Very Late Antigen-4: A Novel Receptor for Club Cell Secretory Protein 16 to Control Inflammation

In this issue of the *Journal*, Johnson and colleagues (pp. 1410–1418) have provided an exciting novel mechanism by which CC16 (club cell secretory protein 16) inhibits leukocyte adherence to endothelial cells and subsequently reduces migration and accumulation of leukocytes to the mouse lungs infected with an atypical bacterium *Mycoplasma pneumoniae* (1). Specifically, they identified a leucine–valine–aspartic acid (LVD) motif in CC16 protein that directly binds to α4β1 integrin or VLA-4 (very late antigen-4), one of the integrin family members involved in leukocyte migration. The LVD motif is critical to the role of CC16 in reducing lung inflammation and airway hyperresponsiveness (AHR) during acute bacterial infection.

CC16, also known as uteroglobin, SCGB1A1 (secretoglobin family 1A member 1), and CC10 (club cell 10 kD protein), is primarily produced by the club cells in human small airway (diameter <2 mm) epithelium. In mice, CC16 is produced in epithelium of entire conducting airways (from the trachea to the terminal bronchioles). CC16 is abundant in both human and mouse airways. CC16 deficiency has been reported under various pathological or disease conditions, including cigarette smoke exposure, chronic obstructive pulmonary disease (COPD) (2), asthma (3), acute lung injury, and idiopathic pulmonary fibrosis (4). The decreased levels of CC16 have been associated with exaggerated proinflammatory responses and worsening of clinical outcomes such as acute exacerbations of COPD and pulmonary functions (5, 6). CC16 has been shown to exert several functions that are beneficial to the host to cope with injury, repair, and respiratory pathogen infections. These include its role in the antiinflammatory response (7) and tissue repair such as inhibition of airway mucus production induced by IL-13 and LPS (8).

The role of CC16 has been carefully examined in various *in vitro* and *in vivo* models, including a respiratory syncytial virus–induced mouse model of lung inflammation and AHR (9), cigarette smoke–induced COPD model (10), and mouse model of airway allergic inflammation (11). So far, the underlying mechanisms of the antiinflammatory function of CC16 remain elusive. Previous studies suggest that CC16 may inhibit NF-κB activation (12), the release of proinflammatory cytokines TNF-α and IFN-γ, and the expression of chitinase 3–like 1, a proeosinophilic mediator (13). As these proposed mechanisms are mostly indirect, there is an urgent need to study how CC16 exactly attenuates the proinflammatory response. The study by Johnson and colleagues timely addressed this important question by discovering VLA-4 as a receptor for CC16. VLA-4 is expressed by leukocytes, including monocytes, lymphocytes, natural killer cells, basophils, and eosinophils. Interestingly, VLA-4 expression on human blood neutrophils is controversial. However, VLA-4 can be expressed or induced on mouse neutrophils and is critical to lung neutrophil recruitment following bacterial infection (14). The ligands for VLA-4 include VCAM-1 (vascular cell adhesion molecule-1) expressed by endothelial cells, fibronectin found in extracellular matrix, MAdCAM-1 (mucosal vascular addressin cell adhesion molecule-1), and JAM-B (junctional adhesion molecule-B). The VLA-4/VCAM-1 pathway activation contributes to inflammatory diseases such as asthma (15), as it is involved in the migration and activation of $CD4^+$ (cluster of differentiation 4–positive) T cells, B cells, monocytes, bone marrow neutrophils, basophils, and eosinophils. Thus, the discovery of CC16 binding to VLA-4 could have broad implications to target VLA-4–mediated inflammatory responses.

