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EDITORIAL COMMENT

Integrating Integrins

A New Way to Increase Our COVID-19 Armamentarium?*

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As I write this piece, the coronavirus disease-2019 (COVID-19) pandemic remains the focal point on the front page of the world's newspapers and as a centerpiece of each evening's news programs. The daily numbers continue to rise astronomically, states and countries move into a repeat lockdown, and "the curve" clearly has not flattened. At this moment, according to the Johns Hopkins Coronavirus Resource Center, there are 58,456,049 global cases with over 1.38 million deaths; there are 12,177,301 cases in the United States alone, with deaths exceeding 250,000. Numbers have increased dramatically, by over 1,000,000 in the United States, just in the last week. Thus, with this unfortunate data at hand, we all look to gather as much scientific evidence to advance our ability to treat and potentially prevent the effects of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, even as we receive tremendously encouraging news about 2 vaccine trials.

Early in the pandemic, Zhou et al. (1) published a seminal piece outlining key clinical aspects of the epidemic that had begun a short time before in China. They defined the genomic sequence of the causative virus along with confirmation of the receptor on cells which allowed viral entry, namely the angiotensin-converting enzyme 2 (ACE2) receptor. ACE2 expression is wide and includes cells of the vascular system such as endothelial and vascular smooth muscle cells, and also ones in the myocardium, where it is detected in fibroblasts, myocytes, and pericytes, among other cell types. ACE2 function has been linked to a range of cardiac diseases, including heart failure, hypertension, pulmonary hypertension, and myocardial infarction. In particular, ACE2 has been recognized for its protective role as a negative regulator of the activated renin-angiotensin system so instrumental in cardiac pathologies. Yet, potentially with SARS-CoV-2 infection, ACE2 is endocytosed with the bound virus, leading to loss of its protective role.

Much attention has been on the role of ACE2 in viral entry and potential roles of ACE inhibitors and angiotensin receptor blockers in COVID-19. Though still in evolution, data support if anything, that ACE inhibitors and angiotensin receptor blockers may well play a protective role against COVID-19.

Along with these studies has come others on what other mechanism and proteins might also facilitate viral entry into cells, including work that was recently published in *JACC: Basic to Translational Science* by Bristow et al. (2). This prior work sets the stage for the subject of this Editorial Comment, the paper by Beddingfield et al. (3) in this issue of *JACC: Basic to Translational Science*, and therefore is worth briefly reviewing. Bristow et al. (2) set out to explore the concept that increased ACE2 as found in severe cardiac disease, such as end-stage heart failure, might be a link to virally mediated heart damage and increased mortality when COVID-19 infects patients with underlying heart disease. They evaluated samples from patients with only mild-to-moderate cardiac dysfunction who were not treated with renin-angiotensin system inhibitors, as opposed to patients with end-stage disease, and found that ACE2 expression was also up-regulated in the mild-to-moderate patient samples, thereby potentially predisposing these individuals to myocardial injury and increased mortality from COVID-19. Pertinent to the

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Beddingfield et al. (3) paper is that Bristow et al. (2) keyed in on prior work on cellular receptors termed *integrins*. Integrins had been previously shown to facilitate internalization of a number of viruses (including SARS-CoV-2) (4) through their RGD (arginine, glycine, and aspartate) motif, and also had been shown to bind ACE2. Although not conclusive, the Bristow et al. (2) work suggested that integrins binding to the SARS-CoV-2 spike protein may serve as a co-receptor for virus, increasing viral binding and entry into myocardium.

Before discussing the paper by Beddingfield et al. (3), we must understand a bit about integrins and what relevance they have in the cardiovascular system, aside from a potential role in COVID-19.

Integrins are heterodimeric, transmembrane glycoprotein receptors composed of α and β subunits that are capable of bidirectionally transmitting signals between the intracellular and extracellular environments (5). Traditionally, in addition to their role in binding to viruses, they are thought of as adhesive receptors that attach cells to extracellular matrix, and in some cases, to other cells. They constitute a large family of proteins, with mammals expressing more than 18 α- and 8 β-integrin subunits that heterodimerize to form 24 different receptors. Integrins are expressed in most cells and can bind varied extracellular ligands such as laminin, collagen, fibrinogen (FN), tenascin-C, and cadherin. The RGD sequence noted previously as being important for viral internalization was first recognized as being present in FN, laminin, and vitronectin matrix substances.

