CD30 Ligand Is a Target for a Novel Biological Therapy against Colitis Associated with Th17 Responses

Xun Sun, Hisakata Yamada, Kensuke Shibata, Hiromi Muta, Kenzaburo Tani, Eckhard R. Podack, Yoichiro Iwakura and Yasunobu Yoshikai

*J Immunol* 2010; 185:7671-7680; Prepublished online 10 November 2010; doi: 10.4049/jimmunol.1002229

http://www.jimmunol.org/content/185/12/7671

References This article cites 44 articles, 17 of which you can access for free at: http://www.jimmunol.org/content/185/12/7671.full#ref-list-1

Subscription Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
CD30 Ligand Is a Target for a Novel Biological Therapy against Colitis Associated with Th17 Responses

Xun Sun,*† Hisakata Yamada,* Kensuke Shibata,* Hiromi Muta,‡ Kenzaburo Tani,‡ Eckhard R. Podack,§ Yoichiro Iwakura,§ and Yasunobu Yoshikai*‡

We have previously found that CD30 ligand (CD30L; CD153)/CD30 signaling executed by the T–T cell interaction plays a critical role in Th17 cell differentiation, at least partly via downregulation of IL-2 production. In this study, we investigated the role of CD30L in the development of colitis experimentally induced by dextran sulfate sodium (DSS), in which IL-17A is involved in the pathogenesis. CD30L−/− mice were resistant to both acute colitis induced by administration of 3 to ∼5% DSS and to chronic colitis induced by administration of 1.5% DSS on days 0–5, 10–15, and 20–25 as assessed by weight loss, survival rate, and histopathology. The levels of IFN-γ, IL-17A, and IL-10 were significantly lower but the IL-2 level higher in the lamina propria T lymphocytes of CD30L−/− mice than those in lamina propria T lymphocytes of wild-type mice after DSS administration. Soluble murine CD30- Ig fusion protein, which was capable of inhibiting Th17 cell differentiation in vitro, ameliorated both types of DSS-induced colitis in wild-type mice. Modulation of CD30L/CD30 signaling by soluble CD30 could be a novel biological therapy for inflammatory diseases associated with Th17 responses. *Journal of Immunology, 2010, 185: 7671–7680.

CD30 ligand (CD30L; CD153) is a 40-kDa type II membrane-associated glycoprotein belonging to the TNF superfamily (1) and is expressed on effector CD4+ T cells (2–4). CD30, the receptor for CD30L and belonging to TNF receptor superfamily, is expressed preferentially by effector or memory Th cells (5–7). There are several lines of evidence that CD30L/CD30 signaling is involved in both Th1 and Th2 cell responses and their associated diseases, such as diabetes in young NOD mice (8), mycobacterial infection (9), and allergic inflammation (10). It has recently been reported that CD30L/CD30 signaling plays a role in naturally occurring regulatory T cell (Treg) response in a CD4+ T cell-mediated lethal graft-versus-host disease model (11). We have recently found that CD30L/CD30 signaling executed by the T–T cell interaction plays a critical role in Th17 cell differentiation in vitro and in vivo (12). Thus, CD30L/CD30 signaling may not be directly linked to a physiological step for the differentiation of a specific Th cell subset but may be important for amplification and/or activation of any subset of effector Th cells.

Human inflammatory bowel diseases (IBDs), including Crohn’s disease and ulcerative colitis, are characterized by inflammation in the large and/or small intestine associated with uncontrolled innate and adaptive immunity against normal constituents, including commensal bacteria and various microbial products (13–15). Among various experimentally induced colitis models in mice, dextran sulfate sodium (DSS)-induced colitis, trinitrobenzene sulfonic acid (TNBS)-induced acute colitis, and colitis in immune-deficient mice to which CD4+ CD45RBhigh T cells have been transferred were thought of as Th1-type colitis animal models (16–18). Recently, it has been reported that a novel subset of helper CD4+ T cells producing IL-17A, namely Th17 cells, is involved in progression (19–22) or regulation of these colitis models (23–25). However, Th2-like responses are closely associated with pathogenesis of another hapten-induced colitis caused by intrarectal administration of oxazolone (OXA) (26) and with spontaneous colitis in IL-2–deficient mice (27) or in TCR-α–deficient mice (28).

