There are about 1000 different GPCRs, many of them labeled GPR123 and so forth, so perhaps, one wonders, why was time on two obscure ones—GPR65 and GPR174? Well, these two receptors share a fascinating feature—they are widely expressed by immune cells and appear to be important regulators of immunity, i.e. anti-inflammatory. Indeed, the brakes applied to immune responses by these two receptors put them up there with Treg cells, and metabolite-sensing receptors such as GPR43, as regulators of immunity. In support of this, genetic polymorphisms in GPR65 associate with many inflammatory diseases, sometimes more so than the MHC (particularly inflammatory bowel diseases).

GPR65 is a proton sensor, so it senses pH, hence conditions such as acidosis or high acid exposure (for instance in the gastrointestinal tract) control its activity, and signaling contributes to gut and immune homeostasis. Mutation in another proton-sensing Gpr65 family member, Gpr132, leads to the development of a lupus-like disease further emphasizing the importance of proton sensing for immune regulation. GPR174 on the other hand is a receptor for a phosphatidylserine metabolite, lysophosphatidylserine (LysoPS). Not much is known about this metabolite and why its presence may be immuno-regulating.

What connects GPR65 and GPR174 is that they appear to be two of the most widely expressed GPCRs that signal through the G-protein, $G_a$ (see Figure 1). Signaling through $G_a$ leads to cAMP production, which is (usually) anti-inflammatory. Few GPCRs expressed on immune cells signal through $G_a$—most like the chemokine receptors signal through $G_i$.

$G_a$-mediated activation of adenylate cyclase leads to accumulation of cAMP, particularly in inflammatory immune cells like neutrophils and eosinophils. cAMP can do various things, one of which is lead to phosphorylation of the transcription factor CREB (cAMP response element-binding protein), which controls many things, including numerous inflammatory cytokines. A convenient way to remember this important dichotomy in GPCR signaling, and immune suppression versus incitement, is as follows:

\[
G_a = \text{Adenylate Cyclase stimulation} \\
= \text{cAMP supply} \\
= \text{immune cell suppression}
\]

Wirasingha et al.\textsuperscript{4}, in a paper recently published in *Immunology & Cell Biology*, found that GPR65 plays an important role in demyelinating autoimmune disease. They used the typical murine model of MS, experimental autoimmune encephalomyelitis (with all its admitted limitations), and found that Gpr65-deficient mice developed exacerbated disease. They used a green fluorescent protein reporter mouse (Gpr65gfp/gfp) to show that GPR65 is likely expressed at very high levels by invariant natural killer T (iNKT) cells. They showed that experimental autoimmune encephalomyelitis severity in Gpr65-deficient mice was normalized in the absence of iNKT cells (using CD1d-deficient mice), suggesting that GPR65 signals in iNKT cells were particularly important for suppressing this autoimmune condition. These results in experimental autoimmune encephalomyelitis follow several other studies that have shown that absence of GPR65 exacerbates inflammation, particularly in colitis models, which may relate to pH sensing in the lower gastrointestinal tract.
A fuzzy picture was emerging, at this point, that maybe GPR65 was the main Gαs-coupled GPCR expressed on immune cells, and that this receptor alone was the immunosuppressive, cAMP supplying GPCR for immune cells. And that acidosis was a major mechanism for suppression of immune cell functions. However, in a paper published in this issue of Immunology & Cell Biology, Barnes and Cyster report on the impressive immune-suppressive activity of LysoPS and its receptor GPR174. They used in vivo models of T-cell proliferation, which were induced by sublethal irradiation, or depletion of Tregs. When GPR174 was expressed on T cells, T-cell proliferation was constrained, whereas GPR174 deletion resulted in much greater T-cell proliferation. When GPR174 was expressed on T cells, T-cell proliferation was constrained, whereas GPR174 deletion resulted in much greater T-cell proliferation. They next showed that GPR174 couples with Gαs to mediate suppression of T-cell proliferation. They showed that LysoPS and GPR174/Gαs suppressed IL-2 production by activated T cells, and limited upregulation of CD25 and CD69. Interestingly, the IL-2 promoter contains a CREB binding site, which presumably is the mechanism whereby IL-2 production is suppressed. Indeed, LysoPS has been known for some years to be involved in inflammation resolution. LysoPS does various things in addition to its effects on T cells. It enhances the clearance of apoptotic neutrophils from inflammatory sites—all consistent with inflammation suppression or resolution.

What lies ahead for GPR65 and GPR174? Probing the associations of the minor allele of Gpr65, (the IBD-associated missense variant rs3742704, which encodes an isoleucine-to-leucine substitution at amino acid 231) which leads to less signaling and cAMP production, with other inflammatory diseases is an obvious next step. Does rs3742704 associate with other major human inflammatory diseases? Does GPR65 play a role in anti-tumor responses? Presumably something is driving the maintenance of the minor allele in humans, because it is present at a reasonably high frequency across all ethnicities. Poor GPR65 signaling and less cAMP production, which associate with the minor allele, may aid anti-tumor or anti-pathogen immune responses. The flip-side is over-zealous inflammatory disease. It appears that GPR65 may be one of those pivotal molecules for immune responses (think immune response gene), which determines the quality of an immune response and the balance between anti-pathogen versus autoimmune responses. GPCRs are good drug targets, but should one agonize or antagonize GPR65? It probably depends on the indication, and as Wirasinha et al. show, GPR65 agonism may aid in treatment of experimental autoimmune encephalomyelitis/MS. Agonism of GPR65 would presumably also be of benefit for inflammatory bowel diseases. Design of small molecule agonists for topical delivery to the GI tract may be a good strategy, and polymorphisms in GPR65 (which limit Gαs signaling) associate with both Crohn’s disease and ulcerative colitis. Agonist small molecule drugs for GPR65 have been produced but are yet to advance to human clinical trials (as far as we know). For GPR174, knowledge on the biology of LysoPS is lacking, and why this metabolite is a natural suppressor of T-cell responses is unknown.

---

**Figure 1.** Gαs-coupled GPCRs and the basics of downstream events. Some of the main Gαs-coupled GPCRs on immune cells are GPR65, GPR174 and A2aR. These selectively signal through Gαs which activates adenylate cyclase and results in cAMP increase. cAMP via protein kinase A (PKA) leads to phosphorylation of CREB (cAMP response element-binding protein) a transcription factor that binds to DNA sequences in promoter or enhancer regions, thereby increasing or decreasing the transcription of downstream genes. Notably, immune genes affected by CREB include IL-2, and certain inflammatory cytokines.
Finally, we would like to make some speculative predictions. One is that immune modifying drugs will emerge that target GPR65 or GPR174, and so modify immune responses for inflammatory diseases, or cancer. These receptors appear to be great targets. Agonists would find use for suppressing inflammatory diseases, whereas antagonists might be of use in boosting immune responses, for immuno-oncology. Another Gαs-coupled GPCR, Adenosine A2a receptor, is a target for new immuno-oncology therapeutics. A second is that GPR65 polymorphisms (genotype) will become a major factor for prediction of inflammatory disease. A third is that pH will enter our consciousness as a major determinant for immune responses. Very little has been published on GPR65 and GPR174 and both of these obscure receptors appear to be fertile ground for future revelations on the workings of the immune system, and human disease.

REFERENCES