Critical for the cardiovascular system is that integrin receptors also transmit mechanical signals into and out of the cell and enable conversion of mechanical information into chemical signals. Work in our group using dominantly mouse models showed that when cardiac myocytes were made deficient in β1 integrins, myocardial development or function was dramatically affected. If the myocytes were made deficient early in cardiogenesis, ventricular compaction and reduced cardiomyocyte proliferation were seen along with a ventricular fibrosis, resulting in 50% lethality by weaning. If deficiency was delayed until the perinatal period, progressive myocardial fibrosis and development of a dilated cardiomyopathy was produced, and the mice were intolerant of hemodynamic loading and ischemic challenges. Conversely, when the dominant laminin-binding α7β1D cardiomyocyte integrin was overexpressed in myocytes, the heart was protected from ischemia-reperfusion injury. Therefore, as we contemplate manipulating integrins for therapeutic purposes, these varied functions must be considered.

In this issue of *JACC: Basic to Translational Science*, Beddingfield et al. (3) studied the potential role of using an integrin binding peptide, termed ATN-161, as a means to reduce or prevent SARS-CoV-2 infection. ATN-161 is derived from a domain of FN. Capitalizing on the knowledge as outlined previously that integrins may act as a co-receptor with ACE2 for SARS-CoV-2 via its RGD binding domain, the authors explored the ability of the SARS-CoV-2 spike protein to bind to a specific integrin heterodimer (α5β1) that is recognized as an FN receptor. In line with this was the study by Bristow et al. (2), which found that α5 was a key integrin subunit modulated by cardiac remodeling, and they posited that it is important for SARS-CoV-2 viral binding and internalization, thus pre-disposing individuals with integrin and ACE2 upregulation, to myocardial damage and untoward disease outcomes. First, Beddingfield et al. (3) used in vitro studies to show that ATN-161 could bind the viral spike protein and could inhibit ACE2 or ACE2/α5β1 binding in a dose-dependent manner on a plastic tissue-culture plate alone. Then, using VeroE6 kidney cells of African green monkey origin that are known to express α5β1 integrin, they further showed that ATN-161 could reduce the viral load of infected cells, as well as inhibit cell damage or lysis. Molecular modeling suggested that the ATN-161 inhibitor would disrupt the integrin-ACE2 complex formation, and in doing so, reduce viral spike binding to this complex and thus viral uptake into cells. Beddingfield et al. (3) note the potential themselves that the integrin inhibitor they used might well have lesser specificity than implied, and bind not only α5β1 integrin, but also other integrins, such as another co-receptor integrin αvβ3, which is a vitronectin receptor, is highly expressed on endothelial cells, and binds a number of other matrices, growth factors, and metalloproteinases. Still, Beddingfield et al. (3) suggest that because ATN-161 has been characterized in numerous studies over many years, including trials that have studied its role as an antitumor therapeutic, they hypothesize that it could be “fast-tracked” as a novel therapeutic for COVID-19.

The current work by Beddingfield et al. (3) clearly advances our understanding of how SARS-CoV-2 may enter cells and even how it may prevent cell death. Beddingfield et al. provide a provocative path forward using the inhibitor they evaluated as a therapeutic for COVID-19. Still, limitations are evident in that the study is fully reliant on biochemical and molecular work in vitro, combined with cell culture. Further studies will clearly require evolution first to small animal work and ultimately to clinical trials. This is particularly true if one were to deliver this

inhibitor systemically, as one must consider the plethora of integrin receptors that exist in this large family, the specificity of the ATN-161 inhibitor, and that integrins as briefly discussed previously have multiple functions beyond facilitating viral attachment. Also, despite the fact that Beddingfield et al. (3) have outlined how this inhibitor has already been used in clinical trials and thus will have an accelerated path forward, only 2 prior trials are noted on ClinicalTrials.gov. Both date back to 2007 to 2008 and are focused on treatment of cancers. Still, let us remain optimistic. In addition to treatments that may reduce severity of disease or vaccines that hopefully will reduce it, some other creative proposals have suggested that beyond standard public health measures such as masking and social distancing, use of nasal drops might prove useful as a preventive measure. Perhaps a morning "squirt" of ATN-161 into each nostril is in our future! Stay tuned.

AUTHOR DISCLOSURES

Dr. Ross has reported that he has no relationships relevant to the contents of this paper to disclose.

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