Biochemical and Biophysical Research Communications 464 (2015) 647–653

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

MAR binding protein SMAR1 favors IL-10 mediated regulatory T cell function in acute colitis

Bhalchandra Mirlekar [a], Sachin Patil [a], Ramanamurthy Bopanna [b], Samit Chattopadhyay [a, *]

[a] Chromatin and Disease Biology Laboratory, National Centre for Cell Science, Ganeshkhind, Pune 411007, India
[b] Experimental Animal Facility, National Centre for Cell Science, Ganeshkhind, Pune 411007, India

ARTICLE INFO

Article history:
Received 30 June 2015
Accepted 6 July 2015
Available online 10 July 2015

Keywords:
Colitis
DSS
IL-10
IL-17
SMAR1
T_{reg}

ABSTRACT

T_{reg} cells are not only crucial for controlling immune responses to autoantigens but also prevent those directed towards commensal pathogens. Control of effector immune responses by T_{reg} cells depend on their capacity to accumulate at inflammatory site and accordingly accommodate to inflammatory environment. Till date, the factors associated with maintaining these aspects of T_{reg} phenotype is not understood properly. Here we have shown that a known nuclear matrix binding protein SMAR1 is selectively expressed more in colonic T_{reg} cells and is required for their ability to accumulate at inflammatory site and to sustain high levels of Foxp3 and IL-10 expression during acute colitis. Elimination of anti-inflammatory subsets revealed a protective role for IL-10 producing T_{reg} cells in SMAR1$^{-/-}$ mice. Moreover, a combined action of Foxp3 and SMAR1 restricts effector cytokine production and enhance the production of IL-10 by colonic T_{reg} cells that controls acute colitis. This data highlights a critical role of SMAR1 in maintaining T_{reg} physiology during inflammatory disorders.

1. Introduction

Each microenvironment requires a precise set of regulatory mechanism that is finely and always tuned to maintain local homeostasis. Various populations of regulatory T cells (T_{reg}) contribute to the maintenance of this balance and organization of controlled immune responses [1]. In particular, T_{reg} cells limit the magnitude of effector responses, which may result in failure to adequately control infection and inflammation [2]. However, T_{reg} cells also help to limit the inflammation caused by various immune responses against pathogenic microbes as well as commensals [3]. Recent reports highlight the pathogenic role of T and B lymphocytes in the development of intestinal inflammation. A subset of CD4$^+$ T cell such as Th17 cells are clearly involved in the development of inflammation by secreting pro-inflammatory cytokines and activating adaptive immune responses [4,5]. In addition to that, they play a crucial role in the induction of IgA secreting B cells and causes subsequent immune activation [6,7]. Such an activated pathogenic inflammatory cascade may be important in enhancing intestinal permeability, where there is increased exposure to luminal microbial pathogens [8].

Upon activation, naïve CD4$^+$ T cells differentiate into different lineages of helper T (Th) cells that are characterized by its developmental regulation and distinct biological functions. T_{reg} cells have been identified as a new lineage of CD4$^+$ T cells, and they have shown to play an important role in various immune responses like infections, inflammation and autoimmune diseases [1,9]. The combination of immunoregulatory cytokine TGF-β and the pro-inflammatory and pleotropic cytokine, IL-2 is required to induce T_{reg} cells and massive amount of IL-10 from naïve T cells [10–12]. Activation of naïve T cells with TGF-β and IL-2 in absence of IL-6 induces a distinct transcription factor, Foxp3, which dictates the cell towards immune regulatory T_{reg} cells [13,14]. Foxp3 is also reported to bind to IL-10 promoter and activate its gene transcription [15]. Thus, IL-10 gene transcription in an activated naïve T cell is under the tight control of specific co-operative external stimuli, contributed by TGF-β and IL-2. Abnormal levels of distinct transcriptional factors and nuclear matrix proteins are noted in the progression of many pathological and proliferative diseases [16]. The manifestations of these diseases are correlated mainly with the disturbances in the conformation of chromatin, facilitated by the

* Corresponding author. National Centre for Cell Science, Pune University Campus, Ganeshkhind, Pune 411007, India.
E-mail address: samit@nccs.res.in (S. Chattopadhyay).

http://dx.doi.org/10.1016/j.bbrc.2015.07.028
0006-291X/

nuclear matrix proteins [17,18]. Thus, unraveling the nature and functions of these proteins has a great importance in understanding the T_{reg} cell biology and disease manifestations.

The role of T_{reg} cells, being an indispensable in sustaining intestinal homeostasis, factors maintaining their appropriate phenotype consistent with regulatory functions and their accumulation in inflamed site is a need to understand. In the current study, we have shown that the matrix attachment region (MAR) binding protein (MARBP) scaffold/matrix attachment binding protein (SMAR1) plays a critical role in maintaining T_{reg} physiology by limiting the polarization towards an effector phenotype and enhancing their regulatory capacity by producing IL-10 and accumulate at inflamed site.

2. Materials and methods

2.1. Mice

For the generation of T cell specific conditional knock-out mice, $SMAR1^{f/f}$ mice were crossed with Lck–Cre mice to form *Lck–Cre* $SMAR1^{f/f}$ ($SMAR1^{-/-}$) mice and the chimeras were screened for the transgene [19] (generated by Ozgene, Australia). Wild-type C57BL/6J and all other mice were inbred in the experimental animal house facility of the institute. Animal experiments were done with mice 6–8 weeks of age and protocols were approved by the Institutional Animal Ethical Committee.

