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Megakaryocytes and platelet homeostasis in diffuse alveolar damage

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Abstract

Platelet homeostasis reflects a balance between the production of platelets via cytoplasmic fragmentation of megakaryocytes in the pulmonary microvasculature and their catabolism. Increased numbers of megakaryocytes are entrapped in the injured lung, potentially affecting circulating platelet counts. We enumerated pulmonary megakaryocytes and blood platelets in patients with diffuse alveolar damage (DAD) in order to determine their association with clinical outcome. Lung biopsies were examined from 21 patients with histologically documented DAD in its proliferative phase and secondary to a variety of causes. Blood platelet counts were determined within 24 h prior to lung biopsy, and CD61+ pulmonary megakaryocytes were localized in *in situ* immunohistochemical stains. The overall mortality in this series was 67%. Patients with DAD attributable to drug toxicity (DAD-D) had higher mortality (80%) and greater number of intrapulmonary CD61+ megakaryocytes than those with DAD due to other causes (23 ± 7, 10 ± 2, $p < 0.05$). Patients with blood platelet counts $=350$ th/cm^3 showed increased survival ($p < 0.05$). The findings support the hypothesis that abnormal platelet homeostasis is associated with increased mortality in acute lung injury and indicate that thrombocytosis in ARDS is associated with improved survival. The mechanisms of altered platelet homeostasis in DAD merit further investigation.

Keywords: Adult respiratory distress syndrome; Diffuse alveolar damage; Megakaryocytes; Platelets

Introduction

The acute respiratory distress syndrome (ARDS) is a clinical disorder characterized by radiographic evidence of pulmonary edema and respiratory failure (Piantadosi and Schwartz, 2004). The mortality of ARDS in the United States approaches 60%, with protective ventilation therapy remaining as the mainstay of treatment (Piantadosi and Schwartz, 2004; Ware, 2005). The pathophysiology of ARDS is complex and includes activation of the pulmonary endothelium, capillary injury, and plasma protein leakage (Piantadosi and Schwartz, 2004). Common associated causes of ARDS include sepsis, aspiration, trauma, pancreatitis, smoke inhalation, and drug toxicity. A variety of clinical parameters have been associated with outcome of ARDS, including age, comorbid illness, and multiorgan system failure (Ware, 2005).

The histopathologic correlate of ARDS, in most cases, is diffuse alveolar damage (DAD). Lung biopsies establish the diagnosis of DAD and exclude other potentially treatable etiologies of ARDS, including infection (Kao et al., 2006). The sine qua non of DAD is the hyaline membrane, composed of fibrin and necrotic epithelial cell debris lining alveolar septae. The later fibroproliferative phase is characterized by the proliferation of lung cell precursors and by fibroplasia. Intravascular fibrin thrombi are seen in both small and large pulmonary vessels in ~50% of cases.

Abnormalities of platelet homeostasis have been observed in ARDS (Carvalho et al., 1987). These include increased platelet turnover rate and increased sequestration of platelets in the lung, liver, and spleen (Schneider et al., 1980). Disseminated intravascular coagulation (DIC) may develop in ARDS and has been shown to be more prevalent in those dying with the disorder (Bone et al., 1976). However, in patients with ARDS, thrombocytopenia has been associated with decreased survival even in the absence of DIC (Bone et al., 1976, 1992).

Platelets are generated normally via the cytoplasmic fragmentation of megakaryocytes within the pulmonary capillary bed

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Table 1
Patient demographics

