The Pathology of Transfusion-Related Acute Lung Injury

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Key Words: Transfusion-related acute lung injury; TRALI; Granulocyte aggregation; Anti-HLA antibodies; Antigranulocyte antibodies

Abstract

Transfusion-related acute lung injury is an uncommon condition characterized by the rapid onset of respiratory distress soon after transfusion. Our understanding of its pathophysiology is based on animal models of complement (C5a) and antibody-induced lung injury and a limited number of autopsies. These models suggest that transfusion-related acute lung injury is induced by granulocytes that aggregate in the pulmonary microvasculature after activation by transfusion-derived antibodies or biologically active lipids. The published autopsy reports provide little support for this model, as they are invariably confounded by underlying pulmonary infection, preexisting disease, and resuscitation injury. We report the case of a previously well 58-year-old man who died of transfusion-related acute lung injury within 2 hours of the onset of pulmonary distress; autopsy showed evidence of massive pulmonary edema with granulocyte aggregation within the pulmonary microvasculature and extravasation into alveoli. Electron microscopy revealed capillary endothelial damage with activated granulocytes in contact with the alveolar basement membranes. These findings provide direct support for the proposed model of transfusion-related acute lung injury pathogenesis.

Transfusion-related acute lung injury is an uncommon syndrome typified by the rapid onset of pulmonary edema and respiratory distress during or shortly after transfusion.[1,2] Diagnosis is made, after the exclusion of circulatory overload and other cardiac and pulmonary etiologies, by the demonstration of donor or recipient anti-HLA and/or anti-granulocyte antibodies capable of causing leukoagglutination in vivo.[3–9] Transfusion-related acute lung injury generally responds to supportive care within 24 to 48 hours of onset, but it is fatal in 5% to 7% of cases. Our understanding of the histopathologic features of transfusion-related acute lung injury is based on findings in animal models of complement- and/or antibody-mediated acute lung injury and a limited number of autopsy reports in the early literature.[10–19] These postmortem findings correlate poorly with the later animal models as they are confounded by the delay between the onset of transfusion-related acute lung injury and death of the patient, underlying pulmonary disease, and damage caused by resuscitative efforts. We describe the pulmonary disease of a patient with no history of pulmonary disease who succumbed rapidly to transfusion-related acute lung injury. These findings are contrasted with the previously published autopsy data and are reviewed in the light of our present understanding of the pathogenesis of transfusion-related acute lung injury.

Case Report

The patient was a 58-year-old man admitted for elective coronary artery bypass surgery. His medical history was notable for adult-onset diabetes mellitus and 12 months of increasingly severe angina pectoris. An exercise stress test showed mild stress-induced ischemia. Cardiac catheterization revealed an anomalous right coronary artery that originated in the left aortic sinus, passing between the aortic and pulmonary outflow tracts, to reach the right atrioventricular groove. Mild (50%) distal circumflex stenosis was documented but thought to be clinically insignificant. A single-vessel bypass operation

was recommended, with grafting of the right internal mammary artery to the right coronary artery.

At surgery, the bypass graft was performed without difficulty. The patient was placed on cardiac bypass for 54 minutes with an aortic cross-clamp time of 34 minutes. Intraoperatively and before transfer to the intensive care unit, the patient was transfused with 3,000 mL of crystalloid, 4 units of fresh frozen plasma (FFP), and 2 pooled units of platelets. He also received intravenous aminocaproic acid (Amicar), 2 doses (1 g) of cefazolin (Ancef), and 3 units of autologous blood salvaged intraoperatively using a Haemonetics Cell-saver device (Haemonetics, Braintree, MA).

