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GPR15-mediated homing controls immune homeostasis in the large intestine mucosa

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Abstract

Lymphocyte homing, which contributes to inflammation, has been studied extensively in the small intestine, but there is little known about homing to the large intestine, the site most commonly affected in inflammatory bowel disease. GPR15, an orphan G-protein coupled receptor, controlled the specific homing of T cells, particularly FOXP3⁺ regulatory T cells (Tregs), to the large intestine lamina propria (LILP). GPR15 expression was modulated by gut microbiota and transforming growth factor- β 1, but not by retinoic acid. GPR15-deficient mice were prone to develop more severe large intestine inflammation, which was rescued by the transfer of GPR15-sufficient Tregs. Our findings thus describe a T cell homing receptor for LILP and indicate that GPR15 plays a role in mucosal immune tolerance largely by regulating the influx of Tregs.

The microbiota of the human gut have coevolved with the host(1–3), and their coexistence is due in large part to an equilibrium established with the host immune system (4). In the gastrointestinal tract, the large intestine harbors significantly more microbiota than the small intestine (5) and contains higher frequencies of FOXP3⁺ regulatory T cells (Tregs) (6–8). Disruption of the equilibrium between the host immune system and microbiota can trigger inflammatory bowel disease in mouse models and, in humans, likely contributes to Crohn's disease and ulcerative colitis (9), in which the large intestine is the primary site of inflammation. Although T cell responses have critical roles in inflammatory bowel diseases (9), it remains unclear how T cells migrate to the large intestine (10–12). Retinoic acid (RA) regulates lymphocyte migration to the small but not to the large intestine (10, 11), indicating that there is a separate mechanism for this process.

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Supporting Online Material

Materials and Methods

Figs. S1 to S13

Table S1

Human GPR15 (also known as BOB) was originally cloned as a co-receptor for HIV/SIV (13, 14). To study the physiological function of its murine ortholog, we made knock-in mice in which endogenous *Gpr15* was replaced with the sequence for GFP (fig. S1). In humans, *GPR15* mRNA is highly expressed in the colon, peripheral blood lymphocytes (PBL), and spleen (13). Similarly, in mice, GFP expression was detected in gut tissues and lymphoid organs, where it was largely restricted to TCR β ⁺ cells (fig. S2A–B). T cells in the large intestine lamina propria (LILP) exhibited the highest percentage of GFP⁺ cells, whereas GPR15 expression was minimal in other immune system cells in the LILP (fig. S2, C–F). To determine the functional characteristics of GPR15⁺ cells, we analyzed the transcriptomes of GFP[−] and GFP⁺ CD4⁺ T cells from the LILP by microarray (Table S1). Many of the genes highly expressed in GFP⁺ cells compared to GFP[−] cells were characteristic of FOXP3⁺ Tregs (*Foxp3* (15), *Eos* (16), *Il-10* (17), *Cd25* (18)) (Table S1). We confirmed the preferential expression of GPR15 in Tregs by analyzing *Foxp3* reporter expression in *Gpr15^{gfp/+} Foxp3^{ires-mrfp}* mice (19) (Fig. 1A) and also staining for FOXP3 protein (fig. S2G–H). Approximately 60–70% of LILP CD4⁺FOXP3⁺ cells expressed *Gpr15*, compared to only 7–20% of CD4⁺FOXP3[−] cells, in mice of two different genetic backgrounds (Fig. 1A, fig. S2H).

We next determined if disproportionate expression of GPR15 in Tregs could affect their presence in the gut. We observed a reduction in the Treg percentage in the LILP, but not in the small intestine lamina propria (SILP) or spleen of *Gpr15* KO compared to *Gpr15* Het mice (Fig. 1B, fig. S3A). Both thymus-derived and peripherally derived Tregs were equally affected (fig. S3B). In cell numbers, only Tregs, CD8⁺ T cells, and double-negative (DN) T cells, all of which showed significant GPR15-GFP expression, were reduced in the LILP of *Gpr15* KO mice (fig. S3C). These populations were unaffected in the SILP (fig. S3D). There was a significant, but much smaller, reduction in FOXP3[−] CD4⁺ T cells (fig. S3C), such that there was an overall decrease in Treg percentage among total CD4⁺ T cells in the LILP (Fig. 1B, fig. S3A).

