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Molecules and Cells

Role of TNFR-Related 2 Mediated Immune Responses in Dextran Sulfate Sodium-Induced Inflammatory Bowel Disease

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Previous work has suggested that the LIGHT-TR2 co-stimulatory pathway plays a role in the acute and chronic stages of dextran sulfate sodium (DSS)-induced colitis [Steinberg et al. (2008); Wang et al. (2005)]. To clarify the role of TNFR-related 2 (TR2) signaling in the maintenance of intestinal homeostasis, we generated a TR2 knock-out (KO) mouse. Using DSS to induce colitis, we compared the colitic symptoms and pathological changes in wild type (WT) and TR2 KO mice, and the production of cytokines by the diseased colons. We also studied the role of TR2 in suppressing innate and adaptive immunity in the DSS model. TR2 deficient mice were characterized by reduced symptoms of intestinal inflammation compared with wild-type mice, and reduced production of cytokines. We therefore generated a monoclonal antibody against mouse TR2 which was specific to TR2 and capable of blocking TR2 signals. With this antibody, we demonstrated that antagonizing TR2 during the development of DSS-induced colitis reduced the symptoms of inflammation. Our findings suggest that TR2 is an important mediator in colitis, and may serve as a therapeutic target in inflammatory bowel disease.

INTRODUCTION

Inflammatory bowel disease (IBD), which includes ulcerative colitis and Crohn's disease (CD), leads to long term, and sometimes irreversible, impairment of gastrointestinal structure and function (Bouma and Strober, 2003). Impaired mucosal barrier function, innate and adoptive immunity, pro-inflammatory and immunoregulatory cytokines, and activation of lymphocytes and macrophages, have all been implicated in the pathogenesis of IBD (Axelsson et al., 1996; Blumberg et al., 1999; Chae et al., 2010).

Proteins belonging to the TNF/TNFR superfamily play multiple roles in the activation and homeostasis of immune cells. LIGHT is a member of the TNF family that is capable of binding to herpes virus entry mediator (HVEM), lymphotoxin beta receptor (LTβR), and soluble decoy receptor 3 (DcR3/TR6), all of which are members of TNFR family. LIGHT is expressed on immature dendritic cells (DC) and activated T cells (Wan et al., 2002). TNFR-related 2 (TR2, HVEM, or TNFRSF-14) is expressed on many immune cells, including T, B, and NK cells, as well as endothelial cells; it is a receptor for three TNF superfamily ligands; LIGHT, glycoprotein D (gD) of herpes simplex virus (HSV) and lymphotoxin alpha (LTα) (Mauri et al., 1998).

Signaling through TR2 promotes growth and proliferation and the production of cytokines such as interleukin-2 (IL-2), interferon-γ (IFN-γ), and TNF-α (Croft, 2003; Wan et al., 2002). Costimulation by LIGHT modulates the effect of TR2 on T cell-mediated immune responses in tumors, graft-versus-host disease (GVHD), and graft rejection (Tamada et al., 2000).

The dextran sulphate sodium (DSS)-induced colitis model is a well-characterized animal model of colitis. It has been shown to have histological relevance to human ulcerative colitis, including focal crypt lesions, mucosal and submucosal inflammation, and granulocyte infiltration (Bauma and Strober, 2003). Although the cause of DSS-induced colitis is unclear, it may result from the toxic effects of DSS on colonic epithelial cells and alterations in luminal bacterial flora. In the acute stages of DSS-induced colitis, the T cell response consists of a polarized T helper type 1 (T_H1) response, but during the later and more chronic phase of inflammation, a mixed T_H1/T_H2 response occurs (Egger et al., 2000).

Recent studies show that LIGHT is involved in DSS-induced colitis. Transgenic mice (Tg) with enhanced LIGHT expression on T cells developed colitis (Wang et al., 2001). Also, when LIGHT Tg mesenteric lymph node cells were adoptively transferred to RAG-/- mice, the recipients developed CD, and T_H1 cytokines including IFN-γ, IL-12, IL-2, and TNF-α were the main cause of the symptoms (Wang et al., 2005). Effects of the interaction of LIGHT and its receptor LTβR (Stopfer et al., 2004), and of TR2, on the acute and chronic stages of DSS-induced colitis have been suggested by previous studies (Steinberg et al., 2008; Wang et al., 2005). However, the precise role of TR2

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is unknown.

