inside blood

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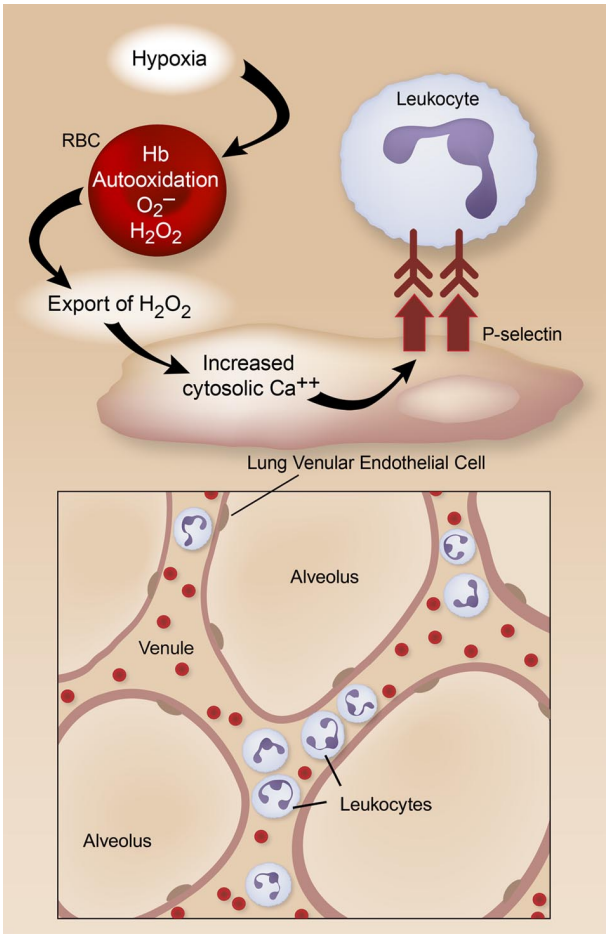
RED CELLS

Comment on Kiefmann et al, page 5205

Hypoxic erythrocytes spark lung leukocyte adhesion

Guy A. Zimmerman UNIVERSITY OF UTAH

Erythrocytes are generally expected to passively transit microvascular beds, carrying oxygen but not delivering ROS. In contrast, Kiefmann and colleagues report in this issue of *Blood* that, in hypoxic lungs, erythrocytes release ROS, activate endothelium, and trigger leukocyte accumulation.



In hypoxic rodent lungs, H_2O_2 is generated in RBCs by auto-oxidation of membrane-bound Hb and is exported to microvascular endothelial cells. This triggers increased cytosolic calcium, translocation of P-selectin to the EC plasma membrane, and leukocyte adhesion in venules and septal capillaries. Illustration by Diana Lim.

The study utilized elegant real-time fluorescent imaging of venular and capillary endothelial cells (ECs) to show that, when isolated rat and mouse lungs are perfused with red blood cells (RBCs), hypoxia triggers increased endothelial reactive oxygen species (ROS) and elevated levels of EC cytosolic calcium. Few laboratories are performing analysis of in situ endothelium in the pulmonary microvasculature with this precision. Biochemical and inhibitor studies, and interesting experiments using RBCs and lungs from genetically altered mice, indicate that the mechanism involves auto-oxidation of hemoglobin (Hb) with resultant generation of hydrogen peroxide (H_2O_2), likely from superoxide. Membrane-bound Hb appears to be particularly important, and this localization may partially isolate ROS generated by Hb auto-oxidation from cytosolic RBC antioxidant enzymes. H_2O_2 is then exported to EC by the hypoxic RBCs, inducing entry of external Ca^+. This leads to translocation of the adhesion molecule P-selectin to endothelial surfaces, and rolling and tight adhesion of leukocytes in lung venules and septal capillaries (see figure). Because P-selectin display, rolling, and "sticking" are initiating events in the emigration of leukocytes into many tissues,[1] these studies indicate that hypoxic RBCs have the potential to deliver sparks that can ignite the fire of acute inflammation. The role of endothelial P-selectin in the accumulation of myeloid leukocytes in lung alveoli is unclear, but there is evidence that it can mediate intravascular leukocyte sequestration in models of lung injury.[2]

Earlier studies of cultured human ECs demonstrate that H_2O_2 induces endothelium-dependent leukocyte adhesion,[3] consistent with results in hypoxic lungs (see figure). Importantly, ROS generation by RBCs did not occur under normoxia. This provides reassuring evidence that pathologic hypoxia (blood pO_2 ~22 mmHg in the current experiments) is required and that these events are unlikely to occur in physiologic breathing or tissue perfusion. The mechanism(s) linking RBC-derived H_2O_2 to Ca^{++} entry in lung endothelium under hypoxic conditions was not established, but there is emerging evidence that H_2O_2 has important signaling roles in addition to cytotoxic effects.[4]

Where might this model have clinical relevance? The authors suggest that it may be an amplifying event in acute lung injury, which is a common syndrome of lung inflammation complicated by hypoxia. In addition, ROS generation by hypoxic RBCs might contribute to transfusion-related acute lung injury (TRALI),[5] and might be a specific risk in transfusion of erythrocytes that have been stored for prolonged periods, resulting in reduced levels of RBC antioxidants. There is also an inflammatory component in some cases of altitude-induced lung dysfunction, especially if the subject has a concomitant lung

infection. Finally, hypoxia is an important priming event for vascular dysfunction in a variety of systemic inflammatory syndromes.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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IMMUNOBIOLOGY

