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Published in:
Gastroenterology

DOI:
10.1053/j.gastro.2019.07.007

Publication date:
2019

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

Gastroenterology 2019;157:1293–1309

Inhibiting PGGT1B Disrupts Function of RHOA, Resulting in T-cell Expression of Integrin $\alpha4\beta7$ and Development of Colitis in Mice

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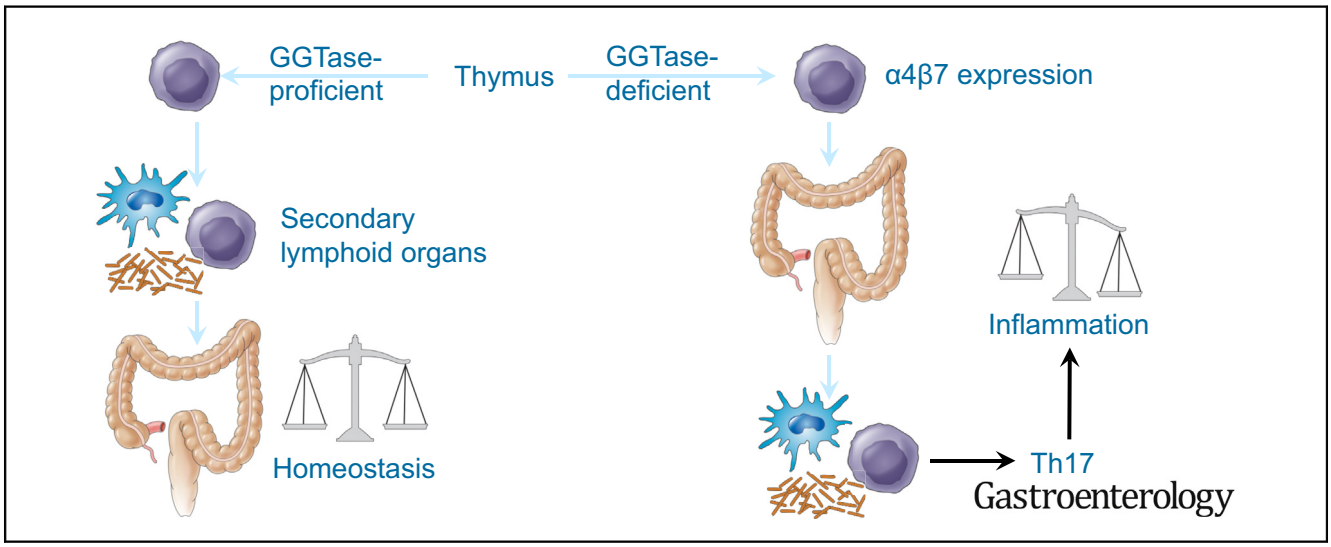


BACKGROUND & AIMS: It is not clear how regulation of T-cell function is altered during development of inflammatory bowel diseases (IBD). We studied the mechanisms by which geranylgeranyltransferase-mediated prenylation controls T-cell localization to the intestine and chronic inflammation. **METHODS:** We generated mice with T-cell–specific disruption of the geranylgeranyltransferase type I, beta subunit gene (*Pggt1b*), called $Pggt1b^{\Delta CD4}$ mice, or the ras homolog family member A gene (*Rhoa*), called $Rhoa^{\Delta CD4}$ mice. We also studied mice with knockout of CDC42 or RAC1 and wild-type mice (controls). Intestinal tissues were analyzed by histology, multiphoton and confocal microscopy, and real-time polymerase chain reaction. Activation of CDC42, RAC1, and RHOA were measured with G-LISA, cell fractionation, and immunoblots. T cells and lamina propria mononuclear cells from mice were analyzed by flow cytometry or transferred to $Rag1^{-/-}$ mice. Mice were given injections of antibodies against integrin alpha4beta7 or gavaged with the RORC antagonist GSK805. We obtained peripheral blood and intestinal tissue samples from patients with and without IBD and analyzed them by flow cytometry. **RESULTS:** $Pggt1b^{\Delta CD4}$ mice developed spontaneous colitis, characterized by thickening of the intestinal wall, edema, fibrosis, accumulation of T cells in the colon, and increased expression of inflammatory cytokines. Compared with control CD4+ T cells, PGGT1B-deficient CD4+ T cells expressed significantly higher levels of integrin alpha4beta7, which regulates their localization to the intestine. Inflammation induced by transfer of PGGT1B-deficient CD4+ T cells to $Rag1^{-/-}$ mice was blocked by injection of an antibody against integrin alpha4beta7. Lamina propria of $Pggt1b^{\Delta CD4}$ mice had increased numbers of CD4+ T cells that expressed RORC and higher levels of cytokines produced by T-helper 17 cells (granulocyte-macrophage colony-stimulating factor, interleukin [IL]17A, IL17F, IL22, and tumor necrosis factor [TNF]). The RORC inverse agonist GSK805, but not antibodies against IL17A or IL17F, prevented colitis in $Pggt1b^{\Delta CD4}$ mice. PGGT1B-deficient CD4+ T cells had decreased activation of RHOA. $RhoA^{\Delta CD4}$ mice had a similar phenotype to $Pggt1b^{\Delta CD4}$ mice, including development of colitis, increased numbers of CD4+ T cells in colon, increased expression of integrin alpha4beta7 by CD4+ T cells, and increased levels of IL17A and other inflammatory cytokines in lamina propria. T cells isolated from intestinal tissues from patients with IBD had significantly lower levels of PGGT1B than tissues from individuals without IBD.

CONCLUSION: Loss of PGGT1B from T cells in mice impairs RHOA function, increasing CD4+ T-cell expression of integrin alpha4beta7 and localization to colon, resulting in increased expression of inflammatory cytokines and colitis. T cells isolated from gut tissues from patients with IBD have lower levels of PGGT1B than tissues from patients without IBD.

Keywords: Mouse Models; Prenylation; IBD; Immune Regulation.

Tissue homeostasis in the gut is guaranteed by a complex interaction between the different cellular compartments of the intestinal mucosa.[1] As a first immunological barrier, the epithelium segregates the luminal content from the plethora of immune cells in the subepithelial space.[2] However, altered local immune responses within the *lamina propria* trigger an overwhelming activation of inflammatory immune cells, which might promote chronic inflammatory disorders, like in inflammatory bowel disease (IBD).[1] In particular, the function of gut-infiltrating T cells has been identified as a key player in IBD pathogenesis. Accordingly, interfering with exacerbated effector T-cell responses could successfully be established as therapeutic approaches in the clinical management of patients with IBD.[3]

Prenylation is defined as the posttranslational attachment of isoprenoids to target proteins, relevantly affecting protein function.[4] The recent identification of prenylation as a novel intrinsic regulator of epithelial integrity[5] implicates that therapy strategies that are able to improve the prenylation status of proteins in intestinal epithelial cells or to trigger the function of specific downstream target proteins may allow epithelial restoration in IBD.[6] Therefore, an improved understanding of prenylation-dependent signaling cascades in nonepithelial cells, such as the T cells, and their impact on the chronic inflammatory process needs to be achieved.

Recent mouse studies demonstrated that lymphocyte-specific genetic deletion of prenylation upstream regulators (HMGCoR)[7] or downstream targets (CDC42, RAC1, RHOA)[8–10] influence T-cell function. However, it remained unclear whether the fate of T cells in the context of chronic inflammation might depend on prenylation. We now analyzed mice lacking the prenylation-catalyzing enzyme PGGT1B[11] within T cells, and could demonstrate that prenylation controls localization and effector function of lymphocytes. The development of a spontaneous colitis in mice carrying PGGT1B-deficient T cells indicates that gut mucosal homeostasis depends on prenylation in T cells. Among prenylated proteins, we could demonstrate that RHOA plays a crucial role in localization to the intestine and inflammatory capacity of T cells. Together with our previous study, this strongly supports the idea that patients with IBD might benefit from a therapeutic induction of prenylation or improved RHOA function within the gut mucosa, which could potentially carry a double beneficial effect by acting on adaptive and innate immune cells.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Although T cells have been identified as crucial regulators of chronic intestinal inflammation in inflammatory bowel diseases, the molecular factors driving altered T cell responses in IBD are incompletely understood.

NEW FINDINGS

GGTaseI-mediated prenylation was identified as a new regulator of T cell trafficking and effector function. The small GTPase RhoA was able to link defects in GGTaseI-mediated prenylation with an augmented $\alpha4\beta7$-dependent colon tropism and pro-inflammatory effector function of T lymphocytes.

LIMITATIONS

Further studies are needed in order to describe the molecular mechanism behind local activation of GGTaseI-deficient T cells within the gut mucosa.

IMPACT

Of particular relevance in the context of colitis gut homeostasis and IBD pathogenesis, *Pggt1b*$^{\Delta CD4}$ mice spontaneously developed a chronic colitis and lamina propria T cells of IBD patients could be characterized by decreased GGTaseI expression.

Materials and Methods

Animals

Pggt1b^{flx},[12] *Cdc42*^{flx},[13] *Rac1*^{flx},[14] and *Rhoa*^{flx}[15] mice were crossbred with CD4Cre mice,[16] to generate T-cell–specific deletion of *Pggt1b*, *Cdc42*, *Rac1,* or *Rhoa*. *Pggt1b*$^{\Delta CD4}$ mice were crossbred with ROSA26-tdTomato mice[17] to generate reporter mice. Animal studies were conducted in a gender- and age-matched manner using littermates as control.

Human Samples

Human blood and tissue samples were obtained from Department of Medicine 1, University Hospital Erlangen (Germany).

Mouse Endoscopy

Mouse gut status was scored by high-resolution video endoscopy as described recently.[18]

Thymocyte Transfer

Isolated thymocytes (mice $\leq$ 10 weeks of age) were stained with carboxyfluorescein succinimidyl ester and injected

§ Authors share co-senior authorship.

Abbreviations used in this paper: GGTI, geranylgeranyltransferase inhibitor; GTP, guanosine-triphosphate; IBD, inflammatory bowel disease; IL, interleukin; LPMCs, lamina propria mononuclear cells; SP, single positive.

Most current article

0016-5085
https://doi.org/10.1053/j.gastro.2019.07.007

intravenously into *Rag1*$^{-/-}$ mice (10^7 cells/mouse). Two hours later, tissues were collected and analyzed. In the repetitive adoptive thymocyte transfer, thymocytes were injected twice per day on 3 consecutive days; tissues were collected the day after the last injection.

