Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol10Issue4abst155P.pdf

Activation of purinergic P2Y receptors modulates C-C chemokine signalling in human monocytes

Hinnah Campwala, Samuel Fountain. University of East Anglia, Norwich, Norfolk, UK

Monocytes are key effector cells in orchestrating immunological and inflammatory responses. Driven by chemical mediators in the external milieu, monocyte recruitment remains an essential component for host defence however, left uncontrolled, can also play a dominant role in the pathogenesis of chronic inflammatory diseases such as atherosclerosis. C-C (C-C motif) chemokines are chemotactic cytokines that modulate monocyte function through mobilisation of intracellular Ca^{2+} by activating G-protein coupled receptors (GPCRs). Although chemokines play a critical role in the recruitment of monocytes and other leukocytes, extracellular nucleotides such as adenosine-5'-triphosphate (ATP) are also known to drive immune cell recruitment following purinergic receptor (ion channel and GPCR) activation. Understanding how such signalling pathways co-exist may facilitate identification of new pharmacological strategies for immunomodulation and resolution of inflammation. Changes in intracellular Ca^{2+} were measured in THP-1 cells and PBMCs loaded with the Ca^{2+} reporter dye Fluo-4 AM. Agonist induced changes in Fluo-4 fluorescence are expressed as a percentage of maximal responses following digitonin (40µM) permeabilisation. Our initial studies examined the effect of ATP/ADP scavenging with the enzyme apyrase (2U/mL) on CCL2 (50ng/mL) evoked Ca^{2+} responses in both THP-1 cells and PBMCs. Further studies in THP-1 cells were conducted examining the effect of the ectonucleotidase inhibitor ARL 67156 (100µM), adenosine deaminase (ADA) (2U/mL) and a range of P1 and P2 receptor antagonists (1-100µM) on CCL2 (10-50ng/mL) Ca^{2+} responses. To support Ca^{2+} mobilisation studies, the effect of apyrase and purinergic receptor antagonists on THP-1 migration towards CCL2 (50ng/mL) were studied using transwell assays. ATP secretion in THP-1 cells in response to CCL2 (50ng/mL) was tested using the luciferase-luciferin assay. Additionally, CCL2-mediated lysosomal exocytosis in THP-1 cells was also evaluated using beta-hexosaminidase release assays. Scavenging ATP/ADP inhibited CCL2 Ca^{2+} responses in THP-1 cells (n=6) and PBMCs (n=3) by 59% and 47%, respectively. Apyrase caused a 4.3-fold shift in the CCL2 EC_{50} response (15±3ng/mL to 65±8ng/mL; n=3, $p<0.05$). CCL2 (50ng/mL, 2 hours) induced a 5.9-fold change in THP-1 migration over that of control cells (n=5, $p<0.05$). Apyrase inhibited CCL2 evoked migration by 71±11% (n=5, $p<0.05$). A role for purinergic signalling in modulating chemokine responses was supported in reciprocal experiments with ARL-67156 which potentiated CCL2 responses by 19±3% (n=9, $p<0.05$). Ca^{2+} mobilisation studies with ADA and the P1 antagonist CGS-15943 did not support a role for adenosine or P1 receptors. The broad-spectrum P2 receptor antagonist suramin (100µM) demonstrated a 94±1% inhibition of CCL2 Ca^{2+} responses (n=3, $p<0.05$). Selective antagonism of $P2Y_6$ receptor with MRS-2578 produced a significant inhibition of CCL2 Ca^{2+} responses (73±7%, n=3, $p<0.05$) as well as an impairment in THP-1 chemotaxis (82±2%, n=4, $p<0.05$). Additionally, CCL2 was shown to evoke a 2.7-fold secretion of ATP over basal levels (n=21, $p<0.05$). Lysosomal studies with beta-hexosaminidase however, did not support a lysosomal mechanism of ATP release by CCL2 (n=3, $p>0.05$). This study identifies agonist stimulated ATP release and purinergic co-signalling as a novel mechanism controlling C-C chemokine induced Ca^{2+} signalling and migration in human leukocytes.