We next examined Treg frequency in the LILP during an antigen-specific T cell response. *Rag2*^{−/−}, OT-II TCR transgenic mice that were heterozygous or homozygous for the *Gpr15^{gfp}* allele were fed with chicken ovalbumin (OVA). Without antigen exposure, all T cells maintained a naïve phenotype (CD44^{lo}) and no Treg or GFP⁺ T cells were observed (fig. S4A). After OVA exposure of heterozygous mice, there was a small influx in the LILP of GFP⁺ T cells (2–5%) (fig. S4A) that were enriched for FOXP3 expression (fig. S4B). There was a significant reduction in the number and frequency of Tregs but not in the number of FOXP3[−] CD4⁺ T cells in the LILP of KO mice (Fig. 1C and fig. S4C). Thus, GPR15 preferentially contributes to Treg frequency in the LILP at steady state and during an antigen-specific T cell response.

To determine whether GPR15 functions as a homing receptor for the LILP, we performed a short-term competitive homing assay by co-injecting T cells transduced with a control or a GPR15-encoding retrovirus into congenic hosts (fig. S5A). When GPR15⁺ cells and control cells were mixed at a 1:1 ratio and transferred into C57BL/6 mice, all tissues examined exhibited a 1:1 ratio of the donor-derived cells, except for the LILP, where there was a ~10-fold enrichment for GPR15⁺ cells (Fig. 2A, fig. S5B). There was minimal homing of transferred cells to the small intestine (fig. S5B). When GPR15⁺ cells were treated with the G α i inhibitor pertussis toxin (PTX) before transfer, they were no longer enriched in the LILP (Fig. 2B), indicating that GPR15 likely signals through G α i like other lymphocyte homing receptors. Many GPCRs have in their second intracellular loop a conserved DRY motif that is important for downstream signaling through its interactions with heterotrimeric G proteins (20). To ensure that active signaling through GPR15 was required for homing, we mutated the GPR15 DRY motif to DAY (R131A). Although both wild-type and mutant

proteins were similarly expressed at the cell surface (fig. S5C), only cells expressing the wild-type fusion protein migrated to the LILP (Fig. 2C, fig. S5D).

Preferential homing of GPR15⁺ cells to the LILP was observed as early as 2h after cell transfer, suggesting that activation of this GPCR may promote integrin-dependent interaction of T cells with the endothelium in the target organ (fig. S5E). Indeed, blocking antibodies against either subunit of the $\alpha 4 \beta 7$ integrin inhibited GPR15-mediated homing to the LILP (fig. S5F). Unlike $\alpha 4 \beta 7$ and CCR9 (21), GPR15 was not induced by RA (fig. S6). However, GPR15 was induced in T cells treated with a combination of TGF- β 1 and either interleukin (IL)-6 or IL-21 (fig. S7), and there was a marked decrease in *Gpr15* mRNA in T cells of *Tgfb1*^{C33S/C33S} mice that have reduced TGF- β 1 in vivo (22) (fig. S8A–B). In contrast, *Il21r*^{-/-}*Il6*^{-/-} mice crossed to *Gpr15*^{tgfp/+} mice had a similar level of GFP expression as control mice (fig. S8C), suggesting that TGF- β 1 is a key regulator of GPR15 expression in vivo. Cells from *Gpr15* Het and KO mice were treated with these cytokines to induce GPR15 expression in vitro and were used in the short-term competitive homing assay (Fig. 2D). The results confirmed the importance of endogenously-expressed GPR15 in the homing of T cells to the LILP.