2.2. Flow cytometry

Anti-CD4, anti-CD62L, anti-CD44, anti-CD25, anti- IFN-γ, anti-IL-17, anti-IL-4, anti-Foxp3, anti-RORγt, anti-TNF-α, anti-IL-10, anti-CD45B/220, anti-CD19, anti-GATA3, anti-IgA were purchased from BD Bioscience. Anti-SMAR1 was purchased from Bethyl Laboratories. For intracellular cytokine staining, *in vitro* cultured cells were restimulated with PMA (Sigma, 50 ng/mL) and ionomycin (Sigma, 400 ng/mL) for 4 h. Golgistop (BD Bioscience) was added during the last 3 h of restimulation. After staining for the extracellular markers, cells were fixed, permeabilized, and stained for intracellular specific cytokines. Cells were acquired on a FACS CANTO flow cytometer (BD) and data analyzed with FACS DIVA software. For purification of T cell subsets, $CD4^+$ T cells were first enriched by magnetic-activated cell sorting beads (autoMACS; Miltenyi Biotec) and then further purified with a FACS ARIA (BD Bioscience). Purity of sorted cells was higher than 98%.

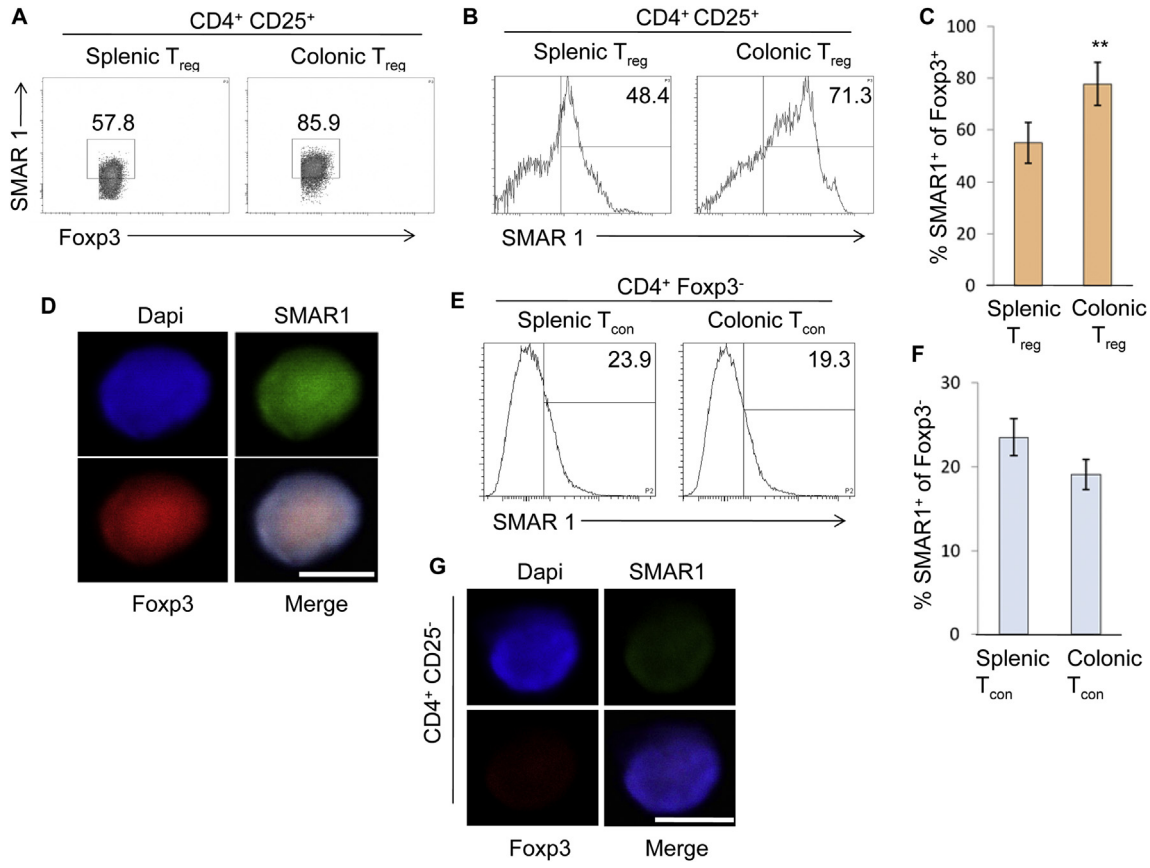

Fig. 1. Expression of SMAR1 by T_{reg} and T_{con} cells. (A) Colonic T_{reg} express Foxp3 and SMAR1. $CD4^+CD25^+$ T cells were harvested from colonic LP, stained for Foxp3, SMAR1 and assessed by flow cytometry; isotype for Foxp3. Number in plots refer to percentage of $SMAR1^+Foxp3^+$ cells gated on live $CD4^+$ cells. (B) SMAR1 is highly expressed by colonic T_{reg} cells. $CD4^+CD25^+$ T_{reg} cells were sorted from spleen and colon LP and stained for SMAR1. Number refers to frequency of $SMAR1^+$ cells. Plots are gated on $CD4^+CD25^+$ T cells. Data shown is representative of 3 independent experiments. (C) Colonic T_{reg} expresses high level of SMAR1 than splenic T_{reg}. Percentage of $CD4^+$ $Foxp3^+$ T_{reg} cells expresses SMAR1 in spleen and colon LP. n = 6 mice per group. $^{**}p < 0.01$. Data is represented as mean ± SEM. (D) Nuclear localization of SMAR1. $CD4^+CD25^+$ T_{reg} cells were purified by sorting from the colonic LP and stained for Foxp3 (red), SMAR1 (green) and dapi (blue) and localization of proteins was assessed by confocal microscopy. Scale bar: 5 μm. (E) Cells were harvested from spleen and colon LP and stained for the expression of CD4, Foxp3 and SMAR1 and assessed by flow cytometry. Histogram shows SMAR1 expression on $CD4^+Foxp3^-$ cells. (F) Percentage of $CD4^+Foxp3^-$ cells expressing SMAR1 in spleen and colonic LP. n = 6 mice per group. Data is represented as mean ± SEM. (G) $CD4^+CD25^-$ T cells were purified by sorting from colonic LP and stained for Foxp3 (red), SMAR1 (green) and dapi (blue) and localization of proteins was assessed by confocal microscopy. Scale bar: 5 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