In the present study we used DSS to induce colitis in WT and TR2 KO mice, and compared the resulting colitic symptoms and pathological changes, as well as the production of cytokines by the diseased colons.

MATERIALS AND METHODS

Mice

Wild type C57BL/6 mice (8-12 weeks old) were purchased from the Jackson Laboratory. All mice were maintained under specific pathogen-free conditions in the animal facilities of the University of Ulsan.

Production of TR2 knock-out (KO) mice

A mouse genomic chromosome clone containing the TR2/HVEM locus was isolated from a 129/SvEv bacteriophage library using a TR2-specific probe derived from the TR2 mRNA sequence (muTR2: 5′-ATGGCCTGAGCAAGTGTCTGC-3′; and Int3Rev: 5′-ACACACCCTGAGAACCTGCCCAC-3′). In the targeting vector, a 4.8-kb fragment extending from the *Kpn*I site in intron 4 to the *Hin*dIII site in intron 9 was replaced by the *neo* gene in pJNS2 (Goulet et al., 1994). This construct was electroporated into ES cells as described previously, and neomycin- and ganciclovir-resistant colonies were identified (Goulet et al., 1994). DNA was isolated from surviving colonies, digested with *Kpn*I, and analyzed by Southern blotting using a probe downstream of the targeting construct (Supplementary Fig. 1A). Chimeric mice were generated from the TR2-targeted ES cell lines and bred with B6D2 mice. The offspring were genotyped by Southern blot analysis of tail DNA, using the probe described above, and heterozygous mice were backcrossed 12 times into the C57BL/6 strain.

Production of anti-mTR2 antibody

Mouse TR2 (145-645 bp; extra cellular domain, ECD) was cloned into pGEX-6T-1 vector from cDNA using the primer pair (sense; ccctcatgcagaca, antisense; tgggaggagcaggt). The cloned DNA was transformed into *E. coli* BL21. Glutathione-S-transferase (GST)-TR2 fusion protein was induced by IPTG and purified with a glutathione-agarose column. Sprague-Dawley rats were subcutaneously immunized with purified GST-TR2 protein emulsified in complete Freund's adjuvant, and intravenous (i.v) immunized with the same purified GST-TR2 protein for the last 7 days. Spleen cells from the immunized rat were fused with mouse myeloma cells (SP20) and hybridoma cells were tested by ELISA for production of antibodies against recombinant TR2 protein. The TR2 mAb (9A3) was identified as Rat IgG2b, κ using an Immunopure Monoclonal Antibody Isotyping kit I (Pierce). To measure TR2 expression on spleen and lymph node cells *in vitro*, cells from TR2 WT and TR2 KO mice were stained with fluorescein isothiocyanate (FITC)-conjugated anti-TR2 and phycoerythrin (PE)-conjugated anti-$CD4^+$ (L3T4; BD Phamingen™) after blocking with Fc receptor-blocking mAb 2.4G2 for 10 min at 4°C. TR2 expression was then analyzed with a FACS Caliber (Becton Dickinson. USA).

T cell proliferation assay

$TR2^{+/+}$ $CD4^+$ and $TR2^{-/-}$ $CD4^+$ T cells were collected as single-cell suspensions of splenocytes or lymph node (LN) cells and enriched by positive selection using magnetic anti-CD4 microbeads and a VarioMACS™ magnetic cell sorter. The $CD4^+$ T cells (2×10^5 cells/well) were incubated in round plates for 70 hr in the presence of 0.25 μg/ml anti-CD3 mAb alone, or in combination with 0.8, 1.6, 3.2, 6.4 and 12.8 μg/ml anti-TR2 mAb. The cells were labeled with 0.5 μCi/well (^{3}H)-thymidine for the final 16 h, harvested, and counted in a liquid scintillation counter (Packard, USA).

