

Pulmonary Megakaryocytes in Coronavirus Disease 2019 (COVID-19): Roles in Thrombi and Fibrosis

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Coronavirus disease 2019 (COVID-19) has already claimed many lives and continues to do so in different parts of the world. Autopsy reports of patients who succumbed to this viral infection have been published despite concerns about health care professional safety. One of the unusual findings in COVID-19 lung autopsy reports is the increase in pulmonary megakaryocytes.^{1,2} Although the presence of megakaryocytes in the lungs is a well-established concept in the medical literature, it is still not widely accepted in the clinical fraternity. In this article, we discuss the role of lung megakaryocytes in relation to the clinicopathological findings in COVID-19 and discuss how this may impact on our understanding of acute respiratory distress syndrome (ARDS), pulmonary thrombi, and lung fibrosis, in general.

Do Megakaryocytes Exist in the Lungs?

Platelets are produced from megakaryocytes by the process of pseudopod formation.³ During this process, megakaryocyte cytoplasm elongates to form pseudopods, and the tips of these structures, which contain the characteristic granular constituents, get broken off in the vascular milieu to become platelets.³ Although the assumption is that this production process occurs in the bone marrow perisinusoidal region, there is evidence that at least part of the production occurs in the lungs.⁴ Interestingly, the presence of lung megakaryocytes was noted more than six decades ago.⁵ There have even been studies that demonstrate the megakaryocytes entering the marrow sinusoids and traveling in the circulation to become lodged in tiny capillaries of the pulmonary vasculature.⁶ Lungs as representing a factory for platelet production has been depicted by human experiments showing higher platelet counts in the pulmonary arterial circulation compared with the venous side.⁷ Despite these facts, proponents arguing against mega-

karyocyte presence in the lungs assert that lung megakaryocytes just represent a gravity phenomenon noted at autopsy. The most elegant (and latest) study for validating the lung origin of platelets comes from Lefrançois et al who directly imaged the lung microcirculation in mice to provide definite proof for the existence of lung megakaryocytes.⁸ They also proved that approximately half of the total number of platelets or 10 million platelets per hour would be produced by these cells.⁸

Is There a Specific Role for the Pulmonary Megakaryocytes?

Lungs are one of the commonest sites for pathogen invasion, and respiratory compromise from infectious diseases is the commonest cause of mortality worldwide. Evolution has for this reason prepared the human lungs with sufficient armamentarium to deal with the constant pathogen invasion, which happens with each breath of air we take. Since platelets are one of the most abundant antiseptic cells available, it makes sense for a large number of platelets to be released in the vicinity of possible pathogen entry (i.e., the alveolar-vascular interface). In this respect, Lefrançois et al showed that the lung megakaryocytes behave differently to their marrow counterparts.⁸ These researchers used RNA sequencing analysis to demonstrate an innate immunity function for lung megakaryocytes in comparison with megakaryocytes from the bone marrow, with alterations identifiable in cases of bacterial pneumonia.⁸ Nonhematopoietic roles of megakaryocytes also involve surface expression of toll-like receptors.⁹ Surface adhesion molecules expressed on megakaryocytes can assist in retention of these cells in the lungs, particularly when the pulmonary endothelium is activated by inflammation or infection.¹⁰ Megakaryocytes also phagocytose bacteria and viruses and use their lysosomal granules as pathogen-eradicating bullets.^{10–12} An

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additional immune role for megakaryocytes is reflected by their interaction with neutrophils, which occurs through the unique process called “emperipolesis.”¹³ During emperipolesis, these leukocytes are engulfed by the megakaryocytes with no membrane disruption but allowing cellular material transfer between the cells.¹³ Although not shown specifically with megakaryocytes in the lung fields, in response to acute inflammation, megakaryocytes become activated and manufacture large quantities of platelets, which can then play a role in inflammatory conditions.¹⁴ In the context of COVID-19, thrombocytosis has been observed as part of the inflammatory response, with very few patients having subnormal platelet count pointing toward this very active megakaryocyte function.¹⁵