Lamina Propria Mononuclear Cell (LPMC) transfer

Freshly isolated LPMCs (mice $\leq$ 10 weeks of age) were injected intraperitoneally into *Rag1*$^{-/-}$ mice (0.5×10^6 cells/mouse). Tissues were analyzed 4 weeks after cell transfer.

Anti-$\alpha 4\beta 7$ Treatment

Mice were treated with 200 μg of LPAM-1 (BE0034; BioXCell, West Lebanon, NH) or corresponding isotype control antibodies via intraperitoneal injection 3 times per week from 4 until 15 weeks of age. In the LPMC transfer model, treatment with LPAM-1 started on the day of adoptive cell transfer.

GSK805 Treatment

Mice were treated with the RORC inverse agonist GSK805 (10 mg/kg; Merck-Millipore, Burlington, MA) or corresponding vehicle control via oral gavage 3 times per week from 4 until 15 weeks of age. GSK805 was dissolved in dimethyl sulfoxide and then suspended in corn oil.

Intravital Microscopy

The intestine of anaesthetized mice was prepared for intravital microscopy, as described recently.[19] Time sequential Z-stacks images were taken with a confocal microscope (SP8; Leica, Wetzlar, Germany).

Cell Culture

Cells incubated in RPMI-1640 with GlutaMAX medium containing FBS (10%) and 100 U mL^{-1} penicillin, were stimulated with anti-CD3 and anti-CD28 (1 μg/mL) or PMA (50 ng mL^{-1}) and ionomycin (750 ng mL^{-1}) with or without brefeldin A (10 μg mL^{-1}). Geranylgeranyltransferase inhibitor (GGTI)-298 (3 μM) or simvastatin (5 μM) were added 1 hour before stimulation; RHO (CN03) or RAC1/CDC42 (CN02) activators (Cytoskeleton, Inc, Denver, CO) were added 10 minutes before GGTI-298 treatment.

Flow Cytometry

Cells were stained in the presence of anti-mouse CD16/32 (Biolegend, San Diego, CA) or FcR block (Miltenyi, Bergisch Gladbach, Germany), with specific antibodies (Supplementary Table 1). The Foxp3 Transcription Factor Staining Buffer Set (eBioscience, San Diego, CA) was used for intracellular staining. Cells were acquired using a BD LSRFortessa (Beckton Dickinson, Franklin Lakes, NJ) or MQ10 (Miltenyi Biotec, Bergisch Gladbach, Germany).

Histology

Hematoxylin-eosin–stained tissue samples were scored in a blinded fashion, taking into account "inflammatory cell infiltrate" (0–4), "epithelial changes" (0–5), and "mucosal architecture" (0–5); resulting in a total score from 0 to 14.

Immunofluorescence Staining

Tissue cryosections were stained with specific antibodies (Supplementary Table 2) and biotinylated-Streptavidin antibody pairs. Pictures were taken with upright or inverted confocal microscopes (Leica). Image quantification; cells per field; 3 fields per sample; $\times$20 magnification.

Label-free Multiphoton Microscopy

Multiphoton microscopy with spectral separation was performed with an upright system (TriM-Scope II; LaVision BioTec GmbH, Bielefeld, Germany) equipped with a femtosecond Ti-Sa-laser and a HC Fluotar L25x/0.95 W Visir objective (Leica). The emission spectrum was separated by dichroic mirrors and filters to detect the specific tissue signals in 3 photomultiplier tubes. Second harmonics generation signals of collagen were collected at ranges 395 to 415 nm (blue) and autofluorescence of NADH at 415 to 485 nm (green). tdTomato emission and autofluorescence of flavins were detected at 540 to 580 nm (red). The field of view was 400 μm $\times$ 400 μm. The excitation wavelength was 800 nm.

Gene Expression Analysis

Total RNA was isolated (NucleoSpin RNA; Macherey-Nagel, Duren, Germany) and retro-transcribed into complementary DNA (Bio-Rad, Hercules, CA). Gene expression was measured by real-time polymerase chain reaction (Bio-Rad) with SyberGreen and Quantitect primers (Qiagen, Hilden, Germany). Gene expression was normalized to HPRT expression.

Immunoblotting

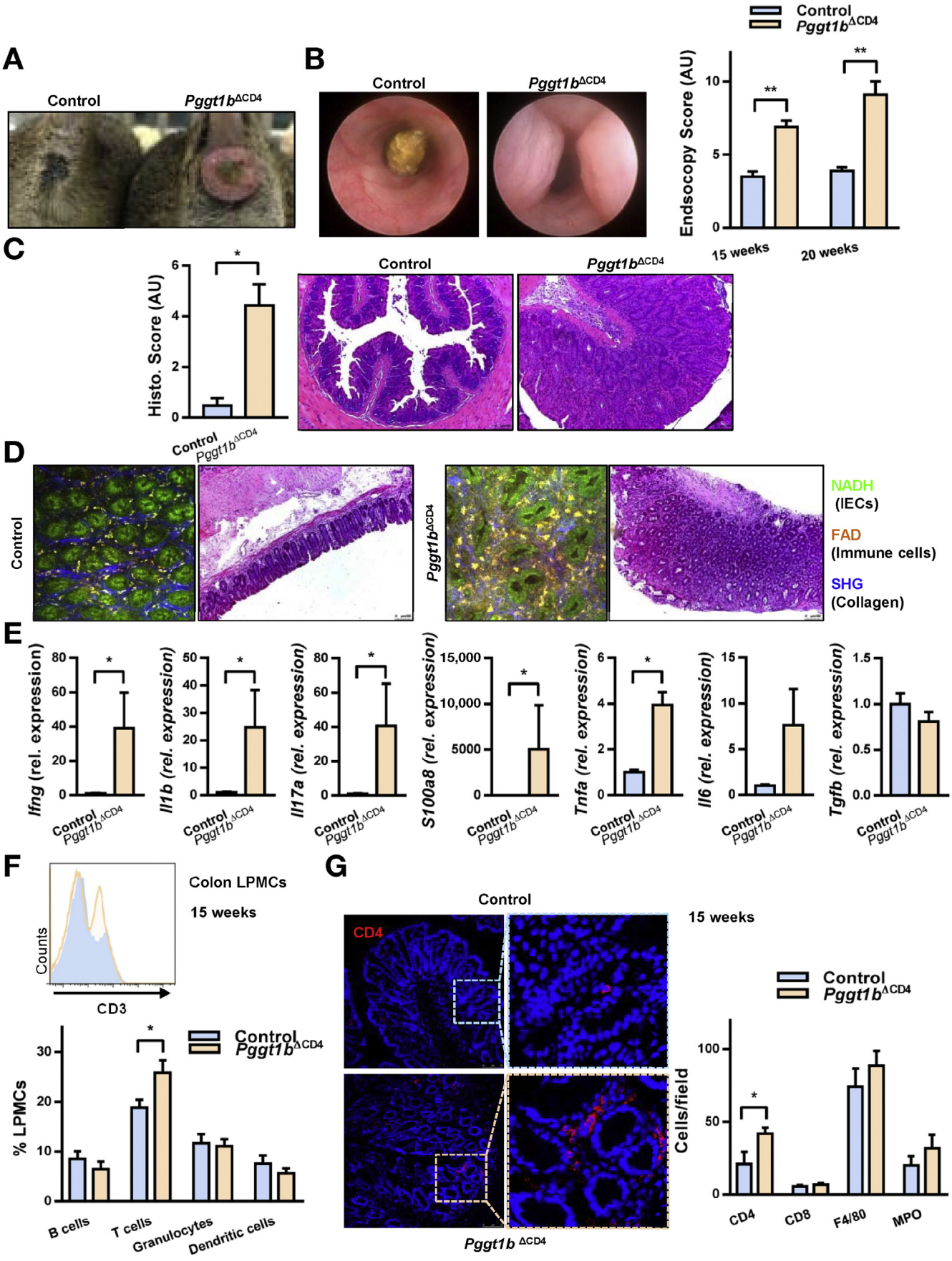
Protein extracts were obtained by incubation in mammalian protein extraction reagent (Thermo Scientific, Waltham, MA). For membrane fraction separation, lysis buffer without detergents, and high-speed centrifugation steps were used. Denaturized proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto nitrocellulose membranes. Specific (Supplementary Table 3) and horseradish peroxidase–linked antibodies were used for chemoluminescence detection. Protein expression was compared with the level of beta-actin.

Small Guanosine-Triphosphate (GTP)ases Activation Assay

CDC42, RAC1, and RHOA activation status was measured with G-LISA Activation Assay (Cytoskeleton).

Statistics

Data are expressed as mean $\pm$ SEM. Significance analysis was performed using 1-tailed Student t test, except where indicated. For multiple comparisons, data were analyzed by 1-way analysis of variance and least significance difference post hoc test. *$P \leq .05$; **$P \leq .001$; ***$P \leq .0001$ versus corresponding control.


A
Control
Pggt1b ΔCD4
B
Control
Pggt1b ΔCD4
Control
Pggt1b ΔCD4
Endsocopy Score (AU)
15 weeks
20 weeks
C
Histo. Score (AU)
Control
Pggt1b ΔCD4
Control
Pggt1b ΔCD4
D
Control
Pggt1b ΔCD4
NADH
(IECs)
FAD
(Immune cells)
SHG
(Collagen)
E
Ifng (rel. expression)
Il1b (rel. expression)
Il17a (rel. expression)
S100a8 (rel. expression)
Tnfa (rel. expression)
Il6 (rel. expression)
Tgfb (rel. expression)
F
Colon LPMCs
15 weeks
Counts
CD3
% LPMCs
B cells
T cells
Granulocytes
Dendritic cells
G
Control
15 weeks
CD4
Pggt1b ΔCD4
Cells/field
CD4
CD8
F4/80
MPO

Results

PGGT1B-deficient T Cells as Potent Inducers of Chronic Colitis

To analyze the role of prenylation in adaptive immune responses, we generated T-cell–specific conditional knockout mice of geranylgeranyltransferase type I, beta subunit gene (*Pggt1b*$^{\Delta CD4}$ mice). Cre-mediated *Pggt1b* deletion in *Pggt1b*$^{\Delta CD4}$ mice affects specifically the T-cell compartment (Supplementary Figure 1*A*), leading to downregulated targeted gene expression in thymocytes (Supplementary Figure 1*B*). Consistently, we could show decreased PGGT1B protein expression and inhibition of prenylation in thymus (Supplementary Figure 1*C*). Thus, we confirmed that *Pggt1b*$^{\Delta CD4}$ mice carry a T-cell–specific deficiency of PGGT1B.