Induction of acute colitis

For induction of acute colitis mice received 3% DSS (MW 36,000-50.000; MP Biomedicals, Inc.) dissolved in drinking water for 7 days. For therapeutic studies, mice were injected with intraperitoneally (i.p.) with 500 μg blocking anti-TR2 mAb or rat IgG as a control on days 0, 2, 4 and 6. Mice were sacrificed five or seven days after DSS treatment, after which the colons were removed and submitted for histological examination, and isolation of cytokines. The body weights of the animals were monitored daily for 3 weeks.

Histological analysis

Colons were snap-frozen in isopentane (2-methylbutane, Sigma-Aldrich) and embedded in Tissue-Tek optimal cutting temperature (OCT) compound (Sakura Finetek USA, Inc.) in liquid nitrogen. Cryostat sections of 6 μm were prepared, stained with hematoxylin and eosin (H&E), and mounted in glycerol-vinyl-alcohol (GVA) mounting solution (Zymed).

In vivo labeling and *in situ* staining of proliferating intestinal epithelial cells

5-Bromo-2′-deoxyuridine (BrdU, 20 mg/kg body weight) was injected intraperitoneally (i.p) five times at 6-h intervals to mice that had been administered 3% DSS for 7 days, and to control mice. The colons were removed and fixed in 4% paraformaldehyde. Sections of 6 μ were then cut with a cryostat in OCT compound and frozen at -80°C. The slides were stained using a BrdU In-Situ Detection Kit (BD Pharmingen™), following the manufacturer's instructions. Briefly, the slides were treated in a coplin jar containing the working solution of BD Retrievagen A, and heated to 89°C in a microwave oven. Binding of biotinylated anti-BrdU antibody was detected with Streptavidin-HRP. Color was developed with DAB and counterstained with hematoxylin.

Cytokine assays

Supernatants were obtained from pooled colon extracts of 0, 5 and 7 day DSS-induced mice. Each group contained three mice. Cytokines were quantified using a cytometric bead array kit (CBA; BD Biosciences) with a FACSCaliber cytometer equipped with Cell-QuestPro and CBA software (BD Biosciences).

Calculation of absolute numbers of lamina propria cells, and staining of intracellular cytokines

Large bowel colons were collected either untreated or after 5 or 7 days of 3% DSS administration. Lamina propria lymphocytes (LPL) were purified as previously described (Kang et al., 2002). In brief, stool was removed, and samples were washed with RPMI 1640 (Hyclone) and cut into 1 mm lengths. Then they were digested with 0.75 mM EDTA in RPMI 1640 and collagenase Type 2 (GIBCO) (2 mg/ml collagenase in RPMI 1640). The supernatants of the collagenase digests were collected, resuspended in 40% Percoll, and loaded onto 75% Percoll gradients (Sigma-Aldrich). The interfaces were collected after 20-min centrifugation at room temperature with the brake off, and the cells were washed and used for FACS analysis or intracellular cytokine staining. After washing, total cell numbers were counted by hemocytometer, and aliquots were stained with FITC-conjugated anti-$CD8^+$ (Ly-2; BD Pharmingen™), or anti-MQ (F4/80; eBioscience) and PE-conjugated anti-$CD4^+$ (L3T4; BD Pharmingen™) or anti-Gr-1 (RB6-8C5; BD Pharmin-

