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The TRPA1 ion channel is expressed in CD4⁺ T cells and restrains T cell-mediated colitis through inhibition of TRPV1

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Abstract

Objective—Transient Receptor Potential Ankyrin-1 (TRPA1) and Vanilloid-1 (TRPV1) are calcium (Ca²⁺)-permeable ion channels mostly known as pain receptors in sensory neurons. However, growing evidence suggests their crucial involvement in the pathogenesis of IBD. We explored the possible contribution of TRPA1 and TRPV1 to T cell-mediated colitis.

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Contributors

SB, ANA and ER designed the study; SB, YA-N, JL, P-Rd-J, PK, TH, TY, KT and NT did most experiments and data analysis; MC designed the genotyping and MC, ER and SH supervised the intercrossing and strain maintenance; AF provided the human PBMCs and SS help designing TRPA1/V1 knockdown experiments; BSB, JTC and SBH provided the human colonic biopsies and consultation on the IBD clinical cohort; SB, YA-N and JL performed the Ca²⁺ imaging experiments with the technical assistance of HD; ANA performed the electrophysiological experiments and related data analysis, and SB, ANA and ER wrote the paper.

Conflicts of interest

HD is cofounder of AddexBio. BSB receives research funding from Takeda and Janssen. The other authors disclose no conflicts.

Materials and Methods

See online supplementary Materials.

Design—We evaluated the role of *Trpa1* gene deletion in two models of experimental colitis (i.e., interleukin-10 knockout and T cell adoptive transfer models). We performed electrophysiological and Ca^{2+} imaging studies to analyze TRPA1 and TRPV1 functions in CD4^{+} T cells. We used genetic and pharmacological approaches to evaluate TRPV1 contribution to the phenotype of *Trpa1*^{−/−} CD4^{+} T cells. We also analyzed *TRPA1* and *TRPV1* gene expression and $\text{TRPA1}^{+}\text{TRPV1}^{+}$ T cell infiltration in colonic biopsies from IBD patients.

Results—We identified a protective role for TRPA1 in T cell-mediated colitis. We demonstrated the functional expression of TRPA1 on the plasma membrane of CD4^{+} T cells and identified that *Trpa1*^{−/−} CD4^{+} T cells have increased T-cell receptor (TCR)-induced Ca^{2+} influx, activation profile and differentiation into Th1-effector cells. This phenotype was abrogated upon genetic deletion or pharmacological inhibition of the TRPV1 channel in mouse and human CD4^{+} T cells. Finally, we found differential regulation of *TRPA1* and *TRPV1* gene expression as well as increased infiltration of $\text{TRPA1}^{+}\text{TRPV1}^{+}$ T cells in the colon of IBD patients.

Conclusions—Our study indicates that TRPA1 inhibits TRPV1 channel activity in CD4^{+} T cells, and consequently restrains CD4^{+} T cell activation and colitogenic responses. These findings may therefore have therapeutic implications for human IBD.

Keywords

Experimental colitis; T cells; TRP channels; calcium signaling; IBD

Introduction

Inflammatory bowel diseases (IBD) are initiated by abnormal cell-mediated immune responses, primarily by CD4^{+} T cells to luminal microbial products in genetically susceptible hosts.¹ The two most common forms of IBD are Crohn's disease (CD) and ulcerative colitis (UC).^{1, 2} CD is characterized as a T helper (Th) 1-mediated inflammatory response with overproduction of interferon (IFN)- γ and tumor necrosis factor (TNF)- α , whereas UC is considered a Th2-mediated immune disease with massive production of interleukin (IL)-5, IL-9 and IL-13.^{2, 3} Th17 cells and their related cytokines are also involved in the pathogenesis of CD and UC, but their function is more complex than originally thought as both proinflammatory and tissue-protective effects have been described.^{2, 4} One of the most advanced therapeutic strategies in IBD patients is the inhibition of effector CD4^{+} T cell homing and functions, and hence intestinal inflammation.⁵ Despite recent progress in IBD treatment, many current approaches have undesirable side effects and new therapeutic targets aiming to suppress colitogenic CD4^{+} T cell responses are needed.

Growing evidence suggests that members of the Transient Receptor Potential (TRP) family of ion channels are functionally expressed in immune cells, including T cells.^{6, 7} Indeed, we recently demonstrated that the TRP Vanilloid 1 (TRPV1) channel is expressed in CD4^{+} T cells and that it increases their proinflammatory properties in models of colitis.⁸ Mechanistically, we identified that TRPV1 contributes to T cell receptor (TCR)-induced calcium (Ca^{2+}) influx and that phosphorylation by the lymphocyte-specific protein tyrosine kinase Lck is a possible gating mechanism for the TRPV1 channel in CD4^{+} T cells upon

TCR stimulation.^{8,9} Like other TRPs, the TRPV1 channel functions as a homo- and heterotetramer with other TRP channel subunits.¹⁰ Since TRPV1 is predominantly co-expressed with the TRP Ankyrin 1 (TRPA1) channel in sensory neurons¹¹⁻¹³ and because these two channels can form heterotetrameric channel complexes,¹⁴⁻¹⁶ we evaluated in the present study TRPA1 function in CD4⁺ T cells and its potential role in T cell-mediated colitis.

TRPA1 is a Ca²⁺-permeable cation channel that is involved in various cellular processes.¹⁷ It was originally identified in sensory neurons to respond to noxious cold temperatures (<17°C) and certain pungent compounds such as allyl isothiocyanate (the pungent compound in mustard oil).^{11,18} Since neuronal TRPA1 is activated by a variety of exogenous and endogenous stimuli that cause pain and inflammation, it is considered to play a proinflammatory role.^{12,17,19} For example, intracolonic administration of the TRPA1-specific agonist mustard oil (MO) or 2,4,6-trinitrobenzene sulfonic acid (TNBS) in mice leads to a severe colitis that is associated with activation of TRPA1 in gut sensory afferent nerves and with the subsequent release of neuropeptides that trigger neurogenic inflammation.^{20,21}

Here, we report an unexpected anti-inflammatory role for the TRPA1 channel in CD4⁺ T cells. In contrast to the role of TRPV1,⁸ we found that TRPA1 decreases CD4⁺ T cell activation and proinflammatory properties. Indeed, *Trpa1*^{-/-} CD4⁺ T cells display increased TCR-induced Ca²⁺ influx, CD4⁺ T cell activation and differentiation into Th1-effector cells. As presented below, we identified that TRPA1 restrains TRPV1 channel activity in CD4⁺ T cells, and consequently controls CD4⁺ T cell activation and proinflammatory responses in two different models of T cell-mediated colitis. Finally, we observed differential regulation of *TRPA1* and *TRPV1* gene expression as well as increased infiltration of TRPA1⁺TRPV1⁺ T cells in colonic biopsies of patients with IBD.

Results

TRPA1 deficiency aggravates colitis in *Il10*^{-/-} mice

To investigate the role of TRPA1 in the development of T cell-mediated colitis, we crossed *Trpa1*^{-/-} mice with the *Il10*^{-/-} strain.²² Between 8- to 10-weeks of age *Il10*^{-/-}*Trpa1*^{-/-} mice developed severe spontaneous colitis (i.e., body weight loss, diarrhea, rectal prolapse, shortening and thickening of the colon), whereas *Il10*^{-/-} control mice developed very mild disease under the same housing conditions (figure 1A, B). The exacerbated colitis in *Il10*^{-/-}*Trpa1*^{-/-} mice was not transmissible to co-housed *Il10*^{-/-} mice and was not likely the result of an altered gut microbiota composition (see online supplementary text and supplementary figure S1). Histological analysis of *Il10*^{-/-}*Trpa1*^{-/-} mice colons revealed epithelial hyperproliferation, massive crypt loss, and marked infiltration of mononuclear cells in the mucosal layer (figure 1C). The gut barrier was also disrupted in these mice as shown by the increased levels of albumin detected in the feces (figure 1D). Consistent with the aforementioned macroscopic alterations in the colon, we found increased transcript levels of proinflammatory cytokines (*Ifng*, *Il17a*, and *Il1b*), as well as T cell markers (*Cd4* and *Cd3d*) in colon tissues from *Il10*^{-/-}*Trpa1*^{-/-} mice (figure 1E). Furthermore, *ex vivo* colonic explants (CEs) from *Il10*^{-/-}*Trpa1*^{-/-} mice produced significantly higher amounts of

IFN- γ compared to *Il10*^{-/-} control mice (figure 1F). These results indicate that TRPA1 deficiency accelerates the development of colitis and exacerbates intestinal inflammation in this model.