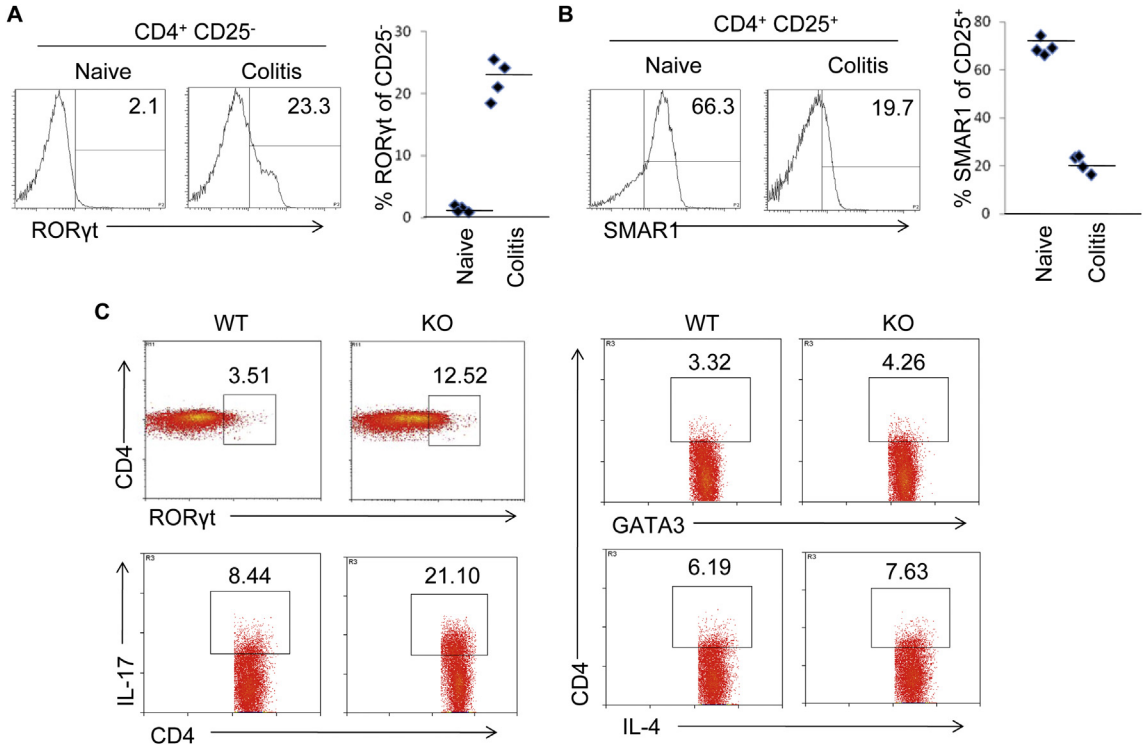

Fig. 2. Altered expression of SMAR1 in colonic LP T_{reg} differentiates towards Th17 cells during acute colitis. (A) Flow cytometric analysis of intracellular transcription factor RORγt in colonic LP $CD4^+CD25^-$ T cells. Cells were obtained from 6 week old WT mice after DSS induced colitis, or left untreated (Naïve). Data is representative of 3 independent experiments with 6 mice per group. (B) Flow cytometric analysis of intracellular SMAR1 in colonic LP $CD4^+CD25^+$ T cells. Cells were obtained from 6 week old WT mice after DSS induced colitis, or left untreated (Naïve). Data is representative of 3 independent experiments with 6 mice per group. (C) SMAR1 deficient $CD4^+$ T cells preferentially differentiate towards Th17 cells but not towards Th2 cells during acute DSS colitis. Cells were harvested from the colonic LP and then stained for CD4, RORγt, IL-17, GATA3 and IL-4 or stimulated for 4 h with PMA/ionomycin and then analyzed by flow cytometry. Plots are gated on CD4. Number in quadrant refers to the percentage of each subset. n = 6 mice per group. Data shown is representative of 3 independent experiments.

2.3. Immuno-fluorescence staining

Cells were harvested and fixed in 4% paraformaldehyde, permeabilized with 0.1% Triton X-100 and incubated with different specific antibodies. Signals were amplified by using the anti mouse and anti rabbit fluorescent labeled secondary antibodies (Millipore, US). For nuclear staining, cells were treated with dapi (Fluka). Coverslips were mounted with dako fluorescent mounting media and examined with a Zeiss LSM-510 Meta Confocal Laser Scanning Microscope (Carl Zeiss MicroImaging, Thornwood, NY).

2.4. DSS induced colitis

Mice were provided 3% (w/v) DSS (Mol. Wt. 36–50 kDa, MP biomedical) for 6–8 days, and then colon was assessed for length, weight and histology. IL-10 depletion prior to DSS treatment was carried out by performing two 100 μg injections i.p. at day 5 and day 2 prior to DSS treatment using anti-IL-10 (JES5-2A5) monoclonal antibody (Bio X Cell). Daily clinical assessment of animal includes detection of blood in stool, evaluation of stool consistency and body weight loss measurement. Disease activity index (DAI) ranging from 0 to 5 was calculated using each of these parameters [20].

2.5. Bacterial count

Organs were weighed and colon length was measured. For MLN, the whole organ was homogenized in 1 ml sterile PBS. For ileum, a piece (100 mg) was collected and homogenized. For colonic bacterial count, fecal content were removed by rinsing opened colon with sterile PBS, then cut in to 1 cm segments and epithelial cells were removed by vigorous horizontal shaking at 37 °C for 30 min in HBSS containing 5% FCS, 10 mM HEPES and 2 mM EDTA. Samples were homogenized in 1 ml sterile PBS using an Omni-prep homogenizer. Serial dilutions were then plated onto blood agar plates and incubated at 37 °C for 48 h, and cfu were calculated.

