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Review Article

G protein signaling modulator-3: a leukocyte regulator of inflammation in health and disease

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Abstract: G protein signaling modulator-3 (GPSM3), also known as G18 or AGS4, is a member of a family of proteins containing one or more copies of a small regulatory motif known as the GoLoco (or GPR) motif. GPSM3 interacts directly with Gα and Gβ subunits of heterotrimeric G proteins to regulate downstream intracellular signals initiated by G protein coupled receptors (GPCRs) that are activated via binding to their cognate ligands. GPSM3 has a selective tissue distribution and is highly expressed in immune system cells; genome-wide association studies (GWAS) have recently revealed that single nucleotide polymorphisms (SNPs) in GPSM3 are associated with chronic inflammatory diseases. This review highlights the current knowledge of GPSM3 function in normal and pathologic immune-mediated conditions.

Keywords: GoLoco, G protein coupled receptor, GPSM3, chemokine, migration, rheumatoid arthritis

Introduction

Elucidating the function and regulation of G protein coupled receptors (GPCRs) was recently recognized with a 2012 Nobel Prize, and these proteins continue to be the largest class of cell-surface receptors successfully targeted for the treatment of human disease [1-3]. GPCRs are functionally associated with heterotrimeric G proteins composed of Gα, Gβ, and Gγ subunits. When the receptor is inactive, Gα is bound to guanosine diphosphate (GDP) in its own inactive state; GDP binding to Gα promotes its association with the Gβγ dimer, which inhibits its spontaneous GDP release by Gα and also assists in receptor coupling. After ligand binding, the GPCR acts as a guanine nucleotide exchange factor (GEF) promoting the replacement of GDP with guanosine triphosphate (GTP) and leading to conformational changes that result in dissociation of Gβγ from the heterotrimeric complex. Both GTP-bound Gα and free Gβγ initiate signal cascades to downstream effectors. Hydrolysis of GTP to GDP returns Gα to its inactive state, allowing re-association with Gβγ and signal termination [4, 5].

Chemokine receptors are GPCR family members that signal through $G\alpha_i$-containing heterotrimers to regulate cellular migration, survival, and angiogenesis in inflammatory conditions [6, 7]. However, as drug therapy targets in autoimmunity, chemokine receptors have had mixed results in various neutralization strategies [8] and, in particular, in rheumatoid arthritis (RA) therapeutics development [9, 10]. This failure has been attributed, in part, to redundancy of chemokine receptor/ligand interactions in inflammation [6], and so it has been proposed that a more efficacious strategy should target a shared pathway regulator of chemokine receptor signaling rather than neutralization of a specific chemokine or its receptor(s) *per se* [11].

GoLoco motif-containing proteins are regulators of GPCR signaling that share a signature 19-amino-acid sequence [12, 13] as well as a hallmark biochemical activity of inhibiting $G\alpha_i$

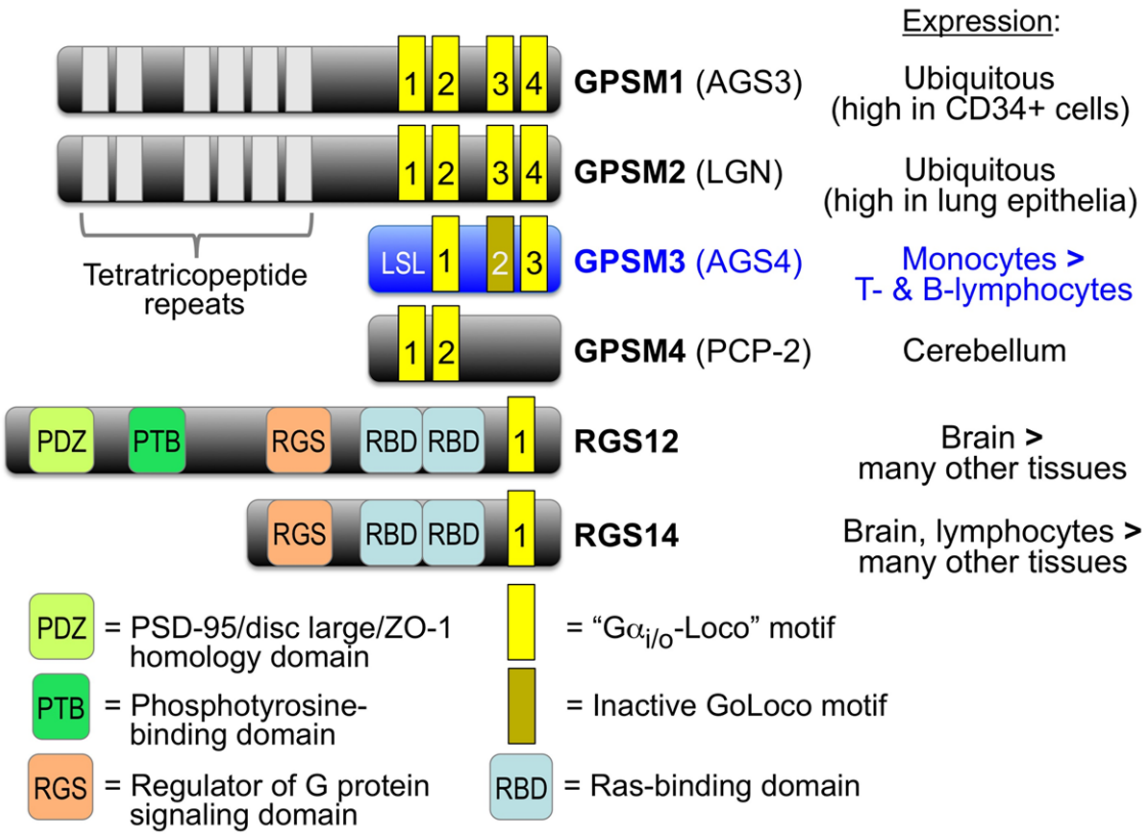

Figure 1. GoLoco motif-containing proteins. Expression pattern as reported by the BioGPS gene expression atlas (http://biogps.org; [59]).