The patient left the operating room at 9:00 PM in stable condition; he received an additional pooled unit of platelets at 9:20 PM and 2 units of FFP shortly before 10:00 PM, for oozing at the incision site. Shortly after 10:00 PM, fulminant pulmonary edema with copious bronchial secretions and respiratory collapse developed, requiring mechanical ventilation. During the next 30 minutes, his arterial oxygen saturation fell from 98% to 78% despite receiving 100% oxygen; arterial pH dropped from 7.35 to 7.17; hematocrit rose from 28.6% to 47.3%; and the WBC count fell from 17,200/μL (17.2×10^9/L) to 8,900/μL (8.9×10^9/L). Although his cardiac function seemed normal at the onset of respiratory distress, maximal cardiac pressor drugs were administered, and the patient's chest was reopened with institution of manual cardiac massage and ventricular pacing. All resuscitative efforts failed, and he was declared dead at midnight, approximately 2 hours after the onset of pulmonary edema.

Materials and Methods

Antigranulocyte antibody assays were performed by Specialty Laboratories, Santa Monica, CA, using an indirect immunofluorescence technique. Microcytotoxicity panel reactive antibody (PRA) screens were performed at the clinical laboratories of Brigham and Women's Hospital, Boston, MA, or the American Red Cross Laboratories, Dedham, MA, using standard techniques, screening commercial panels of 55 to 60 cell lines.[20]

Pathologic Findings

Autopsy

The autopsy was performed 8 hours after death and was limited to the thoracic cavity. The lungs were diffusely edematous, weighing approximately 3 times normal (right lung, 1,610 g [expected, 360–570 g]; left lung, 1,410 g [expected, 325–480 g]), and a substantial amount of clear serous fluid oozed from the trachea on removal of the heart-lung block. No areas of consolidation or pulmonary emboli were grossly apparent. The heart showed left ventricular hypertrophy with 40% to 50% stenosis within the left circumflex and left anterior descending arteries, without acute plaque change or thrombosis. The right internal mammary artery graft to the posterior descending artery was widely patent with good distal flow by postmortem angiography. No evidence for acute myocardial infarction was found.

A striking finding on microscopic examination was the increased number of granulocytes within the pulmonary capillary vasculature and small pulmonary vessels in all lobes of the lungs. In several areas, the granulocyte/RBC ratio approached 1:1. The alveolar air spaces contained amorphic, lightly eosinophilic, proteinaceous material in a diffuse pattern **Image 1** and **Image 2**. There was a positive correlation between the degree of capillary leukostasis and the amount of proteinaceous fluid within the alveolar air spaces. Focally, granulocytes were observed within the alveolar air spaces, as were desquamated epithelial cells. There also was a substantial degree of proteinaceous material within pulmonary septa, consistent with the pulmonary edema observed grossly. Leukostasis was not evident in small vessels associated with large bronchi, which are likely of the systemic circulation. There was no evidence of pneumonia, hyaline membrane formation, hemorrhage, emboli, or long-standing congestive failure.

Ultrastructural examination similarly disclosed granulocyte aggregation within pulmonary capillaries and amorphous proteinaceous material within the adjacent alveolar air spaces **Image 3**. There was gross loss of alveolar epithelium and

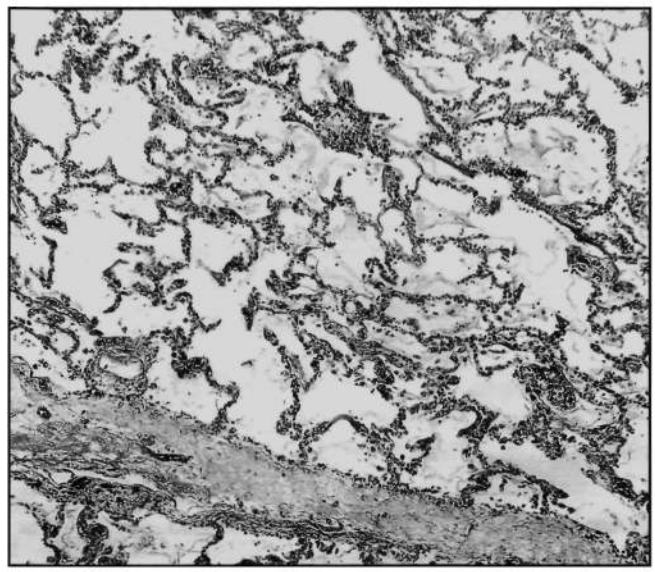

Image 1 Section of lung tissue showing capillaries congested with RBCs and granulocytes. Intra-alveolar proteinaceous material and desquamating epithelium are seen, without evidence of prominent alveolar infiltrates or hemorrhage (H&E, ×40).

