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Phosphatidylserine suppresses T cells through GPR174, and co-inhibition of adenosine receptors and GPR174 synergistically enhances Th1 cytokine production

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Abstract

Background

Extracellular phosphatidylserine (PS) is a potent modulator of immune responses. In addition to exposure during apoptosis, PS is observed on activated platelets, leukocytes, endothelial cells, tumor cells, and exosomes. While PS exposed during apoptosis is known to suppresses inflammatory responses in phagocytic cells, whether either form of exposed PS acts directly on T lymphocytes has not been extensively studied.

Methods

HEK293 cells expressing GPR174 and GloSensor were used to detect GPR174 agonism. Immune cells were stimulated with anti-CD3/CD28 with GPCR inhibitors, NECA, or PS liposomes; and cytokines in media were measured. WT or GPR174-KO mice inoculated with syngeneic tumor cells and treated with anti-GITR (DTA-1) were evaluated for tumor growth.

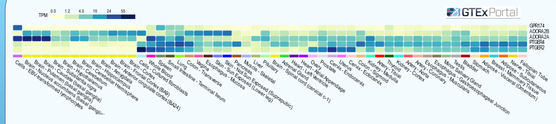
Results

Here we show that PS suppresses T cells through GPR174, a Gαs-coupled GPCR. PS liposomes were more potent than lyso-PS in stimulating GPR174, and PS exposed on various cell types agonized GPR174. Several GPR174 inhibitors of different chemical classes were identified. PS liposomes attenuated Th1 cytokine production from human T cells and WT but not GPR174-KO mouse T cells, and GPR174 inhibitors reversed this suppression. Th1 cytokines were increased by GPR174 inhibition in the presence of tumor exosomes. GPR174 inhibition or genetic deletion also reduced CTLA-4 expression, an immune checkpoint known to be induced by cAMP. Compared to WT mice, GPR174-KO mice significantly controlled tumor growth when Treg were transiently depleted with anti-GITR. GPR174 is similar to A2A/B adenosine receptors in that both suppress Th1 immunity through cAMP in response to products of cell stress and death abundant in the tumor microenvironment. Inhibition of GPR174 and A2A/B synergistically increased cytokine production, GPR174 and A2A/B agonists suppressed T cells to the same extent as both combined, and A2A/B inhibition was more effective on GPR174-KO T cells vs. WT T cells.

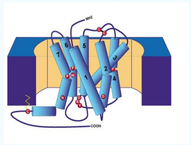
Conclusion

Our findings suggest that for T cells to effectively overcome cAMP-mediated immunosuppression in the tumor microenvironment, both GPR174 and the adenosine pathway must be inhibited.

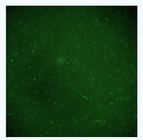
GPR174 is an immune cell-specific Gαs-coupled GPCR



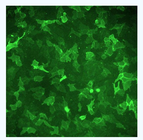
GPCR ligand discovery with the Cellular Redistribution Assay (CRA)



Normal GPCR

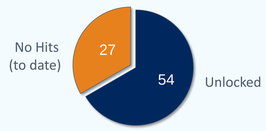


Modified GPCR



Modified GPCR + Ligand

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

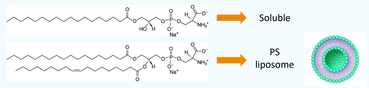


Numerous GPR174-specific hits identified, covering several chemical classes

Class A orphan GPCRs (81)

PS-exposing membranes stimulate GPR174

Lysophosphatidylserine (LysoPS), a catabolite of phosphatidylserine (PS), is the only reported GPR174 agonist.



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.

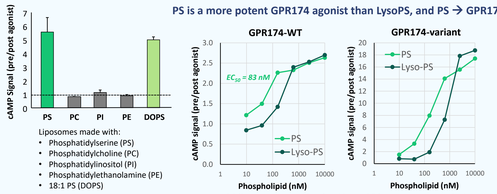
WT or GPR174-deficient mouse T cells were stimulated in anti-hamster IgG coated plates in the presence of 10 ng/mL anti-CD3 (2C11) and 100 ng/mL anti-CD28, in the presence of PS liposomes (1 µM), GPR174-i (1 µM), or vehicle control.

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).

Human PBMC were stimulated in the presence of the indicated concentrations of GPR174-I and TAPI-1 (to inhibit AREG shedding), and CTLA-4 or AREG levels were measured by flow cytometry following 24 hrs culture.

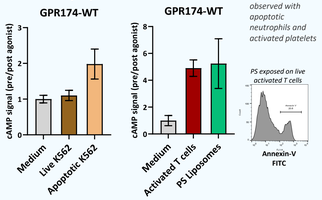
PS is a more potent GPR174 agonist than LysoPS, and PS → GPR174 agonism is inhibited by GPR174-i



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

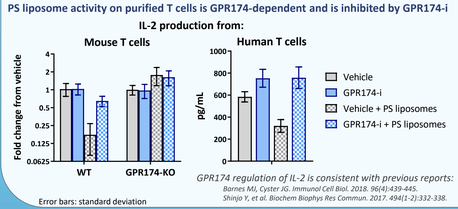
PS liposomes contained ~1% LysoPS by mass spec analysis.