Although they did not demonstrate any modification of their general status up to 10 weeks of age, *Pggt1b*$^{\Delta CD4}$ mice develop a rectal prolapse (Figure 1*A*), which was associated with spontaneous development of colitis starting at 10 weeks of age and being evident in all 15-week-old *Pggt1b*$^{\Delta CD4}$ mice. High-resolution mini-endoscopy pictures showed thickening of the intestinal wall, edema, and tissue fibrosis (Figure 1*B*), whereas immune cell infiltration in the mucosa and crypt elongation became evident in colon histology from *Pggt1b*$^{\Delta CD4}$ mice (Figure 1*C*). Label-free multiphoton microscopy confirmed the massive colonic infiltration of immune cells and severe tissue architecture distortion (Figure 1*D*). Despite high-grade inflammation in the colon, we could not observe any alterations in the small intestine of *Pggt1b*$^{\Delta CD4}$ mice (Supplementary Figure 1*D*). The colitis in *Pggt1b*$^{\Delta CD4}$ mice went along with increased gene expression of inflammatory mediators (interferon-γ, IL1β, IL17A, S-100A8, and tumor necrosis factor [TNF]) (Figure 1*E*; Supplementary Figure 1*E*).

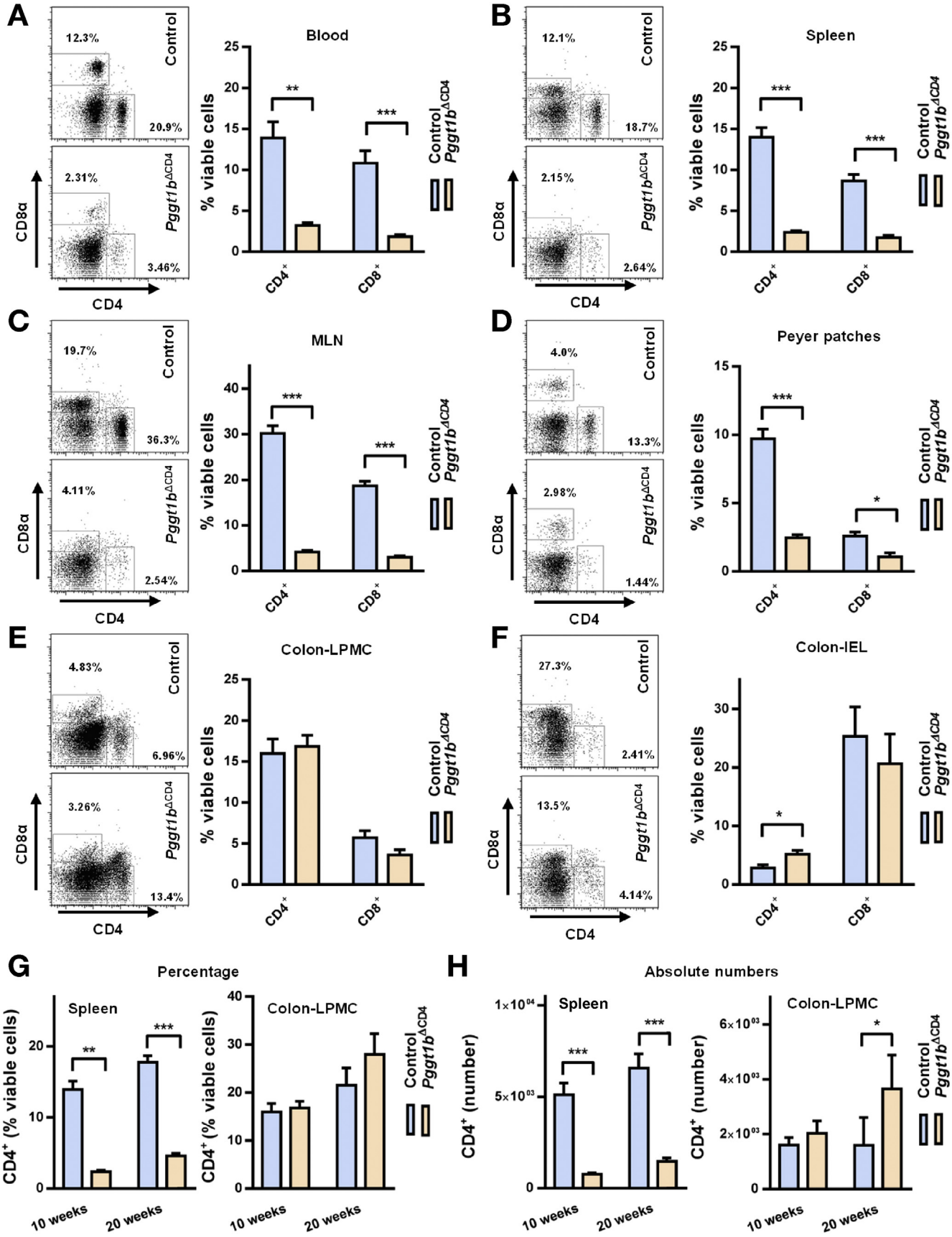
To characterize the infiltrating immune cells in the *lamina propria* of colitic *Pggt1b*$^{\Delta CD4}$ mice, specific cell populations were quantified. We noted a significant accumulation of CD3+ T cells in the colon of *Pggt1b*$^{\Delta CD4}$ mice as compared with wild-type controls (Figure 1*F*); however, no significant changes in the number of B cells, granulocytes, and dendritic cells were noted (Figure 1*F*; Supplementary Figure 1*F*). Immunostaining analysis demonstrated increased numbers of colon-infiltrating CD4+ rather than CD8+ T cells in *Pggt1b*$^{\Delta CD4}$ mice (Figure 1*G*; Supplementary Figure 1*G*). Taking advantage of reporter *Pggt1b*$^{\Delta CD4}$-tdTomato mice, we could demonstrate that colitis in *Pggt1b*$^{\Delta CD4}$ mice is associated with an accumulation of tdTomato-expressing PGGT1B-deficient T cells in the intestinal *lamina propria* (Supplementary Figure 1*H*). Finally, monitoring of *Pggt1b*$^{\Delta CD4}$ mice indicated that the colonic inflammation increased over time (Supplementary Figure 1*I*–*K*) and pointed to the spontaneous development of an age-dependent chronic colitis mediated by gut-infiltrating PGGT1B-deficient CD4$^+$ T cells.

Altered Lymphocyte Localization in Young *Pggt1b*$^{\Delta CD4}$ Mice

To identify cellular and molecular events, which might mechanistically underlie the spontaneous development of colitis in *Pggt1b*$^{\Delta CD4}$ mice, we analyzed organ histology and immune cell distribution in young animals ($\leq$10 weeks of age). Although endoscopic and histology images did not yet show any inflammatory alterations of colon, ileum, kidney, liver, and lung (Supplementary Figure 2*A* and *B*), some alterations could already be identified in thymus and spleen of young *Pggt1b*$^{\Delta CD4}$ mice (Supplementary Figure 2*C*). Enlarged cortex areas in the thymus along with disorganized and smaller T-cell regions in the spleen of *Pggt1b*$^{\Delta CD4}$ mice were associated with significantly decreased numbers of CD4+ and CD8+ T cells in the peripheral blood and the spleen, and a slight accumulation of mature CD4+ in the thymic medulla (Figure 2*A* and *B*; Supplementary Figure 2*D*). We excluded impaired thymic T-cell development in the absence of PGGT1B, because we detected normal numbers of CD4-CD8 double-positive and double-negative cells, with even a tendency toward an increased percentage of fully developed CD4–single-positive (SP) T cells (*P* value = .136) (Supplementary Figure 2*E*). Moreover, increased expression of CD62L on thymocytes from *Pggt1b*$^{\Delta CD4}$ mice indicated that PGGT1B-deficient T cells are able to acquire a mature phenotype (Supplementary Figure 2*F*). In contrast to recent publications describing a massive induction of cell death in thymocytes upon indirect inhibition of prenylation,[7] we could only identify a small increase in Annexin$^+$PI$^-$ early apoptotic cells in thymus from *Pggt1b*$^{\Delta CD4}$ mice compared with control mice (Supplementary Figure 2*G*). Thus, our data implicate that *Pggt1b*$^{\Delta CD4}$ mice are not characterized by a major defect in lymphocyte maturation, but altered trafficking properties of thymus-egressed T cells.

To validate this hypothesis and verify its causative involvement in the intestinal pathology of *Pggt1b*$^{\Delta CD4}$ mice, we next analyzed the capacity of PGGT1B-deficient T cells to infiltrate into the gut and gut-associated lymphoid tissue. Despite significantly decreased T-cell populations in mesenteric lymph nodes, Peyer patches, and in small intestine (Figure 2*C* and *D* and Supplementary Figure 2*H*), the percentage of T cells in the colonic lamina propria and intraepithelial compartment were normal in *Pggt1b*$^{\Delta CD4}$ mice (Figure 2*E* and *F*). In accordance with our analyses in elder mice (Figure 1*F* and *G*), an age-dependent colonic accumulation of PGGT1B-deficient CD4+ T cells, but not CD8+ T cells could be observed (Figure 2*G* and *H*;

Figure 1. Spontaneous colitis in *Pggt1b*$^{\Delta CD4}$ mice. Colonic phenotype in *Pggt1b*$^{\Delta CD4}$ mice ($\geq$15 weeks). (*A*) Rectal prolapse. (*B*) Endoscopy (n $\geq$ 5). (*C*) Hematoxylin-eosin (H&E) staining (n $\geq$ 5). (*D*) Label-free multiphoton microscopy; H&E staining (same area). (*E*) Gene expression in colon tissue (n $\geq$ 7). Nonparametric Mann-Whitney *U* test. (*F*) Immune cell infiltration in LPMCs (gated on viable cells) (n $\geq$ 4). (*G*) CD4, CD8, F4/80, and myeloperoxidase (MPO) immunostaining (n $\geq$ 9). AU, arbitrary units.


