Royal Netherlands Academy of Arts and Sciences (KNAW)

The adhesion G protein-coupled receptor GPR56/ADGRG1 in cytotoxic lymphocytes

Hsiao, Cheng-Chih; Vos, Els; van Gisbergen, Klaas P J M; Hamann, Jörg

2023

DOI (link to publisher)
10.1111/bcpt.13841

document version
Publisher's PDF, also known as Version of record

Link to publication in KNAW Research Portal

citation for published version (APA)
Hsiao, C.-C., Vos, E., van Gisbergen, K. P. J. M., & Hamann, J. (2023). The adhesion G protein-coupled receptor GPR56/ADGRG1 in cytotoxic lymphocytes. *Basic & clinical pharmacology & toxicology*, *133*, 286-294. https://doi.org/10.1111/bcpt.13841

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the KNAW public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the KNAW public portal.

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:
pure@knaw.nl

Download date: 27. Apr. 2025

Received: 2 November 2022 | Revised: 1 February 2023 | Accepted: 2 February 2023
DOI: 10.1111/bcpt.13841

FOCUSED REVIEW

The adhesion G protein-coupled receptor GPR56/*ADGRG1* in cytotoxic lymphocytes

Cheng-Chih Hsiao[1,2] | **Els Vos**[1,2] | **Klaas P. J. M. van Gisbergen**[3] | **Jörg Hamann**[1,2]

[1]Department of Experimental Immunology, Amsterdam Institute for Infection and Immunity, Amsterdam University Medical Centers, Amsterdam, The Netherlands

[2]Neuroimmunology Research Group, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands

[3]Department of Hematopoiesis, Sanquin Research and Landsteiner Laboratory, Amsterdam University Medical Centers, Amsterdam, The Netherlands

Correspondence
Cheng-Chih Hsiao and Jörg Hamann, Department of Experimental Immunology, Amsterdam Institute for Infection and Immunity, Amsterdam University Medical Centers, Amsterdam, The Netherlands.
Email: c.hsiao@nin.knaw.nl and j.hamann@amsterdamumc.nl

Funding information
German Research Foundation, Grant/Award Number: FOR 2149

Abstract

GPR56/*ADGRG1* is an adhesion G protein-coupled receptor connected to brain development, haematopoiesis, male fertility, and tumorigenesis. Nevertheless, expression of GPR56 is not restricted to developmental processes. Studies over the last years have demonstrated a marked presence of GPR56 in human cytotoxic NK and T cells. Expression of GPR56 in these cells is driven by the transcription factor HOBIT, corresponds with the production of cytolytic mediators and the presence of CX_3CR1 and CD57, indicates a state of terminal differentiation and cellular exhaustion, and disappears upon cellular activation. Functional studies indicate that GPR56 regulates cell migration and effector functions and thereby acts as an inhibitory immune checkpoint. We here discuss the current state of knowledge regarding GPR56 in cytotoxic lymphocytes.

KEYWORDS
adhesion G protein-coupled receptor, cytotoxic lymphocytes, immune checkpoint

1 | INTRODUCTION

The adhesion family of G protein-coupled receptors (GPCRs) comprises 32 molecules on the cell surface of human cells.[1] Adhesion GPCRs consist of a seven-transmembrane C-terminal fragment (CTF) that, through a juxtamembranous GPCR-autoproteolysis-inducing (GAIN) domain, couples to an extended extracellular N-terminal fragment (NTF).[2] Central in the GAIN domain poses a GPCR-proteolysis site (GPS), which enables autocatalytic processing in some but not all adhesion GPCRs. Notably, the separated NTF and CTF remain non-covalently attached, thereby increasing structural flexibility of the receptor.[3] The NTFs of adhesion GPCRs possess various molecular folds involved in matricellular interactions[4] and the perception of mechanical forces.[5] Key to the signalling of adhesion GPCRs is the N-terminus of the CTF underneath the GPS, also known as *Stachel* sequence, that as a tethered agonist enables intracellular G protein-dependent signalling.[6,7] Recently, a quartet of cryogenic electron microscopy studies resolved the structure of adhesion GPCRs in their active

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2023 The Authors. *Basic & Clinical Pharmacology & Toxicology* published by John Wiley & Sons Ltd on behalf of Nordic Association for the Publication of BCPT (former Nordic Pharmacological Society).

17427843, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/bcpt.13841 by Cochrane Netherlands, Wiley Online Library on [01/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

state and thereby provided insight into the mechanism by which contact between the *Stachel* sequence and the transmembrane domain activates the receptors.[8–11]

Like canonical GPCRs, adhesion GPCRs have a wide cellular distribution and regulate a range of (patho)physiological functions. GPR56, encoded by *ADGRG1*, is a prototypical adhesion GPCR implicated in developmental processes in the central nervous system, the immune system, and the male reproductive system as well as in tumorigenesis.[12–16] GPR56 is well known for its causative role in the severe human brain malformation bilateral frontoparietal polymicrogyria (BFPP).[12,17] Moreover, the involvement of GPR56 in oligodendrocyte development[18,19] and haematopoietic stem cell formation[14,20] has been studied in detail. Given the link of GPR56 with developmental processes, the finding of abundant GPR56 expression on terminally differentiated NK and T cells[21,22] was unexpected and has become a focus of research.[23] We here discuss the current knowledge on the role of GPR56 in cytotoxic lymphocytes.

