Identification of a vasculo-protective circuit centred on Lipoxin A_4 and operative in mouse mesenteric vascular ischaemia

V. Brancaleone[1,2], T. Gobbetti[2], N. Cenac[3], P. Le Faoudier[3], B. Colom[2], R. J. Flower[2], N. Vergnolle[3], S. Nourshargh[2] and M. Perretti[2]
[1]Dept. of Science, University of Basilicata, Potenza, Italy
[2]William Harvey Research Institute, Queen Mary University of London, United Kingdom
[3]Université Paul Sabatier, Centre de Physiopathologie de Toulouse, INSERM U1043, France

Endogenous protective pathways mitigate the overshooting of inflammation following sterile or infectious injury. Among those pathways the active resolution of inflammation is a relatively novel concept that represents the response of innate immunity to inflammatory stimuli. This response involves various mediators, including Annexin A1 (AnxA1) and lipoxin A_4 (LXA_4), that exert their actions by activating a specific G-protein coupled receptor of the formyl-peptide receptor family (FPR1, FPR2 and FPR3), with particular efficacy on FPR2 (Perretti et al., 2002; Takano et al., 1997). In order to study the role of FPR2 in inflammatory process, our lab generated mice lacking formyl peptide receptor 2/3 (Fpr2/3-/-), mouse counterpart for FPR2 (Dufton et al., 2010). We used these animals to investigate their inflammatory phenotype, performing intravital microscopy (IVM) and confocal imaging of mesenteric microcirculation undergoing ischemia-reperfusion (I/R) protocol. Mice were anesthetised and the superior mesenteric artery was occluded and re-opened (I=30min; R=90min); blood aliquots were collected by cardiac puncture at specific times (pre- and post-I and post-I/R) for further analysis.
Pharmacological treatments with LXA_4 (1-100ng/mouse i.v.) and the pan-Fpr antagonist Boc-2 (10mg/mouse i.p.) were also performed immediately before occlusion or before re-opening. In some cases, platelet/neutrophil aggregates were determined by flow cytometry, labelling cells with anti-CD41 (MWReg30) for platelet and Ly6G (1A8) for neutrophils. Experiments have been performed by using 6-8 mice per group.
Fpr2/3-/- mice display a major phenotype with exacerbated vascular inflammation observed post-ischemia reperfusion (IR) injury, characterised by marked neutrophil adhesion and extravasation, as visualised by intravital microscopy and confirmed by confocal microscopy. Analysis of endogenous agonists for Fpr2/3 revealed that LXA_4 levels were lower after 30 min ischemia in Fpr2/3-/- and this event was associated with augmented vascular inflammation in the reperfusion phase (45-180 min). Since, LXA_4 is normally generated by platelet/neutrophil aggregates formation (Papayianni et al., 1995), we tested whether such event was affected by ischaemia. Our results showed that this cellular response was attenuated in Fpr2/3-/- mice, where platelet/neutrophil aggregates were reduced compared to the ones observed in Fpr2/3+/+. Exogenous delivery of LXA_4 attenuated IR-mediated inflammation in Fpr2/3+/+ but not Fpr2/3-/- mice; conversely, administration of Fpr2/3 antagonist skewed the Fpr2/3+/+ mice vascular phenotype to that of Fpr2/3-/- animals.
Collectively, our data show mice lacking functional Fpr2/3-/- have a more inflamed phenotype and this is associated with a reduced production of LXA_4. We therefore propose that, during ischaemia, neutrophil Fpr2/3 controls formation of platelet/neutrophil aggregates that trigger the rapid generation of circulating LXA_4, which in turn modulates downstream vascular inflammation evident during the reperfusion phase.
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