2.6. Histology

Histological examination was performed on whole colons of experimental and control mice. Colonic sections were fixed in 10% formalin and embedded in paraffin. 5 μm sections were stained with H and E. Histological parameters were quantified in an unbiased way by using parameters as previously described [21].

2.7. Statistical analysis

For all experiments, unless stated otherwise, the unpaired student's t test was applied with GraphPad Prism software to all data points. Correlation between the mice data was tested with pearson's correlation test. *p value* of less than 0.05 was considered significant.

3. Results and discussion

3.1. $Foxp3^+$ T_{reg} cells from colonic site express high level of SMAR1

Large number of infiltrating effector Th17 cells plays a pathogenic role at site undergoing constitutive inflammation such as colon [22,23]. Our preliminary observation showed a critical role of SMAR1 in the differentiation of $CD4^+$ T cells to Th1 subtype by the regulation of T-bet promoter. The expression level of SMAR1 is

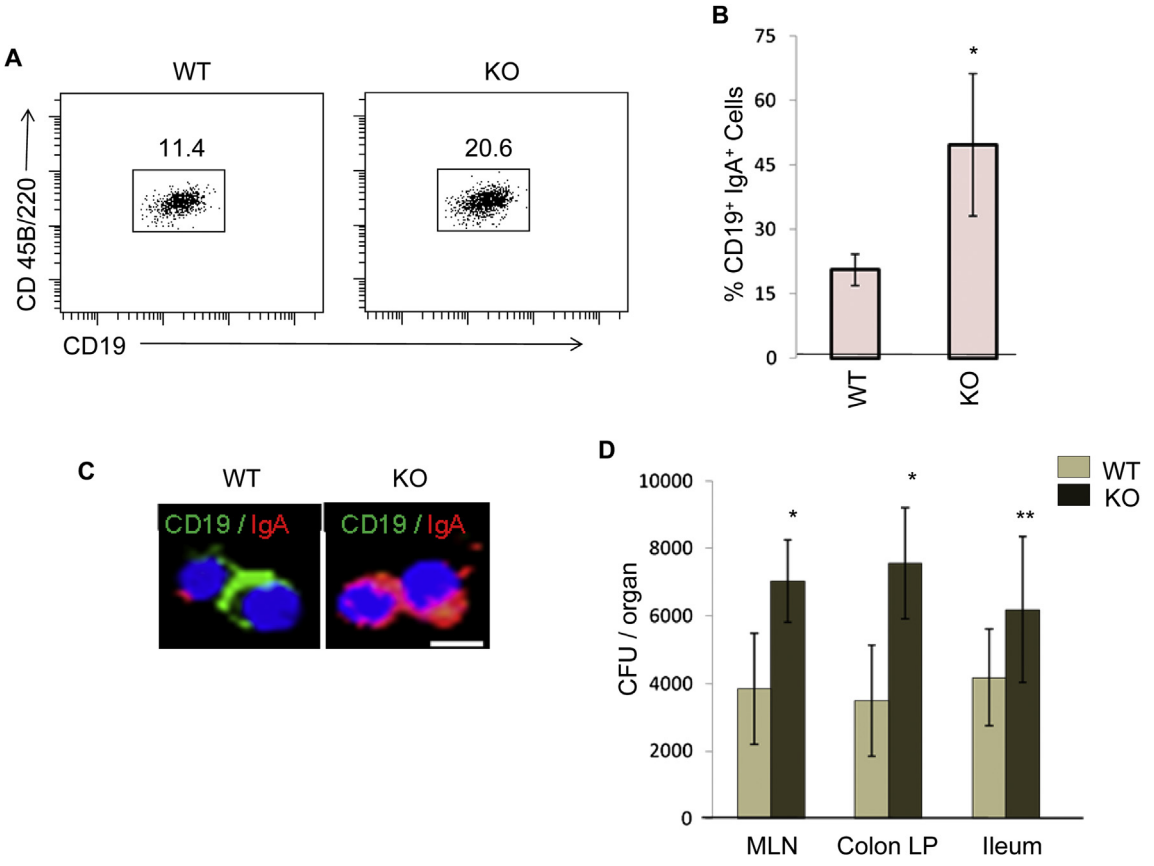

Fig. 3. Increased CD19$^+$ B cell levels and bacterial translocation in SMAR1$^{-/-}$ mice. (A) Representative FACS analysis of colonic B cells from DSS treated WT and SMAR1$^{-/-}$ mice. Staining was done with anti-B220 and anti-CD19 antibodies and analyzed by flow cytometry. Number in plots is the percentages of B cells (n = 6 mice per group). (B) WT and SMAR1$^{-/-}$ mice treated with DSS. Cells were harvested from colonic LP and their expression of CD19 and IgA was evaluated by flow cytometry. Cells were stained for CD19 and IgA. Graph depicts the frequency of CD19$^+$IgA$^+$ cells in the colonic LP. $^*p < 0.005$ (n = 6 mice per group). (C) Representative images of colonic B cells from DSS treated WT and SMAR1$^{-/-}$ mice showing CD19/IgA double positive cells. Confocal images showing CD19 staining (green) and IgA staining (red) on representative double positive cells. Cells were counterstained with dapi (blue). Scale bar: 5 μm. (D) Bacterial cfu count from MLN, colonic LP and Ileum samples from DSS treated WT and SMAR1$^{-/-}$ mice (n = 6). Organs were collected and tissue homogenates were prepared. Serial dilutions were plated into blood agar medium and the numbers of bacterial cfu were calculated. $^*p < 0.01$, $^{**}p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

