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Regulator of G protein signalling-1 (RGS1) selectively regulates gut T cell trafficking and colitic potential

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Abstract

The Regulator of G Protein Signaling 1 [*RGS1*] gene is associated with celiac disease, multiple sclerosis (MS) and Type I diabetes (T1D), which are all T cell-mediated pathologies. And yet there is no reported analysis of RGS1 biology in human T cells. This study shows that RGS1 expression is substantially higher in T cells from human gut *versus* peripheral blood, and that this can be exaggerated in intestinal inflammation. Elevated RGS1 levels profoundly reduce T cell migration to lymphoid-homing chemokines, whereas RGS-1 depletion selectively enhances such chemotaxis in gut T cells, and impairs their colitogenic potential. These findings provide a revised framework in which to view the linkage of RGS1 to inflammatory disease.

Introduction

For T cells to make regulated responses to infections within tissues, they must enter, reside within, and then egress from relevant sites. Chemokines play pivotal roles in such processes. In chronic inflammation, it may be argued that T cell egress in response to lymphoid-homing chemokines is impaired. Hence, the value of elucidating the factors regulating the selective chemotaxis of T cells. Being G-protein coupled receptors (GPCRs), chemokine receptors may be regulated by RGS molecules which bind Gα proteins, increasing their intrinsic GTPase activity up to 100 fold, and thereby attenuating signaling (1). *Rgs1* mRNA is highly enriched in murine gut *versus* lymphoid T cells (2-4), suggesting that it might likewise be enriched in human gut T cells where it might selectively regulate chemokine responses. However, while RGS1 was shown to regulate B cell chemotaxis, there has been no substantive study of it in human T cells.

Perhaps discouraging such analysis, splenic and lymph node T cells from *Rgs1*$^{-/-}$ mice show seemingly normal chemotaxis (5). Nonetheless, the strong associations of *RGS1* SNPs with T cell-mediated diseases including T1D, celiac disease, and MS (4,6-8) argue for it being an important T cell regulator. Therefore, this study investigates *RGS1* biology in human

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intestinal T cells, with supporting studies of *Rgs1*$^{-/-}$ mice. The data argue for a pivotal role of *RGS1* in T cell trafficking and tissue immunopathologies.

Materials and Methods

Human Samples

Mucosal biopsies and paired blood samples were from consented patients with ulcerative colitis (UC) or Crohn's disease (CD) undergoing ileocolonoscopy, or from control patients undergoing colonoscopy for polyp follow-up or diagnosis. PBMC were isolated using Ficoll (GE Healthcare) and gut intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) isolated as described (9). Cells were stained and flow-sorted into discrete populations. RNA was isolated, cDNA synthesis performed, and *RGS1*, *RGS2*, and *RGS10* mRNA assayed by Q-RT-PCR relative to cyclophilin A, using the following primers:

RGS1: TGTGCATTCAGATGCTGCTA and CTCGAGTGCGGAAGTCAATA;

RGS2: AAGATTGGAAGACCCGTTTG and GATGAAAGCTTGCTGTTTGC;

RGS10: CCCTGGAGAATCTGCTGGAAC and TTTCCTGCATCTGAGTCTTA;

cyclophilin A: TGGTGGACCCAACACAAATC and TGCCATCCAACCACTCAGTCT.

Mice

Wild type (WT) and *Rgs1*$^{-/-}$ C57BL/6 mice have been described (5); C57BL/6 *Rag2*$^{-/-}$ mice were from Taconic Farms. Mice were age-matched in all experiments.

T cell transfer model of colitis

0.5×10^6 naïve, CD4$^+$CD45RB^{hi} cells from either WT or *Rgs1*$^{-/-}$ C57BL/6 mice were injected intraperitoneally into *Rag2*$^{-/-}$ mice. Mice were weighed weekly, and culled 7-8 weeks post injection. Colons were removed, measured and subject to histology.