***Il10*^{-/-}*Trpa1*^{-/-} mice display increased Th1-mediated inflammatory responses**

Since the spontaneous colitis in *Il10*^{-/-} mice depends predominantly on CD4⁺ T cells,²³ we analyzed their inflammatory profile in *Il10*^{-/-} and *Il10*^{-/-}*Trpa1*^{-/-} mice. Spleens (SP) and mesenteric lymph nodes (MLN) were enlarged in 12-week old *Il10*^{-/-}*Trpa1*^{-/-} mice compared to age-matched *Il10*^{-/-} control mice (figure 2A, overview insets). SP and MLN CD4⁺ T cells isolated from *Il10*^{-/-}*Trpa1*^{-/-} mice produced significantly higher levels of Th1-type cytokines (IFN- γ and IL-2) compared with those isolated from *Il10*^{-/-} mice (figure 2A, B). To study the colonic T cells of these mice, we isolated lamina propria lymphocytes (LPLs) and analyzed their inflammatory profile by q-PCR. Transcript levels of *Ifng* and *Il2* as well as the transcription factors *Tbet*, *Nfat1*, and *Nfkb* were upregulated in *Il10*^{-/-}*Trpa1*^{-/-} LPLs compared to *Il10*^{-/-} LPLs (figure 2C). We next analyzed the cytokine production of naïve SP CD4⁺ T cells isolated from 6-week old mice without clinical signs of colitis. Interestingly, these cells also showed increased IFN- γ and IL-2 production compared to naïve CD4⁺ T cells isolated from age-matched *Il10*^{-/-} mice (figure 2D). These results indicate that CD4⁺ T cells isolated from *Il10*^{-/-}*Trpa1*^{-/-} mice have an increased proinflammatory profile and a Th1 bias.

***Il10*^{-/-}*Trpa1*^{-/-} naïve CD4⁺ T cells display exacerbated colitogenic properties**

To evaluate whether TRPA1 deficiency in CD4⁺ T cells was responsible for the phenotype of *Il10*^{-/-}*Trpa1*^{-/-} mice, we employed the T cell transfer model of colitis.²⁴ We isolated naïve (CD4⁺CD45RB^{high}CD25⁻) SP T cells from young (6-week old), colitis-free, *Il10*^{-/-} and *Il10*^{-/-}*Trpa1*^{-/-} donor mice and adoptively transferred these cells to *Rag1*^{-/-} recipient mice. As a control, we co-transferred wild-type C57BL/6 (WT) Tregs (CD4⁺CD45RB^{low}CD25⁺) together with WT naïve CD4⁺ T cells, and monitored the development of the colitis in the different experimental groups. Interestingly, naïve CD4⁺ T cells isolated from *Il10*^{-/-}*Trpa1*^{-/-} mice induced a significantly greater body weight loss and a higher Disease Activity Index (DAI) in the recipients compared to naïve CD4⁺ T cells isolated from *Il10*^{-/-} mice (figure 3A, B). As expected, the control group (WT naïve T cells + Treg) did not show any clinical signs of colitis. Histological analysis of the colons confirmed the induction of severe colitis in the recipients of *Il10*^{-/-}*Trpa1*^{-/-} naïve CD4⁺ T cells, whereas only a mild colitis was observed in the recipients of *Il10*^{-/-} naïve CD4⁺ T cells (figure 3C, D). The mRNA levels of several proinflammatory markers (e.g., *Cd4*, *Tbet*, *Ifng*) were also significantly increased in the colon homogenates of *Il10*^{-/-}*Trpa1*^{-/-} T cell recipients compared to *Il10*^{-/-} T cell recipients (figure 3E). Finally, CD4⁺ T cells isolated both from the spleen and MLN of *Il10*^{-/-}*Trpa1*^{-/-} T cell recipients produced higher levels of IFN- γ when compared to *Il10*^{-/-} T cell recipients (figure 3F). These results indicate that *Il10*^{-/-}*Trpa1*^{-/-} CD4⁺ T cells display increased colitogenic properties and suggest that TRPA1 deficiency in CD4⁺ T cells is responsible for the exacerbated colitis observed in *Il10*^{-/-}*Trpa1*^{-/-} mice.

CD4⁺ T cells express a functional TRPA1 channel

To evaluate TRPA1 expression in CD4⁺ T cells, we isolated spinal cord, SP CD4⁺ T cells and CD11c⁺-bone marrow-derived dendritic cells (BMDCs) from WT and *Trpa1*^{-/-} mice and analyzed *Trpa1* mRNA expression by q-PCR. *Trpa1* transcripts were found in WT spinal cord and WT CD4⁺ T cells but not in WT BMDCs or in *Trpa1*^{-/-} samples (figure 4A, B). We confirmed TRPA1 protein expression in CD4⁺ T cells by immunofluorescence staining and identified by confocal microscopy that TRPA1 is mainly expressed at the plasma membrane of CD4⁺ T cells (figure 4C). Indeed, the fluorescence signals of TRPA1 and CD4 (a transmembrane protein) were largely co-localized (Pearson's correlation coefficient, $r = 0.86$) (figure 4D).

We next demonstrated TRPA1 channel functionality in CD4⁺ T cells by recording whole-cell currents evoked by MO (a specific TRPA1 channel agonist)¹⁸ at the holding potential (V_{holding}) of -60 mV in the perforated patch clamp configuration, which leaves the cell intracellular environment intact.²⁵ MO-gated currents (I_{MO}) were recorded in WT CD4⁺ T cells (10.5 ± 1.2 pA; $n = 12$) but not in *Trpa1*^{-/-} CD4⁺ T cells (1.8 ± 0.3 pA; $n = 6$) or in vehicle-treated WT CD4⁺ T cells (1.6 ± 0.3 pA; $n = 5$) (figure 5A, B). We confirmed these findings by recording MO-gated currents from pieces of plasma membrane of CD4⁺ T cells using the cell attached single-channel configuration at $V_{\text{holding}} = -60$ mV (see online supplementary figure S2).

Finally, we performed single-cell Ca^{2+} imaging to further demonstrate TRPA1 channel functionality in CD4⁺ T cells. We found that MO significantly increased the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) in WT but not in *Trpa1*^{-/-} CD4⁺ T cells (figure 5C). In contrast, equal rises in $[\text{Ca}^{2+}]_i$ were observed in WT and *Trpa1*^{-/-} CD4⁺ T cells following stimulation with ionomycin, a Ca^{2+} ionophore used as positive control (figure 5D). Collectively, these results indicate that the TRPA1 channel is functionally expressed on the plasma membrane of CD4⁺ T cells.

