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Role of the inflammatory response in the hemorrhagic syndrome induced by the hemolymph of the caterpillar *Lonomia achelous*

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ABSTRACT

Introduction: Contact with the caterpillar of *Lonomia achelous* causes a hemorrhagic syndrome in humans prompted by two processes, an initial mild DIC that is later masked by overwhelming fibrinolytic activity. Although the venom affects both the hemostatic and inflammatory systems separately, it is not clear whether the hematological and hemostatic disturbances may in part be due to an indirect effect via inflammatory mediators. Here we report results on the crosstalk between these systems, particularly the effect of the pro-inflammatory cytokine TNF-α on hemostatic parameters. *Materials and Methods*: the nitric oxide and TNF-α responses, as well as activation of the coagulation and fibrinolytic systems, were measured in macrophages and endothelial cells treated with *Lonomia achelous* hemolymph (LAH). The same responses were then determined, in a mouse model of LAH envenomation, after treatment with an anti-TNF-α antibody. *Results*: Both macrophages and endothelial cells responded strongly to LAH in terms of pro-inflammatory mediator release and fibrinolytic activities as well as pro-coagulant activity (TF activity) in endothelial cells. Treatment with antibody against TNF-α decreased both TNF-α and NO_3^-/NO_2^- serum levels in the mice, after LAH injection. Blocking TNF-α also modified significantly the serum levels of plasminogen, fibrinogen and FXIII in mice, as well as decreased TF activity in endothelial cells. *Conclusions*: LAH may induce a hemostatic effect through endothelial and macrophage activation. These activated cell release hemostatic enzymes as well as pro-inflammatory mediators, principally TNF-α, that potentiate this release in an autocrine fashion, amplifying the fibrinolytic effect, which may in turn exacerbate the hemorrhagic manifestations. As far as we are aware, this is the first report of the relationship between the hemostatic system and the inflammatory responses in a hemorrhagic syndrome induce by animal secretions.

1. Introduction

Contact with the caterpillar of the moth *Lonomia achelous* (LA) causes a hemorrhagic syndrome clinically characterized in the affected subjects by hematomas, ecchymosis, hematuria and digestive, pulmonary, peritoneal and cerebral hemorrhages that may be fatal (Arocha-Piñango et al., 1977; Arocha-Pinango et al., 1992). In some cases, recently formed wounds reopen and begin to bleed. Hematological tests show: mild anemia with leukocytosis; prolonged prothrombin (PT), partial thromboplastin (aPTT) and thrombin (TT) times; decreased fibrinogen (Fg), factor V (FV), factor XIII (FXIII), plasminogen (Pg) and α2-antiplasmin (AP) levels; increased factor VIII:c (FVIII:C), von Willebrand factor (vWF), and fibrin degradation products/D-dimers levels with normal antithrombin III and platelets. Factor VII (FVII), prothrombin and protein C levels vary (Arocha-Pinango et al., 2000).

Arocha-Piñango et al., demonstrated that the hair secretion of

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Abbreviations

LA	*Lonomia achelous*
LAH	*Lonomia achelous hemolymph*
PT	prothrombin time
aPTT	partial thromboplastin time activated
TT	thrombin time
Fg	fibrinogen
Pg	plasminogen
FV	Factor V
FVII	Factor VII
FVIII:C	Factor VIII:c
vWF	von Willebrand factor
NO_2^-	nitrites
NO_3^-	nitrates
NO	nitric oxide
iNOS	inducible nitric oxide synthase
TNF-α	tumor necrosis factor alpha
FXIII	Factor XIII
tPA	tissue plasminogen activator
PAI-1	plasminogen activator inhibitor type 1
uPA	urokinase plasminogen activator
AP	α2-antiplasmin
TF	tissue factor
IL-1, IL-2, IL-6 e IL-8	Interleukins 1, 2, 6 and 8
DIC	disseminated intravascular coagulation
LEB	*Lonomia obliqua bristle*
Lopap	*Lonomia obliqua* prothrombin activator protease
LO	*Lonomia obliqua*
Losac	*Lonomia obliqua* Stuart-factor activator
FX	Factor X

the LA caterpillar is almost identical in terms of fibrinolytic activity to the hemolymph itself (Arocha-Piñango et al., 1973). Several activities similar to, or directed against, blood clotting factors have been identified in the *L. achelous* hemolymph (LAH) including urokinase-like fibrinolytic enzymes which induce plasmin generation producing abnormal fibrinogen/fibrin degradation products (Lonomin I) and two serine proteases, achelase I and II (Lonomin II), which in turn exert direct fibrinolytic activity, leading to a very rapid degradation of the fibrinogen α-chain. Additionally, an FXIII proteolytic/urokinase-like enzyme (Lonomin I/V), two types of prothrombin activators, one direct (Lonomin III) and another FXa-like (Lonomin IV), were also demonstrated (Arocha-Pinango et al., 2000).

