Prostaglandin E_2 activates the mitogen-activated protein kinase ERK1/2 via the Colony-Stimulating Factor-1 (CSF-1) receptor and synergizes with CSF-1 in the induction of cell migration in macrophages.

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Prostaglandins (PG) play an important role in the immune response by modulating the complex interactions between leukocytes and tissue cells under inflammatory conditions. PGE_2 exerts its autocrine/paracrine effects on target cells by binding to the subtypes 1-4 of the EP G-protein-coupled receptors. The complexity of PGE_2-induced signaling is increased by the existence of cross-talk between EP and receptor tyrosine kinases (RTK) such as the Fibroblast Growth Factor Receptor 1 (FGFR1) or Epidermal Growth Factor Receptor (EGFR). Monocytes/macrophages (Mf) are an essential component of innate immunity and play a central role in inflammation and host defense. Mf express EP2 and EP4 and, PGE_2 increases via these receptors the Mf response to chemotactic proteins such as SDF-1alpha. Colony-Stimulating Factor-1 (CSF-1), by binding to its receptor CSF-1R, sustains Mf survival, proliferation and differentiation, as well as spreading, extension of lamellipodia, membrane ruffling, cell polarization and motility. CSF-1 triggers the autophosphorylation of several intracellular tyrosine residues, and thereby activation, of CSF-1R that leads to the activation of several downstream signaling pathways, including those of ERK1/2, PI3K and ERK5.
The aim of the present study was to examine the crosstalk between PGE_2 and CSF-1R in Mphi. To this purpose, we used two murine macrophage cell lines, BAC-1.2F5, which are CSF-1-dependent for survival and proliferation, and RAW264.7. We assessed first whether PGE_2 is able to induce CSF-1R phosphorylation/activation. To this end, BAC-1.2F5 cells were incubated for 18 h in the absence of CSF-1, and RAW264.7 cells for 24 h in the absence of FBS, before being stimulated or not with 1 μM PGE_2 for 5, 10, 15, 20, 30 or 60 min. Cells were then lysed and protein lysates subjected to SDS-PAGE and immunoblotting. PGE_2-induced CSF-1R phosphorylation was rapid, being detectable as early as 5 min after PGE_2 administration, and reached peak levels after 10-15 min. PGE_2 also induced rapid ERK1/2 phosphorylation (after 5 min), that was sustained up to 15 min.
Previous reports indicated that PGE_2 induces ERK1/2 activation via either EP-coupled G proteins or RTK. To determine whether CSF-1R is necessary for PGE_2-induced ERK1/2 activation, we inhibited CSF-1R genetically (by specific siRNA) or pharmacologically (by a CSF-1R specific drug, GW2580). Either treatment partially prevented PGE_2-induced ERK1/2 phosphorylation, indicating that CSF-1R plays a role in PGE_2-inuced ERK1/2 activation. We then addressed the possibility of a synergism between CSF-1 and PGE_2. CSF-1-starved BAC-1.2F5 cells or serum-starved RAW264.7 cells were stimulated or not with low doses of CSF-1 (12,5 ng/ml) or PGE_2 (100 nM) alone or with their combination for 5, 10, or 15 min. As determined by SDS-PAGE and immunoblotting, CSF-1 or PGE_2 alone induced weak ERK1/2 phosphorylation, but the combined treatment induced marked phosphorylation, indicating a synergism between CSF-1 and PGE_2. In light of the above results and on the fact that ERK1/2 is known to be relevant for cell migration, we tested the effect of combined treatment on cell migration in Boyden chambers. Low doses of PGE_2 (1 and 10 nM) or CSF-1 (5 and 12,5 ng/ml) alone did not induce macrophage migration, but the combined treatment markedly induced cell migration.
In conclusion, our results indicated that CSF-1 and PGE_2, besides playing important roles in inflammation independently of each other, can act synergistically in inflammation sites where they are simultaneously present, as it certainly happens frequently.

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