P18 Anaphylatoxinreceptor C3aR on platelets contributes to arterial thrombosis - intersection between innate immunity and thrombosis

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Platelets play distinct roles in the vascular system: they are the major mediator of thrombosis, critical for restoration of tissue integrity and players in vascular inflammatory conditions. In close spatiotemporal proximity, the complement system acts as the first line of defense against invading microorganisms and is also a key mediator of inflammation. Whereas the fluid phase crosstalk between the complement and coagulation systems is well appreciated, the understanding of the pathophysiological implications of such interactions is still scant.

We analyzed co-expression of the anaphylatoxin receptor C3aR with activated GPIIb/IIIa on platelets of 501 coronary artery disease patients using flow cytometry, detected C3aR expression in human or murine specimen by RT-PCR, immunofluorescence, western blotting or flow cytometry and examined the importance of platelet C3aR by various in vitro platelet function tests, by in vivo bleeding time and intravital microscopy. To shed light on the pathophysiological relevance of C3aR, disease models of myocardial infarction and stroke were applied. To approach underlying molecular mechanisms, we identified the platelet small GTPase Rap1b using immunoprecipitation followed by nanoscale liquid chromatography coupled to tandem mass spectrometry.

Interestingly, we found a strong positive correlation of platelet complement C3aR expression with activated GPIIb/IIIa in coronary artery disease patients and co-expression of C3aR with GPIIb/IIIa in thrombi obtained from patients with myocardial infarction. Our results demonstrate that the C3a/C3aR axis on platelets regulates distinct steps of thrombus formation such as platelet adhesion, spreading and Ca2+ influx. Using C3aR-/- mice or C3-/- mice with re-injection of C3a, we uncovered that the complement activation fragment C3a regulates bleeding time after tail injury and thrombosis. Notably, C3aR-/- mice were less prone to experimental stroke and myocardial infarction. Further, reconstitution of C3aR-/- mice with C3aR+/+ platelets and platelet depletion experiments demonstrated that the observed effects on thrombosis, myocardial infarction and stroke were specifically caused by platelet C3aR. Mechanistically, C3aR-mediated signaling regulates the activation of Rap1b and thereby bleeding arrest after injury and in vivo thrombus formation.

CONCLUSIONS:
Overall, our findings uncover a novel function of the anaphylatoxin C3a for platelet function and thrombus formation, highlighting a detrimental role of imbalanced complement activation in cardiovascular diseases.