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The microbubble or the microparticle?

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DECOMPRESSION SICKNESS (DCS) has long been attributed to physical forces exerted by inert gas bubbles that may form in tissues, resulting in vascular occlusion and tissue disruption. Bubble formation occurs when a decrease in ambient pressure exceeds the rate at which soluble inert gas (e.g., nitrogen) can be eliminated from the tissues. Modern theory asserts that many signs and symptoms of DCS are due to complex vascular and other biological processes that occur in response to the bubbles, including inflammation and activation of coagulation, which lead to microvascular damage and thrombosis. Could it be that small biological vesicles are at the heart of the matter?

In this issue of the *Journal of Applied Physiology*, Thom et al. (13) report that decompression stress in mice results in increased numbers of circulating microparticles (MPs), which they implicate in neutrophil activation and vascular injury. MPs are small (0.1–1 μm) membrane vesicles shed in coordinated processes from the surface of a number of different cells in the vasculature. MPs are formed in response to stimuli that activate the cell or lead to apoptosis (5), and have proinflammatory and prothrombotic properties, making them candidates to mediate vascular injury in DCS. Thom and colleagues (13) report that MPs of platelet origin cause neutrophil activation and vascular injury, and that circulating neutrophils express high levels of antigens from other cell types, which they attribute to transfer from MPs. The findings reported in this study are quite novel, and if substantiated may improve our understanding of DCS.

In addition to proposing pathogenic aspects for DCS, this paper reports several new observations about MP biology. This is the first report (to our knowledge) that individual MPs exhibit surface markers suggesting origin from multiple cells types. This finding could be explained by membrane fusion of MPs from various sources. Such a scenario has been proposed to explain otherwise enigmatic data in other settings, as for example in the case of the cellular origin of tissue factor bearing MPs in cancer (9). Second, the authors report that MPs expressing platelet surface markers interact with circulating neutrophils, leading to neutrophil degranulation and further MP production. Although other groups have reported the capacity of MPs to interact with and activate target cells (4), this paper proposes a novel mechanism by which MPs do this. Finally, and most importantly, the authors use innovative interventions to diminish MP concentrations in vivo and show that their approach attenuates decompression-induced intravascular neutrophil activation, neutrophil sequestration, and tissue injury.

Recently, MPs derived from hematopoietic and vascular sources have been enumerated and characterized in a number of systemic illnesses with vascular pathobiology, including acute coronary syndromes, vasculitis, sepsis, and metabolic syndrome (6, 8). These small vesicles have been implicated in the pathophysiology of these diseases, although causation has yet to be unequivocally established. In this decompression stress model, Thom et al. (13) use two different approaches to diminish circulating MP levels in mice in vivo, and a third strategy to diminish platelet counts, which as expected, also resulted in reduced numbers of circulating platelet-derived MPs. This is the first report to experimentally test the importance of MPs using a depletion strategy. The data presented indicate successful reduction in MP counts in both the control and decompression-stressed mice, and assign causation by showing diminished injury in the treated animals. Broad application of an effective MP depletion strategy would allow investigation into the importance of MPs in a range of disease conditions and pathological processes. Thus it is important that the MP depletion strategies reported here be validated by other groups.

The effectiveness of an antibody to annexin V for MP depletion is curious because it implies that circulating MPs have annexin V bound to their surface in vivo. A large proportion of MPs express negatively charged phosphatidylserine (PS) residues on their membrane surface, which allows ex vivo labeling techniques to take advantage of the affinity of annexin V for PS. The detection of appropriately sized particles that have bound fluorescently tagged annexin V is thereby utilized to differentiate MPs from background events using flow cytometry. It is not clear whether and to what degree endogenous annexin V binds to PS on the surface of MPs in the circulation, although another PS-binding protein—lactadherin—has been shown to associate with platelet-derived MPs in vivo (3). Moreover, MPs that fail to bind annexin V (presumably because they lack PS exposure) have been described both in vitro (10) and in vivo (1, 12); thus it is possible that an antibody to annexin V may deplete only a subpopulation of MPs that appear to be important to vascular injury in this decompression stress model.

Flow cytometry is currently the most commonly used technique for enumerating and identifying cell parentage of microparticles, but there are several inherent technical challenges in studying these very small particles using instruments designed to investigate whole cells (11). There are marked differences in sensitivity of different flow cytometers, and the measurements are influenced by preanalytical practices in preparing and storage of plasma (11). These limitations have resulted in a broad range of reported "normal" numbers as well as cellular origin of plasma MPs. As a first step toward standardizing the measurement of MPs, the International Society of Thrombosis and Hemostasis recently published the result of a standardized flow cytometry protocol designed to enumerate platelet-derived MPs in human plasma (7). During this exercise, it was demonstrated that the ability of various flow cytometers to adequately discriminate particles of 0.5 μm and 0.9 μm was

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often suboptimal. Thus the routine measurement of MPs in plasma continues to pose a significant technical challenge.

Thom and coauthors (13) acknowledge these challenges and adopted a rigorous flow cytometry protocol for their measurements. However, in the control mice, a relatively small fraction (<20 %) of total MPs could be attributed to a specific vascular parent cell type, raising the question as to the origin of the majority of MPs. Published data show that the majority of MPs in the circulation of mice and humans are derived from circulating cells such as platelets, leukocytes, and erythrocytes, as well as vascular endothelial cells (2, 5). The thrombocytopenic mice in this study have roughly half as many MPs as untreated control animals, suggesting either a significant contribution of platelets to MP numbers in control animals, or a contribution of platelets to MP formation by other cell types that could not be characterized according to their cellular origin in the control mice.

Overall, Thom et al. (13) report several new findings that are of potential importance in understanding the pathophysiology of DCS. Further studies are needed to confirm their observations. We have highlighted the methodological issues that can arise in characterizing microparticles in order to emphasize the need for careful evaluation of new data in this rapidly evolving field.

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DISCLOSURES

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