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Epithelial CaSR deficiency alters intestinal integrity and promotes proinflammatory immune responses

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ABSTRACT

The intestinal epithelium is equipped with sensing receptor mechanisms that interact with luminal microorganisms and nutrients to regulate barrier function and gut immune responses, thereby maintaining intestinal homeostasis. Herein, we clarify the role of the extracellular calcium-sensing receptor (CaSR) using intestinal epithelium-specific *Casr*$^{-/-}$ mice. Epithelial CaSR deficiency diminished intestinal barrier function, altered microbiota composition, and skewed immune responses towards proinflammatory. Consequently, *Casr*$^{-/-}$ mice were significantly more prone to chemically induced intestinal inflammation resulting in colitis. Accordingly, CaSR represents a potential therapeutic target for autoinflammatory disorders, including inflammatory bowel diseases.

1. Introduction

The single layer of epithelium composing the intestinal mucosa acts as a barrier to impede the passage of toxins, pathogens, and foreign antigens, while selectively allowing the transport of electrolytes, essential nutrients, and water from the intestinal lumen into peripheral circulation [1]. Molecules pass through the intestinal epithelial monolayer by two routes: transcellular, through the apical and basolateral membranes of a cell; and paracellular, via the intercellular space between adjacent cells [2]. It is the function of apical junctional complexes to seal off the paracellular pathway of transport. The tight junction (TJ) and the adherens junction (AJ) comprise the apical junctional complex. In addition, desmosomes, located basolaterally beneath the AJ, strengthen the bond between adjacent epithelial cells by facilitating cellular proximity and TJ assembly [3]. These epithelial barriers are not absolutely impermeable, and intestinal epithelia have TJs with lower resistance than do other types of epithelia, such as that of the gallbladder, which compartmentalize irritating bile acids from the rest of the abdominal cavity [3]. Unfortunately, these low resistance TJs are prone to leakage, and emerging data suggest that the pathogenesis of inflammatory bowel disease (IBD) is related to three fundamental and self-perpetuating circumstances: compromised intestinal barrier function; exposure of intestinal luminal contents to leukocytes in the lamina propria; and uncontrolled inflammatory immune responses [1].

Importantly, it has recently been shown that the gut epithelium serves as a "communicator" between the luminal flora and the subepithelial immune system comprised of innate and adaptive immune components, including dendritic cells (DCs) and lymphocytes [4]. The epithelium expresses an array of pattern recognition receptors (PRRs), and disruption of these epithelium-derived bacteria-sensing/modulating mechanisms can result in uncontrolled

Abbreviations: CaSR, calcium-sensing receptor; IBD, inflammatory bowel disease; DCs, dendritic cells; TJ, tight junction; AJ, adherens junction; PRRs, pattern recognition receptors; GI, gastrointestinal; DSS, dextran sulfate sodium; TEER, transepithelial electrical resistance; I_{SC}, short-circuit current; G_T, trans-epithelial conductance; LP, lamina propria; Tregs, regulatory T cells; PD1, programmed cell death 1; PTH, parathyroid hormone; TNFR1, tumor necrosis factor receptor 1

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immune responses [5–9]. Resident gut commensal bacteria can also shape local mucosal and systemic immunity by providing critical signals that maintain gut homeostasis [10]. Previous studies have demonstrated that nutrients can modulate the composition of the gut microbiota, epithelial cell function, and host immunity [11–19]. For nutrient-dependent signaling, the gut epithelium is equipped with various nutrient-sensing mechanisms; however, their importance in gut bacteria-sensing, epithelial cell function, and immune homeostasis remains largely unknown.

One such nutrient-sensing receptor is the extracellular calcium-sensing receptor (CaSR) [20]. CaSR is a G protein-coupled nutrient-sensing receptor that is widely expressed in a range of tissues and species [21,22] to regulate calcium homeostasis [20] and osmotic balance [22–24]. Epithelial cells along the entire gastrointestinal (GI) tract express the CaSR [25–30]. Although previous investigations have studied the role of GI CaSR in fluid transport, intestinal epithelial differentiation, growth, and nutrient sensing [30,31], local and systemic consequences of alterations in CaSR signaling have not been detailed.

Here, we investigated additional roles of the CaSR in the intestinal mucosae using intestinal epithelium-specific receptor knockout mice (*Casr*$^{-/-}$). We show that CaSR plays a key role in the maintenance of intestinal barrier function, gut microbiome composition, and in the control of intestinal and systemic immune responses.

2. Materials and methods

2.1. Mice

C57BL/6 mice lacking *Casr* expression in intestinal epithelial cells (*Casr*$^{-/-}$) and their *Casr*$^{+/+}$ littermates were bred and maintained in-house at the University of Florida Communicore Animal Facility. *Casr*$^{-/-}$ mice were generated as previously described [31]. Briefly, *Casr*$^{flox/flox}$ mice were bred with transgenic mice expressing Cre Recombinase under the control of the villin 1 promoter and genotyped prior to all experiments after a minimum of two generations. Mice were used at 5–10 weeks of age in accordance with the Animal Welfare Act and the Public Health Policy on Humane Care. All procedures were approved by the Institutional Animal Case and Use Committee (IACUC) at the University of Florida.