Transient transfections

Jurkat cells were transiently transfected with IRES-EGFP (Clontech) or RGS1-EGFP by electroporation, alone or with CCR7-tomato (pMIT-tomato, kind gift of H. Jumaa, Freiburg). Cells were left for 24-48hrs and washed before use. Primary human T cell blasts were transfected with IRES-EGFP, RGS1-EGFP or RGS10-EGFP (B isoform) using Amaxa II nucleofection.

Migration assays

Transfected Jurkat cells were placed in Transwell migration assays (5μM pore, Corning) for 2-3hrs at 37°C, and migrated cells enumerated by FACS Canto II (BD) analysis of 150μl harvested from the bottom chamber. Cells migrated to 200ng/ml human CXCL12 (ImmunoTools) or 500ng/ml human CCL19 (R&D Systems). Percentages of migrated cells were calculated by dividing the number of transmigrated EGFP^{hi}, EGFP^{low}, or EGFP$^{(-)}$ cells by the number of input cells with comparable EGFP levels. Murine intestinal T cells were isolated and placed in Transwell migration assays, and migrated cells (CD45$^+$, TCR$^+$) enumerated as above in response to recombinant murine CCL19 (50ng/ml), CCL20 (50ng/ml), CXCL12 (100ng/ml) all from ImmunoTools or CCL25 (50ng/ml, R&D Systems).

Confocal imaging

Transfected primary human T cell blasts were incubated overnight, washed and equilibrated on an ICAM-1 pre-coated μVI slide (Ibidi, Germany). 2×10^5 cells were added to each slide

chamber and imaged every 10 seconds for 20 minutes. Prior to image acquisition, 10μl of medium or recombinant human CXCL12 (10μg/ml) (R&D Systems) was applied to filter paper at one end of the chamber. Time-lapse films were analysed by Volocity (Improvision, UK), and $EGFP^+$ cells tracked automatically.

Results and Discussion

RGS1 over-expression in normal and inflamed gut

To determine whether human intestinal T cells, like their mouse counterparts, are enriched in *RGS1* expression, *RGS1* RNA was quantified in purified $TCR\alpha\beta^+CD4^+$ and $TCR\alpha\beta^+CD8\alpha\beta^+$ cells from peripheral blood (light diamonds) or gut biopsies (dark diamonds) (Fig 1A). Notwithstanding inter-individual variation common in human studies, *RGS1* was over-expressed in human gut T cells by an average of ~100 fold, as occurs in mice (2-4). *RGS2* was less overtly over-expressed (~15-fold), and *RGS10* was generally under-represented in gut T cells (Supplemental Figure S1A,B), again as is true in mice. RGS1 can regulate B cell migration to chemokines by targeting $G\alpha_i$ (10) whereas RGS2 preferentially targets $G\alpha_q$ (11), not commonly utilised by chemokine receptors. Hence, RGS1 became the primary focus for further studies.

Conventional ($CD4^+$; $CD8\alpha\beta^+$) and pooled unconventional ($CD4^{(-)}CD8^{(-)}$ "double negative" [DN], and $CD4^{(-)}$ $CD8\alpha\alpha^{(+)}$) αβ T cells from normal, CD, and UC gut mucosa (Fig 1B) revealed consistently high *RGS1* levels, and while many CD/UC samples fell within the normal range, some showed highly elevated expression, particularly for intestinal $CD4^+$ T cells ($p<0.04$). This is noteworthy, given that many such cells will have infiltrated the inflamed gut from the blood and lymphoid organs where *RGS1* levels are low (Fig 1A). Although such cells will be activated in the gut, activation alone of peripheral blood T cells evoked only a transient increase in RGS1 RNA, with peak levels ~10-fold less than in gut T cells (Supplemental Figure S1C). Moreover, RGS1 expression was not significantly different in naive *versus* memory CD4 T cells (Supplemental Figure S1D).