Trpa1^{-/-} CD4⁺ T cells are hyperactivated and display a Th1 bias

We next evaluated whether *Trpa1* gene deletion affects TCR-induced Ca^{2+} influx and the subsequent activation of CD4⁺ T cells. Interestingly, TCR stimulation induced a higher and more sustained Ca^{2+} influx in *Trpa1*^{-/-} CD4⁺ T cells compared with WT CD4⁺ T cells (figure 5E). Similarly to what observed with ionomycin (figure 5D), no differences in Ca^{2+} influx were identified between WT and *Trpa1*^{-/-} CD4⁺ T cells following stimulation with the sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA) pump inhibitor, thapsigargin (figure 5F), which bypasses proximal TCR signaling and induces store-operated Ca^{2+} entry (SOCE).²⁶ In line with their increase in TCR-induced Ca^{2+} influx and analogous to our observations for *Il10*^{-/-} *Trpa1*^{-/-} CD4⁺ T cells (figure 2A), *Trpa1*^{-/-} CD4⁺ T cells produced higher amounts of the Th1-type cytokines IFN- γ and IL-2 (figure 6A) and showed increased *T-bet* expression (see online supplementary figure S3A) after stimulation with anti-CD3/28 Abs, compared to WT CD4⁺ T cells. However, TCR-independent activation of *Trpa1*^{-/-} and WT CD4⁺ T cells with PMA/ionomycin resulted in a similar secretion of these cytokines (figure 6B). *Trpa1*^{-/-} CD4⁺ T cells stimulated with anti-CD3/28 Abs also showed enhanced expression of surface activation markers (i.e., CD25, CD69, OX40 and CD44) (see online

supplementary figure S3B) and increased proliferative responses (see online supplementary figure S3C, D) when compared with WT CD4⁺ T cells. As shown in figure 6C, *Trpa1*^{-/-} naïve CD4⁺ T cells displayed an increased capacity to differentiate *in vitro* into Th1 effectors, but not Th17 effectors or Tregs (data not shown), compared with WT naïve CD4⁺ T cells. We confirmed this increased Th1-cytokine production by using an antigen-specific model and found that *Trpa1*^{-/-} OT-II CD4⁺ T cells stimulated by ovalbumin (OVA)-loaded WT BMDCs display significantly increased IFN- γ and IL-2 production compared with control (*Trpa1*^{+/+}) OT-II CD4⁺ T cells (figure 6D-F). Importantly, as observed for *Il10*^{-/-}*Trpa1*^{-/-} naïve CD4⁺ T cells (figure 3), we found that *Trpa1*^{-/-} naïve CD4⁺ T cells have increased colitogenic properties *in vivo* (see online supplementary figure S4).

Since visceral afferent sensory neurons and intestinal epithelial cells express functional TRPA1 channels that could affect the severity of the colitis,^{20, 27-30} we generated *Rag1*^{-/-}*Trpa1*^{-/-} mice and compared the colitis induced by the transfer of WT naïve CD4⁺ T cells into *Rag1*^{-/-} and *Rag1*^{-/-}*Trpa1*^{-/-} recipients. We found that both recipient groups had comparable body weight loss, colonic inflammation, and displayed similar CD4⁺ T cell responses (see online supplementary figure S5). Collectively, these data suggest that cell-intrinsic expression of TRPA1 in CD4⁺ T cells regulates their activation, their differentiation into Th1-effectors and their colitogenic properties in an adoptive transfer model.

TRPV1 hyperactivation is responsible for the phenotype of *Trpa1*^{-/-} CD4⁺ T cells

We then investigated the mechanism underlying the phenotype of *Trpa1*^{-/-} CD4⁺ T cells. Since we have previously shown that CD4⁺ T cells express the TRPV1 channel⁸ and because TRPA1 and TRPV1 can regulate each other's activities,¹³⁻¹⁶ we evaluated TRPV1 expression and function in WT and *Trpa1*^{-/-} CD4⁺ T cells. We first performed TRPA1 and TRPV1 double immunofluorescence staining and assessed the potential co-localization of these two proteins by confocal microscopy. Interestingly, the staining showed a high degree of TRPA1 and TRPV1 co-localization at the plasma membrane of WT CD4⁺ T cells (figure 7A, B), suggesting a potential direct interaction between these two channels. However, the expression of *Trpv1* mRNA or TRPV1 protein in either total cell lysates (i.e., both active and inactive TRPV1 channels) or plasma membrane extracts (i.e., active TRPV1 channels) was similar in WT and *Trpa1*^{-/-} CD4⁺ T cells (see online supplementary figure S6). In contrast, TRPV1 channel activity was increased in *Trpa1*^{-/-} CD4⁺ T cells. As shown in figure 7C and D, the whole-cell perforated patch current evoked by capsaicin (I_{CAP}), a specific TRPV1 agonist,³¹ was significantly increased in *Trpa1*^{-/-} CD4⁺ T cells (18.7±1.9 pA; n = 8) as compared to WT CD4⁺ T cells (9.9±1.2 pA; n = 8). As expected, no I_{CAP} could be recorded in *Trpv1*^{-/-} CD4⁺ T cells (1.8±0.3 pA; n = 6) and in vehicle-treated WT CD4⁺ T cells (1.6±0.3 pA; n = 5). Consequently, CAP-induced Ca²⁺ influx was significantly higher in *Trpa1*^{-/-} CD4⁺ T cells when compared to WT cells (see online supplementary figure S7A, B). To verify that this effect was mediated by TRPV1, we treated *Trpa1*^{-/-} CD4⁺ T cells with BCTC, a selective TRPV1 antagonist.³² BCTC almost completely inhibited CAP-induced Ca²⁺ influx in these cells (see online supplementary figure S7A, B). In line with their increased TCR-induced Ca²⁺ influx (figure 5E), *Trpa1*^{-/-} CD4⁺ T cells also displayed increased TCR-gated currents compared to WT CD4⁺ T cells (figure 7E, F). Importantly, both genetic deletion and pharmacological inhibition of TRPV1 in *Trpa1*^{-/-}

CD4⁺ T cells (i.e., *Trpa1*^{-/-}*Trpv1*^{-/-} CD4⁺ T cells and BCTC treatment, respectively) significantly decreased their elevated TCR-gated currents (figure 7E, F) and IFN- γ production (see online supplementary figure S7C).

To evaluate the role of TRPV1 in the proinflammatory phenotype of *Il10*^{-/-}*Trpa1*^{-/-} CD4⁺ T cells, we treated *Il10*^{-/-}*Trpa1*^{-/-} mice with SB366791, a specific TRPV1 inhibitor effective *in vivo*,^{8,33} and compared the spontaneous colitis occurrence with vehicle-treated *Il10*^{-/-}*Trpa1*^{-/-} mice or untreated *Il10*^{-/-} control mice. SB366791 treatment significantly decreased the colitis severity in *Il10*^{-/-}*Trpa1*^{-/-} mice (figure 8A, B). To confirm the cell-intrinsic role of TRPV1 in *Trpa1*^{-/-} CD4⁺ T cells, we adoptively transferred *Trpa1*^{-/-} and *Trpa1*^{-/-}*Trpv1*^{-/-} naïve CD4⁺ T cells to *Rag1*^{-/-} recipients and compared the severity of the colitis induced in the different groups. As expected, *Trpa1*^{-/-} naïve T cells induced severe colitis in the recipients, as reflected by clinical and histological signs of colonic inflammation. However, a significantly delayed and attenuated inflammatory response was observed in the recipients of *Trpa1*^{-/-}*Trpv1*^{-/-} naïve CD4⁺ T cells (figure 8C-F).

