

Platelets interact with Coxsackieviruses B and have a critical role in the pathogenesis of virus-induced myocarditis

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Introduction

Although recent studies have shown that platelets have a more active role in the pathogenesis of viral infection as they interact with several viruses ^{1,2}, either for destruction ³ or to protect them from the host immune system ⁴.

Coxsackieviruses B (CVB) are ssRNA viruses which cause the majority of enterovirus-related myocarditis cases ⁵. Considering that CVB specific receptor (CAR) is expressed on platelets ⁶, its reproductive cycle is short and the myocarditis murine model has been extensively used in pathogenesis studies ^{5,7,8}, we here explored platelet interaction with CVB to further understand the role of platelets in the pathogenesis of viral infections. Our main findings are described below and the full version of this study was published in *J Thromb Haemost* ⁹.

Materials and Methods

Platelets or Vero cells (positive control) were infected with the CVB1 or CVB3 strains at a MOI of 10. The presence of viral particles was detected by RT-PCR, immunofluorescence and immunoblotting. Infectious particles were quantified by the infectivity titration assay on Vero cells. P-selectin expression, phosphatidylserine (PS) exposure and heterotypic aggregate formation between platelets (CD61+) and granulocytes (Ly6G+) were analyzed by flow cytometry.

To establish the murine model of myocarditis, C57BL/6J mice were infected with 1x10⁴ UFP of the myocarditis variant CVB3. For platelet depletion, animals received a daily dose of a polyclonal antiserum against platelets. IgG and vWF plasma levels were quantified by ELISA. Hearts were processed to analyze the levels of infectivity virus, to score myocarditis lesions by histology ¹⁰ and to measure type I IFN levels by qPCR ¹¹.

Results and Discussion

Our in vitro investigations showed that CVB1 and 3 bind to human platelets in a CAR-independent manner and the virus does not replicate in platelets, but triggers P-selectin and, to a lesser degree, PS membrane expression. Our initial detection of infectious CVB associated with both platelet supernatants and pellets prompted us to explore whether the association was mediated by the CVB receptor CAR. While treatment with the anti-CAR blocking Ab significantly reduced (>2 logs) the titers in Vero cells, viral titers associated with platelet supernatants or pellets were not changed following pre-incubation with the anti-CAR Ab, strongly suggesting that the association between CVB and platelets was CAR-independent. Of note, the presence of CAR on platelets' surface is still controversial ^{6,12,13}. Although platelets have the molecular machinery required to enable RNA virus replication ¹⁴, no studies have been published demonstrating that platelets support viral replication. Although we found viral particles in the supernatants and pellets of CVB-infected platelets, the observation that their levels did not increase over time strongly suggests that CVB does not replicate in platelets. Nevertheless, we cannot exclude the possibility that the in vitro strategies used here were not

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sensitive enough or that this event can take place in vivo.

Our results showed that CVB binding to platelets induces activation since P-selectin and PS levels in the surface membrane were higher in platelets incubated with virus compared to the non-infected cells. This effect seems to be restricted to some viruses since similar results have been reported with the human adenovirus group C serotype 5 and Dengue virus^{13,15,16}, but not with the Japanese encephalitis virus¹⁶.

To further understand these in vitro data, we analyzed the role of platelets in the pathogenesis of CVB3-infected mice. Our in vivo studies show that CVB3 infection triggered platelet PS but not P-selectin expression or vWF release. These effects were accompanied by an increase in the formation of platelet-leukocyte aggregation and thrombocytopenia. Thrombocytopenia is a common feature of viral infections. Among the different mechanisms proposed, the formation of mixed platelet-leukocyte aggregates in adenovirus infection¹³ as well as platelet apoptosis and macrophage phagocytosis due to PS expression in DENV infections have been shown¹⁷. Our data confirmed that both events occur in CVB3-infected mice, making them both plausible causes of the reduced platelet count. The formation of platelet-mixed aggregates independent of platelet P-selectin expression or vWF was somewhat unexpected, however, since both molecules were reported to mediate this process¹³, although at least in mice, it has been proposed that not only P-selectin but also other cell adhesion molecules support platelet-leukocyte interactions¹⁸.

Our studies in CVB3-infected and platelet-depleted mice showed higher mortality rates, viremia levels, heart viral titers and myocarditis severity, similar type I IFN mRNA levels but decreased IgG serum levels than CVB3-infected mice. Other viruses such as EMCV and LCMV, platelet depletion has been associated with higher mortality^{18,19}. Our study confirmed the critical role of platelets during viral infection for early host survival and extended this to a human virus. The higher viremia with similar levels of type I IFN in the heart of CVB3-infected and depleted mice at 3 dpi, together with the selective enhanced expression of platelet PS, suggests that platelets may have a role in early viral dissemination independently of type I IFN by binding circulating viral particles and directing them towards macrophage phagocytosis and subsequent destruction. Moreover, the enhanced formation of platelet-neutrophil aggregates might accelerate this process. In the absence of such mechanism, more viruses reach target organs, as noted here in the heart homogenates of infected mice, producing more severe myocarditis. Moreover, our observation of decreased levels of IgG in platelet-depleted and infected animals reveals a critical role of platelets in the later adaptive immune response.

In conclusion, our results showed that CVB interact with platelets, decreasing their number, which promotes viral dissemination and impacts on the severity of associated disease and subsequent immune response. Future studies are necessary to confirm these pathogenic mechanisms in other viral diseases.

References

1. Zucker-Franklin D, et al. *Blood*. 1990;75:1920-1923.
2. Onlamoon N, et al. *Blood*. 2010;115:1823-1834.
3. Youssefian T, et al. *Blood*. 2002;99:4021-4029.
4. Zahn A, et al. *J Gen Virol*. 2006;87:2243-2251.
5. Kim KS, et al. *Rev Med Virol*. 2001;11:355-368.
6. Gupalo E, et al. *Virol J*. 2011;8:456.
7. Whitton JL. *Springer Semin Immunopathol*. 2002;24:201-213.
8. Fairweather D, et al. *Curr Opin Rheumatol*. 2012;24:401-407.



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This research was supported with a grant from the International Society for Infectious Diseases (ISID).

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9. Negrotto S, et al. *J Thromb Haemost.* 2014.
10. Cifuentes JO, et al. *J Med Virol.* 2011;83:1571-1581.e
11. Solana ME, et al. *Muscle Nerve.* 2012;46:582-587.
12. Shimony N, et al. *Viol J.* 2009;6:25.
13. Othman M, et al. *Blood.* 2007;109:2832-2839.
14. Rowley JW, et al. *Curr Opin Hematol.* 2012;19:385-391.
15. Gupalo E, et al. *Platelets.* 2013;24:383-391.
16. Ghosh K, et al. *J Electron Microsc (Tokyo).* 2008;57:113-118.
17. Hottz ED, et al. *J Thromb Haemost.* 2013;11:951-962.
18. Koupenova M, et al. *Blood.* 2014.
19. Iannaccone M, et al. *Proc Natl Acad Sci U S A.* 2008;105:629-634.