We previously reported that CD30L−/− mice were susceptible to Th2-type OXA-induced colitis but resistant to Th1-type TNBS-induced acute colitis (29). Furthermore, we have recently found that colitis was attenuated in SCID mice to which CD30L−/− CD4+CD45RBhigh T cells have been transferred, suggesting that CD30L is involved in the pathogenesis of Th1-type colitis (12). In this study, we show that CD30L−/− mice were resistant to DSS-induced acute colitis (30) and to chronic colitis induced by multiple cycles of DSS (16). The levels of IFN-γ and IL-17A were significantly lower in the lamina propria T lymphocytes (T-LPLs) of CD30L−/− mice than those in the T-LPLs of wild-type (WT) mice. In vivo administration of a soluble CD30-Ig ameliorated both types of DSS-induced colitis in WT mice with downregulation of Th1 and Th17 cell activation. Modulation of CD30L signaling could be a novel biological therapy for inflammatory diseases associated with Th1 and Th17 cell activation.

*Division of Host Defense and †Division of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; ‡Department of Immunology, China Medical University, Shenyang, China; §Department of Microbiology and Immunology, University of Miami, Miami, FL 33101; and *Center for Medical Science, Institute of Medical Science, University of Tokyo, Tokyo, Japan

Received for publication July 8, 2010. Accepted for publication October 11, 2010.

This work was supported by the Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases and was launched as a project commissioned by the Ministry of Education, Culture, Sports, Science, and Technology, Japan (to Y.Y.), a Grant-in-Aid for Scientific Research on Priority Areas from the Japan Society for the Promotion of Science (to Y.Y.), grants from the Japanese Ministry of Education, Science and Culture (to Y.Y.), a Grant-in-Aid for the Japan Society for the Promotion of Science Fellows (20-08462) (to X.S.), and by the Yakult Bioscience Foundation (to Y.Y.).

Address correspondence and reprint requests to Yasunobu Yoshikai, Division of Host Defense, Center for Prevention of Infectious Disease, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, Japan. E-mail address: yoshihakai@bioreg.kyushu-u.ac.jp

Abbreviations used in this paper: CD30L, CD30 ligand; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; LPL, lamina propria lymphocyte; mCD30-Ig, murine CD30-Ig; MLN, mesenteric lymph node; n.d., not detected; OXA, oxazolone; T-LPL, lamina propria T lymphocyte; TNBS, trinitrobenzene sulfonic acid; Treg, regulatory T cell; WT, wild-type.

Copyright © 2010 by The American Association of Immunologists, Inc. 0022-1767/10/$16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1002229
Materials and Methods

Mice

Age- and sex-matched BALB/c mice and C.B.17 scid/scid male mice were purchased from Japan KBT (Shizuoka, Japan). The generation and preliminary characterization of CD30L−/− mice have been described previously (11, 31), and the CD30L−/− mice were back-crossed onto the BALB/c strain more than eight times. The progeny of a cross of CD30L−/− mice and SCID mice was intercrossed to generate SCID/CD30L−/− mice. IL-17A−/− mice with a BALB/c background have been described previously (32). All mice were maintained under specific pathogen-free conditions, were offered food and water ad libitum, and were used at 6–8 wk of age. This study was approved by the Committee of Ethics on Animal Experiment of the Faculty of Medicine, Kyushu University (Fukuoka, Japan). Experiments were carried out under the control of the Guidelines for Animal Experiments of Kyushu University.

Abs and reagents

Abs for FACS analysis, Fc receptor-blocking mAb (CD16/32; 2.4G2), anti-CD3ε (145-2C11), anti-CD4 (RM4-5), anti-CD62L (MEL-14), anti-CD44 (IM7), anti-CD153 (RM153), anti–IL-17A (TC11-18H10.1), anti–

