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# ACE2: The Only Thing That Matters?

In December 2019, cases of a respiratory disease were reported in Hubei Province, China, caused by a positive-sense RNA virus from the family *Coronaviridae* (1). Subsequently, the disease was called coronavirus disease (COVID-19) and the virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The outcome of infection with SARS-CoV-2 is highly variable; on one hand, the virus has been responsible for more than 360,000 deaths worldwide, and on the other hand, there is a diverse range of clinical outcomes in different people (2). For any virus, infection depends on the ability to *1*) enter, *2*) evade cellular defenses, *3*) hijack host machineries to express viral genes, *4*) replicate new genomes, *5*) assemble viral particles, and *6*) exit. Virus tropism, the ability to infect particular cell types, is defined by the differential expression of host factors the virus subverts or evades during these processes. The earliest determinant is binding and entry via a cell surface receptor.

For SARS-CoV-2 entry, the primary receptor is ACE2 (angiotensin I–converting enzyme 2), which serves as receptor for SARS-CoV and a human seasonal coronavirus, human coronavirus NL63 (HCoV-NL63) (1). The physiological role of ACE2 is the regulation of the renin-angiotensin hormone system, regulating blood volume, systemic vascular resistance, and cardiovascular homeostasis (3). ACE2 is abundantly expressed in intestine, liver, kidney, and testis (proteinatlas.org). Because COVID-19 is primarily a respiratory disease with obvious virally induced lesions in the lung, there has been intense interest to characterize ACE2 expression in the respiratory tract.

In the current issue of the *Journal*, Zhang and colleagues (pp. 219–229) have analyzed a broad range of preexisting RNA expression microarray data from human trachea and small and large airway epithelium (SAE/LAE) (4). They confirm ACE2 expression in these tissues and report higher levels of ACE2 in the trachea and LAE as compared with SAE. Similarly, Sungnak and colleagues recently reported at a single-cell level that upper airway cell types, including ciliated cells, express ACE2 mRNA (5). Lee and colleagues confirmed this at the protein level, showing ACE2 expression on the motile cilia by immunofluorescent staining (6). Together, these findings imply that because of abundant ACE2 expression, respiratory cells in the upper respiratory tract, particularly ciliated cells, can be infected by SARS-CoV-2 and that they may be more susceptible to infection than those in deep lung. Indeed, Hou and colleagues employed an elegant reverse genetic approach in which recombinant SARS-CoV-2 viruses expressing GFP (green fluorescent protein) were used to infect cells from different levels of the respiratory tract and showed that the gradient of decreased expression of ACE2 from nose to alveolus is mirrored by a decrease in permissiveness to virus infection (7).

However, ACE2 expression may not be the only factor determining SARS-CoV-2 permissivity.

Not all cells that express ACE2 are susceptible to SARS-CoV-2 infection. Re-evaluating single-cell RNA sequencing allowed Zhang and colleagues to identify expression of ACE2 in all SAE cell types (even if at reduced expression relative to LAE), including club cells. Others have confirmed the presence of ACE2 protein and the surface activating protease TMPRSS2 (transmembrane protease, serine 2) in club cells (7). Nevertheless, club cells do not get productively infected by SARS-CoV-2 (7). Club cells have a stem cell–like function in the respiratory epithelium and potentially express intrinsically high levels of some antiviral IFN-stimulated genes, such as IFITMs (IFN-induced transmembrane proteins) and Ly6E (lymphocyte antigen 6E) (8), both described as coronavirus restriction factors (9, 10).

Just as expression of cell surface proteins used for SARS-CoV-2 entry does not always confer susceptibility to infection, different expression levels of ACE2 between individuals do not necessarily determine disease outcome. One key question in the field is why children are less affected by SARS-CoV-2 infection despite similar seroprevalence rates. Some studies have shown an age-dependent direct correlation between levels of ACE2 expression in nasal epithelium and age (11), but in other studies, this pattern did not hold up (6).

Another example is the effect of smoking. When the pandemic first started, smoking was considered a risk factor for COVID-19, as it is for many other respiratory virus infections. Zhang and colleagues were able to categorize their analysis of SAEs according to the smoking status and identified that male smokers had an increased expression of ACE2. This is complementary with other studies that have reported the same observations at the protein level (12). Strikingly, numerous epidemiological reports have found that smokers are actually underrepresented for COVID-19 complications (2, 13). Notably, a study with 1,099 individuals showed that smokers represented only 12.6% of COVID-19 cases while representing 30% of the Chinese population (2). These observations are inconsistent with the increased expression of the virus receptor and emphasize that receptor abundance is not the only factor important for severe disease progression.