A
12.3%
Control
20.9%
2.31%
Pggt1b^ΔCD4
3.46%
CD8α
CD4
Blood
% viable cells
**
***
Control
Pggt1b^ΔCD4
CD4+
CD8+
B
12.1%
Control
18.7%
2.15%
Pggt1b^ΔCD4
2.64%
CD8α
CD4
Spleen
% viable cells
***
***
Control
Pggt1b^ΔCD4
CD4+
CD8+
C
19.7%
Control
36.3%
4.11%
Pggt1b^ΔCD4
2.54%
CD8α
CD4
MLN
% viable cells
***
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Control
Pggt1b^ΔCD4
CD4+
CD8+
D
4.0%
Control
13.3%
2.98%
Pggt1b^ΔCD4
1.44%
CD8α
CD4
Peyer patches
% viable cells
***
*
Control
Pggt1b^ΔCD4
CD4+
CD8+
E
4.83%
Control
6.96%
3.26%
Pggt1b^ΔCD4
13.4%
CD8α
CD4
Colon-LPMC
% viable cells
Control
Pggt1b^ΔCD4
CD4+
CD8+
F
27.3%
Control
2.41%
13.5%
Pggt1b^ΔCD4
4.14%
CD8α
CD4
Colon-IEL
% viable cells
*
Control
Pggt1b^ΔCD4
CD4+
CD8+
G
Percentage
Spleen
CD4+ (% viable cells)
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10 weeks
20 weeks
Colon-LPMC
CD4+ (% viable cells)
10 weeks
20 weeks
Control
Pggt1b^ΔCD4
H
Absolute numbers
Spleen
CD4+ (number)
1×10^04
5×10^03
***
***
10 weeks
20 weeks
Colon-LPMC
CD4+ (number)
6×10^03
4×10^03
2×10^03
*
10 weeks
20 weeks
Control
Pggt1b^ΔCD4

Supplementary Figure 2*I*), and implicated that downregulation of prenylation determines colon localization of T-helper cells. On the other hand, decreased frequencies of T cells in the periphery in *Pggt1b*$^{\Delta CD4}$ mice were associated with increased cell death in spleen but not in colon tissue (Annexin V/PI assay) (Supplementary Figure 2*J*) and TUNEL staining (Supplementary Figure 2*K*). We can assume that the increased percentages of apoptotic CD4-SP and CD8-SP thymocytes (Supplementary Figure 2*G*) and the cell death induction in the spleen (Supplementary Figure 2*J* and *K*) was not functionally relevant for T-cell gut trafficking.

Thymus-egressing PGGT1B-deficient T Cells Are Primed for Selective Localization to the Intestine

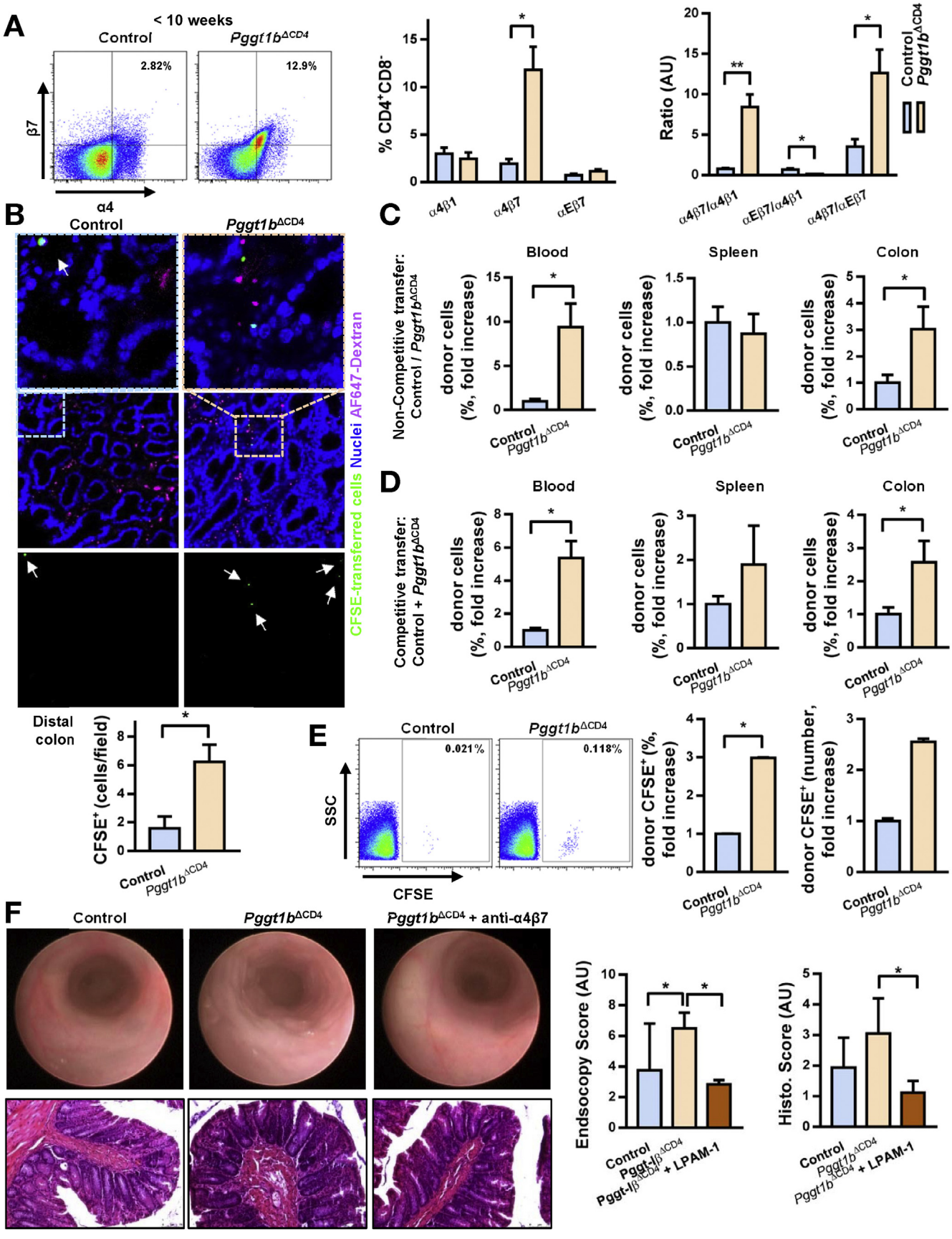
Our data so far suggested that thymus-egressing PGGT1B-deficient lymphocytes are already primed for selective localization to the colon. Indeed, PGGT1B-deficient CD4-SP thymocytes showed significantly increased expression levels of the gut homing marker $\alpha4\beta7$ integrin, but not $\alpha E\beta7$ or CCR9 (Figure 3*A*; Supplementary Figure 3*A* and *B*). As the $\alpha4\beta1$ integrin has been found to control localization of CD4+ T cells to the small intestine,[20] we also compared the presence of $\alpha4\beta7$ and $\alpha4\beta1$ integrins and found a significantly elevated $\alpha4\beta7/\alpha4\beta1$ ratio in CD4+ T cells from *Pggt1b*$^{\Delta CD4}$ mice (Figure 3*A*). We next analyzed KLF2-mediated expression of S1P1R as the main pathway regulating maturation and exit of thymocytes. The expression of S1P1 on cells from thymus or blood did not differ between control and *Pggt1b*$^{\Delta CD4}$ mice (Supplementary Figure 3*C*), and *Klf2* gene expression was even increased in thymus from *Pggt1b*$^{\Delta CD4}$ mice (Supplementary Figure 3*D*). Interestingly, the expression of SP1/KLF transcription factors has been linked to the regulation of integrin expression.[21] Moreover, a previous study could show that inhibition of PGGT1B-mediated prenylation led to RHOA-dependent activation of the transcription factor SP1.[22] Hypothetically, KLF2/SP1-mediated transcription activation could then explain the molecular mechanism behind increased expression of $\beta7$ integrin in PGGT1B-deficient T cells. Indeed we could demonstrate that upregulated KLF2 expression in the thymus of *Pggt1b*$^{\Delta CD4}$ mice was associated with an increased expression and phosphorylation of SP1 (Supplementary Figure 3*D*).

These data supported the idea that mature thymus-egressing PGGT1B-deficient T cells express $\alpha4\beta7$ integrin and are thus predisposed for selective localization to the colon. Thus, we adoptively transferred fluorescence-labeled thymocytes into *Rag1*$^{-/-}$ mice and monitored their colon localization capacity by intravital microscopy. An increased number of T cells could be retrieved in the distal colon mucosa of recipient animals receiving thymocytes from *Pggt1b*$^{\Delta CD4}$ mice compared with those adoptively transferred with wild-type thymocytes, but not in the proximal colon (Figure 3*B*; Supplementary Figure 3*E*). Taking advantage of *Pggt1b*$^{\Delta CD4}$-tdTomato reporter mice, we were able to track transferred PGGT1B-deficient carboxyfluorescein succinimidyl ester-positive^{+}-tdTomato^{+} thymocytes in recipient mice (Supplementary Figure 3*F*). Higher numbers of PGGT1B-deficient than GGTase-I-proficient donor cells could be detected in colon and blood, but not in spleen of recipient animals (Figure 3*C*). These data also could be confirmed when thymocytes from *Pggt1b*$^{\Delta CD4}$-tdTomato and control mice were transferred competitively into the same recipient mouse (Figure 3*D*). Finally, to mimic the physiological continuous T-cell influx from the thymus, we performed a repetitive thymocyte transfer. We could demonstrate accumulation of PGGT1B-deficient T cells in blood and colon, but not in spleen (Figure 3*E*; Supplementary Figure 3*G*). In summary, the in vivo observed organ distribution of transferred PGGT1B-deficient thymocytes was in line with the assumption that downregulation of prenylation promotes the capacity of thymus-egressed T cells to localize to the colon. To validate the functional relevance of altered T-cell localization for the intestinal inflammation in *Pggt1b*$^{\Delta CD4}$ mice, we performed an antibody-mediated in vivo blockade of $\alpha4\beta7$ in *Rag1*$^{-/-}$ mice adoptively transferred with LPMC derived from *Pggt1b*$^{\Delta CD4}$ mice. Indeed, the pro-colitogenic capacity from LPMC containing PGGT1B-deficient T cells could efficiently be dampened by administration of the $\alpha4\beta7$ blocking antibody LPAM-1 (Figure 3*F*). These data convincingly confirmed the increased $\alpha4\beta7$ expression of PGGT1B-deficient T cells as a key driver of colitis development in *Pggt1b*$^{\Delta CD4}$ mice.