Finally, to explore the relevance of the data generated in the mouse to the human condition, we performed siRNA-mediated knockdown of *TRPA1* and *TRPV1* in human primary CD4⁺ T cells isolated from healthy blood donors. Similarly to our observations with mouse *Trpa1*^{-/-} and *Trpa1*^{-/-}*Trpv1*^{-/-} CD4⁺ T cells, *TRPA1* knockdown increased IFN- γ and IL-2 production by human CD4⁺ T cells upon stimulation with anti-CD3/28 Abs, and this effect was abolished by double knockdown of *TRPA1* and *TRPV1* genes (see online supplementary figure S8). To evaluate the clinical significance of these findings, we analyzed *TRPA1* and *TRPV1* transcript levels in colonic biopsies from patients with IBD. We found that *TRPV1* gene expression was significantly downregulated in patients with active UC and CD compared with healthy controls (see online supplementary figure S9). Interestingly, *TRPA1* gene expression showed the opposite trend and was upregulated in IBD patients, although the differences observed did not reach statistical significance with the sample size used in this study. To determine whether this effect resulted from a differential infiltration by TRPA1⁺ and TRPV1⁺ T cells in the colonic mucosa of IBD patients, we performed triple immunofluorescence staining of colon sections for TRPA1, TRPV1 and CD3 (see online supplementary text and supplementary figure S10). We found that the vast majority of infiltrating CD3⁺ cells were also TRPA1⁺ and TRPV1⁺ (i.e., TRPA1⁺TRPV1⁺ T cells) and the number of these cells was significantly increased in the colonic mucosa of both UC and CD patients compared with control subjects (figure 9A, B).

In summary, these results suggest that TRPV1 channel hyperactivation is responsible for the increased TCR-gated currents, the Th1 bias and the exacerbated colitogenic properties of *Trpa1*^{-/-} CD4⁺ T cells. Moreover, the differential regulation of *TRPA1* and *TRPV1* gene expression and the increased infiltration of TRPA1⁺TRPV1⁺ T cells in inflamed colon sections of patients with UC and CD suggest that TRPA1 and TRPV1 channels may play important roles in the pathogenesis of IBD.

Discussion

Growing evidence suggests the crucial involvement of the TRPA1 and TRPV1 ion channels in the inflammatory response observed in IBD.^{19, 27-30} Most studies have focused on the role played by these two channels in peripheral neurons innervating the gut and on their contribution to neurogenic inflammation. However, TRPA1 and TRPV1 have a wide tissue distribution and therefore can play diverse roles in a variety of non-neuronal cells.^{17, 34} For example, TRPA1 mRNA and protein were detected in many organs and tissues, including brain, heart, L intestine, lung, skeletal muscle, pancreas and spleen. In this study, we demonstrated for the first time the functional expression of TRPA1 in CD4⁺ T cells. We found that TRPA1 restrains TRPV1 channel activity in CD4⁺ T cells and consequently, limits their activation and differentiation into Th1-effector cells in two different models of T cell-mediated colitis.

Previous studies have demonstrated a key role for Th1 responses in T cell-mediated intestinal inflammation, including the colitis development in *Il10*^{-/-} mice.^{22, 35} Indeed, we found that *Il10*^{-/-}*Trpa1*^{-/-} mice developed accelerated and exacerbated colitis associated with an increased Th1-mediated inflammatory response. In line with our earlier observations in other *Il10*^{-/-} mutant mice,^{36, 37} the colitis in *Il10*^{-/-}*Trpa1*^{-/-} mice appears to be exacerbated by IFN- γ , but not IL-17A, produced by CD4⁺ T cells. The Th1 bias was observed in three different strains of mice (i.e., *Il10*^{-/-}*Trpa1*^{-/-}, *Trpa1*^{-/-} and *Trpa1*^{-/-} OT-II) and the T cell-intrinsic role of TRPA1 was confirmed by employing the T cell adoptive transfer model of colitis. However, in contrast to *Il10*^{-/-}*Trpa1*^{-/-} mice, *Trpa1*^{-/-} and *Trpa1*^{-/-} OT-II mice housed under specific pathogen-free (SPF) conditions do not develop spontaneous colitis, presumably due to proper regulatory T cell function.

In accordance with the higher and more sustained Ca²⁺ influx required in Th1 cells compared to other Th subsets,^{38, 39} we identified that *Trpa1*^{-/-} CD4⁺ T cells have increased TCR-gated currents and subsequently, TCR-induced Ca²⁺ influx. Furthermore, we found that *Trpa1*^{-/-} CD4⁺ T cells have increased TRPV1 channel activity and that genetic deletion or pharmacological inhibition of TRPV1 in *Trpa1*^{-/-} CD4⁺ T cells (i.e., *Trpa1*^{-/-}*Trpv1*^{-/-} CD4⁺ T cells and BCTC treatment, respectively) rescued their elevated TCR-gated Ca²⁺ currents and their increased Th1 cytokine production. Since BCTC has also been shown to block TRPM8,⁴⁰ we have confirmed the data generated with BCTC *in vitro* and the contribution of TRPV1 to the phenotype of *Il10*^{-/-}*Trpa1*^{-/-} mice by using another TRPV1 antagonist (i.e., SB366791) *in vivo*. We found that treatment with SB366791 significantly decreased the colitis severity in *Il10*^{-/-}*Trpa1*^{-/-} mice. However, we could not exclude that inhibition of TRPV1 in other cell types (e.g., visceral sensory neurons) and its consequences (e.g., inhibition of neuropeptide release) may also have contributed to this effect when SB366791 is given systemically. Therefore, we employed the T cell adoptive transfer model to address the effect of cell-intrinsic inhibition of TRPV1 in *Trpa1*^{-/-} CD4⁺ T cells. We found that *Trpa1*^{-/-}*Trpv1*^{-/-} CD4⁺ T cells have decreased colitogenic properties compared with *Trpa1*^{-/-} CD4⁺ T cells. Furthermore, we identified that genetic deletion of *Trpa1* or *Trpv1*⁸ in the *Rag1*^{-/-} recipients (i.e., *Rag1*^{-/-}*Trpa1*^{-/-} or *Rag1*^{-/-}*Trpv1*^{-/-} mice, respectively) did not affect the course of the colitis in this model. Taken together, these findings suggest that TRPV1 hyperactivation is responsible for the phenotype of *Trpa1*^{-/-}

CD4⁺ T cells and that cell-intrinsic expression and function of TRPA1 and TRPV1 in CD4⁺ T cells regulates the colitis severity in this model.

The TRPV1 hyperactivation observed in TRPA1-deficient CD4⁺ T cells can be achieved by different mechanisms leading to increased: (i) TRPV1 mRNA and/or protein expression, (ii) TRPV1 recruitment to the plasma membrane, or (iii) TRPV1 phosphorylation which reduces its activation threshold.⁴¹⁻⁴³ Our data suggest that TRPV1 hyperactivation is not likely due to increased TRPV1 expression or increased TRPV1 trafficking to the plasma membrane. Therefore, modification of TRPV1 phosphorylation status or TRPV1 quaternary structure (e.g., channel multimerization) might account for the observed TRPV1 channel hyperactivation and the resultant phenotype of *Trpa1*^{-/-} CD4⁺ T cells. In line with this hypothesis, we and others previously reported that TRPA1 and TRPV1 can form heterotetrameric channel complexes¹⁴⁻¹⁶ in which TRPA1 functionally inhibits TRPV1.¹⁵ Hence, the increased formation of TRPV1 homotetramers in absence of TRPA1 could potentially result in the increased TRPV1 channel activity observed in *Trpa1*^{-/-} CD4⁺ T cells.