We also tested the effect of gut microbiota on GPR15-mediated homing of T cells to the LILP. Treatment of *Gpr15*^{tgfp/+} mice with a combination of broad-spectrum antibiotics led to a decrease in GPR15 expression (fig. S9A). In contrast, GPR15-overexpressing T cells preferentially migrated to the LILP even in germ-free or antibiotics-treated recipients (fig. S9B–C). Therefore, microbiota can affect GPR15 expression, but are unlikely to produce ligand(s) for GPR15.

Because GPR15 deficiency affected Treg homing to the LILP, we next investigated its role in immune homeostasis in the large intestine. We first examined cytokine production by CD4⁺ T cells in the large intestines of *Gpr15* Het and KO mice. At steady state there was an increased proportion of interferon (IFN)- γ - and IL-17A-producing cells among total CD4⁺ T cells in the LILP of *Gpr15* KO mice on a 129/B6 mixed background (fig. S10A) but not in C57BL/6-backcrossed mice. However, when C57BL/6-backcrossed mice were injected with CD40 antibody (which induces acute colitis in *Rag2*^{-/-} mice (23)), inflammatory cytokine expression in the large intestine (but not spleen) was higher in *Gpr15* KO mice than in littermate controls (fig. S10B–C). We next tested the physiological consequences of this inflammatory phenotype in an infection-induced colitis model. When mice were infected with *Citrobacter rodentium*, the majority of wild-type mice resolved inflammation and survived. In contrast, most *Gpr15* KO mice suffered severe weight loss and died (Fig. 3A–B). KO mice also exhibited increased inflammation, tissue damage and inflammatory cytokine expression (Fig. 3C–D, fig. S10D), and Treg numbers in the LILP were reduced compared to Het mice (fig. S10E). The large intestine and spleen of Het and KO mice had a similar pathogen load (fig. S10F), indicating that GPR15 is not required for controlling the infection, but rather for dampening the immune response in the large intestine.

To confirm that sensitivity to *Citrobacter* infection was due to a role of GPR15 in Tregs rather than other T cells, we infected lethally irradiated mice that received mixed bone marrow from *Foxp3*^{tgfp} and from either wild-type or *Gpr15* KO mice. In *Foxp3*^{tgfp} x *Gpr15* KO mixed chimeras, Tregs will develop only from *Gpr15* KO bone marrow and will thus lack GPR15 expression, whereas other T cells can develop from GPR15-sufficient *Foxp3*^{tgfp} bone marrow. Indeed, *Foxp3*^{tgfp} x *Gpr15* KO mixed chimeric mice exhibited more severe inflammation and tissue damage than did chimeras generated with WT bone marrow (Fig. 3E–F), indicating that GPR15 expression in Tregs is required to prevent severe colitis following *Citrobacter* infection. This phenotype was not due to a role of GPR15 in regulating Treg function, as WT Tregs and KO Tregs suppressed naïve T cell proliferation

equally well (fig. S11A). These results indicate that GPR15 is critical for preventing pathological inflammation in the large intestine during colitis, most likely by regulating Treg homing.

We next used a non-infectious model of colitis to determine the role of GPR15 in suppressing local inflammation in vivo. CD40 stimulation in the absence of adaptive immunity induces innate immune cell-mediated colitis (23) that can be rescued by introduction of Tregs (24, 25). We therefore transferred Tregs from *Gpr15* WT or KO mice into *Rag2*^{-/-} mice that were subsequently treated with CD40 antibody. The transfer of WT Tregs, but not KO Tregs, reduced colitis severity and tissue damage (Fig. 4A–B). We also determined the ability of naïve T cells from *Gpr15* WT and KO mice to induce colitis after *Helicobacter hepaticus* infection (24). In this T cell transfer colitis model, which is dependent on the absence of Tregs, KO T_{naïve} cells induced colitis as well as their WT counterpart (fig. S11B), consistent with a preferential role for GPR15 in regulating the homing of Tregs.

