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Serum levels of the IgA isotype switch factor TGF-β1 are elevated in patients with COVID-19

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We previously observed enhanced immunoglobulin A (IgA) responses in severe COVID-19, which might confer damaging effects. Given the important role of IgA in immune and inflammatory responses, the aim of this study was to investigate the dynamic response of the IgA isotype switch factor TGF-β1 in COVID-19 patients. We observed, in a total of 153 COVID-19 patients, that the serum levels of TGF-β1 were increased significantly at the early and middle stages of COVID-19, and correlated with the levels of SARS-CoV-2-specific IgA, as well as with the APACHE II score in patients with severe disease. In view of the genetic association of the TGF-β1 activator *THBS3* with severe COVID-19 identified by the COVID-19 Host Genetics Initiative, this study suggests TGF-β1 may play a key role in COVID-19.

Keywords: COVID-19; IgA; TGF-β1; SARS-CoV-2

Abbreviations
IgA, immunoglobulin A; COVID-19, coronavirus disease 2019; SARS-CoV, severe acute respiratory syndrome coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TGF-β1, Transforming growth factor beta-1; ICU, intensive care unit; sIgA, secretory IgA; MCP, monocyte chemoattractant protein; GM-CSF, granulocyte–macrophage colony-stimulating factor; NHLFs, normal human lung fibroblasts; FcαRI, Fc alpha receptor; ADCC, antibody-dependent cellular cytotoxicity.

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The pandemic of coronavirus disease 2019 (COVID-19) has caused serious damage to the world. Despite its similarity to other coronaviruses, such as severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) virologically, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is closer to influenza in epidemiology and virulence [1]. There is the possibility that SARS-CoV-2 and COVID-19 are here to stay, similarly to influenza [1]. Therefore, mechanistic studies on severe COVID-19 are critically important for the development of effective therapies to prevent lethal complications. In our recent study, we observed enhanced immunoglobulin A (IgA) responses in severe COVID-19, which might confer damaging effects in severe COVID-19, and be responsible for the common organ injuries observed in COVID-19, for example, acute pulmonary embolism and kidney injury, related to IgA deposition and vasculitis [2]. Transforming growth factor beta-1 (TGF-β1) has been established as an IgA isotype switch factor in a previous study [3]. In addition, TGF-β1 has critical function in immune suppression and establishing immunological tolerance [4]. In SARS [5,6] and MERS [7,8], TGF-β1 has also been shown to induce the proliferation of fibroblasts resulting in pulmonary fibrosis. In COVID-19, the exact role of TGF-β remains to be defined. The aim of this study was to investigate the dynamic response of serum TGF-β in COVID-19 patients, as well as its relationship with SARS-CoV-2-specific IgA, and to evaluate its relationship with the course of the disease and disease severity.

Methods

Subjects

A total of 153 COVID-19 patients were recruited from 4 hospitals in the Guangdong and Hubei Provinces. Patients' average age was 56.5 ± 18.3 years. The 153 COVID-19 patients included 71 males (43%) and 82 females (57%). All the patients tested positive for SARS-CoV-2 nucleic acids. According to the Guidelines for the Diagnosis and Treatment of COVID-19 Pneumonia (7th edition) [9], the COVID patients were classified into three different groups: 47 severe (18.5%), 70 moderate (58%), and 36 mild (23.5%). The severe group included both severely and critically ill patients. Patients in the mild group showed no abnormal CT findings. The moderate group included patients with fever and/or typical respiratory symptoms, as well as those with typical CT images of viral pneumonia. In addition, severe or critically ill patients met at least one of the following conditions[9]: (a) shortness of breath, respiratory rate (RR) > 30 times·min^{-1}; (b) blood oxygen saturation (SpO_2, resting state) < 93%; (c) PaO_2/FiO_2 < 300 mmHg; (d) respiratory failure requiring mechanical ventilation; (e) shock; and (f) multiple organ failure requiring intensive care unit (ICU) admission. A panel of trained physicians confirmed all the clinical diagnoses. Blood samples were collected during the admission for all patients. In addition, 20 healthy and uninfected control subjects with an average age of 39 ± 9.7 years were recruited at Shenzhen University. Blood samples of 34 convalescent patients (21 males and 13 females, average age of 48 ± 12.3 years) were also compared. This study was approved by the Ethics Committee of Wuhan Central Hospital (Medical Research Ethics No. 1, 2020) and the First Affiliated Hospital of Guangzhou Medical University (Medical Research Ethics No. 44, 2020).