The phenotype of *Trpa1*^{-/-} CD4⁺ T cells is not likely the result of a developmental defect in *Trpa1*^{-/-} mice since it was reproduced by knockdown of TRPA1 in primary, mature human CD4⁺ T cells. Indeed, the increased Th1 cytokine production was also observed after *TRPA1* gene knockdown in human primary CD4⁺ T cells and was abolished by double knockdown of *TRPA1* and *TRPV1* genes. In addition, we identified reciprocal regulation of *TRPA1* and *TRPV1* gene expression in colonic biopsies from patients with active IBD. These findings are consistent with previous reports, which showed differential expression of TRPV1 and TRPA1 in inflamed colon sections of patients with IBD.^{29, 44, 45} However, some of these studies suggested an increase in TRPV1 expression by sensory neurons innervating the colon of IBD patients.^{44, 45} In agreement with our findings, Kun *et al* recently reported a decrease in *TRPV1* expression and an increase in *TRPA1* expression in colon biopsies from patients with IBD. Interestingly, the authors of this study also observed strong TRPA1- and TRPV1-immunopositivity within mononuclear cells infiltrating the colonic mucosa of IBD patients.²⁹ In line with these observations, we found an increased infiltration of TRPA1⁺TRPV1⁺ T cells in the colonic mucosa of both UC and CD patients. Collectively, these results suggest that changes in gene expression of TRPA1 and TRPV1 and/or in infiltration of TRPA1⁺TRPV1⁺ T cells in the colon may contribute to the pathophysiology of IBD.

In summary, we demonstrated in this study the functional expression of TRPA1 in CD4⁺ T cells and reported an unexpected anti-inflammatory role for this channel in T cell-mediated colitis. These findings therefore challenge the current dogma that TRPA1 plays a detrimental role in inflammatory responses that occur in colitis and suggest that TRPA1 in CD4⁺ T cells has an opposite function to TRPA1 in visceral sensory neurons.^{19, 20, 27} While neuronal TRPA1 increases acute (e.g., neurogenic) inflammation, TRPA1 in CD4⁺ T cells appears to decrease the severity of chronic (e.g., T cell-mediated) colitis. Our results are in agreement with another recent study reporting a protective role for TRPA1 in colitis²⁹ and with our previous observations regarding the role of TRPV1 in CD4⁺ T cell activation and its proinflammatory function.⁸ Therefore, additional studies will be necessary to determine the

relative contribution of TRPA1 signaling in visceral sensory neurons and in CD4⁺ T cells, respectively to the pathogenesis of human IBD. Finally, our study further reinforces the idea that Ca²⁺ is a critical regulator of T cell inflammatory responses in colitis ^{46, 47} and suggests that Ca²⁺-permeable channels, such as TRPA1 and TRPV1, could represent new therapeutic targets in IBD and potentially in other T cell-mediated diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BM	bone marrow
BMDCs	bone marrow-derived dendritic cells
[Ca²⁺]_e	extracellular calcium concentration
[Ca²⁺]_i	intracellular calcium concentration
CAP	capsaicin
CD	Crohn's disease
CE	colonic explants
DAI	Disease Activity Index
Iono	ionomycin
LPL	lamina propria lymphocytes
MLN	mesenteric lymph nodes
MO	mustard oil
MPO	myeloperoxidase
OVA	ovalbumin

SP	spleen
TCR	T-cell receptor
TG	thapsigargin
TRPA1	Transient Receptor Potential Ankyrin-1
TRPV1	Transient Receptor Potential Vanilloid-1
UC	ulcerative colitis

References

1. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature*. 2007; 448:427–34. [PubMed: 17653185]
2. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. *Annu Rev Immunol*. 2010; 28:573–621. [PubMed: 20192811]
3. Gerlach K, Hwang Y, Nikolaev A, et al. TH9 cells that express the transcription factor PU.1 drive T cell-mediated colitis via IL-9 receptor signaling in intestinal epithelial cells. *Nat Immunol*. 2014; 15:676–86. [PubMed: 24908389]
4. Troncone E, Marafini I, Pallone F, et al. Th17 cytokines in inflammatory bowel diseases: discerning the good from the bad. *Int Rev Immunol*. 2013; 32:526–33. [PubMed: 24041379]
5. Monteleone G, Caprioli F. T-cell-directed therapies in inflammatory bowel diseases. *Clin Sci (Lond)*. 2010; 118:707–15. [PubMed: 20350293]
6. Schwarz EC, Wolfs MJ, Tonner S, et al. TRP channels in lymphocytes. *Handb Exp Pharmacol*. 2007; 179:445–56.
7. Nohara LL, Stanwood SR, Omilusik KD, et al. Tweepers, Woofers and Horns: The Complex Orchestration of Calcium Currents in T Lymphocytes. *Front Immunol*. 2015; 6:234. [PubMed: 26052328]
8. Bertin S, Aoki-Nonaka Y, de Jong PR, et al. The ion channel TRPV1 regulates the activation and proinflammatory properties of CD4(+) T cells. *Nat Immunol*. 2014; 15:1055–63. [PubMed: 25282159]
9. Bertin S, de Jong PR, Jefferies WA, et al. Novel immune function for the TRPV1 channel in T lymphocytes. *Channels (Austin)*. 2014; 8:479–80. [PubMed: 25530461]
10. Venkatachalam K, Montell C. TRP channels. *Annu Rev Biochem*. 2007; 76:387–41. [PubMed: 17579562]
11. Story GM, Peier AM, Reeve AJ, et al. ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. *Cell*. 2003; 112:819–2. [PubMed: 12654248]
12. Bautista DM, Jordt SE, Nikai T, et al. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell*. 2006; 124:1269–82. [PubMed: 16564016]
13. Salas MM, Hargreaves KM, Akopian AN. TRPA1-mediated responses in trigeminal sensory neurons: interaction between TRPA1 and TRPV1. *Eur J Neurosci*. 2009; 29:1568–7. [PubMed: 19419422]
14. Staruschenko A, Jeske NA, Akopian AN. Contribution of TRPV1-TRPA1 interaction to the single channel properties of the TRPA1 channel. *J Biol Chem*. 2010; 285:15167–7. [PubMed: 20231274]
15. Fischer MJ, Balasuriya D, Jeggle P, et al. Direct evidence for functional TRPV1/TRPA1 heteromers. *Pflugers Arch*. 2014; 466:2229–41. [PubMed: 24643480]
16. Sadofsky LR, Sreekrishna KT, Lin Y, et al. Unique Responses are Observed in Transient Receptor Potential Ankyrin 1 and Vanilloid 1 (TRPA1 and TRPV1) Co-Expressing Cells. *Cells*. 2014; 3:616–2. [PubMed: 24921186]
17. Nilius B, Appendino G, Owsianik G. The transient receptor potential channel TRPA1: from gene to pathophysiology. *Pflugers Arch*. 2012; 464:425–58. [PubMed: 23001121]