FIGURE 1. IL-17A−/− mice are resistant to DSS-induced acute colitis. A, Weight loss, survival rate, macroscopic changes, histological score, and H&E staining of colon (original magnification ×200) are shown (n = 10). B, TNF-α, IL-6, and IL-1β production by LPLs without simulation was measured by ELISA (n = 5). C, IFN-γ– and IL-17A–producing CD4+ T-LPLs were analyzed by intracellular cytokine FACS (n = 5). D, Absolute numbers of IFN-γ+CD4+ and IL-17A+CD4+ T-LPLs were shown (n = 5). E, IFN-γ, IL-17A, IL-17F, IL-21, and IL-22 production by T-LPLs was measured by ELISA (n = 5). Data indicate mean ± SD obtained from a representative of three independent experiments. The difference in survival rates was evaluated by the log-rank test (Mantel–Cox). Statistical differences in other data were evaluated by a Student t test. A–E: n.d., not detected; *p < 0.05; **p < 0.01.
IL-17A−/− mice are resistant to DSS-induced chronic colitis. A, Weight loss, survival rate, macroscopic changes, histological score, and H&E staining of colon (original magnification ×200) are shown (n = 20). B, TNF-α, IL-6, and IL-1β production by LPLs without simulation was analyzed by ELISA (n = 10). C, IFN-γ– and IL-17A–producing CD4+ T-LPLs were analyzed by intracellular cytokine FACS (n = 10). D, Absolute numbers of IFN-γ+CD4+ and IL-17A+CD4+ T-LPLs are shown (n = 10). E, IFN-γ, IL-17A, IL-17F, IL-21, and IL-22 production by T-LPLs was analyzed by ELISA (n = 10). Data indicate mean ± SD obtained from a representative in two independent experiments. The difference in survival rates was evaluated by the log-rank test (Mantel–Cox). Statistical differences in other data were evaluated by a Student t test. A–E: n.d., not detected; *p < 0.05; **p < 0.01.
IL-2 (JES6-5H4), anti-Foxp3 (FJK-16s), and anti–IFN-γ (XMGI1.2) were purchased from BD Biosciences (San Diego, CA). Purified anti-CD3, anti-CD28, anti-CD28 (37.51), anti–IFN-γ (R4-6A2), and anti–IL-4 (11B11) mAbs were obtained from ebioscience (San Diego, CA). Recombinant cytokines including IL-6, TGF-β, IL-12, and IL-4 were purchased from PeproTech (Princeton, NJ).

**Induction of acute or chronic colitis by DSS**

For acute colitis induction by DSS, mice were administered 3% (w/v) DSS (molecular mass 36–50 kDa; ICN Biomedicals, Aurora, OH) in their drinking water. For chronic colitis induction by DSS, mice were administered 1.5% DSS on days 0–5, 10–15, and 20–25. Mice were sacrificed on day 5 in acute colitis or on day 25 in chronic colitis. The tissues of colons were removed and cleaned. Sections were taken for cell culture, flow cytometry, and histology. In some experiments, SCID and SCID × CD30L−/− mice were treated with 3 or 5% DSS drinking water every day to induce acute colitis.

**Histology assessment of colitis**

The middle parts of colons were removed and fixed with 10% neutral buffered formalin and then embedded in paraffin. Five-micrometer thick sections were stained with H&E. Histology was scored as described previously (33).