Inflammatory Gut-Infiltrating PGGT1B-deficient T Cells Produce Th17 Cytokines and Express RORC

Despite the observed causative link between the colonic accumulation of PGGT1B-deficient CD4+ T lymphocytes and the spontaneous colitis in *Pggt1b*$^{\Delta CD4}$ mice, it was still unclear whether the inflammatory effector function of colon-infiltrating lymphocytes might also depend on prenylation. To focus on primary inflammation-independent effects, the following analyses were mainly performed in *Pggt1b*$^{\Delta CD4}$ mice not yet suffering from colitis. Despite a comparable activation status of PGGT1B-proficient and -deficient lamina propria CD4+ T cells in the colon (CD69/CD62L expression) (Supplementary Figure 4*A*), colon-infiltrating CD4+ T cells in *Pggt1b*$^{\Delta CD4}$ mice were characterized by a significantly increased expression of CD38, which has been described as a potential marker for recently activated T cells[23]

Figure 2. T-cell compartment in *Pggt1b*$^{\Delta CD4}$ mice. (*A–F*) CD4+ and CD8+ cells (gated on viable cells). (*A*) Blood ($n \geq 14$). (*B*) Spleen ($n \geq 10$). (*C*) Mesenteric lymphoid nodes (MLN) ($n \geq 11$). (*D*) Peyer patches ($n \geq 8$). (*E*) Colon LPMC ($n \geq 14$). (*F*) Colon-intraepithelial lymphocytes IEL ($n \geq 8$). (*G*, *H*) Analysis of 10- and 20-week-old mice. (*G*) Percentage of CD4+ in spleen (left) and colon LPMC (right) (gated on viable cells) ($n \geq 9$). (*H*) Absolute numbers of CD4+ in spleen (left) and LPMC (right). *Pggt1b*$^{\Delta CD4}$ mice up to 10 weeks of age, except *G* and *H*.


A
< 10 weeks
Control
Pggt1b^ΔCD4
2.82%
12.9%
β7
α4
% CD4+CD8-
α4β1
α4β7
αEβ7
Ratio (AU)
α4β7/α4β1
αEβ7/α4β1
α4β7/αEβ7
Control
Pggt1b^ΔCD4
B
Control
Pggt1b^ΔCD4
CFSE-transferred cells Nuclei AF647-Dextran
Distal colon
CFSE+ (cells/field)
C
Non-Competitive transfer: Control / Pggt1b^ΔCD4
Blood
Spleen
Colon
donor cells (%, fold increase)
D
Competitive transfer: Control + Pggt1b^ΔCD4
E
SSC
CFSE
0.021%
0.118%
donor CFSE+ (%, fold increase)
donor CFSE+ (number, fold increase)
F
Control
Pggt1b^ΔCD4
Pggt1b^ΔCD4 + anti-α4β7
Endoscopy Score (AU)
Histo. Score (AU)
Pggt-1β^ΔCD4 + LPAM-1

(Supplementary Figure 4*B*). Moreover, PGGT1B-deficient CD4+ T cells in the colonic lamina propria produced increased amounts of the inflammatory cytokine IL17A, being even more pronounced in elder *Pggt1b*$^{\Delta CD4}$ mice (≥ 15 weeks of age) (Figure 4*A*), implicating that the colitis further potentiates this phenomenon. Besides IL17A, we were not able to describe a significant PGGT1B-dependent regulation for any of other analyzed cytokines (interferon-γ, IL-4, IL-9, IL-10, TNF) (Supplementary Figure 4*C* and *D*). According to the observed cytokine profile, we detected an increased frequency of RORC expressing CD4+ cells in the lamina propria of *Pggt1b*$^{\Delta CD4}$ mice (Figure 4*B*; Supplementary Figure 4*E*) and several Th17-related cytokines (granulocyte-macrophage colony-stimulating factor, IL17A, IL17F, IL22, and TNF) turned out to be upregulated in the inflamed colon tissue of mice carrying PGGT1B-deficient T cells (Figure 4*C*). Interestingly, in vivo treatment of *Pggt1b*$^{\Delta CD4}$ mice with the RORC inverse agonist GSK-805[24] was able to significantly ameliorate intestinal inflammation (Figure 4*D*), while antibody-mediated blockade of IL17A/IL17F alone was unable to dampen colitis development (Supplementary Figure 4*F*). These data thus identified Th17 lineage differentiation of colonic T cells as another key mechanism underlying the colitis development in *Pggt1b*$^{\Delta CD4}$ mice. In contrast to colon, thymocytes of *Pggt1b*$^{\Delta CD4}$ mice did not show an augmented production of IL17A (Supplementary Figure 4*G*), suggesting that the intestinal milieu and the local activation of T cells within the colonic lamina propria play a crucial role in the polarization of PGGT1B-deficient T cells toward inflammatory effector cells. Overall, we assumed that the spontaneous development of colitis in *Pggt1b*$^{\Delta CD4}$ mice is driven by a massive colonic accumulation of IL17A-producing CD4+ T cells.

RHOA as a Key Downstream Target of PGGT1B in T-Cell Biology

We aimed at the identification of key target proteins of prenylation for T-cell localization. Previously suggested to control integrin expression,[25] Rho proteins appeared as attractive candidates. We took advantage of *Cdc42*$^{\Delta CD4}$, *Rac1*$^{\Delta CD4}$, and *RhoA*$^{\Delta CD4}$ mice (Supplementary Figure 5*A*) to compare the phenotype in the absence of defined Rho proteins with the described results in *Pggt1b*$^{\Delta CD4}$ mice. Interestingly, *Cdc42*$^{\Delta CD4}$ and *RhoA*$^{\Delta CD4}$ mice exhibited decreased numbers of CD4+ and CD8+ T cells in blood, spleen, and mesenteric lymphoid nodes, whereas *Rac1*$^{\Delta CD4}$ mice showed normal T-cell infiltration (Supplementary Figure 5*B*). Moreover, comparable levels of infiltrating CD4+ T cells could be observed in the colon lamina propria of *Cdc42*$^{\Delta CD4}$ mice, *RhoA*$^{\Delta CD}$ mice, and control littermates, which mimicked the phenotype observed in *Pggt1b*$^{\Delta CD4}$ mice (Supplementary Figure 5*C*); but only in the case of *RhoA*$^{\Delta CD}$ mice, increased absolute numbers of CD4+ could be observed in the colon (Figure 5*A*). Interestingly, the increased numbers of CD4-SP and CD8-SP and decreased frequency of double-positive cells in the thymus of *RhoA*$^{\Delta CD}$ mice (Supplementary Figure 5*E*), overlapped with a milder but similar effect in *Pggt1b*$^{\Delta CD4}$ mice (Supplementary Figure 2*E*). RHOA-deficient CD4+ thymocytes, by analogy with PGGT1B-deficient thymocytes, showed a marked increase in the $\alpha4\beta7/\alpha4\beta1$ expression ratio (Figure 5*B*; Supplementary Figure 5*F*), which might underlie their colon localization. In accordance with these findings, adoptively transferred RHOA-deficient thymocytes showed an improved ability to home into the colon of Rag1$^{-/-}$ recipient animals (Figure 5*C*), which was not the case for CDC42-deficient thymocyte (Supplementary Figure 5*G*). Finally, only in *RhoA*$^{\Delta CD4}$ mice were we able to detect spontaneous development of colitis, as demonstrated by increased endoscopy and histology scores (Supplementary Figure 5*H*). Of note, the spontaneous development of colon inflammation in *RhoA*$^{\Delta CD}$ mice could be significantly inhibited on treatment with the $\alpha4\beta7$ neutralizing LPAM-1 antibody (Figure 5*D*; Supplementary Figure 5*I*). Similarly to *Pggt1b*$^{\Delta CD4}$ mice, lamina propria CD3+CD4+ cells from *RhoA*$^{\Delta CD4}$ mice showed increased expression of IL17A (Figure 5*E*; Supplementary Figure 5*J*), and colon tissue of *RhoA*$^{\Delta CD}$ mice was characterized by augmented levels of Th17-related cytokines (Supplementary Figure 5*K*). In summary, these observations underlined the functional relevance of $\alpha4\beta7$-mediated T-cell localization to the gut for the intestinal phenotype of *RhoA*$^{\Delta CD}$ mice and strongly implicated the involvement of colonic Th17-polarized effector T cells.

Overall, the fact that *RhoA*$^{\Delta CD}$ and *Pggt1b*$^{\Delta CD4}$ mice displayed striking parallels with regard to integrin marker expression on thymocytes, T-cell localization, expression of inflammatory cytokines, and intestinal phenotype strongly suggested RHOA as a key downstream target protein of PGGT1B in T-cell biology. Indeed, the absence of PGGT1B in CD4-SP thymocytes was associated with a decreased activation status of RHOA protein, demonstrated by the decreased ratio between activated and total RHOA in CD4-SP cells from *Pggt1b*$^{\Delta CD4}$ mice (Supplementary Figure 5*L* and *M*). Moreover, we could demonstrate a marked decrease of membrane-bound RHOA, but not CDC42, in thymocytes from *Pggt1b*$^{\Delta CD4}$ mice (Supplementary Figure 5*N*), leading to decreased activation of RHOA-downstream pathway, as indicated by impaired phosphorylation of MLC2

Figure 3. Localization of PGGT1B-deficient T cells. (*A*) Gut homing marker expression in CD4-SP thymocytes (gated on CD4+CD8−). Representative dot plots (left); $\alpha4\beta7$, $\alpha4\beta1$, and $\alpha E\beta7$ expression (middle); and $\alpha4\beta7/\alpha4\beta1$, $\alpha E\beta7/\alpha4\beta1$, and $\alpha4\beta7/\alpha E\beta1$ ratios (right) (n ≥ 13). (*B–E*) Adoptive thymocyte transfer into *Rag1*$^{-/-}$ mice. (*B*) Postmortem confocal microscopy of the distal colon (carboxyfluorescein succinimidyl ester-positive cells/field; ×20 magnification; 2 stacks/mouse; 2 pictures/stack) (n = 5). (*C–E*) Fluorescence-activated cell sorting of blood, spleen and colon LPMC (gated on singlets; lymphoid cells). (*C*) Noncompetitive cell transfer (n ≥ 5). (*D*) Competitive cell transfer (n ≥ 9). (*E*) Repetitive cell transfer; dot plots (left), and quantification in percentage and absolute numbers (right) (n = 3, control; and n = 2, *Pggt1b*$^{\Delta CD4}$ mice). (*F*) Anti-$\alpha4\beta7$ antibody treatment in the adoptive LPMC transfer model. Endoscopy and histology (n = 2, control; n = 4, *Pggt1b*$^{\Delta CD4}$; and n = 3, *Pggt1b*$^{\Delta CD4}$+ anti-$\alpha4\beta7$). CFSE, carboxyfluoresceinsuccinimidylester.