Lung Megakaryocytes and Pulmonary Thrombi

Microthrombi are a feature of many pulmonary diseases where there may be associated inflammation. It has been suggested that these clots assist in preventing microbes (in the case of infection) or damage-associated proteins (in the case of inflammation) by escaping into the blood circulation and causing systemic effects, which may be harmful to the host.¹⁶ Platelets are, of course, important in this microthrombi formation, and having the platelet production factory to churn out a large amount of these cells locally in the lungs may indeed be beneficial.¹⁷ An intriguing observation in this regard was made by Sharma and Talbot, who postulated pulmonary megakaryocytes as the “missing link” between cardiovascular and respiratory disease.¹⁸ They suggested that the number of pulmonary megakaryocytes increased in association with atherosclerotic disease because of enhanced thrombopoiesis following increased platelet adherence to atheromatous plaques.¹⁸ Increase in lung megakaryocytes has been linked to pulmonary and cardiovascular disease and suggested to be a reason for preponderance of these diseases in men.^{18,19} Wells et al noted a significant correlation between the lung megakaryocytes and fibrin thrombi counts within the lungs in patients dying from burns.²⁰ The authors went far enough to postulate that any condition that may cause disseminated intravascular coagulation could be associated with increased numbers of pulmonary megakaryocytes.²⁰ Animal studies have suggested that the thrombi that may be noted in those with tumor necrosis factor- α -mediated septic shock could possibly be megakaryocytes occluding the pulmonary vessels.²¹

Lung Megakaryocytes and Acute Respiratory Distress Syndrome

ARDS is a common complication of several infectious and inflammatory conditions. Lung biopsy specimens from 21 patients with diffuse alveolar damage demonstrated increased microvascular CD61+ megakaryocytes compared with normal lungs, reflecting injury to the pulmonary microvasculature.²² The possible mechanism described for this increased number is damage to the lung microvasculature causing impaired mega-

karyocyte fragmentation and may also be a reason for thrombocytopenia commonly noted in ARDS.²² There have been several publications detailing the role of platelets in ARDS, although very few in relation to megakaryocytes.^{23,24} This is possibly due to the lack of awareness of the circulating and lung-specific megakaryocytes and also the fact that “sourcing” these large cells (50–100 μ m in diameter, which are more than 10 times the size of typical red cells) through venepuncture needles would not be practical. One of these overlooked roles is in relation to platelet granular constituents including vasoconstrictors such as serotonin, which have been implicated in the pathogenesis of ARDS.²⁴ However, these granular constituents are also present inside megakaryocytes and as such their exact origin can be debated. Similarly, much interest has been shown in the interaction between platelets and the now well-known neutrophil extracellular traps (NETs).²³ However, Frydman et al have shown that megakaryocytes can generate histone-decorated chromatin webs, which mimics the NETs.¹⁰ This report also demonstrated increased CD61+ staining in the kidneys and lungs from patients with sepsis, which correlated with the development of organ dysfunction, an accompaniment often present in ARDS.

Megakaryocytes and Neovascularization

One of the groundbreaking papers in the field of hemostatic factors in tumor angiogenesis is the discovery of differential release of angiogenesis mediators by the Battinelli et al.²⁵ These were experiments using platelets and not megakaryocytes. They elegantly demonstrated that adenosine diphosphate stimulation of platelets triggered the release of vascular endothelial growth factor (VEGF), whereas thromboxane A2 stimulation released endostatin and not VEGF.²⁵ The former condition facilitated in vitro formation of capillary structures by human umbilical vein endothelial cells, whereas endostatin inhibited the same process.²⁵ Neoangiogenesis is a feature noted in COVID-19 lung pathology specimens and could be induced by the vasogenic factors released by the activated platelets produced by the lung megakaryocytes.²⁶ But how can we link megakaryocytes and neovascularization? It may be useful to recall the clinical sign of digital clubbing (bulbous enlargement of the tips of fingers or toes with sponginess of the nail base), often seen in patients with cardiorespiratory diseases. It has been shown that the characteristic clinical sign is a result of megakaryocyte fragments, which get lodged in the peripheral circulation after escaping the lung circulation due to right-to-left shunting.²⁷ These fragments release vasogenic mediators, which lead to the clinical changes in the skin of the fingers and toes causing the clubbing. Although definite proof is wanting, it is possible that the lung megakaryocytes rather than the platelets are the prime mediators of neovascularization in COVID-19 and other inflammatory conditions.