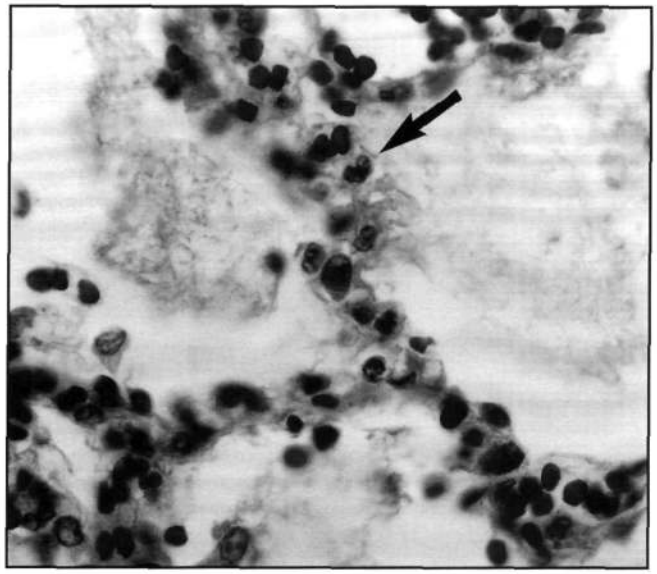

Image 2 Alveolar proteinaceous material and alveolar capillaries congested with granulocytes (arrow) and RBCs. A moderately congested area was selected to better demonstrate granulocyte nuclear morphologic features (H&E, ×1,000).

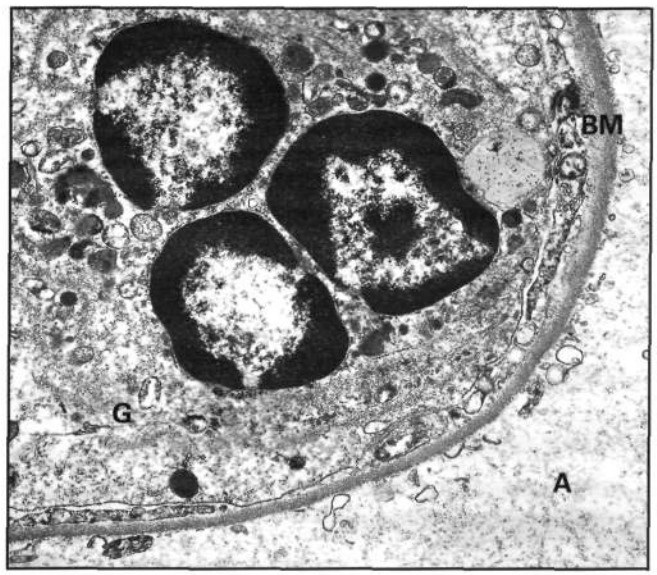

Image 3 Electron micrograph of alveolar basement membrane (BM) with an intravascular, adherent granulocyte (G), desquamated alveolar epithelium, and amorphous proteinaceous alveolar material (A) (×10,000).

features suggestive of endothelial cell injury: granulocytes within the pulmonary capillaries were degranulated, and membrane-bound cytoplasmic fragments were present between the granulocytes and the endothelium. Focally, granulocytes could be seen in direct contact with denuded stretches of capillary wall (Image 3) **Image 4**.