2 | CYTOLYTIC LYMPHOCYTES EXPRESS GPR56/*ADGRG1*

The initial finding that human NK cells express GPR56 dates from 2010. In search for novel human NK-cell markers, Della Chiesa et al. generated two monoclonal antibodies against a hitherto unknown transmembrane molecule on $CD56^{dim}CD16^{+}$ mature NK cells, which they identified as GPR56.[21] Independently, the laboratory of Hsi-Hsien Lin raised a panel of monoclonal antibodies against GPR56 that we showed to bind $CD56^{dim}$ cytotoxic NK cells.[22] GPR56 staining is inversely correlated with the presence of CD94, indicating that GPR56 expression increases during NK-cell maturation.[22] Another marker distinguishing mature NK cells is CD57, corresponding with high cytotoxicity. Indeed, $CD56^{dim}CD57^{+}$ NK cells express the highest levels of GPR56 as well as CX_3CR1, a marker defining migratory lymphocytes with high cytotoxic activity.[24]

In our initial paper, we reported that next to NK cells, also $CD8^{+}$ and $CD4^{+}$ effector memory T cells re-expressing CD45RA (T_{EMRA}) upregulate GPR56.[22] T_{EMRA} cells are circulating, terminally differentiated memory T cells that expand in response to viruses, such as human cytomegalovirus (CMV), and hitherto vary in frequency between individuals depending on their infection history. A hallmark of anti-CMV $CD8^{+}$ T-cell responses is the maintenance of large populations of CMV-specific T_{EMRA} cells as a result of their gradual accumulation during life.[25] In line herewith, GPR56 was the second-most upregulated gene associated with chronological age in a whole-blood gene expression meta-analysis in 14,983 individuals of European ancestry.[26]

$CD4^{+}$ and $CD8^{+}$ T_{EMRA} cells expressing GPR56 show clonal expansion, cytotoxic capacity, and killer-like receptor expression.[27–29] Like mature circulating NK cells, they jointly express CX_3CR1, GPR56, and CD57,[30,31] which also indicated as C–G–C signature. Recent single-cell RNA sequencing data illuminate gene co-expression of this signature with genes encoding cytotoxic mediators, in particular *GZMB* encoding granzyme B, across human lymphocytes (Figure 1).[32] The close association between expressions of GPR56 and granzyme B is supported by their co-expression at protein level in human peripheral blood lymphocytes.[22]

In line with the ability of circulating cytotoxic lymphocytes to survey non-lymphoid tissues, GPR56-expressing NK cells were detected not only in peripheral blood but also in mucosal biopsies obtained from patients with oral lesions.[21] Likewise, adipose tissue and coronary plaques contain $CD4^{+}$ T_{EMRA} cells expressing GPR56.[33,34] The number of these cells is increased in people with auto-inflammatory conditions, such as children progressing rapidly to type 1 diabetes[35] and seropositive rheumatoid arthritis (RA) patients.[36] Moreover, in a wide range of tumour types, infiltrating T cells express GPR56.[37] While human $CD56^{dim}CD16^{+}$ NK and T_{EMRA} cells have the ability to retain granzyme B protein expression into the memory phase, mice may not have an exact correlate of these cells. Accordingly, mice lack circulating NK and T cells expressing GPR56 (www.immgen.org) (Figure 2A).

Next to circulating T_{EMRA} cells, non-circulating $CD4^{+}$ and $CD8^{+}$ tissue-resident memory T cells (T_{RM}) with a $CCR7^{-}CD69^{+}CD103^{+/-}$ phenotype survey non-lymphoid tissues. A subset of $CD4^{+}$ $PD\text{-}1^{hi}CXCR5^{-}$ T_{RM} cells, known as peripheral helper T (T_{PH}) cells, is enriched in the synovial fluid of seropositive RA patients. These cells display a cytotoxic profile and express GPR56.[36] In the brain, $CD8^{+}$ T_{RM} cells express GPR56,[41] and $CD8^{+}$ $CD103^{+}$ T_{RM} cells in multiple sclerosis (MS) lesions upregulate GPR56.[42] Finally, breast cancer $CD8^{+}$ T_{RM} cells express GPR56, and the expression is increased in the $CD103^{+}$ fraction of T_{RM} cells.[43] Similarly in mice, $CD8^{+}$ T-cell populations responding to viral infections express GPR56. In particular, GPR56 is upregulated in herpes simplex virus (HSV)-specific T_{RM} cells of the skin and in lymphocytic choriomeningitis virus (LCMV)-specific T_{RM} cells across several organs (Figure 2A).[40,44,45]

Cellular exhaustion is a characteristic feature of T cells arising in persistent viral infections and in tumours.[46] The dysfunctional state of these antiviral or anti-tumour T cells corresponds with the expression of a multitude of

17427843, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/bcpt.13841 by Cochrane Netherlands, Wiley Online Library on [01/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

