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Inflammation-associated changes in lipid composition and the organization of the erythrocyte membrane

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ABSTRACT

Background: Reduced erythrocyte survival and deformability may contribute to the so-called anemia of inflammation observed in septic patients. Erythrocyte structure and function are affected by both the membrane lipid composition and the organization. We therefore aimed to determine whether these parameters are affected during systemic inflammation.
Methods: A sensitive matrix-assisted laser desorption and ionization time-of-flight mass spectrometric method was used to investigate the effect of plasma components of 10 patients with septic shock and of 10 healthy volunteers subjected to experimental endotoxemia on erythrocyte membrane lipid composition.
Results: Incubation of erythrocytes from healthy control donors with plasma from patients with septic shock resulted in membrane phosphatidylcholine hydrolysis into lysophosphatidylcholine (LPC). Plasma from volunteers undergoing experimental human endotoxemia did not induce LPC formation. The secretory phospholipase A_2 IIA concentration was enhanced up to 200-fold in plasma of septic patients and plasma from endotoxin-treated subjects, but did not correlate with the ability of these plasmas to generate LPC. Erythrocyte phosphatidylserine exposure increased up to two-fold during experimental endotoxemia.
Conclusions: Erythrocyte membrane lipid remodeling as reflected by LPC formation and/or PS exposure occurs during systemic inflammation in a secretory phospholipase A_2 IIA-independent manner.
General significance: Sepsis-associated inflammation induces a lipid remodeling of the erythrocyte membrane that is likely to affect erythrocyte function and survival, and that is not fully mimicked by experimental endotoxemia.

1. Introduction

In patients with inflammation, anemia is associated with poor patient outcome. Next to changes in iron homeostasis and defective erythropoiesis [1,2], reduced erythrocyte lifespan contributes to "anemia of inflammation" [3–5]. This condition is common in patients suffering from sepsis [6]. Erythrocyte shape, deformability, and aggregability were shown to be altered in these patients [7,8]. Such structural and functional changes may contribute to the microcirculatory alterations that are linked to untimely erythrocyte removal in the spleen [9] and to a poor outcome [8].

Erythrocyte structure and function depend on plasma membrane lipid composition [10,11]. Sepsis-associated alterations in membrane lipid composition may contribute to alterations in erythrocyte function. Indeed, incubation of erythrocytes from healthy volunteers with plasma of septic patients has been shown to induce phosphatidylserine (PS) exposure and membrane ceramide formation [12], both with functional consequences [10,13].

Phospholipids constitute the majority of the erythrocyte membrane lipids, with the glycerophospholipid (GPL) phosphatidylcholine (PC) and the sphingolipid sphingomyelin (SM) dominating the outer membrane of the lipid bilayer [10,11]. Secretory phospholipase A_2 ($sPLA_2$) and sphingomyelinase (SMase) catalyze the hydrolysis of GPLs into lysophospholipids (LPLs) and free fatty acids, and SM into ceramide and choline, respectively. The activity of both lipases is enhanced in the

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Table 1
Demographic characteristics of septic patients.

Pt Nr.	Sex (M/F)	Age (years)	Diagnosis	APACHE II	Hb (mmol/L)	Transfusion history
1	V	54	Pneumosepsis	28	4.9	None
2	M	68	Abdominal sepsis after intestinal ischemia		5.1	Day before blood drawing: 3 thrombocyte transfusions 3 plasma transfusions Day of blood drawing: 2 thrombocyte transfusions 8 plasma transfusions 9 erythrocyte transfusions
3	V	81	Abdominal sepsis in ulcerative colitis	28	6.0	None
4	M	59	Infected hip prosthesis		4.9	Day before blood drawing: 1 erythrocyte transfusion Day of blood drawing: 1 erythrocyte transfusion
5	M	75	Cholangitis	23	8.6	Day before blood drawing: 2 thrombocyte transfusions 2 plasma transfusions Day of blood drawing: 1 thrombocyte transfusion
6	M	82	Abdominal sepsis after intestinal ischemia	23	7.5	Day before blood drawing: 3 erythrocyte transfusions
7	M	34	Pneumosepsis	14	7.8	None
8	M	84	Urosepsis	16	6.3	None
9	V	51	Pneumosepsis	27	5.9	None
10	V	65	Toxicoderma	23	6.6	None
Mean ± SD		66.3 ± 16.1		22.8 ± 5.3	6.4 ± 1.3	