Megakaryocytes and Fibrosis

In addition to angiogenic factors, several growth factors relevant to fibrotic diseases are stored within megakaryocytes.²⁸ These include fibroblast growth factor, transforming growth

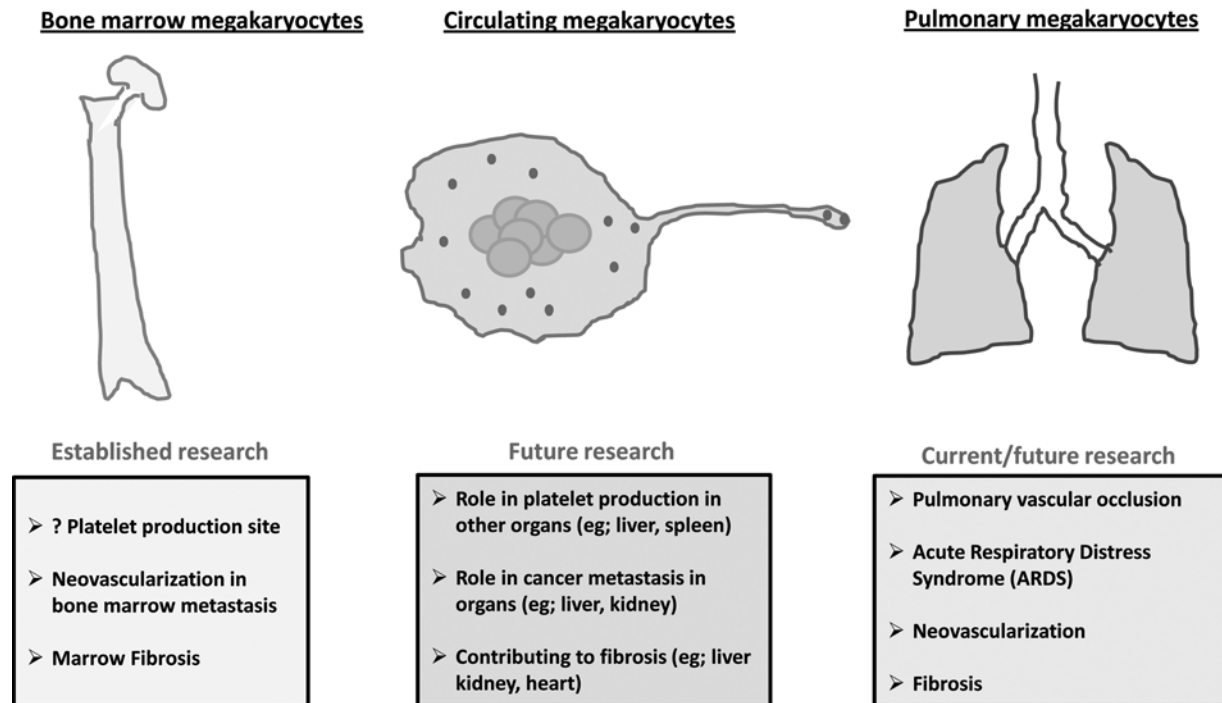
Megakaryocytes and pathological conditions

Fig. 1 The role of megakaryocytes in platelet production and various pathological conditions are detailed. These roles are well-established in the case of the bone marrow; currently they are ongoing in relation to the lung and are likely to be interesting areas of research in other organs with respect to circulating megakaryocytes.

factor, and platelet-derived growth factors. The growth factor cargo inside megakaryocytes and platelets may be present to facilitate the process of wound healing as part of the inflammatory process. During the clotting process, once the clot is formed at the site of endothelial injury, the next step is wound closure, and the same cells active in clot formation (platelets) could be the source of angiogenic and fibrogenic factors that are crucial in this healing process.²⁹ However, intense and continued inflammation would mean continuous release of these mediators, which can, of course, lead to fibrosis. It is also possible that the release of these fibrosis-inducing cytokines could lead to the development of lung fibrosis in COVID-19 patients as a long-term complication, and anecdotes of this feature have already been made in publications.³⁰ There is also considerable literature implicating the thrombotic process as a precursor for pulmonary and liver fibrosis, but the role of megakaryocytes in these organs as the contributor has not yet been explored.^{31,32} Unfortunately, any therapeutic intervention is unlikely to limit the development of the fibrotic process since it is part and parcel of the inflammation/wound healing process. This may explain why the management of pulmonary fibrosis is still a frustrating area for drug developers.

Conclusion

Megakaryocytes may play an important role in the pathogenic process of COVID-19 and possibly other infectious and inflammatory diseases. Their role may have been overlooked

since “obtaining” these large cells for experimental studies from patients is difficult and thus far, research in this area has predominantly used platelets, which may act as a surrogate marker for its parent. Future studies should focus on methods to examine megakaryocytes in various parts of the body including the lungs (but also liver, gut, heart, and kidneys) to elucidate their role beyond a platelet factory (–Fig. 1). Circulating megakaryocytes may be producing platelets locally in these different organs and may be responsible for fibrosis of these organs in pathologic cases.

Conflicts of Interest

None

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