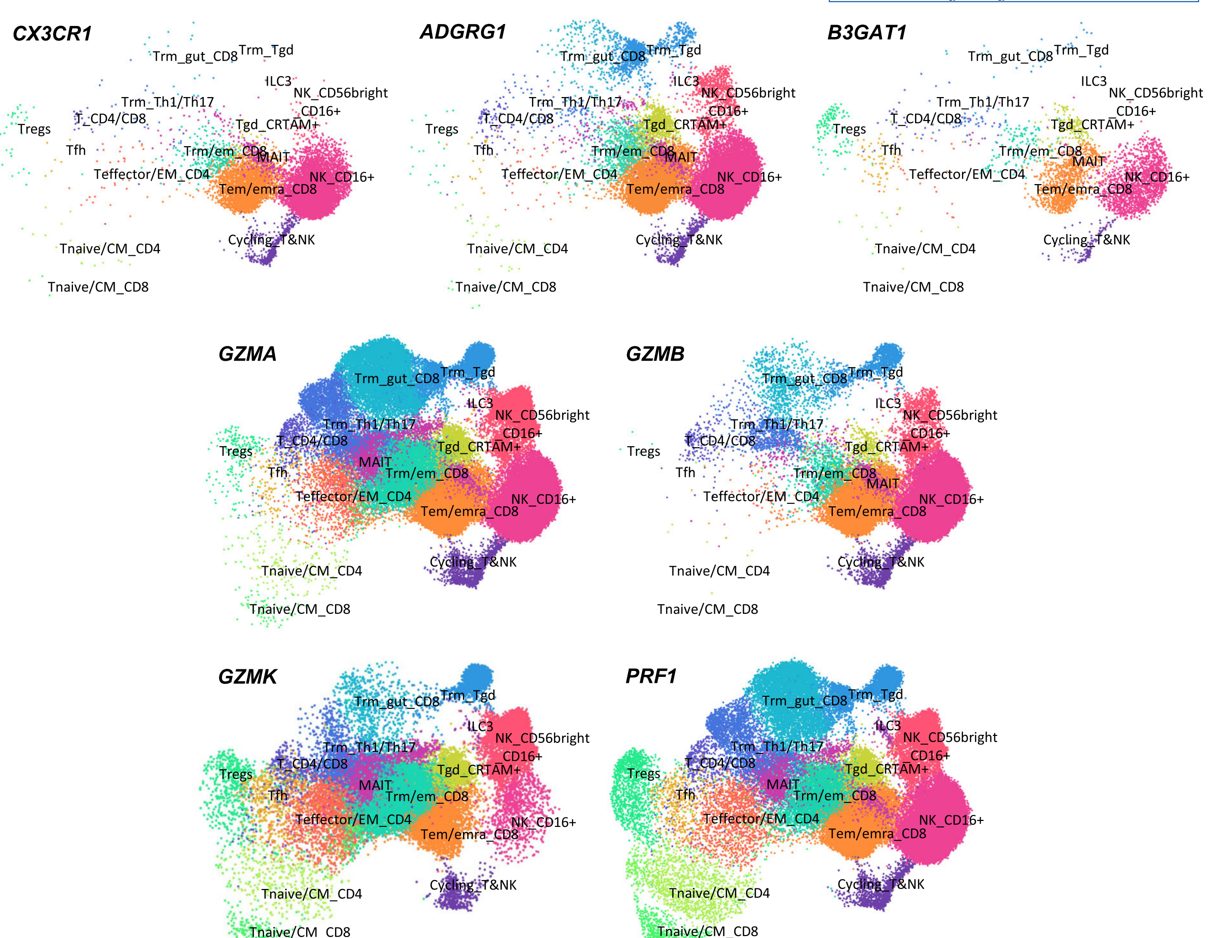

FIGURE 1 Expression of GPR56 in tissue-resident immune cells. UMAP (uniform manifold approximation and projection) visualization of CX3CR1/*CX3CR1*, GPR56/*ADGRG1*, CD57/*B3GAT1*, granzyme A/*GZMA*, granzyme B/*GZMB*, granzyme K/*GZMK*, and perforin/*PRF1* expression in T cells and innate lymphoid cells, including NK cells. Single-cell RNA sequencing data[32] were processed using www.tissueimmunecellatlas.org.

inhibitory receptors, including PD-1, TIM-3, and LAG-3, and prevents them from clearing the infection or combating the tumour. Different studies revealed that during chronic LCMV infection, *Adgrg1* is expressed much higher in T cells possessing an advanced state of exhaustion (Figure 2B).[44,45,47] In line herewith, *ADGRG1* expression is upregulated in terminally exhausted $CD8^+$ tumour-infiltrating lymphocytes in human.[37] Thus, in sum, T_{EMRA} and T_{RM} cells express GPR56 and further upregulate the receptor when entering a state of exhaustion.

3 | REGULATION OF GPR56/*ADGRG1* EXPRESSION

The specific expression of GPR56 by circulating human cytotoxic lymphocytes and mouse T_{RM} cells raises questions regarding the underpinning transcriptional mechanisms. Transcription factors figuring prominently in the terminal differentiation of cytotoxic lymphocytes are HOBIT/*ZNF683*, BLIMP1/*PRDM1*, and RUNX3. We showed that expression of GPR56 in human NK cells is driven by HOBIT, which also is expressed in circulating cytotoxic $CD8^+$ and $CD4^+$ T cells.[48] Knockdown of *ZNF683* largely prevented GPR56 induction in NK-92 cells, which upregulate GPR56 when cultured without IL-2. Reciprocally, ectopic expression of HOBIT in Jurkat cells, which express neither GPR56 nor HOBIT, induced expression of GPR56. HOBIT might actually not be the only transcription factor driving the expression of GPR56. Mature NK and $CD8^+$ T_{EMRA} cells, next to HOBIT, express BLIMP1 and RUNX3, and indeed, ectopic expression of RUNX3 in mouse $CD4^+$ T cells upregulates GPR56.[49]

As HOBIT and BLIMP1 collaborate in the transcriptional regulation of T_{RM} cells,[50] we analysed *Adgrg1* expression in T_{RM} cells of *Znf683*-deficient and *Prdm1-deficient* mice. We found a reduction of *Adgrg1*

17427843, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/bcpt.13841 by Cochrane Netherlands, Wiley Online Library on [01/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

(A)

Source	Cell type	Human	Mouse
B	Stem and progenitor cells	■	■
Circulating	Platelets	■	■
	Basophils		
	Eosinophils		
	Neutrophils		
	Classical monocytes		
	Non-classical monocytes		
	Myeloid DCs		
	Plasmacytoid DCs		
	Naive B cells		
	Plasmablasts		
	Plasma cells		
	Mucosal-associated invariant T cell		
	Naive CD4$^+$ T cells		
	CD4$^+$ T helper 1/2/17 cells		
	Regulatory T cells		
	Folicular T helper cells		
	Central memory CD4$^+$ T cells		
	Effector memory CD4$^+$ T cells	■	
	RA$^+$ effector memory CD4$^+$ T cells	■	
	Naive CD8$^+$ T cells		
	Central memory CD8$^+$ T cells		
	Effector memory CD8$^+$ T cells	■	
	RA$^+$ effector memory CD8$^+$ T cells	■	
	γδ T cells	■	
	CD16$^-$ NK cells		
	CD16$^+$ NK cells	■	
Tissue	Mast cells		
	Macrophages		
	Microglia	■	■
	Tissue-resident memory T cells	■	■
	Exhausted T cells	■	■
	Tissue-resident NK cells	■	■

(B)

