The role of hemostasis in inflammation induced by Influenza A virus

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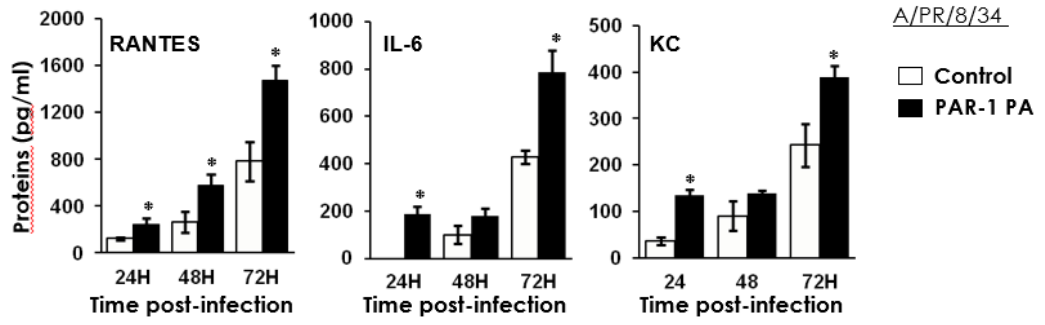
Detrimental inflammation of the lungs is a hallmark of severe influenza virus infections, although the mechanisms underlying these inflammatory processes are poorly understood. The endothelium, which lines the interior surface of the blood vessels, is thought to orchestrate cytokine amplification and accumulation during influenza virus infection (1). Upon endothelial injury, the physiological process of hemostasis (platelet activation, coagulation and fibrinolysis) is activated in order to allow the wound healing. In many inflammatory diseases, the only dysregulation of hemostasis is directly linked to inflammatory disorder.

We hypothesized that hemostasis could be a cause of deleterious inflammation during severe influenza infection. In particular, the role of two factors strongly involved in hemostasis and inflammation: the thrombin activated receptor, PAR-1 (*Protease Activated Receptor 1*) and plasminogen/plasmin (key fibrinolytic protease) in deleterious lung inflammation and in influenza virus pathogenicity.

Using pharmacological and gene deletion approaches, we investigated the role of PAR1 in influenza A virus (IAV) infection, *in vivo*. Our results showed that PAR1 contributed to the deleterious inflammatory response after influenza virus infection in mice. Activating PAR1 by administering the specific PAR1 agonist decreased survival and increased lung inflammation after influenza infection (***Figure 1)***. In addition, both administration of a PAR1 antagonist and PAR1 deficiency protected mice from infection with influenza A viruses (IAVs). PAR1 agonist did not alter survival of mice deficient in plasminogen (PLG). Thus, the deleterious role of PAR1 in the pathogenicity of IAV is, in part, PLG-dependent. Interestingly, intranasal administration of PAR1 antagonist (SCH79797) protected mice from IAV induced mortality, independently of influenza strain, this protection was also correlated with inhibition of lung inflammation (2).

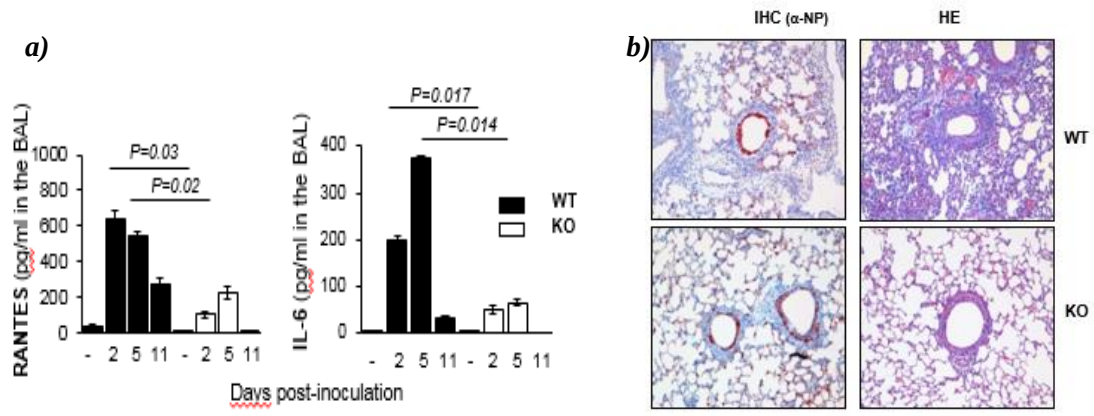
Figure 1: PAR1-AP increases inflammation during influenza virus infection in mice.
Cytokines in the BAL of infected mice treated or not with PAR1-AP were measured by ELISA.