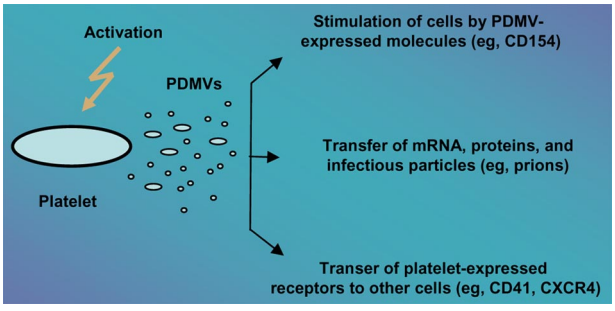
Comment on Sprague et al, page 5028

Microvesicles as immune orchestra conductors

Mariusz Z. Ratajczak STEM CELL INSTITUTE AT JAMES GRAHAM BROWN CANCER CENTER

In this issue of *Blood,* Sprague and colleagues report that platelet-derived membrane vesicles (PDMVs) orchestrate an immune response sufficient to deliver CD154 signals, which stimulate antigen-specific IgG production and modulate germinal-center formation, by cooperating with responses elucidated by $CD4^+$ T cells.

Attention has been focused recently on circular membrane fragments called membrane vesicles (MVs), which for many years have been largely overlooked. MVs are shed from the surface membranes of cells, as well as secreted from the endosomal compartment as circular membrane fragments.[1] They contain numerous proteins and lipids similar to those present in the membranes of the cells from which MVs originate. Furthermore, because they engulf some cytoplasm during membrane blebbing, they may also contain proteins and mRNA.[2] Thus, MVs may stimulate target cells directly via surface-expressed ligands acting as a kind of "signaling complex." It has been postulated that this MV-mediated cell-cell communication system emerged very early in evolution and served as a template for the development of cell-cell interaction mechanisms involving soluble bioactive mediators and fine-tuned ligand-receptor interactions. MVs may, in addition, transfer surface receptors from one cell to another; deliver proteins, mRNA, bioactive lipids, and even whole organelles (eg, mitochondria) into target cells; and finally, serve as a vehicle to transfer infectious particles such as HIV or prions between cells (through a "Trojan horse" mechanism).



Different mechanisms by which PDMVs may interact with target cells. PDMVs may (1) stimulate target cells directly via surface-expressed ligands acting as a kind of signaling complex (eg, CD154), (2) transfer surface receptors from one cell to another (eg, CD41, CXCR4), or (3) deliver proteins, mRNA, and bioactive lipids, and even serve as a vehicle ("Trojan horse" mechanism) to transfer infectious particles between cells (eg, prions).

It is well known that activated platelets are a rich source of MVs released both from their surface as well as from endosomal compartments (exosomes). These PDMVs may (1) directly stimulate hematopoietic cells, lymphocytes, and endothelium[3]; (2) transfer platelet-expressed receptors (eg, CD41 or CXCR4)[4,5] to the surface of other cells; and even (3) transfer mRNA, proteins, and infectious particles (eg, prions) between cells. Thus, PDMVs may exert several pleiotropic effects that are relevant to hematopoiesis and/or lymphopoiesis (see figure).

In this issue of *Blood*, Sprague and colleagues report that PDMVs orchestrate immune responses by being able to deliver a CD154 signal that stimulates antigen-specific IgG production and modulates germinal-center formation through cooperation with $CD4^+$ T cells. CD154 is a ligand for CD40 receptor, which is critical to the initiation and propagation of the adaptive immune response and is expressed on several cell types, including various subsets of T lymphocytes and platelets. Humans and mice lacking functional CD154 fail to isotype-switch from the IgM antibody isotype, which leads to hyper-IgM syndrome, and are also unable to mount the germinal-center response necessary for differentiation of memory B lymphocytes and plasmocytes.

It has been known for many years that platelets modulate inflammatory responses. Because PDMVs released from platelets circulate in peripheral blood, the data published by Sprague et al in this issue of *Blood* explain why platelets may modulate inflammation and adaptive immunity at sites distant from the location of activation. In a set of elegant experiments, the authors challenged a well-established paradigm: that the CD154 signal is delivered solely by $CD4^+$ cells to B cells.

This paper is an important step toward understanding the complexity of cell-cell communication, in which MVs released from activated cells play an important and still underappreciated role. Not only platelets, but all other cells present in the hematopoietic microenvironment or at sites of tissue injury/inflammation, secrete MVs; thus, the MV-related network seems to modulate several important biological responses. Thus, MVs should not be envisioned any longer as biologically irrelevant cell dust. Accumulating evidence demonstrates that they are important mediators of intercellular communication (eg, by delivering CD154 signal, as shown by Sprague and colleagues). Based on these findings, further studies are required to determine whether, in addition to CD154, other components of PDMVs directly or indirectly affect the immune response.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■