2.2. Ex vivo transepithelial electrical resistance (TEER), short-circuit current (I_{SC}), transepithelial conductance (G_T), and permeability measurements of intestinal tissues

Differences in electrogenic ion transport in the colons of *Casr*$^{+/+}$ and *Casr*$^{-/-}$ mice were quantified by measuring the short circuit current responses of freshly isolated colonic tissues mounted in modified Ussing chambers (Physiologic Instruments, San Diego, CA), as previously described [32,33]. Intestinal permeability was assessed using 4 kDa fluorescein isothiocyanate dextran, as described previously [34].

2.3. Lamina propria leukocyte (LPL) preparation

Colonic lamina propria (LP) cells were isolated as previously described [35], with minor modifications. Digestion buffer consisted of DMEM (GIBCO®, Life Technologies) containing 0.25 mg/mL collagenase type VII (Sigma–Aldrich), 0.125 U/mL Liberase TM Research Grade (Roche Applied Science, Indianapolis, IN), 10 mM HEPES, 0.1 M $CaCl_2$ (Sigma–Aldrich), and 5% FBS (3 × 10 min digestions). Cells obtained from the digestions were combined and stained for flow cytometry-based analyses.

2.4. Flow cytometry and antibodies

Single cell suspensions obtained from processed spleens and MLNs, and LP lymphocytes were stained with LIVE/DEAD Aqua Dead Cell Stain Kit® (Molecular Probes®, Life Technologies). Mouse Fc Blocking Reagent (Miltenyi Biotec, Auburn, CA) was used prior to staining with combinations of the following antibodies or their corresponding isotype controls from eBioscience (San Diego, CA), Biolegend (San Diego, CA), BD Pharmingen, or R&D Systems (Minneapolis, MN): CD45-(30-F11)-eFluor650NC, CD11c-(N418)-BV605, CD11b-(M1/70)-PE-Cy7, CD11b-(M1/70)-APC-Cy7, F4/80-(BM8)-PB, GR1-(RB6-8C5)-APC-Cy7, I-A/I-E MHCII-(2G9)-FITC, I-A/I-E MHCII-(2G9)-PE, CD3-(145-2C11)-APC-Cy7, CD4-(RM4-5)-BV605, CD8-(53-607)-PE-Cy7, PD-1-(29F.1A12)-BV421, Pro-IL-1β-(NJTEN3)-APC, TNFα-(MP6-XT22)-PerCP-eFluor710, IL-6-(MP5-20F3)-FITC, IFNγ-(XMG1.2)-PerCP-Cy5.5, IL-17A-(TC11.18H10.1)-PE, FoxP3-(FJK-16A)-PE, IL-1R-(JAMA-147)-PE. Prior to intracellular staining, cells were fixed and permeabilized with BD Cytofix/Cytoperm™ (BD Biosciences). A BD LSRFortessa™ (BD Biosciences) cell analyzer was used to acquire stained, fixed cells. Data were analyzed with FlowJo software (Tree Star, Ashland, OR).

2.5. Real-time PCR

For global gene expression changes, RNA was isolated from the tissues specified with Aurum™ Total RNA Kit (Bio-Rad). iScript™ Select cDNA Synthesis Kit (Bio-Rad) was used for reverse transcription and the obtained cDNA used for quantitative PCR by SYBR® Green Dye gene expression assay on a Bio-Rad CFX96™ Real time system; $n = 8$/group. The reaction was carried out in 10 μl final volume with an initial denaturation at 95 °C, followed by temperature cycling of 95 °C 30 s, 60 °C 30 s and 72 °C 30 s; 40 cycles were totally performed. For microbiota composition, ZR Fecal DNA MiniPrep™ Kit (Zymo Research, Irvine, CA) was used to extract total fecal DNA per the manufacturer's instructions. Real-time PCR analysis was performed on 2 ng of total DNA template (SsoAdvanced™ SYBR® Green Supermix, Bio-Rad) to target variable regions of bacteria group-specific 16S rRNA sequences [36]; $n = 8$/group. Groups were normalized to the housekeeper Eubacteria group to determine the relative abundance. Primers used can be found in Table S1.

2.6. 16S ribosomal DNA sequencing

Fecal DNA was amplified by Illumina Miseq compatible primers, targeting the 16S rDNA V4-V5 region for microbiome analyses. Amplicons were purified by QIAquick Gel extraction kit (Qiagene, Madison, WI) and quantified by Qubit® 2.0 Fluorometer (Invitrogen, Grand Island, NY) and Kapa SYBR fast qPCR kit (Kapa Biosystems, Inc., Woburn, MA). Equal amounts of amplicons were pooled with 10% of Phix control. Miseq v2 reagent kit (Illumina, Inc., San Diego, CA) was used to run the pooled samples on the Illumina Miseq. Data were analyzed as previously described [37].

2.7. Dextran sulfate sodium (DSS)-induced colitis

Casr$^{+/+}$ and *Casr*$^{-/-}$ mice received 3% DSS in the drinking water for 7 days to induce colitis. Disease progression, including weight loss and diarrhea, was monitored throughout the study. Body condition scoring (BCS) of the mice, as determined by a veterinarian, was used as criteria for early termination of the experiment. Despite severe colitis, none of the mice reached a BCS requiring euthanasia. Mice were sacrificed at day 13 and the colons isolated for analyses. Tissues were fixed, sectioned, and stained with hematoxylin and eosin (H&E) by Histology Tech Services (Gainesville,

FL). Sections were analyzed/scored blindly by a boarded veterinary pathologist (JLO). Colitis was graded based on 7 parameters (0–17). Namely, degree of inflammation in the LP (0–3), goblet cell loss (0–2), abnormal crypts (0–3), crypt abscess (0–1), mucosal erosion and ulceration (0–1), submucosal spread to transmural involvement (0–3), and neutrophil numbers at 40x magnification (0–4). Stool consistency was scored as follows: 0 = normal, 1 = pasty, 2 = watery, 3 = watery with perianal staining.