downregulated in effector CD4$^+$ T cells like Th1 and Th17 [21]. On the other hand, a substantial fraction of CD4$^+$ T$_{reg}$ cells express SMAR1. Interestingly, T$_{reg}$ cells from large intestinal lamina propria (LP) expressed higher level of SMAR1 as compared to splenic T$_{reg}$ cells (Fig. 1A and B). Evaluation of SMAR1 at colonic LP site revealed that 80% of T$_{reg}$ cells expressed SMAR1 (Fig. 1C). Confocal imaging of purified CD4$^+$CD25$^+$ T cells confirmed the nuclear localization of Foxp3 and SMAR1 in large intestinal LP T$_{reg}$ cells (Fig. 1D). In contrast, the expression of SMAR1 in CD4$^+$CD25$^-$ Foxp3$^-$ conventional T (T$_{con}$) cells was 3–4 fold less in comparison to T$_{reg}$ cells (Fig. 1E and F). Comparatively, confocal imaging of purified CD4$^+$CD25$^-$ T cells showed lower amount of SMAR1 in the nucleus of effector T cells (Fig. 1G). We found that SMAR1 is highly expressed in colonic T$_{reg}$ cells and confocal analysis confirms the co-expression of SMAR1 and Foxp3 in colonic T$_{reg}$. Also, a significant co-expression of SMAR1 and Foxp3 was observed in considerable fraction of T$_{reg}$ cells from colonic LP.

3.2. Altered expression of SMAR1 in colonic LP T$_{reg}$ differentiates towards Th17 cells during acute colitis

As the reduced expression of SMAR1 in Foxp3$^-$ T$_{con}$ cells leads to their effector phenotype, it indicates reciprocal control of effector T cells and regulatory T cells by SMAR1. We observed, a substantial fraction of CD4$^+$CD25$^-$ T cells expressed Th17 lineage specific transcription factor, RORγt during colonic inflammation (Fig. 2A). To further evaluate the role of SMAR1 in acute colitis, CD4$^+$CD25$^+$ T$_{reg}$ cells from dextran sodium sulphate (DSS) treated wild type (WT) mice were assessed for the expression of SMAR1. CD4$^+$CD25$^+$ T$_{reg}$ cells from diseased mice showed 3.5–4 fold downregulation of SMAR1 in comparison with untreated naïve mice (Fig. 2B). Additionally, SMAR1 deficient T$_{reg}$ cells expressed 2.5–3.5 fold higher level of RORγt and effector cytokine IL-17 compared with WT T$_{reg}$ cells during DSS induced acute colitis. While, we did not observe altered expression of Th2 lineage specific transcription factor GATA3 and cytokine IL-4 in CD4$^+$ T$_{reg}$ cells from SMAR1$^{-/-}$ and WT mice (Fig. 2C). In support to our data, we found increased expression of RORγt and IL-17 in SMAR1$^{-/-}$ colonic LP T$_{reg}$ cells compared to WT during colonic inflammation (Fig. 2C). Thus, under homeostatic proliferative situation, CD4$^+$ T$_{reg}$ cells expressed lower amount of SMAR1 and produced effector cytokine IL-17. SMAR1$^{-/-}$ mice showed a perturbed Th17 response during inflammatory diseases. SMAR1 expression in T$_{reg}$ cells control the expression of pro-inflammatory cytokines and is required to maintain T$_{reg}$ phenotype. During acute intestinal inflammation, loss of SMAR1 led to altered regulatory phenotype and hence generation of RORγt$^+$IL-17$^+$ pathogenic Th17 cells. Reports on plasticity of T$_{reg}$ cells in inflammatory response showed the control of T$_{reg}$ cells by specific transcription factors in polarized condition and loss of this polarity leads to expression of effector cytokines [24–26]. We found that