Understanding the wide spectrum in severity of COVID-19 disease in different individuals infected by SARS-CoV-2 is important but challenging because disease outcome is determined by a combination of exposure levels, virus, and host responses. A first and crucial step is to understand how expression levels of genes we know



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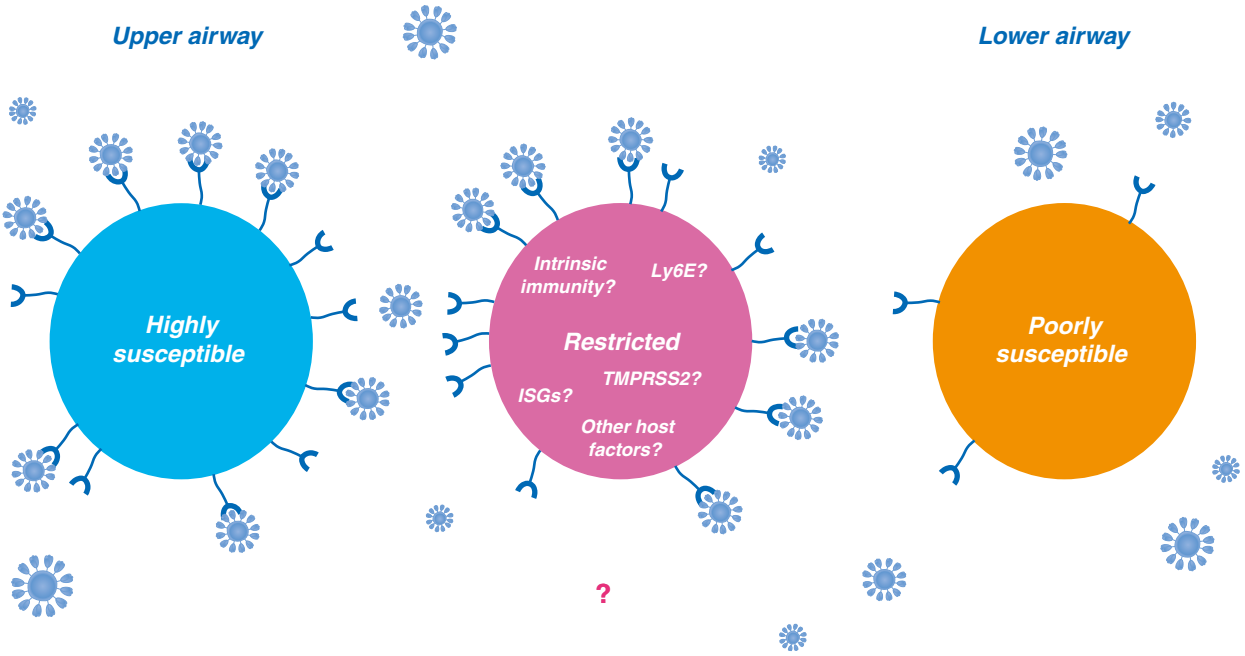


**Figure 1.** In some cells, ACE2 expression is higher (left cell), whereas in other cells, ACE2 expression is lower (right cell). In the case of the airways, this renders the more highly expressing upper airway cells inherently more permissive to infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Nevertheless, in other cells (central cell), ACE2 expression is high, and yet the population remains resistant to SARS-CoV-2 infection. This phenotype is potentially dictated by the expression levels of additional proviral factors, or restrictive host factors such as IFN-stimulated genes (ISGs).

to be involved in virus replication might vary within and between hosts. The paper from Zhang and colleagues makes an important contribution to this body of knowledge and underscores again the key role of ACE2 as the virus receptor. However, taken in the wider context of accumulating cell biological and epidemiological information, the correlates appear to be less clear cut than expected (Figure 1). Other host genes that correlate with cell permissivity to SARS-CoV-2 infection now need to be defined in an unbiased manner. In addition, coupling epidemiological observations with this knowledge, for example to understand why different cohorts have different risks of disease progression, might reveal a novel Achilles heel for the virus. ■

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Raul Y. Sanchez-David, Ph.D.
Olivia C. Swann, B.A.
Thomas P. Peacock, Ph.D.
Wendy S. Barclay, Ph.D., F.Med.Sci.
*Section of Molecular Virology*
*Imperial College London*
*London, United Kingdom*

ORCID IDs: 0000-0001-7110-0189 (R.Y.S.-D.); 0000-0002-0155-7818 (O.C.S.); 0000-0001-7077-2928 (T.P.P.); 0000-0002-3948-0895 (W.S.B.).