2.8. Statistical analyses

Unless stated otherwise, representative data indicate mean ± S.E.M. Significance was determined by two-tailed unpaired *t* tests for two group comparisons (GraphPad Prism 6 for Mac OS X, La Jolla, CA).

3. Results

3.1. Intestinal CaSR contributes to intestinal barrier function integrity

The evaluation of intestinal permeability *ex vivo* using Ussing chambers revealed that *Casr*$^{-/-}$ mice showed reduced transepithelial resistance (TEER), and higher transepithelial conductance (G_T) and passive transport of FITC-conjugated dextran (Fig. 1A–C). Interestingly, significant alterations in short-circuit current (I_{SC}) and ion transporter transcript measurements, indicative of defective transcellular transport, were not observed between the two groups (Fig. 1D), suggesting that the CaSR exclusively affects the paracellular transport pathway. Apical junctional complexes, such as TJs, primarily maintain the epithelial barrier; TJs involve complex interactions between approximately 40 proteins, including the transmembrane proteins, occludins and claudins. These proteins are anchored to the actin filaments and myosin light chain through the zonula occludens (ZO) family. Consistent with a defective intestinal epithelial barrier, *Casr*$^{-/-}$ mice had a decreased colonic expression of TJ molecules, particularly claudin-2, a major component of TJs (Fig. 1E). We then investigated a range of ion transporter-associated genes and found that *Casr*$^{-/-}$ mice also exhibited a significantly increased expression of myosin light-chain kinase-1, an enzyme that controls contractility of the perijunctional actomyosin rings and epithelial permeability (Fig. 1F).

3.2. Defect in epithelial CaSR signaling leads to gut microbe imbalance

A mutual interaction exists between the gut microbiota and the epithelial cells comprising the intestinal barrier, and both populations can influence the other. To assess if the breakdown of intestinal epithelial integrity in *Casr*$^{-/-}$ mice alters the distribution of microbiota between either side of the epithelial barrier, we used a combination of real-time PCR and Illumina Miseq to analyze the microbiota of steady-state *Casr*$^{-/-}$ and wild-type mice. No significant differences were observed between the overall richness and diversity of the two gut microbial communities (Fig. 2A and B). However, deeper analyses indicated significant changes in composition. For instance, at the phylum level, we noted an outgrowth in the minor group Deferribacteraceae (Fig. 2C), which was previously found to correlate with inflammatory responses in the colons of *Citrobacter rodentium*-infected mice [38], a model of bacterial colitis. Concurrently, beneficial lactobacilli were decreased in *Casr*$^{-/-}$ mice (Fig. 2D). Moreover, the relative abundance and distribution of the Gram-positive *Clostridium coccoides* was significantly altered in *Casr*$^{-/-}$ mice, with depletion noted in the lumen and enrichment in the subepithelium (Fig. 2E). Consistent with enhanced bacteria translocation and dissemination in host tissues, *Casr*$^{-/-}$ mice had significantly decreased epithelial expression of *Reg3b* and *Reg3g*, which encode secreted C-type lectins that bind and protect against translocation and dissemination of Gram-negative [39] and Gram-positive bacteria [40,41], respectively (Fig. 3B).

3.3. Enhanced intestinal inflammation and immune cell activation in *Casr*$^{-/-}$ mice

Dysbiosis of the intestinal microbiota may lead to pathogenic inflammatory immune responses locally and systemically. Indeed, gene array analyses of the distal colon of wild-type and *Casr*$^{-/-}$ mice demonstrated a marked increase in the expression of a range of PRRs and cytokine-encoding genes in the colons of *Casr*$^{-/-}$ mice (Fig. 3). To distinguish between proinflammatory responses in the intestinal epithelium due to attenuated CaSR signaling versus responses induced in resident immune cells, we examined colonic leukocytes by flow cytometry. Data show that colonic CD11b$^+$ DCs upregulated their costimulatory molecules and proIL-1β and its receptor in *Casr*$^{-/-}$ mice (Fig. 4A andB). As further evidence of chronic intestinal inflammation in *Casr*$^{-/-}$ mice, higher IL1R and programmed cell death (PD)1 were significantly expressed on CD4$^+$ and CD8$^+$ T cells (Fig. 5A andB).

We then investigated differences in systemic immune responses in these mice. We found a selective expansion of CD11b$^+$ DCs in the mesenteric lymph nodes (MLNs) and spleens of *Casr*$^{-/-}$ mice (Fig. S1A and B). Moreover, higher numbers of Gr-1$^+$CD11b$^+$ neutrophils were noted in the spleens of *Casr*$^{-/-}$ mice (Fig. S1C). Similar to the colon, splenic CD11b$^+$ DCs were