**Culture of lamina propria lymphocytes for assay of cytokine secretion**

Lamina propria lymphocytes (LPLs) were isolated as described elsewhere (34). Isolated LPLs and mononuclear cells from spleen and mesenteric lymph node (MLN) were incubated with an FcγR-blocking mAb and stained with mAbs against mouse CD4, CD153, and CD3. For intracellular cytokine staining, LPLs were stimulated with PMA (25 ng/ml; Sigma-Aldrich, St. Louis, MO) and ionomycin (1 μg/ml; Sigma-Aldrich) for 5 h at 37°C. Brefeldin A (10 μg/ml; Sigma-Aldrich) was added for the last 4 h of incubation. These cells were harvested, washed, and stained with mAbs against mouse Foxp3, IL-17, or IFN-γ for 30 min at 4°C. The intracellular expression of Foxp3, IL-17A, or IFN-γ in CD4+ T-LPLs was analyzed using Flow Cytometry FACS using a Cytoburner/Cytoperm Kit Plus (BD Biosciences, San Jose, CA) according to the manufacturer’s instructions. The data were analyzed using CellQuest software (BD Biosciences). To measure spontaneous cytokine production by colon LPLs, 2 × 105 LPLs were cultured without any stimulation for 24 h at 37°C under 5% CO₂ in 96-well flat-bottom plates in a volume of 0.2 ml RPMI 1640 (Wako, Osaka, Japan) containing 10% FBS (Cell Culture Technologies, Tokyo, Japan). To measure cytokine production by T-LPLs in LPLs, LPLs were cultured with 96-well flat-bottom plates (Falcon; BD Biosciences, Japan). To measure cytokine production by T-LPLs in LPLs, LPLs were cultured without any stimulation for 24 h at 37°C under 5% CO₂ in 96-well flat-bottom plates in a volume of 0.2 ml RPMI 1640 (Wako, Osaka, Japan) containing 10% FBS (Cell Culture Technologies, Tokyo, Japan). To measure cytokine production by T-LPLs in LPLs, LPLs were cultured with 96-well flat-bottom plates (Falcon; BD Biosciences) coated with anti-CD3 (10 μg/ml) and soluble anti-CD28 mAbs (1 μg/ml) for 48 h. IFN-γ, IL-17A, IL-4, IL-10, IL-2, IL-21, and IL-17F (R&D Systems, Minneapolis, MN), or IL-22 (ebioscience) secretion in the culture supernatant was measured by using an ELISA kit (R&D Systems) according to the manufacturer’s instructions.

**In vivo treatment with soluble murine CD30-Ig**

Murine CD30-Ig (mCD30-Ig) was obtained using a method described previously (7). The mCD30-Ig fusion cDNA in expression vector pBGMNeo (35) was transfected in NIH 3T3 cells. The mCD30-Ig protein was secreted by growing the NIH 3T3 cells in serum-free medium (medium 101; Nissui Pharmaceutical, Tokyo, Japan) and was purified by HiTrap Protein G HP (Amersham Biosciences) and analyzed by SDS-PAGE for purity. mCD30-Ig, diluted to 1 mg/ml in PBS, was stored at −80°C until use. For in vivo neutralization, 200 μg mCD30-Ig or isotype control murine IgG1 (Biolegend, San Diego, CA) was i.p. injected into WT mice with DSS-induced chronic colitis on days 3, 6, 9, 12, 15, 18, 21, and 24 or i.p. injected into mice with acute colitis on days 1, 3, and 5. Body weight and survival rate were monitored daily. Mice were sacrificed on day 6 or day 25 in acute colitis or chronic colitis, respectively.

**Cell culture**

CD4+CD25+CD62Llo CD4+ T cells were sorted and cultured as described previously (19). In brief, purity of naive CD4+ T cells was confirmed by FACS and was consistently higher than 98%. Naive CD4+ T cells (1 × 10⁶) were cultured in wells of a 96-well plate coated with anti-CD3 (10 μg/ml) and soluble anti-CD28 (1 μg/ml) mAb in the presence or absence of IL-12 (5 ng/ml) and anti–IL-4 (10 μg/ml) for Th1 cell differentiation, IL-4 (25 ng/ml) and anti–IFN-γ (20 μg/ml) for Th2 cell differentiation, and TGF-β (5 ng/ml), IL-6 (25 ng/ml), anti–IL-4 (10 μg/ml), and anti–IFN-γ (10 μg/ml) for Th17 cell differentiation in the presence or absence of mCD30-Ig (25 μg/ml) or murine IgG1. After 4 d, supernatants were harvested and used to analyze cytokine production by ELISA. Intracellular staining was performed with mAbs against IL-17A and IL-2.

**Statistical analysis**

The difference in survival rates was evaluated by the log-rank test (Mantel–Cox). Differences in parametric data were evaluated by a Student t test. Differences of p < 0.05 were considered statistically significant.

