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B45

Phosphatidylserine suppresses T cells through GPR174, and co-inhibition of adenosine receptors and GPR174 synergistically enhances T cell responses

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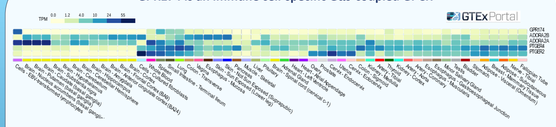
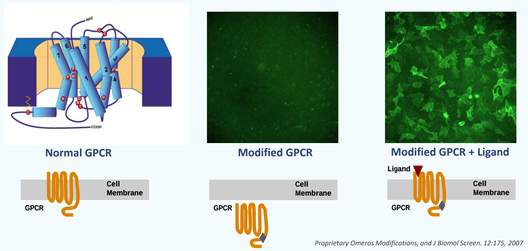
Abstract

Extracellular phosphatidylserine (PS) is a potent modulator of immune responses. Various phospholipid scramblases respond to different cellular processes to expose PS either during apoptosis or during activation of multiple cell types, including platelets, lymphocytes, endothelial cells, and tumor cells. While it is well established that PS exposed during apoptosis suppresses inflammatory responses in phagocytic cells during efferocytosis, whether either form of exposed PS acts directly on T lymphocytes has not been extensively studied. Here we show that PS suppresses T cells through GPR174, a Gαs-coupled GPCR previously described as a receptor for lysophosphatidylserine (lysoPS), a soluble catabolite of PS. PS liposomes were found to be 5x more potent than lysoPS in promoting GPR174-dependent cAMP generation, and PS exposed on apoptotic cells, platelets, and activated T cells all induced GPR174 signaling in a reporter cell line. Consistent with the well described immunosuppressive nature of cAMP signaling, PS liposomes suppressed human T cell IL-2 production and mouse IL-2 production from WT but not GPR174-KO T cells. Leveraging a novel GPCR-modulating chemical library screen, we have identified several GPR174 inhibitors covering multiple chemical classes, and a GPR174 inhibitor reversed PS liposome-mediated suppression of human and WT mouse T cells while having no effect on GPR174-KO mouse T cells. In a syngeneic mouse tumor model, GPR174-deficiency significantly increased control of tumor growth in the presence of sub-optimal anti-GITR co-therapy. In many respects, GPR174 is similar to the A2A/A2B adenosine receptors in that both suppress T cells through cAMP signaling in response to products of cell stress and death abundant in the tumor microenvironment, and we have found that both pathways work synergistically to restrain T cell responses. In mouse splenocyte cultures containing endogenous levels of adenosine and PS, an A2A/A2B inhibitor enhanced T cell responses more effectively in GPR174-knockout cells than WT cells. In similar cultures of human PBMC, GPR174 and A2A/A2B inhibitors, or GPR174 inhibitors and adenosine deaminase, synergistically enhanced IL-2 production. Our findings suggest that inhibition of both GPR174 and the adenosine pathway will be important for effectively overcoming cAMP-mediated immunosuppression in the tumor microenvironment.

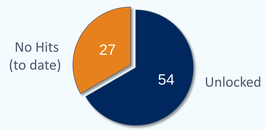
GPR174 is an immune cell-specific Gαs-coupled GPCR



GPCR ligand discovery with the Cellular Redistribution Assay (CRA)



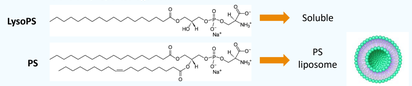
Proprietary Omeros Modifications, and J Biomol Screen. 12:175, 2007

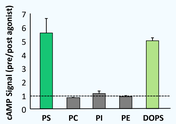


Numerous GPR174-specific hits identified, covering several chemical classes

PS-exposing membranes stimulate GPR174

Lysophosphatidylserine (LysoPS), a catabolite of phosphatidylserine (PS), is the only reported agonist