Serologic Results

Before the onset of respiratory compromise, the patient received 6 units of FFP and 3 pooled units of random donor platelets derived from 12 donors. All units were negative on microbial culture and were ABO compatible, with no anti-RBC antibodies detected by routine screen. All units, as well as the patient's pretransfusion and posttransfusion serum samples, were screened for antigranulocyte antibodies using an indirect immunofluorescence technique and for lymphocytotoxic antibodies using a standard PRA assay with commercial panels of HLA-typed cells. The results indicated that the patient had a negative PRA screen before transfusion but showed 30% panel reactivity after transfusion **Table 1**. Pretransfusion and posttransfusion serum samples showed weak antigranulocyte antibody activity. Two male platelet donors were shown to have strongly positive antigranulocyte antibodies; 1 male platelet donor showed 39% and 1 female FFP donor showed 60% PRA activity. In all cases, the PRA showed broad specificity and no particular HLA specificity could be discerned. The products from these donors were transfused approximately 1 to 1½ hours before the development of fulminant pulmonary edema. A direct crossmatch test between the donor serum samples and

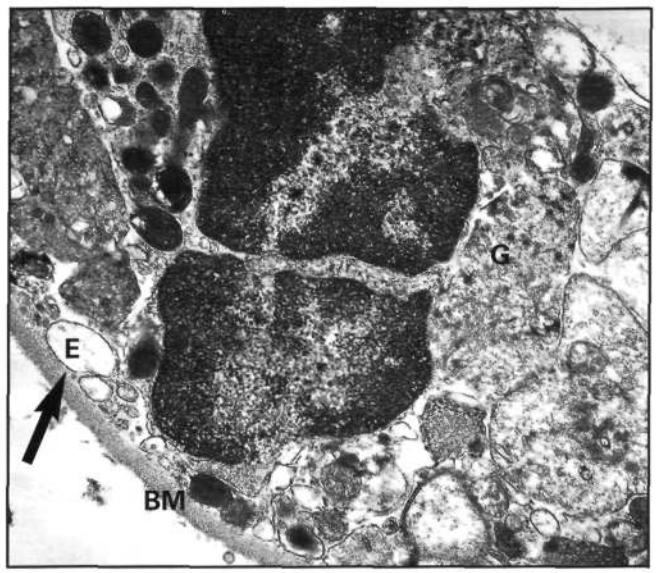

Image 4 Electron micrograph of adherent degranulated granulocyte (G) in contact with the basement membrane (BM) and with vesicular "blebbing" of an endothelial cells (E) (×14,000).

the patient's granulocytes was not performed, as the patient's leukocytes were not available postmortem.

Discussion

Transfusion-related acute lung injury is characterized by the rapid onset of fever, chills, tachycardia, cough, and

Table 1
Time Line for Transfused Products and Testing Results

Time of Transfusion	Product	ABO Type	Unit	Donor Sex	Lymphocytotoxic PRA (%)*	Antigranulocyte Antibodies
Pretransfusion		O, Rh positive			0	±
7:13 PM	Pooled platelets	A, Rh positive				
			1	M	2	–
			2	M	0	±
			3	M	0	+++
			4	M	0	±
7:53 PM	FFP	O, Rh positive		M	0	±
	FFP	O, Rh positive		F	0	–
	FFP	O, Rh positive		M	0	–
	FFP	O, Rh positive		M	0	–
	Pooled platelets	Rh positive†				
			1	M	0	–
			2	M	0	+++
			3	M	0	±
			4	M	39	–
9:20 PM	FFP	O, Rh positive		M	2	–
	FFP	O, Rh positive		F	60	–
	Pooled platelets	Rh positive†				
			1	M	0	–
			2	M	4	±
			3	F	2	–
			4	M	0	–
10:00 PM; onset of respiratory distress						
10:55 PM; posttransfusion					30	±

FFP = fresh frozen plasma; ± = extremely weak; – = negative; +++ = strong reactivity.
* Microcytotoxicity panel-reactivity assay (PRA) on panels of 55 to 60 cell lines.
†Containing a mixture of ABO types.

various degrees of respiratory distress, usually within 4 hours of transfusion.[1,2] Chest radiography reveals bilateral patchy pulmonary infiltrates in the absence of cardiac enlargement or pulmonary arterial engorgement. Severe pulmonary edema and hypoxemia may ensue, requiring intubation and vigorous respiratory support. Normal or low pulmonary wedge pressures and normal central venous pressure help rule out cardiogenic or circulatory overload causes. Unlike acute respiratory distress syndrome (ARDS), most transfusion-related acute lung injury cases improve dramatically within 24 to 48 hours of onset, and the pulmonary infiltrates resolve in 1 to 4 days; however, transfusion-related acute lung injury is fatal in 5% to 7% percent of cases.