**Results**

**IL-17A is involved in the pathogenesis of both acute and chronic types of DSS-induced colitis**

The role of the Th17 cell is still controversial in DSS-induced colitis, depending on each experimental protocol and the genetic background of mice used (20, 25). Therefore, we first determined the precise role of IL-17A in the pathogenesis of DSS-induced intestinal inflammation using IL-17A−/− mice with a BALB/c background. Acute or chronic colitis was induced by oral administration of 3% DSS or 1.5% DSS drinking water every day or on days 0–5, 10–15 and 20–25, respectively. IL-17A−/− mice showed impaired development of both types of DSS-induced colitis as indicated by weight loss, survival rate, macroscopic changes, and histopathology (Fig. 1A and Fig. 2A). The levels of inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 decreased in DSS-treated IL-17A−/− mice on day 5 in acute colitis (Fig. 1B) and on day 25 in chronic colitis (Fig. 2B). IFN-γ–producing CD4+ T-LPLs were significantly lower in IL-17A−/− mice than those in WT mice on day 5 in acute colitis (Fig. 1C, 1D) and on day 25 in chronic colitis (Fig. 2C, 2D). IFN-γ production by T-LPLs in colon of IL-17A−/− mice with DSS-induced acute or chronic colitis also decreased compared with that in WT mice (Fig. 1E and Fig. 2E). There were no differences in the levels of other Th17 cytokines including IL-17F, IL-21, and IL-22

**FIGURE 3.** Increased CD30L expression on CD4+ T-LPLs in DSS-induced colitis. A, FACS analysis for CD30L expression on CD4+ T cells of spleen, MLN, and lamina propria from naive mice and mice with DSS-induced acute or chronic colitis (n = 5). The results are presented as typical profiles after an analysis gate had been set on CD3+ cells. B, Absolute number of CD4+CD30L+ T cells in spleen, MLN, and lamina propria (n = 6). Data indicate mean ± SD obtained from a representative of three separate experiments. Statistical differences in other data were evaluated by a Student t test. A and B: *p < 0.05; **p < 0.01.
between IL-17A−/− mice and WT mice with DSS-induced acute or chronic colitis (Fig. 1E and Fig. 2F). These results elucidate that IL-17A of intestinal mucosa contributes to the development of both types of DSS-induced colitis in our system.

**Increased CD30 ligand expression on CD4+ T cells in DSS-induced colitis**

To measure the expression of CD30L in DSS-induced colitis, we analyzed the levels of CD30L expression on cells of spleen, MLN, and lamina propria by flow cytometry. Consistent with a previous report (36), a few but significant number of CD4+ T cells of spleen, MLN, and LPLs expressed CD30L in naive mice. CD30L expression on CD4+ T cells, especially in LPLs, was significantly increased on day 5 in DSS-induced acute colitis and on day 25 in DSS-induced chronic colitis (Fig. 3A). The absolute numbers of CD4+CD30L+ T cells in the LPLs of DSS-treated mice of both groups were greater than those in naive mice (Fig. 3B).

**CD30L−/− mice are resistant to DSS-induced acute colitis**

We next examined the role of CD30L in the pathogenesis of acute colitis induced by oral administration of 3% DSS. As shown in Fig. 4A, CD30L−/− mice treated with 3% DSS drinking water every day developed wasting disease of less severity than WT mice did. The levels of TNF-α, IL-6, and IL-1β were significantly lower on day 5 in CD30L−/− mice with DSS-induced acute colitis (Fig. 4B). The number of IFN-γ– or IL-17A–producing CD4+ T-LPLs decreased in DSS-treated CD30L−/− mice (Fig. 4C, 4D). IFN-γ, IL-17A, and IL-10 secretions by T-LPLs were reduced in CD30L−/− mice whereas IL-2 production was enhanced in CD30L−/− mice compared with those in WT mice (Fig. 4E). There was no difference in the frequency of Foxp3+ Tregs between WT mice and CD30L−/− mice (18.6 ± 1.3% in WT mice versus 12.4 ± 6% in CD30L−/− mice). DSS-induced acute colitis was originally considered to be a T cell-independent model, although T cell-mediated immunity is somewhat involved in the pathogenesis of DSS-