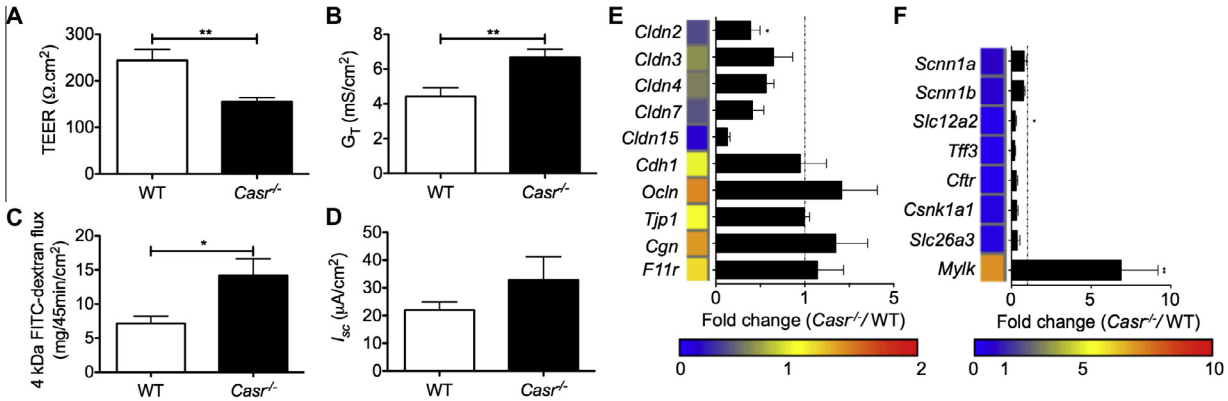

Fig. 1. (2 columns) Gastrointestinal epithelial barrier dysfunction in *Casr*$^{-/-}$ mice. Intestinal barrier integrity (A–D) and gene expression measured by Real-Time PCR (E and F) of steady-state *Casr*$^{-/-}$ mice and wild-type controls; $n = 10$ mice/group. Heat maps were developed from the mean fold change in expression (*Casr*$^{-/-}$/WT) calculated. TEER (A), transepithelial conductance (B), passive transport of FITC-conjugated dextran (C), and short-circuit current (D) of colons measured *ex vivo*. Tight junction- and ion transporter-associated gene expression of isolated colon tissues measured by Real-Time PCR (E and F). Data are shown as mean +/− S.E.M. $^*P < 0.05$, $^{**}P < 0.01$ compared with controls.