release of GDP [5, 14-16]. One such member of this protein family with a highly-restricted expression pattern (**Figure 1**), GPSM3 (*a.k.a.* AGS4 [17] or G18 [18]) possesses three GoLoco motifs (but only two are functional; [18]) and an additional LSL motif required for Gβ subunit binding [19]. The functional GoLoco motifs of GPSM3 allow the protein to bind $G\alpha_i$·GDP and act as a GDP dissociation inhibitor (GDI) [17, 18] independent of $G\alpha_i$·GDP interaction with the Gβγ dimer [16, 20], while the LSL motif interaction with monomeric Gβ subunits is suspected to regulate GPCR/G-protein heterotrimer association at the level of Gβγ dimer assembly [19] (**Figure 2**). The cellular and biologic effects of GPSM3 continue to be explored as recent research points to its important regulatory functions in chemokine receptor signaling, monocyte phenotypes, and autoimmune disease development.

GPSM3 regulation of GPCR signaling

Recent studies by Giguere *et al.* have illustrated that GPSM3 deficiency affects the chemotactic response of myeloid cells activated through the chemokine receptors CCR2, CX3CR1, and CMKLR1 [21]. Ongoing biochemical and cell biological research is helping to define the specific role that GPSM3 plays in the intracellular sequelae of chemokine receptor signaling and how this role ultimately affects cellular chemotaxis and viability.

Not only does GPSM3 interact with $G\alpha_i$·GDP and monomeric Gβ subunits, but studies of local energy transfer between fusion proteins expressed in cells suggest that GPSM3 may also help position Gα subunits at the cell membrane, proximal to GPCRs, and thereby help provide a signaling substrate to ligand-activated receptors [22]; however, these latter results from ectopic overexpression of recombinant fusion proteins have yet to be confirmed in an endogenous expression context. More controversial is the suggestion that GPSM3 may regulate GPCR signaling pathways via GoLoco motif interactions that directly promote the dissociation of Gα and Gβγ independent of GPCR/ligand-stimulated GEF activity [14]; evidence