18. Bandell M, Story GM, Hwang SW, et al. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron*. 2004; 41:849–57. [PubMed: 15046718]
19. Bautista DM, Pellegrino M, Tsunozaki M. TRPA1: A gatekeeper for inflammation. *Annu Rev Physiol*. 2013; 75:181–200. [PubMed: 23020579]
20. Engel MA, Leffler A, Niedermirtl F, et al. TRPA1 and substance P mediate colitis in mice. *Gastroenterology*. 2011; 141:1346–58. [PubMed: 21763243]
21. Kimball ES, Prouty SP, Pavlick KP, et al. Stimulation of neuronal receptors, neuropeptides and cytokines during experimental oil of mustard colitis. *Neurogastroenterol Motil*. 2007; 19:390–40. [PubMed: 17509021]
22. Kuhn R, Lohler J, Rennick D, et al. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell*. 1993; 75:263–74. [PubMed: 8402911]
23. Roers A, Siewe L, Strittmatter E, et al. T cell-specific inactivation of the interleukin 10 gene in mice results in enhanced T cell responses but normal innate responses to lipopolysaccharide or skin irritation. *J Exp Med*. 2004; 200:1289–97. [PubMed: 15534372]
24. Powrie F, Leach MW, Mauze S, et al. Phenotypically distinct subsets of CD4⁺ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int Immunol*. 1993; 5:1461–7. [PubMed: 7903159]
25. Leech CA, Holz GG 4th. Application of patch clamp methods to the study of calcium currents and calcium channels. *Methods Cell Biol*. 1994; 40:135–51. [PubMed: 8201974]
26. Hogan PG, Lewis RS, Rao A. Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu Rev Immunol*. 2010; 28:491–533. [PubMed: 20307213]
27. Engel MA, Becker C, Reeh PW, et al. Role of sensory neurons in colitis: increasing evidence for a neuroimmune link in the gut. *Inflamm Bowel Dis*. 2011; 17:1030–3. [PubMed: 20722067]
28. Holzer P. Transient receptor potential (TRP) channels as drug targets for diseases of the digestive system. *Pharmacol Ther*. 2011; 131:142–70. [PubMed: 21420431]
29. Kun J, Szitter I, Kemeny A, et al. Upregulation of the transient receptor potential ankyrin 1 ion channel in the inflamed human and mouse colon and its protective roles. *PLoS One*. 2014; 9:e108164. [PubMed: 25265225]
30. Zielinska M, Jarmuz A, Wasilewski A, et al. Role of transient receptor potential channels in intestinal inflammation and visceral pain: novel targets in inflammatory bowel diseases. *Inflamm Bowel Dis*. 2015; 21:419–27. [PubMed: 25437822]
31. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*. 1997; 389:816–24. [PubMed: 9349813]
32. Valenzano KJ, Grant ER, Wu G, et al. N-(4-tertiarybutylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine - 1(2H)-carbox-amide (BCTC), a novel, orally effective vanilloid receptor 1 antagonist with analgesic properties: I. in vitro characterization and pharmacokinetic properties. *J Pharmacol Exp Ther*. 2003; 306:377–86. [PubMed: 12721338]
33. Varga A, Nemeth J, Szabo A, et al. Effects of the novel TRPV1 receptor antagonist SB366791 in vitro and in vivo in the rat. *Neurosci Lett*. 2005; 385:137–42. [PubMed: 15950380]
34. Fernandes ES, Fernandes MA, Keeble JE. The functions of TRPA1 and TRPV1: moving away from sensory nerves. *Br J Pharmacol*. 2012; 166:510–21. [PubMed: 22233379]
35. Davidson NJ, Leach MW, Fort MM, et al. T helper cell 1-type CD4⁺ T cells, but not B cells, mediate colitis in interleukin 10-deficient mice. *J Exp Med*. 1996; 184:241–5. [PubMed: 8691138]
36. Gonzalez-Navajas JM, Fine S, Law J, et al. TLR4 signaling in effector CD4⁺ T cells regulates TCR activation and experimental colitis in mice. *J Clin Invest*. 2010; 120:570–81. [PubMed: 20051628]
37. Bertin S, Lozano-Ruiz B, Bachiller V, et al. Dual-specificity phosphatase 6 regulates CD4⁺ T-cell functions and restrains spontaneous colitis in IL-10-deficient mice. *Mucosal Immunol*. 2015; 8:505–1. [PubMed: 25227984]
38. Robert V, Triffaux E, Savignac M, et al. Calcium signalling in T-lymphocytes. *Biochimie*. 2011; 93:2087–94. [PubMed: 21712067]

39. Toldi G, Kaposi A, Zsembery A, et al. Human Th1 and Th2 lymphocytes are distinguished by calcium flux regulation during the first 10 min of lymphocyte activation. *Immunobiology*. 2012; 217:37–43. [PubMed: 21899918]
40. Weil A, Moore SE, Waite NJ, et al. Conservation of functional and pharmacological properties in the distantly related temperature sensors TRPV1 and TRPM8. *Mol Pharmacol*. 2005; 68:518–27. [PubMed: 15911692]
41. Cerny AC, Huber A. Regulation of TRP signalling by ion channel translocation between cell compartments. *Adv Exp Med Biol*. 2011; 704:545–72. [PubMed: 21290316]
42. Planells-Cases R, Garcia-Sanz N, Morenilla-Palao C, et al. Functional aspects and mechanisms of TRPV1 involvement in neurogenic inflammation that leads to thermal hyperalgesia. *Pflugers Arch*. 2005; 451:151–9. [PubMed: 15909179]
43. Yao X, Kwan HY, Huang Y. Regulation of TRP channels by phosphorylation. *Neurosignals*. 2005; 14:273–80. [PubMed: 16772730]
44. Yiangou Y, Facer P, Dyer NH, et al. Vanilloid receptor 1 immunoreactivity in inflamed human bowel. *Lancet*. 2001; 357:1338–9. [PubMed: 11343743]
45. Szallasi A. Vanilloid (capsaicin) receptors in health and disease. *Am J Clin Pathol*. 2002; 118:110–21. [PubMed: 12109845]
46. Schwarz A, Tutsch E, Ludwig B, et al. Ca²⁺ signaling in identified T-lymphocytes from human intestinal mucosa. Relation to hyporeactivity, proliferation, and inflammatory bowel disease. *J Biol Chem*. 2004; 279:5641–7. [PubMed: 14585840]
47. Di Sabatino A, Rovedatti L, Kaur R, et al. Targeting gut T cell Ca²⁺ release-activated Ca²⁺ channels inhibits T cell cytokine production and T-box transcription factor T-bet in inflammatory bowel disease. *J Immunol*. 2009; 183:3454–62. [PubMed: 19648266]

Significance of this study

What is already known on this subject?

- CD4⁺ T cells are essential mediators of the inflammatory response occurring in IBD.
- Therapeutic targeting of aberrant CD4⁺ T cell responses is currently the most promising strategy for treating IBD and other chronic inflammatory diseases.
- The Transient Receptor Potential Vanilloid-1 (TRPV1) ion channel is expressed in CD4⁺ T cells and regulates their activation and proinflammatory properties.
- TRPV1 and TRP Ankyrin 1 (TRPA1) channels in sensory neurons inter-regulate each other's activity and function.

What are the new findings?

- *Tpa1* gene deletion leads to exacerbated T cell-mediated colitis in mice.
- The TRPA1 channel is functionally expressed on the plasma membrane of CD4⁺ T cells.
- TRPA1 inhibits TRPV1 channel activity and consequently restrains TCR-induced Ca²⁺ influx, CD4⁺ T cell activation and colitogenic responses.
- TRPA1⁺TRPV1⁺ T cell infiltration is increased in colonic biopsies of IBD patients.

How might it impact on clinical practice in the foreseeable future?

- The novel functions for TRPA1 and TRPV1 in CD4⁺ T cells suggest that these two ion channels could represent new therapeutic targets in T cell-mediated inflammatory diseases such as IBD.

Figure 1. TRPA1 deficiency aggravates colitis in *Il10^{-/-}* mice

(A) Time course of Disease Activity Index (DAI; i.e., the combined score of weight loss and presence of blood in the stools) for *Il10^{-/-}* and *Il10^{-/-}Trpa1^{-/-}* mice. (B) Representative pictures of the rectal prolapse and the colon of *Il10^{-/-}* and *Il10^{-/-}Trpa1^{-/-}* mice. The % of mice that develop rectal prolapse at 12-weeks of age is indicated. (C) Left panel: Representative pictures of colon sections stained with H&E. Scale bar = 500 μm. *Il10^{-/-}Trpa1^{-/-}* mice developed severe colonic inflammation as judged by colonic wall thickening (black arrowheads), massive crypt loss (black asterisk), and marked infiltration of

mononuclear cells in the mucosa and lamina propria (black arrows). Right panel: Colitis score of 12-week old *Il10*^{-/-} and *Il10*^{-/-}*Trpa1*^{-/-} mice. (D) Albumin levels in the stools (feces) analyzed by ELISA. (E) The relative mRNA expression level of several proinflammatory cytokines (top panel) and cell markers (bottom panel) was analyzed by q-PCR in colon homogenates from *Il10*^{-/-}*Trpa1*^{-/-} and *Il10*^{-/-} mice and was normalized to *Gapdh* housekeeping gene. The level in the *Il10*^{-/-} control group was used as reference and assigned to 1. (F) Proinflammatory cytokine production by colonic explants after 24h of culture (ELISA). One representative experiment out of three is shown. Results are expressed as mean $\pm$ SEM (n = 9-10 [A, B] or 6-8 [C-F] mice/group). n.s.: not significant; **p* < 0.05; ***p* < 0.01; ****p* < 0.001 (two-way ANOVA with post hoc Bonferroni's test [A] or two-tailed Student t-test [B-F]).