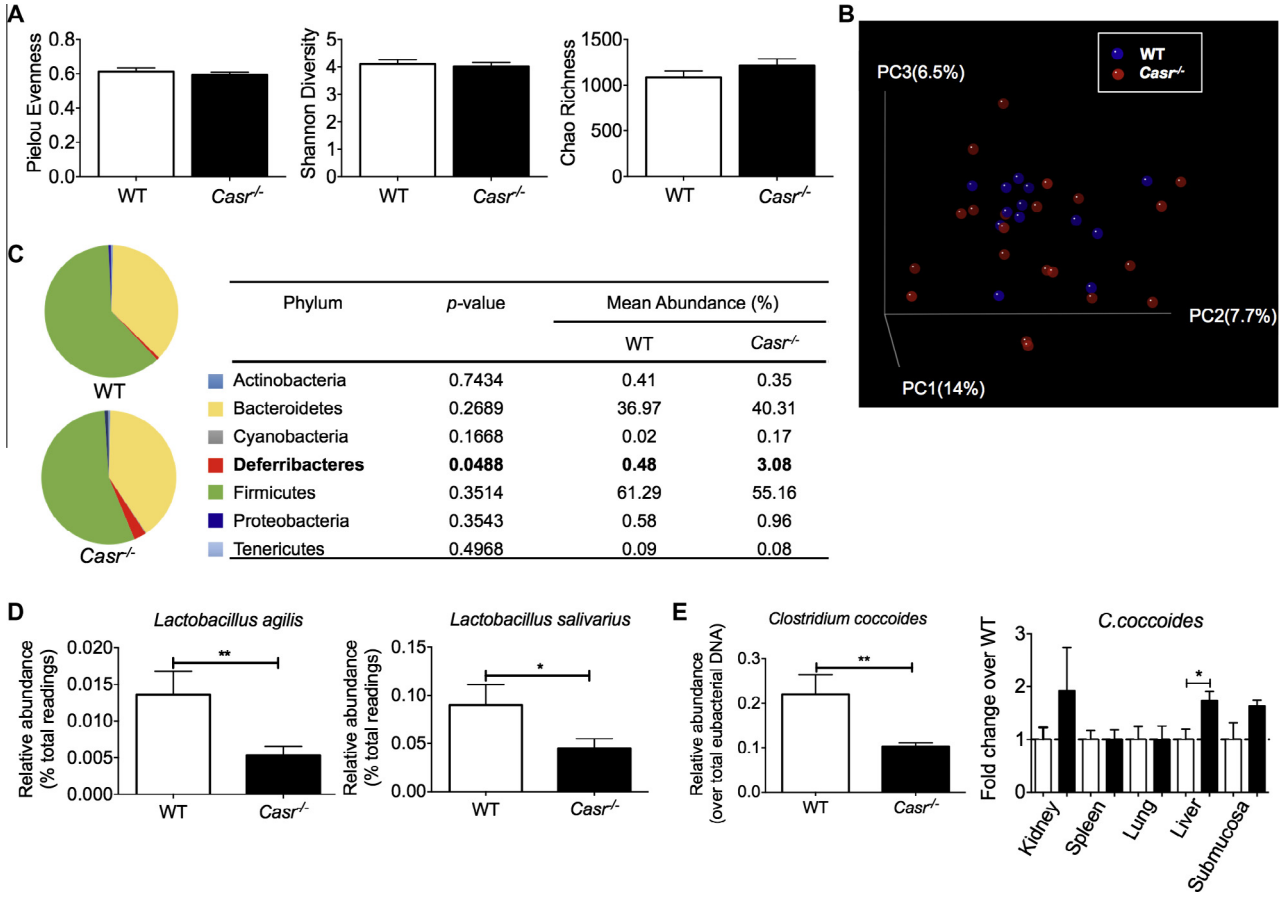

Fig. 2. (2 columns) Gut microbiota composition of *Casr*$^{-/-}$ mice. Microbiota composition alterations due to CaSR deficiency in healthy mice; $n = 10$ control mice and $n = 20$ *Casr*$^{-/-}$ mice. (A) Microbial evenness, diversity, and species richness in mice tested. Left: The species evenness index was calculated using the formula $J' = H'/H'_{max}$, where H' is the Shannon diversity index and H'_{max} is the maximal value of H'. Middle: The Shannon diversity index was used to estimate microbial diversity for each group. Right: The Chao richness index was used as a measure of species richness. (B) Unweighted UniFrac analyses were used to calculate distances between samples obtained from the two groups and three-dimensional scatterplots were generated by using principal coordinate analysis (PCoA). (C) Bacteria genera most enriched or depleted in *Casr*$^{-/-}$ mice, as measured by linear discriminant analysis (LDA). (D and E) Changes in abundance of specific bacteria and potential dissemination were measured by Real-Time PCR. Data are shown as mean +/− S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control mice.

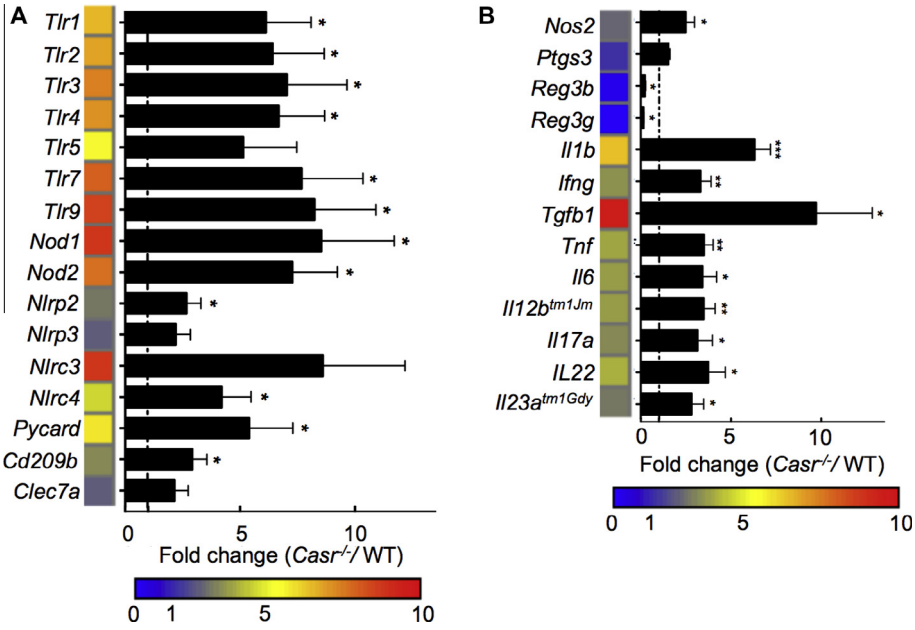

Fig. 3. (1.5 columns) Increased inflammation in the colons of *Casr*$^{-/-}$ mice. Gene expression profile of the distal colons of *Casr*$^{-/-}$ and *Casr*$^{+/+}$ littermate controls; $n = 10$ control mice and $n = 20$ *Casr*$^{-/-}$ mice. Data are shown as mean +/− S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with WT. Heat maps were developed from the mean fold change in expression (*Casr*$^{-/-}$/WT) calculated.

