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**Megakaryocyte-derived microvesicles, please stand up!**

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In this issue of *Blood*, Flaumenhaft and colleagues report that circulating MVs isolated from murine blood express markers that suggest their megakaryocytic origin. They also report that MegMVs are CD62− LAMP-1− and express full length filamin in contrast to PMVs that are CD62+ LAMP-1+ filamin−.

Microvesicles (MVs), small circular membrane fragments shed from the cell surface or released from the endosomal compartment, play an important and underappreciated role in cell-to-cell communication.1 This intriguing MV-mediated communication system emerged very early during evolution and served as a template for the further development of cell-to-cell interaction mechanisms involving soluble bioactive mediators and finely tuned ligand–receptor interactions. The biologic significance of MVs has been largely overlooked for many years, and MVs were regarded just as simple cellular debris/fragments or part of apoptotic bodies. However, evidence is accumulating that these tiny membrane fragments orchestrate several biologic responses. MVs contain numerous bioactive proteins and lipids similar to those present in the membranes of the cells from which they originate. Furthermore, since they engulf some cytoplasm during membrane blebbing, they may also contain proteins and mRNA.2 Thus, MVs may stimulate target cells directly by surface-expressed ligands acting as a kind of “signaling complex.” In addition, they may transfer surface receptors from one cell to another and deliver proteins, mRNA, bioactive lipids, and even whole organelles (eg, mitochondria) into target cells. Finally, they may also serve as a vehicle to transfer infectious particles between cells, such as prions or HIV (“Trojan horse” mechanism of infection).

It is widely accepted that MVs circulating in peripheral blood originate from activated blood platelets, endothelial cells, and leukocytes. Platelet-derived microvesicles (PMVs) are released both from the platelet surface or from the endosomal compartments (exosomes) and, as shown in the figure, may (1) directly stimulate other cells (eg, hematopoietic cells, lymphocytes and endothelium),3 (2) transfer platelet expressed receptors (eg, CD41 or CXCR4)4,5 to the surface of other cells and, in some situations, or (3) transfer mRNA, proteins, and even infectious particles (eg, prions, HIV) to the target cells. Interestingly, in healthy donors, the majority of circulating CD41+ MVs do not express surface activation marker CD62P, suggesting that they do not originate from activated platelets.

Here, Flaumenhaft et al report that a significant number of circulating CD41+ MVs in healthy individuals are derived directly from megakaryocytes (Megs). In a sequence of very elegant studies, the authors first demonstrate this via electron microscopy of spontaneous
formation of Meg-derived MVs (MegMVs) from cultured murine Megs. Next, studies with cytoskeleton inhibitors reveal that the mechanism of MegMV formation is an active process different from platelet formation. Furthermore, both CD41 positive MegMVs and PMVs could be distinguished according to unique phenotype of surface markers. Accordingly, MegMVs are CD62L−LAMP-1− and express full-length filamin in contrast to PMVs that are CD62L+ LAMP-1+ and express little, if any, full-length filamin. Finally, direct studies on human Megs expanded from umbilical cord–blood CD34+ cells confirm that human Megs can also secrete MVs and suggest that CD41+ MVs circulating in healthy donors are similarly, as in mice, Meg–derived.

In conclusion, this article adds a complexity to our overall view on the population of MVs circulating in peripheral blood. Since new markers have been established to distinguish and potentially separate a population of CD41+ PMV from CD41+ MegMVs, further work is needed to look for functional differences between PMVs and MegMVs. This opens a new area for further investigations. For example, in addition to differences in expression of surface makers, both types of MVs may differ in (1) mRNA content, (2) ability to stimulate target cells (eg, endothelium), (3) the role they play in supporting inflammatory reactions, and (4) complement activation. Finally, we can also envision that measuring both types of MVs in peripheral blood could have novel diagnostic and prognostic applications for several cardiovascular disorders, illnesses in which a fraction of PMVs released from activated platelets increases, changing the overall value of the MegMVs/PMVs ratio.

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

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**Bone marrow cells can manipulate healing**

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The mechanical fragility and healing problems in patients with EB provide interesting paradigms for tissue integrity and wound healing. In this issue of *Blood*, Tolar and colleagues report on how RDEB can be helped by specific subpopulations of cells (CD150+ CD48−) derived from bone marrow.

Epidermolysis bullosa (EB) is a very heterogeneous genetic disease. Careful genetic analysis, immunomapping, and electron microscopy have yielded very detailed knowledge about the epithelial, basement membrane zone, and dermal molecules responsible for anchoring the cutaneous and mucosal epithelium to the underlying cells and structures. For example, intraepidermal mutations in keratins 5/14, plectin, can lead to EB simplex, with cleavage through the epidermis and milder disease. Mutations in laminin-332 (formerly laminin-5), type XVII collagen, and α6β4 integrin cause an often lethal form (junctional EB), with cleavage in the lamina lucida. Recessive dystrophic EB (RDEB) is due to mutations in type VII collagen (COL7A1), a molecule produced by keratinocytes and fibroblasts that comprises most of the fibrils anchoring the epidermis to the underlying dermis.

In individuals with RDEB, even mild trauma leads to the rapid development of blisters and wounds. Severe scarring is the rule, and invasive, metastasizing squamous cell carcinomas are common. Treatments are palliative and remain unsatisfactory.

During the past few years, very exciting developments have occurred in the potential therapeutic approach to RDEB. Many of these findings have been surprising in that, when administered intradermally or systemically, the corrective collagen type VII protein appears to find its way to the subepidermal area of anchoring fibrils. However, Tolar et al make a valid point in stating that, because of the generalized nature of RDEB, the focus should remain on a systemic treatment approach. They provide a useful proof of principle that the administration of congenic bone marrow CD150+ CD48− cells in a murine model of RDEB can partially rescue the affected animals, which normally die within 2 weeks after birth. The results are intriguing. The authors demonstrate clinical efficacy in RDEB mice and histologic evidence that this hematopoietic cell (HC) subpopulation, or possibly cells copurified with it, homes to the subepidermal area and helps reconstitute the anchoring fibrils. Mesenchymal stem cells (MSCs), which also produce type VII collagen, were not able to achieve positive results in RDEB mice. However, this conclusion is weakened by the fact that 8 to 10 times more CD150+ CD48− cells as compared to MSCs were used. A similar argument can be made with the negative results the authors obtained with epidermal stem cells and transient-amplifying cells because these cell types were also used at a lower dose. The authors readily acknowledge these shortcomings; in some cases, cell size may have precluded larger doses because of infusional toxicity. Of course, one could make the argument that cell number, and not necessarily the cell subpopulation, is the critical factor. In fact, we have found that the dose of cultured autologous MSCs delivered topically to human chronic leg wounds directly correlates with healing; doses lower than 1.5×10^6 cells/cm² are not effective. The possibility of a dose threshold for engraftment and effectiveness is real. Still, Tolar et al do show very interesting evidence for engraftment of CD150+ CD48−, a subpopulation that contains true stem cells and is capable of giving rise to both HC and non-HC progeny.

More work is needed to expand on these promising findings.
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