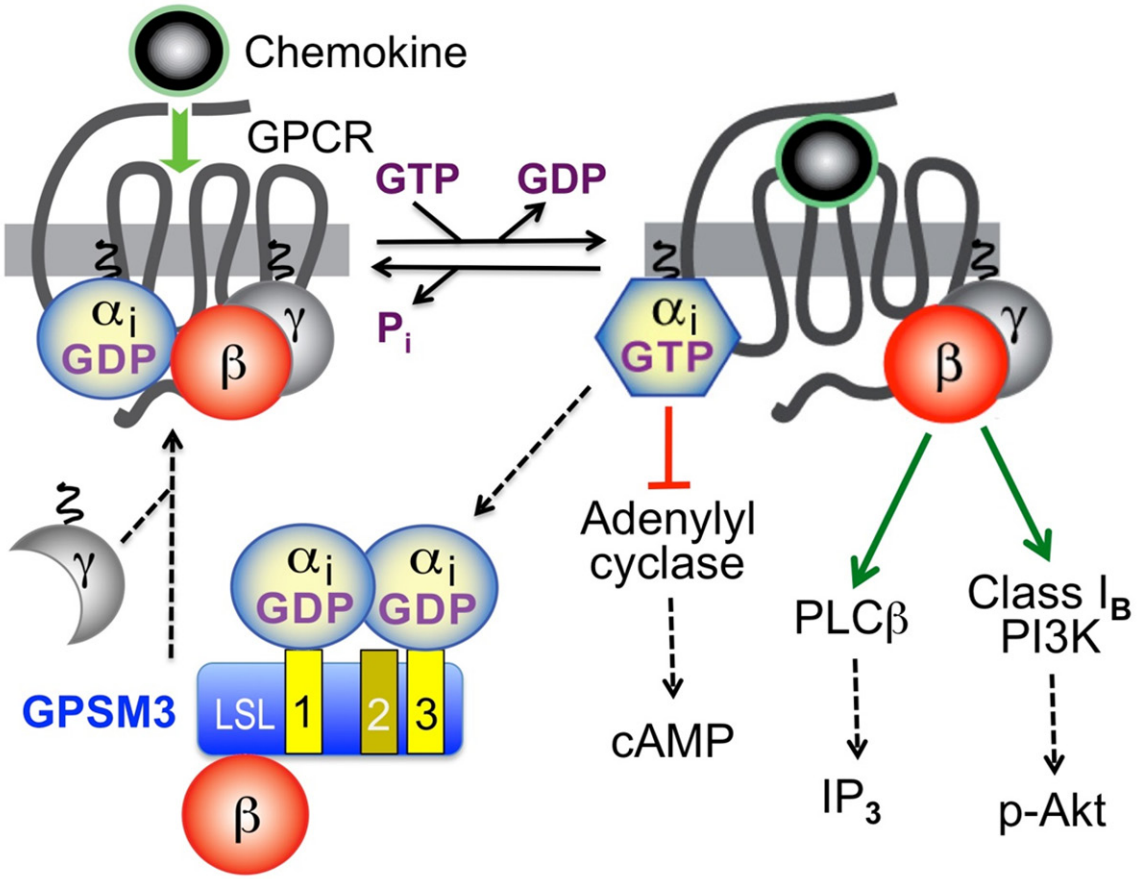

Figure 2. Model for GPSM3 function in regulating Gi-coupled chemokine receptors. Two of the three GoLoco motifs within GPSM3 (yellow) bind to $G\alpha_i$·GDP subunits (Cao et al., 2004; Kimple et al., 2004). Additionally, the LSL motif within GPSM3 interacts with Gβ subunits toward their biosynthetic pathway in forming Gβγ dimers (Giguere et al., 2012). These interactions likely regulate ligand-activated chemokine receptor signaling pathways, including $G\alpha_i$-mediated inhibition of cAMP production by adenylyl cyclase, inositol phosphate production from Gβγ-mediated activation of phospholipase-Cβ, and/or cell survival signaling by Gβγ-mediated activation of class IB phosphatidylinositol-3'-kinase (PI3K) leading to PKB/Akt phosphorylation.

from both structural biology studies [16] and electrophysiological recordings of Gβγ-gated ion channels [20] suggest that the GoLoco motif/$G\alpha_i$·GDP interaction is mutually exclusive to the assembly of a traditional $G\alpha_i$·GDP/Gβγ heterotrimer, but that the GoLoco motif cannot itself displace a preformed $G\alpha_i$·GDP/Gβγ heterotrimer.

A common outcome of chemokine receptor signaling is the mobilization of internal calcium stores via Gβγ-mediated activation of phospholipase-Cβ (PLCβ) (**Figure 2**). Ectopic GPSM3 expression is seen to negatively affect GPCR signaling by decreasing PLCβ-mediated generation of the second messenger inositol trisphosphate (IP_3) [21]. This inhibitory effect requires GPSM3 interaction with Gβ subunits, given that a specific loss-of-function mutation to the Gβ-interaction site within GPSM3 (*i.e.*, mutation of the "LSL" motif) abrogates inhibition of IP_3 accumulation after agonist stimulation; it is important to note that mutation to the LSL mutation does not affect the GPSM3/$G\alpha_i$·GDP interaction [21]. Indeed, endogenous Gβ subunits are seen to co-immunoprecipitate with the endogenous GPSM3 expressed in the human monocytic THP-1 cell line; moreover, endogenous Gβ subunits co-localize with endogenous GPSM3 at the plasma membrane in THP-1 cells [21]. This subcellular colocalization is consistent with the proteomic detection of GPSM3 within cholesterol-rich, detergent-resistant, plasma membrane fragments from bovine leukocytes [23], as well as an independent report of ectopically-expressed GPSM3

Figure 3. The Ensembl database predicts two distinct wild-type human GPSM3 proteins that have different N-terminal polypeptide sequences and that correlate with different mRNA transcripts.

being able to form a G protein-dependent complex in proximity to membrane-delimited GPCRs [22]. Additional evidence that GPSM3 interacts with Gβ has been obtained by examining the direct activation of PLCβ2 via co-expression of free Gβ1γ2 subunits; overexpression of wild-type GPSM3 (but not the loss-of-function LSL mutant) is seen to inhibit PLCβ2 activation by Gβ1γ2 [21].

Another common Gβγ-mediated signaling target is activation of the pro-survival kinase PKB/Akt pathway (**Figure 2**) - an event that is often measured by its serine-473 phosphorylation status [24]. When GPSM3 levels are decreased by RNA-interference in the monocytic THP-1 cell line, activation of PKB/Akt by serum addition is diminished [21]. These data mirror the decreased survival observed in GPSM3-deficient THP-1 cells [21] and are consistent with the known role of GPCR/Gβγ-mediated activation of class I_B PI3K and resultant PIP_3-dependent activation of PKB/Akt and downstream survival signaling in leukocytes ([25, 26]; see **Figure 2**).

GPSM3 in immune cell function and cancer

Myeloid-derived inflammation mediators and suppressors

GPSM3 expression is highly regulated during monocyte/macrophage differentiation *ex vivo,* and GPSM3 deficiency affects monocyte survival as well as migration to specific chemokine ligands [21]. $Ly6C^{high}CD11b^{+}$ monocytes are known to mobilize rapidly in response to infection and inflammation [27]; flow cytometry of splenocytes from GPSM3-deficient mice indicates that this population of $Ly6C^{high}CD11b^{+}$ monocytes is significantly reduced in comparison to wild-type mouse controls [21]. These observations suggest a role for GPSM3 in survival and/or differentiation of particular myeloid-lineage cells, but the molecular mechanisms underlying this role remain to be fully elucidated.