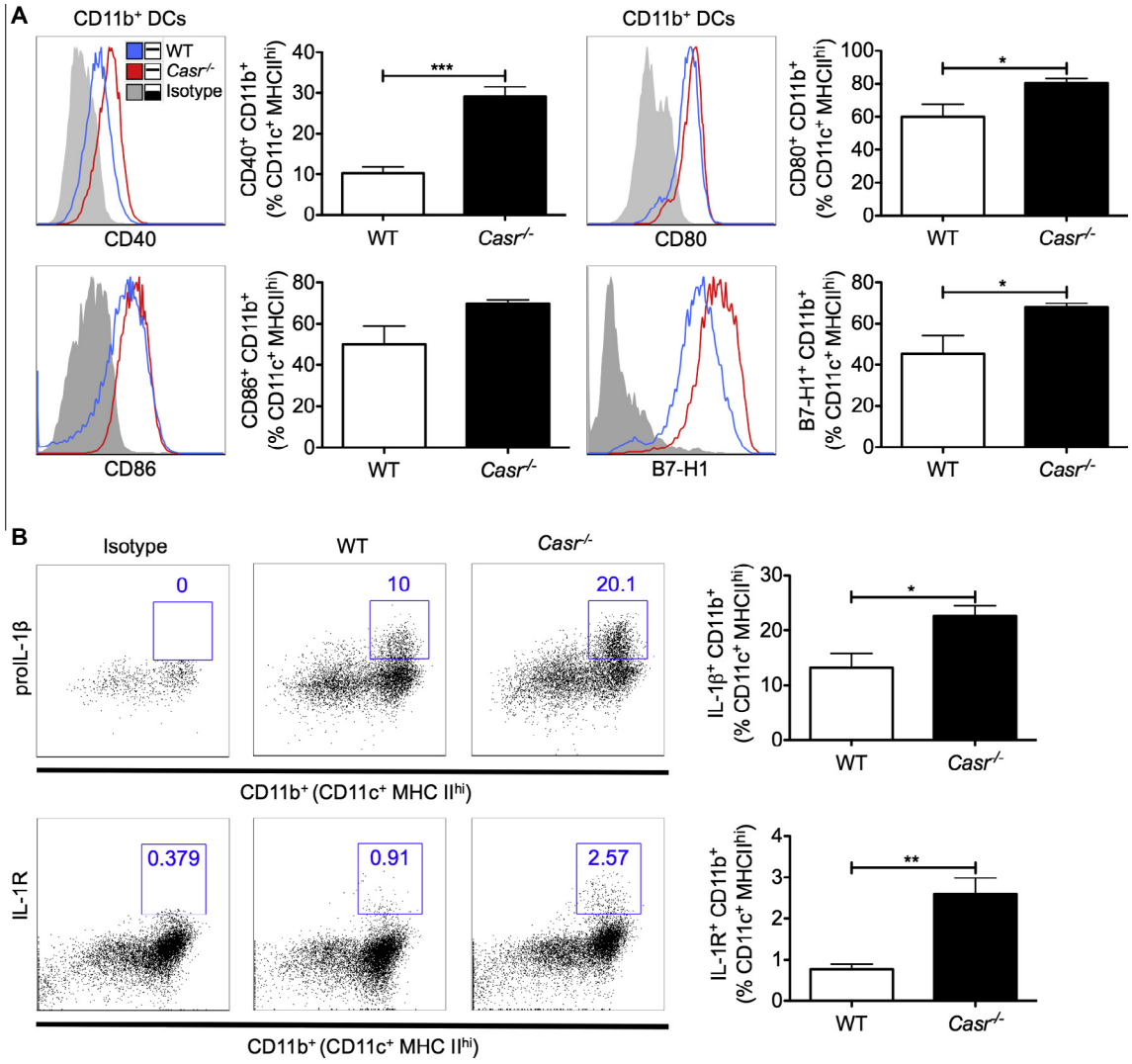

Fig. 4. (1.5 columns) Activation of proinflammatory innate immune responses in *Casr*$^{-/-}$ mice. (A) Cell surface expression of CD40, CD80, CD86, and B7-H1 in CD45^{+}MHCII^{hi}CD11c^{+}F4/80^{-}CD11b^{+} colonic DCs was analyzed by flow cytometry. Gray tinted line = isotype control; blue line = WT; red line = *Casr*$^{-/-}$ mice. (B) Production of the proinflammatory cytokine IL-1β and expression of its receptor in colonic DCs. Data represent observations from two independent experiments and are shown as mean +/− S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with WT. $n = 5$ mice/group.

significantly activated, as evident by proIL-1β production (Fig. S2A and B). In addition, IL-10^{+} was significantly reduced in splenic DCs of *Casr*$^{-/-}$ mice (Fig. S2B). DCs isolated from the MLNs of *Casr*$^{-/-}$ mice yielded similar trends, but no statistically significant differences (Fig. S3). Also, as in the colon, CaSR deficiency resulted in IL1R and PD1 upregulation in splenic and mesenteric CD4^{+} T cells (Fig. S4).

Intestinal IL1R signaling in CD4^{+} T cells has previously been found to promote Th17 responses [42]. We found that IL-17A^{+} as well as IFNγ^{+} CD4^{+} T cells were increased in the MLNs of *Casr*$^{-/-}$ mice (Fig. S5). Notably, despite the chronic intestinal inflammation observed in intestinal epithelium-specific *Casr*$^{-/-}$ mice, the frequency of FoxP3^{+} regulatory T cells (Tregs) was not decreased in these mice (Fig. S6); in fact, the number of Tregs was significantly increased in the MLNs of *Casr*$^{-/-}$ mice (Fig. S6B), perhaps as a compensatory mechanism to counteract the preexisting inflammation.

3.4. *Casr*$^{-/-}$ mice are more susceptible to DSS-induced colitis

Considering the notion that intestinal barrier dysfunction and imbalanced microbiota may contribute to enhanced susceptibility of *Casr*$^{-/-}$ mice to autoinflammatory diseases (e.g., IBD), the DSS acute induced colitis model was employed [43]. DSS promotes mucosal destruction independent of the intestinal microbiota, but the intestinal microbiota is thought to modify responsiveness and susceptibility to this chemical [44,45]. *Casr*$^{-/-}$ mice demonstrated more severe colitis with delayed recovery compared to their littermate counterparts, as demonstrated by weight changes and stool consistency at the endpoint of the experiment (Fig. 6A and B). Consistently, wild-type mice were able to recover from colitis, as shown by the gross morphology and histopathology of the colons at day 13 post-DSS (Fig. 6C and D). In contrast, *Casr*$^{-/-}$ mice still displayed severe colonic inflammation and mucosal damage (Fig. 6C and D), indicating that the intestinal CaSR plays a key role in the regulation of induced-inflammation.