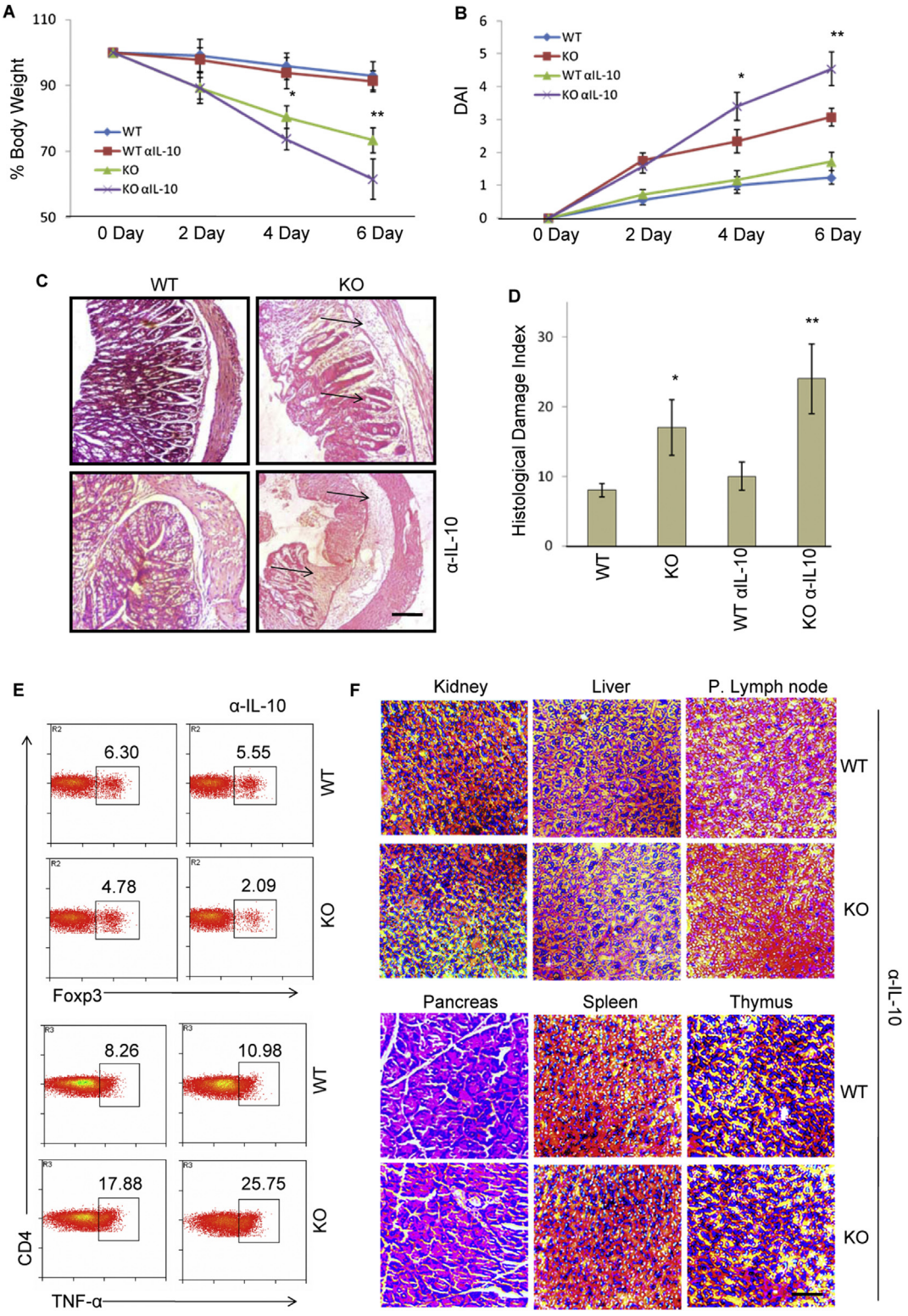

Fig. 4. CD4 dependent IL-10 production suppresses acute colitis in SMAR1$^{-/-}$ mice. (A and B) Body weight changes (A) and disease activity index score (DAI) (B) from WT and SMAR1$^{-/-}$ mice treated with 100 μg anti-IL-10 neutralizing antibodies at day 5 and day 2 prior to DSS treatment, or left untreated. Data is represented as mean ± SEM and are pooled from 3 independent experiments (n = 6). (C) Photomicrographs of representative H and E stained colon sections from WT and SMAR1$^{-/-}$ mice treated with anti-IL-10 neutralizing antibodies or untreated control groups at day 6 following 3% DSS treatment. Images show similar region (arrow) of colon sections highlighting predominant histological changes in each of the experimental group. Scale bar represents 100 μm. (D) Histological damage index scores of whole mouse colons collected after only DSS treatment and DSS plus anti-IL-10 treatment (horizontal axis). Data are from 6 mice per group and represented as mean ± SD. $^{*}p < 0.01$, $^{**}p < 0.005$. (E) Flow cytometry of intracellular Foxp3 and TNF-α in CD4^{+} T cells from the colonic LP of WT and SMAR1$^{-/-}$ mice treated with anti-IL-10 neutralizing antibodies or untreated control groups at day 6 following 3% DSS treatment. Numbers in plot indicate percent Foxp3^{+} and TNF-α^{+} cells among gated CD4^{+} T cells. (F) Macroscopic and histological analysis of the kidney, liver, peripheral lymph node (P. lymph node), pancreas, spleen and thymus of 6 week old WT and SMAR1$^{-/-}$ mice treated with 100 μg anti-IL-10 neutralizing antibodies at day 5 and day 2 prior to DSS treatment. Scale bar represents 100 μm. One representative picture per group is shown.

expression of SMAR1 in T_{reg} cells is downregulated during colonic inflammation and SMAR1 deficient T_{reg} cells produced large amount of proinflammatory cytokine IL-17 compared to WT. It clearly shows effector cytokine production by T_{reg} cells occurred in a condition, in which, SMAR1 was reduced or absent.

3.3. Increased IgA and bacterial translocation in SMAR1$^{-/-}$ mice during acute colitis

We previously demonstrated that $CD4^+$ T cells, which mainly composed of IL-17 secreting Th17 cells, are increased in large intestine from SMAR1$^{-/-}$ mice compared to WT mice during DSS colitis. Therefore, we examined whether this could be associated with increased number of $CD19^+B220^+$ B cells leading to increased IgA production. By FACS staining we found 2 ± 0.2 fold elevated level of $CD19^+B220^+$ B cells in colonic mucosa from SMAR1$^{-/-}$ mice compared to WT mice (Fig. 3A). Consistent with elevated levels of $CD19^+B220^+$ B cells in colonic mucosa, there was 2.5 ± 0.3 fold abundance of LP $CD19^+IgA^+$ plasma cells in SMAR1$^{-/-}$ mice (Fig. 3B). Furthermore, quantification of $CD19^+IgA^+$ plasma cells revealed a significant increase in SMAR1$^{-/-}$ mice compared to WT mice (Fig. 3C). Because SMAR1 deficient T cells preferentially differentiate towards Th17 phenotype leading to more infiltration of Th17 cells and $CD19^+IgA^+$ plasma cells, we examined whether the increased IgA levels correlates with enhanced translocation of luminal bacteria into the colonic LP. Bacterial count was determined by plating serial dilutions and measuring colony-forming units (cfu) in colonic LP tissue homogenates derived from mucosa. We found significantly higher number of bacteria in the colonic LP from SMAR1$^{-/-}$ mice compared to control WT mice with an enormous difference in number of cfu (Fig. 3D). However, there was no significant difference of total bacterial count between WT and SMAR1$^{-/-}$ mice in colonic LP without DSS treatment. In addition, bacterial growth was also significantly increased in the mesenteric lymph nodes (MLNs) and ileum of DSS treated SMAR1$^{-/-}$ mice compared to WT mice (Fig. 3D), suggesting that bacteria translocated into LP in SMAR1$^{-/-}$ mice, which further disseminated into secondary lymphoid organs and peripheral tissues. Our data is also consistent with a critical role of Th17 cells in IgA isotype class switching in $CD19^+$ B cells through developing IgA mediated immune responses in the gut induced by antigen exposure [6,7]. Hence, IgA associated responses are important for lymphocyte mediated compensation during enhanced intestinal inflammation. The exact mechanism by which IgA antibodies functions in colonic inflammation in SMAR1$^{-/-}$ mice remains unclear, but may involve increased transport of antigens across the epithelium, subsequent recognition and phagocytosis by antigen presenting cells.