The second objective was to study the role of PLG/plasmin, in influenza infections. It has been reported that fibrinolysis was activated in mice during influenza infections (3). However, this phenomenon releases fibrin degradation products (FDP), which are potent pro-inflammatory molecules (4). We therefore hypothesized that dysregulation of this process could contribute to deleterious inflammation of the lungs during influenza virus infection.
In vivo, we infected mice deficient in PLG or inhibited pharmacologically the activation of PLG into plasmin. We found that PLG deficient mice were protected from harmful lung inflammation induced by influenza infection (***Figure 2***), independently of the viral strain, compared with wild type mice. This effect was abolished when the mice were pretreated with Ancrod, a defibrinogenating agent. Thus, these results demonstrated that hyper-fibrinolysis mediated by activation of PLG into plasmin plays an important role in the deleterious lung inflammation and in the pathogenicity of influenza viruses. Interestingly, treatment with an anti-fibrinolytic molecule, 6-AHA (6-Aminohexanoic Acid, Amicar) protect mice from mortality induced by IAV (5).

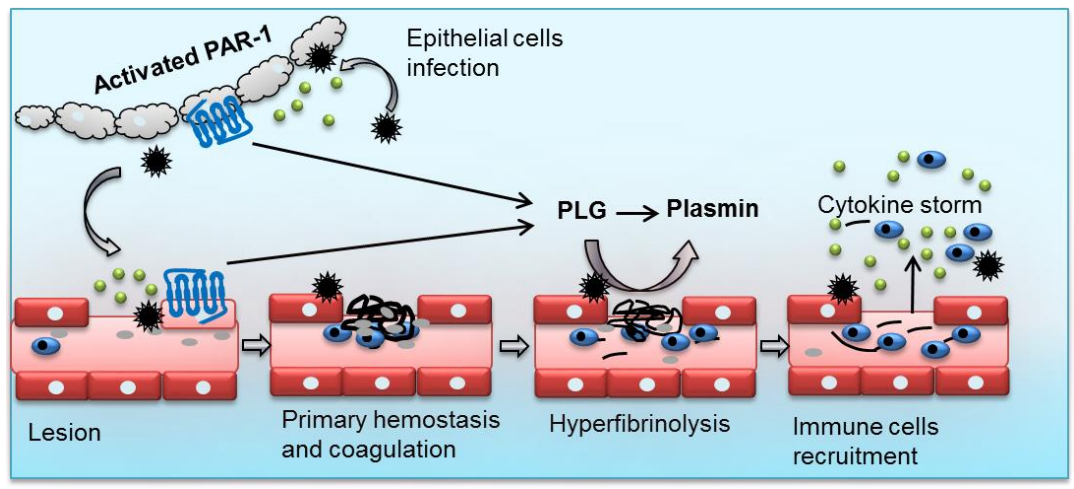
Figure 2: Plasminogen-deficiency prevents severe lung inflammation.

a) *Cytokine levels in Broncho-alveolar lavage of WT (black bars) and PLG-KO mice (white bars) infected with IAV A/PR/8/34.* ***b)*** *Histopathological analysis of lungs from infected WT and PLGKO mice inoculated with A/PR/8/34 virus.*



Proposed model of influenza induced cytokine storm.

During severe influenza infection, endothelial cells are injured or activated. Hemostasis is activated, and deregulation of fibrinolysis through hyperactivation of plasminogen/plasmin promotes excessive and deleterious inflammation. PAR1, which is also expressed at the surface of the endothelium, cooperates with plasminogen and further exacerbates inflammation and injury.



We have also showed that the influenza virus incorporates cellular proteins in the viral envelope, allowing it to evade the immune system, contributing to inflammatory disorder (6). All the results obtained allowed to better understand the mechanisms underlying immune response dysregulation during influenza infection and suggest new therapeutic targets to fight against severe influenza infection.

1)Teijaro JR., et al, 2011. Endothelial cells are central orchestrators of cytokine amplification during influenza virus infection. *Cell* 146: 980-91
2)Khoufache K, **Berri F**, et al, 2013. PAR1 contributes to influenza A virus pathogenicity in mice. *J Clin Invest.*
3)Keller TT, 2006. Effects on coagulation and fibrinolysis induced by influenza in mice with a reduced capacity to generate activated protein C and a deficiency in plasminogen activator inhibitor type 1. *Circ Res.*
4) Leavell et al, 1996. The role of fibrin degradation products in neutrophil recruitment to the lung. *American journal of respiratory cell and molecularbiology* 14: 53-60
5)**Berri F**, et al, 2013. Plasminogen Controls Inflammation and Pathogenesis of Influenza Virus Infections via Fibrinolysis. PLoS Pathog.
6)**Berri F**, et al, 2014. Annexin V incorporated into influenza virus particles inhibits gamma interferon signaling and promotes viral replication. *J Virol.*