The exclusion of cardiogenic causes and the demonstration of leukoagglutinating antibodies indicate a diagnosis of transfusion-related acute lung injury, in the appropriate clinical setting. In 89% of cases, anti-HLA and/or anti-granulocyte (anti NB2, anti-NA2, and anti 5b) antibodies are found in the donor plasma that cause leukoagglutination of recipient granulocytes.[2] In rare cases, the recipient may harbor antibodies reactive for donor granulocytes.[2,9] Infusion of leukoagglutinating antibodies has been shown to cause transient marked leukopenia[15,16] and the localization of indium 111–labeled granulocytes to the pulmonary circulation in humans.[14] These antibodies also cause acute pulmonary edema in the presence of a complement source, in a rabbit ex vivo lung perfusion model.[17] Our understanding of the probable histopathogenesis of transfusion-related acute lung injury is based on animal models of ARDS triggered by infusion of activated complement (C5a).[10–13] In these studies, C5a promotes the activation, aggregation, and sequestration of granulocytes in the pulmonary microvasculature. The local release of proteases, oxygen radicals, and acidic lipids causes endothelial damage and the extravasation of protein-rich fluid into the adjacent interstitium and alveoli, leading to pulmonary edema and respiratory distress.

Recently, Silliman et al[21] noted that animal models of ARDS require at least 2 pulmonary insults. These authors suggested an alternative mechanism for transfusion-related acute lung injury in which the first insult may be the underlying clinical condition (eg, infection, hypoxia, after surgery), and the second may be bioactive lipids that accumulate in stored blood products. Bioactive lipids trigger granulocyte activation in vitro and are detected in posttransfusion specimens in patients with transfusion-related acute lung injury. While it is unclear whether these lipids are the cause of transfusion-related acute lung injury or just an effect of the lung injury, the proposed final mechanism of injury is direct granulocyte-mediated damage to the pulmonary blood vessels, as outlined.

Histologic evidence in support of either model of transfusion-related acute lung injury in humans is sparse, given the lack of availability of diagnostic tissue specimens. The role of

complement in transfusion-related acute lung injury is unclear, and it is notable that transfusion-related acute lung injury differs from classic ARDS in that it is generally a transient rather than persistent cause of pulmonary infiltration and distress.

Autopsy reports are conflicting, probably owing to variable delays between the onset of transfusion-related acute lung injury and death and the confounding factors of infection, underlying disease, and resuscitative efforts. **Table 2** summarizes the findings in 8 published autopsies. Popovsky and Moore[1] described 2 cases that showed resolving pneumonitis and pulmonary edema. Wolf and Canale[7] described a 13-year-old girl with thalassemia who died of transfusion-related acute lung injury within 12½ hours of transfusion. Autopsy showed congested lungs (800 g total) with dilated subpleural lymphatics and abundant edema fluid. Marked accumulation of intra-alveolar inflammatory cells was noted, as were hyaline membranes and congested alveolar capillaries. Kernoff et al[6] described a case of a 9-year-old patient with hemophilia who died 13 hours after transfusion; the lungs were grossly edematous with basal atelectasis. Extensive hemorrhagic edema and areas of agonal acute pneumonitis characterized by alveolar polymorphonuclear exudate and fibrin deposition were present. Eastlund et al[9] described a case of a 55-year-old HLA-B35-positive woman who died within 2 hours of a transfusion containing a nonleukoagglutinating, anti-HLA B35 antibody. On autopsy, no pulmonary edema, leukostasis or other cause of pulmonary failure was noted. Flury and Reutter[18] described a case of massive hemorrhagic pulmonary edema without evidence of intravascular leukocyte agglutinates or thrombi. Felbo and Jensen[19] similarly reported a case with pulmonary edema, as well as "fairly large numbers of leukocytes in pulmonary vessels and collections of granulocytes in the alveolar spaces."