Measurement of immunoglobulins and cytokines

The level of TGF-β1 was detected using ELISA kits from Sizhengbo Company (Beijing, China) and Yiqiao Shenzhou Company (Beijing, China), and the experiment followed the manufacturers' protocols. The repetition rate by the two kits was > 99%. Virus-specific antibodies were detected using the magnetic chemiluminescence enzyme immunoassay kit (Bioscience Inc. Tianjin, China).

Statistical analysis

The results were presented as the means ± SEM, and statistical tests were performed using the GraphPad prism 6.0 (San Diego, CA, USA). The values were compared among patients with severe, moderate, and mild infections, and healthy controls. When the data were not normally distributed, the Kruskal–Wallis H-test was used for comparing multiple groups. Bonferroni's *t*-test was used to adjust for

type I errors in multiple comparisons. The correlation between groups was evaluated using Pearson's test, while the repeated-measures analysis of variance was used to test for the kinetic trend for the levels of TGF-β1 and SARS-CoV-2-specific IgA antibodies in patients with different disease severities.

Results

Levels of serum TGF-β1 and SARS-CoV-2-specific antibodies

We first analyzed the levels of serum TGF-β1 and SARS-CoV-2-specific antibodies in patients with COVID-19. Our results show that the serum TGF-β1 levels in patients with severe and moderate COVID-19 are significantly increased from 0 to 10 days since the onset of symptoms to the end of the disease course (Fig. 1A), while in those subjects at convalescence, the levels of circulating TGF-β1 were similar to that of the healthy subjects. Compared with patients with severe and mild COVID-19, the level of TGF-β1 in patients with moderate COVID-19 increased most significantly in the first 30 days ($P < 0.01$) and decreased in the following 10 days ($P < 0.05$). During the course of COVID-19 disease, we observed that the TGF-β1 level was lower in mild patients than both moderate and severe groups of patients. The simultaneous levels of serum antibodies in patients with COVID-19 or at convalescence were further investigated (Fig. 1B–D). Consistent with the previous study [2], we found that the level of SARS-CoV-2-specific IgA increased significantly at the early stage of COVID-19 and decreased dramatically during the late course of COVID-19, with further declining observed during the convalescence period. In approximately 50% of the convalescent patients, SARS-CoV-2-specific IgA level was undetectable. The SARS-CoV-2-specific IgM and IgG levels were also decreased, but with a slower kinetic change than that of IgA.

Correlation and kinetics of TGF-β1 and IgA in patients with different severities of COVID-19

Because TGF-β is known to induce IgA isotype class switch, we further examined the correlation between the levels of TGF-β1 and different SARS-CoV-2-specific antibody isotypes, including IgA. We found that, within 30 days after symptom onset, the TGF-β1 levels were significantly correlated with the levels of SARS-CoV-2-specific IgA in patients with moderate and severe illness, and the correlation was stronger in patients with moderate COVID-19 than that in severe patients (Fig. 2A,B), suggesting the possibility that increased levels of virus-specific IgA may be as the result of increased levels of TGF-β, which promotes antibody isotype switching as the consequence SARS-CoV-2 infection. No correlation was detected between TGF-β1 levels and SARS-CoV-2-specific IgG or IgM levels in severe, moderate, or mild patients. Moreover, we found that the TGF-β1 level in severe patients was positively correlated with the APACHE II score (Fig. 2C).