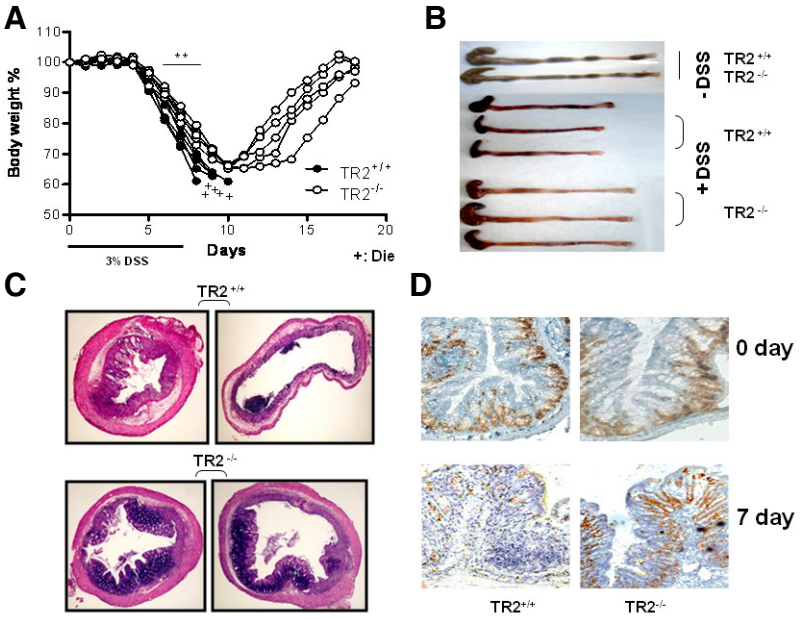

Fig. 1. Establishment of an experimental murine model of colitis. (A) TR2 WT and TR2 KO mice were administered 3.0% DSS water for 7 days. Body weight was recorded daily from day 1 to day 20. (B) Macroscopic appearance of the dissected colons from TR2 WT (+DSS/TR2$^{+/+}$) and TR2 KO (+DSS/TR2$^{-/-}$) mice, or control (-DSS) mice on day 7 of treatment with 3.0% DSS water. (C) Microscopic views of H&E-stained sections of colons from TR2 WT and TR2 KO mice administered DSS for 7 days (magnification, 20X). (D) Proliferating cells were detected using BrdU incorporation and anti-BrdU immunostaining. Positive cells in the crypts are reddish brown. (magnification, 100X) Values are means ± s.d. of triplicate samples. **, P < 0.01, WT versus KO mice.

gen™) after blocking with Fc receptor-blocking mAb 2.4G2 for 10 min at 4C. Intracellular cytokine staining was performed as described in the BD Cytofix/Cytoperm™ Kit Manual (BD Biosciences). PE-conjugated IFN-γ (BD Pharmingen) was used for intracellular cytokine staining. LPLs purified from WT or TR2 KO mice were stimulated with 50 ng/ml PMA plus 500 ng/ml ionomycin for 4 h at 37°C in the presence of BD GolgiPlug (BD Pharmingen).

Statistical analysis

Student's *t*-test was used for analysis of *in vitro* and *in vivo* data.

RESULTS

Generation and analysis of TR2 KO mice

To characterize the immunological functions of TR2 *in vivo*, we generated TR2 KO mice by genetic targeting as illustrated in Supplementary Fig. 1A. Southern blot analysis confirmed disruption of the gene encoding TR2 in both ES cells and mice, after germ-line transmission of the TR2 mutation (Supplementary Fig. 1B). Northern blot analysis of RNA from TR2/wild type (TR2$^{+/+}$) and KO (TR2$^{-/-}$) bone marrow cells (Supplementary Fig. 1C), and flow cytometric analysis (Supplementary Fig. 1D) of lymph nodes and splenocytes confirmed that the targeted TR2 gene contained a null mutation. We also generated an anti-TR2 antibody, 9A3, and examined its effect on T cell proliferation. When CD4$^+$ T cells from TR2$^{+/+}$ mice were stimulated with anti-CD3 mAb plus various concentrations of anti-TR2 mAb, measurements of [^{3}H]-thymidine incorporation showed that 9A3 acted as a blocking antibody for TR2 signal (Supplementary Fig. 1E). Since the results relating to the generation of TR2 KO mouse and mAb against TR2 were very similar to those reported previously (Harrop et al., 1998; Wang et al., 2001), we present them in supplementary figures.

