Origin of Pulmonary Megakaryocytes

Richard M. Kaufman, Romano Airo, Simeon Pollack, William H. Crosby and Raymond Doberneck

Seventeen decades ago Aschoff described the presence of megakaryocytes in the lungs. It was his opinion that these cells migrated from the bone marrow under chemotactic stimulation and lodged in the pulmonary capillaries. A few years later Foa suggested that this occurrence was a normal, physiologic event which was exaggerated (and therefore more readily observed) under pathologic conditions.

A controversy regarding the origin of these cells was ignited in 1937 when Howell and Donahue, observing giant cells in pulmonary tissue and finding more platelets in blood leaving the lung than in blood which enters it, concluded that megakaryocytes and platelets are generated in the lung. This view was quickly challenged. Nevertheless, Howell and Donahue's work had called attention again to the relationship of the megakaryocyte to the lung. Since 1948, studies have revealed that megakaryocytes are almost always found in animal and human lungs. Infections and other conditions which stimulate megakaryocytopenesis are associated with increased numbers of pulmonary megakaryocytes. It has now become evident, as Foa long ago had surmised and as Schwarz has more recently re-emphasized, that the presence of megakaryocytes in the lung is a physiologic phenomenon.

Does the lung itself produce megakaryocytes (and, therefore, platelets), or are these cells carried to that organ in the blood? Studies in dogs, described below, indicate the latter to be the case. Moreover, the experiments suggest that many platelets may be released in the lung, corroborating, in part, the postulations of Howell and Donahue.

Materials and Methods

Eleven healthy male and female mongrel dogs, weighing from 15 to 25 Kg., were used in this study. The principles of laboratory care as promulgated by the National Society for Medical Research were observed. Six of these animals were anesthetized with pentobarbital sodium, intubated, and placed on a Drinker respirator. A left thoracotomy was performed. Three dogs (Group I) underwent the following procedure: the left main pulmonary artery was ligated and divided; the left subclavian artery was divided and the proximal portion anastomosed, end to end, to the distal portion of the left pulmonary artery (fig. 1A). Thus, while the right lung received venous, right-heart blood, the left lung received only arterial, left-heart blood. The 3 other dogs (Group II) had the following procedure performed upon them: the left subclavian artery was ligated and divided; the proximal portion was then anastomosed, end to side, to the intact left main pulmonary artery (fig. 1B). Thus, the left lung was perfused by a mixture of arterial and venous blood, while the right lung was perfused almost entirely by venous blood. Immediately after each shunt was opened a strong thrill was felt throughout all portions of the lung.
Fig. 1.—Schematic illustrations of the vascular shunts. (A) End-to-end shunt. The left pulmonary artery is ligated and divided. The left subclavian artery is then divided and the proximal portion anastomosed, end-to-end, to the distal portion of the left pulmonary artery. Thus, the left lung receives only arterial blood. (B) End-to-side shunt. The left subclavian artery is ligated and divided, and the proximal portion anastomosed, end-to-side, to the intact left pulmonary artery. The left lung then receives a mixture of venous and arterial blood.
Fig. 2.—Section from the right lung of dog (Y48) with an end-to-end shunt killed 1 week postoperatively. Note 7 megakaryocytes (circled) in this one field alone (0.14 cm²). Counts averaged 65.7 megakaryocytes cm⁻² in this dog's lung, over 25 times the normal maximum count of 2.5 cells cm⁻² of the left lung. The thrill remained palpable through the chest wall when the thoracotomy was closed. A persistent machinery murmur was easily heard over the left thorax in all animals. The dogs fared well postoperatively. Although several had wound infections which were drained, all incisions remained intact.

One, 2 and 4 weeks after surgery, a dog from each group was killed by intravenous pentobarbital injection. All shunts were fully patent. Two dogs, (OX6, 4X4), 1 each from Groups I and II, had mottled hemorrhagic infiltrates measuring from 0.25 to 1 cm² in area on the pleural surfaces of both lungs. Lung tissue was fixed in 10 per cent buffered formalin, and sections were made from representative portions of each lobe of both lungs. These were stained with hematoxylin and eosin, and Periodic-acid-Schiff (PAS). Megakaryocytes were counted by systematically examining each section at a magnification of 430X. These cells are readily identified in pulmonary tissue by their characteristic size and distinctive, lobulated nuclei which contain thick, clumped, deeply basophilic chromatin (fig. 2). The counts were expressed as the number of megakaryocytes per square centimeter of lung tissue. Similar counts were made on pulmonary tissue of 3 unoperated dogs (Group III), and of 2 dogs (Group IV), who underwent simple left thoracotomy in order to assess the effect of surgery alone on the incidence and location of pulmonary megakaryocytes. The latter dogs were killed 1 and 2 weeks postoperatively; neither developed a wound infection.