Discussion

In this study, we observed significant elevation in the serum levels of TGF-β1 in samples from patients of any severity (mild, moderate, or severe) with early- and mid-stage COVID-19 disease, whereas the levels decreased significantly thereafter in the later stages of the disease. In addition to the findings of this study, the COVID-19 Host Genetics Initiative [10] identified a genetic association from an important gene in the TGF-β1 signaling pathway in 5582 patients with very severe COVID-19 disease vs. 709 010 population controls. The association signal was tagged by a nonsynonymous single nucleotide variant (SNV) p.S159G (rs35154152) at the thrombospondin 3 gene (*THBS3*), with $P = 4.12E-07$. According to the Genotype-Tissue Expression (GTEx) database, the alternative allele of rs35154152 corresponding to increased risk of severe COVID-19 is associated with increased expression levels of *THBS3* in multiple human tissues, including peripheral blood ($P = 1.4E-10$), lung ($P = 6.3E-07$), and spleen($P = 7.4E-05$; https://www.gtexportal.org/home/snp/rs35154152). Thrombospondins are adhesive glycoproteins that mediate cell-to-cell and cell-to-matrix interactions [11]. Thrombospondins activate TGF-β1 by direct binding with the inactive precursor complex of TGF-β1, known as latent TGF-β [12]. This presents further evidence showing the role of TGF-β1 in severe COVID-19, with not only increased levels of TGF-β1 but also elevated activation.

As a mucosal targeted virus, SARS-CoV-2 may induce strong mucosal immunity, including the generation of secretory IgA (sIgA). It is known that sIgA is synthesized and secreted in intestine, respiratory tract, mammary gland, salivary gland, and lacrimal gland [13]. IgA is involved in local mucosal immunity and plays an important role in mucosal antiviral immunity, at least in part, by combining with corresponding pathogenic microorganisms to prevent pathogens from adhering to the cell surface [14]. Also, mucosal IgA in the respiratory mucosa has been shown to be protective of infections caused by other respiratory viruses [15]. However, recent studies also found that sIgA is able to induce