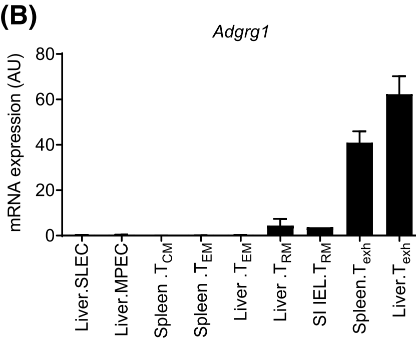

FIGURE 2 Expression of GPR56 in cytotoxic lymphocytes. (A) Profiling by flow cytometry and RNA sequencing of GPR56 expression in circulating and tissue-resident immune cells in human and mice. Data were compiled from different sources; see text,[38,39] and www.immgen.org. B, bone marrow; DC, dendritic cell. (B) Expression of *Adgrg1*, determined by RT-qPCR, in mouse lymphocyte populations in liver, spleen, and intestine. Short-lived effector cells (SLECs) and memory precursors effector cells (MPECs) were isolated at Day 8, and memory and exhausted T cells were obtained at Day 30+ after chronic infection with LCMV. The latter included central memory (T_{CM}), effector memory (T_{EM}), tissue-resident memory (T_{RM}), and exhausted (T_{exh}) T cells. Data were adapted from Hsiao et al.[40]

expression in mouse T_{RM} cells lacking HOBIT and, to a lesser extent, in those lacking BLIMP1 and a further reduction in *Znf683* and *Prdm1* double-deficient T_{RM} cells.[40] TGF-β establishes part of the residency-specific transcriptional profile of T_{RM} cells in various tissues. TGF-β strongly upregulates expression of *Adgrg1*, and reciprocally, expression of *Adgrg1* is reduced in skin T_{RM} cells of mice lacking a functional TGF-β receptor II.[40] Thus, the transcription factors HOBIT and the T_{RM} cell-inducing cytokine TGF-β contribute to the expression of *Adgrg1* in cytotoxic lymphocytes.

Proliferative arrest of the NK-92 cell line by IL-2 withdrawal correlates with an upregulation of GPR56.[48] In line herewith, Della Chiesa et al. showed that activation of NK cells by the cytokines IL-2, IL-12, IL-15, and IL-18 or monocyte-derived dendritic cells leads to a downregulation of GPR56.[21] The downregulation of GPR56 on NK cells by inflammatory cytokines is fast and caused by a combination of shedding of the NTF and inhibition of gene transcription.[48] Thus, expression of GPR56 is restricted to terminally differentiated, non-proliferating cells and rapidly lost upon their cellular activation.

4 | FUNCTIONAL IMPLICATIONS OF GPR56/*ADGRG1* EXPRESSION

Previous reports have linked GPR56 with the regulation of neural progenitor and melanoma cell migration.[51,52] In line herewith, ectopic expression of GPR56 reduces spontaneous and SDF-1-driven trans-well migration of NK-92 cells,[22] a finding that recently has been confirmed also for primary T cells overexpressing GPR56.[37] A further role of GPR56 in cytotoxic lymphocytes was elucidated when studying BFPP patients with a GPR56 mutation that prevents surface expression of the receptor. NK and T cells developed fairly normally and had no obvious changes in their surface marker profile. Nevertheless, they produced enhanced amounts of TNF and IFNγ upon contact with K562 target cells, resulting in more efficient killing.[48] NK-92 cells overexpressing GPR56 showed an opposite behaviour. They possessed less killing activity against K562 cells, as indicated by reduced target cell apoptosis, NK-cell degranulation, and production of TNF and IFNγ.[48] Thus, lack of GPR56 did not hamper normal NK-cell development but appeared to enhance their functional capacity, indicating that the physiological role of GPR56 in cytotoxic lymphocytes may be to limit the potentially harmful cytotoxic effects of granzyme B and pro-inflammatory cytokines under homeostatic conditions.

Notably, the presence of GPR56 in immune cells is not confined to cytotoxic lymphocytes. Also, a distinct myeloid cell population expresses GPR56. Others and we

17427843, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/bcpt.13841 by Cochrane Netherlands, Wiley Online Library on [01/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