RESULTS

Microscopic examination revealed that none of the animals had entirely normal lungs. Interstitial infiltrates consisting of macrophages and polymorphonuclear leukocytes were randomly distributed in some lung sections
Small areas of frank interstitial hemorrhage were observed in other sections, even in those from the normal dogs, however, less in extent. The incidence and location of pulmonary megakaryocytes appeared unrelated to either the infiltrated or hemorrhagic areas.

Megakaryocytes were observed in all sections; only mature cells or naked nuclei were noted (figs. 2-4). Approximately 10 per cent of all megakaryocytes contained discernible cytoplasm. The majority of cells appeared to lie intravascularly, although the relationship of some cells to vascular channels was difficult to ascertain.

The megakaryocyte counts are shown in table 1-4. In normal dogs the number varied less than twofold from one lung to the other in each animal studied (table 1). The maximum count was 2.5 cells/cm² In dogs with end-to-end shunts, from 11 to 73 times more megakaryocytes were found in the right lung (perfused by venous blood) than in the left lung (perfused by arterial blood) (table 2). Furthermore, the count in the left lung did not exceed 1.5 cells/cm² even when the count in the right lung ranged as high as 65 cells/cm² (26 times the maximum observed in normal lungs). On the other hand, in 2 dogs with end-to-side shunts the right lung contained only 4 to 5 times as many megakaryocytes as the left lung, and in one dog contained slightly more (table 3). Quite significantly, and in marked contrast to dogs with end-to-end shunts, the count in the left lung of 2 of these shunted dogs ranged above normal, as high as 20 cells/cm².

In the 2 dogs which underwent simple left thoractomy there was absolutely no difference in the incidence of megakaryocytes from one lung to the other.
Fig. 4.—Pulmonary megakaryocyte with intact cytoplasm stained by PAS reaction. The cytoplasm is PAS positive. Note similar staining material filling a nearby capillary and assuming the shape of that vessel. It has been pointed out that the occasional elongated platelet forms seen on peripheral blood smears may actually be derived from platelets delivered in the lungs, as this photomicrograph suggests.

Not unexpectedly, the cell count was increased postoperatively, for at least 7 days, but returned to normal levels by the end of the second postoperative week.

**Discussion**

These results reveal that the number of megakaryocytes in a lung is related to the flow of venous blood to that lung. Thus, in dogs with end-to-end shunts where the left lung receives no venous blood very few megakaryocytes are observed in that lung. But when venous blood is allowed to enter the left lung, as in dogs with end-to-side shunts, megakaryocytes are found in abundant numbers there. The possibility that surgery might cause megakaryocytes to be concentrated in the right lungs of dogs with shunts may be reasonably excluded because dogs who underwent thoracotomy without shunt (Group IV) showed no such unilateral localization of cells. These findings support

<table>
<thead>
<tr>
<th>Dog (Group III)</th>
<th>Megakaryocytes/cm.²</th>
<th>Ratio of Incidence Right : Left</th>
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<tbody>
<tr>
<td></td>
<td>Right Lung</td>
<td>Left Lung</td>
</tr>
<tr>
<td>A935</td>
<td>1.7</td>
<td>.98</td>
</tr>
<tr>
<td>A937</td>
<td>1.1</td>
<td>1.9</td>
</tr>
<tr>
<td>Z34</td>
<td>2.5</td>
<td>2.1</td>
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</tbody>
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Table 2.—Incidence of Pulmonary Megakaryocytes in Dogs with End-to-End Shunts

<table>
<thead>
<tr>
<th>Dog</th>
<th>Megakaryocytes/cm.²</th>
<th>Ratio of Incidence</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Right Lung</td>
<td>Left Lung</td>
</tr>
<tr>
<td>Y48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Killed 1 week postsurgery</td>
<td>65.7</td>
<td>0.9</td>
</tr>
<tr>
<td>4X4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Killed 2 weeks postsurgery</td>
<td>7.6</td>
<td>0.4</td>
</tr>
<tr>
<td>43Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Killed 4 weeks postsurgery</td>
<td>17.1</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Table 3.—Incidence of Pulmonary Megakaryocytes in Dogs with End-to-Side Shunts

