Evidence for an anti-inflammatory loop centred on PMN FPR2/ALX and operative in the inflamed microvasculature

Vincenzo Brancaleone\textsuperscript{1,2}, Stefania Bena\textsuperscript{1}, Jesmond Dalli\textsuperscript{1}, Roderick J Flower\textsuperscript{1}, Giuseppe Cirino\textsuperscript{2}, Mauro Perretti\textsuperscript{1}

\textsuperscript{1}Centre for Biochemical Pharmacology, WHRI, Bart’s and The London School of Medicine and Dentistry, Queen Mary University of London, EC1M 6BQ, United Kingdom, \textsuperscript{2}Dip. di Farmacologia Sperimentale, Universita’ degli Studi di Napoli FEDERICO II, 80131, Italy

The importance of proresolving mediators in the overall context of resolution of acute inflammation is accepted and prominent, though little is known on whether these anti-inflammatory and pro-resolving molecules act in concert. Here we focused on lipoxin A\textsubscript{4} (LXA\textsubscript{4}) and annexin A1 (AnxA1) since these two very different mediators converge on a single receptor, formyl peptide receptor type 2 (acronym FPR2/ALX). Addition of LXA\textsubscript{4} (10-100nM, 10-60minutes) to human PMN provoked a concentration-and time-dependent mobilization of AnxA1 onto the plasma membrane, as determined by western blotting and flow cytometry analyses. This property was shared by another FPR2/ALX agonist, antiflammin-2 (AF2), but not by fMLP or peptide Ac2-26 (an AnxA1 derivative which can activate all three members of the human FPR family). Selective FPR2/ALX antagonist WRW\textsubscript{4} (10\textmu M, 10minutes) blocked AnxA1 mobilization activated by LXA\textsubscript{4} (30nM) and AF2 (10\textmu M). Analysis of PMN degranulation patterns has been performed to uncover whether or not neutrophils granules, whose specific markers are CD35, CD66b and CD63, were involved in mobilization of AnxA1. Results clearly showed that AnxA1 released was not delivered from granules store, so we address our attention on the phospho-AnxA1 (phosphoSer\textsuperscript{27}) status induced by treatment with FPR2/ALX agonists (5-10minutes). Relevant outcomes from this experiments suggested a model where the two FPR2/ALX agonists mobilize the cytosolic (and not the granular) pool of AnxA1 through an intermediate phosphorylation step.

In order to give to these findings a functional role, we set intravital microscopy investigations of the inflamed mesenteric microvasculature of wild type and AnxA1\textsuperscript{-/-} mice. This study revealed that LXA\textsubscript{4} (1\textmu g, i.v.) provoked leukocyte detachment from the post-capillary venule endothelium in the former (>50% within 10 min; P<0.05), but not in the latter genotype (~15%; NS). Furthermore, recruitment of Gr1\textsuperscript{+/+} cell into dorsal air pouches inflamed with IL-1 was significantly attenuated by LXA\textsubscript{4} in wild type (~50% reduction in cell infiltrate), but not in AnxA1\textsuperscript{-/-}, mice.

Collectively, these data prompt us to propose the existence of an endogenous network in anti-inflammation centred on PMN and AnxA1 and activated by selective FPR2/ALX agonists that could also represent a useful target to develop a new category of anti-inflammatory drugs.