-059-007), Seoul National University Hospital (1509-103-705 and 1511-117-723), Seoul National University Boramae Medical Center (26-2016-8), Seoul Medical Center (Seoul2015–12–102), and Dankook University Hospital (DKHU2016-02-014). The present study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and all subsequent revisions. All the participants provided written informed consent for participating in the study.

Enzyme-linked immunosorbent assay

To detect the levels of MERS-CoV spike (S1 domain)-specific IgGs, semi-quantitative ELISA was performed using an anti-MERS-CoV S1 ELISA kit (EUROIMMUN, Lubeck, Germany) according to the manufacturer’s instructions. Optical density (OD) ratios were calculated by comparing the extinction values of the samples and the calibrator. OD ratios under 0.7 were considered negative, those over 1.4 were considered positive, and those between 0.7 and 1.4 were considered intermediate.

Statistical analysis

Statistical analysis among different groups was performed using a two-tailed Student’s t-test or one-way analysis of variance (ANOVA), followed by the Newman–Keuls t-test. The correlation between variables was assessed using the Pearson correlation test. A p value < 0.05 was considered statistically significant. All data analyses were performed using GraphPad Prism Software.

Results

The participants were classified into three groups according to their disease severity during the 2015 MERS outbreak in Korea [79]. Group I (G-I) included 18 participants who were asymptomatic or had mild fever without developing pneumonia. Group II (G-II) included 33 participants who developed mild pneumonia without hypoxemia. Group III (G-III) included 18 participants who recovered after developing more prolonged and severe pneumonia. The participants in G-III experienced hypoxemia and were treated with oxygen during hospitalization (Table S1). Humoral immune responses against the MERS-CoV spike antigen were assessed in all the serum samples collected from the participants up to 60 months after symptom onset (Fig. 1A). The geometric mean OD ratios against the spike antigen (S1) were generally maintained for 48 months in all the samples analysed (geometric mean ± SD 0.95 ± 1.17, 1.06 ± 1.52, 1.01 ± 1.42, and 1.15 ± 1.17 at 12, 24, 36 and 48 months, respectively) and decreased thereafter (0.84 ± 1.02 at 60 months). The seropositivity rate also persisted for up to 48 months after infection (50.7%, 53.0%, 54.9%, and 56.1% at 12, 24, 36 and 48 months, respectively) and decreased to 35.9% (14/39 participants) at 60 months (Fig. 1B). The seropositivity rates were generally higher in participants who had more severe disease, as reported previously [7]. In particular, no participant in G-I was seropositive from 36 months after infection (Fig. 1C). In G-II, 54.6–65.0% of the participants were persistently seropositive for up to 48 months, while 35.3% (6/17 participants) were seropositive at 60 months. In addition, 81.3–83.3% of the participants in G-III showed seroconversion and persistent
antibody responses for up to 48 months, while 61.5% (8/13) remained seropositive at 60 months. Thus, more severe pneumonia is associated with significantly more persistent anti-spike IgG responses and seropositivity for 4 years after symptom onset, with a decrease being noted thereafter.

Neutralizing activity against MERS-CoV (PRNT\textsubscript{50}) was assessed using serum samples from the participants in G-II and G-III for up to 60 months. The neutralizing antibody titres against MERS-CoV gradually decreased in the participants and significantly dropped from 48 months after infection (Fig. 2A). The geometric mean
PRNT$_{50}$ value was reduced by 16.7% in the second year and 35.8% in the third year (mean ± SD 1541 ± 2503 and 1187 ± 2070, respectively) compared with the value in the first year (1850 ± 2961). The value was further reduced by 72.2% in the fourth year and 67.0% in the fifth year (mean ± SD 514 ± 1425 and 611 ± 816, respectively). Nevertheless, the neutralizing antibody titres were generally higher in patients with more severe pneumonia (G-III) than in those with milder disease (G-II) (Fig. 2A, right panels). In addition, the OD ratios against the S1 antigen correlated well with the neutralizing antibody titres against MERS-CoV in the plasma samples collected during the fourth and fifth years (Fig. 2B).