Myeloid cells, particularly macrophages and neutrophils, are known mediators of inflammation; conversely, a heterogenous, immature myeloid cell population, termed "myeloid-derived suppressor cells" (MDSCs) are able to *suppress* T cell and macrophage responses in inflammatory disease [28, 29]. Work by Yan *et al.* has shown that in lysosomal acid lipase (*lal*) knockout mice, which exhibit expanded MDSC development, *GPSM3* expression in these cells is upregulated two-fold (along with other G protein-related genes) as part of a broader perturbation of myeloid development and homeostasis [30]. MDSCs have been shown to expand as a functional cell population in multiple pathological conditions including infection, autoimmunity, inflammation, and tumor burden [29]. In the collagen-induced arthritis (CIA) mouse model of rheumatoid arthritis (RA), MDSCs are seen to accumulate in the spleen during peak arthritis, inhibit production of the inflammatory cytokines IFN-γ, IL-2, TNF-α, and IL-6, and suppress T helper 17 (Th17) pathogenic T cells [31].

MDSCs also affect tumor angiogenesis and metastasis, as well as promote the development of FOXP3+ regulatory T-lymphocytes (Tregs) [32]. MDSCs expand in numbers in many tumor models and are believed to be recruited from the bone marrow by tumor-derived factors [33]; these increased levels of MDSCs can interfere with innate and adaptive anti-tumor responses by depressing immune responses to tumor burden, leading to the advancement of malignancy [34]. Data from mouse tumor models suggest that two populations of $CD11b^{+}$ MDSCs predominate: "granulocytic" $Ly6G^{+}/Ly6C^{lo}$ MDSCs and "monocytic" $Ly6G^{-}/Ly6C^{+}$ MDSC [29]. Both of these MDSC subsets are decreased in the spleens of GPSM3-deficient mice [21]. This genetic ablation of *GPSM3* was shown to protect mice from a monocyte-driven model of acute inflammatory arthritis [21]; however, it remains unexplored whether these GPSM3-deficient mice possess a differential response to tumor burden.

Although *GPSM3* appears to be most highly expressed in developing monocytes and is also expressed in MDSCs, Lapan *et al.* have shown that prostatic cancer cells also increase *GPSM3* expression greater than two-fold when co-cultured *ex vivo* with endothelial cells, in a model mimicking angiogenesis within the tumor microenvironment [35]. As a regulator of GPCR and G protein function, GPSM3 could therefore also influence invasive or migratory phenotypes in different cancers, or their responses to survival or angiogenic factors that might be secreted as tumors grow. Therefore, it needs to be examined whether tumor progression in GPSM3-deficient mice may be influenced by the decreased prevalence of MDSCs or by changes that occur in adaptive immune cells or the tumor cells themselves.

Lymphocytes

Although GPSM3 has its highest expression in monocytes, B- and T-lymphocytes also have detectable GPSM3 expression [17, 21], suggesting that GPSM3 could also regulate GPCR/G protein signaling outcomes such as survival and chemotactic trafficking within these key lymphocyte classes required for full immune function. For example, in a study by Reif and Cyster [36], activated B cells were shown to dynamically regulate the expression of multiple different regulators of G protein signaling that alter responses to local chemokine gradients.

Lymphocyte involvement in inflammatory diseases also involves the development of ectopic (tertiary) lymphoid structures. The architecture of these germinal center-like structures requires chemokine-driven organization and intercellular interactions [37] likely to be impacted by GPSM3 function on GPCR signaling *in vivo*. While it is currently unknown exactly how GPSM3 functions to regulate GPCR signaling in lymphocytes, it is possible that the activity of this GPCR signaling modulator could influence inflammatory function, adaptive immune responses, and/or be involved in the protection against or exacerbation of lymphocyte-mediated inflammatory disease.

GPSM3 in genetic association studies of immune-mediated diseases

Compelling genome-wide association studies (GWAS) have identified protective alleles located within the *GPSM3* gene locus that are significantly less prevalent in patients with several autoimmune diseases [38-40]. In the most notable examples to-date, protective alleles of two *GPSM3* single-nucleotide polymorphisms (SNPs rs204989, rs204991) have been associated with a decreased incidence of RA [39, 40]. Importantly, these same SNPs associate significantly with decreased incidence of developing other autoimmune diseases that share similar immunologic mechanisms with RA: namely, ankylosing spondylitis (AS), systemic lupus erythematosus (SLE), and multiple sclerosis (MS) [39]. Conversely, *GPSM3* SNPs show risk association with type I diabetes and autoimmune thyroid disease [39, 40], and *GPSM3* genetic polymorphisms have also been linked with atopic dermatitis and childhood obesity, two diseases also characterized by chronic inflammation [41, 42]. The *GPSM3* locus on chromosome 6 is located very near to the *Notch4* gene locus and is found within the densely-packed and disease-relevant human leukocyte antigen (HLA) region [39, 42], raising the possibility that *GPSM3* SNP variants mark functional polymorphisms in other closely associated genes. However, increasing research into GPSM3 expression and function is revealing a consistent pattern of potential physiological involvement in immune system cell types relevant to inflammatory diseases. Therefore, it is conceivable that sequence variation within *GPSM3* could functionally alter transcript expression or splicing, and/or GPSM3 protein sequence differences could contribute directly to immune disease pathogenesis.