To determine whether the function of GPR15 is conserved between human and mouse, we examined *GPR15* mRNA expression in different cell types from various human tissues. Similar to mice, *GPR15* expression was minimal in lymphocytes from the blood and the small intestine; however, it was expressed at high levels in lymphocytes from the large intestine (fig. S12). In contrast, we did not detect elevated *GPR15* mRNA expression in the Treg-enriched CD25⁺CD4⁺ T cell population relative to other LILP T cell populations. Rather, there was more *GPR15* mRNA in CD25⁻CD4⁺ T cells than in CD25⁺CD4⁺ T cells. All colon samples were from colorectal carcinoma patients, thus the pattern of GPR15 expression in colonic lymphocytes from normal subjects, IBD patients and patients with HIV-mediated enteropathy (26, 27) remains to be determined.

Our results indicate that the small and the large intestine utilize different homing cues and different homing receptors for adaptive immune cells (fig. S13A), and thereby compartmentalize immune tolerance mediated by Tregs (fig. S13B). GPR83 is also preferentially expressed in Tregs, and has been suggested to contribute to Treg cell differentiation in inflammatory conditions (28). We cannot rule out a similar role for GPR15, in addition to its function in directing migration or retention of T cells in the large intestine. It will thus be interesting to examine the role of other inflammatory signals in GPR15-dependent Treg function. Our results improve understanding of immune homeostasis in the intestinal mucosa and could potentially lead to new therapeutic strategies to treat inflammatory diseases by combining in vitro expansion of Tregs and GPR15 induction for reintroduction into patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and notes

1. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell*. 2005; 122:107–118. [PubMed: 16009137]
2. Kamada N, et al. Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. *Science*. 2012; 336:1325–1329. [PubMed: 22582016]
3. Ley RE, et al. Evolution of mammals and their gut microbes. *Science*. 2008; 320:1647–1651. [PubMed: 18497261]
4. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science*. 2012; 336:1268–1273. [PubMed: 22674334]
5. McGuckin MA, Lindén SK, Sutton P, Florin TH. Mucin dynamics and enteric pathogens. *Nat Rev Microbiol*. 2011; 9:265–278. [PubMed: 21407243]
6. Atarashi K, et al. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science*. 2011; 331:337–341. [PubMed: 21205640]
7. Lathrop SK, et al. Peripheral education of the immune system by colonic commensal microbiota. *Nature*. 2011; 478:250–254. [PubMed: 21937990]
8. Geuking MB, et al. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity*. 2011; 34:794–806. [PubMed: 21596591]
9. Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature*. 2011; 474:298–306. [PubMed: 21677746]
10. Agace WW. T-cell recruitment to the intestinal mucosa. *Trends in Immunology*. 2008; 29:514–522. [PubMed: 18838302]
11. Mora JR, Iwata M, von Andrian UH. Vitamin effects on the immune system: vitamins A and D take centre stage. *Nat Rev Immunol*. 2008; 8:685–698. [PubMed: 19172691]
12. Sigmundsdottir H, Butcher EC. Environmental cues, dendritic cells and the programming of tissue-selective lymphocyte trafficking. *Nat Immunol*. 2008; 9:981–987. [PubMed: 18711435]
13. Deng HK, Unutmaz D, Kewalramani VN, Littman DR. Expression cloning of new receptors used by simian and human immunodeficiency viruses. *Nature*. 1997; 388:296–300. [PubMed: 9230441]
14. Farzan M, et al. Two orphan seven-transmembrane segment receptors which are expressed in CD4-positive cells support simian immunodeficiency virus infection. *J Exp Med*. 1997; 186:405–411. [PubMed: 9236192]
15. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol*. 2003; 3:330–336. [PubMed: 12612578]
16. Pan F, et al. Eos mediates Foxp3-dependent gene silencing in CD4+ regulatory T cells. *Science*. 2009; 325:1142–1146. [PubMed: 19696312]
17. Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med*. 1999; 190:995–1004. [PubMed: 10510089]
18. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol*. 1995; 155:1151–1164. [PubMed: 7636184]
19. Wan YY, Flavell RA. Identifying Foxp3-expressing suppressor T cells with a bicistronic reporter. *Proc Natl Acad Sci USA*. 2005; 102:5126–5131. [PubMed: 15795373]
20. Rovati GE, Capra V, Neubig RR. The highly conserved DRY motif of class A G protein-coupled receptors: beyond the ground state. *Molecular Pharmacology*. 2007; 71:959–964. [PubMed: 17192495]
21. Iwata M, et al. Retinoic acid imprints gut-homing specificity on T cells. *Immunity*. 2004; 21:527–538. [PubMed: 15485630]