---

**FIGURE 4.** CD30L−/− mice are resistant to DSS-induced acute colitis. A, Weight loss, survival rate, macroscopic changes, and H&E staining of colon (original magnification ×200). B, Inflammatory cytokine production by LPLs without stimulation was analyzed by ELISA. C, IFN-γ and IL-17A–producing CD4+ T-LPLs were analyzed by intracellular cytokine FACS. D, Absolute numbers of IFN-γ+CD4+ and IL-17A+CD4+ T-LPLs are shown. E, IFN-γ, IL-17A, IL-2, IL-10, and IL-4 production by T-LPLs was analyzed by ELISA. Data indicate mean ± SD of eight mice of each group obtained from a representative of two independent experiments. The difference in survival rates was evaluated by the log-rank test (Mantel–Cox). Statistical differences in other data were evaluated by a Student t test. A–E: *p < 0.05; **p < 0.01.
induced acute intestinal inflammation (30). To determine whether CD30L is involved in DSS-induced acute colitis in a T cell-dependent manner, we examined DSS-induced acute colitis in SCID mice and SCID×CD30L−/− mice. As shown in Fig. 4F, severe intestinal inflammation and high mortality were found in SCID mice after 5% DSS treatment, confirming that DSS-induced acute colitis occurred in a T cell-independent manner. Notably, there were no differences in both weight loss and survival rate between SCID mice and SCID×CD30L−/− mice in DSS-induced acute colitis (Fig. 4F). Similar results were found in SCID mice and SCID×CD30L−/− mice after 3% DSS treatment (data not shown). These results suggest that CD30L plays an important role in DSS-induced acute colitis driven by T cells but not by the innate immune system.

**CD30L−/− mice are resistant to DSS-induced chronic colitis**

We next examined DSS-induced chronic colitis in CD30L−/− mice by oral administration of 1.5% DSS drinking water on days 0–5, 10–15, and 20–25. As shown in Fig. 5A, DSS-induced chronic colitis was attenuated in CD30L−/− mice as indicated by weight loss, survival rate, macroscopic changes, and histopathology. Macroscopic inspection showed a significantly longer colon in CD30L−/− mice than that in WT mice on day 25 after DSS administration. Histopathologically, CD30L−/− mice showed mild infiltration of inflammatory cells to the mucosa, minimal loss of crypts and reduction of goblet cells, and histological scores of the colon were significantly decreased in CD30L−/− mice compared with those in WT mice. Thus, CD30L−/− mice are largely protected against the development of DSS-induced chronic colitis. Inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, decreased significantly on day 25 in DSS-treated CD30L−/− mice (Fig. 5B). The frequency and number of IFN-γ- or IL-17A-producing CD4+ T-LPLs decreased in DSS-treated CD30L−/− mice (Fig. 5C, 5D). IFN-γ, IL-17A, and IL-10 secretions by CD4+ T cells in LPLs were lower in DSS-treated CD30L−/− mice than those in WT mice upon stimulation with anti-CD3/anti-CD28 mAbs, whereas the levels of IL-2 and IL-4 increased in DSS-treated CD30L−/− mice compared with those in WT mice (Fig. 5E). There was no difference in the frequency of Foxp3+ Tregs between WT mice and CD30L−/− mice (20.3 ± 1.0% in WT mice versus 19.7 ± 2.4% in CD30L−/− mice). These results show that CD30L plays an important role in the pathogenesis of DSS-induced chronic colitis.
Soluble CD30-Ig inhibits Th17 cell differentiation in vitro

We have recently reported that naïve CD44lowCD62LhighCD4+ T cells from CD30L−/− mice exhibited impaired differentiation into Th17 cells but an increased ability to produce IL-2 after in vitro culture with Th17-polarizing condition (12). To determine whether mCD30-Ig can suppress Th cell differentiation by blocking of CD30L/CD30 signaling, naïve CD4+ T cells were sorted from splenocytes of WT mice and cultured with mCD30-Ig in polarizing conditions for Th1, Th2, or Th17 cell differentiation. As shown in Fig. 6A, addition of mCD30-Ig significantly inhibited Th17 differentiation and simultaneously increased IL-2 production. FACS analysis further confirmed that mCD30-Ig decreased the fraction of IL-17A+CD4+ cells and increased IL-2+CD4+ cells in Th17 cell differentiation (Fig. 6B).