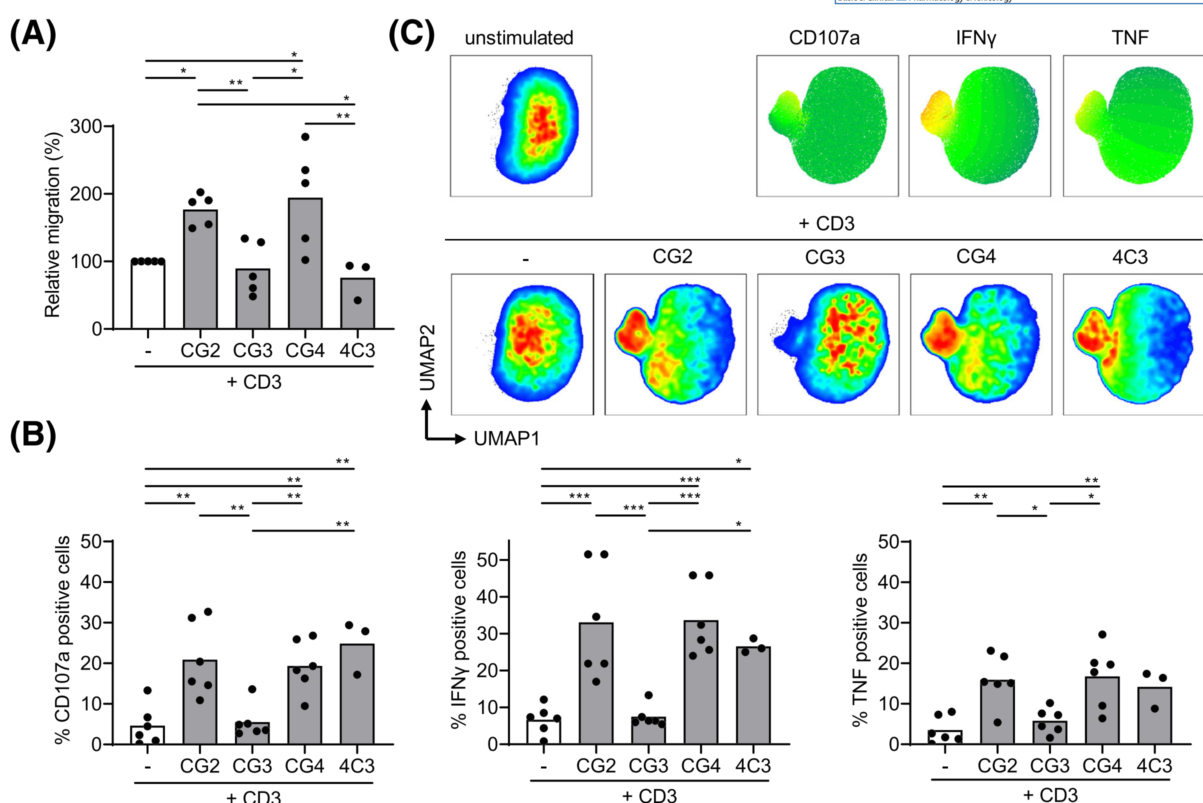

FIGURE 3 GPR56 antibodies enhance T-cell migration and effector functions. (A) Peripheral blood lymphocytes (PBLs) were exposed to plate-bound anti-CD3 monoclonal antibody (coated at a concentration of 0.1 μg/ml; BioLegend) in the absence or presence of anti-GPR56 monoclonal antibodies (clones CG3, CG2, CG4, and 4C3 at a concentration of 2 μg/ml) for 2 h. Subsequently, cells were left to transmigrate through 5-μm trans-well filters (corning) in the presence of 100 ng/ml SDF-1 (R&D systems) in the lower compartment for 3 h. Cells migrated through the filter were quantified by flow cytometry. (B) PBLs exposed to plate-bound anti-CD3 monoclonal antibody (coated at a concentration of 0.1 μg/ml) in the absence or presence of anti-GPR56 monoclonal antibodies (clones CG3, CG2, CG4, and 4C3 at a concentration of 2 μg/ml) for 5 h were analysed for the expression of CD107a, IFNγ, and TNF in granzyme B^+ $CD8^+$ T cells by flow cytometry. All data are presented as means ± S.D. of three to six independent experiments. One-way ANOVA with Tukey's range test, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$. Antibodies to GPR56 have kindly been provided by Dr. Lin (CG2/3/4) or purchased from BioLegend (4C3). (C) UMAP visualization of PBLs with different stimulation, based on expression of CD107a, IFNγ, and TNF (bottom). UMAP plot of CD107a, IFNγ, and TNF expression (top). UMAP was generated by FlowJo (Tree Star).

showed that GPR56 is part of the core cellular signature of microglia, the specialized yolk sac-derived macrophages of the central nervous system (CNS).[53,54] A recent study reported elevated TNF production in microglia in knockout mice with a conditional deletion of microglial *Adgrg1*.[55] This interesting finding matches the proposed role of GPR56 as inhibitory receptor in lymphocytes. Other myeloid cells, including monocyte-derived and tissue macrophages, do not express GPR56.[56]

We have recently addressed the contribution of GPR56 in the regulation of mouse pathogen-specific T_{RM} cells.[40] Analysis of pathogen-specific $CD8^+$ T-cell responses in *Adgrg1* knockout animals after acute infection with LCMV and *Listeria monocytogenes* did not reveal an essential inhibitory role of GPR56 in the regulation of proliferative, cytokine, or cytotoxic responses of $CD8^+$ T_{RM} cells. While T_{RM} cells specifically acquire GPR56 expression, the impact of this receptor on T_{RM} cells after acute infection does not appear essential to regulate their effector functions. Actually, the moderate expression of inhibitory receptors, like GPR56, by T_{RM} cells after acute infection in comparison with their strong upregulation on exhausted $CD8^+$ T cells after chronic infection may indicate a more important function of GPR56 during persistent, chronic viral infection, which remains to be clarified.

How GPR56 exerts its inhibitory activities at the molecular level is only partly understood. Trans interactions of GPR56 with tissue transglutaminase, collagen III, and heparin have been described[16,57,58] but not confirmed yet for GPR56 on lymphocytes. In cis, full-length GPR56 associates with the chaperone tetraspanin CD81, and while recruitment of $G\alpha_{q/11}$ has been shown,[59] this complex has not been linked yet with inhibition of effector functions of lymphocytes. Pharmacological studies

17427843, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/bcpt.13841 by Cochrane Netherlands, Wiley Online Library on [01/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

FIGURE 4 Facts and open questions regarding the presence of GPR56 in cytotoxic lymphocytes. See text for details.

revealed that truncation of the NTF causes significantly increased signalling of the CTF of the receptor via the $G\alpha_{12/13}$/RhoA pathway, commonly associated with cell activation.[7,60,61] Several monoclonal antibodies binding to the NTF of GPR56 mimic this activity by dissociating the $GPR56^{NTF}$–$GPR56^{CTF}$–CD81 complex. The cytolytic function and cytokine secretion of NK-92 cells overexpressing GPR56 and human primary NK cells are greatly enhanced by these antibodies, and we here show that they also enhance SDF-1-stimulated migration, degranulation, and cytokine secretion of human primary T cells (Figure 3A–C). Together, dissociation of the NTF of GPR56 turns the inhibitory full-length receptor into an activating CTF.

5 | CONCLUSIONS AND FUTURE PERSPECTIVES

This review focusses on the adhesion GPCR GPR56 in lymphocytes (Figure 4). Initiated by HOBIT in concert with other transcription factors, GPR56 is expressed by terminally differentiated NK and T cells. In particular, three types of T cells express GPR56 – T_{EMRA}, T_{RM}, and exhausted T cells. The common denominator of the expression of GPR56 is the presence of granzyme B protein indicating that GPR56 functions in lymphocytes with direct cytotoxic potential. Functional studies in circulating NK and T cells indicate a role of GPR56 as inhibitor of cell migration and effector functions, including cytokine secretion and cell killing. Despite growing insight in the expression, regulation, and function of GPR56, key questions remain to be answered regarding its mechanism of action. Remarkably, GPR56 acts as a checkpoint inhibitor in immune cells but activates these cells upon antibody binding-mediated release of the NTF and subsequent self-activation of the CTF. These seemingly opposite activities of the receptor may indicate biased signalling, indicating that different ligands, receptor conformations, and/or cis interactions affect the signalling behaviour of a receptor.[62] While biased signalling is a common phenomenon in canonical GPCRs, it requires closer investigation in

17427843, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/bcpt.13841 by Cochrane Netherlands, Wiley Online Library on [01/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

adhesion GPCRs. The wide presence in developing as well as in differentiated cells, the insight into the structure and pharmacology, and the availability of molecular tools and in vivo models make GPR56 an ideal molecule to address such basic questions with exciting spin-offs in disciplines, such as immunology.