RGS1 decreases T cell migration to lymphoid-homing chemokines

To assess the functional significance of RGS1 over-expression, transwells were employed to assay T cell migration to chemokines. Because two human RGS1 transcripts, differing in coding potential by 13 amino acids, have been reported (5), Jurkat cells (which do not express RGS1) were independently transfected with each species linked *via* an internal ribosome entry site (IRES) to EGFP. Relative to control, $EGFP^+$, empty-vector transfectants, $EGFP^+$ RGS1 transfectants showed dose-dependent inhibition of migration to CXCL12, a lymphoid chemokine for the receptor CXCR4. Moreover, transfectants expressing lower levels of RGS1 ($EGFP^{low}$) were less compromised than $EGFP^{hi}$ cells expressing higher RGS1 (Fig 2A). From data collated from ten independent experiments, RGS1 significantly decreased migration to CXCL12 by >35% ($p<0.01$). Primary human peripheral blood T cells which express very little RGS1 were then engineered to over-express either RGS1 or RGS10, and their migration assessed by time-lapse microscopy (Fig 2B). Addition of CXCL12 to the bottom of the ICAM-1-coated slide redirected the random migration of empty-vector control or RGS10-transfected cells along the chemokine gradient (Fig 2B), with coincident decreases in migration velocity ($p\sim0.001$, Fig 2C). Conversely, RGS1-transfected cells did not align with the chemokine gradient (Fig 2B,C). Thus, elevating RGS1 limited CXCL12 responsiveness without grossly affecting normal kinesis.

To assess the capacity of RGS1 to regulate CCR7-mediated chemotaxis to CCL19, that promotes T cell egress from tissues to lymph nodes, Jurkat cells (which express very low levels of CCR7) were transfected with CCR7 (upstream of a tomato-tracker gene), resulting

in CCR7 surface expression (Fig 2D) and migration to CCL19 (Fig 2E). Co-transfection with RGS1 did not effect CCR7 levels (Fig 2D), but across ten independent experiments reduced migration to CCL19 by ~54% ($p<0.003$) (Fig 2E).

Having established that RGS1 is elevated in human gut T cells and can limit directional chemotaxis, the requirement for RGS1 in T cell migration was re-examined. Of note, the comparable migration to CCL19 reported for WT and *Rgs1*$^{-/-}$ splenic T cells (5) might simply reflect the negligible expression of RGS1 by splenic T cells. Consistent with this, intestinal T cells from *Rgs1*$^{-/-}$ mice showed significantly enhanced migration to CCL19 and CXCL12 relative to WT intestinal T cells. By contrast, migration to the gut-homing chemokine CCL25 (CCR9 ligand) or to CCL20 (CCR6 ligand) was unaffected (Figure 3). Hence, RGS1 specifically limited gut T cell responsiveness to the lymphoid-homing chemokines tested. Also, in two housing facilities, the gut-associated integrin, CD103, was found on significantly more CD4^{+} T cells ($p<0.008$) (Supplemental Figure S2A) at significantly higher levels ($p<0.037$), in the spleens of *Rgs1*$^{-/-}$ *versus* WT mice, perhaps reflecting enhanced intestinal egress.

To determine if these effects extrapolated to a role for *Rgs1* in intestinal immunopathology, three experiments in two housing facilities on two continents compared the colitogenic potentials of *Rgs1*$^{-/-}$ *versus* WT T cells after transfer to *Rag2*$^{-/-}$ mice (12). Invariably, colitis was ameliorated by *Rgs1* deficiency, as judged by significantly less increase in colon weight/thickness and by histopathology (Fig 4A, B). Recipients of *Rgs1-/-* cells usually lost less weight (Supplemental Fig 2B) although as has been noted previously (13) overall weight loss does not correlate well with colitis in this system. Reduced disease did not reflect generally impaired engraftment, since by contrast to the gut, spleens of recipient mice showed comparable accumulation of *Rgs1*$^{-/-}$ and WT T cells (Supplemental Figure S2C).