mCD30-Ig treatment ameliorates DSS-induced colitis

The effect of inhibition of CD30L signaling by administration of mCD30-Ig in vivo was examined in the course of both types of DSS-induced colitis in WT mice. Mice given mCD30-Ig had reduced loss of body weight and increased survival rate in both types of DSS-induced colitis in WT mice. Mice given mCD30-Ig had reduced loss of body weight and increased survival rate in both types of DSS-induced colitis. The colon was markedly shorter in mice treated with control Ig than in mice treated with mCD30-Ig on day 6 in acute colitis (Fig. 7A) and on day 25 in chronic colitis (Fig. 7A and Fig. 8A). Histological analysis showed that inflammation of the colon was impaired in mCD30-Ig–treated mice (Fig. 7A and Fig. 8A). The levels of inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, decreased in mCD30-Ig–treated mice on day 6 in acute colitis and on day 25 in chronic colitis (Fig. 7A and Fig. 8A). The levels of IFN-γ and IL-17A production by T-LPLs decreased but the levels of IL-2, IL-10, and IL-4 increased in mCD30-Ig–treated mice (Fig. 7C and Fig. 8C). The frequency of Foxp3+ Tregs did not increase in CD30-Ig–treated T cells (data not shown). Thus, in vivo blocking of CD30L signaling by soluble mCD30-Ig significantly protected against the development of both types of DSS-induced intestinal inflammation in WT mice.

Discussion

Identification of the key molecule that critically regulates pathogenic T cell responses is important for the development of novel treatment for IBD. In the current study, using CD30L−/− mice, we showed an important role of CD30L in development of T cell-mediated colitis associated with Th17 cell activation. It is noteworthy that therapeutic application of soluble mCD30-Ig ameliorated DSS-induced colitis in WT mice. Modulation of CD30L signaling by soluble CD30 Ig could be a novel biological therapy for IBD and other inflammatory diseases associated with Th17 cells.

DSS-induced acute colitis was originally considered to be a T cell-independent model (30). This has been proved using immunodeficient mice, such as SCID mice. However, there is evidence for involvement of T cell-mediated immunity in the exacerbation of DSS-induced acute colitis in WT mice (19). We found in the current study that SCID mice developed acute colitis by DSS administration, confirming that DSS-induced acute colitis occurred in a T cell-independent manner. However, there was no difference in severity of DSS-induced acute colitis between SCID mice and SCID×CD30L−/− mice. These results suggest that CD30L plays an important role in exacerbation of DSS-induced acute colitis in a T cell-dependent manner. CD30L/CD30 signaling was widely examined and discussed over the years, and most in vitro experimental models were set to describe the interactions between CD30+ T cells and CD30L+ APCs (4). In the current study, CD30L was expressed mainly by CD4+ T cells in mice with DSS-induced colitis. We have recently found that CD30L+ T−CD30+ T cell interactions quite possibly existed in induction of colitis in SCID mice transferred with naive CD4+ T cells (12). Thus, CD30L/CD30 signaling executed by CD4+ T−CD30+ T cell interaction may play an important role in exacerbation of DSS-induced acute colitis.

T cell-mediated immunity is associated with pathogenesis of chronic colitis induced by multiple cycles of DSS or in the recovery phase of exaggerated colitis induced by DSS (16). Effector CD4+ T cells in DSS-induced chronic colitis have long been considered as a Th1-type colitis animal model resembling Crohn’s disease, but recent studies challenged this paradigm by demonstrating that Th17 cells were responsible for the development of DSS-induced colitis (19, 20). However, it has recently been shown that IL-17A suppressed Th1-mediated colitis by directly inhibiting Th1 cell activation (25). Thus, the role of IL-17A in colitis is controversial, depending on the experimental protocol and genetic background of mice used in each experiment (19–23, 37–39). In the current study, we confirmed an importance of IL-17A in the pathogenesis of both types of DSS-induced colitis using mice lacking IL-17A, which developed milder forms of both types of DSS-induced colitis. CD30L−/− mice are resistant to both types of DSS-induced colitis accompanied by impaired production of IL-17A but increased IL-2 production by T-LPLs. We have recently found that reverse signal via CD30L downregulates IL-2 production by activated CD4+ T cells (12). IL-2 was recently shown to inhibit Th17 differentiation through STAT5 activation (40). Taken together, we speculate that lack of CD30L signaling suppressed Th17 differentiation and attenuated DSS-induced colitis at least partly via upregulation of IL-2 production by CD30L−/− CD4+ T cells.

