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COVID-19 and asthma, the good or the bad?

To the Editor,

> "Misfortune might be a blessing in disguise."
> – Tao-Te-Ching (Book of the Way) by Lao-Tzu, 350 BCE

Since its first report in Wuhan, China, in December 2019, the novel pandemic COVID-19, caused by SARS-CoV-2 virus, has rampaged throughout the world.[1] People with asthma and allergies are usually at greater risk of more severe outcomes with virus infections. However, recent reports have accumulated evidences that the prevalence of allergic diseases and asthma in patients with COVID-19 is lower than expected among other comorbidities and risk factors of the severe form of COVID-19 (Appendix S1 for additional reference 1-3). Why are then allergic diseases and asthma underrepresented as co-morbid risk factors in patients with COVID-19? Is this a sampling bias in the currently published clinical reports or is there a real discrepancy in the prevalence of asthma among COVID-19-infected patients that may glean a light for us to fight this pandemic? Here, we hypothesize the plausible mechanisms in asthmatics based on available publications (Appendix S1) that may have effects in determining their susceptibility to and disease severity with SARS-CoV-2 infection.

Firstly, coronavirus recognition and infection are dependent on the cellular receptors, the angiotensin-converting enzyme 2 (ACE2) for docking spike protein of SARS-CoV-2, and transmembrane protease serine 2 (TMPRSS2) to cleave the docked spike protein for virus entry by membrane fusion (Appendix S1, Reference 4, 5). The gene expression levels of ACE2 and TMPRSS2 are influenced by the genetic variants of the host and microbial infections, and induced by innate immune response, such as the production of interferons and mucins.[2] In asthma patients, the expression of these two molecules in the respiratory epithelial cells during SARS-CoV-2 infection is determined by the age, gender, comorbidity, and type 2 allergic inflammation. Jackson et al[3] found asthma and respiratory allergy are associated with reduced ACE2 expression in airway cells based on their patient cohorts. In contrast, Sajuthi et al[4] demonstrated that TMPRSS2 is part of a mucus secretory network, highly upregulated by type 2 allergic inflammation mainly by interleukin-13. Peters et al[5] found the gene expression for ACE2 and TMPRSS2 in the cells obtained from induced sputum did not differ between healthy subjects and asthmatics. Therefore, these reports did not reveal convincing information, regarding whether asthmatic patients have lower expression of ACE2 and serine protease TMPRSS2 for SARS-CoV-2 based on their allergic status and/or chronic lung inflammation. Particularly, these studies lack the effect of SARS-CoV-2-specific analysis and observations in asthmatic patients to be able to reach a conclusion in the real world.

Secondly, viral load and immune response of the host determine the final outcome and/or the severity of ARDS and multiple organ failure in COVID-19-infected patients. For asthmatic patients, the innate immune response to COVID-19 infection may be impaired due to lower levels of IFNγ in their bronchial epithelial cells, but it may also be favourable in reducing ACE2 expression, which is depended on IFNγ production.[4] In addition to IFNs, there are other molecules of innate immunity in the respiratory tract that may also have anti-viral functions, such as mannose-binding lectin (MBL), and surfactant protein A (SP-A) and D (SP-D) that are produced by alveolar type 2 cells in the lung, which are also largely infected by SARS coronavirus. These molecules, MBL and SP-D, found in higher concentrations in the BALF of patients with asthma and respiratory allergy and are increased due to chronic inflammation, have been identified to bind spike protein of SARS coronavirus, inhibit its binding of the ACE2 cellular receptor and are thereby able to protect the alveolar macrophages from virus-induced activation.[6,7] Recently, trained immunity found in the myeloid cells and alveolar macrophages of asthmatics may provide anti-viral immunity in specific organs such as the lungs (Appendix S1, reference 6-8). Although this hypothesis has not been validated in patients with COVID-19, clinical trials to boost trained innate immunity by BCG vaccination to protect against COVID-19 have been initiated in several countries.[8]