22. Yoshinaga K, et al. Perturbation of transforming growth factor (TGF)- β 1 association with latent TGF- β binding protein yields inflammation and tumors. *Proc Natl Acad Sci USA*. 2008; 105:18758–18763. [PubMed: 19022904]
23. Uhlig HH, et al. Differential Activity of IL-12 and IL-23 in Mucosal and Systemic Innate Immune Pathology. *Immunity*. 2006; 25:309–318. [PubMed: 16919486]
24. Maloy KJ, et al. CD4+CD25+ T(R) cells suppress innate immune pathology through cytokine-dependent mechanisms. *J Exp Med*. 2003; 197:111–119. [PubMed: 12515818]
25. Garrett WS, et al. Communicable Ulcerative Colitis Induced by T-bet Deficiency in the Innate Immune System. *Cell*. 2007; 131:33–45. [PubMed: 17923086]
26. Brenchley JM, Douek DC. HIV infection and the gastrointestinal immune system. *Mucosal immunology*. 2008; 1:23–30. [PubMed: 19079157]
27. Chase AJ, et al. Severe depletion of CD4+ CD25+ regulatory T cells from the intestinal lamina propria but not peripheral blood or lymph nodes during acute simian immunodeficiency virus infection. *J Virol*. 2007; 81:12748–12757. [PubMed: 17855517]
28. Hansen W, et al. G protein-coupled receptor 83 overexpression in naive CD4+CD25– T cells leads to the induction of Foxp3+ regulatory T cells in vivo. *J Immunol*. 2006; 177:209–215. [PubMed: 16785516]
29. Brunkow ME, et al. Disruption of a new forkhead/winged-helix protein, scurfy, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet*. 2001; 27:68–73. [PubMed: 11138001]
30. Niess JH, et al. CX3CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance. *Science*. 2005; 307:254–258. [PubMed: 15653504]
31. Mitchell TC, et al. Immunological adjuvants promote activated T cell survival via induction of Bcl-3. *Nat Immunol*. 2001; 2:397–402. [PubMed: 11323692]
32. Collison LW, Vignali DAA. In vitro Treg suppression assays. *Methods Mol Biol*. 2011; 707:21–37. [PubMed: 21287326]
33. Fox JG, et al. *Helicobacter hepaticus* sp. nov., a microaerophilic bacterium, isolated from livers and intestinal mucosal scrapings from mice. *J Clin Microbiol*. 1994; 32:1238–1245. [PubMed: 8051250]
34. Mora JR, et al. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science*. 2006; 314:1157–1160. [PubMed: 17110582]
35. Rivera-Nieves J, et al. L-selectin, α 4 β 1, and α 4 β 7 integrins participate in CD4+ T cell recruitment to chronically inflamed small intestine. *J Immunol*. 2005; 174:2343–2352. [PubMed: 15699171]
36. Cassani B, et al. Gut-tropic T cells that express integrin α 4 β 7 and CCR9 are required for induction of oral immune tolerance in mice. *Gastroenterology*. 2011; 141:2109–2118. [PubMed: 21925467]

Summary

GPR15 controls homing of Tregs specifically to the large intestine mucosa and thereby regulates local immune homeostasis.