We previously found that pulmonary viral loads and mortality were significantly reduced upon lethal MERS-CoV challenge in a transgenic mouse model therapeutically treated with human plasma containing a high neutralizing antibody titre (PRNT$_{50}$ > 1/5000); however, treatment with plasma containing a moderate neutralizing antibody titre (approximately 1/1000) failed to provide any clinical benefit [7]. In the third year after infection, the plasma samples of three out of 39 participants (7.7%) in G-II and G-III had a high neutralizing antibody titre (> 1/5000). In the fourth year, one out of 28 participants (3.6%) had a high neutralizing antibody titre; however, this was not detected in any of the participants in the fifth year. Assessment of the half-life of antibody responses and neutralizing activity in 19 participants without negative seroconversion during the study period (Fig. 2C) revealed that the OD ratios against the S1 antigen decreased gradually (estimated half-life: 61 months), while the neutralizing antibody titres decreased more rapidly (half-life: 20 months). Only five (21.1%) participants had neutralizing antibody titres greater than 1/1000 (range 1/1001–1/2927) of PRNT$_{50}$ at 5 years after infection.

**Discussion**

In this 5-year follow-up study, the characteristics and maintenance of humoral immunity against the MERS-CoV spike antigen were analysed in 70 participants who had recovered from MERS. In our previous study, we confirmed that specific IgG responses, especially neutralizing antibody responses, were sustained for up to three years after infection and that this was particularly noted in MERS patients who developed viral pneumonia [7]. These findings are consistent with those of other tracer studies that were conducted with smaller sample sizes and for shorter periods of less than 3 years after the initial MERS-CoV infection [10–12]. Sustained antibody responses were found to be significantly associated with clinical severity and the duration of viral continuity during the acute phase [9,12–15]. We also confirmed the presence of significant correlations between the antibody response levels and fever duration, viral shedding periods and maximum viral loads [7]. In the present study, we observed that the seropositivity rate persisted for up to 4 years after infection and dropped in the fifth year (Fig. 1). In contrast, the neutralizing antibody titres against MERS-CoV decreased more rapidly and were significantly reduced in the participants from 4 years after infection (Fig. 2). A previous study also revealed that both specific antibody responses and the count of specific memory B cells gradually decreased and reached undetectable levels in SARS patients at 6 years after infection [16]. Thus, long-lasting MERS-specific humoral immunity is potentially sustained for 4 years after infection and substantially declines thereafter.

Passive antibody therapy using plasma from convalescent patients has been urgently used in epidemics when there is a lack of time or resources to develop specific immunoglobulin therapy: this is also the case in the current COVID-19 pandemic [17]. Several studies have reported a reduction in viral loads and an improvement in clinical symptoms in patients with emerging coronavirus infections, such as those caused by SARS-CoV-1, MERS-CoV, and SARS-CoV-2 [4,18–22]. Previously, we showed that treatment with sera containing a high neutralizing antibody titre (1/7046) significantly reduced viral loads and increased the survival of human DPP4 transgenic mice challenged with a lethal dose of MERS-CoV; however, treatment with sera containing a moderate neutralizing antibody titre (1/1081) failed to provide any clinical benefit [7]. Nevertheless, high-titre plasma therapy did not reduce pulmonary pathogenesis, as confirmed by pathological changes in the lungs and by the initial weight loss. We therefore concluded that only high neutralizing antibody titres in sera can suppress viral replication and subsequent viral spread but are still not effective in treating pulmonary inflammatory lesions during fatal MERS-CoV infection [7]. In addition, in a recent study, a patient treated with convalescent plasma developed acute respiratorv distress syndrome, suggesting the chances of transfusion-related acute lung injury after treatment with convalescent plasma in COVID-19 patients [23]. Moreover, neutralization-escaping mutants of SARS-CoV-2 were recently detected following treatment with convalescent human sera [24]. Taken together, these findings suggest that a narrow repertoire of neutralizing antibodies produced by coronavirus infection may exert pressure for the selection of single amino acid substitutions in the viral spike protein and could thus constrict neutralizing antibody responses [24]. The detailed characterization of antibody responses against emerging coronaviruses is necessary for clarifying the nature of antiviral immune responses and for preparing effective countermeasures against viral diseases [25]. Therefore, the application of immune plasma therapy needs to be carefully considered in ongoing clinical trials [26].

**Transparency declaration**

The authors declare that they have no conflicts of interest. The present study was supported by a grant (2017N-ER5307/2017-NG47003) from the Korea Disease Control and Prevention Agency, funded by the Ministry of Health and Welfare, and by a grant (2017M3A9E4061998) from the National Research Foundation of Korea (NRF). U.P., H.P., Y.K., Y.T.H.N. and A.A. received a scholarship from the BK21 PLUS education programme provided by the NRF.

**Author contributions**

S.C., U.P. and H.P. contributed equally to this work. Y.S.K. and N.H.C. conceived and designed the study and analysed the results. S.C., U.P., Y.S.K. and N.H.C. wrote the manuscript. All other authors conducted the study, collected data, revised the manuscript and approved the final version.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2021.06.009.

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