Two different protein isoforms of GPSM3, with different N-terminal polypeptide sequences (**Figure 3**), have been predicted to date in the human genomic sequence database curated by Ensembl (http://ensembl.org) [43], as encoded by three different *GPSM3* transcripts: *GPSM3-001, -002*, and *GPSM3-004*. It is presently unclear whether these different isoforms exert differential effects on GPCR signaling, cellular function, or disease. However, it is intriguing to note that Zhao *et al. [44]* suggest that GPSM3 differentially affects the nucleotide state of $G\alpha_{i}$ *vs* $G\alpha_{o}$ subunits via its N-terminal proline-rich region - a region significantly different in the open-reading frames encoded by transcripts *GPSM3-001* and *-002* versus *GPSM3-004* (**Figure 3**).

GPSM3 and autoimmune arthritis

RA is a chronic, autoimmune polyarthritis that, if left untreated, leads to deformity, chronic pain, and disability for patients through a process of synovial inflammation, synovial cell proliferation with angiogenesis, and joint destruction [7]. Additional extra-articular disease manifestations such as vascular disease from unchecked inflammation further lead to increased mortality [45-47]. Improved understanding of immune system-mediated RA pathogenesis has led to new biologic treatments that target selective elements of the RA inflammatory response; however, there are still patients who are non-responders or have adverse effects limiting their treatment [48].

Pre-clinical evidence suggests that neutralization of proinflammatory, monocyte-attracting chemokines, or their cognate cell-surface receptors, would be beneficial for the treatment of RA [6, 49]; however, clinical trials have revealed disappointing results both for neutralization of chemokines or for antagonism of their GPCR counterparts [10, 50-52]. One explanation for this apparent discrepancy between pre-clinical evidence and clinical trial results has focused on the known functional redundancy of the chemokine system in inflammation, whereby many chemokines within a structural class can bind to multiple chemokine receptors sharing similar structural motifs [6, 7]. Thus, targeting monocyte/macrophage recruitment in RA may be desirable, but current strategies using single chemokine or chemokine receptor blockade have produced limited results.

GPSM3 appears restricted in its tissue expression to cells of hematopoietic origin [17, 21], suggesting a potentially crucial role in inflammatory cell function. In particular, monocyte viability and chemotaxis are found to be affected in a GPSM3-deficient acute inflammatory arthritis model [21], and GWAS data suggest that *GPSM3* SNPs are associated differentially between healthy persons and those with inflammatory arthritis from either RA, SLE, or AS [39, 40]. Thus, understanding the regulatory roles of GPSM3 as a GPCR signaling modulator may help to promote this protein as a desirable therapeutic target for patients with autoimmune arthritis.

In an acute, collagen antibody-induced arthritis (CAIA) mouse model of inflammatory arthritis, GPSM3-deficient mice (those either homozygous or heterozygous for the ablated *GPSM3* allele) exhibit decreased clinical disease as well as reduced histopathology with less inflammation in cartilage, less synovial damage, and less bone erosion [21]. Additionally, a decreased overall disease incidence was observed in *Gpsm3-/-* mice (34% for *Gpsm3-/-* mice *vs* 95% for control mice) [21]. Independent studies by Schmidt *et al*. have shown that *GPSM3* expression decreases in the joints after anti-inflammatory treatment with dexamethasone in the chronic, collagen-induced arthritis (CIA) model [53]. Thus, both chronic and acute mouse models of inflammatory arthritis reveal a compelling role for GPSM3 involvement.

Our group has recently shown that GPSM3 deficienct mice have reduced CAIA that is associated with a decreased transcript expression of the proinflammatory, monocyte-derived cytokines *IL-6* and *IL-1β*, as well as of monocyte-specific chemokine receptors in the joints [21]. TNF-α, IL-1β, and IL-6 are all produced by monocytes/macrophages, which are recruited to the inflamed synovium by chemokines such as CCL2/MCP-1 and CX3CL1/fractalkine, among numerous others [6, 7, 54, 55]. GPSM3 deficiency is seen to reduce monocyte chemotaxis toward CCL2, CX3CL1 and chemerin, and to enhance etoposide-induced apoptosis *ex vivo* [21], suggesting that GPSM3 affects monocytes through the regulation of chemokine-receptor signaling and its downstream intracellular sequelae. The critical nature of monocyte infiltration and pathogenesis in autoimmune arthritis [56, 57] is further underscored by the direct correlation between disease severity and the accumulation of monocyte-targeting nanoparticles in the inflamed joint synovium, as observed recently by Kim *et al* [58].

Summary

As a newly-characterized regulator of $G\alpha_i$ GPCR signal transduction, GPSM3 may represent a novel biomarker and/or therapeutic target for inflammatory arthritis. Coupled with compelling human SNP-association data, our recent findings of blunted disease sequelae in a mouse strain with targeted deletion of *Gpsm3* have helped to validate the status of GPSM3 as a

protein worthy of future development as a drug discovery target that could transcend the present roadblocks observed with single chemokine or chemokine receptor neutralization strategies.

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References

[1] Jacoby E, Bouhelal R, Gerspacher M and Seuwen K. The 7 TM G-protein-coupled receptor target family. ChemMedChem 2006; 1: 761-782.

[2] Lagerstrom MC and Schioth HB. Structural diversity of G protein-coupled receptors and significance for drug discovery. Nat Rev Drug Discov 2008; 7: 339-357.

[3] Overington JP, Al-Lazikani B and Hopkins AL. How many drug targets are there? Nat Rev Drug Discov 2006; 5: 993-996.