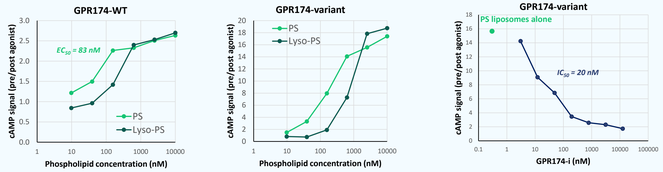


Liposomes made with:
- Phosphatidylserine (PS)
- Phosphatidylcholine (PC)
- Phosphatidylinositol (PI)
- Phosphatidylethanolamine (PE)
- 18:1 PS (DOPS)

Methods
HEK293 cells were transfected with plasmids expressing cAMP biosensor pGlo22F (Promega) and wild-type GPR174 or a GPR174 mutant with lowered basal activity (GPR174-variant). 5-6 hours after transfection, cells were trypsinized and plated in 96-well plates, and the following day, culture medium was replaced with 100 µL/well X-vivo15 (Lonza), 2% GloSensor Assay reagent (Promega). Plates were incubated for 1 hour at room temperature and measured in a luminometer to obtain the pre-stimulation values. The indicated reagents or cells were added to wells, and plates were incubated for 15 minutes at room temperature, afterwhich plates were measured in a luminometer to obtain the post-stimulation measurement. For GPR174-i activity, the inhibitor was added to cells before PS liposome addition. The ratio of post-compound addition to pre-compound addition measurements (i.e. the extent of Glosensor signal induction by an agonist) was determined.

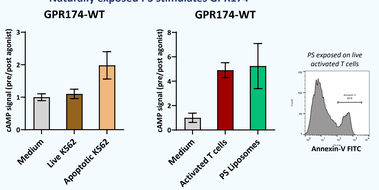
WT or GPR174-deficient mouse T cells were stimulated in anti-hamster IgG coated plates in the presence of 10 ng/mL anti-CD3 (2C11) and 100 ng/mL anti-CD28, in the presence of PS liposomes (1 µM), GPR174-i (1 µM), or vehicle control.
Human T cells were stimulated in the presence of K562 cells (FcR-expressing artificial antigen presenting cells), 0.5 µg/mL anti-CD3 (UCHT1), 2 µg/mL CD80.Fc (R&D Systems), and PS liposomes (1 µM), GPR174-i (3 µM), or vehicle control.
IL-2 was measured in supernatants after 24 hrs (MesoScale Discovery).

PS is a more potent GPR174 agonist than LysoPS, and PS → GPR174 agonism is inhibited by GPR174-i



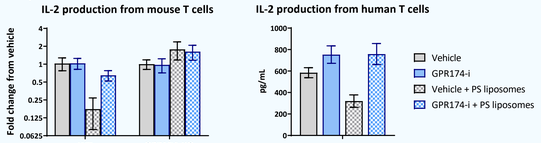
PS liposomes contained ~1% LysoPS by mass spec analysis.

Naturally exposed PS stimulates GPR174



Signaling also observed with apoptotic neutrophils and activated platelets

PS liposome activity on purified T cells is GPR174-dependent and is inhibited by GPR174-i

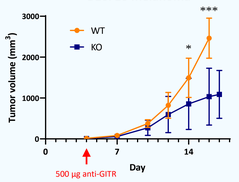


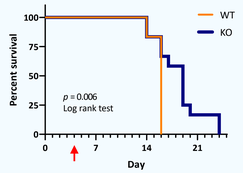
GPR174 regulation of IL-2 is consistent with previous reports:
Barnes MJ, Cyster JG. Immunol Cell Biol. 2018. 96(4):439-445.
Shinjo Y, et al. Biochem Biophys Res Commun. 2017. 494(1-2):332-338.