ACKNOWLEDGEMENTS

We thank Dr. Joost Smolders for his comments on this paper.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ORCID

Cheng-Chih Hsiao https://orcid.org/0000-0003-3572-1046

Klaas P. J. M. van Gisbergen https://orcid.org/0000-0003-3623-7555

Jörg Hamann https://orcid.org/0000-0002-9448-1727

REFERENCES

1. Hamann J, Aust G, Araç D, et al. International Union of Basic and Clinical Pharmacology. XCIV. Adhesion G protein-coupled receptors. *Pharmacol Rev*. 2015;67(2):338-367. doi:10.1124/pr.114.009647
2. Araç D, Boucard AA, Bolliger MF, et al. A novel evolutionarily conserved domain of cell-adhesion GPCRs mediates autoproteolysis. *EMBO j*. 2012;31(6):1364-1378. doi:10.1038/emboj.2012.26
3. Lin HH, Chang GW, Davies JQ, Stacey M, Harris J, Gordon S. Autocatalytic cleavage of the EMR2 receptor occurs at a conserved G protein-coupled receptor proteolytic site motif. *J Biol Chem*. 2004;279(30):31823-31832. doi:10.1074/jbc.M402974200
4. Hamann J, Vogel B, van Schijndel GM, van Lier RA. The seven-span transmembrane receptor CD97 has a cellular ligand (CD55, DAF). *J Exp Med*. 1996;184(3):1185-1189. doi:10.1084/jem.184.3.1185
5. Scholz N, Gehring J, Guan C, et al. The adhesion GPCR latrophilin/CIRL shapes mechanosensation. *Cell Rep*. 2015;11(6):866-874. doi:10.1016/j.celrep.2015.04.008
6. Liebscher I, Schön J, Petersen SC, et al. A tethered agonist within the ectodomain activates the adhesion G protein-coupled receptors GPR126 and GPR133. *Cell Rep*. 2014;9(6):2018-2026. doi:10.1016/j.celrep.2014.11.036
7. Stoveken HM, Hajduczok AG, Xu L, Tall GG. Adhesion G protein-coupled receptors are activated by exposure of a cryptic tethered agonist. *Proc Natl Acad Sci USA*. 2015;112(19):6194-6199. doi:10.1073/pnas.1421785112
8. Xiao P, Guo S, Wen X, et al. Tethered peptide activation mechanism of the adhesion GPCRs ADGRG2 and ADGRG4. *Nature*. 2022;604(7907):771-778. doi:10.1038/s41586-022-04590-8
9. Ping YQ, Xiao P, Yang F, et al. Structural basis for the tethered peptide activation of adhesion GPCRs. *Nature*. 2022;604(7907):763-770. doi:10.1038/s41586-022-04619-y
10. Qu X, Qiu N, Wang M, et al. Structural basis of tethered agonism of the adhesion GPCRs ADGRD1 and ADGRF1. *Nature*. 2022;604(7907):779-785. doi:10.1038/s41586-022-04580-w
11. Barros-Álvarez X, Nwokonko RM, Vizurraga A, et al. The tethered peptide activation mechanism of adhesion GPCRs. *Nature*. 2022;604(7907):757-762. doi:10.1038/s41586-022-04575-7
12. Piao X, Hill RS, Bodell A, et al. G protein-coupled receptor-dependent development of human frontal cortex. *Science*. 2004;303(5666):2033-2036. doi:10.1126/science.1092780
13. Bae BI, Tietjen I, Atabay KD, et al. Evolutionarily dynamic alternative splicing of GPR56 regulates regional cerebral cortical patterning. *Science*. 2014;343(6172):764-768. doi:10.1126/science.1244392
14. Solaimani Kartalaei P, Yamada-Inagawa T, Vink CS, et al. Whole-transcriptome analysis of endothelial to hematopoietic stem cell transition reveals a requirement for Gpr56 in HSC generation. *J Exp Med*. 2015;212(1):93-106. doi:10.1084/jem.20140767
15. Chen G, Yang L, Begum S, Xu L. GPR56 is essential for testis development and male fertility in mice. *Dev Dyn*. 2010;239(12):3358-3367. doi:10.1002/dvdy.22468
16. Xu L, Begum S, Hearn JD, Hynes RO. GPR56, an atypical G protein-coupled receptor, binds tissue transglutaminase, TG2, and inhibits melanoma tumor growth and metastasis. *Proc Natl Acad Sci U S A*. 2006;103(24):9023-9028. doi:10.1073/pnas.0602681103
17. Chiang NY, Hsiao CC, Huang YS, et al. Disease-associated GPR56 mutations cause bilateral frontoparietal polymicrogyria via multiple mechanisms. *J Biol Chem*. 2011;286(16):14215-14225. doi:10.1074/jbc.M110.183830
18. Giera S, Deng Y, Luo R, et al. The adhesion G protein-coupled receptor GPR56 is a cell-autonomous regulator of oligodendrocyte development. *Nat Commun*. 2015;6(1):6121. doi:10.1038/ncomms7121
19. Ackerman SD, Garcia C, Piao X, Gutmann DH, Monk KR. The adhesion GPCR Gpr56 regulates oligodendrocyte development via interactions with $G\alpha_{12/13}$ and RhoA. *Nat Commun*. 2015;6(1):6122. doi:10.1038/ncomms7122
20. Maglitto A, Mariani SA, de Pater E, et al. Unexpected redundancy of Gpr56 and Gpr97 during hematopoietic cell development and differentiation. *Blood Adv*. 2021;5(3):829-842. doi:10.1182/bloodadvances.2020003693
21. Della Chiesa M, Falco M, Parolini S, et al. GPR56 as a novel marker identifying the $CD56^{dull}$ $CD16^{+}$ NK cell subset both in blood stream and in inflamed peripheral tissues. *Int Immunol*. 2010;22(2):91-100. doi:10.1093/intimm/dxp116
22. Peng YM, van de Garde MDB, Cheng KF, et al. Specific expression of GPR56 by human cytotoxic lymphocytes. *J Leukoc Biol*. 2011;90(4):735-740. doi:10.1189/jlb.0211092
23. Lin HH, Hsiao CC, Pabst C, Hébert J, Schöneberg T, Hamann J. Adhesion GPCRs in regulating immune responses and inflammation. *Adv Immunol*. 2017;136:163-201. doi:10.1016/bs.ai.2017.05.005
24. Collins PL, Cella M, Porter SI, et al. Gene regulatory programs conferring phenotypic identities to human NK cells. *Cell*. 2019;176(1-2):348-360.e12. doi:10.1016/j.cell.2018.11.045
25. Kuijpers TW, Vossen MT, Gent MR, et al. Frequencies of circulating cytolytic, $CD_{45}RA^{+}CD27^{-}$, $CD8^{+}$ T lymphocytes depend on infection with CMV. *J Immunol*. 2003;170(8):4342-4348. doi:10.4049/jimmunol.170.8.4342