Reduced susceptibility in of TR2 deficient mice to DSS-induced colits

To determine the role of TR2 in the development of colitis, we induced colitis with DSS in 8 to10 weeks old TR2$^{+/+}$ and TR2$^{-/-}$ mice. Administration of 3% DSS in a drinking water for 7 days resulted in epithelial crypt loss, presence of infiltrated cells throughout the mucosa, ulceration and mucosal bleeding in both TR2$^{+/+}$ and TR2$^{-/-}$ mice; however, the TR2$^{-/-}$ mice displayed reduced and delayed clinical symptoms compared with the TR2$^{+/+}$ mice. The loss of body weight was less severe in the TR2$^{-/-}$ mice compared with that of TR2$^{+/+}$ mice. Around 7-9 days after DSS injection, all of the TR2$^{+/+}$ mice died whereas all the TR2$^{-/-}$ mice survived (Fig. 1A). The TR2$^{-/-}$ mice began to gain weight from day 13 and their weight returned to normal by 20 days after DSS administration. Similar with the survival rate, the colons were severely shortened by treating DSS in TR2$^{+/+}$ mice, but moderately in TR2$^{-/-}$ mice. The colons of the TR2$^{-/-}$ mice were less reduced in length (Fig. 1B). Histological analysis again showed that the crypt distortion, the loss of goblet cells, and inflammation were more severe in the colons of TR2$^{+/+}$ mice compared with that of TR2$^{-/-}$ mice (Fig. 1C). The inflammation in the colons of the TR2$^{+/+}$ mice included massive inflammatory response, epithelial erosions and a destruction of the crypt structure in the colon, whereas the TR2$^{-/-}$ mice displayed epithelial damage but less inflammation and largely intact crypt structure (Fig. 1C).

In a separate experiment, when the TR2$^{+/+}$ and TR2$^{-/-}$ mice were labeled with BrdU 7 days after the DSS administration, we found more abundant BrdU-positive cells in the colons of TR2$^{-/-}$ mice compared with that of TR2$^{+/+}$ mice (Fig. 1D).

Delayed infiltration of leukocytes into the lamina propria of DSS-administered TR2$^{-/-}$ mice

As the severity of colitis is co-related with the infiltration of immune cells, the cellular composition of the inflamed colon was analyzed in the lamina propria. The number of macrophages (F4/80), granulocytes (Ly-6G+) and T-cell subsets (CD4$^+$ and CD8$^+$) in the lamina propria of colon was assessed on days 0, 5 and 7. Before the administration of DSS, there were no differences in the frequencies of any of the cell types between TR2$^{+/+}$ and TR2$^{-/-}$ mice; the lamina proprias of both mice contained comparable levels of macrophages and granulocytes and low numbers of lymphocytes. Five days after the DSS administration, there were marked increases of macrophages and granulocytes in the lamina propria of TR2$^{+/+}$ mice and also T-cells

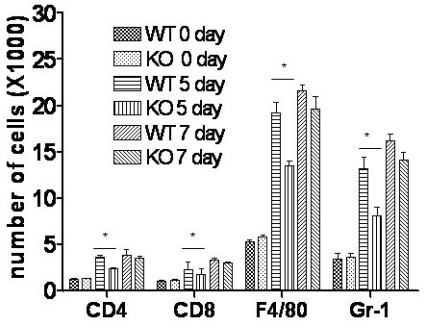

Fig. 2. The DSS-treated TR2 WT and TR2 KO mice were infiltrated by leukocytes and granulocytes. Epithelial cells were prepared from mice on days 5 and 7 of DSS treatment. The cells were stained with FITC-conjugated anti-CD4 and anti-Gr-1, PE-conjugated anti-CD8 and anti-F4/80, and were quantified by FACS. Values are means ± s.d. of 3 independent experiments. *, $P < 0.05$, cells from WT versus KO mice.

although they were less frequent compared to macrophages and granulocytes. Those innate and adaptive immune cells were also significantly increased in the laminar propria of $TR2^{-/-}$ mice by treating DSS, but the numbers of the infiltrated immune cells were significantly less than that of $TR2^{+/+}$ mice (Fig. 2). However, since the leukocyte infiltration into laminar propria was swiftly increased in $TR2^{-/-}$ mice, the differences in the infiltration between $TR2^{+/+}$ and $TR2^{-/-}$ mice were no longer sustained 7 days after the DSS administration (Fig. 2).