Most autopsy cases have demonstrated histologic evidence of moderate to severe pulmonary edema, as well as alveolar changes that are most commonly associated with infection and ARDS. Few have revealed pulmonary intravascular leukoagglutination, the presumed mechanism of transfusion-related acute lung injury hypothesized from animal and clinical studies.

We report a case report of a 58-year old man who died of acute fulminant pulmonary edema within 5 hours of the first transfusion and within 2 hours of the onset of symptoms. The patient had no underlying pulmonary disease, and the diagnosis was confirmed by the exclusion of cardiac causes and the demonstration of anti-HLA and antigranulocyte antibodies in the transfused products. Because we did not perform a crossmatch of the patient's granulocytes with donor serum samples, we were unable to implicate a particular donor in this reaction; rather, the possibility arises that transfusion-related acute lung injury was due to the unfortunate interaction of antibodies from multiple donors. In keeping with the histopathologic model of transfusion-related acute lung injury developed from animal studies of complement and antibody-mediated pulmonary injury,[10–17] on autopsy, we found evidence of massive pulmonary edema with prominent leukostasis within pulmonary capillaries. This was associated with proteinaceous material within alveolar air spaces and interstitial tissues and focal granulocytes within alveolar air spaces. We did not see macrophages within alveolar air spaces, as have been described 24 to 48 hours after activated complement infusion in animals.[12] This lack of macrophages is not surprising, however, given that our patient died only 2 hours after initial clinical signs of pulmonary edema.

Ultrastructural analysis further supported the animal models for transfusion-related acute lung injury, although we are unable to exclude the possibility that some of these findings were due to postmortem changes, as approximately 8 hours elapsed between the patient's death and tissue sampling. We noted diffuse denudation of the alveolar epithelium on electron microscopy that may reflect a postmortem change. We do not believe the same explanation can be applied to our observations of widespread degranulation of granulocytes, membrane-bound cytoplasmic fragments between the granulocytes and endothelium, and the focal finding of granulocytes directly contacting denuded capillary walls. These

Table 2
Summary of Pulmonary Findings on Autopsy in Published Cases of Transfusion-Related Lung Injury

Report	No. of Cases	Pulmonary Edema	Intravascular Leukocyte Aggregates	Pneumonitis	Hyaline Membrane	Intra-alveolar Leukocytes	Intra-alveolar Hemorrhage	Atelectasis	Interstitial Inflammation
Popovsky and Moore[1]	2	+	NR	+	NR	NR	NR	NR	NR
Wolf and Canale[7]	1	+	–	NR	+	+	NR	NR	NR
Kernoff et al[6]	1	+	NR	+	NR	+	+	+	NR
Eastlund et al[9]	1	–	–	NR	NR	NR	NR	NR	NR
Silliman et al[21]	1	+	NR	NR	+	NR	+	NR	–
Flury and Reutter[18]	1	+	–	NR	NR	NR	+	NR	NR
Felbo and Jensen[19]	1	+	+	NR	NR	NR	NR	NR	NR
Present report	1	+	+	–	–	+	–	–	–

+ = present; NR = not reported; – = absent.

latter findings, together with the histologic, gross pathologic, and clinical features of this case, argue strongly for a similar mechanism for transfusion-related acute lung injury and the lung injury shown in animal models. Whether this implies that complement is implicated in transfusion-related acute lung injury, possibly through antibody activation, or that complement and leukoagglutinating antibodies act through a common final pathway of granulocyte activation, is unclear.

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Acknowledgments: We thank Doreen Sese, Brian J. Cunningham, and Anthony R. Jackson for performing lymphocytotoxicity panel reactive antibody assays.

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