Figure 2. *Il10*^{-/-}*Trpa1*^{-/-} mice display increased proinflammatory CD4⁺ T cell response
 (A) Representative pictures of the spleen (SP) and the mesenteric lymph nodes (MLN) harvested from 12-week old *Il10*^{-/-} and *Il10*^{-/-}*Trpa1*^{-/-} mice (overview insets). Cytokine production by SP or MLN CD4⁺ T cells isolated from each group, 24h after re-stimulation with anti-CD3/28 Abs (ELISA). (B) SP CD4⁺ T cells were stimulated or not with anti-CD3/28 Abs for 5h. Cells were then stained for CD4 and intracellular staining was performed for IFN- γ . Representative panels of intracellular cytokine production in the different conditions are shown. Mean % ± SEM of CD4⁺IFN- γ ⁺ cells is indicated (right)

panel). (C) The relative mRNA expression level of several proinflammatory cytokines (top panel) and transcription factors (bottom panel) was analyzed in lamina propria lymphocytes (LPLs) isolated from the colon of *Il10^{-/-}Trpa1^{-/-}* and *Il10^{-/-}* mice and was normalized to *Gapdh* housekeeping gene. The level in the *Il10^{-/-}* control group was used as reference and assigned to 1. (D) Naïve CD4⁺ T cells cytokine production. Naïve CD4⁺ T cells were isolated from the spleen of 6-week old *Il10^{-/-}Trpa1^{-/-}* mice that didn't develop colitis yet and didn't have any signs of colonic inflammation and from age-matched *Il10^{-/-}* control mice. IFN- γ , IL-2, IL-17A and TNF- α production was analyzed 24h after re-stimulation with anti-CD3/28 Abs (ELISA). One representative experiment out of three is shown. Results are expressed as mean $\pm$ SEM (n = 6 [A] or 4 [B-D] mice/group). n.s: not significant; * p < 0.05; ** p < 0.01; *** p < 0.001 (two-tailed Student t-test).

Figure 3. $I110^{-/-}Trpa1^{-/-}$ naïve CD4⁺ T cells induce an exacerbated colitis in the adoptive transfer model

(A) Percentage of initial body weight of $Rag1^{-/-}$ recipient mice 4 weeks post-adoptive transfer with 3×10^5 $I110^{-/-}$ or $I110^{-/-}Trpa1^{-/-}$ FACS-sorted naïve (CD4⁺CD45RB^{high}CD25⁻) T cells, or with 3×10^5 WT naïve CD4⁺ T cells + 1.5×10^5 WT Treg (CD4⁺CD45RB^{low}CD25⁺). Statistical analysis compared $I110^{-/-}Trpa1^{-/-}$ T cell group to $I110^{-/-}$ T cell group and $I110^{-/-}$ T cell group to WT T cells + Treg group. (B) Disease Activity Index (DAI) in the three recipient groups. (C) Representative pictures of colon sections stained with H&E. Scale bars = 1 mm (overview insets), 100 μ m (high-power

fields). (D) Colitis score in the three recipient groups. (E) Comparison of mRNA expression of *Cd4*, *Ifng*, and *T-bet* in SP CD4⁺ T cells isolated from *Rag1*^{-/-} recipients. (F) Cytokine production by SP or pooled MLN CD4⁺ T cells isolated from *Rag1*^{-/-} recipients, 24h after re-stimulation with anti-CD3/28 Abs (ELISA). Mean $\pm$ SEM (n = 7 mice/group [*Il10*^{-/-} or *Il10*^{-/-} *Trpa1*^{-/-} T cell groups] or 4 mice/group [WT T cells + Treg group]) of one representative experiment out of two is shown. n.s: not significant; **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001 (one- [B-F] or two-way [A] ANOVA with post hoc Bonferroni's test).

Figure 4. TRPA1 is constitutively expressed in CD4⁺ T cells

(A) Spinal cord, SP CD4⁺ T cells and bone marrow-derived dendritic cells (BMDCs) were isolated from C57BL/6 (i.e., WT) and *Trpa1*^{-/-} mice, and *Trpa1* mRNA expression was analyzed by q-PCR. PCR products from the q-PCR reaction were separated on a 2% agarose gel. *Nfl* (Neurofilament), *Cd3d*, and *Cd11c* were used as markers for neurons, T cells and dendritic cells, respectively. *Gapdh* was used as a loading control. (B) Comparative expression of *Trpa1* in spinal cord, SP CD4⁺ T cells and CD11c⁺ BMDCs by q-PCR. Expression of *Trpa1* transcripts was normalized to *Gapdh* and assigned to 1 for spinal cord

(positive control). Mean $\pm$ SEM of 6 mice is shown. n.s: not significant; * $p < 0.05$; *** $p < 0.001$ (one-way ANOVA with post hoc Bonferroni's test). (C) Immunofluorescence labeling of TRPA1 and CD4 in WT and *Trpa1*^{-/-} SP CD4⁺ T cells. DAPI (left panel), TRPA1-AF488 (mid-left panel), CD4-PE (mid-right panel) and the merge (right panel) are shown. Scale bars = 20 μ m (overview insets), 5 μ m (high-power fields). The yellow color in the merge panel indicates areas of TRPA1 and CD4 co-localization at the plasma membrane. (D) TRPA1 and CD4 co-localization scatter plot was generated using Velocity® software. Data are representative of three independent experiments.

Figure 5. CD4⁺ T cells express a functional TRPA1 channel

(A) Representative traces of whole-cell recordings in WT and *Trpa1*^{-/-} SP CD4⁺ T cells after application of mustard oil (MO, 100 μM) or its vehicle (Veh, 0.01% DMSO) in presence of 2 mM extracellular calcium concentration ([Ca²⁺]_e). (B) Statistical analysis of MO-gated currents (*I*_{MO}) in WT (n = 12), *Trpa1*^{-/-} (n = 6) and Veh-treated WT (n = 5) CD4⁺ T cells as shown in A. (C-F) WT and *Trpa1*^{-/-} SP CD4⁺ T cells were loaded with Fura-2 AM. Changes in [Ca²⁺]_i were monitored by confocal imaging and were calculated as the ratio of Fura-2 emission at 340 versus 380 nm wavelength excitation. (C) Representative

Ca²⁺ influx profiles in WT and *Trpa1*^{-/-} CD4⁺ T cells after application of MO (100 μM) in [Ca²⁺]_e = 2 mM (2Ca). Right panel: statistical analysis of the Ca²⁺ influx peak as indicated by a dashed line in the left panel. (D) Cells were stimulated with ionomycin (Iono, 1 μM) in Ca²⁺-free medium (0Ca) and CaCl₂ (2 mM) was added to the extracellular medium as indicated. Right panel: statistical analysis of the Ca²⁺ influx peak as indicated by a dashed line in the left panel. (E) Cells were stimulated with soluble biotinylated anti-CD3 (10 μg/mL) and cross-linked with streptavidin (10 μg/mL) in Ca²⁺-free medium and CaCl₂ (2 mM) was added to the extracellular medium as indicated. Right panel: statistical analysis of the sustained TCR-induced Ca²⁺ influx as indicated by a dashed line in the left panel. (F) Store-operated Ca²⁺ measurements were performed after passive depletion of intracellular stores using thapsigargin (TG, 1 μM). Right panel: statistical analysis of the sustained Ca²⁺ influx as indicated by a dashed line in the left panel. Statistical analyses of the Ca²⁺ influx profiles (C-F) are shown as the mean ± SEM of 40-50 individual cells. n.s: not significant; ****p* < 0.001; *****p* < 0.0001 (one-way ANOVA with post hoc Bonferroni's test [B] or two-tailed Student t-test [C-F]). Data are representative of three independent experiments.