4. Discussion

We demonstrate that CaSR is a key molecule expressed in gut epithelial cells that contributes to the preservation of intestinal epithelial cell integrity, and maintenance of immune homeostasis in the gut, the disruption of which results in intestinal

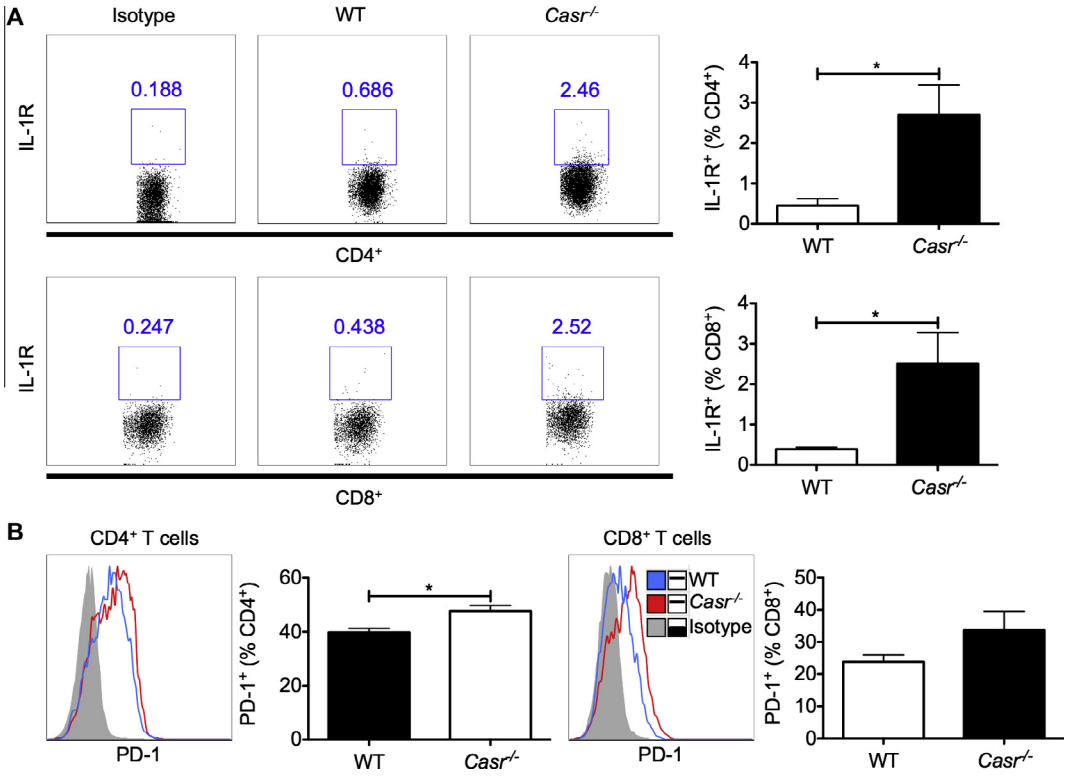

Fig. 5. (1.5 columns) Colonic T cell phenotype in *Casr*$^{-/-}$ mice. Cell surface expression of IL1R (A) and PD-1 (B) on CD4$^+$ and CD8$^+$ T cells was analyzed by flow cytometry. Data represent observations from two independent experiments and are shown as mean +/− S.E.M. $^*P < 0.05$ compared to WT. $n = 5$ mice/group.