[4] Kimple AJ, Bosch DE, Giguere PM and Siderovski DP. Regulators of G-protein signaling and their Galpha substrates: promises and challenges in their use as drug discovery targets. Pharmacol Rev 2011; 63: 728-749.

[5] Siderovski DP and Willard FS. The GAPs, GEFs, and GDIs of heterotrimeric G-protein alpha subunits. Int J Biol Sci 2005; 1: 51-66.

[6] Tarrant TK and Patel DD. Chemokines and leukocyte trafficking in rheumatoid arthritis. Pathophysiology 2006; 13: 1-14.

[7] Szekanecz Z, Koch AE and Tak PP. Chemokine and chemokine receptor blockade in arthritis, a prototype of immune-mediated inflammatory diseases. Neth J Med 2011; 69: 356-366.

[8] Gladue RP, Brown MF and Zwillich SH. CCR1 antagonists: what have we learned from clinical trials. Curr Top Med Chem 2010; 10: 1268-1277.

[9] Proudfoot AE, Power CA and Schwarz MK. Anti-chemokine small molecule drugs: a promising future? Expert Opin Investig Drugs 2010; 19: 345-355.

[10] Haringman JJ, Gerlag DM, Smeets TJ, Baeten D, van den Bosch F, Bresnihan B, Breedveld FC, Dinant HJ, Legay F, Gram H, Loetscher P, Schmouder R, Woodworth T and Tak PP. A randomized controlled trial with an anti-CCL2 (anti-monocyte chemotactic protein 1) monoclonal antibody in patients with rheumatoid arthritis. Arthritis Rheum 2006; 54: 2387-2392.

[11] Hammaker D and Firestein GS. "Go upstream, young man": lessons learned from the p38 saga. Ann Rheum Dis 2010; 69 Suppl 1: i77-82.

[12] Siderovski DP, Diverse-Pierluissi M and De Vries L. The GoLoco motif: a Galphai/o binding motif and potential guanine-nucleotide exchange factor. Trends Biochem Sci 1999; 24: 340-341.

[13] Takesono A, Cismowski MJ, Ribas C, Bernard M, Chung P, Hazard S 3rd, Duzic E and Lanier SM. Receptor-independent activators of heterotrimeric G-protein signaling pathways. J Biol Chem 1999; 274: 33202-33205.

[14] Blumer JB, Oner SS and Lanier SM. Group II activators of G-protein signalling and proteins containing a G-protein regulatory motif. Acta Physiol (Oxf) 2012; 204: 202-218.

[15] Peterson YK, Bernard ML, Ma H, Hazard S 3rd, Graber SG and Lanier SM. Stabilization of the GDP-bound conformation of Gialpha by a peptide derived from the G-protein regulatory motif of AGS3. J Biol Chem 2000; 275: 33193-33196.

[16] Kimple RJ, Kimple ME, Betts L, Sondek J and Siderovski DP. Structural determinants for GoLoco-induced inhibition of nucleotide release by Galpha subunits. Nature 2002; 416: 878-881.

[17] Cao X, Cismowski MJ, Sato M, Blumer JB and Lanier SM. Identification and characterization of AGS4: a protein containing three G-protein regulatory motifs that regulate the activation state of Gialpha. J Biol Chem 2004; 279: 27567-27574.

[18] Kimple RJ, Willard FS, Hains MD, Jones MB, Nweke GK and Siderovski DP. Guanine nucleotide dissociation inhibitor activity of the triple GoLoco motif protein G18: alanine-to-aspartate mutation restores function to an inactive second GoLoco motif. Biochem J 2004; 378: 801-808.

[19] Giguere PM, Laroche G, Oestreich EA and Siderovski DP. G-protein signaling modulator-3 regulates heterotrimeric G-protein dynamics through dual association with Gbeta and Gal-