Figure 6. *Trpa1* $^{-/-}$ CD4 $^{+}$ T cells are hyperactivated and display a Th1 bias

(A) WT and *Trpa1* $^{-/-}$ SP CD4 $^{+}$ T cells were stimulated with anti-CD3/28 Abs for 48h and cytokine production was assessed (ELISA). Mean $\pm$ SEM (n = 4 mice/group) is shown. (B) Cells were stimulated with PMA (25 ng/mL) and ionomycin (500 nM) for 48h and cytokine production was assessed (ELISA) as A. (C) SP naïve CD4 $^{+}$ T cells were isolated from WT and *Trpa1* $^{-/-}$ mice, cultured for 5 days under Th1 and Th17-polarizing conditions and re-stimulated as described in the methods section. Representative panels of intracellular cytokine production in the different differentiated subsets as determined by FACS on gated

CD4⁺ T cells are shown. Right panel: % of IFN- γ ⁺ and IL-17A⁺ CD4⁺ T cells in the Th1- and Th17-inducing conditions, respectively. (D-F) Cytokine production by OT-II or *Trpa1*^{-/-} OT-II CD4⁺ T cells co-cultured with OVA-loaded WT BMDCs for 3 days and re-stimulated with anti-CD3/28 Abs for 6 (intracellular staining, ICS) or 24h (ELISA). (D) Representative panels of intracellular cytokine production in gated CD4⁺ T cells and (E) mean % $\pm$ SEM (3 mice/group) of IL-2⁺, IL-2⁺IFN- γ ⁺ and IFN- γ ⁺ CD4⁺ T cells are shown. (F) For ELISA, supernatants were collected and IFN- γ and IL-2 levels were measured. n.s: not significant; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001 (two-tailed Student t-test [A, B, E] or one-way ANOVA with post hoc Bonferroni's test [F]). Data are representative of two (D-F) or three (A-C) independent experiments.

Figure 7. TRPV1 hyperactivation is responsible for the phenotype of *Trpa1*^{-/-} CD4⁺ T cells
 (A) TRPA1 and TRPV1 co-localization in CD4⁺ T cells as determined by double immunofluorescence labeling. Resting WT SP CD4⁺ T cells were stained with TRPA1 and TRPV1 Abs and analyzed by confocal microscopy. DAPI (left panel) was used as a nuclear counterstain. TRPA1-AF488 (mid-left panel), TRPV1-AF546 (mid-right panel) and the merge (right panel) are shown. The yellow color in the merge panel indicate areas of TRPA1 and TRPV1 co-localization at the plasma membrane. Scale bar = 5 μ m. (B) TRPA1 and TRPV1 co-localization scatter plot was generated using Velocity® software. r , Pearson's

correlation coefficient. (C) Representative traces of whole-cell perforated patch recordings for capsaicin (CAP, 1 μ M) or its vehicle (Veh, 0.01% DMSO) in WT, *Trpa1*^{-/-} and *Trpv1*^{-/-} CD4⁺ T cells in [Ca²⁺]_i = 2mM. (D) Statistical analysis of CAP-gated currents (I_{CAP}) in WT (n = 8), *Trpa1*^{-/-} (n = 8), *Trpv1*^{-/-} (n = 6) and Veh-treated WT (n = 5) CD4⁺ T cells as shown in C. (E) Representative traces of whole-cell recordings for anti-CD3 Ab (10 μ g/mL) in WT, *Trpa1*^{-/-} $\pm$ BCTC and *Trpa1*^{-/-}*Trpv1*^{-/-} CD4⁺ T cells in [Ca²⁺]_i = 2mM. (F) Statistical analysis of CD3-gated currents (I_{CD3}) in unstimulated WT (n = 7) and in anti-CD3 Ab-stimulated WT (n = 12), *Trpa1*^{-/-} (n = 9), *Trpa1*^{-/-} + BCTC (n = 7), and *Trpa1*^{-/-}*Trpv1*^{-/-} (n = 11) CD4⁺ T cells as shown in E. n.s: not significant; **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001 (one-way ANOVA with post hoc Bonferroni's test). Data are representative of three independent experiments.

Figure 8. Genetic deletion or pharmacological inhibition of TRPV1 rescues the proinflammatory phenotype of *Il10*^{-/-}*Trpa1*^{-/-} and *Trpa1*^{-/-} CD4⁺ T cells *in vivo*

(A, B) *Il10*^{-/-}*Trpa1*^{-/-} mice were treated three times per week with the TRPV1 inhibitor SB366791 (3 mg/kg of body weight, i.p) or with its vehicle (Veh, 10% DMSO/90% Saline) starting at 6-weeks of age and until they reached 12 week old. Age- and sex-matched untreated *Il10*^{-/-} mice were used as controls. (A) Representative pictures of colon sections stained with H&E (right panel). Scale bars = 1 mm (overview insets), 100 μ m (high-power fields). (B) Colitis score in the three different groups (n = 6-10 mice/group). (C-F) *Rag1*^{-/-} mice were transferred with 3×10^5 *Trpa1*^{-/-} or *Trpa1*^{-/-}*Trpv1*^{-/-} FACS-sorted naïve (CD4⁺CD45RB^{high}CD25⁻) T cells, or as control with 3×10^5 WT naïve CD4⁺ T cells + 1.5×10^5 WT Treg (CD4⁺CD45RB^{low}CD25⁺). (C) Percentage of initial body weight in the different recipient groups (n = 6 mice/group). Statistical analysis compared *Trpa1*^{-/-} and *Trpa1*^{-/-}*Trpv1*^{-/-} naïve T cells groups. (D) Disease Activity Index (DAI) in the three

recipient groups. (E) Representative pictures of colon sections stained with H&E. Scale bars = 1 mm (overview insets), 100 μ m (high-power fields). (F) Colitis score in the three recipient groups. n.s: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (one- [B, D, F] or two-way [C] ANOVA with post hoc Bonferroni's test).

Figure 9. Increased infiltration of TRPA1⁺TRPV1⁺ T cells in inflamed colon sections of patients with active IBD

(A) Representative pictures of immunofluorescence staining for TRPA1, TRPV1 and CD3 in colon sections from healthy controls (Ctrl), patients with active UC and patients with active CD. Nuclei were stained with Hoechst 33258 (blue). TRPA1 (green), TRPV1 (red), CD3 (white) and merged image are shown. Scale bars = 50 μ m. (B) Quantification of CD3⁺TRPA1⁺TRPV1⁺ cells per high-power field (HPF, average of 3-6 different 600x visual fields) in healthy controls (n = 8), patients with active UC (n = 7) and patients with active CD (n = 6). Each symbol represents an individual subject; horizontal lines indicate the mean $\pm$ SEM. n.s.: not significant; ** p < 0.01 (one-way ANOVA with post hoc Bonferroni's test).