17427843, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/bcpt.13841 by Cochrane Netherlands, Wiley Online Library on [01/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

26. Peters MJ, Joehanes R, Pilling LC, et al. The transcriptional landscape of age in human peripheral blood. *Nat Commun*. 2015;6(1):8570. doi:10.1038/ncomms9570
27. Tian Y, Babor M, Lane J, et al. Unique phenotypes and clonal expansions of human CD4 effector memory T cells re-expressing CD45RA. *Nat Commun*. 2017;8(1):1473. doi:10.1038/s41467-017-01728-5
28. Truong KL, Schlickeiser S, Vogt K, et al. Killer-like receptors and GPR56 progressive expression defines cytokine production of human $CD4^+$ memory T cells. *Nat Commun*. 2019;10(1):2263. doi:10.1038/s41467-019-10018-1
29. Rouers A, Chng MHY, Lee B, et al. Immune cell phenotypes associated with disease severity and long-term neutralizing antibody titers after natural dengue virus infection. *Cell Rep Med*. 2021;2(5):100278. doi:10.1016/j.xcrm.2021.100278
30. Böttcher JP, Beyer M, Meissner F, et al. Functional classification of memory $CD8^+$ T cells by CX3CR1 expression. *Nat Commun*. 2015;6(1):8306. doi:10.1038/ncomms9306
31. Wanjalla CN, McDonnell WJ, Barnett L, et al. Adipose tissue in persons with HIV is enriched for $CD4^+$ T effector memory and T effector memory RA^+ cells, which show higher CD69 expression and CD57, CX3CR1, GPR56 co-expression with increasing glucose intolerance. *Front Immunol*. 2019;10:408. doi:10.3389/fimmu.2019.00408
32. Domínguez Conde C, Xu C, Jarvis LB, et al. Cross-tissue immune cell analysis reveals tissue-specific features in humans. *Science*. 2022;376(6594):eabl5197. doi:10.1126/science.abl5197
33. Wanjalla CN, Mashayekhi M, Bailin S, et al. Anticytomegalovirus $CD4^+$ T cells are associated with subclinical atherosclerosis in persons with HIV. *Arterioscler Thromb Vasc Biol*. 2021;41(4):1459-1473. doi:10.1161/ATVBAHA.120.315786
34. Wanjalla CN, McDonnell WJ, Ram R, et al. Single-cell analysis shows that adipose tissue of persons with both HIV and diabetes is enriched for clonal, cytotoxic, and CMV-specific $CD4^+$ T cells. *Cell Rep Med*. 2021;2(2):100205. doi:10.1016/j.xcrm.2021.100205
35. Bediaga NG, Garnham AL, Naselli G, et al. Cytotoxicity-related gene expression and chromatin accessibility define a subset of $CD4^+$ T cells that mark progression to type 1 diabetes. *Diabetes*. 2022;71(3):566-577. doi:10.2337/db21-0612
36. Argyriou A, Wadsworth MH, Lendvai A, et al. Single cell sequencing identifies clonally expanded synovial $CD4^+$ TPH cells expressing GPR56 in rheumatoid arthritis. *Nat Commun*. 2022;13(1):4046. doi:10.1038/s41467-022-31519-6
37. Bilemjian V, Vlaming MR, Álvarez Freile J, Huls G, De Bruyn M, Bremer E. The novel immune checkpoint GPR56 is expressed on tumor-infiltrating lymphocytes and selectively upregulated upon TCR signaling. *Cancers (Basel)*. 2022;14(13):3164. doi:10.3390/cancers14133164
38. Monaco G, Lee B, Xu W, et al. RNA-Seq signatures normalized by mRNA abundance allow absolute deconvolution of human immune cell types. *Cell Rep*. 2019;26(6):1627-1640.e7. doi:10.1016/j.celrep.2019.01.041
39. Yeung J, Adili R, Stringham EN, et al. GPR56/ADGRG1 is a platelet collagen-responsive GPCR and hemostatic sensor of shear force. *Proc Natl Acad Sci U S A*. 2020;117(45):28275-28286. doi:10.1073/pnas.2008921117
40. Hsiao CC, Kragten NAM, Piao X, Hamann J, van Gisbergen KPJM. The inhibitory receptor GPR56 (Adgrg1) is specifically expressed by tissue-resident memory T cells in mice but dispensable for their differentiation and function in vivo. *Cells*. 2021;10(10):2675. doi:10.3390/cells10102675
41. Hsiao CC, Engelenburg HJ, Jongejan A, et al. Osteopontin associates with brain TRM-cell transcriptome and compartmentalization in donors with and without multiple sclerosis. *iScience*. 2023;26(1):105785. doi:10.1016/j.isci.2022.105785
42. Fransen NL, Hsiao CC, van der Poel M, et al. Tissue-resident memory T cells invade the brain parenchyma in multiple sclerosis white matter lesions. *Brain*. 2020;143(6):1714-1730. doi:10.1093/brain/awaa117
43. Savas P, Virassamy B, Ye C, et al. Single-cell profiling of breast cancer T cells reveals a tissue-resident memory subset associated with improved prognosis. *Nat Med*. 2018;24(7):986-993. doi:10.1038/s41591-018-0078-7
44. Hudson WH, Gensheimer J, Hashimoto M, et al. Proliferating transitory T cells with an effector-like transcriptional signature emerge from PD-1^+ stem-like $CD8^+$ T cells during chronic infection. *Immunity*. 2019;51(6):1043-1058.e4. doi:10.1016/j.immuni.2019.11.002
45. Sandu I, Cerletti D, Oetiker N, et al. Landscape of exhausted virus-specific CD8 T cells in chronic LCMV infection. *Cell Rep*. 2020;32(8):108078. doi:10.1016/j.celrep.2020.108078
46. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T cell exhaustion during chronic viral infection and cancer. *Annu Rev Immunol*. 2019;37(1):457-495. doi:10.1146/annurev-immunol-041015-055318
47. Beltra JC, Manne S, Abdel-Hakeem MS, et al. Developmental relationships of four exhausted $CD8^+$ T cell subsets reveals underlying transcriptional and epigenetic landscape control mechanisms. *Immunity*. 2020;52(5):825-841.e8. doi:10.1016/j.immuni.2020.04.014
48. Chang GW, Hsiao CC, Peng YM, et al. The adhesion G protein-coupled receptor GPR56/ADGRG1 is an inhibitory receptor on human NK cells. *Cell Rep*. 2016;15(8):1757-1770. doi:10.1016/j.celrep.2016.04.053
49. Fonseca R, Burn TN, Gandolfo LC, et al. Runx3 drives a $CD8^+$ T cell tissue residency program that is absent in $CD4^+$ T cells. *Nat Immunol*. 2022;23(8):1236-1245. doi:10.1038/s41590-022-01273-4
50. Mackay LK, Minnich M, Kragten NAM, et al. Hobit and Blimp1 instruct a universal transcriptional program of tissue residency in lymphocytes. *Science*. 2016;352(6284):459-463. doi:10.1126/science.aad2035
51. Iguchi T, Sakata K, Yoshizaki K, Tago K, Mizuno N, Itoh H. Orphan G protein-coupled receptor GPR56 regulates neural progenitor cell migration via a $G\alpha_{12/13}$ and rho pathway. *J Biol Chem*. 2008;283(21):14469-14478. doi:10.1074/jbc.M708919200
52. Chiang NY, Peng YM, Juang HH, et al. GPR56/ADGRG1 activation promotes melanoma cell migration via NTF dissociation and CTF-mediated $G\alpha_{12/13}$/RhoA signaling. *J Invest Dermatol*. 2017;137(3):727-736. doi:10.1016/j.jid.2016.10.031
53. Bennett FC, Bennett ML, Yaqoob F, et al. A combination of ontogeny and CNS environment establishes microglial identity. *Neuron*. 2018;98(6):1170-1183.e8. doi:10.1016/j.neuron.2018.05.014
54. van der Poel M, Ulas T, Mizee MR, et al. Transcriptional profiling of human microglia reveals grey-white matter