Delayed expression of inflammatory cytokines in DSS-administered $TR2^{-/-}$ mice

Since we found that the inflammation was delayed in the $TR2^{-/-}$ mice after the DSS administration, we wondered whether the expression of inflammatory cytokines would be reduced or delayed in the absence of TR2. Therefore, we next examined the effects of TR2 signaling on the inflammatory cytokine profiles in the inflamed colons. Before DSS treatment, there were basal levels of cytokines the $TR2^{-/-}$ and $TR2^{+/+}$ mice. Five days after the DSS administration, the expressions of IL-6, MCP-1, IFN-γ, and TNF-α were significantly lower in the colons of the $TR2^{-/-}$ mice compared with that of the $TR2^{+/+}$ mice. By day 7, the levels of IL-6, MCP-1 and TNF-α were still lower in $TR2^{-/-}$ mice compared with that of $TR2^{+/+}$ mice had much higher amounts of IL-6, MCP-1, TNF-α, IL-10 (Fig. 3).

Since the results were consistent with the survival rate of DSS-administered $TR2^{-/-}$ mice, we concluded that the symptoms of DSS-induced colitis were moderate in $TR2^{-/-}$ mice due to the delayed and reduced inflammation in the absence of TR2 signaling.

Antagonistic mAb against TR2 ameliorates DSS-induced colitis

Since DSS-induced colitis was ameliorated in the absence of TR2 signaling, we further tested the role of TR2 in using the blocking antibody for TR2, 9A3. Specific binding of 9A3 mAb to TR2 was confirmed using $TR2^{+/+}$ and $TR2^{-/-}$ T cells as 9A3 did not bind to any of the lymphocyte gated LN cells or splenocytes from the $TR2^{-/-}$ mice (Supplementary Fig. 1D). Treatment with 9A3 suppressed *in vitro* proliferation of $CD4^+$ T cells in a dose-dependent manner (Supplementary Fig. 1E), suggesting that it is an antagonistic antibody.

C57BL/6 mice were given the 3% DSS in a drinking water

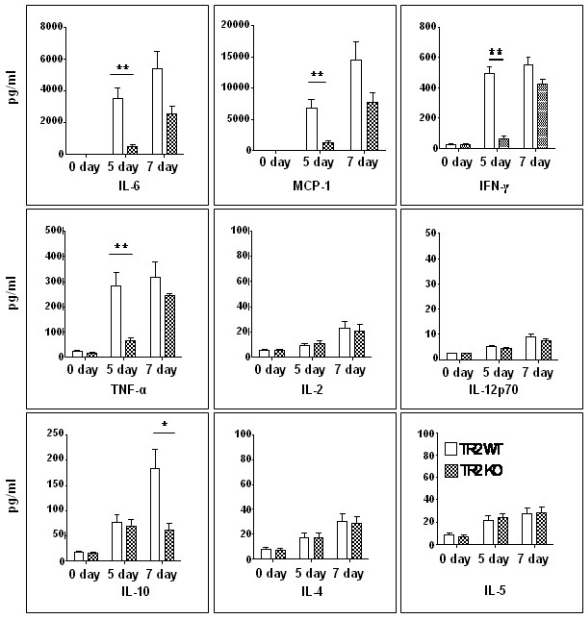

Fig. 3. Enhanced production of cytokines and chemokines in TR2 WT but not inTR2 KO mice, and impaired epithelial proliferation. Epithelia were collected from TR2 WT and TR2 KO mice on day 5 of DSS treatment and amounts of IL-6, MCP-1, IFNγ, TNF-α, IL-4, IL-2, IL-10, IL-5 and IL-12-p-70 were quantified using a cytometric bead array kit (CBA; BD Biosciences) and a FACS Caliber cytometer. Values are means ± s.d. of triplicate samples. **, $P < 0.01$, WT versus KO mice.

and further injected i.p. with the blocking anti-TR2 mAb, 9A3. Most of control mice treated with rat IgG were died around 8 days after the DSS administration while all the mice received blocking anti-TR2 mAb survived from the DSS-induced colitis. In the initial phase of colitis, both rat IgG- and 9A3 treated mice showed comparable level of body-weight loss. However, from the fifth day after DSS administration, the body weight loss of the mice receiving 9A3 was less severe, while the rat IgG-treated mice eventually died around day 8 (Fig. 4A). Again the control mice had shortened colons (Fig. 4B) with severe ulcerations, distortion of crypts and loss of goblet cells in the colon (data not shown). The effects of 9A3 treatment on cytokine levels closely resembled those of TR2 KO (Fig. 4C). These results demonstrate that 9A3 can modulate the pathology associated with TR2 *in vivo*.

