IN VIVO INTERACTIONS OF PLATELETS AND LEUKOCYTES WITH THE ENDOTHELIUM IN ANTIGEN-INDUCED ARTHRITIS OF MICE
ROLE OF P-SELECTIN

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ABSTRACT INTRODUCTION: Growing evidence indicates an important role of platelets in the development and maintenance of rheumatoid arthritis. Clinical symptoms of rheumatoid arthritis were reduced by inhibition of platelet-activating factor (1) and an increase of platelet-leukocyte-complex could be shown in rheumatoid arthritis (2). Activation and adherence of platelets in synovial microcirculation may therefore be responsible for endothelial damage and activation of leukocytes. Although the mechanism responsible for a potential platelet mediated damage remain not fully understood.

We visualized and quantified for the first time in vivo platelet-endothelial cell interactions in Antigen-induced Arthritis (AiA) in mice. In Balb/c mice without AiA compared to animals with AiA, we observed a significant increase of platelet-endothelial cell interactions. The fraction of rolling platelets in animals with AiA was 6-fold elevated and adherent platelets were 12-fold increased. Whereas in mice with AiA leukocyte rolling was 3-fold and adherence 6-fold increased (3). At activation of platelets, P-selectin stored in a-granules and Weibel-Palade bodies is extruded to the cell surface within 5-10 min. This glycoprotein activation of platelets, P-selectin stored in α-granules and adherent platelets were 12-fold increased. Whereas in mice with AiA the fraction of rolling platelets in animals with AiA reached the level of healthy C57/Bl6 (rolling: 0.041 ±0.01; adherence: 174.2±36.3 mm²) and P-selectin deficient controls without AiA (rolling: 0.035±0.01; adherence: 119.0±22.2 mm²).

Leukocyte-endothelial interaction showed the same significant decline of rolling fraction 0.116±0.02 (0.205±0.03 C57/Bl6 with AiA) and adherent cells 387.2±36.7 mm² (1491.8±283.5 mm² C57/Bl6 with AiA) in P-selectin deficient animals. These results are again on the level of C57/Bl6 animals (rolling: 0.074±0.02; adherence: 551.3±148 mm²) and P-selectin deficient mice without AiA (rolling: 0.090±0.01; adherence: 355.4±47.6 mm²).

The histological score describes the morphologic changes in AiA in five grades from 0 to 4, whereas 0 equals a normal knee and 4 cartilage and bone destruction. Both control groups without AiA showed mean changes comparable with score 0, the C57/Bl6 wild-type group showed a mean score of 3 and the P-selectin deficient mice with AiA showed a mean score of 1. Immunostaining in random tissue samplings of B6.129S7-Selptm1Bay/J mice showed no P-selectin expression.

The aim of the present study was to investigate in vivo the influence of P-selectin on the platelet-endothelial cell interaction, leukocyte-endothelial cell interaction and on the histological achieved morphologic changes in AiA. Furthermore we provided a basis to differ between endothelial or platelet depending effects of P-selectin in mice with AiA.

METHODS: C57/Bl6 and B6.129S7-Selptm1Bay/J (P-selectin deficient) mice with AiA and control groups without AiA consisting of the same strains (C57 wild-type or P-selectin deficient animals) we investigate a decline in the interaction of the platelets with the endothelium in animals with AiA lacking endothelial P-selectin, but donated platelets with P-selectin. In these animals rolling fraction 0.122±0.02 and adherent cells 497±58.4 mm² were significant reduced compared to C57/Bl6 arthritic mice with platelet and endothelial P-selectin (rolling: 0.254±0.04; adherence: 1705.6±242.3 mm²). In C57/Bl6 mice with AiA and donated platelets lacking P-selectin rolling fraction 0.173±0.02 and adherence 807.4±198 mm² was significant debased versus C57/Bl6 mice owning platelet and endothelial P-selectin with AiA (rolling: 0.254±0.04; adherence: 1705.6±242.3 mm²).

Second we visualized the differences between effects of platelet or adherent P-selectin in deficient animals a reduction of histological symptoms of the Arthritis. We showed for the first time in vivo a significant decrease of the leukocyte-interaction with the endothelium in animals with AiA. Also leukocyte-endothelial cell interaction in animals with AiA lacking platelet or endothelial P-selectin was declined. Animals without endothelial P-selectin showed a significant reduction in rolling fraction 0.124±0.02 and in adherent cells 612.3±72 mm² vs. C57/Bl6 with AiA (rolling: 0.229±0.02; adherence: 1667.6±222.7 mm²). If donated platelets are absent of P-selectin, leukocytes display a rolling fraction of 0.190±0.02 vs. 0.229±0.02 and in adherent cells a significant decrease 1105.1±107 mm² vs.1667.6±222.7 mm².

DISCUSSION: We showed for the first time in vivo a significant decrease of the interaction of platelets and leukocytes with the endothelium in P-selectin deficient mice with AiA. We additionally observed in P-selectin deficient animals a reduction of histological symptoms of the Arthritis. Second we visualized the differences between effects of platelet or endothelial P-selectin. Most important, we showed the influence of P-selectin on the leukocyte interaction with the endothelium. These findings suggest that the platelets hold a key role in the development and maintenance of arthritis and they are at least partly responsible for the leukocyte tissue damage in Antigen-induced Arthritis. We further provide first in vivo evidence that the inhibition of platelets could be a new option for treatment of arthritis.

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