17427843, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/bcpt.13841 by Cochrane Netherlands, Wiley Online Library on [01/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

heterogeneity and multiple sclerosis-associated changes. *Nat Commun*. 2019;10(1):1139. doi:10.1038/s41467-019-08976-7

55. Yu D, Li T, Delpech JC, et al. Microglial GPR56 is the molecular target of maternal immune activation-induced parvalbumin-positive interneuron deficits. *Sci Adv*. 2022;8(18): eabm2545. doi:10.1126/sciadv.abm2545
56. Hsiao CC, Sankowski R, Prinz M, Smolders J, Huitinga I, Hamann J. GPCRomics of homeostatic and disease-associated human microglia. *Front Immunol*. 2021;12:674189. doi:10.3389/fimmu.2021.674189
57. Luo R, Jeong SJ, Jin Z, Strokes N, Li S, Piao X. G protein-coupled receptor 56 and collagen III, a receptor-ligand pair, regulates cortical development and lamination. *Proc Natl Acad Sci USA*. 2011;108(31):12925-12930. doi:10.1073/pnas.1104821108
58. Chiang NY, Chang GW, Huang YS, et al. Heparin interacts with the adhesion GPCR GPR56, reduces receptor shedding, and promotes cell adhesion and motility. *J Cell Sci*. 2016; 129(11):2156-2169. doi:10.1242/jcs.174458
59. Little KD, Hemler ME, Stipp CS. Dynamic regulation of a GPCR-tetraspanin-G protein complex on intact cells: central role of CD81 in facilitating GPR56-G$\alpha_{q/11}$ association. *Mol Biol Cell*. 2004;15(5):2375-2387. doi:10.1091/mbc.e03-12-0886
60. Paavola KJ, Stephenson JR, Ritter SL, Alter SP, Hall RA. The N terminus of the adhesion G protein-coupled receptor GPR56 controls receptor signaling activity. *J Biol Chem*. 2011;286(33): 28914-28921. doi:10.1074/jbc.M111.247973
61. Kishore A, Purcell RH, Nassiri-Toosi Z, Hall RA. Stalk-dependent and stalk-independent signaling by the adhesion G protein-coupled receptors GPR56 (ADGRG1) and BAI1 (ADGRB1). *J Biol Chem*. 2016;291(7):3385-3394. doi:10.1074/jbc.M115.689349
62. Smith JS, Lefkowitz RJ, Rajagopal S. Biased signalling: from simple switches to allosteric microprocessors. *Nat Rev Drug Discov*. 2018;17(4):243-260. doi:10.1038/nrd.2017.229

How to cite this article: Hsiao C-C, Vos E, van Gisbergen KPJM, Hamann J. The adhesion G protein-coupled receptor GPR56/*ADGRG1* in cytotoxic lymphocytes. *Basic Clin Pharmacol Toxicol*. 2023;1-9. doi:10.1111/bcpt.13841

17427843, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/bcpt.13841 by Cochrane Netherlands, Wiley Online Library on [01/03/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License