Time Course of Hemosiderin Production and Clearance by Human Pulmonary Macrophages*

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Tracheal aspirates from four previously healthy infants with acute pulmonary hemorrhage, and small volume bronchial lavages from children undergoing flexible fiberoptic bronchoscopy were examined for pulmonary alveolar macrophages (PAM) containing hemosiderin. Hemosiderin formation was also studied in vitro. Macrophages containing hemosiderin were first seen in tracheal aspirates 50 hours after an acute pulmonary hemorrhage and after 72 hours in cultured macrophages. A small percentage of the PAM recovered by bronchoalveolar lavage from both adults and children contained hemosiderin. Hemosiderin was rapidly cleared from the lungs following an acute pulmonary hemorrhage.

Pulmonary alveolar macrophages (PAM) process hemoglobin from ingested red blood cells to form ferritin and other degradative products. These are identified as hemosiderin when stained by acid ferrocyanide. Little is known of the time course of hemosiderin formation and clearance from the human lung. Observations of PAM from four previously well infants who developed acute pulmonary hemorrhage and of hemosiderin formation in cultured PAM now afford a basis for estimation of the interval during which hemosiderin-laden PAM can be detected in airway secretions following a single episode of hemorrhage.

Material and Methods

Case 1

A four-week-old previously healthy infant presented with tachypnea and blood in his nose and mouth. He was cyanotic, dyspneic and had crackles in his left chest. A chest roentgenogram showed diffuse alveolar infiltrates, and bronchoscopic examination six hours after the onset of symptoms revealed blood throughout the tracheobronchial tree. Pulmonary alveolar macrophages did not contain hemosiderin. He recovered from this episode and did well until he died suddenly and unexpectedly at eight weeks of age. At autopsy, multiple lung sections showed diffuse, severe interstitial and intra-alveolar pulmonary hemorrhage without evidence of hemosiderin in PAM, suggesting a second, acute hemorrhage into the lung.

Case 2

A four-week-old previously healthy infant developed dyspnea and bloody nasal discharge while eating. He became cyanotic and had fine crackles throughout both lung fields. Chest roentgenogram showed diffuse alveolar infiltrates and a consolidated right upper lobe. Blood was present throughout the respiratory tree at endoscopic examination. He required intermittent positive pressure ventilation for nine days. Bloody secretions virtually disappeared 24 hours after intubation and tracheal secretions were scant after 48 hours. Hemosiderin-laden macrophages were not seen in tracheal aspirates until the 5th day. Lung biopsy on the 12th day showed focal interstitial fibrosis, consistent with the ventilatory assistance the patient had received, and a few hemosiderin-laden macrophages in interlobar septae, but none in airways.

Case 3

A 13-week-old previously healthy girl awoke with grunting respirations and blood around her nose and mouth. She was initially in moderate respiratory distress with crackles bilaterally, but her condition rapidly deteriorated and she required intermittent positive pressure ventilation for three days. At intubation, bloody secretions were noted within the trachea. Tracheal aspirates contained blood for 24 hours and subsequently were scant. Hemosiderin-laden macrophages were not noted in serial tracheal aspirates until 50 hours following intubation.

Case 4

A three-month-old previously healthy boy was brought to the emergency room because of hemoptysis. Direct laryngoscopic examination revealed blood beneath the vocal cords. He was in mild distress and a chest roentgenogram showed mild bilateral interstitial infiltrates. Although initially moderately hypoxemic, he did well and by 72 hours after admission was asymptomatic. Flexible fiberoptic bronchoscopic examination 72 hours following the hemoptysis revealed normal anatomy. Saline lavage of the right lower lobe provided very slightly blood tinged fluid. Forty percent of the PAM in this aspirate stained densely for hemosiderin, and virtually all appeared to have ingested red blood cells.

Smeared from small volume (5-10 ml) bronchial lavages from six children undergoing bronchoscopy for diseases other than pulmonary hemorrhage were examined for hemosiderin. These children were three months to seven years age and had airway obstruction (three pts), recurrent pneumonia due to immune defi-
Hemosiderin was seen in six and 12 percent of the PAM from the patients with recurrent pneumonia, rarely (<0.1 percent) in PAM from one patient with airway obstruction, and not in the other two patients, and in less than 1 percent of the PAM from the patient with aspiration.

A patient with typical idiopathic pulmonary hemorrhage underwent a lung biopsy and a touch preparation was stained for hemosiderin. Greater than 90 percent of the PAM stained for hemosiderin.

To study the time course of hemosiderin formation, we obtained PAM from five healthy, non-smoking, young adult volunteers after informed consent, using five successive 100 ml saline lavages of the lingula through a flexible bronchoscope. Glass-adherent monolayers were prepared as previously described. Four percent sheep red blood cells (SRBC) in Hanks' balanced salt solution (HBSS) were incubated with a 1:80 dilution of anti-SRBC IgG (Corids Lab) for two hours at room temperature, washed twice with cold HBSS and added to two-hour-old macrophage cultures at a final concentration of 0.5 percent in McCoy medium. After one hour, non-adherent SRBC were washed off and the PAM were incubated at 37°C, 100 percent humidity and 5 percent CO₂ in McCoy medium with 10 percent fetal calf serum and antibiotics. Control cultures were not treated with SRBC. Cultured PAM were examined after fixation in ethanol for SRBC adherence by a tetrachrome stain, and for hemosiderin production by the acid ferrocyanide reaction at 0, 24, 48, 72, 96, and 120 hours.