Finally, we highlight that therapeutic medications and biologics used for asthma control may have some beneficial pharmacological effects in COVID-19 infections. From in vitro models, inhaled corticosteroids alone or in combination with bronchodilators have been shown to suppress coronavirus replication and cytokine production (Appendix S1, reference 9). Asthmatic patients using inhaled corticosteroids (ICS) demonstrated lower expression of ACE2 and TMPRSS2 in their bronchial epithelial cells.[5] There is a clinical report of the improvement in three COVID-19-infected patients after using inhaled ciclesonide, although this study did not have proper controls (Appendix S1, reference 10). Clinical observation in children with severe asthma who received anti-IgE monoclonal antibody (omalizumab) have shown decreased duration of human rhinovirus infections, viral shedding and risk of virus-related exacerbation (Appendix S1, reference 11). In vitro, omalizumab attenuated plasmacytoid dendritic cell (pDC) FcεRIα protein expression while simultaneously augmenting pDC IFN-α responses to rhinovirus and influenza virus (Appendix S1, reference 12). Together, these findings provide direct evidence that blocking IgE decreases susceptibility to respiratory viral illnesses through enhanced IFN-α responses in pDCs. More interestingly, azithromycin combined with hydroxychloroquine in an open-label nonrandomized clinical trial for COVID-19-infected patients has significantly decreased the viral load after six

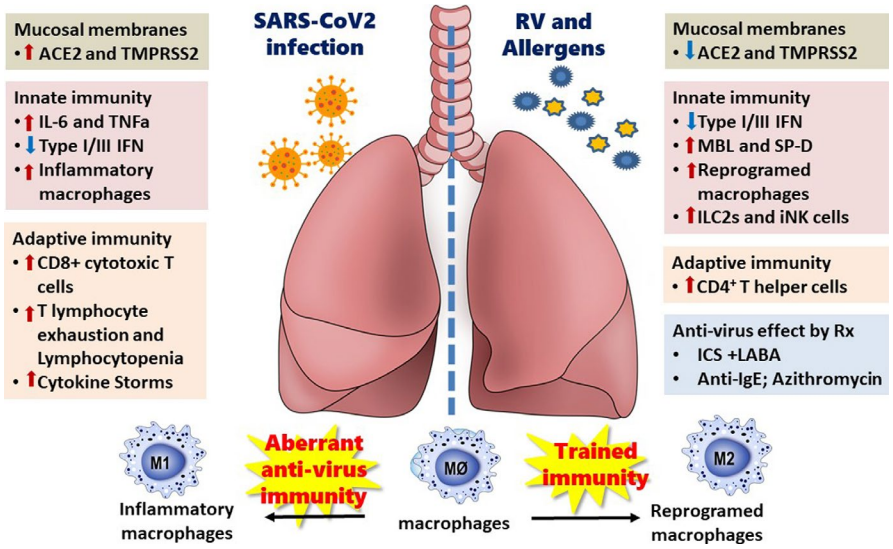

FIGURE 1 The immunopathogenesis of COVID-19 and asthma

days of treatment compared with untreated controls (Appendix S1, reference 13). Azithromycin has been demonstrated to reduce the frequency of asthma exacerbation and improve the quality of life of asthmatic adults and preschool children with asthma that was not adequately controlled on standard inhaler therapy (Appendix S1, reference 14, 15). Although the mechanisms of this anti-inflammatory effect in asthma is still not well defined, Gielen and co-workers (Appendix S1, reference 16) demonstrated that azithromycin augments IFN-β and IFN-λ production and decreasing rhinovirus replication and release from rhinovirus-infected human bronchial epithelial cells in vitro. Other studies also presented reduction levels of IL-6 and TNF-α after azithromycin treatment (Appendix S1, reference 17, 18). More interestingly, it is suggested that azithromycin may have protective effects in reducing SARS-CoV-2 invasion by interfering with ligand/CD147 receptor interactions, a novel SARS-CoV-2 cellular receptors beside ACE2, and decreasing the expression of some metalloproteinases (downstream to CD147) in primary human bronchial epithelial infected with rhinovirus (Appendix S1, reference 19). However, controlled clinical trials using azithromycin to treat patients with COVID-19 (not involving asthmatic subjects) are now registered in several countries with results still pending.