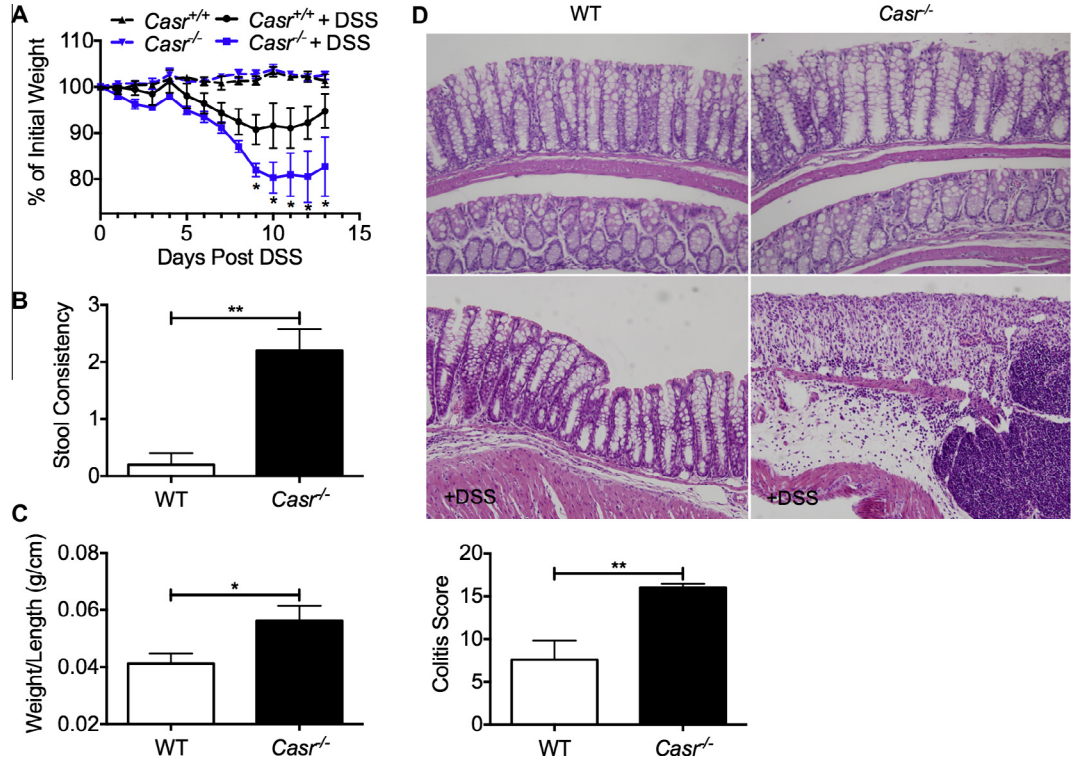

Fig. 6. (1.5 columns)*Casr*$^{-/-}$ mice are highly susceptible to DSS-induced colitis. *Casr*$^{-/-}$ and *Casr*$^{+/+}$ littermate controls were given 3% DSS in the drinking water for 7 days. Disease progression was scored by weight loss (A), diarrhea (B), gross inflammation of the colon (C), and histopathology (D). Data are shown as mean +/− S.E.M. $^*P < 0.05$, $^{**}P < 0.01$ compared to WT. $n = 5$ mice/group. 20× magnification.

inflammation. Recently, it was shown that "global"*Casr*$^{-}$/parathyroid hormone (*Pth*)$^{-}$ double knockout mice had chronic intestinal inflammation, with increased neutrophil infiltration, myeloperoxidase activity, tumor necrosis factor receptor 1(TNFR1) expression [46], and susceptibility to DSS [47]. However, given the global nature and multiple factors involved in that study, it was difficult to ascertain the cause of the inflammation. Nonetheless, the study suggested that intestinal CaSR may be anti-inflammatory. Indeed, activation of CaSR by its dietary agonists, calcium, spermine, or tryptophan, reduced inflammation (Tang & Cheng, unpublished observations), whereas inhibition of the receptor by depletion of dietary calcium enhanced gut inflammation in animal models of induced colitis [48]. Interestingly, while gut-specific CaSR detects nutrients and is potentially anti-inflammatory, the CaSR in murine bone marrow-derived macrophages/monocytes is pro-inflammatory in that it detects the "danger signal" (i.e., Ca released as a result of tissue injury) and activates the NLRP3 inflammasome [49]. Thus, role of CaSR in inflammation appears to be cell-type specific.

Nutrient availability and nutrient-sensing in the host significantly contribute to gut homeostasis and the immune responses induced [48,50–61]. In the present study, we demonstrate that a single deficiency in epithelial CaSR altered the composition of the gut microbiota, with *Casr*$^{-/-}$ mice having significantly increased Deferribacteraceae and reduced abundance of lactobacilli and *C. coccoides* (cluster XIVa of the genus Clostridium), despite identical environmental conditions. Clostridium cluster XIVa species are beneficial commensal bacteria that induce butyrate production, Treg development, maintenance of barrier function, and competition with pathogens in gut colonization. The specific depletion of commensal Clostridia, together with the outgrowth of small groups such as Deferribacteraceae, may thus contribute to the disruption of mucosal homeostasis and subsequent activation of local immune responses seen in *Casr*$^{-/-}$ mice. Indeed, depletion of Clostridia has been consistently associated with chronic inflammation in patients with IBD [62–65] and atopy [66], whereas Deferribacteraceae was shown to correlate with the level of inflammatory responses in *C. rodentium*-infected mice [38]. Whether these changes are a cause or a consequence of inflammation, or consequent to the downregulation of the anti-inflammatory CaSR, requires further investigation.

The immune responses observed in *Casr*$^{-/-}$ mice suggest that intestinal epithelial CaSR deficiency leads to a shift in local and systemic innate and T cell immune responses from a status characterized by regulation to one that is highly stimulated. However, since the expression of PD1, a molecular signature of T cell exhaustion [67], was significantly enhanced on T lymphocytes, the activated immune phenotype displayed in the immune cells of *Casr*$^{-/-}$ mice may not necessarily indicate active, effective immunity, but evidence of chronic intestinal inflammation. In further support of chronic inflammatory responses, Th1 and Th17 responses were elevated in *Casr*$^{-/-}$ mice. Interestingly, FoxP3^{+} Tregs were not decreased, but were increased in *Casr*$^{-/-}$ mice, particularly in the MLNs. Nonetheless, the quality of these Tregs warrants further investigation, as studies have highlighted the plasticity of these cells toward a proinflammatory phenotype [68]. Therefore, considering the heightened state of inflammation in *Casr*$^{-/-}$ mice, it is conceivable that *Casr*$^{-/-}$ mice-derived FoxP3^{+} Tregs may be functionally defective. This state of chronic inflammation rendered *Casr*$^{-/-}$ mice highly susceptible to DSS-induced colitis, indicating that, in addition to defective intestinal barrier function and increased inflammation, CaSR deficiency may also result in impaired wound healing in the gut.

In summary, the present study demonstrates that attenuated CaSR signaling in the gut epithelium leads to enhanced permeability of the epithelial barrier, resulting in translocation and dissemination of luminal bacteria and activation of local and systemic innate and adaptive proinflammatory immune responses. The subsequent excessive gut inflammation disrupts the intestinal milieu and impairs gut homeostasis, thereby contributing to susceptibility to autoinflammatory diseases, such as IBD. Thus, the CaSR may serve as a potential therapeutic target for a range of autoinflammatory intestinal disorders, including IBD and colon cancer.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.05.007.

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