Extracellular Vesicles: Progress and Challenges in the Study of Human Immunodeficiency Virus and Cocaine-associated Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a disease characterized by progressive remodeling of the pulmonary vasculature, resulting in higher vascular resistance and premature death. The incidence of Group I PAH is markedly elevated in patients with chronic infection with the human immunodeficiency virus (HIV) (1). A better understanding of PAH pathology has led to the approval of 14 therapies since the early 1990s (2), but mechanisms leading to increased incidence in a population with associated risk factors such as HIV remain poorly understood. Extracellular vesicles (EVs) have been shown to be elevated in patients with PAH and to contribute to the disease in animal models (3, 4), suggesting a significant role in regulating PAH development and progression.

In this issue of the *Journal*, Krishnamachary and colleagues (pp. 413–429) address the role of EVs in the pathophysiology of PAH in patients infected with HIV with concurrent cocaine abuse (5) (an important and common risk modifier for PAH in the HIV^+ population) (6). In an elegant series of experiments, using clinical samples as well as *in vitro* and *in vivo* models, they define a role for plasma EVs in patients with HIV (especially in the presence of concurrent cocaine exposure) via increased EV expression of transforming growth factor-β1 (TGF-β1), a central mediator of PAH pathophysiology (7). The authors show that endothelial, smooth muscle cell, and right ventricular dysfunction associated with pulmonary vasculopathy are tied to the delivery of TGF-β1 via EVs, whose biogenesis they link, at least in part, to cells of the macrophage–monocyte lineage. There are many aspects of this pathway yet to be explained. However, the authors convincingly demonstrate that TGF-β1 signaling, which is thought to be fundamental to cardiopulmonary complications of HIV and cocaine abuse (as well as to PAH *per se*), can be mediated to a significant degree by EVs and that this pathway is itself modulated by cocaine exposure.

This science carries significance for future research. EVs, long treated as cellular "dust" or detritus (8), have gained increasing prominence as critical mediators of respiratory disease (9, 10), including roles in the pulmonary vasculopathy of both World Health Organization Group 1 (11) (to which HIV-associated PAH pertains) and Group 3 (10) forms of the disease. It seems likely that EVs are involved in a great number of key pathophysiological processes within PAH (including roles downstream of TGF-β signaling [12]) and elsewhere. However, EVs are technically and scientifically challenging to study, and the work of Krishnamachary and colleagues highlights not only the importance but also the complexity of such work. As such, a number of potentially confounding factors must be noted to contextualize these data. First, given the size and density of the virions, as well as the potential presence of self-proteins and lipids carried over through the budding process, one has to consider the presence of viral particles in the EV fraction isolated either by canonical methods such as ultracentrifugation, bead-based pulldown, or other methods (13). Because virion secretion coopts aspects of the exosome-secretion machinery (and corresponding "EV markers"), and because HIV virion secretion exists along a spectrum between complete, functional viruses and incomplete, EV-like particles that simply incorporate one or more viral elements (e.g., viral proteins and/or nucleic acids) (14), the distinction between EV and virus is not clearly demarcated. Indeed, it has been shown that EVs can carry viral proteins as well as the transactivation response element RNA from HIV itself (15, 16), elements which may themselves facilitate TGF-β1 signaling in smooth muscle cells (17). Also, no perfect EV purification method exists, and it is difficult to obtain pure, uncontaminated, and unselected populations of EVs, especially from complex biological fluids like plasma (13). Lastly, administration of cocaine can itself have a strong effect on the rate of EV production as well as the type of cargo that they deliver to target cells (18), adding another layer of complexity to the study of this system. By using the HIV-transgenic rat model, validating their principal findings across strata of viremia, varying EV purification techniques, and using complementary cell culture–based experiments, Krishnamachary and colleagues have attenuated, but not eliminated, some of these potential confounders. Their findings must be viewed with these caveats in mind, and future, more mechanistic interrogation of this system remains needed.

Nonetheless, this work adds to a growing body of research demonstrating that EVs carry powerful biological impact in human disease, and it adds important information on the pathogenesis of PAH in HIV and cocaine use. The outsized power of these nano-sized particles also suggests promise; some EVs may also have protective effects (19), and there may be potential to manipulate EVs for use as either therapeutic targets or delivery of therapeutic cargos (20, 21) in PAH and in other diseases.

In summary, there is much work yet to do before we realize the potential of EV research, but Krishnamachary and colleagues are to be applauded. Careful, well-designed, and intricate studies such as this are critical for advancing our knowledge of the complexities of PAH biology and EV signaling. This can be a challenging, even laborious, process. However, continued examination of these

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pathways fits Teddy Roosevelt's famous maxim of "hard work worth doing," as it sets the stage for potential preventative, monitoring, and treatment strategies that improve the health of patients. ■

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Camilla Margaroli, Ph.D.
Department of Medicine
University of Alabama at Birmingham School of Medicine
Birmingham, Alabama

Derek Russell, M.D.
Department of Medicine
University of Alabama at Birmingham
Birmingham, Alabama
and
Birmingham Veterans Affairs Medical Center
Birmingham, Alabama

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