In this issue of the *Journal*, Johnson and colleagues (pp. 1410–1418) demonstrated the effectiveness of the wild-type CC16 protein in reducing lung neutrophil recruitment and AHR in a mycoplasma infection mouse model, which was dependent on the LVD motif being present within the protein. Given their finding that CC16 significantly reduced the adherence of a monocyte cell line (THP-1 cells) to human endothelial cells, it would be more informative to demonstrate whether lung macrophage recruitment was increased in CC16-deficient (versus wild-type) mice after lung infection with *Mycoplasma pneumoniae*. If so, an exaggerated macrophage inflammatory response in CC16-deficient mice could be ameliorated by the recombinant wild-type intact CC16 protein but not by the mutant CC16 protein with the loss of the LVD motif. The study by Johnson and colleagues has opened the door for the research community to pursue studies to address some key questions that may pave the way to use CC16-derived peptides to treat a broad spectrum of inflammatory diseases related to infection, allergen exposure, tobacco smoke, autoimmunity, and cancer. The research questions may include the following: Does CC16-mediated inhibition of VLA-4 binding of leukocytes to vascular endothelial cells reduce the severity of airway inflammation and AHR in mice with respiratory viral infection such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other strains of bacteria utilizing TLR4 signaling (e.g., *Pseudomonas aeruginosa*) rather than TLR2 signaling utilized by mycoplasma (16)? Does CC16 reduce human blood neutrophil or monocyte adherence to vascular endothelial cells under various pathological conditions? The study by Johnson and colleagues used an acute bacterial infection model to test the prophylaxical effect of CC16 in mice lacking CC16. It may also be important to use a subacute or chronic inflammation model (e.g., asthma or COPD) to test the potential therapeutic effect of CC16 on inflammation as well as tissue remodeling in the host with reduced or impaired production of CC16. Regulation of CC16 generation and secretion remains unclear, but loss or reduction in the number of club cells due to lung tissue injury following the harmful effects of cigarette smoke, oxidants, and others have been proposed. Results from these future studies are expected to provide strong rationale toward the application of CC16-derived protease-resistant products to restore homeostasis

Supported by NIH grants R01AI150082, R01AI152504, and U19AI125357.

Originally Published in Press as DOI: 10.1164/rccm.202012-4467ED on January 15, 2021

and host defense functions in human diseases with an excessive inflammatory response. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Hong Wei Chu, M.D.
Niccolette Schaunaman, M.S.
Department of Medicine
National Jewish Health
Denver, Colorado

ORCID IDs: 0000-0002-8329-4803 (H.W.C.); 0000-0002-2880-4436 (N.S.).

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BMP9 in Acute Respiratory Distress Syndrome: Decades of BMP Studies in Vascular Biology Paying Off?

Roughly 20 years ago, BMPR2 was found to be the causative mutation for most heritable pulmonary arterial hypertension (PAH) (1). This was surprising because until then, the BMP pathway was thought to be extremely important in embryonic development, but it did not yet have a known role in adults. The paradigm of reactivation of developmental pathways in injury repair was not common. The discovery of BMPR2 as the PAH gene thus drove two decades of fascinating science about the role of BMP—and other developmental pathways—in the injury repair process. These findings are now starting to make their way to the clinic.

Originally Published in Press as DOI: 10.1164/rccm.202012-4388ED on December 23, 2020

The original BMPR2 mutations were primarily haploinsufficiency, which means there was still a functional pathway, but there just wasn't enough signaling through it. Why not just add more ligand? Unfortunately, the answer to that was the BMP pathway does too many things in too many places. Add ligand, and you might cause heterotopic ossification; in blast injuries, and in fibrodysplasia ossificans progressiva, the problem is too much BMP signaling (2). Therefore, off-target effects made the approach of just adding ligand apparently too dangerous to try.

For the last decade or so, the Morrell group out of Cambridge has been working on a clever workaround to this—BMP9 (3, 4). Although there are many ligands for BMPR2, BMP9 has two singular properties that make it more suitable—and safer—for this purpose. First, most BMP ligands are relatively short range; they are meant to impact cells within a few dozen microns, not halfway across the body. BMP9, in contrast, is primarily made in the liver and circulates (5); in fact, it is the predominant circulating form of BMP ligand (6). Second, BMP9 does not signal through BMPRIa or BMPRIb, the more common type