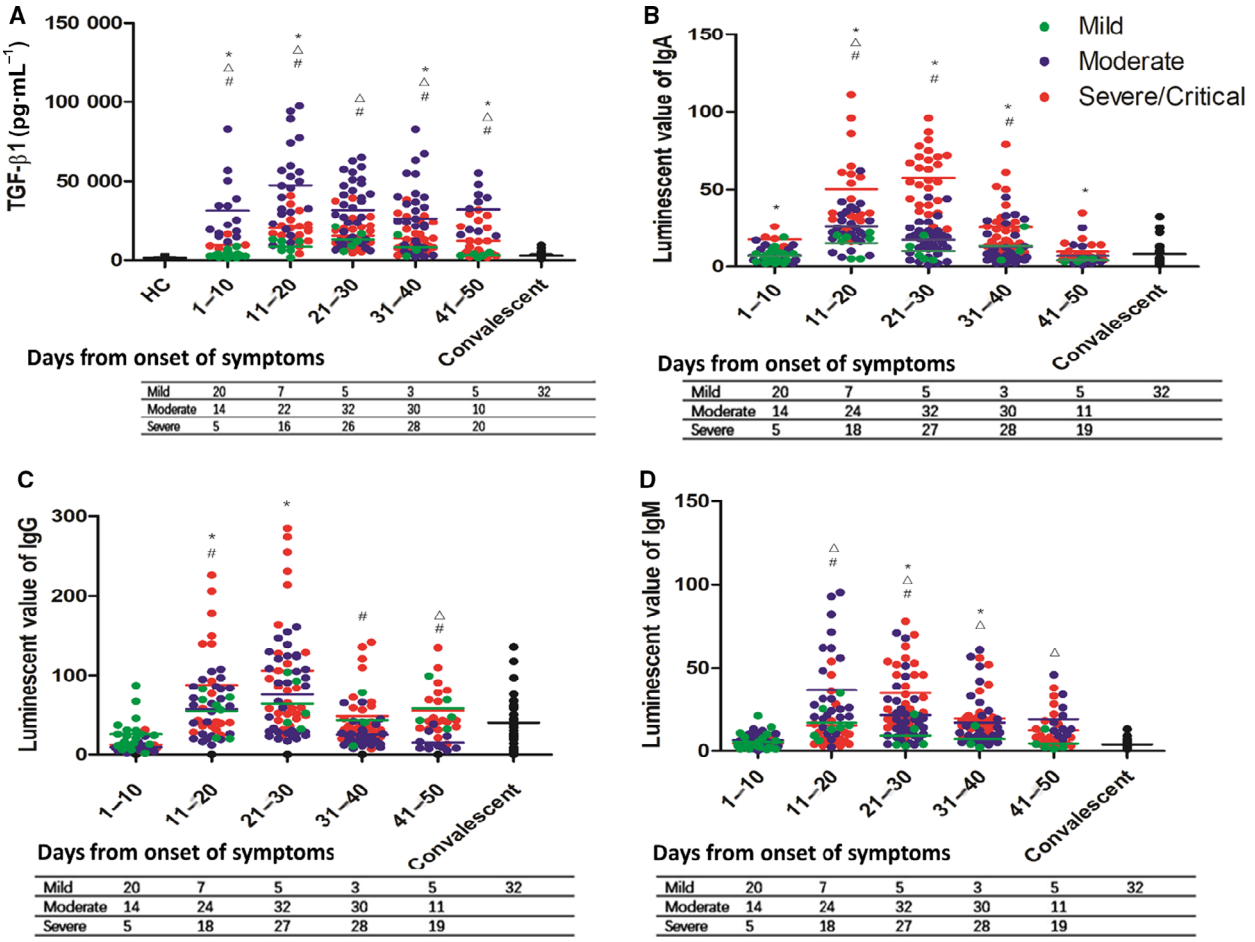

Fig. 1. The kinetic responses of serum TGF-β1 in COVID-19 patients and its correlations with SARS-CoV-2-specific IgA, and the disease course and severities. The values of TGF-β1 in severe/critical, moderate, and mild infection patients and healthy control (HC) subjects in every 10 days of temporal were compared. $^{*}P < 0.05$ for severe/critical vs. moderate; $^{\triangle}P < 0.05$ for severe/critical vs. mild; $^{\#}P < 0.05$ for moderate vs. mild. The levels of TGF-β1 (A), IgA (B), IgG (C), and IgM (D) of COVID-19 patients in the 1st, 2nd, 3rd, 4th, and 5th 10 days from the symptom onset are presented in the plot diagrams. Numbers of cases at each time point are shown under each plot.

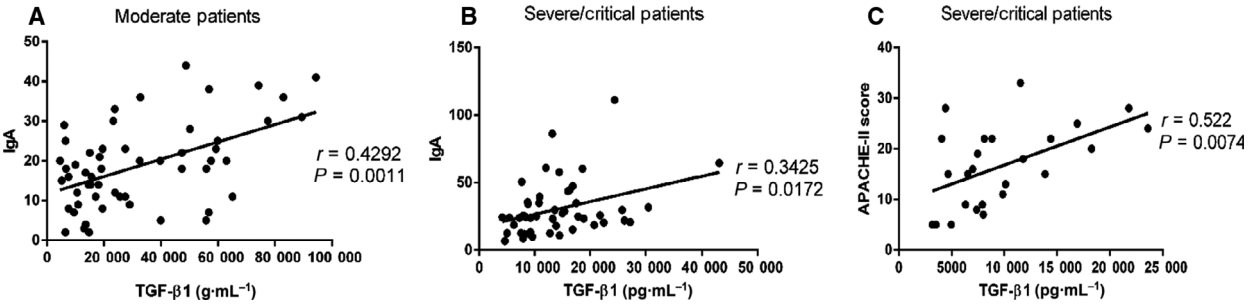

Fig. 2. The correlation of TGF-β1 with SARS-CoV-2-specific IgA in moderate (A) ($n = 48$) and severe/critical patients (B) ($n = 55$) at 30 days after the onset of COVID-19. We found significantly positive correlation of TGF-β1 with APACHE II scores in severe/critical patients (C).