In sum, this first study of RGS1 biology in human T cells has identified overtly high expression in the gut; exaggerated expression in some IBD cases; a significant, non-redundant capacity to limit gut T cell responses lymphoid-homing chemokines, and a profound contribution to the colitogenic potential of T cells. These findings could be consistent with RGS1 ordinarily repressing T cell egress from the gut, possibly to sustain local immunoprotection and/or immunoregulation *vis-à-vis* commensals. Nonetheless, an analogous capacity to limit egress of inflammatory and/or autoimmune cells could clearly promote immunopathology. Such a key contribution of regulated T cell migration to pathology seems consistent with spontaneous colitis occurring in mice lacking $G\alpha_{i2}$, that reportedly opposes the effects of RGS1 in B cells (14,15). Additionally, the selective importance of RGS1 is strongly suggested by evidence that mice transgenic for *Rgs16*, which does not affect responses to CCL19 (16,17) and which is not substantively expressed in the gut (data not shown), display T cell hyper-responsiveness (as do $G\alpha_{i2}$ mutant mice) but no colon pathology.

Notwithstanding practical difficulties, it would seem appropriate to assess whether RGS1 is elevated in relevant tissue-associated T cells in T1D, MS, and celiac disease, all of which are associated with *RGS1* SNPs (4, 6-8). That such SNPs have not yet been associated with IBD may merely reflect the complex, multi-factorial nature of IBD, and should not diminish the prospect of RGS1 regulating disease initiation and/or progression. Indeed, RGS1 has been highlighted as a biomarker for undifferentiated spondyloarthritis (18), often associated with gastrointestinal lesions (19). Clearly, RGS1 appears a valid, tissue-associated target for further biological and clinical investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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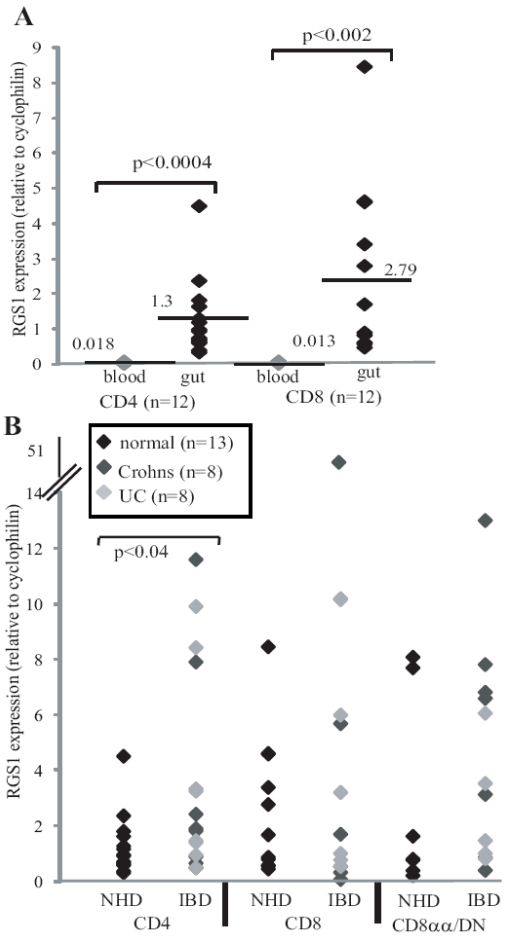

Fig 1.

(A) Paired samples of TCRαβ$^+$ CD4$^+$ or CD8αβ$^+$ cells from blood (light diamonds) or gut (black diamonds) of normal patients, were assessed for *RGS1* mRNA relative to cyclophilin A. Significance (by Student's T test) are shown for comparisons of blood and intestinal T cells. Note that *RGS1* mRNA levels were not statistically different between intestinal IEL and LPL (*data not shown*). **(B)** *RGS1* mRNA levels in gut CD4$^+$, or CD8αβ$^+$ or unconventional CD8αα$^+$/DN cells from normal donors (NHD, black diamonds) or patients with UC (light grey diamonds) or CD (dark grey diamonds).