phai protein subunits. J Biol Chem 2012; 287: 4863-4874.
[20] Webb CK, McCudden CR, Willard FS, Kimple RJ, Siderovski DP and Oxford GS. D2 dopamine receptor activation of potassium channels is selectively decoupled by Galpha-specific Go-Loco motif peptides. J Neurochem 2005; 92: 1408-1418.
[21] Giguere PM, Billard MJ, Laroche G, Buckley BK, Timoshchenko RG, McGinnis MW, Esserman D, Foreman O, Liu P, Siderovski DP and Tarrant TK. G-protein signaling modulator-3, a gene linked to autoimmune diseases, regulates monocyte function and its deficiency protects from inflammatory arthritis. Mol Immunol 2013; 54: 193-198.
[22] Oner SS, Maher EM, Breton B, Bouvier M and Blumer JB. Receptor-regulated interaction of activator of G-protein signaling-4 and Galphai. J Biol Chem 2010; 285: 20588-20594.
[23] Nebl T, Pestonjamasp KN, Leszyk JD, Crowley JL, Oh SW and Luna EJ. Proteomic analysis of a detergent-resistant membrane skeleton from neutrophil plasma membranes. J Biol Chem 2002; 277: 43399-43409.
[24] Sarbassov DD, Guertin DA, Ali SM and Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 2005; 307: 1098-1101.
[25] Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, Struewing JP, Morrison J, Field H, Luben R, Wareham N, Ahmed S, Healey CS, Bowman R, Meyer KB, Haiman CA, Kolonel LK, Henderson BE, Le Marchand L, Brennan P, Sangrajrang S, Gaborieau V, Odefrey F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Peto J, Fletcher O, Johnson N, Seal S, Stratton MR, Rahman N, Chenevix-Trench G, Bojesen SE, Nordestgaard BG, Axelsson CK, Garcia-Closas M, Brinton L, Chanock S, Lissowska J, Peplonska B, Nevanlinna H, Fagerholm R, Eerola H, Kang D, Yoo KY, Noh DY, Ahn SH, Hunter DJ, Hankinson SE, Cox DG, Hall P, Wedren S, Liu J, Low YL, Bogdanova N, Schurmann P, Dork T, Tollenaar RA, Jacobi CE, Devilee P, Klijn JG, Sigurdson AJ, Doody MM, Alexander BH, Zhang J, Cox A, Brock IW, MacPherson G, Reed MW, Couch FJ, Goode EL, Olson JE, Meijers-Heijboer H, van den Ouweland A, Uitterlinden A, Rivadeneira F, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Hopper JL, McCredie M, Southey M, Giles GG, Schroen C, Justenhoven C, Brauch H, Hamann U, Ko YD, Spurdle AB, Beesley J, Chen X, Mannermaa A, Kosma VM, Kataja V, Hartikainen J, Day NE, Cox DR and Ponder BA. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007; 447: 1087-1093.
[26] Barberis L and Hirsch E. Targeting phosphoinositide 3-kinase gamma to fight inflammation and more. Thromb Haemost 2008; 99: 279-285.
[27] Sunderkotter C, Nikolic T, Dillon MJ, Van Rooijen N, Stehling M, Drevets DA and Leenen PJ. Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. J Immunol 2004; 172: 4410-4417.
[28] Fujii W, Ashihara E, Hirai H, Nagahara H, Kajitani N, Fujioka K, Murakami K, Seno T, Yamamoto A, Ishino H, Kohno M, Maekawa T and Kawahito Y. Myeloid-derived suppressor cells play crucial roles in the regulation of mouse collagen-induced arthritis. J Immunol 2013; 191: 1073-1081.
[29] Gabrilovich DI and Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 2009; 9: 162-174.
[30] Yan C, Ding X, Dasgupta N, Wu L and Du H. Gene profile of myeloid-derived suppressive cells from the bone marrow of lysosomal acid lipase knock-out mice. PLoS One 2012; 7: e30701.
[31] Akata K, Yatera K, Aoki T, Fujii M, Kinoshita S, Kawanami T, Ishimoto H, Hisaoka M, Korogi Y and Mukae H. Disseminated Pulmonary Penicilliosis in a Rheumatoid Arthritis Patient. J Thorac Imaging 2014; 29: W14-6.
[32] Corzo CA, Cotter MJ, Cheng P, Cheng F, Kusmartsev S, Sotomayor E, Padhya T, McCaffrey TV, McCaffrey JC and Gabrilovich DI. Mechanism regulating reactive oxygen species in tumor-induced myeloid-derived suppressor cells. J Immunol 2009; 182: 5693-5701.
[33] Montero AJ, Diaz-Montero CM, Kyriakopoulos CE, Bronte V and Mandruzzato S. Myeloid-derived suppressor cells in cancer patients: a clinical perspective. J Immunother 2012; 35: 107-115.
[34] Bunt SK, Clements VK, Hanson EM, Sinha P and Ostrand-Rosenberg S. Inflammation enhances myeloid-derived suppressor cell cross-talk by signaling through Toll-like receptor 4. J Leukoc Biol 2009; 85: 996-1004.
[35] Lapan P, Zhang J, Hill A, Zhang Y, Martinez R and Haney S. Image-based assessment of growth and signaling changes in cancer cells mediated by direct cell-cell contact. PLoS One 2009; 4: e6822.
[36] Reif K and Cyster JG. RGS molecule expression in murine B lymphocytes and ability to down-regulate chemotaxis to lymphoid chemokines. J Immunol 2000; 164: 4720-4729.
[37] Corsiero E, Bombardieri M, Manzo A, Bugatti S, Uguccioni M and Pitzalis C. Role of lymphoid chemokines in the development of functional ectopic lymphoid structures in rheumatic auto-