Fig. 1. GPR15 is preferentially expressed in and regulates the frequency of FOXP3⁺ regulatory T cells in the LILP

(A) *Gpr15^{gfp/+}* mice were bred to *Foxp3^{ires-mrfp}* mice. GFP and mRFP expression was examined in T cell subsets from different tissues (SILP: Small intestine lamina propria; DN T: CD4⁻CD8^β⁻ T cells). Results shown are representative of at least three independent experiments. (B) Percentage of FOXP3⁺ Tregs among CD4⁺ T cells in different tissues of *Gpr15^{gfp/+}* mice (Het) and *Gpr15^{gfp/gfp}* (KO) mice (B6N10 (C57BL/6-backcrossed 10 times): n=9; combined from at least two independent experiments). (C) Numbers of FOXP3⁺ (left panel) and FOXP3⁻ cells (right panel) in the LILP were compared between OT-II *Rag2^{-/-}* *Gpr15^{gfp/+}* (Het) and OT-II *Rag2^{-/-}* *Gpr15^{gfp/gfp}* (KO) mice after OVA administration (n=12, combined from four independent experiments). * p<0.05 (t-test).

Fig. 2. GPR15 mediates T cell homing to the LILP

(A) Ratio of *Gpr15*-transduced and control-transduced donor cells in different tissues (MLN: Mesenteric lymph nodes; PLN: Inguinal, Brachial, and Axillary lymph nodes) at 10h after transfer of an equal number of cells (n=6, combined from three independent experiments). (B) Ratio of *Gpr15*-transduced cells treated with PTX and untreated control-transduced cells after co-transfer (n=5). (C) Ratio of cells transduced with control vector and the R131A mutant *Gpr15* fused with *gfp* (GPR15mut-GFP) (n=7, combined from three independent experiments). (D) Ratio in different tissues of CD4⁺ T cells from *Gpr15*^{gf/+} (Het) and *Gpr15*^{gf/gf} (KO) mice after in vitro culture in GPR15-inducing conditions and transfer of equal numbers of cells into recipients (n=5, combined from two independent experiments). *p<0.05 (t-test).

Fig. 3. GPR15-deficient mice are prone to inflammation of the large intestine due to a defect in Tregs
(A–F) Results after infection of mice with *C. rodentium*. **(A)** Kaplan-Meier survival curve of *wild-type* (WT) and KO mice (WT: n=9; KO: n=22, combined from three independent experiments). **(B)** Weight change (n=6–8, representative of three independent experiments) **(C, E)** H&E staining of colon sections (Bar=70 μ m) of *C. rodentium*-infected Het and KO mice **(C)** or of chimeric mice reconstituted with bone marrow progenitors for GPR15-sufficient (WT) or –deficient (KO) Tregs **(E)**. **(D, F)** Histology score and inflammation index of colons of Het and KO mice **(D)** or of mixed bone marrow chimeras **(F)** (n=5–6 per group). *p<0.05 (t-test), **p<0.05 (Log-rank test).

Fig. 4. Tregs from GPR15-deficient mice cannot rescue colitis

(A, B) *Rag2*^{-/-} mice received 5×10^5 mRFP⁺ Tregs transferred from either *Foxp3*^{ires-mrfp} or *Gpr15*^{gfp/gfp} *Foxp3*^{ires-mrfp} mice that were subsequently injected with CD40 antibody (FGK45). (A) H&E staining of proximal and mid colon section of mice without any treatment, with colitis induction alone, or with colitis induction and rescue by WT or KO Tregs (Bar=70 μm) (B) Histology scores (n=7–12, combined from two independent experiments). * p<0.05 (t-test).