DISCUSSION

Oral administration of DSS is believed to cause colitis by inducing initial epithelial damage that breaks the important barrier function of this cell layer for the luminal contents of the intestine, and leads to an inflammatory response. LIGHT/TR2 signaling is believed to be involved in the subsequent development of colitis. However, the role of TR2 in the colitis has not been clearly identified. In this study, we examined the role of TR2 in DSS-induced colitis using TR2 KO mouse and a TR2 antagonistic antibody.

DSS-treated $TR2^{-/-}$ mice displayed delayed and reduced clinical symptoms compared to wild type mice (Fig 1). Histological analysis demonstrated that the $TR2^{-/-}$ mice seemed to be protected from the extensive mucosal destruction, including

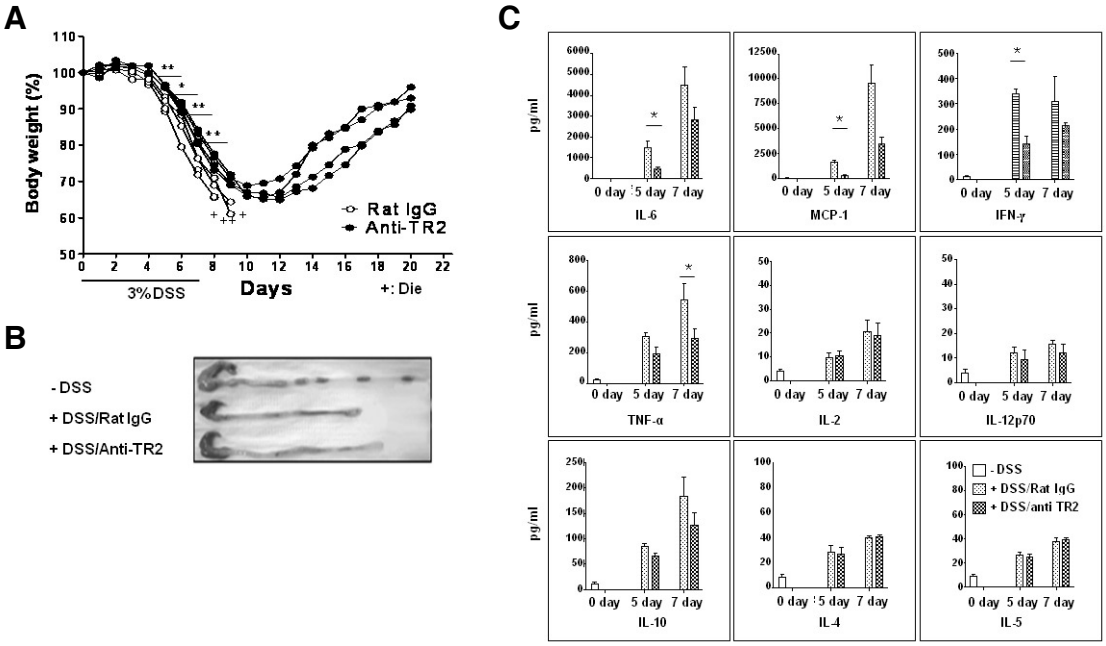

Fig. 4. DSS-induced colitis and the influence of blocking TR2 mAb. (A) DSS-treated mice were injected with 500 μg/ml blockingTR2 mAb i.p. on days 0, 2, 4 and 6. Rat IgG was used as control. The mice were administered 3.0% DSS water. Body weight was recorded daily from days 1 to 20. (B) Photographs of dissected colons of mice treated with rat IgG and antagonist TR2 mAb mice on day 7 after 3.0% DSS water. The mice were killed by cervical dislocation, and large intestines with ceca were removed. (C) DSS-induced cytokine expression in mice receiving rat IgG or antagonist TR2 mAb. Values are means ± s.d. of triplicate samples. *, $P < 0.05$, **, $P < 0.01$, WT versus KO mice.