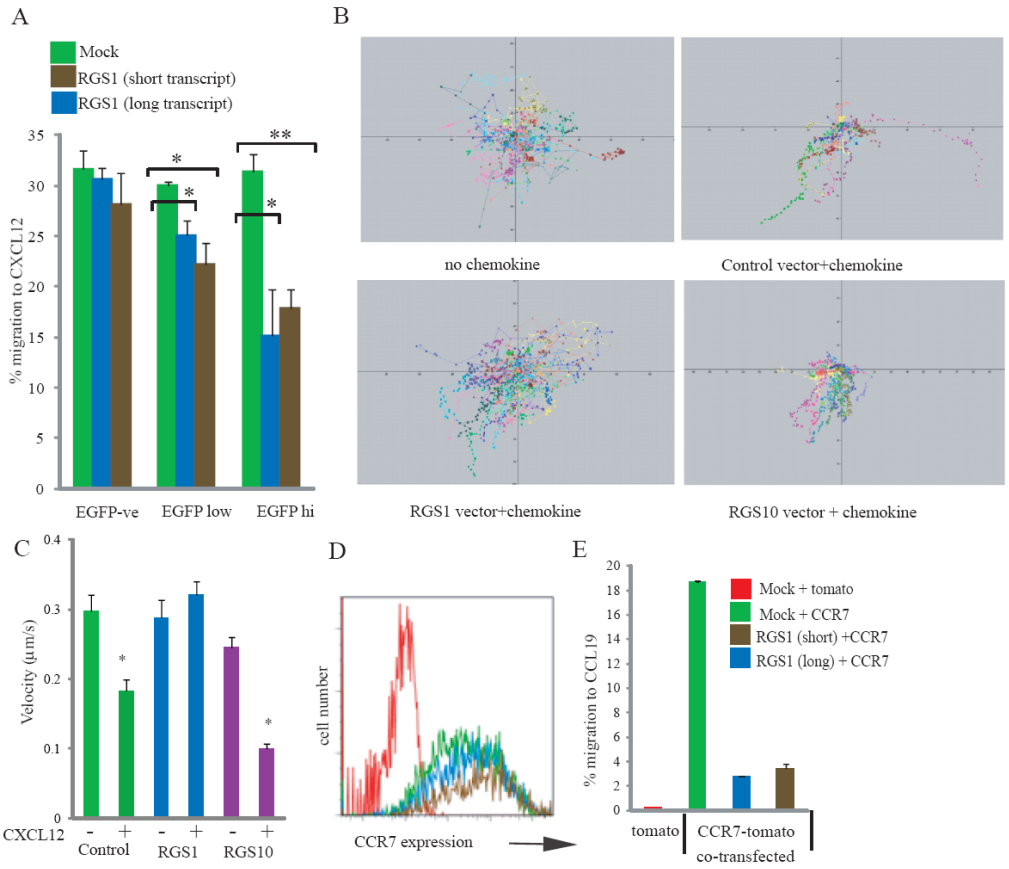

Fig 2.

(A). Jurkat cells transfected with EGFP control vector (green bars), short RGS1-EGFP (brown bars), or long RGS1-EGFP (blue bars) assessed in duplicate CXCL12-responsive migration assays. Specific migration for EGFP^{-ve}, EGFP^{low} or EGFP^{high} cells is shown from a representative experiment. Statistically significant differences (Student's T test) are highlighted: *$p<0.05$, **$p<0.02$.

(B, C) Peripheral blood T cells nucleofected with RGS1-EGFP, RGS10-EGFP, or control vector and assessed for CXCL12 responses: (**B)** migration tracks measured by time-lapse confocal imaging- cells migrated to chemokine added to the base of the slide; **(C)** velocities of cells transfected with the different vectors in the presence or absence of CXCL12. Statistics were performed using Kolmogorov-Smirnov comparisons, *$p<0.001$. **(D)** CCR7 expression levels on Jurkat cells transfected with control tomato vector and control EGFP vector (red line); CCR7-tomato and control EGFP vector (green); or CCR7-tomato co-transfected with either "short-RGS1"-EGFP (brown) or "long-RGS1"-EGFP (blue). **(E)** Specific migration to CCL19 of Jurkat cells transfected with CCR7-tomato in the presence or absence of *RGS1* isoforms.