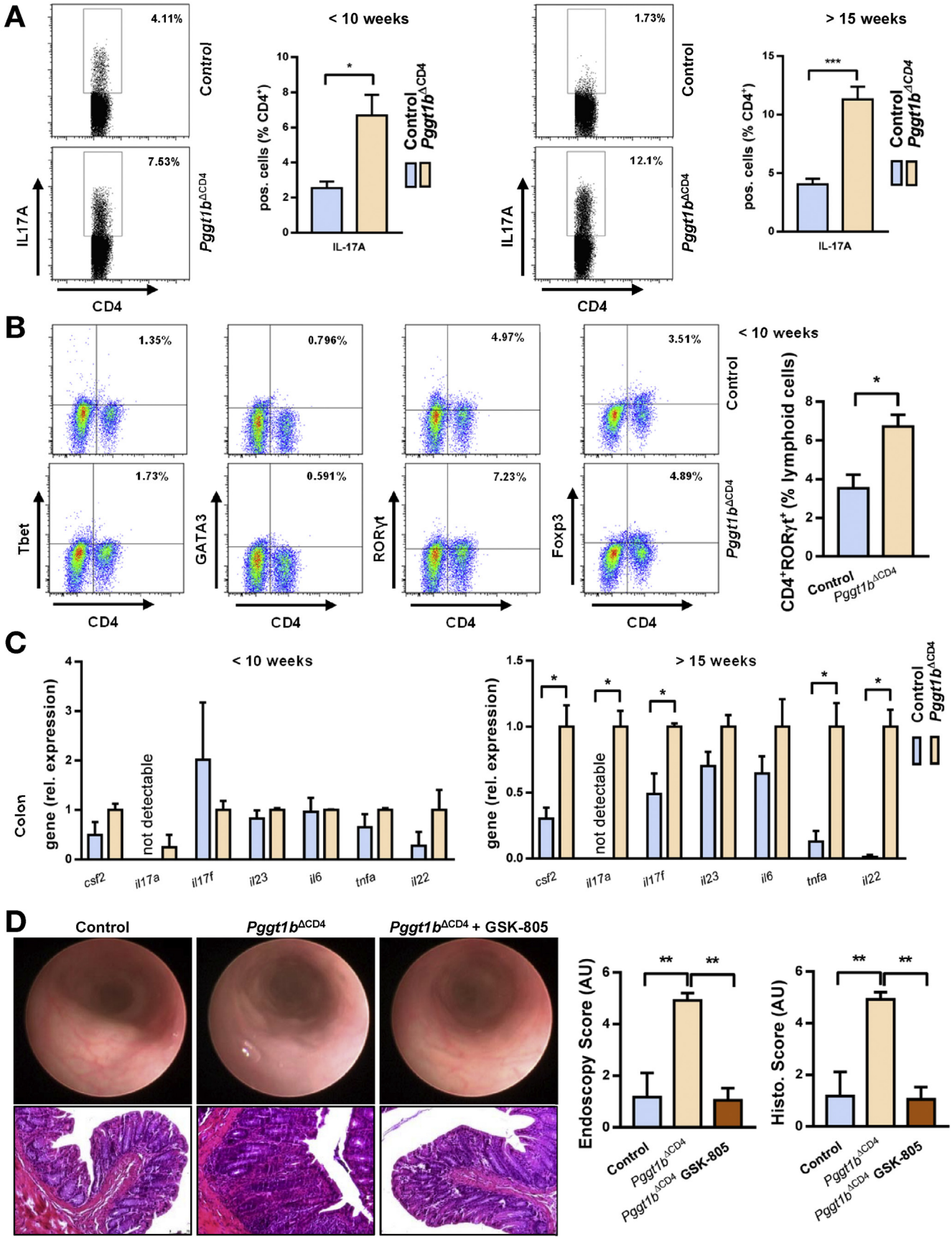


Figure 4. Effector function of PGGT1B-deficient T cells. (*A*) IL17A expression from CD4+ LPMC; dot plots, and quantification (gated on singlets-lymphoid cells, CD4+); mice ≤ 10 (left); and ≥15 weeks of age (right) (n ≥ 7;). (*B*) Analysis of transcription factors in LPMC (n = 4); mice ≤10 weeks of age. (*C*) Gene expression of Th17-related cytokines in colon tissue (n ≥ 4); mice ≤10 (left), and ≥15 weeks of age (right). (*D*) Treatment of *Pggt1b*$^{\Delta CD4}$ mice with GSK805 (n = 2, control; n = 3, *Pggt1b*$^{\Delta CD4}$; and n = 4, *Pggt1b*$^{\Delta CD4}$ + GSK805).

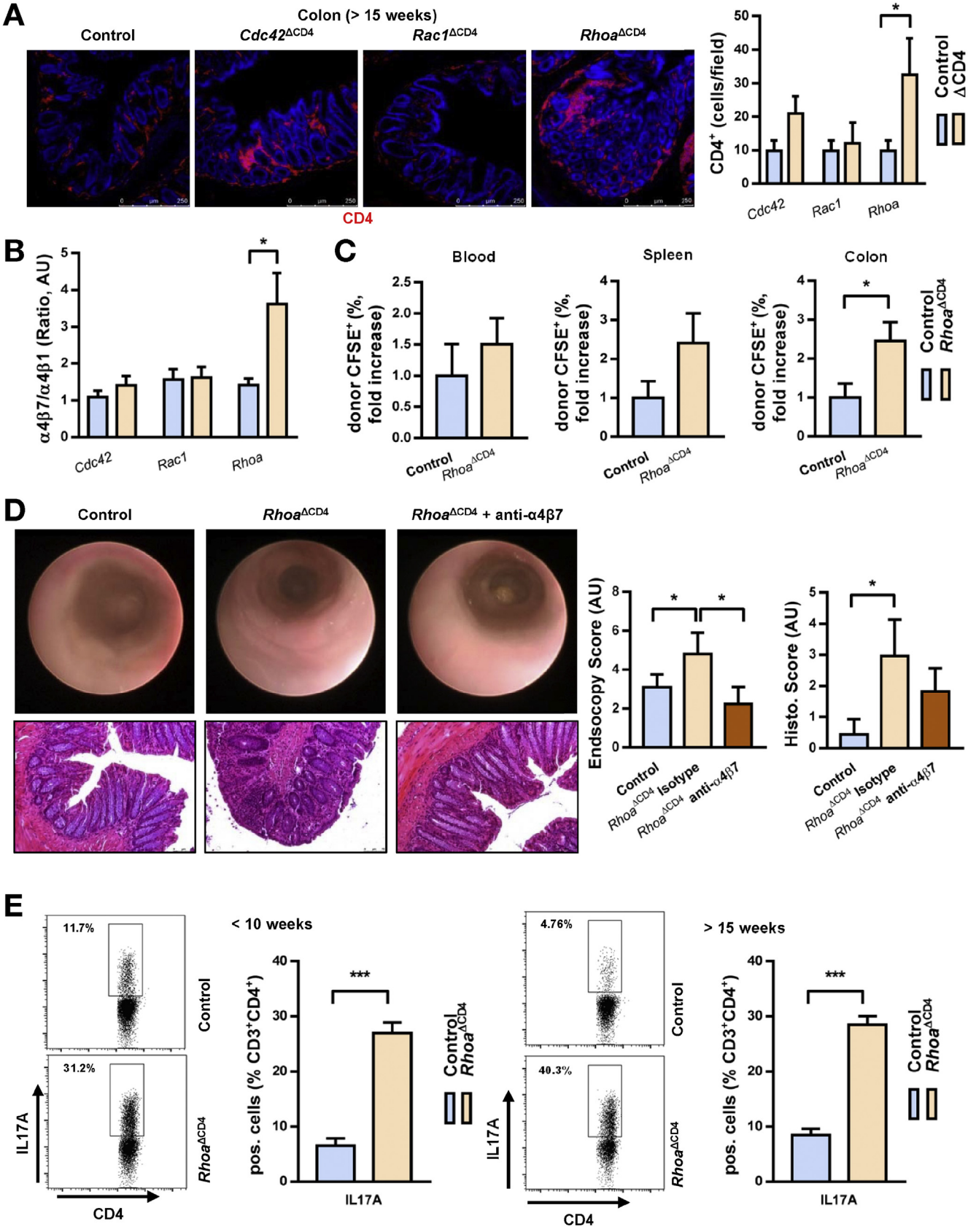


Figure 5. Role of PGGT1B-mediated prenylation targets for T-cell localization. (*A*, *B*) *Cdc42*$^{\Delta CD4}$, *Rac1*$^{\Delta CD4}$, and *RhoA*$^{\Delta CD4}$ mice. (*A*) CD4 immunostaining in colon tissue from 20-week-old mice ($n \geq 6$). (*B*) $\alpha4\beta7/\alpha4\beta1$, $\alpha E\beta7/\alpha4\beta1$, and $\alpha4\beta7/\alpha E\beta1$ ratios in mice $\leq$10 weeks of age ($n \geq 7$). (*C*) Noncompetitive cell transfer of RHOA-deficient thymocytes into *Rag1*$^{-/-}$ mice. Fluorescence-activated cell sorting analysis of colon LPMC, spleen, and blood (gated on singlets; lymphoid cells) ($n \geq 5$). (*D*) Treatment of *RhoA*$^{\Delta CD4}$ mice with anti-$\alpha4\beta7$ antibody ($n = 5$, control; $n = 5$, *RhoA*$^{\Delta CD4}$; and $n = 4$, *RhoA*$^{\Delta CD4}$ + anti-$\alpha4\beta7$). (*E*) IL17A expression from CD3+CD4+ colon LPMC (gated on CD3+CD4+). Mice $\leq$10 (left); and $\geq$15 weeks of age (right) ($n \geq 6$).

(Supplementary Figure 5*O*). Thus, these data confirmed that the absence of PGGT1B-mediated prenylation in T cells results in an impaired function of RHOA, which in turn might drive the development of spontaneous colitis.