Error bars: standard deviation

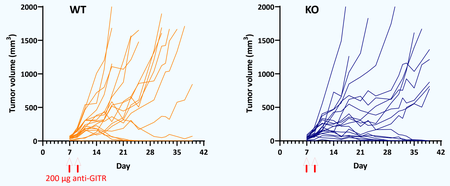
GPR174 deficiency synergizes with anti-GITR-mediated Treg attenuation to activate anti-tumor immunity

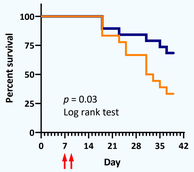
B16F10 melanoma





CT26.cl25 colon carcinoma

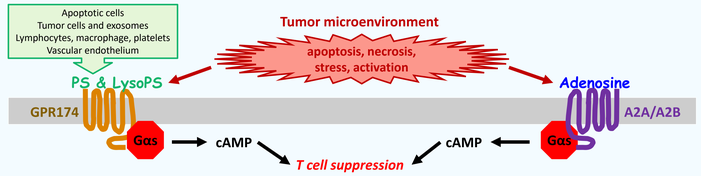




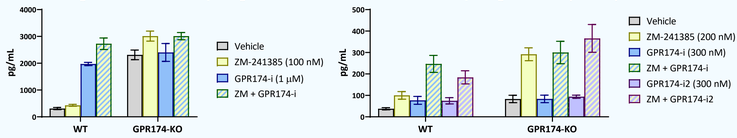
B16F10: 75K cells ID into C57BL/6 x 129 male GPR174-WT or KO F1 mice; 500 µg anti-GITR IP on day 4.

CT26.cl25: 500K cells SC into BALB/c x 129 male GPR174-WT or KO F1 mice; 200 µg anti-GITR IP on d7, d9.

Release from GPR174 and A2A/A2B adenosine receptor signaling facilitates high Th1 cytokine production

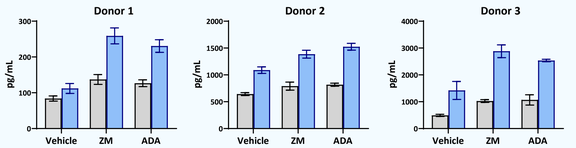


Synergistic IL-2 production from anti-CD3/CD28-stimulated mouse splenocytes with combined GPR174 and A2A/A2B inhibition



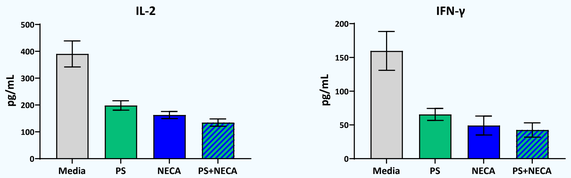
Methods
In 96-well flat-bottom plates, 10^5 mouse splenocytes were cultured in quadruplicate with anti-CD3, anti-CD28 (each 100 ng/mL), the indicated concentrations of GPCR inhibitors, and 100 nM NECA (right). 48 hr supernatants were analyzed for IL-2 (MesoScale Discovery).

Synergistic IL-2 production from anti-CD3/CD28-stimulated human PBMC with combined GPR174 and A2A/A2B inhibition, in the absence of exogenous NECA



Methods
In 96-well flat-bottom plates, 10^5 human PBMC were cultured in quadruplicate with anti-CD3 (UCHT1; 1 µg/mL) and anti-CD28 (100 ng/mL), and GPR174-i (1 µM), ZM-241385 (100 nM), or adenosine deaminase (ADA; 750 ng/mL). 24 hr supernatants were analyzed for IL-2 (MesoScale Discovery).

Parallel suppression of Th1 cytokines from purified human T cells by PS liposomes and NECA



Methods
In 96-well flat-bottom plates, 10^5 purified human T cells were cultured in quadruplicate with ImmunoCult™ Human CD3/CD28 T Cell Activator (StemCell) (1:50 dilution), with PS liposomes (1 µM), NECA (100 nM), or media control. 24 hr supernatants were analyzed for IL-2 and IFN-γ (MesoScale Discovery).

Error bars: standard deviation

Conclusions

- **GPR174 is an immune system-restricted Gαs-coupled GPCR.**
- **PS exposed on liposomes and cellular membranes stimulates GPR174, supporting a model of active GPR174-mediated immune suppression in the tumor microenvironment.***
- **GPR174-deficiency enhances anti-tumor immune responses in mice.**
- **If both PS/LysoPS and adenosine are present, inhibition of both axes is necessary for effective potentiation of T cell function.**

*Park M, Kang KW. Arch Pharm Res. 2019. 42(7):617-628.