<table>
<thead>
<tr>
<th>Dog</th>
<th>Megakaryocytes/cm.²</th>
<th>Ratio of Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right Lung</td>
<td>Left Lung</td>
</tr>
<tr>
<td>0X6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Killed 1 week postsurgery</td>
<td>17.2</td>
<td>19.8</td>
</tr>
<tr>
<td>A280</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Killed 2 weeks postsurgery</td>
<td>60.9</td>
<td>15.8</td>
</tr>
<tr>
<td>A297</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Killed 4 weeks postsurgery</td>
<td>3.1</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Table 4.—Incidence of Pulmonary Megakaryocytes in Dogs Following Simple Thoracotomy

<table>
<thead>
<tr>
<th>Dog</th>
<th>Megakaryocytes/cm.²</th>
<th>Ratio of Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right Lung</td>
<td>Left Lung</td>
</tr>
<tr>
<td>C176</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Killed 1 week postsurgery</td>
<td>14.4</td>
<td>14.8</td>
</tr>
<tr>
<td>C054</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Killed 2 weeks postsurgery</td>
<td>3.2</td>
<td>2.8</td>
</tr>
</tbody>
</table>

the concept that pulmonary megakaryocytes do not originate in the lung, but elsewhere in the body, and are carried to the lung in the venous blood to be filtered by the pulmonary capillaries. Certain clinical observations and studies in humans support this conclusion. In a case of primary thrombocytopenia sections of the bone marrow revealed megakaryocytes in the venules but not in the arterioles. In a case of myelofibrosis with megakaryocyte production in the spleen, but not in the bone marrow, there were no megakaryocytes noted in the lung; the liver was interposed between the lung and the source of the megakaryocytes. And, very significantly, megakaryocytes
have now been demonstrated to circulate in the venous blood of normal persons \textsuperscript{17-20} (as well as in persons afflicted with cancer or bone marrow disorders). These cells are far more numerous in blood draining areas rich in bone marrow (e.g., lower vena caval blood) than in blood draining a bone marrow-poor area (e.g., antecubital vein blood).\textsuperscript{21-23} Finally, the fact that only mature forms and naked nuclei are seen in the lungs also speaks against the lung's being a site of megakaryocyte production.

Although the lung is an efficient filter it is not a perfect one, and some megakaryocytes are apparently able to traverse the pulmonary capillary network. Thus, a few megakaryocytes are seen in the left lung of dogs with end-to-end shunts, the cells having first successfully passed through the filter of the right lung. In humans, megakaryocytes have been observed in pulmonary vein blood,\textsuperscript{24} these cells having passed through the lungs, obviously embolize peripherally, which no doubt explains, as has been previously suggested,\textsuperscript{6,9} why an occasional megakaryocyte is found in such organs as the liver, heart and kidney.

Surgery appears to be a potent stimulus to megakaryocytopoiesis. The megakaryocyte count in both groups of dogs with shunts was elevated for at least 2 weeks following surgery. However, most of these dogs had infected wounds which would tend to enhance megakaryocyte production. But, the 2 dogs who underwent simple thoracotomy did not develop wound sepsis. Pulmonary megakaryocytosis was present for at least 7 days postoperatively but had subsided by 14 days after surgery in these dogs.

Besides functioning as a platelet repository\textsuperscript{25} the lungs may also serve as a site of entry into the circulation for new platelets.\textsuperscript{26,27} In dogs, 10 per cent of the megakaryocytes seen in the lung contain cytoplasm. Doubtless, this cytoplasm will be shorn from these cells, producing platelets (fig. 4). Thus, for some megakaryocytes at least, the normal life cycle includes birth and maturation in the bone marrow, entrance into the circulation, passage to the lung, and finally, the yielding of platelets in the pulmonary capillaries.

**Summary**

1. Evidence is presented which indicates that pulmonary megakaryocytes do not originate in the lungs but elsewhere in the body and are carried to the lungs in the venous blood.
2. Some megakaryocytes in the lungs evidently deliver platelets to the blood.
3. Surgery is a potent stimulus to megakaryocyte production; the numbers of megakaryocytes found in the lung postoperatively is significantly increased.

**Summario in Interlingua**

1. Es presentate observationes que suggere que megacaryocytos ha lor origine non in le pulmones sed alterubi in le corpore e que illos es portate verso le pulmones per le sanguine venose.
2. Un certe numero de megacaryocytos in le pulmones ha evidentemente le function de delivrar plachettas ad in le sanguine.
3. Interventiones chirurgic es un potente stimulo del production de mega-
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Caryocytos. Le numero de megacaryocytos trovate in le pulmones post un operation es augmentate de maniera significative.

REFERENCES

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