T Cells in the Inflamed Intestinal Mucosa of Patients With IBD Show a Decreased Expression of PGGT1B

Our findings in *Pggt1b*$^{\Delta CD4}$ mice demonstrated that PGGT1B-mediated prenylation crucially impacts on colonic immune homeostasis. We then assumed that chronic intestinal inflammation in patients with IBD might also be influenced by potential alterations within the prenylation machinery of T cells. Using an indirect flow cytometric staining, no significant differences in the signal intensity or percentage of PGGT1B^{+} T cells could be observed in peripheral blood mononuclear cells isolated from patients with IBD and healthy donors (Supplementary Figure 6*A* and *B*). However, similar analyses revealed that lamina propria CD3+ or CD3+CD4+ T cells derived from patients with clinically manifest IBD are characterized by decreased percentages of PGGT1B^{+} cells compared with patients in remission (Figure 6*A* and *B*). Accordingly, *Pggt1b* gene expression was decreased in LPMCs isolated from inflamed gut areas of patients with IBD compared with unaffected control tissue (Supplementary Figure 6*C*). Interestingly, the decreased PGGT1B expression was restricted to patients with mild to moderate inflammation (Figure 6*C*). We hypothesized that exacerbated intestinal inflammation might trigger secondary prenylation-independent lymphocyte recruitment. Despite only minor differences between UC and CD patients (Figure 6*D*), the decrease in PGGT1B^{+} cells became more obvious in colonic than small intestine samples from patients with IBD (Figure 6*E*). This is in line with the fact that PGGT1B-deficient T cells in *Pggt1b*$^{\Delta CD4}$ mice showed a selective colon tropism and might therefore be of particular relevance in patients with IBD with a colon-restricted disease manifestation. Together, we could demonstrate that T cells infiltrating the inflamed gut of patients with IBD show a decreased expression of PGGT1B, suggesting that the newly discovered functional link between PGGT1B-mediated prenylation and intestinal homeostasis in mice is relevant for IBD pathogenesis.

PGGT1B-mediated Prenylation Regulates Gut Homing Marker Expression and Effector Function of Human T Cells

Next, we analyzed the cellular consequence of experimentally modified prenylation levels in primary human T cells. We took advantage of the specific GGT inhibitor GGTI-298, which inhibited prenylation in human peripheral blood CD4+ T cells (Supplementary Figure 7*A*). GGTI-298 treatment was not associated with induction of cell death in human T cells (Supplementary Figure 7*B*) or altered activation status (Supplementary Figure 7*C*). In agreement with our findings in *Pggt1b*$^{\Delta CD4}$ mice (Figure 3*A*), we could indeed detect an increased expression of $\alpha4\beta7$ integrin after exposure of stimulated human CD4+ T cells to GGTI-298 compared with untreated cells (Figure 7*A*). The GGTI-298-induced increase of $\alpha4\beta7$ expression occurred as a specific consequence of GGTase inhibition and could not be observed in PGGT1B-deficient thymocytes (Supplementary Figure 7*D*). In donors characterized by a GGTI-298–induced $\alpha4\beta7$ upregulation, a similar $\alpha4\beta7$-inducing effect in T cells could be described for simvastatin, which limits the cellular availability of isoprenoids and thereby represents an indirect prenylation inhibitor (Figure 7*B*). Increased expression of $\alpha4\beta7$ integrin on treatment with GGTI-298 also occurred in LPMC isolated from patients with IBD in remission (Supplementary Figure 7*E*). Furthermore, the GGTI-298–mediated effect on $\alpha4\beta7$ expression could be reverted by previous RHO, but not RAC/CDC42, activation (Figure 7*C*, Supplementary Figure 7*F*). These findings indicated that prenylation and RHOA function markedly affect $\alpha4\beta7$ expression in human T cells, suggesting that RHOA activation in T cells might be a new approach to regulate colonic T-cell localization in IBD.

Discussion

Our study identified for the first time a link between prenylation in T cells and chronic intestinal inflammation in patients with IBD. The abrogation of geranylgeranylation within T cells in mice leads to an age-dependent spontaneous intestinal inflammation mimicking colonic inflammation in IBD. Mechanistically, our data suggest that thymus-egressing PGGT1B-deficient T cells are primed for a selective colon tropism, based on a SP1-dependent induction of $\alpha4\beta7$ integrin expression. Thus, PGGT1B-deficient $\alpha4\beta7$-expressing lymphocytes potentially bypass antigen encountering in secondary lymphoid organs and directly localize to the intestine where they might get activated by local antigen-presenting cells,[26] such as dendritic cells, macrophages, or $\gamma\delta$ T cells. The local activation of PGGT1B-deficient T cells in the intestine was further supported by their increased expression of CD38.[23] Subsequently, PGGT1B-deficient T cells accumulate within the colon mucosa, where they undergo polarization towards a Th17-like inflammatory phenotype promoting the initiation of colitis. Most likely, local antigen presentation in the presence of cytokines, like IL-1β or TNF, and microbial products, contributes to the Th17 polarization of infiltrating PGGT1B-deficient T cells. In accordance with published data in other experimental models of colitis,[24] our analyses in *Pggt1b*$^{\Delta CD4}$ mice revealed that inhibition of RORC suppressed gut inflammation underlining a key role of Th17 cells for colitis development. However, neutralization of IL-17A/F failed to significantly ameliorate colitis in *Pggt1b*$^{\Delta CD4}$ mice, suggesting that blockade of the Th17 lineage via RORC rather than the neutralization of single Th17 cytokines is required for suppression of colitis in *Pggt1b*$^{\Delta CD4}$ mice. Previous studies on inactivation of IL-17A and IL-17F in various colitis models have led to partial contradictory results with beneficial, neutral, or even detrimental effects.[27–33] Thus, the outcome of IL17-targeting strategies

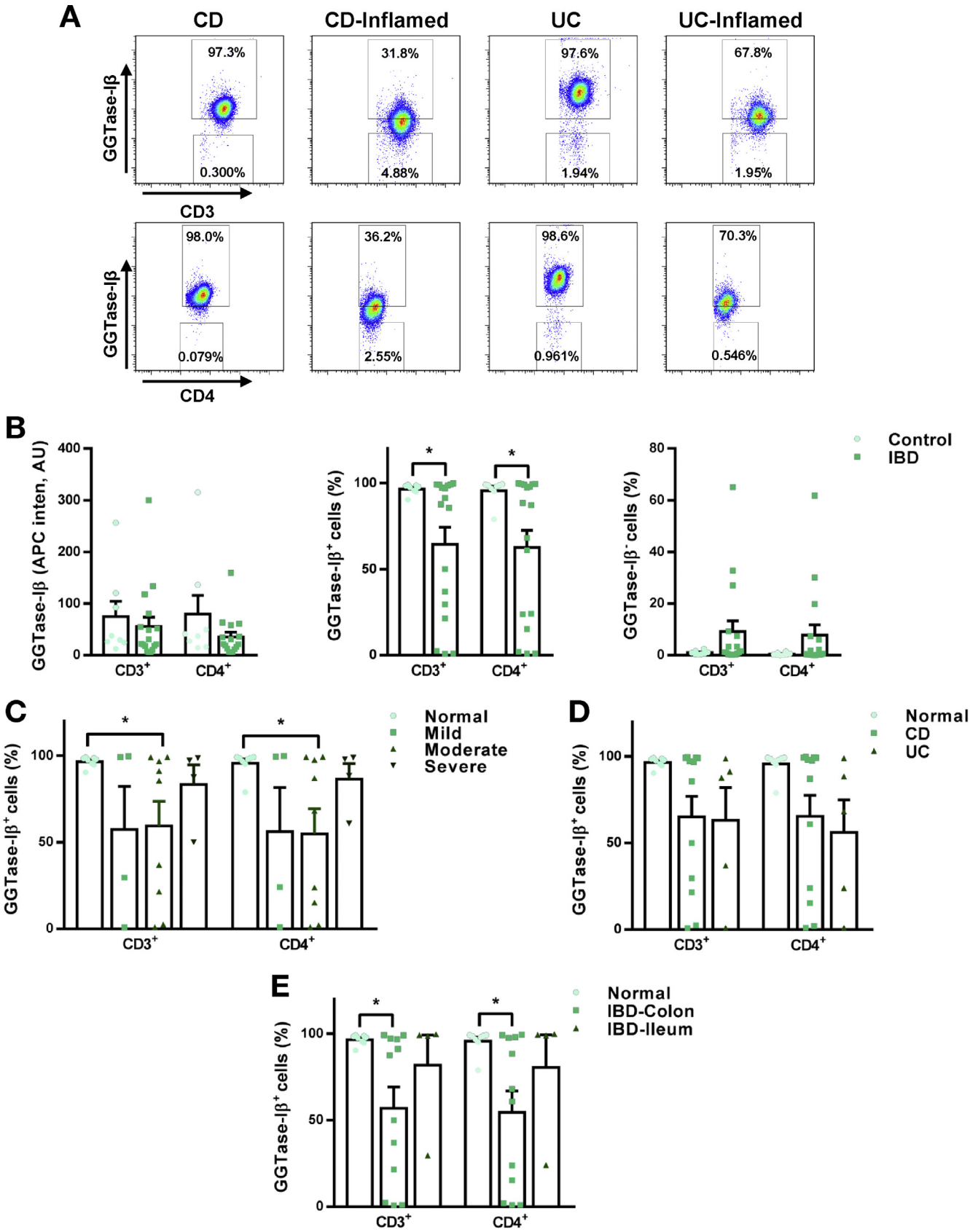
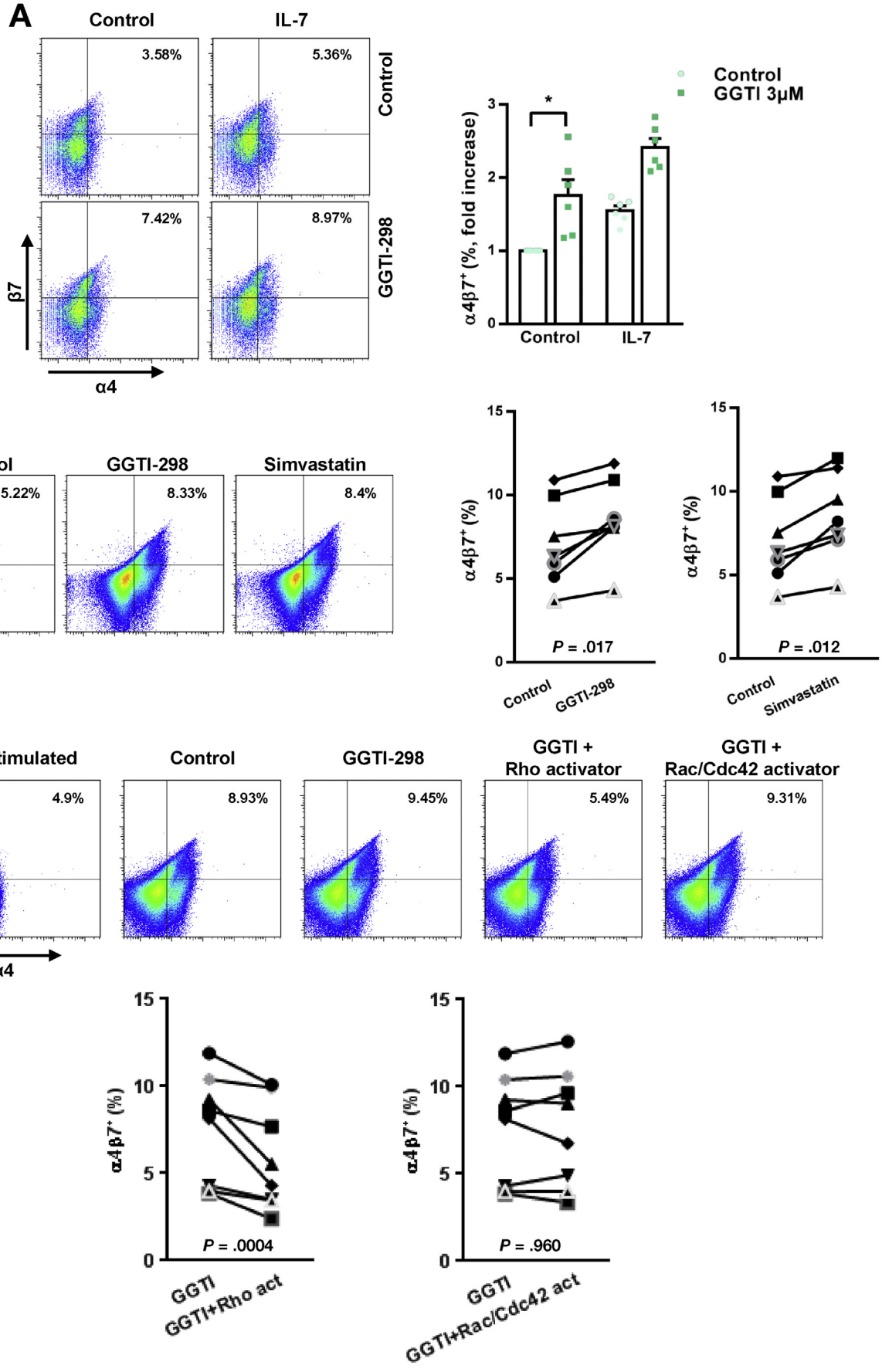