3.4. CD4 dependent IL-10 production suppresses acute colitis in SMAR1$^{-/-}$ mice

The immunosuppressive cytokine IL-10 has also been critically involved in suppression of colitis and it is known to play an important role in T_{reg} suppressive function [27,28]. In addition to colonic LP macrophages, IL-10 producing $CD4^+$ T_{reg} cells are also well known to regulate intestinal immune responses [28,29]. Interestingly, we observed that relative IL-17 level was considerably high in SMAR1$^{-/-}$ T_{reg} cells and it enhanced frequency of $CD19^+IgA^+$ B cells leading to altered regulatory activity in SMAR1$^{-/-}$ mice during acute colitis. To investigate the role of $CD4^+$ T_{reg} cells secreting IL-10 from SMAR1$^{-/-}$ mice, mice were treated with neutralizing IL-10 antibodies at 5 days and 2 days prior to DSS treatment. Antibody treated SMAR1$^{-/-}$ mice were far more susceptible to DSS induced acute colitis than untreated SMAR1$^{-/-}$ mice, beginning to lose weight from day 3 post DSS treatment (10 ± 5%) and losing close to 65% of their initial body weight by day 6 (35 ± 5%) (Fig. 4A). In addition, anti-IL-10 treated SMAR1$^{-/-}$ mice also developed diarrhea at much earlier time point (day 4 post DSS treatment) than their untreated counterparts, which contribute to a significantly higher disease activity index (DAI) (4.5 ± 0.3) (Fig. 4B). Histological analyses revealed extensive ulceration, mucosal injury and inflammation in anti-IL-10 treated SMAR1$^{-/-}$ mice. In contrast, the increased susceptibility to DSS following anti-IL-10 administration was not observed in WT mice (Fig. 4C and D). Because SMAR1$^{-/-}$ mice were observed to have increased mucosal IL-17 production, anti-IL-10 administration led to enhanced susceptibility to DSS. We investigated whether altered function of T_{reg} cells in absence of SMAR1 could lead to enhanced production of proinflammatory cytokines. The first observation was 2.5 ± 0.3 fold reduction in Foxp3 level in anti-IL-10 treated SMAR1$^{-/-}$ mice following DSS treatment (Fig. 4E). Intracellular cytokine staining (ICS) of colonic LP lymphocytes from anti-IL-10 treated SMAR1$^{-/-}$ mice following DSS treatment, which are highly enriched in effector $CD4^+$ T cells, showed augmented induction of TNF-α revealing that lymphocytes are a major source of TNF-α (Fig. 4E). No other organs including lymphoid organs were affected from anti-IL-10 treated WT and SMAR1$^{-/-}$ mice following DSS treatment (Fig. 4F). These results indicate depletion of IL-10 in SMAR1$^{-/-}$ mice causing increased susceptibility to acute colitis compared to WT mice. It shows $CD4^+$ T_{reg} cells, being the major source for IL-10 in SMAR1$^{-/-}$ mice, can help in maintaining the immune homeostasis. Neutralization of IL-10 in SMAR1$^{-/-}$ mice led to striking finding that compensation for T_{reg} cell defects also depend on IL-10 signaling. It is possible that increased IL-17 and TNF-α production in SMAR1$^{-/-}$ mice compared with WT mice produces a secondary immune response to increase bacterial translocation and subsequent immune activation. Foxp3 amount in SMAR1$^{-/-}$ mice are greatly diminished upon anti-IL-10 treatment following DSS administration; we demonstrate that T_{reg} cells are a major source of IL-10 in SMAR1$^{-/-}$ mice. Furthermore, we showed that increased colonic TNF-α production in SMAR1$^{-/-}$ mice during acute colitis is $CD4^+$ T cell dependent. This data propose that IL-10 secreting T_{reg} cells are a critical component of immune mediated protection during increased intestinal inflammation in SMAR1$^{-/-}$ mice.

In this report, we highlight the lymphocytic contribution controlling immune responses in the gut and their mechanisms of control, which is associated with maintenance of intestinal barrier function and suppression of acute inflammation. In this context, $CD4^+$ $Foxp3^+$ T cells play an important role in the maintenance of intestinal homeostasis through IL-10 dependent mechanisms and their action is complemented by additional T and B lymphocytes [30]. Collapse in immune regulatory networks in the intestine leads to inflammatory diseases of the gut such as inflammatory bowel disease (IBD). In this condition, regulatory lymphocytes are an attractive target for therapies of intestinal inflammation. In the context of T_{reg} cell biology, the current study reveals a novel role of SMAR1 in controlling T_{reg} physiology during inflammation. Therefore, the regulation and function of T_{reg} is an interesting target for immunotherapy in inflammatory disorders.

Conflict of interest

The authors have no financial conflicts of interest.

Acknowledgment

We thank our Director Dr. Shekhar Mande for providing us opportunity to work on this project. We thank Dr. L.S. Limaye, Mrs. Trupti Joshi for FACS facility, Dr. Rahul Bankar, Mr. M.L. Shaikh for assistance with animal husbandry. This work is funded by NCCS,

Department of Biotechnology, Indian Council of Medical Research (ICMR), New Delhi. BM is a recipient of fellowship from ICMR, New Delhi.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.07.028.