**FIGURE 6.** Soluble mCD30-Ig inhibits Th17 cell differentiation in vitro. A. Naïve CD4+ T cells were sorted from WT mice and cultured under Th1-, Th2-, or Th17-polarizing conditions for 4 d with murine IgG1 or soluble mCD30-Ig. IFN-γ, IL-4, IL-17A, and IL-2 secretions were analyzed by ELISA. B. Intracellular cytokine FACS analysis of IL-17A or IL-2–producing CD4+ T cells of WT mice after culture in Th17-polarizing conditions with murine IgG1 or soluble mCD30-Ig. Data indicate mean ± SD (five mice per group) obtained from a representative of three separate experiments. Statistical differences in other data were evaluated by a Student t test. A and B: *p < 0.05; **p < 0.01.
We previously reported that CD30L−/− mice were susceptible to Th2-type, experimental OXA-induced colitis but resistant to Th1-type, TNBS-induced acute colitis. The level of Th1-type cytokine IFN-γ in T-LPLs was significantly lower in OXA- or TNBS-treated CD30L−/− mice than that in WT mice (29). We also found that CD30L−/− mice were susceptible to infection with bacillus Calmette-Guérin in association with reduced Th1 responses (36). These results suggested that CD30L/CD30 signaling is linked to Th1 cell responses and Th1-associated diseases. In the current study, IFN-γ-producing Th1 cell response in vivo was impaired in both types of DSS-induced colitis in CD30L−/− mice, although inhibiting effect of mCD30-Ig on Th1 cell differentiation was not apparent unlike Th17 differentiation. We speculate that CD30L/CD30 signaling may be important for amplification of already differentiated effector Th1 cells in synergy with IL-12. Addition of exogenous IL-12 may be sufficient for in vitro Th1 differentiation under Th1-polarizing condition without CD30L/CD30 signaling. Recently, it has been reported that TL1A (TNFSF15) regulates the development of DSS-induced chronic colitis by modulating both Th1 and Th17 activation (20). TL1A is thought to act as a central regulator in the development of T cell-mediated chronic intestinal inflammation by directly enhancing IFN-γ production from Th1 cells and by enhancing IL-17A production from Th17 cells in combination with IL-12 and IL-23. CD30L, a member of the TNF family, has the potential to induce TNF receptor-associated factor 2–mediated NF-κB activation via CD30, which can recruit several TNF receptor-associated factors (41, 42). Therefore, it is possible that, like TL1A, CD30 signaling may contribute directly to Th1 cell differentiation. Both Th1 and Th17 cells play critical roles in the pathogenesis of DSS-induced colitis. We speculate that Th1 cells may promote the entry of Th17 cells to the colon as reported in experimental autoimmune encephalomyelitis in an animal model of multiple sclerosis (43, 44). Further analysis is required to elucidate this possibility.

Treatment of IBD has greatly advanced since the introduction of biological reagents, such as anti–TNF-α mAbs. However, given the enormous number of patients with autoimmune diseases, safety and the cost of current injectable protein-based therapies are a matter of concern. Because the relative importance of Th1 and Th17 cells in many autoimmune diseases has been clarified, we propose from our results that CD30L could be a candidate target molecule for use in treatment. Modulation of CD30L signaling by soluble CD30 could be a novel biological therapy for IBD and other inflammatory diseases associated with both Th1 and Th17 cells.
FIGURE 8. mCD30-Ig treatment ameliorates DSS-induced chronic colitis. Chronic colitis was induced in WT mice by 1.5% DSS, and 200 μg mCD30-Ig or murine IgG1 was i.p. injected as described in Materials and Methods. A, Body weight, survival rate, colon length, and histological analysis (original magnification ×200) are shown (n = 10 mice per group). B and C, Cytokine production with or without stimulation was analyzed by ELISA on day 25 in chronic colitis (n = 10 mice per group). Data indicate mean ± SD obtained from a representative of three separate experiments. The difference in survival rates was evaluated by the log-rank test (Mantel–Cox). Statistical differences in other data were evaluated by a Student t test. A–C: *p < 0.05; **p < 0.01.

Acknowledgments
We thank Kyoumi Akasaki and Akiko Yano for excellent technical assistance.

Disclosures
The authors have no financial conflicts of interest.

References

The Journal of Immunology 7679