Figure 6. PGGT1B-mediated prenylation within T cells in human IBD. Expression of PGGT1B on LPMC ($CD3^+$; $CD3^+CD4^+$) isolated from patients with IBD. (*A*) Dot plots. (*B*) Mean intensity signal (left); % of $PGGT1B^+$ (middle); and % of $PGGT1B^-$ cells (right) (n = 25; n = 8, Control; n = 17, Inflamed). (*C–E*) Distribution analysis considering (*C*) severity of inflammation; (*D*) CD/UC diagnosis (n = 8, normal; n = 12, CD; n = 5, UC); and (*E*) disease localization (n = 8, normal; n = 12, colon; n = 4, ileum).


A
Control
IL-7
3.58%
5.36%
7.42%
8.97%
Control
GGTI-298
β7
α4
Control
GGTI 3μM
α4β7+ (%, fold increase)
*
Control
IL-7
B
Control
GGTI-298
Simvastatin
5.22%
8.33%
8.4%
β7
α4
α4β7+ (%)
P = .017
Control
GGTI-298
α4β7+ (%)
P = .012
Control
Simvastatin
C
unstimulated
Control
GGTI-298
GGTI + Rho activator
GGTI + Rac/Cdc42 activator
4.9%
8.93%
9.45%
5.49%
9.31%
β7
α4
α4β7+ (%)
P = .0004
GGTI
GGTI+Rho act
α4β7+ (%)
P = .960
GGTI
GGTI+Rac/Cdc42 act

may depend on various parameters, such as the selected colitis model and the treatment schedule.

The introduction of biological agents significantly improved the prognosis of patients with IBD; however, there still exist 30% to 50% of anti-TNF nonresponders,[34] indicating the urgent need for optimized treatment regimens. In that framework, strengthening of epithelial barrier function[35] and control of T-cell response[4] represent promising concepts. Strikingly, gut-infiltrating T cells in patients with IBD with active colon disease manifestation showed a decreased expression of PGGT1B, which might underlie their augmented $\alpha4\beta7$ integrin expression[36] and inflammatory cytokine profile.[37] Our data implicate that PGGT1B-deficient T cells potentially bypass antigen presentation in secondary lymphoid organs and might be strictly dependent on local activation in the gut. We then hypothesize that the high level of antigen load in the distal colon[38] might particularly promote colon tropism and local accumulation of $\alpha4\beta7$-expressing PGGT1B-deficient T cells. Together, patients with IBD with a relevant involvement of the colon might in particular benefit from a therapeutic restoration of deficient prenylation in the gut. A potential combination with regulators of prenylation might further improve the clinical performance of recently introduced anti-adhesion drugs.[39] This hypothesis is further supported by the observation that antibody-mediated blockade of $\alpha4\beta7$ integrins was able to significantly ameliorate the spontaneous intestinal pathology in $RhoA^{\Delta CD4}$ mice and the induced colitis in $Rag1^{-/-}$ mice adoptively transferred with PGGT1B-deficient LPMCs (Figure 5*D* and 3*F*). Based on the recently described PGGT1B-mediated maintenance of epithelial integrity in the gut,[5] pharmacological modulation of prenylation might even result in a double beneficial effect, acting both on T cells and epithelium.

Among downstream targets of PGGT1B, Rho proteins play a key role for T-cell biology.[8–10] Of note, it has been suggested that RHOA is activated in gut tissue samples from patients with IBD.[40] However, because RHOA is ubiquitously expressed within the intestinal mucosa, it remained open in how far the activation status of RHOA within the T-cell compartment contributed to this phenomenon. In our current study, we were now able to demonstrate that the absence of PGGT1B-mediated prenylation in T cells results in an impaired RHOA activation and function. Accordingly, RHO activation was associated with ameliorated epithelial alterations in $Pggt1b^{i\Delta IEC}$ mice,[5] and significantly dampened $\alpha4\beta7$ expression of human GGTI-treated CD4+ T cells (Figure 7*C*). These data were in accordance with the concept that GGTase-mediated prenylation and not GTP-loading determines T-cell function and Rho-mediated effects on T-cell migration.[41] Overall, modulation of RHO inhibitors/activators or interference with RHO downstream targets appear as promising strategies for IBD therapy, for instance, blocking intrinsic and GAP stimulated GTPase activity resulting in constitutively active GTPases.[42] The fact that $RhoA^{\Delta CD4}$ mice developed a milder intestinal phenotype than $Pggt1b^{\Delta CD4}$ mice might be because of a potential functional compensation of the RHOA deficiency by other Rho GTPases.[13]

Mechanistic studies suggested that prenylation is not affecting $\beta7$ function directly, but its expression via the RHOA/KLF2/SP1-dependent pathway. Our data strongly implicate that increased $\alpha4\beta7/\alpha4\beta1$ expression ratio of RHOA-deficient thymocytes (Figure 5*B*) underlies their preferential localization to the intestine (Figure 5*A*; Supplementary Figure 5*B* and *C*) and the subsequent intestinal inflammation in $RhoA^{\Delta CD4}$ mice (Figure 5*D* and *E*; Supplementary Figure 5*H–K*). Janagathan et al.[43] postulated that RHOA activation favors retention of human hematopoietic stem/progenitor cells in their niche, which is in line with decreased numbers of T cells in blood or spleen in $RhoA^{\Delta CD4}$ mice (Supplementary Figure 5*B*). In contrast to the spontaneous gut inflammation in $RhoA^{\Delta CD4}$ mice, inhibition of RHOA within T cells has recently been discussed as a therapy strategy for multiple sclerosis, due to reduced T-cell migration.[10] This controversy might be explained by the use of different Cre-deleter strains (Lck-Cre versus CD4Cre), and the occurrence of *Rhoa* deletion at different stages of thymic T-cell maturation. Because we postulate that the preference of PGGT1B-deficient T cells for localization to colon is predetermined within the thymus, the observed differences in thymocyte maturation between both strains might also affect the integrin expression of thymus-egressing lymphocytes. Neither the localization to the intestine nor the behavior of gut T cells in *Rhoa*-LckCre mice was investigated.

Based on the here presented data, PGGT1B-mediated prenylation was identified as a new regulator of T-cell localization and effector function. The functional relevance of this finding for gut homeostasis and IBD pathogenesis was highlighted by the observation that $Pggt1b^{\Delta CD4}$ mice spontaneously developed a chronic colitis and LPMC of patients with IBD could be characterized by decreased PGGT1B expression. Among GGTase-I downstream targets, RHOA was able to link defects in PGGT1B-mediated prenylation with an augmented localization to colon and inflammatory effector function of T lymphocytes. Together, our data support the emerging theory of a prenylation-based therapy in IBD.

Study Approval

Animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Erlangen and the Regierung Mittelfranken (Würzburg, Germany). Collection of human samples was approved by Ethics

Figure 7. Geranylgeranylation-dependent expression of integrins on human T cells. $\alpha4\beta7$ expression on human CD3+CD4+ cells. (*A*) Treatment with GGTI-298 with or without IL7 stimulation (n = 6). (*B*) Treatment with GGTI-298 (3 μM) or Simvastatin (5 μM); selected patients showing increased $\alpha4\beta7$ expression upon GGTI-298 treatment (n = 7). (*C*) Treatment with RHO or RAC/CDC42 activators before GGTI-298 (3 μM) stimulation (n = 8).

Committee of the Department of Medicine of the University of Erlangen-Nuremberg (Erlangen, Germany).

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2019.07.007.

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Received August 23, 2018. Accepted July 7, 2019.

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Acknowledgments
The authors thank Florentine Koppitz (Core Unit Cell Sorting and Immunomonitoring, University Hospital Erlangen) for excellent technical assistance.

Author contributions: RLP, PF, JPI, VT, TK, LCMS, and LSB performed the acquisition of data. RLP, IA, and MFN designed the experimental approach and drafted the manuscript. ASK and SZ provided valuable technical support. SW, CN, CHB, and MOB provided different mouse strains. BC, OF, SS, and MW performed, analyzed and interpreted the label-free multiphoton microscopy experiments. RA provided us with human material. All authors helped shape the manuscript.

Conflicts of interest
The authors disclose no conflicts.

Funding
The research leading to these results received funding from the People Programme (Marie Curie Actions) under REA grant agreement number 302170 of the European Union's Seventh Framework Programme (FP7/2007–2013). The work was funded by the Interdisciplinary Center for Clinical Research (IZKF) of the University Erlangen-Nuremberg, the Collaborative Research Centers TRR241 and SFB1181 and the Research Group FOR2438 of the German Research Council (DFG), the Emerging Fields Initiative ADVENDO-LIFE, and the Ludwig Demling Center.