References

[1] A. Izcue, J. Coombes, F. Powrie, Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation, Immunol. Rev. 212 (2006) 256–271.
[2] L.A. Zenewicz, A. Antov, R.A. Flavell, CD4 T-cell differentiation and inflammatory bowel disease, Trends Mol. Med. 15 (2009) 199–207.
[3] D.A. Vignali, L.W. Collison, C.J. Workman, How regulatory T cells work, Nat. Rev. Immunol. 8 (2008) 523–532.
[4] S. Fujino, A. Andoh, S. Bamba, et al., Increased expression of interleukin 17 in inflammatory bowel disease, Gut 52 (2003) 65–70.
[5] E.V. Acosta-Rodriguez, G. Napolitani, A. Lanzavecchia, F. Sallusto, Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells, Nat. Immunol 8 (2007) 942–949.
[6] P.J. Milpied, M.G. McHeyzer-Williams, High-affinity IgA needs TH17 cell functional plasticity, Nat. Immunol. 14 (2013) 313–315.
[7] K. Hirota, J. Turner, M. Villa, et al., Plasticity of TH17 cells in Peyer's patches is responsible for the induction of T cell–dependent IgA responses, Nat. Immunol. 14 (2013) 372–380.
[8] A. Kaser, S. Zeissig, R.S. Blumberg, Inflammatory bowel disease, Annu. Rev. Immunol. 28 (2010) 573–621.
[9] M. Miyara, S. Sakaguchi, Natural regulatory T cells: mechanisms of suppression, Trends Mol. Med. 13 (2007) 108–116.
[10] S.G. Zheng, J. Wang, P. Wang, J.D. Gray, D.A. Horwitz, IL-2 is essential for TGF-β to convert naïve $CD4^+CD25^-$ cells to $CD25^+Foxp3^+$ Regulatory T cells and for expansion of these cells, J. Immunol. 178 (2007) 2018–2027.
[11] D. Unutmaz, B. Pulendran, The gut feeling of T_{reg} cells: IL-10 is the silver lining during colitis, Nat. Immunol. 10 (2009) 1141–1143.
[12] E. Zorn, E.A. Nelson, M. Mohseni, et al., IL-2 regulates FOXP3 expression in human $CD4^+CD25^+$ regulatory T cells through a STAT dependent mechanism and induces the expansion of these cells *in vivo*, Blood 108 (2006) 1571–1579.
[13] S. Hori, T. Nomura, S. Sakaguchi, Control of regulatory T cell development by the transcription factor Foxp3, Science 299 (2003) 1057–1061.
[14] Y.Y. Wan, R.A. Flavell, Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression, Nature 445 (2007) 766–770.
[15] D.M. Sakib Hossain, A.K. Panda, A. Mann, et al., FoxP3 acts as a cotranscription factor with STAT3 in tumor-induced regulatory T cells, Immunity 39 (2013) 1057–1069.
[16] S. Chattopadhyay, L. Pavithra, MARs and MARBPs: key modulators of gene regulation and disease manifestation, in: Kundu, Dasgupta (Eds.), Chromatin and Disease: Book, Series: Subcellular Biochemistry, vol. 41, Springer, 2006.
[17] L. Pavithra, S. Chattopadhyay, Chromatin and cancer: reprogramming chaos in the cell, Natl. Acad. Sci. 30 (3&4) (2007) 71–82.
[18] S.K. Malonia, S. Sinha, P. Lakshminarasimhan, et al., Gene regulation by SMAR1: role in cellular homeostasis and cancer, Biochim. Biophys. Acta 1815 (2011) 1–12.
[19] S. Chemmannur, A.J. Badhwar, B. Mirlekar, et al., A critical role of the MAR binding protein SMAR1 in regulation of T cell differentiation and allergic airway disease, Mucosal Immunol. (2015), http://dx.doi.org/10.1038/mi.2015.11 (Epub ahead of print).
[20] M.G. Laukoetter, P. Nava, W.Y. Lee, et al., JAM-A regulates permeability and inflammation in the intestine *in vivo*, J. Exp. Med. 204 (2007) 3067–3076.
[21] P. Nava, S. Koch, M.G. Laukoetter, et al., Interferon-gamma regulates intestinal epithelial homeostasis through converging beta-catenin signaling pathways, Immunity 32 (2010) 392–402.
[22] Z. Liu, P.K. Yadav, J. Su, J. Wang, K. Fei, Potential role of Th17 cells in pathogenesis of inflammatory bowel disease, World J. Gastroenterol. 15 (46) (2009) 5784–5788.
[23] O.H. Nielsen, I. Kirman, N. Rudiger, J. Hendel, B. Vainer, Upregulation of interleukin-12 and -17 in active inflammatory bowel disease, Scand. J. Gastroenterol. 38 (2003) 180–185.
[24] D.R. Littman, A.Y. Rudensky, Th17 and regulatory T cells in mediating and restraining inflammation, Cell 140 (2010) 845–858.
[25] G. Oldenhove, N. Bouladoux, E.A. Wohlfert, et al., Decrease of $Foxp3^+$ T_{reg} cell number and acquisition of effector cell phenotype during lethal infection, Immunity 31 (5) (2009) 772–786.
[26] X. Zhou, S.L. Bailey-Bucktrout, L.T. Jeker, et al., Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells *in vivo*, Nat. Immunol. 10 (9) (2009) 1000–1007.
[27] R. Kuhn, J. Lohler, D. Rennick, K. Rajewsky, W. Muller, Interleukin-10-deficient mice develop chronic enterocolitis, Cell 75 (1993) 263–274.
[28] E.G. Schmitt, D. Haribhai, J.B. Williams, et al., IL-10 produced by induced regulatory T cells (iTregs) controls colitis and pathogenic Ex-iTregs during immunotherapy, J. Immunol. 189 (2012) 5638–5648.
[29] Y.P. Rubtsov, J.P. Rasmussen, E.Y. Chi, et al., Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces, Immunity 28 (2008) 546–558.
[30] J. Bollrath, F. Powrie, Controlling the frontier: regulatory T-cells and intestinal homeostasis, Sem. Immunol. 25 (2013) 352–357.