plasma of patients with sepsis [14,15], and the lipids they generate are involved in the pathology of inflammation [16]. *In vitro*, erythrocyte deformability and survival are negatively influenced by $sPLA_2$ and SMase, and by the incorporation of their products into the membrane [9,13,17,18].

The aim of the current study was to investigate the involvement of lipase activity in the erythrocyte-related pathophysiology during systemic inflammation in patients with sepsis and in experimental human endotoxemia. Using sensitive matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry (MS), we investigated the lipid composition of erythrocytes after incubation with the plasma of patients suffering from septic shock and with plasma of subjects undergoing experimental endotoxemia. A mechanistic explanation for the observed changes was explored by measuring plasma $sPLA_2$ IIA levels and erythrocyte PS exposure.

2. Material and methods

2.1. Septic patients

Anticoagulated blood (4 mL) was collected from 10 septic shock patients who resided in the department of Intensive Care Medicine of the Radboud University Medical Center, Nijmegen, the Netherlands, and from ten healthy volunteers. Septic shock was defined as having two or more systemic inflammatory response syndrome criteria [19], in combination with a proven or suspected infection and the need for vasopressor therapy following adequate fluid resuscitation. The study was carried out in accordance with the applicable rules on review by ethics committees and informed consent. Blood from all patients was drawn within 24 h after starting vasopressor therapy. Plasma, erythrocytes and erythrocyte membrane fractions were obtained by differential centrifugation.

Table 2
Characteristics of healthy subjects.

	Endotoxemia subjects	Healthy volunteers
N	10	10
Age (years ± SD)	22.7 ± 2.8	34.7 ± 14.8
Sex (M/F)	10/0	8/2

2.2. Human endotoxemia trial

This study was part of a larger endotoxemia trial (*clinicaltrials.gov identifier*: *NCT01349699*) [20]. Our analyses were performed on data of the ten placebo-LPS-treated subjects only. The trial was approved by the local ethics committee, and carried out according to GCP standards and the declaration of Helsinki. A detailed protocol of the human endotoxemia trial was previously described [20]. Blood was collected at various time points after the injection of purified endotoxin (LPS) prepared from *E. coli* O:113 (Clinical Center Reference Endotoxin, National Institute of Health (NIH), Bethesda, MD, USA) [20].

2.3. $sPLA_2$ IIA and cytokine measurements

$sPLA_2$ IIA concentrations were determined by ELISA (Cayman Chemical, Ann Arbor, MI, USA). Tumor necrosis factor (TNF)-α and interleukin-6 (IL-6) were measured by Luminex (Bio-Plex cytokine assay, Bio-Rad, Hercules, CA, USA).

2.4. Erythrocyte isolation from blood group O, Rhesus-negative donors

EDTA-anti-coagulated blood was collected from several blood group O, Rhesus-negative volunteers, and erythrocytes were isolated using Ficoll (GE Healthcare, Waukesha WI, USA) density centrifugation.

2.5. Plasma incubation

Blood group O, Rhesus-negative erythrocytes were incubated in the plasma of patients or healthy volunteers at 10% hematocrit in a final volume of 500 μL, for 20 h at 37 °C with gentle agitation. Erythrocytes incubated with calcium-containing (2.5 mM $CaCl_2$) Ringer with or without bee venom $sPLA_2$ type III (Cayman Chemical, Ann Arbor, MI, USA) served as positive and negative controls. Absorption at 415 nm was determined to assess the extent of hemolysis.

2.6. Flow cytometry

Erythrocytes were probed for PS exposure with Annexin V-FLUOS (Roche, Basel, Switzerland). Flow cytometry was performed as described previously [21].