RESULTS

Hemosiderin staining of macrophage granules was seen in 0.1 to 3 percent of PAM freshly lavaged from each of the normal volunteers. Tetrachrome staining showed SRBC on the surface of all PAM examined at the end of the two hour adherence period. Cultures not exposed to SRBC had no increase in hemosiderin staining from baseline, whereas more than 90 percent of PAM exposed to SRBC showed a faint blue staining of the cytoplasm first seen at 72 hours, and more intense granular staining by 120 hours.

The pulmonary hemorrhage in the infants described was acute, diffuse, and life threatening. Despite intensive investigation, including pulmonary angiograms, respiratory and gastrointestinal tract endoscopy, echo-cardiograms, investigation for immune disease, platelet counts, prothrombin time, activated partial thromboplastin time, bleeding time, bacterial cultures of the blood and tracheal aspirates, and viral cultures of the nasopharynx, no etiology was discovered. These patients do not fit the usual clinical description of ill newborn infants with massive pulmonary hemorrhage, pulmonar y hemosiderosis associated with milk precipitins, or idiopathic pulmonary hemosiderosis.

The acute nature of the pulmonary hemorrhage in these patients allowed assessment of hemosiderin formation in vivo. Patients 1, 2, and 3 had respiratory tract secretions sampled within hours of the acute bleeding episode. In each case, the secretions were grossly bloody and contained numerous PAM which failed to stain for hemosiderin. Patient 1 was not intubated. Endotracheal intubation of patients 2 and 3 allowed serial sampling of respiratory tract secretions, although these secretions were scant and did not contain large numbers of PAM. Hemosiderin-laden macrophages were seen 50 hours after intubation in patient 3, and after five days in patient 2. Patient 4 had many hemosiderin-positive macrophages in his bronchial lavage at 72 hours. A lung biopsy 12 days after the pulmonary hemorrhage in patient 3 contained no hemosiderin-laden macrophages in Airways, but some were present in the interlobar septae. Hemosiderin-laden macrophages could not be demonstrated at autopsy in patient 1 four weeks after an acute, diffuse bleeding episode. These observations suggest clearance of hemosiderin-laden macrophages from Airways within two weeks, and total clearance of these macrophages from the lung within two to four weeks after an acute hemorrhage.

Pulmonary alveolar macrophages containing hemosiderin were noted in the lavages of all five non-smoking adult volunteers. These subjects had no history of chronic lung disease or recent acute illness. Four of the five subjects had hemosiderin in 0.1-0.5 percent of their PAM. The other subject had 3 percent positive PAM. This subject worked in a research laboratory and was occasionally exposed to strong chemical fumes. These subjects had undergone a large volume bronchoalveolar lavage which may yield a somewhat different population of PAM than normally seen in sputum samples or tracheal aspirates. The small volume lavages from the six pediatric patients without pulmonary hemorrhage demonstrated that PAM containing hemosiderin may also be seen in children. The numbers of PAM with hemosiderin were highest in the children with recurrent pneumonia, suggesting that inflammation and small amounts of bleeding could account for the hemosiderin.

Cultured PAM first demonstrated hemosiderin at 72 hours following exposure to antibody-coated SRBC, and more intense staining at 120 hours. Detection of hemosiderin-laden macrophages by 50 hours after acute pulmonary hemorrhage in patient 3 and dense staining of the macrophages at 72 hours in patient 4 raises the possibility that hemosiderin production occurs somewhat faster in vivo than in vitro. This possibility is supported by data from rabbits whose PAM contained hemosiderin 33 hours after instillation of blood and hypertonic dextrose into the trachea.

DISCUSSION

Our data indicate that the diagnosis of pulmonary hemosiderosis using pulmonary lavage techniques to harvest PAM should be made cautiously. We propose that many of the PAM present should stain intensely for hemosiderin before a diagnosis of pulmonary hemosiderosis can be supported by examination of pulmonary lavage fluid. Further study is warranted before application of this criterion to examination of sputum.
or aspirated tracheal secretions.
Conversely, the absence of hemosiderin in pulmonary secretions does not exclude the possibility of a recent (less than 48 hours) or remote (greater than 12 days) acute pulmonary hemorrhage. Indeed, it may be difficult to demonstrate hemosiderin in PAM at any time following a single pulmonary hemorrhage because of the rapid clearance of blood, scant secretions within 24 hours after hemorrhage, and the limited time span during which hemosiderin-laden macrophages reside in the airways. Serial specimens of tracheobronchial secretions may be required to verify the occurrence, and if tracheal secretions do not yield adequate specimens, small volume bronchial lavages as in patient 4 may be helpful. Persistence of sputum production and abundant hemosiderin-laden macrophages indicates continuing or frequently recurring episodes of pulmonary hemorrhage.

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