loss of crypts, mucosal erosion, ulcers and inflammatory cell infiltration observed in the $TR2^{+/+}$ mice. Consistent with a previous report (Dieleman et al., 1994; Stevceva et al., 2001), we observed significant increases in numbers of lamina propria macrophages, granulocytes, and $CD4^+$ and $CD8^+$ subsets were after exposure, of wild type mice to DSS. These cells also increased in the $TR2^{-/-}$ mice, but, the increase was slower (Fig. 2).

Analysis of cytokines in the colons of DSS-treated wild type mice revealed increased levels of T_H1 cytokines such as IFN-γ, TNF-α, IL-2, and IL-12, and also of T_H2 cytokines such as IL-4, IL-5, and IL-10, as expected (Scaldaferri and Fiocchi, 2007). Although TNF-α, and IFN-γ increases in the colons of $TR2^{-/-}$ mice by the 5th day of treatment, their levels were much lower than in the wild type. Of the T_H1 cytokines, secretion of IL-2 and IL-12 was not much lower in the $TR2^{-/-}$ mice (Fig. 3). These cytokine pattern were different from those in LIGHT Tg mice, in which all of the T_H1 cytokines tested, including IFN-γ, IL-12, IL-2, and TNF-α, were significantly elevated and were responsible for the symptoms (Wang et al., 2005). The results of the cytokine assays suggested that the T_H1 response on its own could not explain the role of TR2 in DSS induced colitis, as suggested for the LIGHT Tg mice. We further analyzed cytokine production in the colon and found lower levels of IL-10, and IL-6, as well as of the chemokine, MCP-1, in the colons of $TR2^{-/-}$mice than in those of wild type mice. IL-6 is reported to be involved in IBD, since it induces T cell accumulation via an antiapoptotic signal (Mudter and Neurath, 2007). IL-10 deficient mouse have been found to display reduced symptom of colitis due to elimination of local macrophages (Watanabe et al., 2003). MCP-1 is also important in colitis since MCP-1 deficient mice showed a significant reduction in the severity of colitis as a consequence of down-regulation of IL-1B, and IFN-γ (Khan et al., 2006). In this report, we have demonstrated that not only T_H1 cytokines such as TNF-α, and IFN-γ (Wang et al., 2005), but also IL-6, MCP-1, and IL-10, are important in the immune responses mediated by TR2 in colitis induced by DSS.

Administration of DSS is known to damage epithelial cells, and the proliferation of cells in colonic crypts is decreased (Strober et al., 2002). In the colon of TR2-deficient mice, superficial epithelial injury induced by DSS adsorption is reduced and the rate of healing is accelerated. To reestablish mucosal integrity, epithelial cells need to proliferate to replace the reduced cell populations. Since measurements of BrdU incorporation showed that BrdU-positive cells were mainly localized to the crypt regions of $TR2^{-/-}$ mice (Fig. 1D), it appears that the epithelial cells of $TR2^{-/-}$ mice recover more efficiently from DSS-induced damage due to stimulation of the proliferation of epithelial cell precursors. These results are consistent with an early report that low concentrations of TNF-α, IFN-γ, IL-6, and IL-10 are important mediators of tissue repair and restitution after nonspecific colonic injury (Dieleman et al., 1996).

Our results demonstrated that a deficiency of TR2 retarded the progress of inflammation in DSS-induced colitis, and this finding was confirmed using an antagonistic antibody against TR2 which was seem to modulate the pathology associated with TR2 *in vivo* (Fig. 4).

In summary, we have clarified the roles of TR2 signaling in the maintenance of intestinal homeostasis. Knockout of TR2 has the effect of protecting mucosal integrity, suppressing innate and adaptive immunity in response to DSS.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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