interleukin (IL)-6, IL-8, monocyte chemoattractant protein (MCP)-1, and granulocyte–macrophage colony-stimulating factor (GM-CSF) production by normal human lung fibroblasts (NHLFs) [16]. Crosslinking of the Fc alpha receptor (FcαRI) by IgA and pathogen is able to transmit activating signals leading to

phagocytosis, respiratory burst, antibody-dependent cellular cytotoxicity (ADCC), increased antigen presentation, degranulation, and cytokine release that can exacerbate inflammatory responses in the host [17]. Consequent to our previous observations of enhanced IgA responses in COVID-19 [2], this is the first study to observe the simultaneous changes in serum levels of TGF-β1 and serum antibodies (IgA, IgG, and IgM) in patients with different severities of COVID-19 disease (including nonsevere, severe, and convalescent patients) with different time courses. The results of this study suggest that increased level of the IgA isotype switch factor TGF-β1 is responsible for abnormal IgA responses. TGF-β1 has also been suggested as an important factor that causes fatal symptoms of pulmonary fibrosis in SARS and MERS. Studies have shown that the levels of TGF-β1 in SARS and MERS increased significantly at the end of the disease course [5,7]. The elevation of TGF-β1 in patients with severe and moderate COVID-19 disease may be due to the large area of infectious lesions in lung tissue. Lung tissue can produce a large amount of TGF-β1 during viral infection [18]. In addition, TGF-β1 is also produced by infiltrated neutrophils activated by SARS-CoV-2 infection, as well as by macrophages recruited by SARS-CoV-2-induced cell apoptosis [19]. It was noted that the TGF-β1 level in severe patients is significantly lower than that in moderate patients. One possible explanation is that most of the severe patients in this study had undergone the ICU care, treated with corticosteroids (such as methylprednisolone), which inhibit effectively TGF-β1 production [20,21]. In contrast, the effect of corticosteroid therapy on IgA levels is not as obvious [22].

The study by Sterlin et al. observed that elevated IgA plasmablasts shortly after the onset of symptoms of COVID-19 with the peak at the third week of the disease [23]. Plasmablast levels were also significantly elevated in COVID-19 convalescent patients [24]. Considering the TGF-β1 results together with our previous finding of serum IgA in COVID-19, the TGF-β1-IgA axis may play an important role in the pathogenesis of COVID-19. The increased TGF-β1 levels in COVID-19 patients may also suggest TGF-β1 as a potential target for clinical intervention, considering new drugs targeting TGF-β1 have been developed [25]. Future research is warranted to evaluate the clinical potential of TGF-β1 in prognosis prediction and therapy of COVID-19.

Author contributions

ZL, HH, and SKH conceptualized the study. EW, HC, BS, HW, and HQQ designed methodology. EW, HC, BS, HW, HQQ, and JQ involved in formal analysis. EW, HC, BS, HW, and HQQ investigated the data. YL, XS, ZF, LT, and YZ provided resources. EW, HC, BS, HW, HQQ, and JQ curated the data. EW, HC, BS, HW, and HQQ wrote the original manuscript. ZL, HH, and SKH wrote, reviewed, and edited the manuscript. ZL, HH, and SKH supervised the data. All authors have read and agreed to the published version of the manuscript.

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Data accessibility

The data presented in this study are available on request from the corresponding author.

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