Age	Sex	Outcome	Blood platelet count (th/cm^3)	Hospital stay days	Etiology
74	M	Dead	333	29	Cytoxan therapy for pulmonary fibrosis
55	F	Dead	126	26	Cisplatin chemotherapy and radiation for small cell carcinoma
66	M	Dead	74	20	Busulfan therapy for lymphoma
67	M	Dead	270	25	Bleomycin therapy for laryngeal carcinoma
63	M	Dead	234	22	Cytomegalovirus and *Candida* sepsis
68	M	Dead	105	67	Cytomegalovirus pneumonitis and Wegener's granulomatosis
82	F	Dead	327	14	Post myocardial infarction and hypoxemia
49	F	Dead	55	1	Ischemic pulmonary infarction following lung transplantation
66	F	Dead	39	12	Fungal sepsis
23	F	Dead	150	36	Bacterial pneumonia following lung transplantation
58	F	Dead	73	10	Pneumonia
58	F	Dead	271	7	Mycobacterial pneumonia
44	M	Dead	61	21	Acute myelogenous leukemia
74	M	Dead	297	15	Steroid therapy for rheumatoid disease
76	M	Alive	387	25	Amiodarone pneumonitis
46	M	Alive	434	62	Post-operative hypoxemia
49	F	Alive	382	14	Diabetic ketoacidosis
57	F	Alive	432	16	Eosinophilic pneumonitis
77	M	Alive	56	48	Neutropenia and Wegener's granulomatosis
49	F	Alive	171	47	Steroid therapy for rheumatoid disease
31	M	Alive	206	72	Prolonged intubation following cerebrovascular accident

as judged by ultrastructural observations both in mice and humans (Zucker-Franklin and Philipp, 2000). Whereas megakaryocytes are rarely identified histologically in the pulmonary microvasculature of normal lungs, pulmonary microvascular megakaryocytes are frequently identified by surgical pathologists in biopsies of DAD. Increased pulmonary megakaryocytes have been described in patients dying from burns (Well et al., 1984) and in experimental models of shock (Sukowski et al., 1999) and may be a generic marker of both localized and diffuse acute pulmonary microvascular injuries. As pulmonary megakaryocytes are commonly seen in DAD, we examined the quantitative relationship between pulmonary megakaryocytes and blood platelet counts in patient with ARDS and determined whether changes in these parameters might predict subsequent mortality.

Materials and methods

Video-assisted thoracoscopic lung biopsies conducted between 1995 and 2006 with a histologic diagnosis of DAD in fibroproliferative phase were retrieved from the files of Massachusetts General Hospital Pathology Department. Blood platelet counts (normal range=150–250 th/cm^3) recorded within 24 h prior to lung biopsy were retrieved from medical records. Sections (5 μM) from paraffin-embedded and formalin-fixed tissues were stained by indirect immunohistochemical methods with anti-CD61, which binds to the IIIa subunit of the glycoprotein heterodimer IIb/IIIa located on the cell surface membranes of both megakaryocytes and platelets (Ventana Medical Systems,

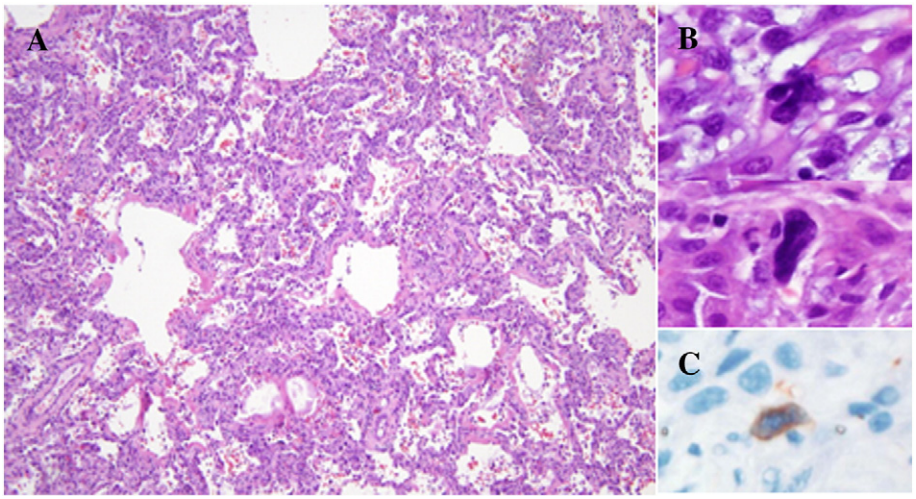

Fig. 1. (A) Diffuse alveolar damage (DAD) showing numerous hyaline membranes and proliferative changes, hematoxylin and eosin, magnification 100×. (B) Intravascular pulmonary megakaryocyte, as seen in H&E stained sections of DAD, magnification 400×. (C) CD61+ megakaryocyte as seen in the lung in DAD, peroxidase stain, magnification 400×.