Naturally exposed PS stimulates GPR174



Signaling also observed with apoptotic neutrophils and activated platelets

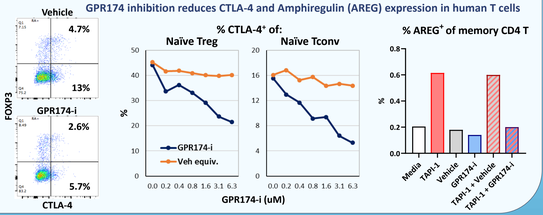
PS liposome activity on purified T cells is GPR174-dependent and is inhibited by GPR174-i



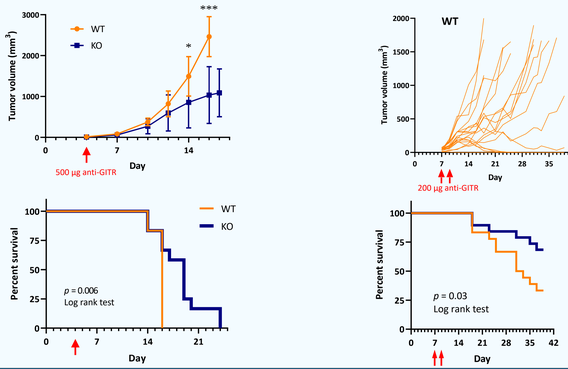
GPR174 regulation of IL-2 is consistent with previous reports:
Barnes MJ, Cyster JG. Immunol Cell Biol. 2018. 96(4):439-445.
Shinjo Y, et al. Biochem Biophys Res Commun. 2017. 494(1-2):332-338.

Error bars: standard deviation

GPR174 inhibition reduces CTLA-4 and Amphiregulin (AREG) expression in human T cells



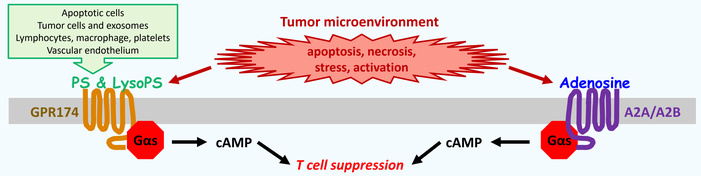
GPR174 deficiency synergizes with anti-GITR-mediated Treg attenuation to activate anti-tumor immunity



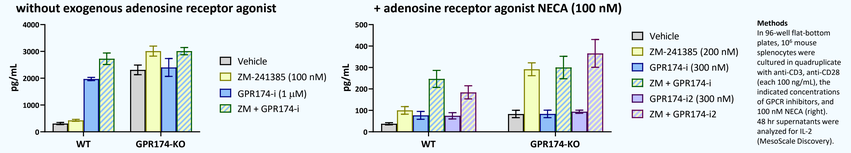
B16F10: 75K cells ID into C57BL/6 x 129 male GPR174-WT or KO F1 mice; 500 µg anti-GITR IP on day 4.

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

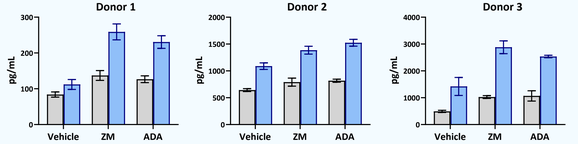


Synergistic IL-2 production from anti-CD3/CD28-stimulated mouse splenocytes with combined GPR174 and A2A/A2B inhibition



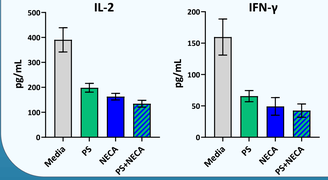
Methods
In 96-well flat-bottom plates, 10^5 mouse splenocytes were cultured in quadruplicate with anti-CD3, anti-CD28 (each 100 ng/mL), the indicated concentrations of GPCR inhibitors, and 100 nM NECA (right). 48 hr supernatants were analyzed for IL-2 (MesoScale Discovery).

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

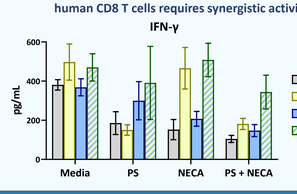


Methods
In 96-well flat-bottom plates, 10^5 human PBMC were cultured in quadruplicate with anti-CD3 (UCHT1; 1 µg/mL) and anti-CD28 (100 ng/mL), and GPR174-i (1 µM), ZM-241385 (100 nM), or adenosine deaminase (ADA; 750 ng/mL). 24 hr supernatants were analyzed for IL-2 (MesoScale Discovery).

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



Reversal of PS+NECA-mediated suppression of IFN-γ production from human CD8 T cells requires synergistic activity of ZM and GPR174-i



Methods
In 96-well flat-bottom plates, 10^5 purified human $CD3^+$ T cells (left) or human $CD8^+$ T cells (right) were cultured in quadruplicate with ImmunoCult™ Human CD3/CD28 T Cell Activator (StemCell) (1:50 dilution), with PS liposomes (1 µM), NECA (100 nM, left; 50 nM, right), or media control. For CD8 T cell cultures, the indicated concentrations of ZM-241385 and GPR174-i were included. 24 hr (left) or 44 hr (right) supernatants were analyzed for cytokines (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.***
- **Reversal of GPR174-mediated T cell suppression increases Th1 cytokine production and reduces CTLA-4 and AREG expression.**
- **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 Th1 cell function.**

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

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