Hindsight

Complement, oxidants, and endothelial injury: how a bedside observation opened a door to vascular biology

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A single encounter with a dialysis patient led to the study of complement and neutrophil aggregation, which in turn spawned our work and the remarkable development of the field of vascular biology. As our understanding of these cellular interactions and the signaling pathways involved in these processes has expanded, so has our appreciation for the broad impact of this work on an array of human diseases.

In the mid 1970s, the late Phillip Craddock was called to see a patient with a low white count, hypoxemia, and chest pain 20 minutes into a hemodialysis session. The neutrophil counts returned to normal within an hour, and the patient’s chest pain dissipated. As a physician-scientist, Craddock returned to his laboratory to test a hypothesis that the patient’s symptoms were initiated when blood circulation over the cellophane dialysis membranes activated complement, which in turn stimulated neutrophils to aggregate in the microcirculation of the lung. These landmark studies revealed that activated complement (C5a) generated during dialysis caused pulmonary hypertension, leukostasis, and pulmonary edema (1). This suggested that pulmonary endothelial cell activation or injury was a consequence of activated neutrophils. In 1978, our group proposed the mechanism of this activation (2). We believed that activated complement could induce endothelial damage — assessed by 51Cr release — through the production of neutrophil-derived oxygen radicals, since superoxide dismutase and catalase inhibited this injury. Tight adhesion was necessary for the neutrophils to damage the endothelium. We and others speculated that not only would this pathophysiology explain pulmonary vascular responses to inflammatory stimuli.

These studies incited prolific basic and clinical research in inflammation and vascular biology. This work led to the discontinuation of cellophane membrane use in dialysis in favor of a more biocompatible material that minimally activated complement. Clinical studies were initiated in ARDS patients using high doses of methylprednisolone to prevent neutrophil aggregation and endothelial injury (4). In the late 1970s, neutrophil biology studies exploded onto the scene, dissecting the roles of the NADPH-oxidase, myeloperoxidase, adhesion molecules such as CD11b/CD18, complement receptors, selectin receptors, and granule components including gelatinase and defensins in inflammation. Nostalgically, we remember that the annual Phagocyte Workshop at the APCR/ASC1/AAP meeting served as a forum for these interdisciplinary discussions as the field developed.

Radical ideas

Interest in oxidant biology dramatically enhanced the understanding of processes including the release of NADPH-oxidase, the myeloperoxidase system, antioxidant defenses, mitochondrial function, iron-catalyzed reactions, and lipid peroxidation. Cell signaling by oxidants has proven integral in the inflammatory response. The discovery of nitric oxide and its role in vascular biology is now fundamental in understanding cardiovascular health.

Complement biology is at the root of autoimmune processes, organ transplantation rejection, inflammation, and acute lung injury (5). For example, paroxysmal nocturnal hemoglobinuria, with its devastating hemolytic episodes and thrombotic events, can today be tamed with an anti-C5 antibody (6). New insights in complement biology have also led to understanding the mechanisms in hemolytic uremic syndrome and macular degeneration.

Sticking together

Our manuscript (2) was one of the first examining neutrophil-endothelial cell interactions. In our study, activated neutrophils injured endothelial cells in the context of close approximation. Decreasing adhesion or scavenging the oxidant species abrogated the injury. This observation was seminal for subsequent studies of neutrophil, adhesion, activation and migration, though Jaffe et al. (7) and Gimbrone et al. (8) pioneered in vitro culture techniques for human endothelial cells that made this possible. Since then, critical roles for molecules involved in these interactions, including P- and E-selectin, integrins VCAM-1 and ICAM-1, platelet-activating factor and IL-8, adhesion molecules including PECAM-1, sphingosine-1-phosphate, and E-cadherin, and of course, nitric oxide, have been described. Our in vitro endothelial cell cultures were crucial to understanding the critical balance of endothelial anticoagulants such as thrombomodulin and procoagulant molecules including von Willebrand factor, PAI-1 tissue factor, etc., which defines the thrombotic state today. Our laboratory has subsequently defined a set of protective responses of the endothelium, such as heme oxygenase-1 and ferritin, in response to heme-driven oxidants that allow adaptation of the vasculature to inflammation (9). Finally, understanding the marked heterogeneity of the vasculature defines organ-specific responses to injury and inflammatory stimuli.

20/20 Hindsight

With modern technology, investigation of these pulmonary vascular responses to inflammatory cell oxidants is even more
fascinating than we realized in the 1970s. For example, we now have a greater appreciation of the heterogeneity of the pulmonary vasculature, the role of nitric oxide in the pulmonary vasculature, the adhesion molecule responses, and the complement regulatory responses, and this knowledge would likely have changed our perspective of the pulmonary vascular response. A ready example is provided by recent findings showing C5a contributes to hemodialysis-associated thrombosis through the expression of functionally active tissue factor in peripheral blood neutrophils (10).

The story of Phillip Craddock’s observation and the science that sprung from it underscores the value of the physician-scientist in our health care system, whose role is now too often focused on algorithms and relative value units. However, the curiosity of physician scientists combined with today’s knowledge and technologies has the potential to lead us toward a brighter future and a healthier population.

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