immune diseases. Immunol Lett 2012; 145: 62-67.
[38] Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007; 447: 661-678.
[39] Sirota M, Schaub MA, Batzoglou S, Robinson WH and Butte AJ. Autoimmune disease classification by inverse association with SNP alleles. PLoS Genet 2009; 5: e1000792.
[40] Corona E, Dudley JT and Butte AJ. Extreme evolutionary disparities seen in positive selection across seven complex diseases. PLoS One 2010; 5: e12236.
[41] Comuzzie AG, Cole SA, Laston SL, Voruganti VS, Haack K, Gibbs RA and Butte NF. Novel genetic loci identified for the pathophysiology of childhood obesity in the Hispanic population. PLoS One 2012; 7: e51954.
[42] Chang WC, Lee CH, Hirota T, Wang LF, Doi S, Miyatake A, Enomoto T, Tomita K, Sakashita M, Yamada T, Fujieda S, Ebe K, Saeki H, Takeuchi S, Furue M, Chen WC, Chiu YC, Chang WP, Hong CH, Hsi E, Juo SH, Yu HS, Nakamura Y and Tamari M. ORAI1 genetic polymorphisms associated with the susceptibility of atopic dermatitis in Japanese and Taiwanese populations. PLoS One 2012; 7: e29387.
[43] Flicek P, Ahmed I, Amode MR, Barrell D, Beal K, Brent S, Carvalho-Silva D, Clapham P, Coates G, Fairley S, Fitzgerald S, Gil L, Garcia-Giron C, Gordon L, Hourlier T, Hunt S, Juettemann T, Kahari AK, Keenan S, Komorowska M, Kulesha E, Longden I, Maurel T, McLaren WM, Muffato M, Nag R, Overduin B, Pignatelli M, Pritchard B, Pritchard E, Riat HS, Ritchie GR, Ruffier M, Schuster M, Sheppard D, Sobral D, Taylor K, Thormann A, Trevanion S, White S, Wilder SP, Aken BL, Birney E, Cunningham F, Dunham I, Harrow J, Herrero J, Hubbard TJ, Johnson N, Kinsella R, Parker A, Spudich G, Yates A, Zadissa A and Searle SM. Ensembl 2013. Nucleic Acids Res 2013; 41: D48-55.
[44] Zhao P, Nguyen CH and Chidiac P. The proline-rich N-terminal domain of G18 exhibits a novel G protein regulatory function. J Biol Chem 2010; 285: 9008-9017.
[45] de Groot L, Posthumus MD, Kallenberg CG and Bijl M. Risk factors and early detection of atherosclerosis in rheumatoid arthritis. Eur J Clin Invest 2010; 40: 835-842.
[46] Kitas GD and Gabriel SE. Cardiovascular disease in rheumatoid arthritis: state of the art and future perspectives. Ann Rheum Dis 2011; 70: 8-14.
[47] Zinger H, Sherer Y and Shoenfeld Y. Atherosclerosis in autoimmune rheumatic diseases-mechanisms and clinical findings. Clin Rev Allergy Immunol 2009; 37: 20-28.
[48] Kekow J, Mueller-Ladner U and Schulze-Koops H. Rituximab is more effective than second anti-TNF therapy in rheumatoid arthritis patients and previous TNFalpha blocker failure. Biologics 2012; 6: 191-199.
[49] Rampersad RR, Tarrant TK, Vallanat CT, Quintero-Matthews T, Weeks MF, Esserman DA, Clark J, Di Padova F, Patel DD, Fong AM and Liu P. Enhanced Th17-cell responses render CCR2-deficient mice more susceptible for autoimmune arthritis. PLoS One 2011; 6: e25833.
[50] Vergunst CE, Gerlag DM, Lopatinskaya L, Klareskog L, Smith MD, van den Bosch F, Dinant HJ, Lee Y, Wyant T, Jacobson EW, Baeten D and Tak PP. Modulation of CCR2 in rheumatoid arthritis: a double-blind, randomized, placebo-controlled clinical trial. Arthritis Rheum 2008; 58: 1931-1939.
[51] Rogers JL, Serafin DS, Timoshchenko RG and Tarrant TK. Cellular Targeting in Autoimmunity. Curr Allergy Asthma Rep 2012; 12: 495-510.
[52] van Kuijk AW, Vergunst CE, Gerlag DM, Bresnihan B, Gomez-Reino JJ, Rouzier R, Verschueren PC, van de Leij C, Maas M, Kraan MC and Tak PP. CCR5 blockade in rheumatoid arthritis: a randomised, double-blind, placebo-controlled clinical trial. Ann Rheum Dis 2010; 69: 2013-2016.
[53] Schmidt N, Art J, Forsch I, Werner A, Erkel G, Jung M, Horke S, Kleinert H and Pautz A. The anti-inflammatory fungal compound (S)-curvularin reduces proinflammatory gene expression in an in vivo model of rheumatoid arthritis. J Pharmacol Exp Ther 2012; 343: 106-114.
[54] Gordon S and Taylor PR. Monocyte and macrophage heterogeneity. Nat Rev Immunol 2005; 5: 953-964.
[55] Iwamoto T, Okamoto H, Toyama Y and Momohara S. Molecular aspects of rheumatoid arthritis: chemokines in the joints of patients. FEBS J 2008; 275: 4448-4455.
[56] De Rycke L, Baeten D, Foell D, Kruithof E, Veys EM, Roth J and De Keyser F. Differential expression and response to anti-TNFalpha treatment of infiltrating versus resident tissue macrophage subsets in autoimmune arthritis. J Pathol 2005; 206: 17-27.
[57] Baeten D, Boots AM, Steenbakkers PG, Elewaut D, Bos E, Verheijden GF, Berheijden G, Miltenburg AM, Rijnders AW, Veys EM and De Keyser F. Human cartilage gp-39+, CD16+ monocytes in peripheral blood and synovium: correlation with joint destruction in rheumatoid arthritis. Arthritis Rheum 2000; 43: 1233-1243.

[58] Kim JS, An H, Rieter WJ, Esserman D, Taylor-Pashow KM, Sartor RB, Lin W and Tarrant TK. Multimodal optical and Gd-based nanoparticles for imaging in inflammatory arthritis. Clin Exp Rheumatol 2009; 27: 580-586.
[59] Wu C, Orozco C, Boyer J, Leglise M, Goodale J, Batalov S, Hodge CL, Haase J, Janes J, Huss JW 3rd and Su AI. BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. Genome Biol 2009; 10: R130.