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# Complement Activation during Critical Illness: Current Findings and an Outlook in the Era of COVID-19

*Esse quam videri*
—Marcus Tullius Cicero

The complement system is often underestimated. Progress of complement research and consequential clinical applications seem slow but steady. The first glimpse of the existence of complement system was obtained by Jules Bordet (a later Nobel prize laureate) during his pioneering work in the late 19th century. Thereafter, it took over one-hundred years until the first complement inhibitor, eculizumab, received approval by the U.S. Food and Drug Administration in 2007. Eculizumab is a humanized anti-C5 (complement component 5) antibody preventing the cleavage of C5 into C5a and C5b, the central converging point of all pathways of complement activation (Figure 1). Eculizumab improves the survival of patients with paroxysmal nocturnal hemoglobinuria (1). It is also effective for atypical hemolytic uremic syndrome and neuromyelitis optica spectrum disorder (2). These three disease entities have in common the fact that before the introduction of anticomplement therapy, either no choices or only very limited choices of other drugs were available. This recent success story of complement inhibition has refueled a broader interest in this ancient system of innate immune defense for prognostic, diagnostic, and therapeutic exploitation.

In their current work in this issue of the *Journal*, Bain and colleagues (pp. 230–240) report on the association between alternative complement pathway activity and better survival in patients with critical illness (3). The alternative pathway hemolytic assay (AH50) and total complement activity (CH50) tests were retrospectively analyzed in a single-center heterogeneous cohort of $n = 321$ patients with acute respiratory distress syndrome (33%), with suspected sepsis (63%), and on mechanical ventilation (96%). Samples from the first 2 days after ICU admission were measured using commercially available, non–U.S. Food and Drug Administration approved tests. Of note, complement diagnostic tests can be challenging, and sophisticated functional assays have limitations. The patients with a depleted AH50 activity (i.e., below the statistical median of the cohort) had a higher probability of 30-day mortality (36% vs. 22%) and lower 1-year survival. These correlations were not observed for CH50. Preserved AH50 activity correlated with higher serum concentrations of alternative pathway proteins (factor B, factor H, and properdin) and a "hypoinflammatory" phenotype (bicarbonate, IL-8, and TNFR1) but did not correlate with the used definition of immune suppression. Survivors of critical illness showed increased transcriptional expression of complement genes in peripheral blood cells. A higher alternative pathway activity was associated with a lower frequency of bacteremia. Lastly, mice with deficiency of C3 or factor B were prone to splenic dissemination of *Klebsiella pneumoniae* infection.

So, what disease mechanisms could explain the described correlation between higher AH50 and better survival of critical illness? The alternative pathway of the complement system is activated by spontaneous hydrolysis of C3 on foreign surfaces of pathogens (Figure 1), which, unlike host cells, lack the presence of complement inhibitory surface proteins (CD46 and CD55). Complement activation mediates pathogen clearance by the formation of the membrane-attack complex, opsonization for phagocytosis, and modulation of inflammation by chemotactic immune-regulatory anaphylatoxins (Figure 1) (4). Hence, alternative pathway activity may provide control of bacterial infections as a protective mechanism of host defense. Survivors of critical illness may simply have higher capacities of complement protein production or a superior ability to rapidly initiate and de-escalate complement activity, as the authors discuss. Another viewpoint is that low AH50 could denote patients after exuberant complement consumption. Inappropriate complement activity may result in the unloading of harmful effector functions on host cells, with the consequence of disease-causing tissue injury and organ dysfunction during critical illness. The harmfulness of complement overactivation is underscored by the fact that cobra venom factor from poisonous snakes hijacks the alternative pathway, with clearly adverse effects for the host. Therefore, it seems premature to consider whether therapeutic infusions of alternative complement proteins could increase the survival of patients with critical illnesses.



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