Furthermore, *in vivo* studies demonstrated that subcutaneous injections of LAH in rabbits induce a dose-dependent decrease in Fg, Pg and FXIII without bleeding manifestations, followed by a rapid return to normal baseline values. No significant changes in PT, aPTT or TT were observed (Marval et al., 1999). Lonomin V, in a rabbit jugular vein thrombosis model, also induced dose-dependent thrombolysis in combination with a decrease in Fg, Pg, AP and FXIII, without an increase in fibrinogen/fibrin degradation products or any bleeding manifestations (Guerrero et al., 2001).

In victims of LA envenomation, clinical deterioration is observed when whole blood or plasma (fresh or frozen) are administered, with a concomitant decrease in platelets, FII, FV, FVII, VIII:C, FXIII, Pg and AP levels and a slight increase in Fg concentration. Some patients died of cerebral hemorrhage, and those who recovered did so very slowly (Arocha-Pinango et al., 1992, 2000).

Correlating clinical and laboratory alterations observed in the patients after contact with LA caterpillars (an intense fibrinolytic activity, but little effect on coagulation and on platelets), and considering the different hemolymph activities, as well as the results observed in animal models, it was concluded that two processes coexisted in the pathogenesis of the bleeding syndrome, an initial mild DIC which is then masked by overwhelming fibrinolytic activity (Arocha-Pinango et al., 2000).

Coagulation disorders, intracerebral bleeding and acute renal failure characterize the pathophysiologic process involved in the hemorrhagic syndrome induced by *Lonomia obliqua* (LO) venom. This hemorrhagic syndrome has been associated with disseminated intravascular coagulation due to thrombin generation by procoagulant toxins (the prothrombin and factor X activators termed Lopap and Losac, respectively), in combination with a secondary activation of the fibrinolytic system linked to a hyperfibrinolytic state (Carrijo-Carvalho and Chudzinski-Tavassi, 2007; Alvarez Flores et al., 2010).

The close relationship between hemostasis and inflammation is well known and is an important relationship as these systems must act together. The inflammatory response activates hemostasis through various mechanisms including endothelial cell activation, platelet activation and aggregation, thrombin generation, and activation of the fibrinolytic system (Cambien et al., 2003; Joseph et al., 2002; Levi et al., 2002), but the hemostatic system may also reciprocally affect the immune system and promote inflammatory activity (O'Brien, 2012; Levi and Van Der Poll, 2005). Many types of venom affect both systems, with a variety of effects on hemostasis as well as the release of pro-inflammatory cytokines and chemokines. The principal inflammatory mediators involved appear to be nitric oxide (NO), tumor necrosis factor alpha (TNF-α) and interleukins 1 and 6 (IL-1/6) (Farsky et al., 2005; Petricevich, 2004).

Spider, scorpion and snake venoms have all been shown to activate endothelial cells, which are pivotal in both hemostasis and inflammation (White, 2005; De Moraes et al., 2008; Patel et al., 1994; Petricevich, 2010). In human umbilical vascular endothelial cells, Lopap increases the secretion of molecules such as NO and prostacyclin PGI_2, both potent vasodilators and inhibitors of platelet activation. Additionally, it was suggested that this enzyme also could contribute to inflammation induced by the venom, since it is able to upregulate interleukin 8, intracellular adhesion molecule-1 and E-selectin (Carrijo-Carvalho and Chudzinski-Tavassi, 2007).

In the case of LA envenomation, although it is evident that components of the venom may affect the hemostatic and inflammatory systems separately, it is not clear whether the hematological disturbances may in part be due to an indirect effect via inflammatory mediators. In a previous paper, we reported results showing that in an animal model of LA envenomation, the hematological response of the C57BL/6 mice was similar to that observed in humans and that venom injection induced an inflammatory response (TNF-α) within 1 h post-injection, followed by a more persistent increase in serum NO levels (Barrios et al., 2012). In the present study, the same mouse model and an *in vitro* model were employed to extend our understanding of the hemolymph's effect on the inflammatory response, specifically TNF-α, in the hematological and hemostatic manifestations of this syndrome; as well as the crosstalk between the inflammatory response and the hemostatic system.