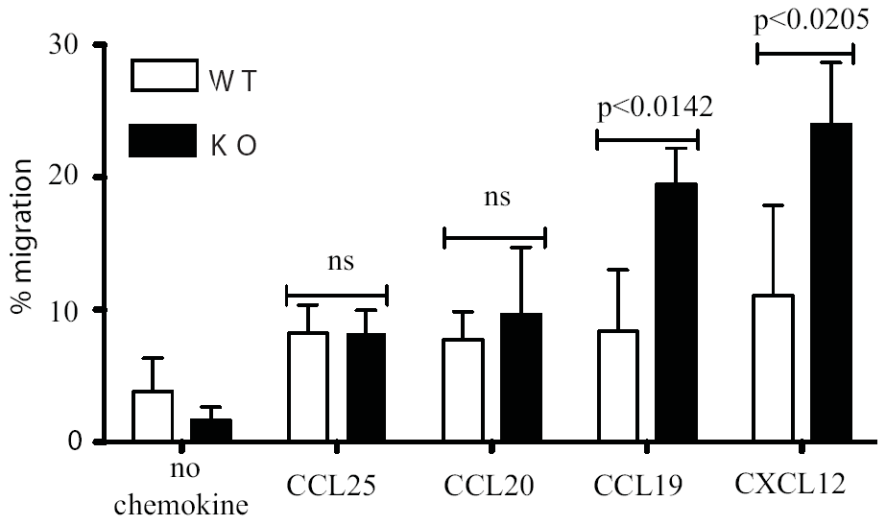

Fig 3.

Migration of intestinal T cells (mean of three independent experiments) isolated from WT (open bars) or *Rgs1*$^{-/-}$ mice (dark bars) in response to different chemokines.

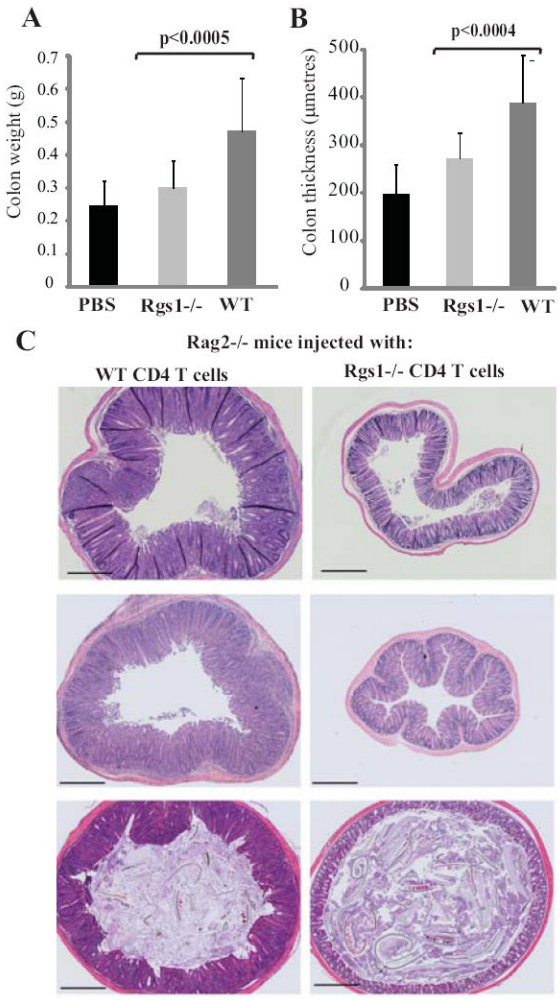

Fig 4.

Rag2$^{-/-}$ mice were injected in three separate experiments with either PBS (n=6) or CD4^{+} CD45RB^{hi} T cells extracted from WT (n=13) or *Rgs1*$^{-/-}$ mice (n=11). Colons were removed 7-8 weeks later, weighed and sectioned. Mean colon weight (**A**) and medial colon thickness **(B)** in recipients of WT (dark grey bars) or *Rgs1*$^{-/-}$ T cells (light grey bars) or PBS (black bars): statistics by Student's T test. **(C)** Representative mid colon histologies at 7 weeks for recipients of WT (left) or *Rgs1*$^{-/-}$ cells (right). One colon for each group is shown from each of three independent experiments. Scale bar =500μm.