In summary, we have seen a new zoonotic coronavirus, SARS-CoV-2, infection that has had a devastating effect on the host immunity via the inhibition of interferons leading to aberrant innate immune response, macrophage inflammation in releasing a cytokine storm and exhaustion of the cellular immunity of T lymphocytes.[9] Fortunately, due to chronic and sustained type 2 immune inflammation in the lungs of asthmatic patients, or by the medications they use for asthma control, it seems asthma may not be a major confounding disease in COVID-19 infection, and this unexpected phenomenon may shed a new light on finding therapies or preventative strategies for SARS-CoV-2 (Figure 1). We still need a more comprehensive and in-depth immune analysis of SARS-CoV-2 infection in the coming days to explore this hypothesis. However, all standard asthma therapies, whether inhaled steroids, combination of inhaled steroid plus long acting bronchodilator therapies or monoclonal antibodies like omalizumab and azithromycin, should be continued to be used to optimize asthma controls. For they can not only substantially reduce the risk of asthma exacerbation, but also will reduce risks and severe outcomes of COVID-19.

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CONFLICT OF INTEREST

All authors declare no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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A novel method for precise detection of allergen-specific IgE via immobilizing His-tagged allergens to paper-based device

To the Editor,

Allergic diseases are characterized by elevated specific IgE (sIgE) serum levels against certain allergens.[1,2] Thus, the fast and precise detection of sIgE is necessary for the diagnosis of allergic diseases. Clinical exist diagnosis methods all utilize allergen's own groups to immobilizing it,[3] with the involved parts often being ambiguous and uncontrollable (Figure 1A). In this mode, sIgE-binding epitopes of allergens located on or beside attached sites could be covered due to the steric hinderance, causing the inaccurate sIgE identification and misdiagnosis of allergic diseases[4-6] (Figure 1A). This is a serious problem that has been overlooked for a long time in the clinical practice for sIgE identification. An alternative method that could overcome this main drawback is emergently needed.

We herein developed a paper-based device with surface modification of Ni-NTA moieties for recombinant allergen protein immobilization to realize fast, low-cost, high-efficiency, and quantitative sIgE detection in serum samples (Figure 1C). Papers are easy to be prepared and surface functionalized, which possess good application prospects in medical detection and diagnosis.[7,8] In this study, we first synthesized the Ni-NTA and subsequently fixed it on the surface of paper through mild and efficient Schiff base reaction (Figures S1-S2). Then, we used His-tagged recombinant Can f 1 (rCan f 1), one of the main dog allergen proteins, as the model allergen to evaluate the performance of this paper-based device in the sIgE detection. His-tag, consisting of six consecutive histidine residues, is a common tag fused in recombinant proteins for the better purification.[9] The His-tagged rCan f 1 was efficiently immobilized by the interaction between His-tag and Ni-NTA on the paper surface (Figure 2A-C). The features of this strategy for allergen immobilization were highlighted with strong and specific binding site, particularly the preservation of sIgE-binding activity without covering any IgE-binding epitopes of allergens (Figure 1B). The presence of IgE in human sera allergic to dog extracts used in these studies was confirmed by SPT and ImmunoCAP (ThermoFisher, Fremont CA). The sIgE levels in reference sera were analyzed by the ImmunoCAP system, using Can f 1 covalently bound to the CAP solid support (kindly supplied by Prof. Jinlv Sun from Peking Union Medical College Hospital). These reference sera were used for the optimization of the paper-based device and establishing calibration curve of sIgE quantitative detection.

To achieve the excellent performance, we firstly optimized the experimental parameters. After a series of experiments, the best parameters were finally confirmed as that each microzone of the paper-based device was incubated with 4 μL of Ni-NTA (2 mg/mL) solution, 3 μL of rCan f 1 (0.06 μg) solution for 5 minutes, 3 μL of 10-fold diluted serum samples for 10 minutes, and 3 μL of 1000-fold diluted horse radish peroxidase (HRP)-labeled